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The need for speed. I. Fast reactions and myelinated axons in copepods

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Abstract A rapid and powerful escape response decreases predation risk in planktonic copepods. Calanoid copepods are sensitive to small and brief hydrodynamic disturbances: they respond with multiple nerve impulses to a vibrating sphere. Some species, such as *Pleuromamma xiphias* and *Labidocera madurae*, respond with very large spikes (1–4 mV), whereas maximum spike heights are an order of magnitude smaller in others, such as *Undinula vulgaris* and *Neocalanus gracilis*. A comparative study of the escape responses showed that all species reacted within 10 ms of the initiation of a hydrodynamic stimulus. However, *U. vulgaris* and *N. gracilis* had significantly shorter reaction times (minimum reaction times: 1.5 ms and 1.6 ms) than the other two, *P. xiphias* (6.6 ms) and *L. madurae* (3.1 ms). Examination of the first antenna and the central nervous system using transmission electron microscopy revealed extensive myelination of sensory and motor axons in the two species with the shorter reaction times. Axons of the other two species resembled typical crustacean unmyelinated fibers. A survey of 20 calanoids revealed that none of the species in two of the more ancient superfamilies possessed myelin, but myelination was present in the species from three more recently-evolved superfamilies.

Key words Crustacean · Escape behavior · Mechanosensitivity · Phylogeny · Myelination

Introduction

Timeliness in an escape response is key to avoiding predation for many organisms. Many zooplankton, inhabiting pelagic environments with little cover, depend on an effective escape reaction for survival. Among these are the calanoid and cyclopoid copepods. These small crustaceans (< 3 mm) usually dominate the marine zooplankton and form a key component of oceanic food webs. During an escape "jump," a copepod propels itself forward at 200–500 body lengths per second through the coordinated power strokes of its four or five pairs of swimming legs, the pereiopods (Storch 1929; Strickler 1975; Fields 1996). This escape response may lower predation risk by as much as 50% compared to less evasive prey (Drenner et al. 1978; Browman et al. 1983; Trager et al. 1994). The rate of work output per gram of muscle during such an escape jump is among the highest in the animal kingdom (Svetlichnyy 1987; Lenz and Hartline 1999).

Although all pelagic copepods exhibit a strong escape response, species-specific variation exists. This may lead to differences in susceptibility to certain predators, which may affect the structure of planktonic communities (Brooks and Dodson 1965). Relative effectiveness of the escape response may contribute to observed copepod distribution and abundance patterns (e.g., Kimmerer 1991; Hays et al. 1997). A critical component of the escape behavior is the time it takes an animal to initiate a response. We have recently measured the reaction time of a calanoid (*Undinula vulgaris*) and have found it to be exceptionally short (< 3 ms; Lenz and Hartline 1999). We also discovered that many, but not all, calanoid copepods have myelin-like sheaths enveloping many of their axons (Davis et al. 1999). The calanoids possessing myelin sheaths may be presumed to have increased nerve impulse conduction velocities by a factor of ten or more (Ritchie 1984). Although increased conduction velocities due to myelination have been well documented in both vertebrates and invertebrates, no studies have linked this physiological advantage to improved behavioral performance. In the present study, we quantify the escape responses in four species of calanoids. We correlate the reaction time with the presence or absence of myelin-like sheaths. We demonstrate that the two species with the

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shortest reaction times are also the two with myelin, while the slower species lack it. It appears that by utilizing myelin, copepods in certain groups have increased their escape effectiveness by improving their response time. A survey of 20 species showed that myelin is present in calanoids belonging to more recently evolved superfamilies. The presence/absence of myelin leads to predictions of the physiological and behavioral patterns in species not yet studied by these methods.

Materials and methods

Collection

Our work focused on four calanoid species from three superfamilies of the suborder Calanoida: *Pleuromamma xiphias* (superfamily Augaptiloidea: family Metridinidae), *Labidocera madurae* (Centropagoidea: Pontellidae), *U. vulgaris* (Megacalanoidea: Calanidae), *Neocalanus gracilis* (Megacalanoidea: Calanidae). *L. madurae* and *U. vulgaris* are common sub-tropical surface-dwelling species. These two species overlap in their distribution, although *L. madurae* is found primarily near shore, and *U. vulgaris* is more widely distributed. *P. xiphias* is a widespread mesopelagic species (daytime occupation of 0.1–1 km depths), which is characterized by extensive diel vertical migration (Haury 1988; Mauchline 1998). *N. gracilis* is a subtropical oceanic species, which inhabits the epipelagic (surface to 0.1 km depth) regions (Ambler and Miller 1987; Mauchline 1998). Subsurface tows with a plankton net were used to collect *U. vulgaris* and *L. madurae* from inside Kaneohe Bay, Oahu, Hawaii. Additional species collected from Kaneohe Bay included *L. pavo*, *Bestiolina similis* and *Acartia fossae*. *N. gracilis*, as well as *Candacia aethiopica*, *Centropages* sp., and *Euchaeta rimana* were collected ca. 2 km offshore from Kaneohe Bay with sub-surface net tows. *Pleuromamma xiphias*, as well as *N. gracilis* and *E. rimana* were collected 4 km offshore from Kailua-Kona, Island of Hawaii with oblique plankton tows between 100 m and the surface at night. Additional *P. xiphias*, *Gaussia princeps* and *Euchirella* sp. were collected at the Natural Energy Laboratory of Hawaii, Keahole Pt., Hawaii, using a net attached to the end of a pipe pumping water from 586 m depth. Calanoids from San Juan Strait, Puget Sound, Washington were collected with oblique plankton tows from ca. 120 m (*Calanus pacificus*, *C. marshallae*, *Eucalanus bungii*, *Pareuchaeta elongata*, *Pseudocalanus moultoni*). *Epilabidocera longipedata* were collected with a bucket off the Friday Harbor Laboratories pier. After collection, adult and late copepodid stages were sorted and maintained in 2- or 4-l jars with fresh seawater prior to the physiological and behavioral experiments. The animals prepared for TEM were either fixed immediately or kept for up to 48 h before fixation.

Experimental set-up – electrophysiological

Impulse traffic in the first antennae (A1) was monitored by clamping copepods in forceps and drawing them into an overlying layer of mineral oil, leaving the antenna protruding into the sea-water bath (Fig. 1A). Extracorporeal recordings from the A1 sensory nerve were made between forceps and bath as described previously (Yen et al. 1992; Lenz and Yen 1993). The amplifier used had a corner frequency of 10 kHz, producing some filtering of the recorded spikes. A1 mechanosensors were stimulated with hydrodynamic disturbances generated by a vertically moving 3-mm sphere placed a few millimeters from the first antenna. The resulting discharge was digitized at 40 kHz and stored on computer (see below; Gassie et al. 1993; Hartline et al. 1996). Sample spike heights and half-amplitude durations were measured from recordings from different experiments. Significant differences between species were tested with the Mann-Whitney *U*-test (Siegel 1956).

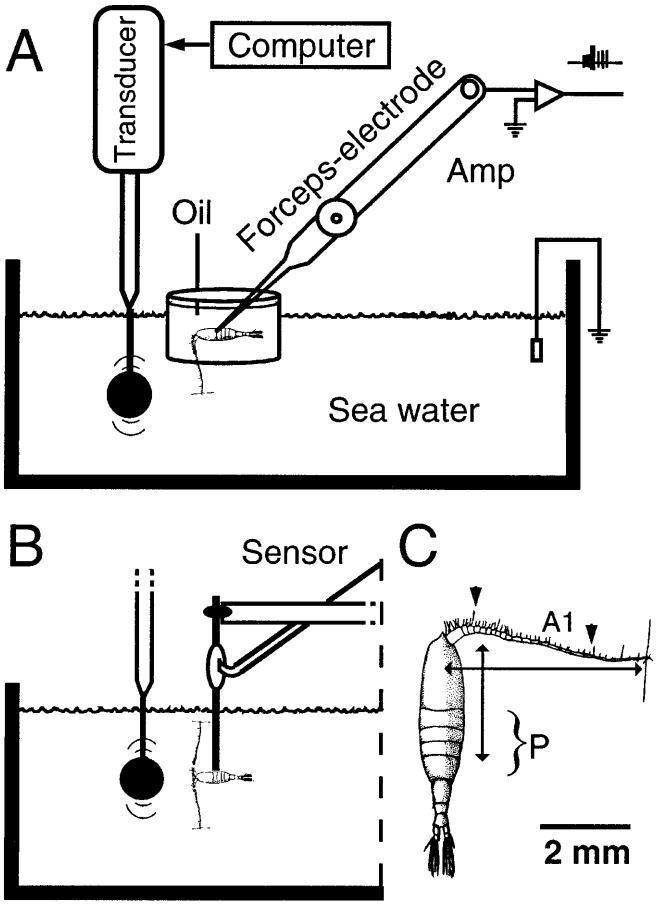

Fig. 1A–C Experimental setups. **A** Setup for recording sensory discharges. Under computer control, water movements are generated by vertical displacement of sphere, activating setal mechanoreceptors located along the first antenna. Copepod is held in oil by stainless steel forceps with the antenna projecting into seawater bath. Amplified neuronal activity picked up by the forceps electrode (Welsh et al. 1968) is digitized and stored on computer. **B** Setup for recording force production during escape reactions. Copepod is fastened to aluminum wire tether, which deflects slightly with applied forces. Force is monitored by a fiber-optic displacement sensor, which measures distance to a small mirror mounted on the wire. **C** Drawing of top view of calanoid copepod (*Pleuromamma xiphias*) showing relative dimensions. *A1* first antenna; *P* position of pereiopods (swimming legs) located on ventral surface. EM sections taken at arrow heads

Experimental set-up – behavioral

Behavioral responses to hydrodynamic disturbances were monitored in individual copepods tethered to a force transducer as described in detail in Lenz and Hartline (1999) (Fig. 1B). Briefly, individual copepods were glued to a stiff wire with cyanoacrylate adhesive (Krazy Glue). During an escape jump, the force produced by the power strokes of the pereiopods was monitored by measuring the slight movement of the wire with a fiberoptic displacement sensor (Philtec 88 N). The hydrodynamic stimulus was produced by the vertical movement (approximately parallel to the receptor-bearing first antennae) of a sphere (3–5 mm diameter) centered 3.5–5 mm from the rostrum of the copepod. Rapid sphere displacements of up to 40 μm were produced under computer control by a piezoelectric transducer (Burleigh PZL-015 or PZL-060). Water movements at the animal were calculated using the equation for the dipole spread of near-field disturbances for this configuration: $d = -\frac{1}{2}D(a/r)^3$, where d is computed water

displacement at the animal, D is the displacement of the sphere, a is sphere radius and r is the distance from the center of the sphere to the animal, as discussed previously (Harris and van Bergeijk 1962; Gassie et al. 1993; Lenz and Hartline 1999). Reaction times were measured from the onset of a stimulus waveform to the onset of a detectable forward propulsion corresponding to commencement of posterior movement of the pereiopods (Lenz and Hartline 1999). Non-parametric statistical comparisons among species were done using the Kruskal-Wallis one-way analysis of variance for multiple independent samples and the Mann-Whitney U-test for pair-wise comparisons (Siegel 1956).

Physiological delays – computation

Minimum expected physiological delays were computed based on standard crustacean parameters. Sensory transduction was estimated at 200 μs, as Yen et al. (1992) reported neural response times in a calanoid copepod under 1 ms recorded ca. 0.5 mm from the sensory setae. Conduction velocities for unmyelinated crustacean axons were calculated from the relation velocity $v = 1.4\sqrt{\rho}$ for fibers of radius ρ, based on the study of lobster medial giant axons by Govind and Lang (1976). An axon diameter of 5 μm was assumed, based on transmission electron micrographs of the largest axons in the first antenna of *Pleuromamma xiphias* (Lenz and Yen 1993), giving theoretical conduction velocities of ca. 1.5 m s^{-1}. Conduction distances were estimated from the tip of the antenna to the central nervous system (CNS) (4 mm for *P. xiphias* and 2 mm for *U. vulgaris*) and from the CNS to the 4th pereiopod (2 mm). In order to compute minimum delays, we assumed all neuron-to-neuron synapses to be electrical with 0.2-ms delays (Roberts et al. 1982) and the delay at the neuromuscular chemical synapse to be 0.4 ms (Katz and Miledi 1964). Activation delay, from muscle depolarization to the onset of contraction, was estimated at 2 ms based on the fastest-known crustacean muscle, the antennular remotor of lobster (Mendelson 1969). While there is good reason to expect differences in quantitative values for these parameters in copepods, they provide an instructive starting point for interpreting measured reaction times.

Transmission electron microscopy

Two methods of fixation were used: conventional chemical fixation with glutaraldehyde and ultrarapid cryofixation. Details are given in Weatherby et al. 2000). Briefly, chemical fixation employed 4% glutaraldehyde in 0.1 mol l^{-1} cacodylate buffer (pH 7.4–7.6) with 0.35 mol l^{-1} sucrose, followed by 1% OsO_4 (protocol of Weatherby 1981). Cryofixation involved plunging specimens into freezing propane (−187 °C; Reichert-Jung KF80 system), followed by transfer to 1% OsO_4 in either acetone or methanol at −190 °C, then placement in a freezer (−80 °C) for 6 days prior to dehydration and infiltration with LX 112 (Ladd) epoxy resin and polymerization. Sections (80–90 nm) were stained with uranyl acetate and lead citrate, and viewed with a Zeiss 10/A TEM at 80 kV and 100 kV.

Results

Species differences in mechanoreceptor spikes suggest differences in physiology

Calanoids are characterized by a prominent pair of first antennae (or antennules, "A1"; Fig. 1C). Detection of potential threats is achieved by the mechanosensory setae located on the anterior edge and distal tip of the first antenna (Gill 1985; Gill and Crisp 1985; Bundy and Paffenhöfer 1993; Weatherby et al. 1994; Lenz et al. 1996; Fig. 1C). Responses to mechanical stimulation of A1 receptors from copepods of two different superfamilies obtained with extracorporeal recordings are shown in Fig. 2. Both species responded with increased neural activity when presented with a 200-Hz sinusoidal stimulus. Large nerve impulses were recorded from two reidentifiable units from mechanoreceptors in *P. xiphias* (as reported by Lenz and Yen 1993; Hartline et al. 1996). The smaller of the two (the "A" unit) ranged in amplitude from 1.5 mV to 2.4 mV in good preparations (three individuals). *U. vulgaris* was also very sensitive to mechanical perturbations, but similar physiological recordings showed units that were over an order of magnitude smaller than those in *P. xiphias*. The largest unitary spike in five preparations ranged in amplitude from 80 μV to 180 μV. In addition to a consistent amplitude difference, there was a 50% difference in spike duration. Durations at half-amplitude were 153 ± 22 μs for *P. xiphias* (mean ± SD, $n = 3$) and 98 ± 17 μs for *U. vulgaris* ($n = 5$). Spike durations were significantly different between the two species ($P \ll 0.0001$, Mann-Whitney U-test, one-tailed). The faster spikes would have been attenuated somewhat more than the slower ones by the amplifier filtering characteristics, but this would account for $<2\%$ of the observed difference. Since the recording situation was the same in these two cases, it suggests that differences in morphology or

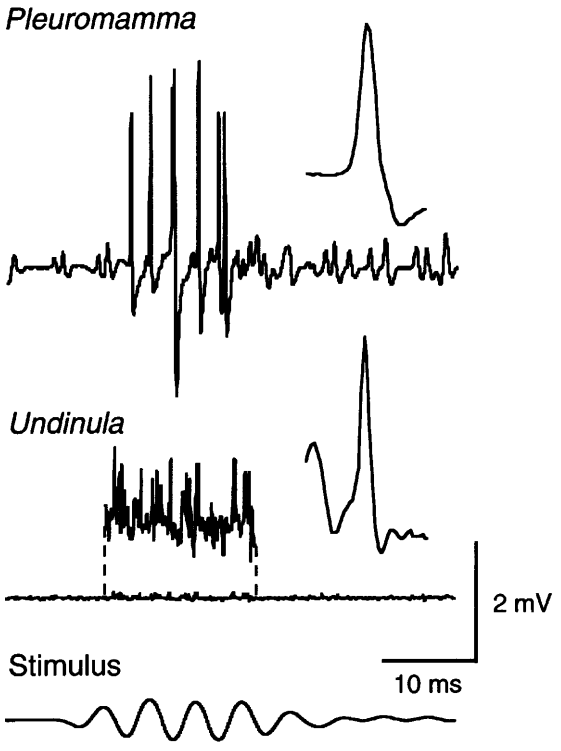

Fig. 2 Extracellular recordings of nerve impulses in the first antenna of *P. xiphias* (*top*) and *Undinula vulgaris* (*middle*) in response to sinusoidal movement of a 3-mm-diameter sphere (*bottom*). Above the lower trace is a tenfold vertical expansion of that portion. Single-spike insets show 1-ms traces with amplitudes normalized. Stimulus magnitude: 3× threshold (to elicit multiple spikes). Expts: PLfb9–18; UN9–15

physiology are responsible for the differences in both spike amplitude and duration in the two species.

Reaction times differ among species

U. vulgaris and *P. xiphias* responded readily to sudden mechanical perturbation with an escape response. In Fig. 3, we show force records of such responses from these two species. To a short sinusoidal (1.5 cycle, 700 Hz) movement of the stimulating sphere commencing at time zero, the calanoids responded with multiple force transients produced by the combined power strokes of the four pairs of swimming legs. The escape responses produced by the two species differed: *U. vulgaris* responded within a few milliseconds with a short burst of kicks (peaks in force record of Fig. 3), the first kick usually being the strongest. The entire behavioral response rarely lasted longer than 70 ms. In contrast, escape responses by *P. xiphias* to a single short stimulus presentation were characterized by long trains of kicks, often exceeding 20 in number (force production in these two species has been analyzed by Lenz and Hartline 1999 and Hartline et al. 1999). The reaction times were longer and the first 10 kicks showed a steady increase in the maximum force produced.

It is instructive to calculate expected minimum reaction times using conventional physiological parameters. A detailed description of the anatomy of a calanoid copepod by Park (1966) indicates that the neural circuit for the escape response is similar to that of other crustaceans: sensory cells synapse onto a giant interneuron that travels in the ventral nerve cord. This cell in turn synapses onto giant motor neurons that innervate the muscle. For a calanoid with this circuitry, the delays contributing to the reaction time for a hydrodynamic stimulus can be estimated (see Materials and methods) as 0.2 ms for sensory transduction, 0.4 ms for two electrical synapses, 0.4 ms for the neuromuscular synapse and 2 ms for muscle tension development. Adding conduction delays computed at 1.5 m s^{-1} along axonal lengths totaling 6 mm for an animal of the dimensions of *P. xiphias*, or 4 mm for *U. vulgaris*, gives total predicted minimum reaction times of about 7 ms and 5.7 ms, respectively.

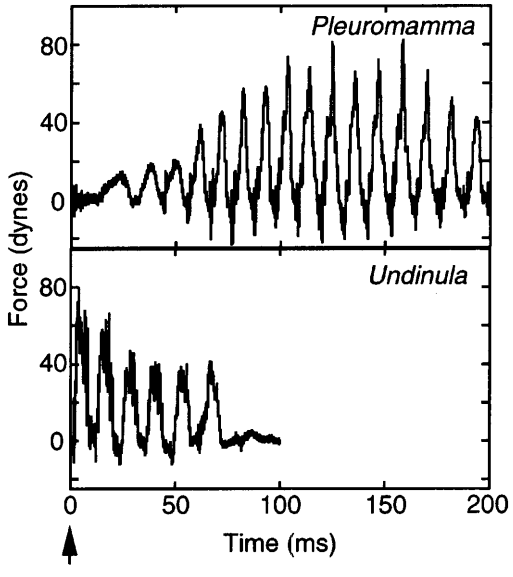

Fig. 3 Comparison of force-transducer traces showing rapid swim response in *P. xiphias* and *U. vulgaris* following a 1.5-cycle, 700-Hz movement of a sphere. *P. xiphias*: 100× threshold (BPL97–5.D01). *U. vulgaris*: 3–6× threshold (UN96–11.D04). Stimulus presented at time zero (*arrow*)

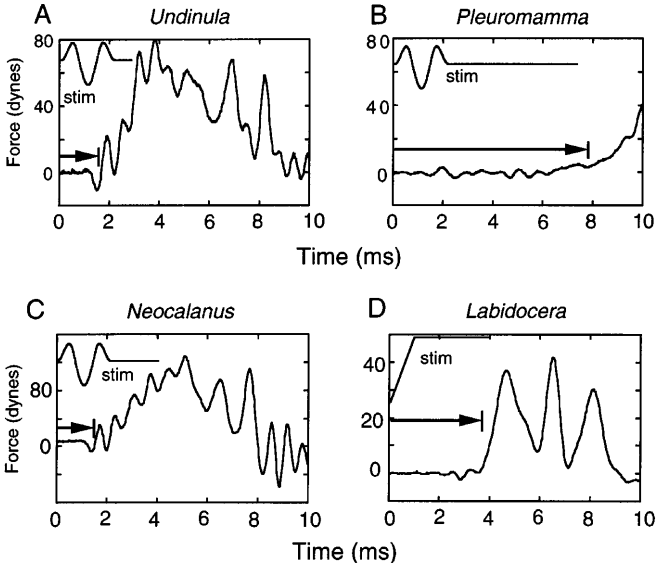

Fig. 4 Force production during escape responses in four calanoid copepod species: **A** *U. vulgaris* (expansion of Fig. 2 record); **B** *P. xiphias* (100× threshold; BPL97–3.D10); **C** *Neocalanus gracilis* (18× threshold; CL97–2.D19); **D** *Labidocera madurae* (threshold not determined; LA97–3.D05). *Arrows* show measurement of reaction time. Note "preparatory movement" preceding onset of pereiopod (*forward*) propulsion (**A, C**). Stimulus: 1.5-cycle, 700-Hz movement of a sphere (**A, B, C**) or a short ramp (**D**) presented at time zero (*insets*)

Fast sweeps showing precise reaction times for the two species are shown in Fig. 4A,B. Histograms for the reaction times are presented in Fig. 5A. The median reaction times were 9.2 ms and 1.9 ms for *P. xiphias* and *U. vulgaris*, respectively. The shortest reaction times measured for these two species were 6.6 ms and 1.5 ms. The observed minimum for *P. xiphias* agreed well with the theoretical estimate. However, all reaction times measured for *U. vulgaris* were significantly shorter than expected (Figs. 4A, 5A).

Differences in reaction times were also seen in other species. Figures 4C and 4D show force records, and Fig. 5B the histograms for reaction times for *N. gracilis* and *L. madurae*, two species that are similar in size to *U. vulgaris* and *P. xiphias*. Median reaction time for *L. madurae* was 5.7 ms with a minimum latency of 3.1 ms. That for *N. gracilis* was similar to *U. vulgaris* with a median of 1.9 and a minimum of 1.6 ms. A Kruskal-Wallis one-way analysis of variance on the minimum and median latencies indicated significant

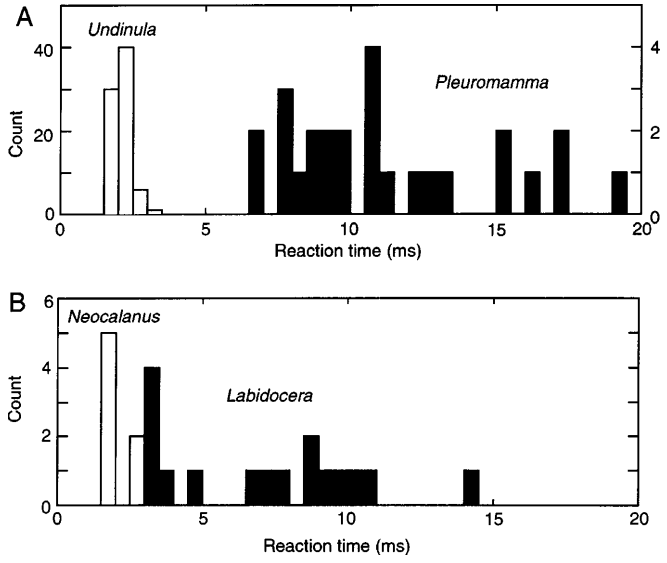

Fig. 5 Reaction time histograms for four calanoid species: **A** *U. vulgaris* (*clear bars: scale on left*) and *P. xiphias* (*filled bars; scale on right*); **B** *N. gracilis* (*clear bars*) and *L. madurae* (*filled bars*). A minimum of 2 and a maximum of 20 measurements were made on each individual. Range of means for individuals were (number of individuals tested in parentheses): *U. vulgaris*: 1.7–2.3 ms ($n = 10$); *P. xiphias* 8.4–17.6 ms ($n = 7$); *N. gracilis*: 1.9–2.2 ($n = 2$); *L. madurae*: 3.4–10.1 ms ($n = 3$)

differences among the four species ($P < 0.002$, two-tailed). The minimum reaction times for all 22 experiments in which they were measured are presented in Fig. 6 as cumulative distributions. For each species, it plots the rank of the minimum reaction time (expressed as a percentage of all experiments on that species) versus its value (in milliseconds). The 50% level corresponds to the median. The distributions of minimum reactions times for *N. gracilis* and *U. vulgaris* superimpose. A statistical comparison of minimum latencies from these two species showed no significance difference (Mann-Whitney U-test, $P \gg 0.05$, one-tailed). *L. madurae* responded more quickly than *P. xiphias*

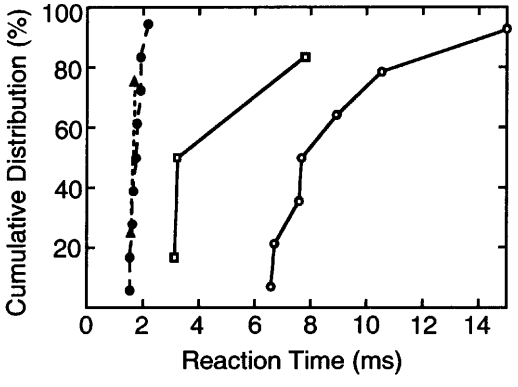

Fig. 6 Cumulative distribution of minimum reaction times for four species measured in each of the experiments in Figs. 4 and 5. *Closed symbols*: myelinated species, *U. vulgaris* (*circles*), and *Neocalanus gracilis* (*triangles*). *Open symbols*: non-myelinated species, *L. madurae* (*squares*) and *P. xiphias* (*circles*)

(Mann-Whitney *U*-test, $P = 0.092$, one-tailed). Comparison between the latencies of *L. madurae* and *P. xiphias* versus those of the two faster species indicated a significant difference between these two pairs of species (Mann-Whitney *U*-test, $P < 0.001$, one-tailed). In fact, the longest minimum reaction time measured in the two faster species was less than the shortest reaction time in the two slower species. Part of the explanation for the differences in these two groups was found in axonal morphology.

Myelin is found in the faster copepods

An examination of the first antenna of these calanoid copepods revealed that *P. xiphias* and *L. madurae*, the two species with the longer reaction times, had axons enveloped by a simple membrane (Fig. 7A, B), as is usual in crustacean nerves in the first and second antennae (antennule and antenna). However, in the two faster species, the axons in the first antenna were enveloped by a multilamellar sheath, resembling myelin (Fig. 7C, D). Cross-sections through the cephalothorax showed a similar pattern: in *U. vulgaris* and *N. gracilis* the giant axons as well as many other axons were enveloped by myelin-like sheaths, whereas in *L. madurae* and *P. xiphias* all were unmyelinated. A survey of temperate and sub-tropical, neritic and oceanic, epipelagic and mesopelagic calanoid species showed that myelinated axons are ubiquitous in some taxa and completely absent from others. Myelinated axons were present in the superfamilies Megacalanoidea, Eucalanoidea and Clausocalanoidea. They were absent from the Augaptiloidea and Centropagoidea (Table 1, Fig. 8). Myelination did not correlate with calanoid size: even the small (< 1 mm) paracalanid species had myelinated axons, yet myelination was absent from the large *G. princeps* (ca. 7–9 mm).

Discussion

Although it is well recognized that myelin conveys a significant advantage in speeding up nervous systems, a direct behavioral demonstration of this is difficult. Among vertebrates, the non-myelinated forms (Agnatha: lamprey) have been driven into restricted ecological refugia, accompanied by modification of body form, owing to competition from advanced (myelinated) species. Calanoids are unusual in that both myelinated and non-myelinated species of similar body form and life history co-exist, allowing a direct comparison. We have found that the megacalanoideans, *U. vulgaris* and *N. gracilis*, have exceptionally short reaction times, which correlate with extensive myelination of sensory, motor and central axons in these two species, including the giant interneurons of the CNS (Weatherby et al. 2000). In contrast, *P. xiphias* and *L. madurae* take longer to respond and these species lacked the multilamellar

Table 1 Survey of calanoid species indicating the presence (+) or absence (−) of myelin, and spike heights recorded from the first antenna. All species belong to the order Calanoida, and their phylogenetic relationships are given

Superfamily[1]	Family	Species	Spike height[2]	Myelin sheath
Augaptiloidea Sars, 1905	Metridinidae	*Pleuromamma xiphias*	Giant[3]	−
		Gaussia princeps	Giant[4]	−
Centropagoidea Giesbrecht, 1892	Acartiidae	*Acartia fossae*	Giant	−
	Candaciidae	*Candacia aethiopica*	Giant	−
	Centropagidae	*Centropages* sp.	Small[5]	−
	Pontellidae	*Labidocera pavo*		−
		Labidocera madurae	Giant	−
		Epilabidocera longipedata		−
	Temoridae	*Temora longicornis*		−[6]
Megacalanoidea Sewell, 1947	Calanidae	*Calanus finmarchicus*		+[7]
		Calanus pacificus		+
		Calanus marshallae		+
		Undinula vulgaris	Small	+
		Neocalanus gracilis	Small	+
	Paracalanidae	*Bestiolina similis*		+
Eucalanoidea Giesbrecht, 1892	Eucalanidae	*Eucalanus bungii*		+
Clausocalanoidea Giesbrecht, 1892	Aetideidae	*Euchirella* sp.	Small	+
	Clausocalanidae	*Pseudocalanus moultoni*		+
	Euchaetidae	*Euchaeta rimana*	Small[3]	+
		Pareuchaeta sp.	Small	+[8]

[1] Nomenclature follows Andronov (1974) and Park (1986), but differs from the one given in Andronov (1991) (Augaptiloidea = Arietelloidea; Centropagoidea = Diaptomoidea; and Megacalanoidea = Calanoidea)
[2] Yen et al. (1992), except as indicated
[3] Lenz and Yen (1993)
[4] Lenz (1993)
[5] Probably incorrect: see Hartline et al. (1996)
[6] Gill (1986)
[7] Lowe (1935) and Barrientos (1980)
[8] *P. elongata*

axonal sheaths. A tenfold higher conduction speed is expected in a myelinated fiber compared to an unmyelinated fiber of the same diameter (Ritchie 1984). In the fast copepods of this study with 4 mm of total axonal length, myelination would shorten reaction times by ca. 2.4 ms. This could account for a significant fraction (but perhaps not all) of the observed differences between myelinated and non-myelinated species.

Spikes in myelinated copepods are smaller and faster

A dichotomy among the calanoids was noted earlier in electrophysiological recordings of mechanosensory units in the first antennae (Yen et al. 1992; Hartline et al. 1996) and confirmed in the present study. Certain species exhibited a mechanically-evoked nerve impulse of several millivolts amplitude (extracellular records; Yen et al. 1992; Lenz and Yen 1993). In contrast to these "giant antennal mechanoreceptor" units, the largest mechanoreceptive units recorded in other calanoids were an order of magnitude smaller. In the present study, we have noted that the giant spikes are also slower than the spikes from the small-spike species. The giant-spike, non-myelinated species possess at least two axons in the first antenna that are especially large in comparison with others (e.g., Fig. 7B; Yen et al. 1992; Lenz and Yen 1993; Hartline et al. 1996). These are likely to underlie or contribute to the giant-sized spikes. Since giant axons are a well-known adaptation for rapid behavioral reactions and are typically involved in escape behavior, it was puzzling that some of the more recently evolved calanoids (see below) seemed to have "lost" the giant units. The correlation between small-spike species and myelination can be understood in terms of the decrease in internodal radial current leakage produced by myelin. This reduces membrane current, restricts it to the nodes, produces major changes in electrical properties of axons, and concomitantly increases conduction speed. Along with any alterations in diameter and membrane properties associated with acquisition of myelin, this might easily explain the decreases observed in amplitude and duration of extracellularly recorded spikes.

Myelination follows phyletic lines

Calanoid evolution is monophyletic (Park 1986; Fig. 8). The "giant-spike species" are comprised of members of the more primitive superfamilies (Augaptiloidea and Centropagoidea), while most of the "small-spike species" are more recently evolved (Megacalanoidea and Clausocalanoidea; Hartline et al. 1996). In the present study, we found that the presence of myelin corresponds to the more recently evolved superfamilies as well (Fig. 8). Myelin is apparently absent not only from the Augaptiloidea and Centropagoidea but also from the one cyclopoid that we have examined (*Sapphirina* sp.), the cyclopoids sharing with these calanoids a still earlier common ancestor. In addition to the 20 species examined so far with electron microscopy (Table 1), several additional species have been categorized according to

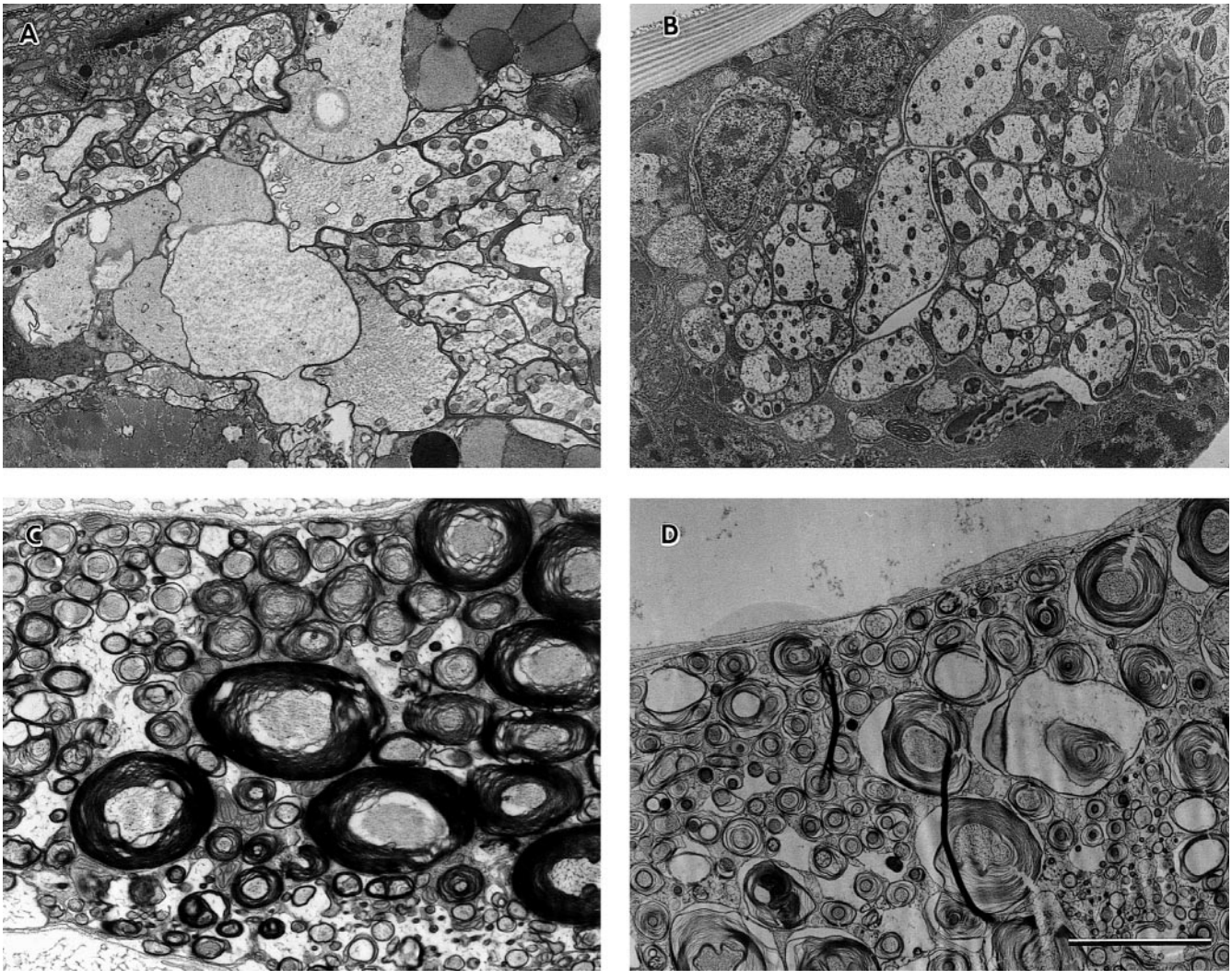

Fig. 7A–D Transmission electron micrographs of cross-sections through nerves of the first antenna of four calanoids. **A** *P. xiphias*, ca. segment XX (chemical fixation). **B** *L. madurae*, ca. segment XX (chemical fixation). Note the two especially large axons putatively corresponding to the giant mechanoreceptor spikes. **C** *U. vulgaris*, sensory nerve, segment V (cryofixation). **D** *N. gracilis*, sensory nerve, segment V (cryofixation). Nomenclature of segments of the first antenna follows Huys and Boxshall (1991). Scale bar 5 μm

spike size in previous work (Yen et al. 1992; Hartline et al. 1996). The large-spike species from the genera *Metridia* (Augaptiloidea) and *Pontellopsis* (Centropagoidea) are thus presumed to be non-myelinated, while the small-spike species from the genera *Cosmocalanus* (Megacalanoidea) and *Aetideus* and *Gaetanus* (Clausocalanoidea) should be myelinated. These conclusions are supported by the phylogenetic status of the genera. The pattern, while still based on a small number out of the ca. 1800 species of calanoid copepods, predicts that: (1) species belonging to the three earliest superfamilies (Platycopioidea, Epacteriscioidea and Pseudocyclopoidea) will lack myelin, and (2) the remaining recent superfamilies, Bathypontioidea, Ryocalanoidea and Spinocalanoidea, will possess it (Fig. 8).

Myelination correlates with ecology

Park (1986) compared the geographic distribution of calanoid superfamilies and noted a correlation between phylogeny and ecology that parallels the phylogenetic distribution of myelin. The Augaptiloidea (non-myelinated) are limited to deep-sea forms. Many are strong vertical migrators, avoiding predators by dwelling in the dark depths by day and ascending to surface waters (upper 100 m) to feed at night (e.g., Ambler and Miller 1987; Hays et al. 1997). The Centropagoidea (non-myelinated) are also restricted, occurring primarily in freshwater or neritic habitats, where environmental variability adds ecological factors that may lower the relative impact of predator pressure. In contrast, the myelinated Clausocalanoidea and Megacalanoidea are more widespread, inhabiting the upper regions of the open oceans, as well as the neritic and deep environments. Ecosystems of the open ocean are environmentally stable and driven by biological interactions: food limitations in the face of dilute resources and susceptibility to predation in the face of high predator diversity (Hayward and McGowan 1979). Various factors have

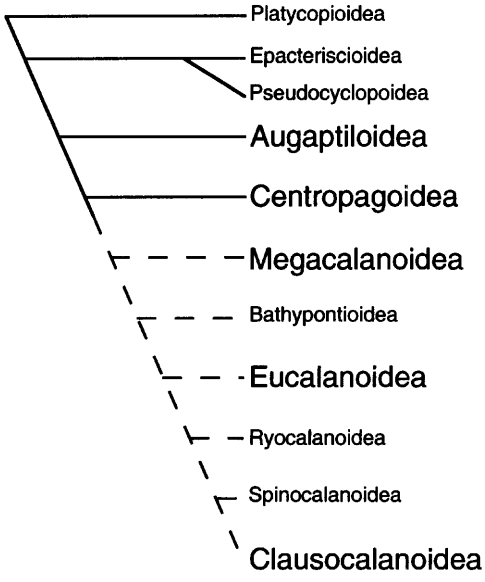

Fig. 8 Cladogram of the Calanoida showing a monophyletic origin for the superfamilies as proposed by Andronov (1974) and Park (1986). Line type indicates absence (*solid lines*) or presence (*broken lines*) of myelin, whether surveyed directly or inferred (predicted). Superfamilies surveyed (see Table 1) indicated in large typeface. Those indicated in small typeface have not been surveyed

been cited as affecting susceptibility to predation, including body size and pigmentation (Hays et al. 1994), but neither of these falls easily along the phyletic lines indicated by Park (1986). Hays et al. (1997) suggested that a superior escape ability (they detected a correlation with body shape) might allow some species (megacalanoids in their study) to remain in surface waters of the open ocean whereas others (augaptiloids) depend on vertical migration to avoid predation. We propose that myelin may be the key difference between these species: escape responses to hydrodynamic disturbances are initiated two to five times faster in the myelinated species than in the non-myelinated ones we have examined. The rapidly reacting animals should withstand predatory attacks better than those reacting more slowly.

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