

---

## Mechanoreception in marine copepods: electrophysiological studies on the first antennae

Jeannette Yen<sup>1</sup>, Petra H.Lenz<sup>2</sup>, Donald V.Gassie<sup>2</sup> and Daniel K.Hartline<sup>2</sup>

*Hawaii Institute of Geophysics, University of Hawaii, Honolulu, HI 96822 and*  
*<sup>2</sup>Békésy Laboratory of Neurobiology, PBRC, 1993 East-West Rd, Honolulu, HI 96822, USA*

*<sup>1</sup>Present address: Marine Sciences Research Center, State University of New York, Stony Brook, NY 11794-5000, USA*

---

**Abstract.** Neural activity was recorded extracellularly at the base of the first antenna in 15 marine copepods. Controlled mechanical stimuli were delivered with a vibrator driven by a waveform generator. Many species exhibited responses characterized by a large number of small spikes, while others were characterized by the presence of a small number of large units. Two bay species, *Labidocera madurae* and *Acartia fossae*, exhibited large units that could be easily distinguished from the background activity of smaller units. In these species, the antennal receptors fired short latency (>5 ms) trains of one to several impulses in response to a brief mechanical stimulus and sustained trains to a prolonged sinusoidal stimulus. They were extremely sensitive to small displacements and sensitivity increased with stimulus frequency. The receptors responded to stimuli between 40 and 1000 Hz and receptors required displacement velocities of 20  $\mu\text{m s}^{-1}$  or more to fire. Displacements as small as 10 nm were capable of triggering spikes. With an increase in the amplitude of the displacement, a decrease in the latency and an increase in the number of units recruited and/or firing frequency was recorded. Phase-locking to oscillatory stimuli was observed over a frequency range of 80-500 Hz. Neural activity increased in response to bending of individual setae. Setae appear innervated and structurally constrained to movements in specific directions. These experiments suggest that (i) some copepod setal receptors may be more nearly velocity detectors than purely displacement sensors, (ii) they may be capable of sensing closely spaced stimuli, (iii) the patterns of response may code for intensity and duration of the stimulus, and (iv) receptors may be capable of supplying directional information.

---

### Introduction

To understand the ecology of pelagic copepods and other planktonic organisms, it is essential to determine how these animals gather information from their environment. Behavioral and morphological analyses have indicated that mechanoreception is used by pelagic copepods to sense prey, avoid predators and recognize mates (Clutter and Anraku, 1968; Strickler and Bal, 1973; Strickler, 1975; Friedman, 1980; Haury *et al.*, 1980; Kerfoot *et al.*, 1980; Buskey, 1984; Gill and Crisp, 1985; Kirk, 1985; Légier-Visser *et al.*, 1986; Yen, 1988). Visual observations have shown that movement by prey is required before some predatory copepods deliver their quick, precise attacks (videotaped observations, J.Yen). However, not much is known about the sensory system of pelagic copepods, nor how these crustaceans detect prey prior to capture and distinguish them from predators or mates.

The setae on the elaborate first antennae of calanoid copepods have been considered the most likely receptors involved with the sensing of fluid disturbances (Fleminger, 1973; Haury *et al.*, 1980; Gill, 1985). Many of the setae are aligned parallel to the streamlines of the flow field (Yen and Nicoll, 1990)

and thus may be optimally positioned to detect deformations caused by an object moving against the flow. These setae may allow the copepods to detect hydrodynamic disturbances such as fluid displacements in the wakes cast off by swimming animals (Strickler, 1975; Strickler and Twombly, 1975; Kerfoot *et al.*, 1980), or oscillatory vibrations created by beating appendages (Kirk, 1985; Price and Paffenhöfer, 1986). Yet despite evidence for the critical role of mechanoreception, the sensory capabilities of antennal setae have not been studied physiologically.

Here, we present results of morphological and electrophysiological studies of the first antennae of several marine copepods showing evidence of mechanosensitivity. Copepods could detect oscillatory movements up to high frequencies and within the nanometer range of displacement. We seek a characterization of neural response patterns to external stimuli, a definition of the environmental signals these sensors are employed to detect, and a description of the behavioral responses to such cues.

## Method

### *Copepod collections*

Shallow water bay copepods were collected in Kaneohe Bay, Oahu, Hawaii, using a 0.5 m diameter, 333  $\mu\text{m}$  mesh net towed slowly near the surface. Oceanic copepods were collected 2 km outside Kaneohe Bay, where depths are  $>200$  m. The same net was towed slowly from 30 m to the surface. Actively swimming animals were selected from the collection, transferred into clean sea water and maintained at 20°C. Oceanic deep water animals were collected at the Ocean Thermal Energy Conversion facility of the Natural Energy Laboratory of Hawai'i at Ke'ahole Pt, Hawaii. Collections were made over 2 h periods, using a 183  $\mu\text{m}$  mesh net attached to the end of a 30.5 cm diameter, 800 m long PVC pipe, pumping water at 88  $\text{m}^3 \text{h}^{-1}$  from 586 m depth, 30 m above the sea floor. Undamaged and actively swimming animals were transferred into clean sea water and could be kept at 4–6°C without food for over 1 month. Animals for physiology experiments were used within 1 week of collection.

### *Morphological studies*

For scanning electron microscopy (SEM), sub-samples of copepods from the three locations were preserved in 2.5% glutaraldehyde in 0.1 M sodium cacodylate-buffered filtered sea water. The copepods were decalcified by incubating the animals for 1.5 h in fixative with 2% disodium EDTA. They were then returned to buffered glutaraldehyde for 0.5 h, before being washed in buffered sea water. This was followed by post-fixation in buffered 1% osmium tetroxide and dehydration in a graded ethanol series. The specimens were critical-point dried, mounted on stubs, grounded with silver paste, sputter coated and examined in a Cambridge Stereoscan S-150 at 10 kV.

For transmission electron microscopy (TEM), 22 *Labidocera madurae* were fixed in either one of two types of fixation: (i) 2.5% glutaraldehyde in buffered

sea water; and (ii) 4% glutaraldehyde in 0.1 M sodium cacodylate with 0.35 M sucrose. Decalcification and post-fixation were carried out as described for the SEM samples, except that the 4% glutaraldehyde specimens were rinsed in 0.45 M sucrose buffer. After dehydration in a graded ethanol series and propylene oxide, the animals were embedded in LX112. Thick and thin sections through the antenna were made on a Reichert Jung Ultracut E ultramicrotome, followed by double staining with uranyl acetate and lead citrate, and viewed and photographed in a Zeiss 10/A TEM at 80 kV.

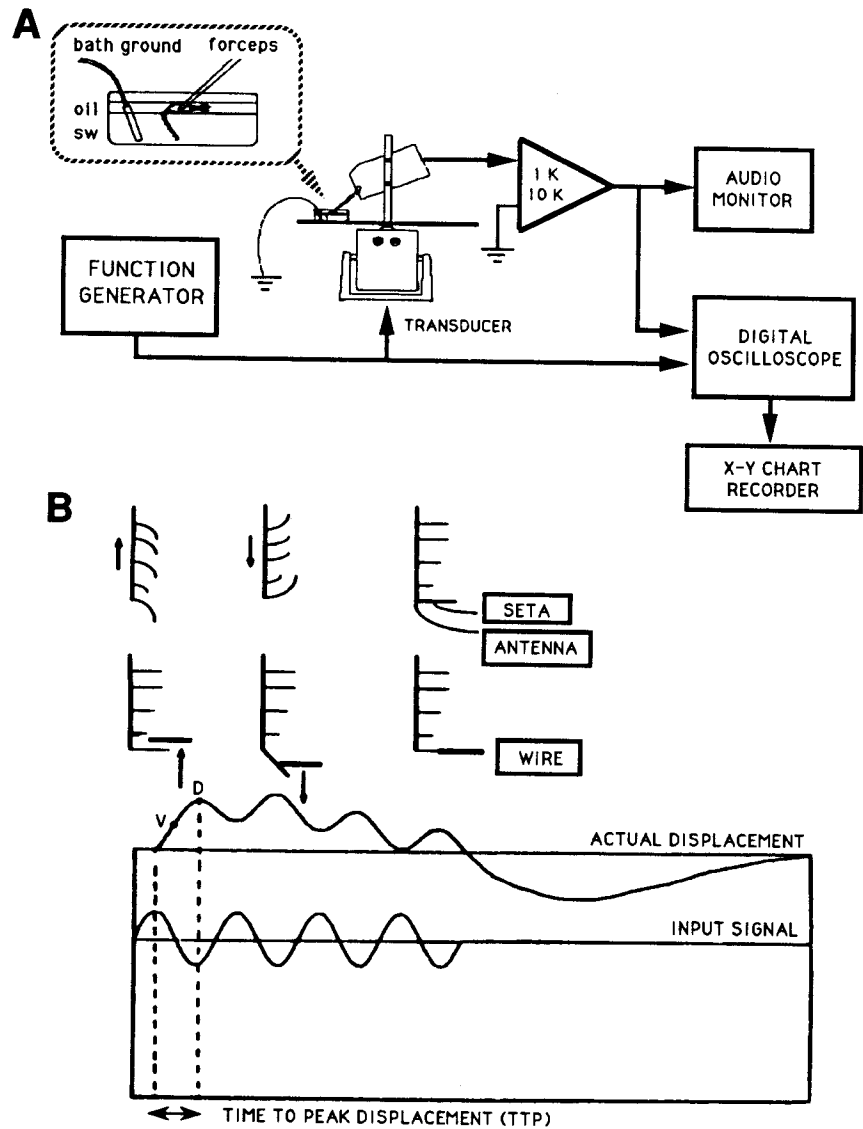
The drawing of the first antenna of *L. maduræ* (Figure 2) was made on a dry mount using a camera lucida on a Nikon Optiphot compound microscope at  $\times 100$  magnification.

#### *Physiological studies*

The detached antenna was clamped at the base in stainless-steel forceps and held, shaft down, in a pool of sea water. A droplet of mineral oil was placed on the water surface, and the forceps and antenna base were drawn up, bringing several segments out of the sea water into the insulating oil. A chlorided silver wire was placed in the seawater and the electrical potential was recorded differentially between this and the forceps. This recording situation picks up nerve impulse traffic at the oil-water interface travelling between antenna and central nervous system (CNS). In the course of the experimental series, it was discovered that the preparations lasted longer (several hours) with whole animal mounts. In these experiments, the animals were held in the forceps by the thorax and drawn up into the oil layer, leaving one antenna, pointing shaft down into a pool of sea water. The other first antenna was cut with iridectomy scissors to prevent it from collapsing against the submerged antenna. Responses were amplified either 1000 or 10 000 times, displayed on a dual-beam oscilloscope and monitored with an audio-monitor. Permanent records of the responses were stored on floppy disks using a Nicolet digital oscilloscope (model 2090). Hard copies were made with an analog X-Y plotter (Figure 1A). These records were analyzed for spike height, latency, firing frequency and temporal patterns.

Controlled mechanical stimuli were delivered with a vibrator with a force rating of 50 lb. (Ling 411), driven by a waveform generator (WaveTek Model 185). Stimuli were delivered by either driving the forceps which held the copepod or antenna, or by holding the antenna fixed and placing a vibrating wire against a seta (Figure 1B). In these arrangements, the stimulus is directly applied to the receptor hair, rather than via a more 'natural' perturbation of the surrounding water.

The sinusoidal stimulus was gated on and off at zero crossings to deliver 1-8 consecutive cycles, followed by a quiescent period of 3-5 s. The stimulus was calibrated with a capacitance-sensitive displacement gauge (ADE No. 3046). The displacement gauge was calibrated by the manufacturer, and this calibration was checked in the laboratory with a dial gauge. The highest resolution of the displacement gauge is 20 nm, so smaller displacements were calculated by linear extrapolation from larger calibrated values. Between 40 and 600 Hz, we



**Fig. 1. (A)** Schematic diagram of neurophysiological set-up: sine wave signal input is produced by the function generator, sent to the transducer and monitored by the digital oscilloscope. Nerve impulses recorded from the antenna by the forceps electrode with respect to the bath ground, amplified and displayed on the digital oscilloscope and fed to the audio monitor. The experimental preparation (enlarged inset) is on a separate stand from the transducer, isolating the antenna from any unwanted vibrations. **(B)** Diagram of the controlled mechanical stimulus. An electrical sinusoidal input signal drives a mechanical vibrator. Amplitude, frequency and duration of the stimulus can be varied. When the vibrator is displaced in the positive direction (upswing), either the antenna is pushed up and all the setae are bent distally (upper drawing), or the single seta resumes its unbent orientation (lower drawing). When the displacement is in the negative direction (downswing), either the entire antenna is pushed down and all the setae are bent proximally, or the single seta is bent distally. Actual displacements were measured by the displacement gauge. On the displacement trace, V marks the maximum velocity and D the maximum displacement.

calibrated the stimulus at the experimental displacements. The electromechanical properties of the stimulation system filtered and distorted the applied waveform so that a 'sinusoidal stimulus' produced an initial displacement followed by a series of peaks of decreasing displacement, and a slow 'undershoot' after stimulus termination (Figure 1B) at the time between just-detectable onset of movement and peak displacement. Time-to-peak displacement (TTP) and maximum velocities were determined empirically from the displacement gauge output (Figure 1B). The relationship between TTP and frequency of the applied sine wave was  $TTP(s) = 0.662 \text{ Freq(Hz)}^{-1} + 0.078$  ( $r^2 = 0.982$ ) in the range 120–1000 Hz. At 40 and 80 Hz the time-to-peak displacement was less than predicted from the relationship.

The threshold stimulus for neural activity was determined by varying the amplitude of the stimulating displacement until a response (one or more impulses) was recorded 50% of the time. Each 20 decibel (db) change in intensity represents a 10-fold change in amplitude of the displacement. Dependence of response (threshold, number of spikes, phase relations) on stimulus parameters [magnitude of the displacement, frequency, duration (number of cycles)] was examined.

## Results

### *Morphological studies*

An examination of setal sensitivity to mechanical stimulation requires a detailed morphological map of the antennae with their receptors. The antennae of nine species [*Aetideus* sp., *Euchirella curticauda*, *Gaetanus* sp., *L. madurae* (see Figure 2), *Metridia* sp., *Pareuchaeta* sp., *Pleuromamma xiphias*, *Scottocalanus securifrons* and *Undeuchaeta major*] were studied under light and scanning electron microscopy. In general, the antennae could be subdivided into three sections: proximal, middle and distal, each bearing setae. These setae were of four types: (i) smooth, tapered, of lengths varying between 200 and 500  $\mu\text{m}$ ; (ii) short (<100  $\mu\text{m}$ ) and tapered; (iii) short with a blunt rounded tip; or (iv) feathered. The setal hinges were asymmetrical as shown in the scanning electron micrographs (Figure 2B and C), so that the hairs appeared structurally constrained with a tendency to bend distally, not proximally.

The proximal section was often crowded with all types of setae. Most setae projected anteriorly. Some copepods, in addition to the usual setae, had a dense collection of short hairs on the posterior side (*L. madurae*, *U. major*, *Pareuchaeta* sp., *S. securifrons*). In the middle section, setal arrangement became more regular, with only two or three setae per segment, usually consisting of one long tapered seta, one feathered seta and, sometimes, one blunt rounded short seta. The distal tip had a cluster of several setae of different morphotypes, where some setae could be as much as 1 mm long (*Gaetanus* sp.). Similar structures have been described on other copepods (Fleminger, 1985; Gill, 1986; Landry and Fagerness, 1988; Yen and Nicoll, 1990).

Besides species-specific differences, sexual differences were noted in setal morphology and arrangement. *Euchaeta* females have long setae on their 3rd,

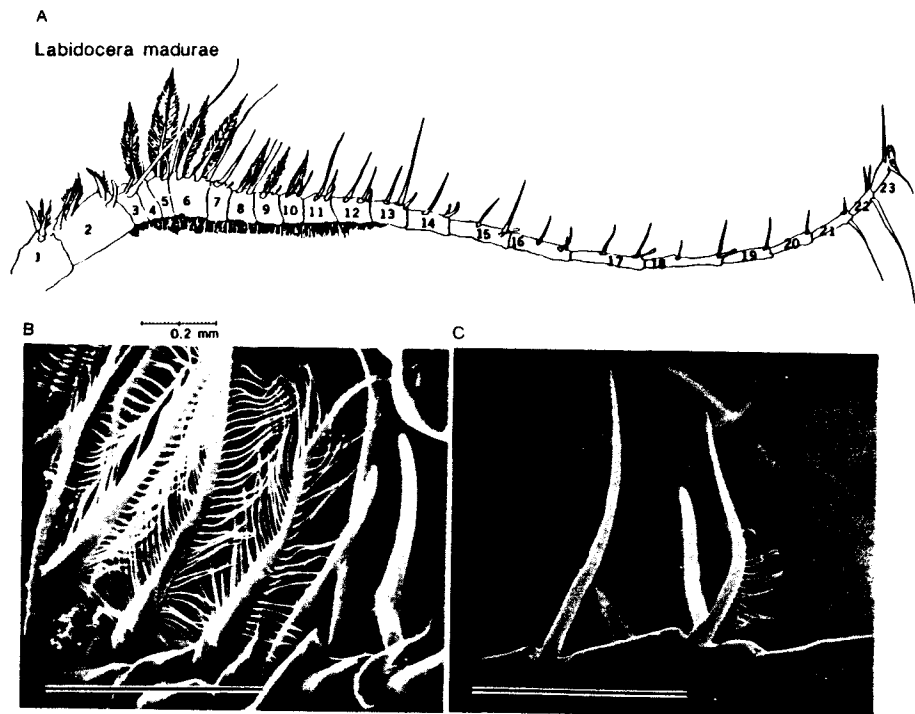
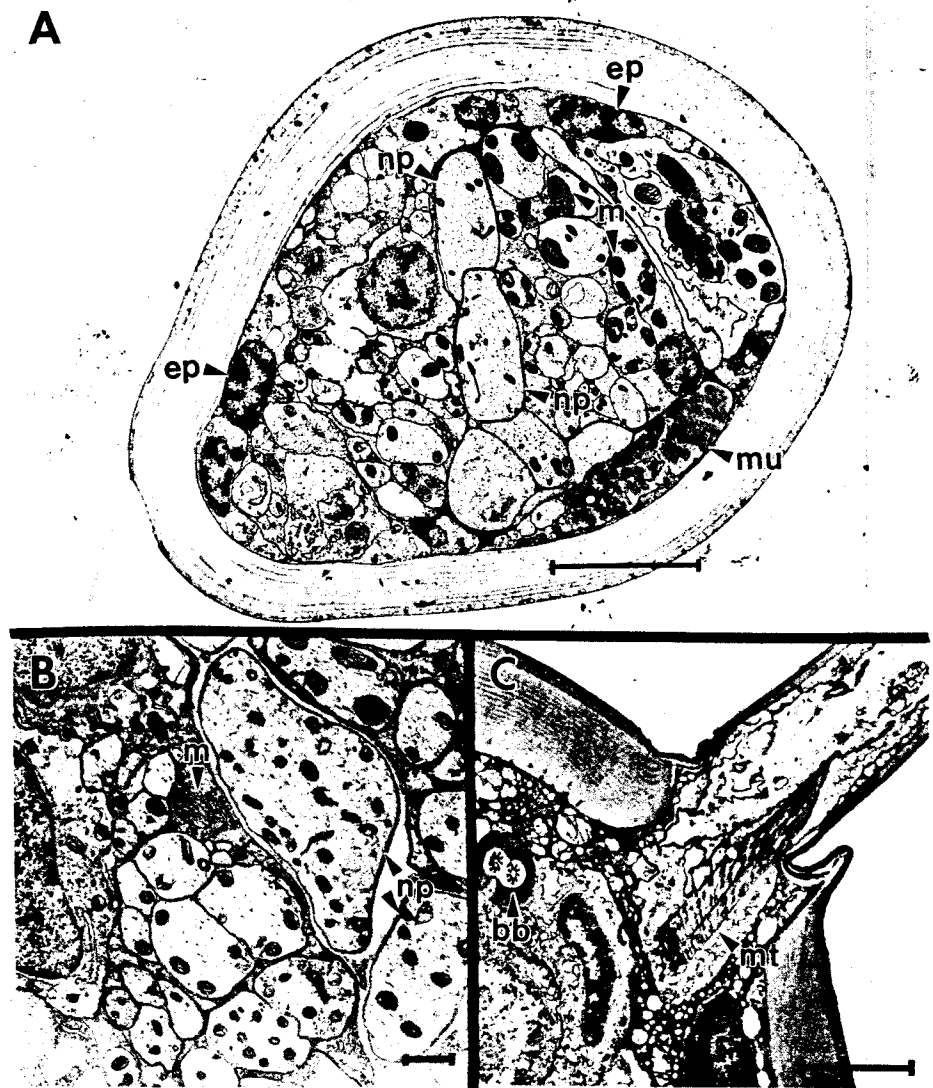


Fig. 2. The first antenna of *L. madurae*. (A) Camera lucida drawing showing 23 antennal segments ranging in length from 30  $\mu\text{m}$  (segment 4) to 200  $\mu\text{m}$  (segment 18) and in diameter from 20  $\mu\text{m}$  (segment 21) to 150  $\mu\text{m}$  (segment 1). Long setae occurred on segments 3, 6, 13, 22 and 23 (the latter two pointing posteriorly) and at least two long feathered setae were observed on the last distal segment. Scanning electron micrographs of the feathered and smooth setae and palps near the base of the antenna (B) and a distal segment bearing two setae and one blunt palp (C). Note that one seta is feathered and that the setal hinge is asymmetrical. Scale bar represents 40  $\mu\text{m}$ .

7th, 8th and 13th segments, while males have a long setae on only their 3rd segment. *Euchirella* females have feathered setae on their first antennal segment, while males have short vermiform setae (nomenclature of Fleminger, 1985). *Pleuromamma* males have these vermiform setae while females do not. Such morphological variations in the antennae between species and sexes as well as within different sectors and locations on the antenna itself suggest different sensitivities to different cues.

The overall internal cellular organization of a *L. madurae* antenna is shown in Figure 3(A). Muscle ( $\mu$ ) and large mitochondria ( $m$ ) associated with the muscle were present in all segments examined, of which the 19th was the most distal examined. Several bundles of neural processes ( $np$ ) were observed containing at least 35 neural processes with a small number of large ones (Figure 3B;  $6 \times 3 \mu\text{m}$ ) and many smaller ones ( $<0.5 \mu\text{m}$  diameter). The seta shown (Figure 3C) was innervated, and a distinct structure ( $mt$ ) was visible at the base. This structure consisted of two bundles of parallel arrays of densely packed microtubules. Pairs of basal bodies ( $bb$ ) with a  $9 + 0$  structure were observed in the antenna.



**Fig. 3.** Transmission electron micrographs of the first antenna and a seta of *L. madurae*. (A) Cross section of the antenna through the 17th segment measuring  $25 \times 20 \mu\text{m}$ . Note muscle (mu), mitochondria (m), neural processes (np) and epithelial cells (ep). Scale bar represents  $5 \mu\text{m}$ . (B) Magnified view of a neural process measuring  $6 \times 2.5 \mu\text{m}$  (17th segment). Scale bar represents  $1 \mu\text{m}$ . (C) Cross section through the base of a seta in the 6th segment. Note basal body (bb) and microtubules (mt). Scale bar represents  $5 \mu\text{m}$ .

#### *Neurophysiological studies*

Neural activity was recorded from the first antennae of 15 calanoid copepod species (Figure 4 and Table I). Background neural activity was observed in all species, and activity increased significantly in response to several types of

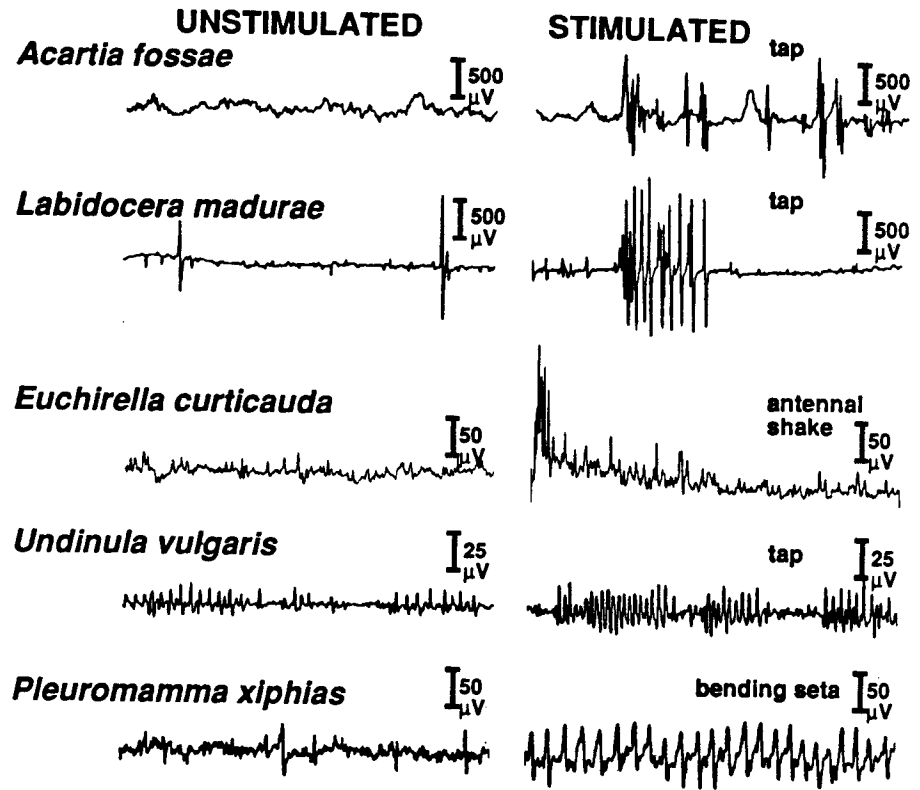


Fig. 4. Neural activity from the first antenna of five copepod species. Left column: spontaneous background activity. Right column: responses to a variety of mechanical stimuli (tap, traces 1, 2 and 4; antennal shake, trace 3; setal bend, trace 5). All recordings are 40 ms long, but the vertical scales vary among traces, as indicated on figure.

Table I. Spontaneous neural responses were recorded from the following marine copepods, collected near the Hawaiian islands

Species	Habitat	Source	SEM (n <sup>a</sup> )	Electrophysiology (n <sup>a</sup> )	
				Detached	Whole
<i>Acartia fossae</i>	Shallow bays	Kaneohe Bay			4
<i>Labidocera madurae</i>	Shallow bays	Kaneohe Bay	2	4	16
<i>Pontellopsis</i>	Surface pelagic	Outside K.Bay reef			2
<i>Undinula vulgaris</i>	Surface pelagic	Outside K.Bay reef		4	1
<i>Cosmocalanus</i>	Surface pelagic	Outside K.Bay reef		1	
<i>Candacia aethiopica</i>	Surface pelagic	Outside K.Bay reef		2	2
<i>Centropages</i>	Surface pelagic	Outside K.Bay reef		2	
<i>Neocalanus</i>	Surface pelagic	Outside K.Bay reef		1	
<i>Euchaeta rimana</i>	Surface pelagic	Outside K.Bay reef		5	7
<i>Pareuchaeta</i>	Deep pelagic	600 m, OTEC	3	1	
<i>Euchirella curticauda</i>	Deep pelagic	600 m, OTEC	4	12	1
<i>Aetideus</i>	Deep pelagic	600 m, OTEC	1	4	
<i>Pleuromamma xiphias</i>	Deep pelagic	600 m, OTEC	2	1	10
<i>Scottocalanus securifrons</i>	Deep pelagic	600 m, OTEC	5		1
<i>Scolecithrix danae</i>	Deep pelagic	600 m, OTEC		1	

<sup>a</sup>Number of specimens examined.

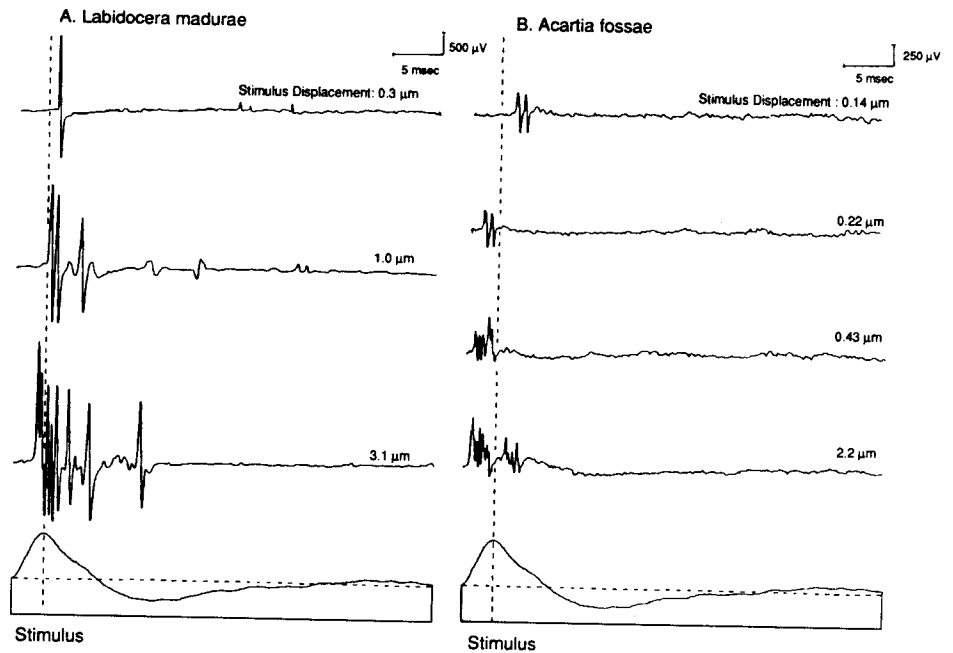


mechanical stimuli (Figure 4, right column). Different species had distinct activity patterns, while two closely related pontellids (*L. madurae* and *Pontellopsis* sp.) had similar spontaneous activity and responses to stimuli. Elevated activity was often followed by a period of inactivity or post-stimulus depression. Maximum spike height among species ranged from  $<25 \mu\text{V}$  (*Undinula vulgaris*) to nearly  $800 \mu\text{V}$  (*L. madurae*). Stimulus-sensitive activity persisted for 1–2 h in detached antennae and for over 3 h in whole animals. We could not detect any differences in neural activity between whole animal and detached antennae recordings. Preparation deterioration was often accompanied by trains of high frequency ( $>2 \text{ kHz}$ ) spikes. These bursts could not be entrained by mechanical stimuli. No spiking was recorded in antennae which were killed with a large electrical voltage (e.g. 25 V, 20 s; a flat baseline was observed).

Along the first antennae of many copepods, one to two long setae per segment are found with a tuft of four to six setae on the distal tip. We tested direction-specific sensitivity to bending of these long setae in four species (*P. xiphias*, *U. vulgaris*, *Euchaeta rimana*, *Cosmocalanus*). In general, when the setae were bent distally, increased neural activity was recorded from the antenna. When setae were bent proximally, only a slight increase in activity of smaller spikes was recorded. However, in *E. rimana*, two setular projections in the distal tip produced an increase in neural activity when bent either anteriorly or posteriorly. These results suggest that the long setae on the first antennae are sensitive to setal contact and bends, and thus may provide important sensory information, including directional cues, to the animal.

Since large spikes were recorded for *Acartia fossae* and the two pontellid species which had some of the best signal-to-noise ratios, we focused our most detailed examination on these species. Responses of their setal receptors to rhythmic movements were elicited by a sinusoidal waveform of one to several cycles driving the vibrator and displacing the antennal setae. Over the frequencies studied (40–1000 Hz), spikes continued to fire as long as the stimulus was on with a decrease in firing rate. In response to increasing the amplitude of the stimulus displacement, the number of spikes increased and latency decreased. This is shown for a single cycle stimulus in Figures 5 and 6. These are characteristics that are typical of many mechanosensory receptors. A short latency in the physiological response may permit a quick reaction time for the behavioural response.

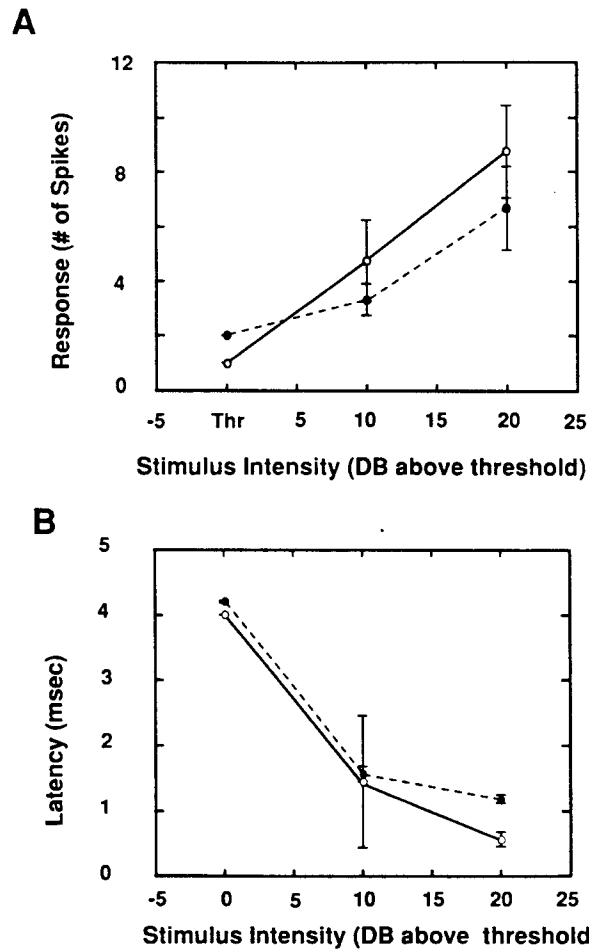
Response latencies of *Pontellopsis* were measured at several times-to-peak (TTP) for a displacement 2.5 times threshold (Figure 7). Latencies tracked peak times over the range tested. At long TTPs ( $\geq 4 \text{ ms}$ ), the receptor fired before peak displacement, while firing occurred at peak displacement at the shortest TTP tested (TTP = 0.65 ms, 1000 Hz input), which corresponded to a latency of 1 ms. A similar latency had been observed for *Labidocera* for a 3.8 ms TTP stimulus where latency decreased from 4 ms at threshold to  $<1 \text{ ms}$  at a displacement 10 times above threshold (Figure 6B). These latencies may be slightly overestimated, since they also include an unknown propagation delay from point of impulse origin to recording site. This suggests that these copepods are capable of responding rapidly to sudden displacements.



**Fig. 5.** Neural activity recorded from the first antenna of (A) *L. madurae* in response to an increase in the amplitude of the stimulus displacement of the entire antenna from 0.3 to 3.1  $\mu\text{m}$  and (B) *A. fossae* in response to an increase in the amplitude of the stimulus displacement from 0.14 to 2.2  $\mu\text{m}$ . Form of the stimulus (output of displacement gauge) is depicted as the bottom displacement trace in both (A) and (B).

Displacement thresholds were determined for *Labidocera* over a range of stimulation TTPs (Figure 8). Similar results were found for three separate individuals, suggesting that this threshold sensitivity curve may be a specific characteristic of the species. Sensitivity increased with a decrease in time-to-peak displacement. The maximum sensitivity, 10 nm, was observed at the shortest TTP (0.65 ms). Below 4 ms, an approximately linear relationship was observed between threshold displacement and TTP (Figure 8). Thus, in this range, the large-spike receptor may be considered a velocity detector, requiring only a  $20 \mu\text{m s}^{-1}$  velocity of fluid with respect to antenna to trigger a neural response. At shorter TTP, a fall-off of sensitivity would be expected; however, our stimulation method has not yet allowed us to establish where this occurs. At long TTPs ( $>3.8$  ms), linearity breaks down, and the receptor is relatively insensitive, requiring a larger displacement and velocity before a response is elicited (Figure 8). This type of sensitivity curve is surprising, since we expected the setal receptors to be sensitive to flow fields and thus be tuned to long TTP (or low frequency) stimuli associated with flow velocities ranging between 1 and  $10 \text{ mm s}^{-1}$  (Strickler, 1982, 1985; Yen *et al.*, 1991).

One way to assess the speed of recovery of the receptor after firing a spike, as well as the potential for the animal to distinguish a time-varying stimulus, is to



**Fig. 6.** Dependence of response characteristics, the number of spikes (A) and latency (B), on stimulus intensity (amplitude: measured in db relative to threshold). Data from two individuals (*L. madurae*) are shown. Threshold displacements were 34 nm (open symbols) and 91 nm (closed symbols). Stimulus was a single cycle of TTP = 3.8 ms. Error bars are sample standard deviations. The latency was measured from the beginning of physical movement of the antenna.

examine the phase-locking of spikes to a sinusoidal stimulus pattern; i.e. the tendency of spikes to fire at a fixed phase in the stimulus interval. Phase locking in *Labidocera* studied by stimulating only the distal seta is shown in Figure 9(A). The stimulus (1  $\mu\text{m}$  displacement) was presented 1, 2, 3, 4 and 8 times. As the number of cycles of movement increased, the number of spikes increased in a direct relationship. The spikes exhibited phase-locking to this 200 Hz oscillation firing approximately one-quarter of the way through the cycle from the peak displacement. Firing occurred only on the distal bends of the seta (downswing of displacement trace). This is because the seta is only being pushed from one side in this method of stimulation. Phase locking when the entire antenna of

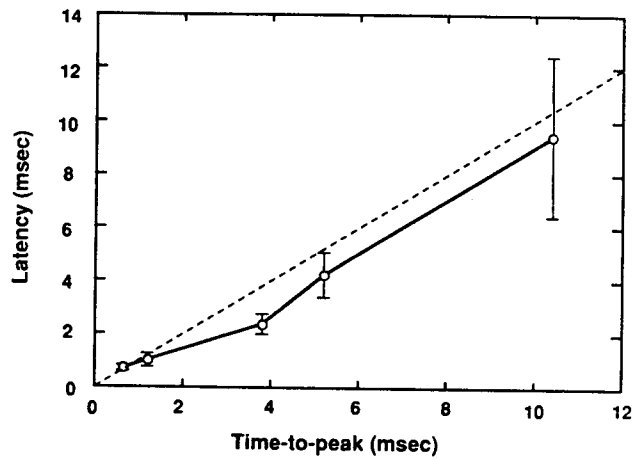


Fig. 7. Latency as a function of TTP (ms) for the initial part of a sinusoidal stimulus 7–8 db above threshold (which was TTP dependent) for *Pontellopsis* sp. Latency was measured from the beginning of antennal movement. The respective TTPs, thresholds and stimulus intensities were: 10.4 ms, thr = 92 db, stim = 85 db; 5.2 ms, thr = 92 db, stim = 85 db; 3.8 ms, thr = 88 db, stim = 80 db; 1.2 ms, thr = 75 db, stim = 68 db; 0.65 ms, thr = 69 db, stim = 62 db). Dashed line shows a slope of 1.0 for comparison.

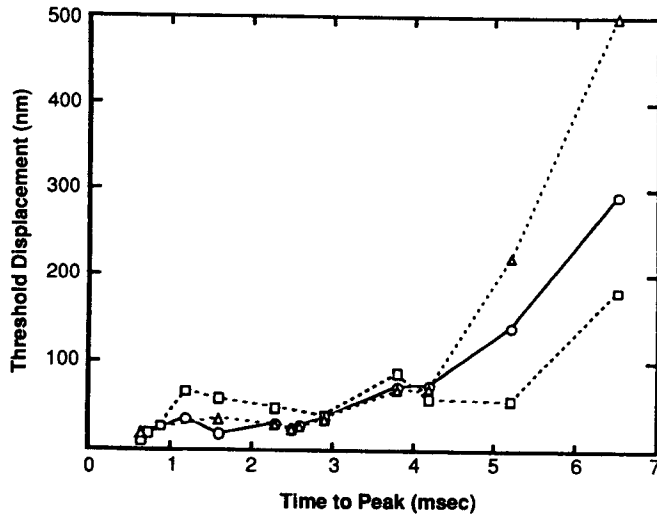


Fig. 8. Threshold sensitivities of the first antenna of *L. maduræ* showing the displacement required at each time to peak displacement (TTP) to elicit a response 50% of the time. Response curves of three individuals (open circle, square, triangle) are shown.

*Labidocera* was vibrated is shown in Figure 9(B). More spikes were observed, occurring with both distal (upswing of displacement trace) and proximal (downswing of displacement trace) setal bends (Figure 9B). A similar symmetrical response was observed in *Acartia fossae* where brief trains of spikes were elicited during both distal and proximal movement phases of a single-cycle

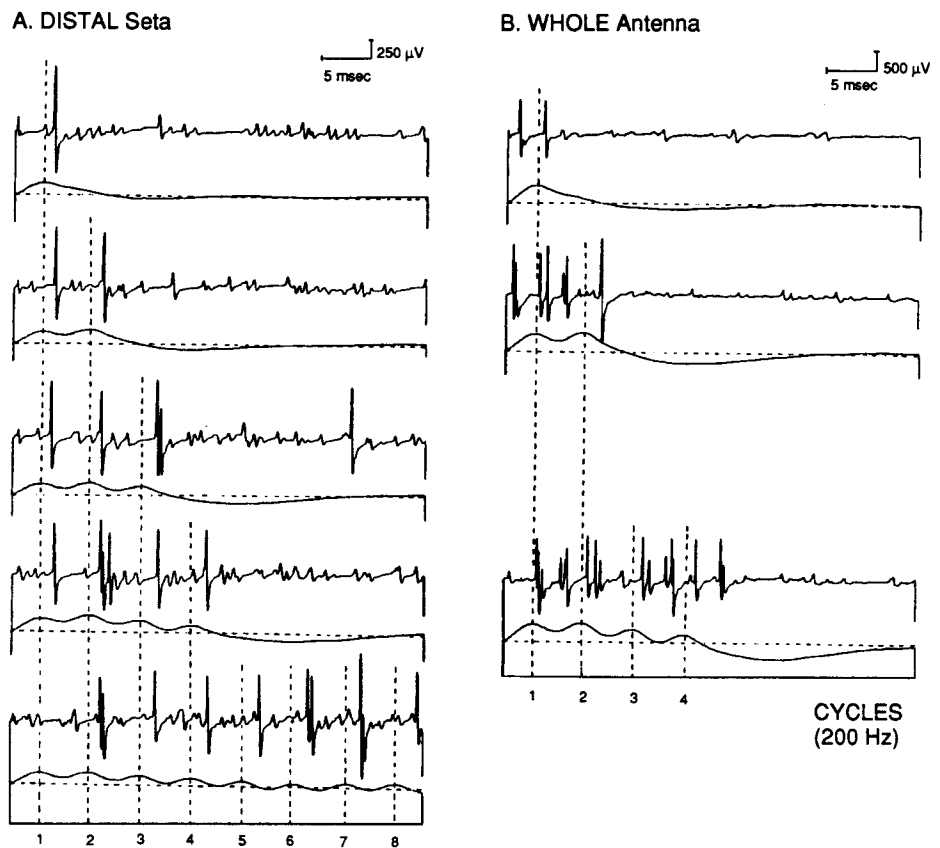
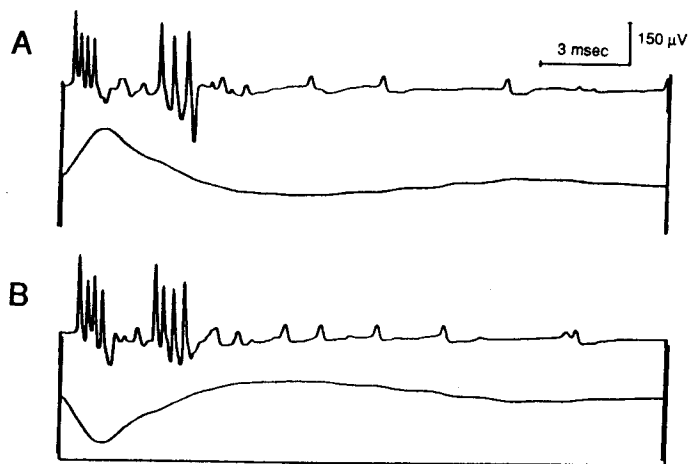


Fig. 9. Neural activity recorded from the first antenna of *L. madurae* in a phase-locked response to  $1.0\ \mu\text{m}$  oscillatory movements of the distal seta (A) and the whole antenna (B). Note that the downward movement of the displacement trace corresponds to a distal bend of setae in (A) and a proximal one in (B).

stimulus (Figure 10A). The response was nearly identical when the initial direction of the displacement was reversed (Figure 10B) suggesting that setae can be sensitive to movements in more than one direction. Phase-locking was also studied in *Pontellopsis* and *A. fossae* and several copepod species with only smaller spikes (*Neocalanus*, *Euchirella curticauda*, *Aetideus*, *Pareuchaeta* and *Euchaeta antarctica*). All copepods tested showed phase-locking. In *E. curticauda*, *Aetideus* and *E. antarctica*, the receptors phase-locked over the entire frequency range tested (80–500 Hz). At the higher frequencies, short latencies were observed, comparable to those measured for *L. madurae*. Thus setal receptors can recover rapidly and, hence may be capable of sensing closely spaced stimuli.

### Discussion

Behavioral studies have indicated that the first antennae in copepods are



**Fig. 10.** Neural activity recorded from the first antenna of *A. fossae* in response to a single-cycle,  $1.76 \mu\text{m}$  displacement of the entire antenna. (A) Initial distal bend followed by a proximal bend of all the setae. (B) Proximal followed by distal bend of all the setae. The lower trace depicts the stimulus.

important sensory structures with mechanoreceptive capabilities (Fleminger, 1973; Strickler and Bal, 1973; Friedman, 1980; Haury *et al.*, 1980; Gill and Crisp, 1985; Gill, 1986). Morphological studies have substantiated these behavioral observations by describing the presence of sensory cells in the first antenna (Strickler and Bal, 1973; Markl, 1978; Friedman, 1980). Setae appeared to be modified from ciliary structures, and it was suggested that they function as mechanoreceptors detecting gravitational and inertial forces transmitted by fluid mechanical means. Strickler and Bal (1973) indicated that the mechanoreceptive hairs on the cyclopid *Cyclops scutifer* were probably derived from two sheathed ciliary neurons that had a  $9 + 0$  basal body structure. Yet no nerve tissue could be detected in the setae. We found similar  $9 + 0$  basal body-like structures in the calanoid copepod *Labidocera*, as well as neural tissue within the seta. Our morphological studies also indicated the presence of muscle tissue throughout the antenna, suggesting that calanoid copepods have substantial control over movement of the antenna.

We have initiated a physiological study of the mechanosensors of the first antenna of calanoid copepods, and here we present the first recordings of the antennal sensors. All 16 copepod species tested were sensitive to mechanical stimuli, showing species-specific responses. The unusual large units and good signal-to-noise ratio in *Labidocera*, *Pontellopsis* and *Acartia* lead us to hypothesize the presence of nerve cells with large axons in the antennae of these copepods. Indeed, transmission electron micrographs of *Labidocera* indicate that the antenna is innervated by at least two large axons. Giant axons are usually associated with rapid response behaviors (e.g. escape). This would suggest that a capability for rapid reaction times to mechanical stimuli received by the first antennae has particular survival value for these animals. The short latencies that we observed for *Labidocera* further support the suggestion that

these copepods depend on fast reaction times. These mechanosensory properties may reflect an adaptation to their ecology. Further study is needed to determine how the properties of the mechanosensors in *Labidocera*, *Pontellopsis* and *Acartia* compare with those in other copepod species.

The first antennae were characterized by the presence of several long and elaborate setae at their distal tips. Gill (1985) suggests that it is reasonable for the tips to be the most sensitive part of the antennae; they are farther away from the body and so can detect stimuli with least interference from the movement of other limbs. In fact, within the flow field created by the second antennae, velocities close to the body can be  $>5$  times the velocity of the water moving past the tips of the first antennae (Yen *et al.*, 1991). As Haury *et al.* (1980) described, 'the fluid in the neighborhood of the copepods undergoes deformation, whereas the copepod itself is relatively rigid'. Thus the antenna may detect differences in the velocity of the fluid adjacent to the body versus near the tip. In our physiological experiments, we found that bending of these distal setae elicited neural responses and that at least some of them may be directionally sensitive. These results support the hypothesis that the distal setae are very important in sensing mechanical stimuli. However, more work will be needed to quantify the contribution of the distal tips relative to other sectors of the antennae in obtaining sensory information for the animal.

Sensory systems in many organisms have special adaptations that can enhance reception of important messages. For example, the resonance frequency of the antennae of certain male Diptera lies in the range 350–400 Hz, which coincides with the wingbeat of the female (Tautz, 1979). The sensors of noctuid moths, katydids and certain other nocturnally flying insects are tuned to ultrasound frequencies ( $\sim 20$  KHz), which corresponds to the echo-location sounds emitted by their predator, the bat (Roeder, 1974; Nolan and Hoy, 1986). The chaetognath, *Spadella cephaloptera*, has sensory structures which could detect oscillatory motions created by a vibrating glass probe; the behavioral response of attack was triggered at frequencies between 9 and 20 Hz at source amplitudes of 100–500  $\mu\text{m}$  (Horridge and Boulton, 1967). Newbury (1972) found that these frequencies corresponded to the beating rates of the feeding appendages of copepods, their natural prey. Ctenophores have simple non-motile cilia up to 100  $\mu\text{m}$  long, which issue from axon-bearing cells, and are sensitive to water displacements (Horridge, 1966). The mechanoreceptors in cnidocytes, comprised of kino- and stereocilia, appear tuned to the movements of swimming prey by chemoreceptors (Watson and Hessinger, 1989). Thus, it can be expected that the specific properties of the mechanoreceptors we have found in copepods are adapted to particular features of natural stimuli which are behaviorally relevant.

Physiological evidence presented here suggests that the copepod sensors can respond to stimuli up to the kHz range. The sensitivity to such high frequencies was surprising. The fish lateral line is tuned to detect frequencies below 200–300 Hz, with peak sensitivities typically at or below 100 Hz (Kuiper, 1967; Fay, 1988; Coombs and Janssen, 1990) and we expected a similar sensitivity curve for the copepods. Furthermore, given that fluid displacements are the suggested

likely mechanical stimuli, receptors ought to be sensitive to low frequency oscillations, as has been found in other crustaceans such as the sensillae of decapods which are tuned, with best frequencies between near-zero and 400 Hz (Wiese, 1976; Barth, 1980; Tautz *et al.*, 1981; Hatt, 1986). It is possible that high frequencies are produced in the flicking of an antenna or urosome, and the sensitivity of copepods to these frequencies may allow early detection of a prey or predator. The type of sensitivity we found in bay calanoids could permit them to detect rapid changes in displacement like those in wakes of darting escapes or predatory lunges. Differences in antennal mechanosensors of copepods from those of decapod crustaceans also may be explained by a difference in their habitats. The sensory needs of benthic decapods can be expected to differ from those of pelagic animals. Sensitivity to high frequencies had been reported in two other pelagic zooplankters: *Euphausia superba* (Wiese and Marschall, 1990) and *Chaoborus trivittatus* larvae (Giguère and Dill, 1979). Giguère and Dill (1979) reported a high frequency (1 kHz) component in the acoustic signals produced by two *Diaptomus* species. However, because only frequencies >400 Hz were analyzed, it is unclear how the amplitude of these high frequency signals compares with the low frequency oscillations (<100 Hz) produced by copepods (e.g. Kirk, 1985; Price and Paffenhöfer, 1986). The mechanosensory capabilities of the calanoid copepod are expected to be a special adaptation to ensure this animal's success in the pelagic habitat.

Unlike previously described crustacean mechanosensors (e.g. Laverack, 1963; Tazaki and Ohnishi, 1974; Wiese, 1976; Tazaki, 1977; Barth, 1980; Tautz *et al.*, 1981; Altner *et al.*, 1983; Hatt, 1986), detection capabilities of the copepod receptor approach that of vertebrate hair cells (1 nm; Ashmore, 1991). We found that in *Labidocera* and other bay species, the large-spike receptors are sensitive to mechanical displacements at the sub-micron level (10 nm), and that their sensitivity increased with frequency from 40 to at least 1000 Hz. These experiments further suggest that, in marked contrast to the evidence for most other crustacean setal mechanoreceptors, these copepod receptors may be more nearly velocity detectors than purely displacement sensors. This modality relates critically to the nature of the natural stimuli which activate the receptor; large static objects in a flow field may be better detected by the displacements they produce, while small oscillating prey objects producing small displacements might be better detected by a velocity sensor. The  $20 \mu\text{m s}^{-1}$  velocity required to elicit a neural response from the antenna of *Labidocera* suggests that velocities present in copepod feeding currents may be detectable (Strickler, 1982; Yen *et al.*, 1991). Further studies of these mechanoreceptors, as well as a better characterization of the pertinent mechanical stimuli, are needed to assess the capability of these sensors to perceive such signals in the aquatic environment.

#### Acknowledgements

We would like to thank the following people for their contribution to this study: A.Kitalong for assistance with the copepod collection, identification and scanning electron microscopic work; B.Lienert for the use of his displacement



gauge and its calibration, and his effort in constructing prey mimics; T. Weatherby for the electron microscope work; W. Akaka, K. Nasu and J. Zhang for assistance with the physiological set-up; M. Valdez for constructing parts of the mechanical stimulator; B. Tomiyasu and M. Andres for laboratory assistance; R. Nakamoto and K. Wong for assistance in preparation of figures; J. Hirota for use of field gear; F. Ferrari for the use of the camera lucida; and I. Cooke for the use of the Nicolet oscilloscope and other physiological equipment. We also would like to thank the Hawaii Institute of Marine Biology for use of their small boats, the Natural Energy Laboratory of Hawaii (Director T. Daniel) for access to the deep-water pump, and the University of Hawaii Research Council for funding this study. J. Y. wishes to thank the Smithsonian Oceanographic Sorting Center for the Visiting Scientist award where the antennal drawings were completed, and the Office of Naval Research N00014-87K-0181 for support. D.K.H. and D.V.G. were partially supported by NIH grant NS15314 to D.K.H. and P.H.L. was partially supported by NSF grant OCE89-18019 to D.K.H.

### References

- Altner, I., Hatt, H. and Altner, H. (1983) Structural properties of bimodal chemo- and mechano-sensitive setae on the pereopod chelae of the crayfish, *Austropotamobius torrentium*. *Cell Tissue Res.*, **228**, 357–374.
- Ashmore, J.F. (1991) The electrophysiology of hair cells. *Annu. Rev. Physiol.*, **53**, 465–476.
- Barth, F.G. (1980) Campaniform sensilla: another vibration receptor in the crab leg. *Naturwissenschaften*, **67**, 201–202.
- Buskey, E.J. (1984) Swimming pattern as an indicator of the roles of copepod sensory systems in the recognition of food. *Mar. Biol.*, **79**, 165–175.
- Clutter, R.I. and Anraku, M. (1968) Avoidance of samplers. In *Zooplankton Sampling. Monographs of Oceanic Methodology*, Vol. 2, UNESCO, pp. 57–76.
- Coombs, S. and Janssen, J. (1990) Behavioral and neurophysiological assessment of lateral line sensitivity in the mottled sculpin, *Cottus bairdi*. *J. Comp. Physiol.*, **A167**, 557–567.
- Fay, R.R. (1988) *Hearing in Vertebrates: a Psychophysics Databook*. Hill-Fay Associates, Winnetka, IL.
- Fleminger, A. (1973) Pattern, number, variability and taxonomic significance of integumental organs (sensilla and glandular pores) in the genus *Eucalanus* (Copepoda, Calanoida). *Fish. Bull. US*, **71**, 965–1010.
- Friedman, M.M. (1980) Comparative morphology and functional significance of copepod receptors and oral structures. In Kerfoot, W.M. (ed.), *Evolution and Ecology of Zooplankton Communities*. New England University Press, Hanover, NH, pp. 185–197.
- Giguère, L.A. and Dill, L.M. (1979) The predatory response of *Chaoborus* larvae to acoustic stimuli, and the acoustic characteristics of their prey. *Z. Tierpsychol.*, **50**, 113–125.
- Gill, C.W. (1985) The response of a restrained copepod to tactile stimulation. *Mar. Ecol. Prog. Ser.*, **21**, 121–125.
- Gill, C.W. (1986) Suspected mechano- and chemosensory structures of *Temora longicornis* (Copepoda: Calanoida). *Mar. Biol.*, **93**, 449–457.
- Gill, C.W. and Crisp, D.J. (1985) Sensitivity of intact and antennule amputated copepods to water disturbance. *Mar. Ecol. Prog. Ser.*, **21**, 221–227.
- Hatt, H. (1986) Responses of a bimodal neuron (chemo- and vibration-sensitive) on the walking legs of the crayfish. *J. Comp. Physiol.*, **A159**, 611–617.
- Haury, L.R., Kenyon, D.E. and Brooks, J.R. (1980) Experimental evaluation of the avoidance reaction of *Calanus finmarchicus*. *J. Plankton Res.*, **2**, 187–202.
- Horridge, G.A. (1966) Some recently discovered underwater vibration receptors in invertebrates. In Barnes, H. (ed.), *Some Contemporary Studies in Marine Science*. Allen and Unwin, London, pp. 395–405.
- Horridge, G.A. and Boulton, P.S. (1967) Prey detection by chaetognaths via a vibration sense. *Proc. Roy. Soc. Lond., Ser. B*, **168**, 413–419.

- Kerfoot, W.C., Kellogg, D.C., Jr and Strickler, J.R. (1980) Visual observations of live zooplankters: evasion, escape and chemical defenses. In Kerfoot, W.C. (ed.), *Evolution and Ecology of Zooplankton Communities*. University Press of New England, Hanover, NH, pp. 10–27.
- Kirk, K.L. (1985) Water flows produced by *Daphnia* and *Diaptomus*: implications for prey selection by mechanosensory predators. *Limnol. Oceanogr.*, **30**, 679–686.
- Kuiper, J.W. (1967) Frequency characteristics and functional significance of the lateral line organ. In Cahn, P.H. (ed.), *Lateral Line Detectors*. Indiana University Press, Bloomington, IN, pp. 105–121.
- Landry, M.R. and Fagerness, V.L. (1988) Behavioral and morphological influences on predatory interactions among marine copepods. *Bull. Mar. Sci.*, **43**, 509–529.
- Laverack, M.S. (1963) Responses of cuticular sense organs of the lobster, *Homarus vulgaris* (Crustacea). III. Activity invoked in sense organs of the carapace. *Comp. Biochem. Physiol.*, **10**, 261–272.
- Légier-Visser, M.F., Mitchell, J.G., Okubo, A. and Fuhrman, J.A. (1986) Mechanoreception in calanoid copepods: a mechanism for prey detection. *Mar. Biol.*, **90**, 529–535.
- Markl, H. (1978) Adaptive radiation of mechanoreception. In Ali, M.A. (ed.), *Sensory Ecology: Reviews and Perspectives*. Plenum Press, NY, pp. 319–344.
- Newbury, T.K. (1972) Vibration perception by chaetognaths. *Nature*, **236**, 459–460.
- Nolan, T.G. and Hoy, R.R. (1986) Phonotaxis in flying crickets. II. Physiological mechanisms of two-tone suppression of the high frequency avoidance steering behavior by the calling song. *J. Comp. Physiol.*, **A159**, 441–456.
- Price, H.J. and Paffenhöfer, G.-A. (1986) Effects of concentration on the feeding of a marine copepod in algal monocultures and mixtures. *J. Plankton Res.*, **8**, 119–128.
- Roeder, K.D. (1974) Responses of the less sensitive acoustic sense cells in the tympanic organs of some noctuid and geometrid moths. *J. Insect Physiol.*, **20**, 55–66.
- Strickler, J.R. (1975) Intra- and interspecific information flow among planktonic copepods: receptors. *Verh., Int. Ver. Limnol.*, **19**, 2951–2958.
- Strickler, J.R. (1982) Calanoid copepods, feeding currents, and the role of gravity. *Science*, **218**, 158–160.
- Strickler, J.R. (1985) Feeding currents in calanoid copepods: two new hypotheses. In Laverack, M.S. (ed.), *Physiological Adaptations of Marine Animals*. Society of Experimental Biology, pp. 459–485.
- Strickler, J.R. and Bal, A.K. (1973) Setae of the first antennae of the copepod *Cyclops scutifer* (Sars): their structure and importance. *Proc. Natl Acad. Sci. USA*, **70**, 2656–2659.
- Strickler, J.R. and Twombly, S. (1975) Reynolds number, diapause, and predatory copepods. *Verh., Int. Ver. Limnol.*, **19**, 2943–2950.
- Tautz, J. (1979) Reception of particle oscillation in a medium—an unorthodox sensory capacity. *Naturwissenschaften*, **66**, 452–461.
- Tautz, J., Masters, W.M., Aicher, B. and Markl, H. (1981) A new type of water vibration receptor on the crayfish antenna. I. Sensory physiology. *J. Comp. Physiol. A*, **144**, 533–541.
- Tazaki, K. (1977) Nervous responses from mechanosensory hairs on the antennal flagellum in the lobster, *Homarus gammarus* (L.). *Mar. Behav. Physiol.*, **5**, 1–18.
- Tazaki, K. and Ohnishi, M. (1974) Responses from tactile receptors in the antenna of the spiny lobster *Panulirus japonicus*. *Comp. Biochem. Physiol.*, **A47**, 1323–1327.
- Watson, G.M. and Hessinger, D.A. (1989) Cnidocyte mechanoreceptors are tuned to the movements of swimming prey by chemoreceptors. *Science*, **243**, 1589–1591.
- Wiese, K. (1976) Mechanoreceptors for near-field water displacements in crayfish. *J. Neurophysiol.*, **39**, 816–833.
- Wiese, K. and Marschall, H.P. (1990) Sensitivity to vibration and turbulence of water, in context with schooling in Antarctic krill, *Euphausia superba*. In Wiese, K., Krenz, W.-D., Tautz, J., Reichert, H. and Mulloney, B. (eds), *Frontiers in Crustacean Neurobiology, Advances in Life Sciences*. Birkhäuser, Basel, pp. 121–139.
- Yen, J. (1988) Directionality and swimming speeds in predator–prey and male–female interactions of *Euchaeta rimana*, a subtropical marine copepod. *Bull. Mar. Sci.*, **43**, 175–193.
- Yen, J. and Nicoll, N.T. (1990) Setal array on the first antennae of a carnivorous marine copepod, *Euchaeta norvegica*. *J. Crust. Biol.*, **10**, 218–224.
- Yen, J., Sanderson, B.G., Strickler, J.R. and Okubo, A. (1991) Feeding currents and energy dissipation by *Euchaeta rimana*, a subtropical pelagic copepod. *Limnol. Oceanogr.*, **36**, 362–369.

Received on December 27, 1990; accepted on August 29, 1991