ELSEVIER

Journal of Experimental Marine Biology and Ecology 314 (2005) 175–186

Journal of EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

www.elsevier.com/locate/jembe

Chemosensory neurons in the mouthparts of the spiny lobsters *Panulirus argus* and *Panulirus interruptus* (Crustacea: Decapoda)

Anders Garm[a,*], Shkelzen Shabani[b], Jens T. Høeg[a], Charles D. Derby[b]

[a]*Biological Institute, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen O, Denmark*
[b]*Department of Biology, Georgia State University, Atlanta, GA 30302-4010, USA*

Received 16 April 2004; received in revised form 28 June 2004; accepted 28 August 2004

Abstract

We studied electrophysiological properties of single chemosensory neurons in the mouthparts of the spiny lobsters *Panulirus argus* and *Panulirus interruptus* to complement our growing understanding of the behavioral roles of mouthparts of decapod crustaceans. Food mixtures and 13 single compounds were used to characterize the response specificity, sensitivity, and time course of individual neurons in the endopods of maxilliped 2 and 3. Additional chemoreceptors were found in the mandibular palp and basis of maxilliped 1 but they were not characterized. Neurons were broadly tuned, with the five most potent single compounds being ammonium, adenosine-5′ -monophosphate, taurine, glutamate, and aspartate. Cluster analysis indicated that the neurons constitute a heterogeneous population that could be placed into seven groups linked according to their most excitatory compound. These neurons in the mouthparts had concentration-dependent responses, with thresholds between 10^{-7} and 10^{-4} M and without saturation even at 10^{-3} or 10^{-2} M. They also quickly adapted when exposed to their best compounds at 10^{-4} and 10^{-3} M. A comparison of the response properties of these neurons in the mouthparts with those of chemosensory neurons in other crustacean appendages shows that neurons in the mouthparts have relatively broad tuning biased toward detecting and resolving high concentrations. Based on these comparisons, we suggest a functional distinction among the chemosensors on the different appendages: long distance detection by the antennae, precise location and collection by the pereiopods, and detailed assessment of quality by the mouthparts.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Chemoreceptors; Chemical senses; Gustation; Electrophysiology; Adaptation

1. Introduction

Behavioral studies indicate that many crustaceans rely on chemical information when detecting and handling their prey (Derby and Atema, 1982a; Atema, 1995; Zimmer and Butman, 2000; Weissburg, 2000; Derby et al., 2001). The sensory organs involved in

* Corresponding author. Department of Cell and Organism Biology, Lund University, Helgonavagen 3, 22362 Lund, Sweden. Fax: +46 46 222 4425.
E-mail address: anders.garm@cob.lu.se (A. Garm).

0022-0981/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jembe.2004.08.016

this process are mainly sensory hairs, called setae or sensilla, found on the antennae, pereiopods, mouthparts, as well as other body surfaces (Paffenhöfer and Loyd, 2000; Cate and Derby, 2001, 2002; Derby et al., 2001; Garm et al., 2003).

Decapod crustaceans, especially the Caribbean spiny lobster *Panulirus argus*, have been especially well studied models of chemosensory biology (Carr et al., 1990; Derby, 2000; Derby et al., 2001; Ache, 2002; McClintock and Xu, 2002). The antenna 1 (antennule) has received particular attention because in many crustaceans it carries specialized olfactory setae, called aesthetascs (Laverack, 1987; Derby, 1989; Hallberg et al., 1992). Aesthetascs are noteworthy because they are unimodal and densely innervated, often with hundreds of neurons per aesthetasc. This is in contrast to the many other types of antenna 1 setae, which are typically bimodal (mechano- and chemosensitive) and are innervated by much fewer neurons (e.g. Cate and Derby, 2001). The antenna 1 chemosensory neurons often have sub-nanomolar response thresholds (Thompson and Ache, 1980; Derby et al., 1991) and play a major role in long distance sensing of and orientation to prey items (Atema, 1995; Derby et al., 2001). The thoracic legs of decapods contain bimodal chemo- and mechanosensory setae (Shelton and Laverack, 1970; Hatt and Bauer, 1980, 1982; Derby and Atema, 1982a; Altner et al., 1983; Weissburg and Derby, 1995; Weissburg et al., 1996; Trott et al., 1997; Weissburg, 1999, 2001). The leg sensors can function as chemotactile organs in detecting nearby food (Derby and Atema, 1982b), although in some decapods they may also aid in distance orientation (Moore et al., 1991; Keller et al., 2003).

The behavioral role of mouthparts and the nature of the sensory innervation of their setae have received relatively little attention. The role of mouthparts in controlling ingestion of food has been experimentally demonstrated for *Homarus americanus* (Derby and Atema, 1982b). Morphological data on the innervation of mouthpart setae are available only from maxilla 2 of the shrimp *Palaemon adspersus* (Garm et al., 2003), maxillipeds of the copepod *Temora stylifera* (Paffenhöfer and Loyd, 1999, 2000), and maxilliped 3 of the shrimp *Penaeus merguiensis* (Alexander et al., 1980). For the shrimps, behavioral observations together with morphological data support the idea that mouthpart setae are mainly bimodal mechano- and chemosensors. Electrophysiological recordings of chemo- and mechanosensory neurons from maxilliped 3 have been made for *H. americanus* (Derby, 1982; Corotto et al., 1992).

The goal of this paper is to examine which mouthparts contain chemosensory neurons and to characterize their response properties, including specificity, threshold, and adaptation, using single-unit extracellular electrophysiological recordings. Special attention is given to maxilliped 2 and 3. We use the mouthparts of two species of spiny lobsters—*P. argus* and *Panulirus interruptus*, since they are model animals for crustacean sensory biology.

2. Materials and methods

Caribbean spiny lobsters *P. argus* (40–80 mm carapace length) and California spiny lobsters *P. interruptus* (80–100 mm CL) were captured in the wild and maintained in our laboratory in 400-l aquaria with artificial seawater (Instant Ocean™, Aquarium Systems, Mentor, OH) at 23–28 °C. They were fed shrimp and squid three times per week.

Mouthparts from *P. argus* examined in this study included mandibular palp (n=3), basis of maxilliped 1 (n=3), endopod of maxilliped 2 (n=13), and endopod of maxilliped 3 (n=9). Only maxilliped 2 was used in *P. interruptus* (n=21).

Immediately before each experiment, a mouthpart was ablated and dissected in cold lobster saline (g/l: 28 NaCl, 0.75 KCl, 3.4 $MgCl_2 \cdot 6H_2O$, 2.5 $CaCl_2 \cdot 2H_2O$, 3 Na_2SO_4, 0.3 glucose, 0.72 HEPES) to gain access to its nerve bundles and artery. For maxilliped 3, both the dactylus and propodus were removed from the endopod. The cuticle was removed from the propodus, and the muscle was removed by carefully cutting the apodeme at its insertion onto the dactylus and then gently removing it with forceps. This dissection left behind only the principal artery flanked by the major nerve. The artery and nerve bundle were separated from each other using fine tungsten needles, and the nerve was divided into four to six bundles. The procedure was similar for the mandibular palp and endopod of maxilliped 2, although the endopod of maxilliped 2 was cut at the

merus-carpus joint and the nerve in the carpus was used instead. For maxilliped 1, the basis was cut from the limb and the cuticle was removed from the proximal half. In this limb, only a small amount of muscle and connective tissue had to be removed to reveal the nerve and artery. When maxilliped 1 was used, the animal was anaesthetized on ice before dissection. Even though involved in food handling, the bases of maxilla 1 and 2 were not used due to their small size.

After the dissection, the preparation was secured in an olfactometer—a stimulating-recording chamber made of two Petri dishes separated by a dental-wax barrier. The preparation was secured in the wax, and the two parts of the mouthpart were separated by a barrier and in different dishes containing different solutions. The distal part of the preparation, which housed undissected cuticle and setae, was in a stimulating dish that contained artificial seawater (g/l: 24.7 NaCl, 0.66 KCl, 4.7 $MgCl_2 \cdot 6H_2O$, 1.9 $CaCl_2 2H_2O$, 6.3 $MgSO_4 \cdot 7H_2O$, 0.18 $NaHCO_3$). The proximal part, which had the exposed nerve and artery, was in a recording dish that contained saline. After the wax barrier was sealed, the artery was cannulated and perfused with pressurized, oxygenated lobster saline at a rate of 0.4–1.1 ml/min (see Derby, 1995, for more details). The time from ablation of the mouthpart to perfusion was 10–15 min, and nerve recordings were initiated 15 min later. The intact part of the limb was then placed inside a stimulation tube that was fabricated from a pipette tip. Artificial seawater flowed through the tube at 5 ml/min.

Chemical stimuli were presented to the preparation in 6-s pulses via a computer-controlled electronic value (Derby, 1995; Steullet and Derby, 1997). To examine the time course and dilution of the chemical stimulus as it passed through the olfactometer and over the appendage, measurements were taken using an IVEC system (In Vitro Electrochemistry) with Axopatch 200B® software. In this system, an electrode was calibrated for serotonin, and measurements were then taken just in front of the appendage and at the exit of the stimulating chamber as a pulse of 1 mM serotonin was introduced. The odor concentration at the exit of the chamber rose to a concentration 60–70% of that injected within 1–2 s, then continued to slowly increase during the stimulation period, eventually reaching 85% of the injected concentration.

Electrophysiological recordings were made *en passant* using fine-tipped extracellular suction electrodes as described by Derby (1995). Chemosensory neurons were identified using shrimp extract as a search stimulus (Carr and Derby, 1986) and afterwards their response to the control, artificial seawater (ASW), was tested. Throughout the experiments, ASW and the search stimulus were tested periodically to ensure that the responsiveness did not change; cells were in general discarded if the response became less than 70% of the initial response. All responses were corrected for response to the control (spontaneous activity), although few cells responded to ASW and when they did the response was very small (1–2 spikes).

We identified which mouthparts of *P. argus* contained chemosensory neurons and determined the response threshold of these neurons. The responses of the chemosensitive neurons were recorded to artificial crab mixture (Steullet et al., 2000) and to several known stimulatory single components of artificial crab mixture. Adenosine-5′-monophosphate (AMP), ammonium chloride (NH_4), taurine (Tau), L-glutamate (Glu), L-aspartate (Asp), L-cysteine (Cys), and L-histidine (His) were tested in random order on all neurons; L-arginine (Arg), betaine (Bet), glycine (Gly), L-lysine (Lys), and, L-proline (Pro) were tested on some neurons. All compounds were initially tested at 10^{-4} M, and the most excitatory compound was tested over at least a three-order of magnitude concentration series. Each stimulus was generally repeated twice. The interstimulus interval was 1 min, during which the system was washed with ASW three times.

In another experimental series, 21 chemosensory neurons from maxilliped 2 of *P. interruptus* were used to test the specificity (response spectrum), sensitivity (concentration–response function), and adaptation (saturation) of the mouthpart chemosensory neurons. These neurons were tested with at least 9 of 11 known stimulatory single compounds at 10^{-4} M in a random order. The compounds were: Tau, Glu, Asp, Cys, Lys, Pro, His, Gly, NH_4, AMP, and urea. In some cases, the most stimulatory compound was also tested at 10^{-3} M. There was an interstimulus interval of 1.5 min during which the system was washed with ASW three times.

All recordings lasted for 10 s (beginning 1 s before the opening of the electronic valve that introduced the chemical stimulus) and had a sampling frequency of 56 kHz using Axoscope 9.0 software (Axon Instru-

ments, Union City, CA). Spike sorting and quantification were performed using Datapac 2000 software (RUN Technologies, Mission Viejo, CA).

3. Results

3.1. Chemosensitivity of mouthpart appendages

Chemically evoked responses were successfully recorded from neurons in all mouthparts of *P. argus* that we examined: mandibular palp, basis of maxilliped 1, and endopods of maxilliped 2 and 3. We analyzed the sensory properties of neurons from maxilliped 2 and 3 of *P. argus* and maxilliped 2 of *P. interruptus*, since only they were recorded for sufficient time to complete protocols.

3.2. Response specificity of individual chemosensory neurons in P. interruptus

The 21 neurons from *P. interruptus* that were tested with up to 11 compounds were found to be rather broadly tuned (Fig. 1). In general, these neurons responded to more than half of the 11 single compounds tested, and for more than half of the neurons the second most excitatory compound produced a response at least 50% of the response magnitude displayed by the most excitatory compound. No more than three of the neurons responded only to a single compound (Fig. 1). One quantitative measure of the breadth of the responses is tuning breadth index, or H values (Smith and Travers, 1979), used previously for several different chemosensory systems including antenna 1 of spiny lobsters (Derby and Ache, 1984; Derby et al., 1991; Derby, 2000). According to this metric, H-values range from 0 to 1, with 0 representing a neuron responsive to only a single stimulus, and 1 representing a neuron equally responsive to all tested stimuli. *P. interruptus* mouthpart chemoreceptor neurons had H values of 0.58 ± 0.064 (mean±S.E.M.).

3.3. Response spectra of individual chemosensory neurons

We used cluster analysis, a multivariate technique that has been used previously on many chemosensory systems (Bieber and Smith, 1986), including antenna 1 chemosensory neurons of spiny lobsters (Derby, 2000), to determine if neurons might be grouped into types based on their response spectra. Cluster analysis of these 21 neurons segregated the chemoreceptors into seven clusters, grouped generally according to the first or second most excitatory compound for the neurons (Fig. 2). The seven clusters are: 2 Asp-best neurons, 1 His- and Urea-best neuron together with 1 Urea-best neuron, 1 AMP-best neuron, 1 Lys-best neuron together with 1 Cys-best neuron having Lys as second best compound, 5 NH4-best neurons together with 1 Glu-best neuron having NH_4 as second best compound, 3 Glu-best neurons together with 1 Tau-best neuron having Glu as second best compound, and 4 Tau-best neurons. Thus, these neurons are not homogeneous in terms of the response spectra; rather, they have different but partially overlapping sensitivities.

3.4. Concentration–response functions: sensitivity, thresholds, and saturation

To determine the sensitivity, threshold, and saturating concentration of neurons in the mouthparts, nine neurons from maxilliped 2 and 3 of *P. argus* were presented with their best-compound over a 3–4 log unit range of concentrations (Figs. 3 and 4). These included 5 NH_4-best neurons and 4-AMP-best neurons. All nine neurons had response thresholds between 10^{-7} and 10^{-4} M (after correction for the dilution in the olfactometer). NH_4- and AMP-best neurons had similar concentration–response functions with slopes of 4–18 spikes/log stimulus concentration (mean=11). All neurons continued to show increased response magnitudes with each successive concentration series. Even at the highest concentration of 10^{-2} M (which was tested on six neurons), the response magnitude increased significantly from that evoked by 10^{-3} M (Figs. 3 and 4). Data pooled from neurons in both species show that neurons are not saturating at the highest concentrations. The mean±S.E.M. interspike interval in the initial first second of the response was 92 ± 21 ms (n=12) at 10^{-3} M, and 39 ± 7 ms (n=6) at 10^{-2} M. The mean interspike interval at 10^{-2} M was statistically smaller than at 10^{-3} M (Student's t-test, $p<0.05$). There were no differences in the mean interspike interval between the

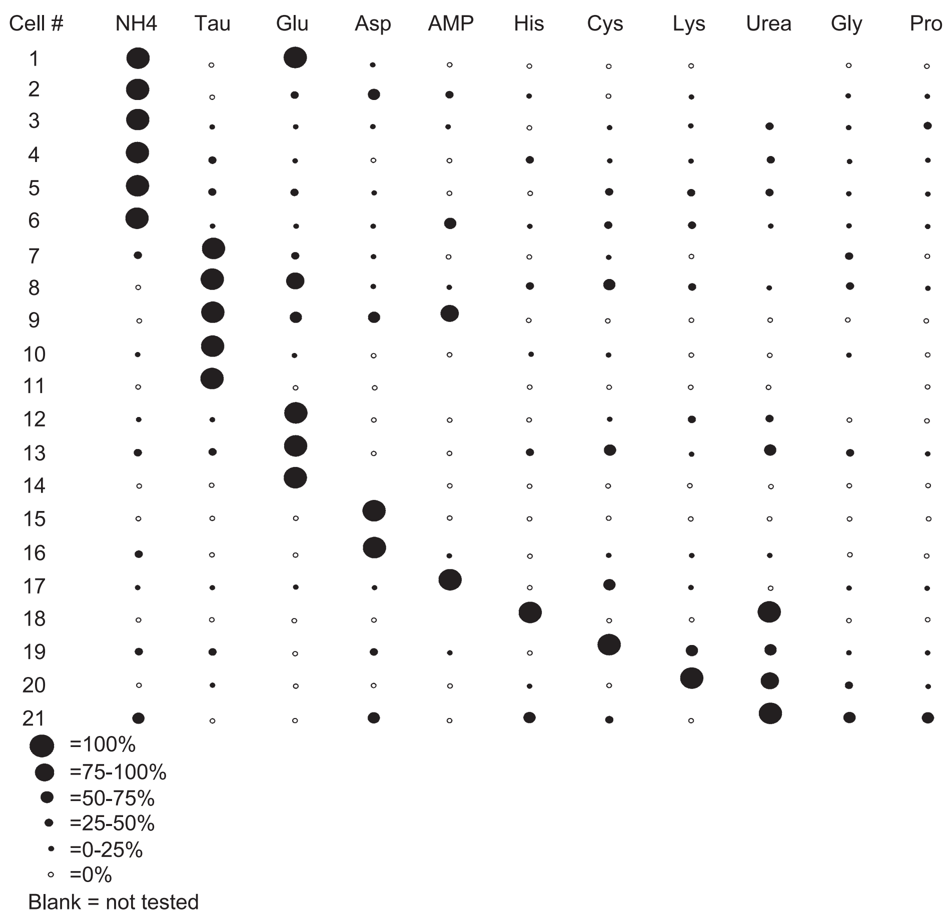

Fig. 1. Responses of 21 *P. interruptus* mouthpart chemosensory neurons to 11 different single compounds at 10^{-4} M. The size of the dots relates in percentage to the response to the "best" compound. See text for a description of the results.

two species (ANOVA, $F_{(1,27)}$=1.32, p=0.20) or between neurons with different best compounds at either concentration (ANOVA, $F_{(1,9)}$=3.15, p=0.37).

3.5. Adaptation rates

The adaptation rate to the best compound was tested at 10^{-4} M for 17 *P. interruptus* neurons and 9 *P. argus* neurons and for 8 *P. interruptus* neurons and 5 *P. argus* neurons at 10^{-3} M. A two-factor ANOVA test showed no significant effect of concentration on the adaptation rates ($F_{(1,35)}$=0.645, p=0.42) but a significant difference between the two species ($F_{(1,35)}$=21.8, p=0.000044). Even though there is a species difference, the mouthpart chemoreceptors from both species display a very fast adaptation rate. The adaptation rate of *P. interruptus* neurons is faster with the response reaching below 20% of the initial response within 1.5 s. *P. argus* neurons reach 20% of the initial response within 3 s (Fig. 5). The species also differ in that *P. interruptus* completely adapts after 4 s whereas the neurons from *P. argus* adapted to a level around 20% of the initial response. It should be noted that 4 of 14 *P. argus* neurons also adapted completely within 4 s, and 8 of them adapted completely within 6 s.

4. Discussion

We have shown that all the mouthparts of the Caribbean spiny lobster *P. argus* which we examined,

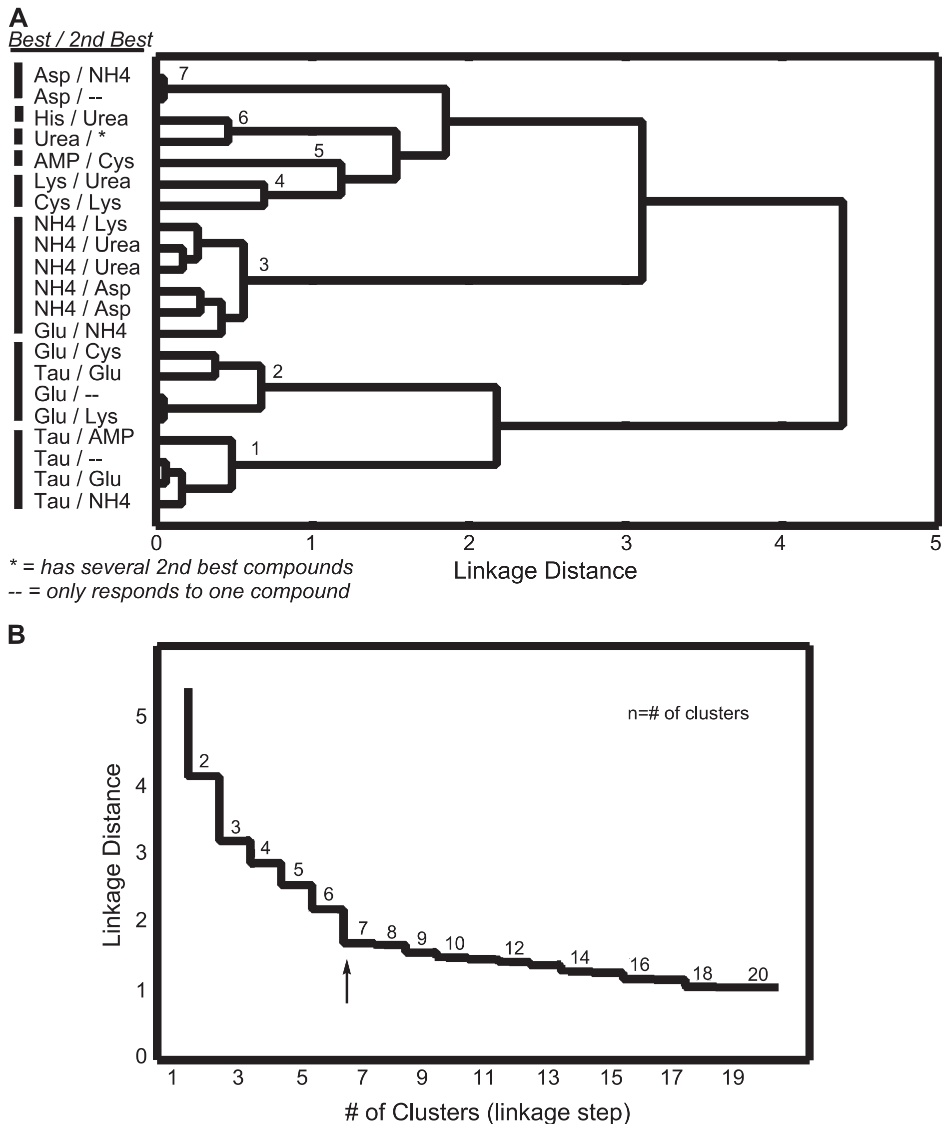

Fig. 2. Cluster analysis of the response spectra of 21 *P. interruptus* mouthpart chemosensory neurons identifies seven clusters or 'neuron types'. (A) Tree diagram of the results of the cluster analysis, which used 1-Pearson *r* as the distance metric and Ward's method as the clustering algorithm (Statistica, StatSoft, Tulsa, OK). The diagram shows that the 21 neurons, each represented by a horizontal line, are best grouped into seven clusters (represented by a number at the top of each cluster). Each cluster is well characterized by the member neurons' 1st or 2nd most excitatory compound, which are given to the left of each neuron. (B) Scree diagram for the cluster solution, demonstrating that the 7-cluster solution is the best one. Best-fit solution is indicated by an inflection point in the scree diagram; i.e. the point at which occurs a sharp decline in the linkage distance at which clusters are formed (Bieber and Smith, 1986). In our analysis, the inflection point (represented by the arrow) occurs after the formation of seven clusters (arrow).

including the mandibular palp, basis of maxilliped 1, propodus and dactylus of the endopod of maxilliped 2, and dactylus of maxilliped 3, contain chemosensory neurons. In addition, we have characterized the specificity, sensitivity, and temporal properties of chemoreceptors neurons in maxilliped 2 and 3 of *P. argus* and *P. interruptus*. Because of many similarities in the neural responses of the mouthparts from these

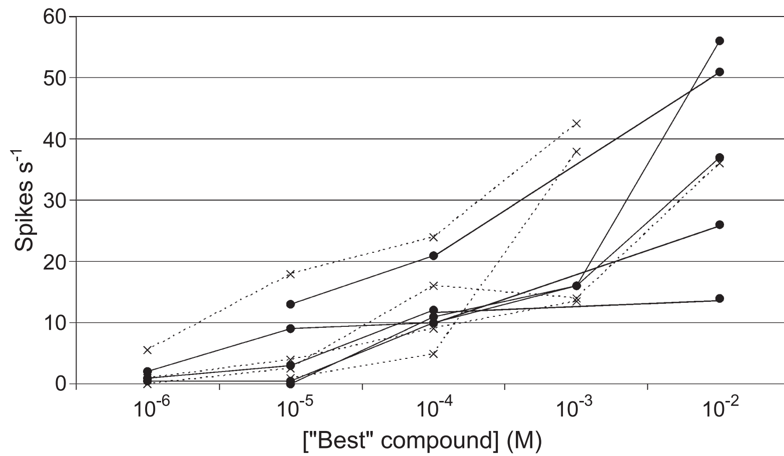

Fig. 3. Concentration–response functions for nine *P. argus* mouthpart chemosensory neurons to their best compound. Included are five ammonium-best neurons (solid lines) and four AMP-best neurons (broken lines). Responses are quantified as the number of spikes in the 1st sec of response. All neurons had response thresholds between 10^{-4} and 10^{-7} M, and no neurons saturated in this concentration range. Data points are typically the mean of two responses.

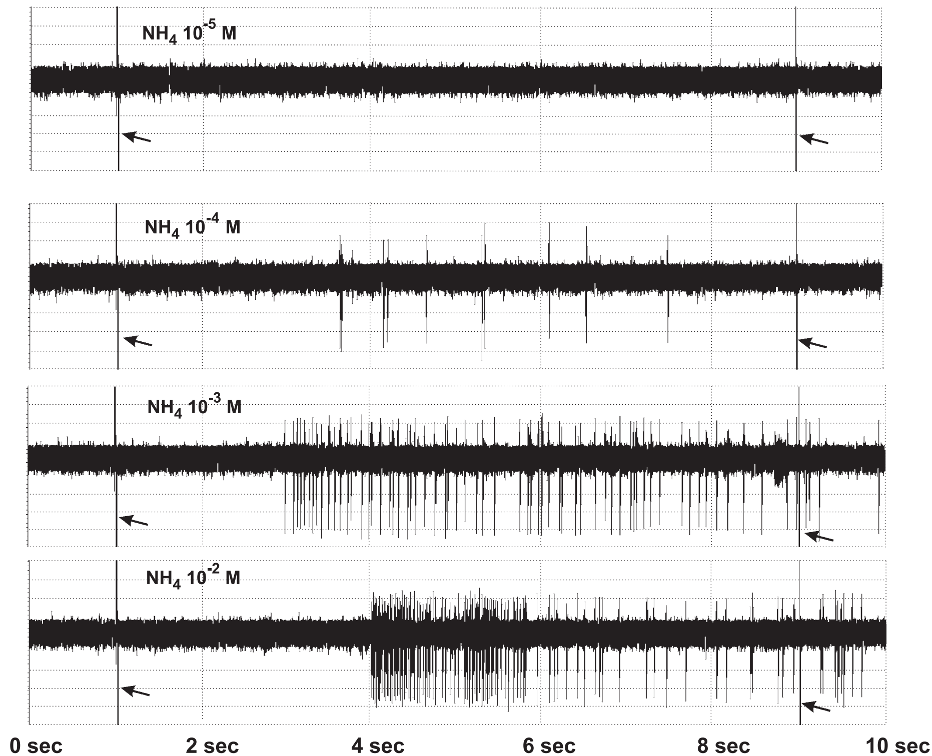

Fig. 4. Example of concentration-dependent responses from an ammonium-best neurons from *P. argus*. This neuron had a response threshold between 10^{-4} and 10^{-5} M and did not saturate even at 10^{-2} M. Arrows indicate on-set (left) and off-set (right) of stimulus.

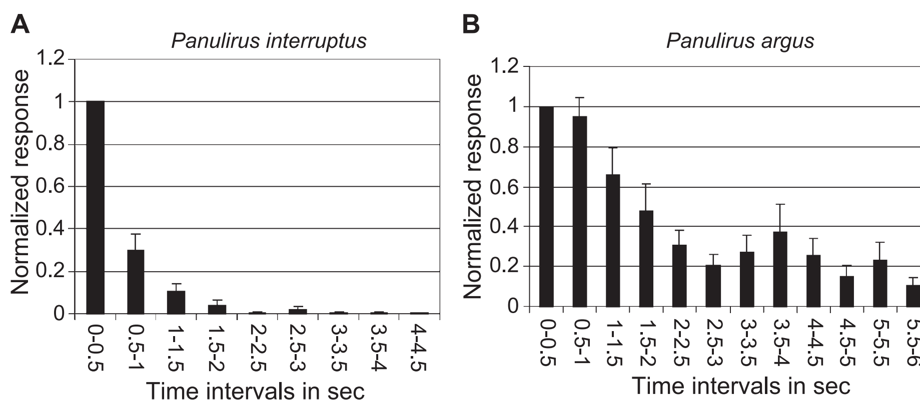

Fig. 5. Adaptation of *P. interruptus* (A) and *P. argus* (B) mouthpart chemosensory neurons when stimulated with their best compound at 10^{-4} M or 10^{-3} M. There was a significant difference in the adaptation rate between the two species but not between the two concentrations (two-factor ANOVA). Responses were normalized to the number of spikes in the 1st 0.5 s of the response. Responses are mean±S.E.M. n=25 in A, 14 in B.

two species, we differentiate between them in the following discussion only in specific situations where our data document clear differences in response properties.

4.1. Neurons in the mouthparts are broadly tuned to food odors and have a diversity of response spectra

Chemoreceptor neurons in the mouthparts of *Panulirus* are broadly tuned relative to most other crustacean chemoreceptor neurons, including those on other appendages of *Panulirus* and those on the mouthparts of *H. americanus*. This is seen from a quantification using the breadth of tuning metric, or *H* values, for a neuron. Our study shows that mouthpart chemoreceptor neurons have an average *H* value of 0.58, compared to *H*=0.27 for neurons from antenna 1 of *P. argus* (Derby and Ache, 1984). Interestingly chemosensory neurons from maxilliped 3 of *H. americanus* are also narrowly tuned (mean *H*=0.25) (Corotto et al., 1992). The functional significance of this difference is not clear, especially since studies on the feeding behavior of these two species do not indicate any distinct differences in the use or function of their mouthparts (Derby and Atema, 1982b; Lavalli and Factor, 1995; Garm, 2004). It should be noted that the *H*-value can be biased towards higher values by challenging neurons with high stimulus concentrations (Derby et al., 1991). We selected concentrations midway in the response–concentration curve, suggesting that the large *H* values reported here are not artifacts of using high concentrations. Chemoreceptor neurons from the minor claw of the fiddler crab *Uca pugnax* are also broadly tuned, some of them with *H*-values as high as 0.8 (Weissburg, 1999). Similar to the mouthparts of lobsters, this claw is also directly involved in food manipulation. This broad tuning is believed to be correlated with the fact that fiddler crabs feed on microorganisms, where the animals are unable to choose some food particles over others (Weissburg, 1999). This does not seem to be the case for the mouthparts of *Panulirus,* where active sorting of food particles occurs frequently (Garm, 2004).

Even though mouthpart chemoreceptor neurons are broadly tuned, they do not have identical response spectra. Cluster analysis revealed that the population of 21 neurons belongs to seven groups, clustered according to their best- and second-best-compounds (Figs. 1 and 2). This analysis can be conservatively interpreted to indicate that the mouthparts contain a heterogeneous population of neurons; the exact nature of these neurons, including differences between them and the existence of ‘neuron types’, will require testing them with a larger and broader array of compounds and even mixtures (see Derby, 2000 for a discussion of ‘neuron types’ in crustaceans).

The five most excitatory compounds for *Panulirus* mouthpart chemoreceptor neurons were (in rank order) ammonium, taurine, AMP, glutamate, and aspartate. Since these compounds are common in the food of spiny lobsters (Carr and Derby, 1986), detection of these compounds by neurons in the

mouthparts likely indicates the presence of food in their mouth. Ammonium, glutamate, and taurine, but not aspartate, are also potent compounds for chemosensory neurons on maxilliped 3 of *H. americanus* (Corotto et al., 1992). AMP was not tested on *H. americanus*, and we did not test hydroxy-L-proline (the most potent compound for *H. americanus*: Voigt and Atema, 1992) on the mouthparts of *P. argus* because it is in very low concentrations in the tissue of prey of *P. argus* and at these concentrations it is at best weakly stimulatory to antenna 1 of *P. argus* (Derby and Ache, 1984).

Ammonium, AMP, taurine, and glutamate are also the most potent compounds for the chemosensory neurons found in the lateral flagellum of antenna 1 of *P. argus* (Derby et al., 1991). This shows that despite the fact that setae on the mouthparts and antenna 1 have different functions, they detect many of the same compounds. In the case of *H. americanus,* the populations of neurons on the antenna 1, antenna 2, maxilliped 3, and pereiopods also respond to a similar set of compounds, but they differ in the percentage distribution of neuron types, especially hydroxy-L-proline best neurons, which were much more abundant on the antenna 1 and 2 (Voigt and Atema, 1992). It is not surprising that the olfactory and gustatory pathways detect the same compounds, since they are probably the ones carrying the most information on prey quality. In some cases though, differences are expected, since some compounds are more suitable for long distance dispersal than others (Zimmer and Butman, 2000). This means that some information containing compounds may be detected by the mouthparts but not by the antennae, since they are not dispersed well over long distances.

4.2. Mouthpart chemoreceptor neurons have high threshold and saturation levels

Concentration–response functions for mouthpart chemosensory neurons revealed thresholds between 10^{-7} and 10^{-4} M, and saturation was at least 10^{-2} M (Figs. 3 and 4), thus conferring a dynamic range of at least 4 log units. These threshold and saturation levels are high compared with those of chemosensory neurons on the antenna 1 of *P. argus*, whose thresholds are nanomolar or even lower (Thompson and Ache, 1980; Derby et al., 1991) and saturation points are often micromolar (Derby et al., 1991; Cromarty and Derby, 1997). Glutamate-best neurons in the maxilliped 3 of *H. americanus* were also found to have thresholds of about 10^{-7} M (Corotto et al., 1992). These thresholds are in correspondence with the functions of the limbs containing the neurons. When an animal tears its prey and the mouthpart setae are in direct contact with the food, the concentrations of the chemicals are extremely high compared to the situation in long distance sensing via antenna 1. The thresholds of neurons in the mouthparts are also higher than those described for the neurons on the pereiopods of some crustacean species (Hatt and Bauer, 1980, 1982; Derby and Atema, 1982a; Altner et al., 1983; Hatt, 1986). Chemoreceptors in the claws of fiddler crabs display high response thresholds, which is correlated with the high background levels of the excitatory compounds (Weissburg, 1999). High background levels could also explain the high thresholds in spiny lobster chemoreceptor neurons, since the tearing of food is likely to result in high concentrations of dissolved compounds around the mouth apparatus. Other reports of chemosensors in crustaceans with similarly high thresholds are from neurons in funnel canal organs on the legs of *Carcinus maenas* (Schmidt and Gnatzy, 1989). High thresholds are also found in setae in the oesophagus of the crayfish *Astacus astacus* (Altner et al., 1986), which are also obviously contact chemosensors that function during food processing after the mouthparts.

A dynamic range of 4 log units is typical of crustacean chemoreceptors. A similar dynamic range was found for chemoreceptors on the claws and legs of *Uca* (fiddler crabs) (Weissburg and Derby, 1995), on the antennae, pereiopods, and maxilliped 3 of *H. americanus* (Borroni and Atema, 1988a; Voigt and Atema, 1992; Corotto et al., 1992), and on antenna 1 of *P. argus* (Derby, 1989; Cate and Derby, 2002). These similarities in the dynamic range suggest common properties of the receptor molecules in the different chemosensory appendages even though they play different behavioral roles.

4.3. Mouthpart chemosensory neurons show rapid adaptation

The chemosensory neurons from maxilliped 2 and 3 rapidly adapt, with the response rate

decreasing significantly within 1–3 s (Fig. 5). Adaptation rates at 10^{-3} and 10^{-4} M did not significantly differ, even though adaptation rate normally increases with higher stimulus concentration. The lack of difference found in this study is probably due to the tested concentrations being only one log unit apart. It is very interesting that the adaptation rates differ between the two species. This could indicate that the mouthpart chemoreceptors and thereby the mouthparts have different functions in the two species. Functional observations on the mouthparts of *P. argus* are available (Garm et al., 2004), but not from *P. interruptus*, and it is therefore not possible at present to test this hypothesis.

The adaptation rates reported here are intermediate to adaptation rates from other crustacean chemoreceptors. Fiddler crabs of the genus *Uca* have very slowly adapting neurons in the dactylus and propodus of their legs and claws, giving a more or less constant response during the first 5 s of exposure to chemical stimuli (Weissburg and Derby, 1995). Chemoreceptors in antenna 1 of *P. argus* are also slowly adapting compared to the mouthpart chemoreceptors (Derby, unpublished data). The chemoreceptors in antenna 1 of *H. americanus* are described as having extremely rapid adaptation rates, completely adapting within 0.5 s (Gomez and Atema, 1996).

The functional significance of rapid adaptation in the mouthpart chemoreceptors is not known. Rapid adaptation has been suggested to be an important temporal filter for antenna 1 chemoreceptors, enabling the animal to detect small concentration differences in turbulent waters (Gomez and Atema, 1996, 1999). Obviously, this function does not apply to the mouthpart receptors. Adaptation is likely to decrease the sensory output from these mouthparts since they are often in prolonged contact with prey items during their handling, sometimes for several minutes in the case of maxilliped 3 (Garm and Høeg, 2001; Garm, 2004). During such periods of food holding by maxilliped 3, the threshold of its neurons should shift to a new level—to the concentration of that chemical in the prey (Borroni and Atema, 1988a,b). The functional implications of the rapid adaptation of the chemosensory neurons are largely dependent on their recovery time, but we did not examine this parameter from the neurons in the mouthparts of *Panulirus*.

4.4. Functional organization

Our observations on chemosensors in the mouthparts of *P. argus* and *P. interruptus* fit well with the earlier suggested functional divisions of the different chemosensitive appendages (Derby and Atema, 1982a,b; Moore et al., 1991; Voigt and Atema, 1992). The antennae serve as long distance detectors and in locating chemicals from a distance. The pereiopods direct local searches until the prey is captured, after which the mouthparts functionally take over. With their high threshold, broad tuning, and fast adaptation, mouthpart chemoreceptors probably perform a final check of the quality of the prey item, i.e. they identify if feeding stimulants are present and deterrents are absent. This last quality check is likely to also include processing of tactile information from the bimodal setae (Garm et al., 2004).

Acknowledgments

The authors thank the staff of the Florida Key Marine Laboratory for supplying lobsters. We also thank Dr. Akira Sakurai, Georgia State University, USA, for assistance with the IVEC measurements in the olfactometer, and Dr. Manfred Schmidt for critical comments on the manuscript. Supported by DC00312 from the National Institute on Deafness and other Communication Disorders, IBN-0077474 from the National Science Foundation, the Georgia Research Alliance, the GSU Research Program Enhancement Fund, a framegrant from the Danish Research Agency (SNF), and the PhD program at the University of Copenhagen. All experiments in this study comply with the "Principles of animal care", Publication No. 86-23, revised 1985 of the National Institute of Health and with the laws of the United States of America. **[SS]**

References

Ache, B.W., 2002. Crustaceans as animal models for olfactory research. In: Wiese, K. (Ed.), Crustacean Experimental Systems in Neurobiology. Springer-Verlag, New York, pp. 189–199.

Alexander, C.G., Hindley, J.P.R., Jones, S.G., 1980. Structure and function of the third maxillipeds of the banana prawn *Penaeus merguiensis*. Mar. Biol. 58, 245–249.

Altner, I., Hatt, H., Altner, H., 1983. Structural properties of bimodal chemo- and mechanosensitive setae on the pereiopod chelae of the crayfish, *Austropotamobius torrentium*. Cell Tissue Res. 228, 357–374.

Altner, H., Hatt, H., Altner, I., 1986. Structural and functional properties of the mechanoreceptors and chemoreceptors in the anterior oesophageal sensilla of the crayfish, *Astacus astacus*. Cell Tissue Res. 244, 537–547.

Atema, J., 1995. Chemical signals in the marine environment: dispersal, detection, and temporal signal analysis. Proc. Nat. Acad. Sci. U. S. A. 92, 62–66.

Bieber, S.L., Smith, D.V., 1986. Multivariate analysis of sensory data: a comparison of methods. Chem. Senses 11, 19–47.

Borroni, P.F., Atema, J., 1988a. Adaptation in chemoreceptor cells: I. Self-adapting backgrounds determine threshold and cause parallel shift of response function. J. Comp. Physiol. A 164, 67–74.

Borroni, P.F., Atema, J., 1988b. Adaptation in chemoreceptor cells: II. The effects of cross-adapting backgrounds depend on spectral tuning. J. Comp. Physiol. A 165, 669–677.

Carr, W.E.S., Derby, C.D., 1986. Behavioral chemoattractants for the shrimp, *Palamonetes pugio*: identification of active compounds in food extracts and evidence of synergistic mixture interactions. Chem. Senses 11, 49–64.

Carr, W.E.S., Gleeson, R.A., Trapido-Rosenthal, H.G., 1990. The role of perireceptor events in chemosensory processes. Trends Neurosci. 13, 212–215.

Cate, H.S., Derby, C.D., 2001. Morphology and distribution of setae on the antennules of the Caribbean spiny lobster *Panulirus argus* reveal new types of bimodal chemo-mechanosensilla. Cell Tissue Res. 304, 439–454.

Cate, H.S., Derby, C.D., 2002. Ultrastructure and physiology of the hooded sensillum, a bimodal chemo-mechanosensillum of lobsters. J. Comp. Neurol. 442, 293–307.

Corotto, F., Voigt, R., Atema, J., 1992. Spectral tuning of chemoreceptor cells of the third maxilliped of the lobster, *Homarus americanus*. Biol. Bull. 183, 456–462.

Cromarty, S.I., Derby, C.D., 1997. Multiple excitatory receptor types on individual olfactory neurons: implications for coding of mixtures in the spiny lobster. J. Comp. Physiol. A 180, 481–491.

Derby, C.D., 1982. Structure and function of cuticular sensilla of the lobster *Homarus americanus*. J. Crust Biol. 2, 1–21.

Derby, C.D., 1989. Physiology of sensory neurons in morphologically identified cuticular sensilla of crustaceans. In: Felgenhauer, B.E., Watling, L., Thistle, A.B. (Eds.), Functional Morphology of Feeding and Grooming in Crustacea. A.A.Balkema, Rotterdam, pp. 27–48.

Derby, C.D., 1995. Single unit electrophysiological recordings from crustacean chemoreceptor neurons. In: Spielman, A.I., Brand, J.G. (Eds.), Experimental Cell Biology of Taste and Olfaction. Current Techniques and Protocols. CRC Press, New York, pp. 241–250.

Derby, C.D., 2000. Learning from spiny lobsters about chemosensory coding of mixtures. Physiol. Behav. 69, 203–209.

Derby, C.D., Ache, B.W., 1984. Electrophysiological identification of the stimulatory and interactive compounds of a complex odorant. Chem. Senses 9, 201–218.

Derby, C.D., Atema, J., 1982a. Chemosensitivity of walking legs of the lobster *Homarus americanus*: neurophysiological response spectrum and thresholds. J. Exp. Biol. 98, 303–315.

Derby, C.D., Atema, J., 1982b. The function of chemo- and mechano-receptors in lobsters (*Homarus americanus*) feeding behaviour. J. Exp. Biol. 98, 317–327.

Derby, C.D., Girardot, M., Daniel, P.C., 1991. Responses of olfactory receptor cells of spiny lobsters to binary mixtures: I. Intensity mixture interactions. J. Neurophysiol. 66, 112–130.

Derby, C.D., Steullet, P., Horner, A.J., Cate, H.S., 2001. The sensory basis of feeding behaviour in the Caribbean spiny lobster, *Panulirus argus*. Mar. Freshw. Res. 52, 1339–1350.

Garm, A., 2004. Mechanical functions of setae from the mouth apparatus of seven species of decapod crustaceans. J. Morphol. 260, 85–100.

Garm, A., Høeg, J.T., 2001. Function and functional groupings of the complex mouth apparatus of the squat lobsters *Munida sarsi* Huus and *M. tenuimana* G.O. Sars (Crustacea: Decapoda). Biol. Bull. 200, 281–297.

Garm, A., Hallberg, E., Høeg, J.T., 2003. The role of maxilla 2 and its setae during feeding in the shrimp *Palaemon adspersus* (Crustacea: Decapoda). Biol. Bull. 204, 126–137.

Garm, A., Derby, C.D., Høeg, J.T., 2004. Mechanosensory neurons in mouthpart setae from the spiny lobster *Panulirus argus* with bend- and osmo-sensitivity. Biol. Bull., in press.

Gomez, G., Atema, J., 1996. Temporal resolution in olfaction: II. Time course of recovery from adaptation in lobster chemoreceptor cells. J. Neurophysiol. 76, 1340–1343.

Gomez, G., Atema, J., 1999. Temporal resolution in olfaction: III. Flicker fusion and concentration-dependent synchronization with stimulus pluse trains of antennular chemoreceptor cells in the American lobster. J. Comp. Physiol. A 185, 427–436.

Hallberg, E., Johansson, K.U.I., Elofsson, R., 1992. The aesthetasc concept: structural variations of putative olfactory receptor cell complexes in Crustacea. Microsc. Res. Tech. 22, 325–335.

Hatt, H., 1986. Responses of a bimodal neuron (chemo- and vibration-sensitive) on the walking legs of the crayfish. J. Comp. Physiol. A 159, 611–617.

Hatt, H., Bauer, U., 1980. Single unit analysis of mechano- and chemosensitive neurons in the crayfish claw. Neurosci. Lett. 17, 203–207.

Hatt, H., Bauer, U., 1982. Electrophysiological properties of pyridine receptors in the crayfish walking leg. J. Comp. Physiol. A 148, 221–224.

Keller, T., Powell, I., Weissburg, M.J., 2003. Identifying the role of olfactory appendages in chemically mediated orientation of blue crabs. Mar. Ecol. Prog. Ser. 261, 217–231.

Lavalli, K., Factor, J., 1995. The feeding appendages. In: Factor, J. (Ed.), Biology of the Lobster. Academic Press, New York, pp. 349–393.

Laverack, M.S., 1987. The nervous system of the Crustacea, with special reference to the organisation of the sensory system. In: Ali, M.A. (Ed.), Nervous System of the Invertebrates. Springer-Verlag, Berlin, pp. 1–30.

McClintock, T.S., Xu, F., 2002. Molecular physiology of G-proteins in olfactory transduction and CNS neurotransmission in the lobster. In: Wiese, K. (Ed.), The Crustacean Nervous System. Springer-Verlag, Berlin, pp. 359–366.

Moore, P.A., Scholtz, N., Atema, J., 1991. Chemical orientation of the lobster, *Homarus americanus* in a turbulent odor plume. J. Chem. Ecol. 17, 1293–1307.

Paffenhöfer, G.A., Loyd, P.A., 1999. Ultrastructure of the setae of the maxilliped of the marine planktonic copepod *Temora stylifera*. Mar. Ecol. Prog. Ser. 178, 101–107.

Paffenhöfer, G.A., Loyd, P.A., 2000. Ultrastructure of cephalic appendages setae of marine planktonic copepods. Mar. Ecol. Prog. Ser. 203, 171–180.

Schmidt, M., Gnatzy, W., 1989. Specificity and response characteristics of gustatory sensilla (funnel-canal organs) on the dactylus of the shore crab, *Carcinus maenas* (Crustacea Decapoda). J. Comp. Physiol. A 166, 227–242.

Shelton, R.G.J., Laverack, M.S., 1970. Receptor hair structure and function in the lobster *Homarus gammarus* (L.). J. Exp. Mar. Biol. Ecol. 4, 201–210.

Smith, D.V., Travers, J.B., 1979. A metric for the breadth of tuning of gustatory neurons. Chem. Senses 4, 215–229.

Steullet, P., Derby, C.D., 1997. Coding of blend ratios of binary mixtures by olfactory neurons in the Florida spiny lobster, *Panulirus argus*. J. Comp. Physiol. A 180, 123–135.

Steullet, P., Krützfeldt, D.R., Hamidani, G., Flavus, T., Ngo, V., Derby, C.D., 2000. Dual antennular chemosensory pathways mediate odor-associative learning and odor discrimination in the Caribbean spiny lobster *Panulirus argus*. J. Exp. Biol. 205, 851–867.

Thompson, H., Ache, B.W., 1980. Threshold determination for olfactory receptors of the spiny lobster. Mar. Behav. Physiol. 7, 249–260.

Trott, T.J., Voigt, R., Atema, J., 1997. Chemoreception by the red-jointed fiddler crab *Uca minax* (LeConte): spectral tuning properties of the walking legs. Mar. Freshw. Behav. Physiol. 30, 239–249.

Voigt, R., Atema, J., 1992. Tuning of chemoreceptor cells of the second antenna of American lobster (*Homarus americanus*) with a comparison of four of its other chemoreceptor organs. J. Comp. Physiol. A 171, 673–683.

Weissburg, M.J., 1999. Tuning breadth and sex-specific sensitivity in chemosensory neurons of male and female *Uca pugnax*. J. Comp. Physiol. A 185, 229–238.

Weissburg, M.J., 2000. The fluid dynamical context of chemosensory behavior. Biol. Bull. 198, 188–202.

Weissburg, M.J., 2001. Sex, sensitivity, and second messengers: differential effect of cyclic nucleotide mediated inhibition in the chemosensory system of fiddler crabs. J. Comp. Physiol. A 187, 489–498.

Weissburg, M.J., Derby, C.D., 1995. Regulation of sex-specific feeding behavior in fiddler crabs: physiological properties of chemoreceptor neurons in claws and legs of males and females. J. Comp. Physiol. A 176, 513–526.

Weissburg, M.J., Pearce, J., Govind, C.K., Derby, C.D., 1996. Sexually dimorphic patterns of neural organization in the feeding appendages of fiddler crabs. Cell Tissue Res. 286, 155–166.

Zimmer, R.K., Butman, C.A., 2000. Chemical signaling processes in the marine environment. Biol. Bull. 198, 168–187.