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Failure to replicate evidence of decapod crustacean nociception

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Introduction

Nociception is the physiological detection of stimuli that are potentially damaging to tissue. It is closely correlated, but not identical, to the psychological experience of pain. Understanding nociception in a particular species has significant implications for the care and welfare of that species, and may create new models for research on human pain.

Invertebrate nociception has been documented in multiple phyla, including annelid worms (i.e., leeches; Pastor et al., 1996), nematode worms (i.e., *Caenorhabditis elegans*; Wittenburg and Baumeister, 1999), mollusks (i.e., sea hares, *Aplysia californica*; Illich and Walters, 1997), and insects (i.e., fruit flies, *Drosophila melanogaster*; Al-Anzi et al., 2006; Tracey et al., 2003).

Recently, Barr and colleagues (2007) published the first behavioral evidence of crustacean nociception using prawns, *Palaemon elegans*. They applied acids or bases to one of an individual's antennae and found that subsequent grooming and rubbing was preferentially directed towards the stimulated antennae. These behaviors were reduced if a local anesthetic was applied to the antennae prior to stimulation. These results are consistent with nociception in better-studied vertebrates.

Here, we attempt to replicate their experiments with two other species of decapod crustaceans that bracket *P. elegans* phylogenetically: white shrimp (*Litopenaeus setiferus*) and Louisiana red swamp crayfish (*Procambarus clarkii*). *Litopenaeus setiferus* have a similar ecology to prawns. *Procambarus clarkii* is widely used in the study of neurobiology, and would be an excellent candidate for further neurophysiological studies.

Methods

Sodium hydroxide (NaOH) was used as a noxious stimulus in all experiments, because it generated the largest effects in prior experiments (Barr et al. 2007). Preliminary trials indicated animals did not respond to sodium hydroxide (NaOH) at concentrations used by Barr et al., however, so the concentration was increased to 6M.

All animals were tested in 17.5×10×9 cm tanks, comparable to those used by Barr et al. (2007). Following application of stimuli, behavior was observed for 10 minutes, compared to 5 minutes in Barr et al. (2007).

Behavior was measured in two ways, based on methods in Barr et al. (2007). "Grooming" was measured by contact of other portions of the body (i.e., mouth, legs) with either antenna. Unlike Barr et al. (2007), we did not include antennae contacting the tank wall in our measure of grooming, as incidental contact seemed highly probable given the small size of the tank and the length of the antennae, particularly in shrimp. Activity ("movement") was measured by counting the number of times the anterior region of the carapace (i.e., eyes) crossed the midline of the tank along its long axis.

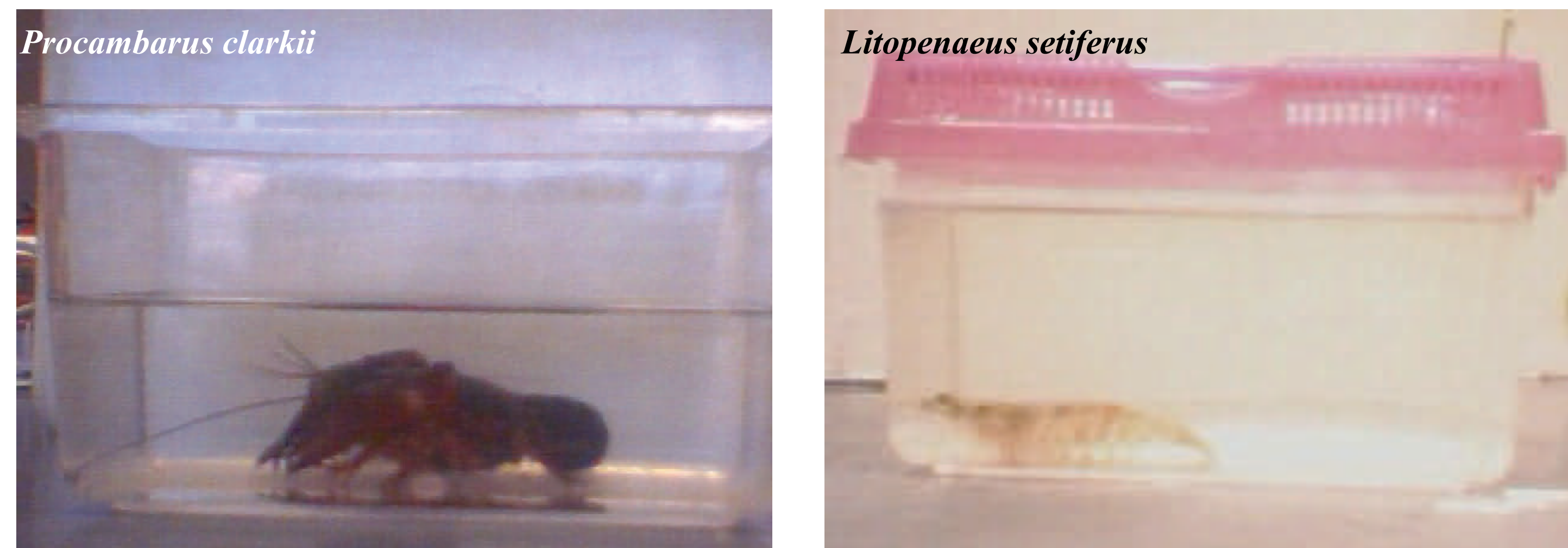


Figure 1. Experimental subjects in testing tanks.

Experiment 1

Procambarus clarkii (Girard, 1852) were bought from commercial suppliers, then transported to The University of Texas-Pan American and housed individually in aquaria. Crayfish of both sexes were placed on a paper towel and one antenna was swabbed at random with deionized water (control) or 6M NaOH. Individual were placed in a tank filled with ~5-8 cm of fresh water (about twice as deep as Barr et al. 2007), and their behaviors were video recorded.

Experiment 2

Litopenaeus setiferus (Linnaeus, 1767) were bought from commercial suppliers and housed in communally in aquaria at The University of Texas-Pan American. Shrimp of both sexes were placed on a paper towel and one antenna was swabbed at random with seawater (control) or 6M sodium hydroxide (NaOH). Individuals were placed in a tank filled with ~5-8 cm of water and their behaviors were video recorded.

Antennae were examined under a dissecting microscope before and after swabbing with water and NaOH to determine if swabbing caused any noticeable alterations in antennal shape, particularly putative sensory hairs.

Experiment 3

Litopenaeus setiferus were bought from the same commercial supplier as Experiment 2. This experiment was conducted on site to minimize any aberrant behavior caused by transport and extended housing in aquaria. Shrimp were placed on a paper towel and one antenna was swabbed at random with tap water (control) or 6M NaOH. Individual were placed in a tank filled with ~3 cm of water (comparable to Barr et al. 2007) and their behaviors were recorded for 10 minutes.

Results

No significant differences were found between control animals and animals exposed to the putative noxious stimulus.

Experiment 1

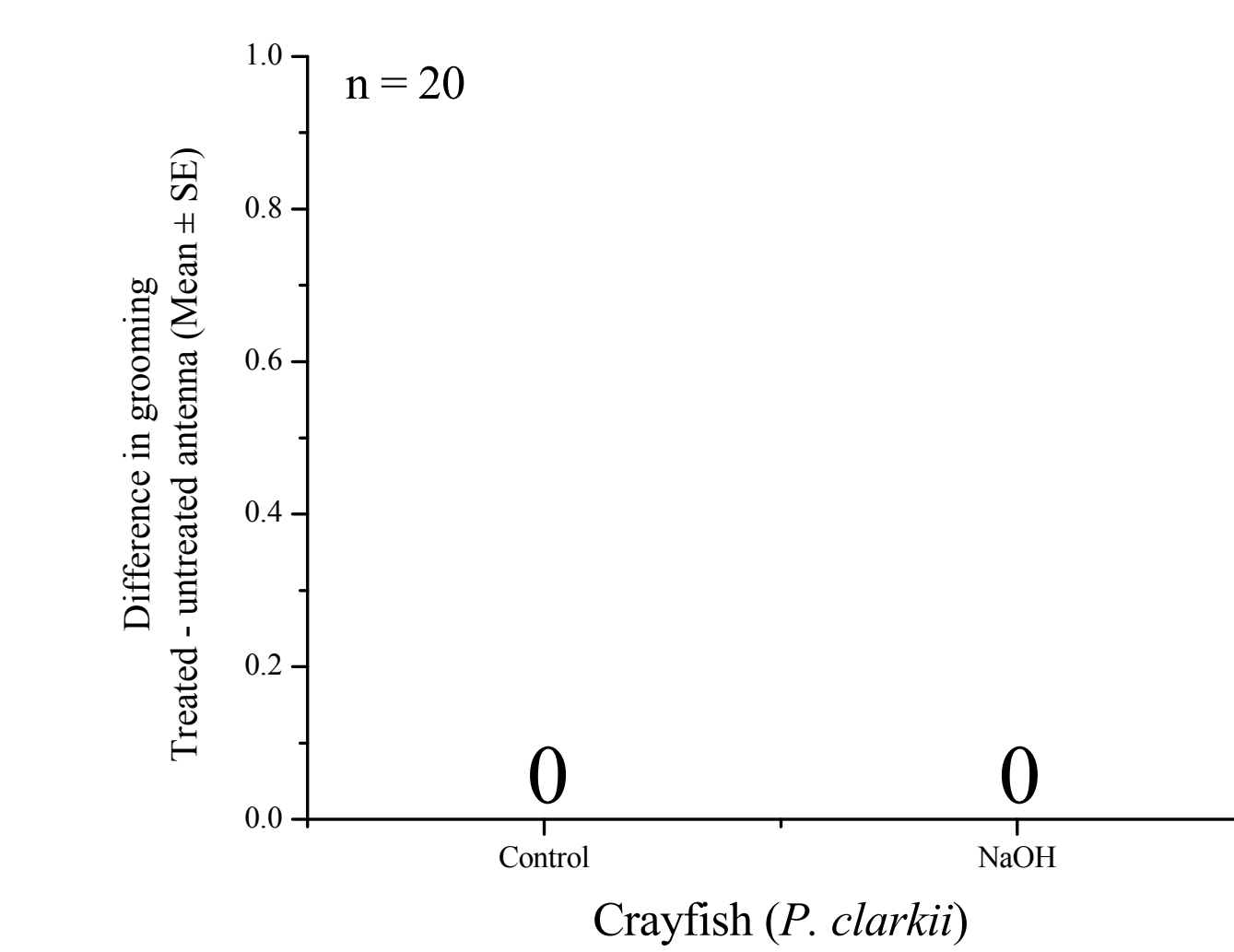


Figure 2a. Crayfish (*P. clarkii*) antennal grooming. No significant difference in grooming between control (deionized water) and noxious stimuli (6M NaOH).

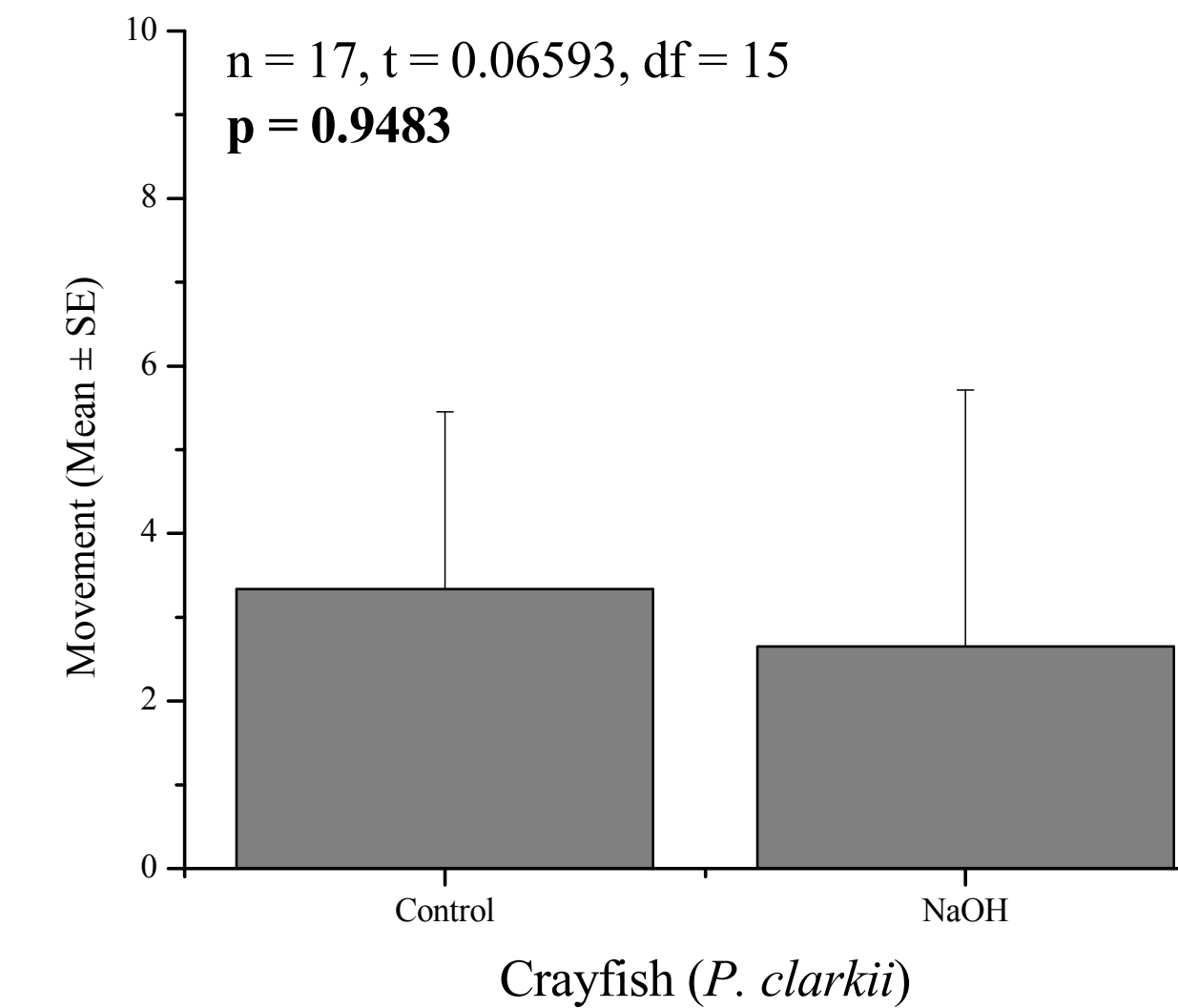


Figure 2b. Crayfish (*P. clarkii*) activity. No significant difference in movement between control (deionized water) and noxious stimuli (6M NaOH).

Experiment 2

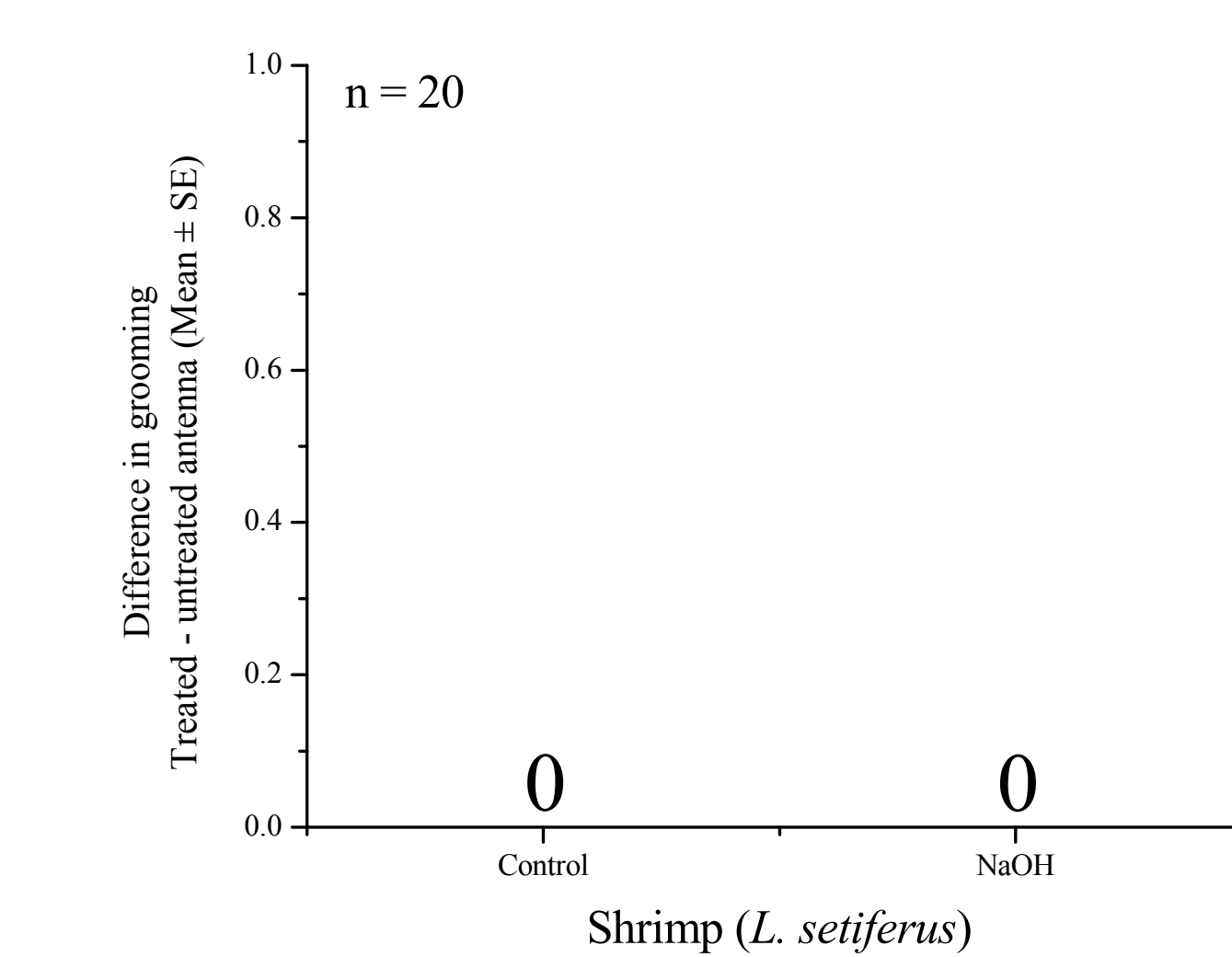


Figure 3a. Shrimp (*L. setiferus*) antennal grooming. No significant difference in grooming between control (sea water) and noxious stimuli (6M NaOH).

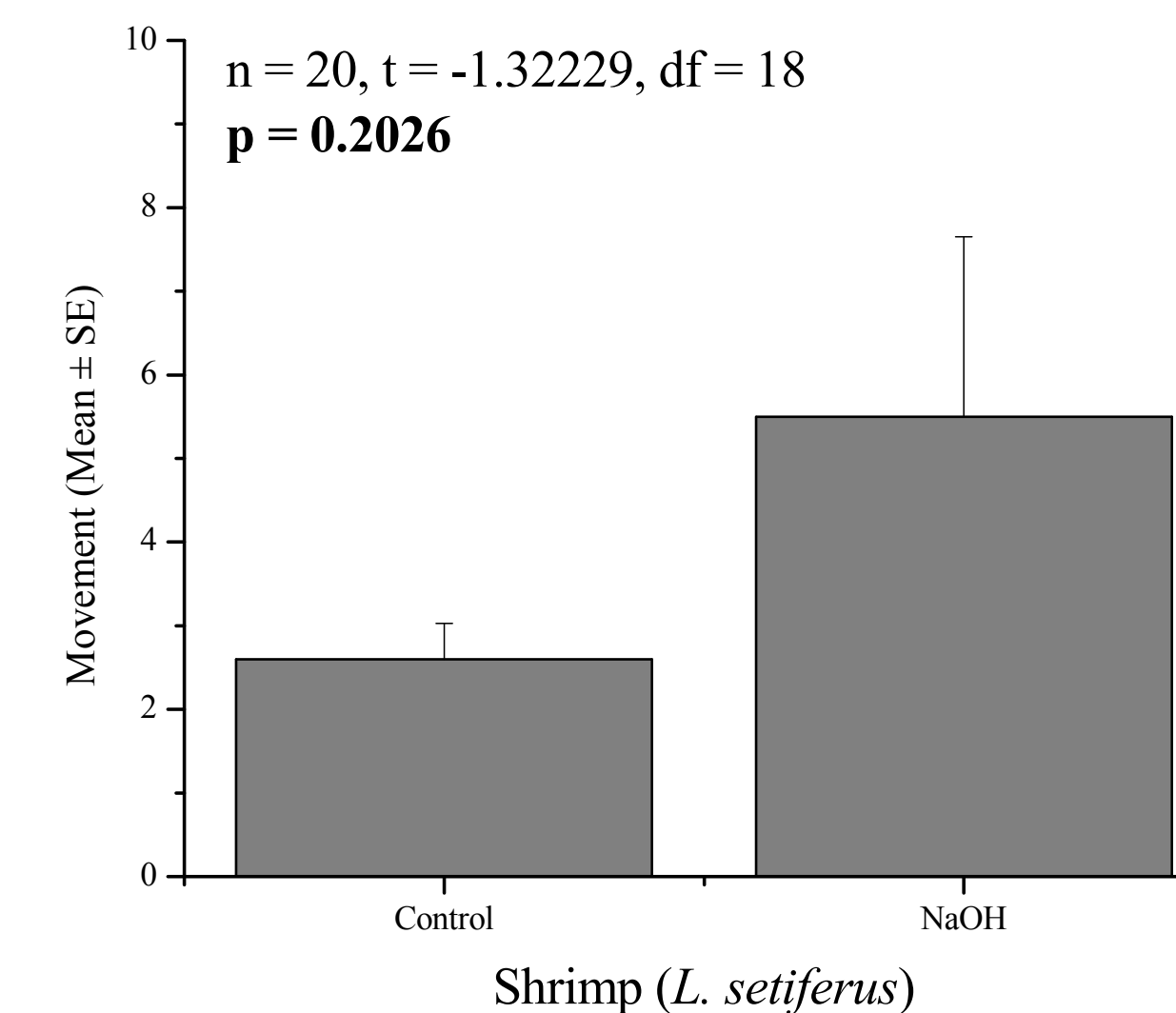


Figure 3b. Shrimp (*L. setiferus*) activity. No significant difference in movement between the control (sea water) and noxious stimuli (6M NaOH).

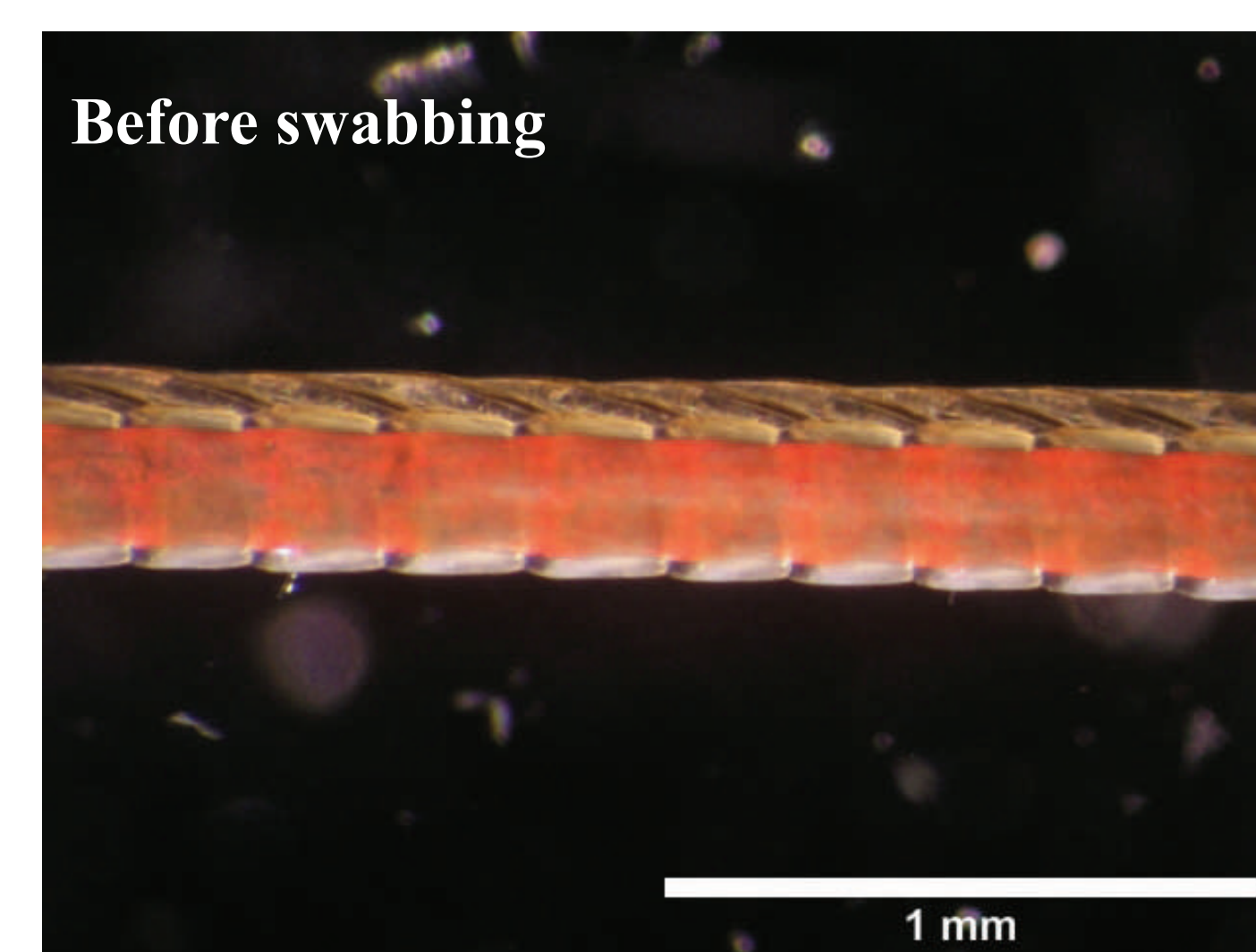


Figure 4a. Shrimp (*L. setiferus*) antennae before stimulus applied by swabbing with cotton swabs.

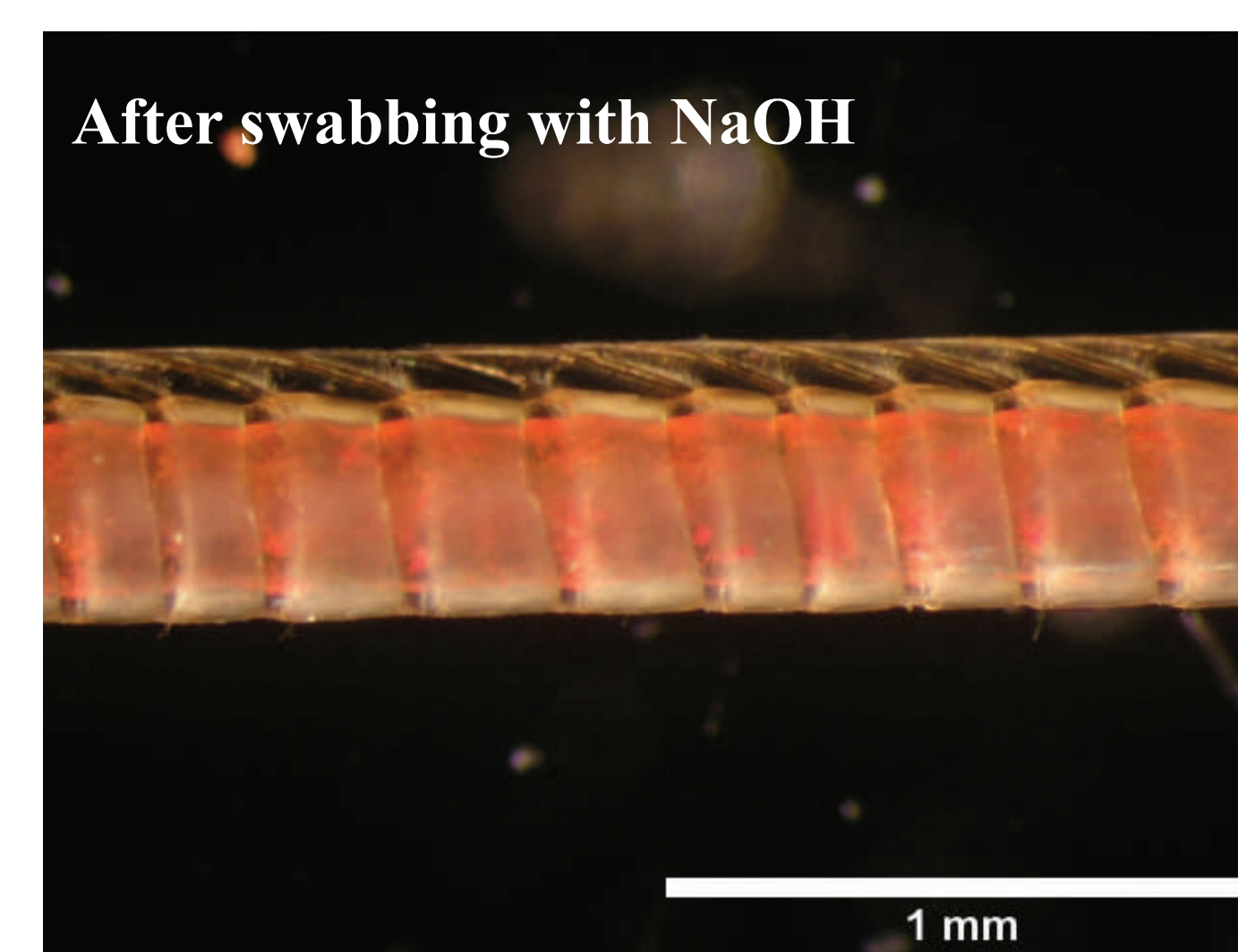


Figure 4b. Shrimp (*L. setiferus*) antennae after noxious stimulus (6M NaOH) applied by swabbing with cotton swabs. No gross damage to putative sensory hairs are visible due to swabbing.

Experiment 3

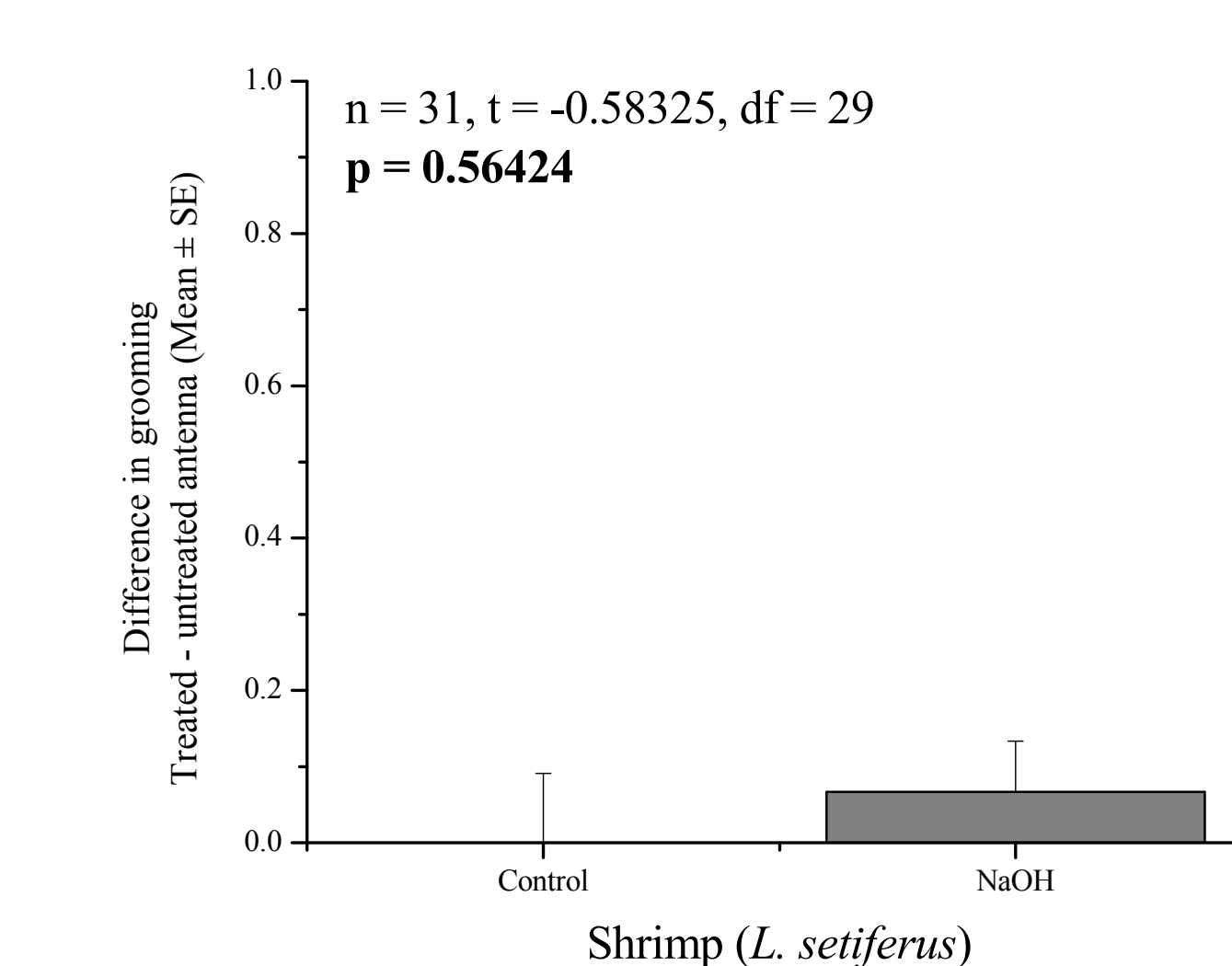


Figure 5a. Shrimp (*L. setiferus*) antennal grooming. No significant difference in grooming between control (sea water) and noxious stimuli (6M NaOH).

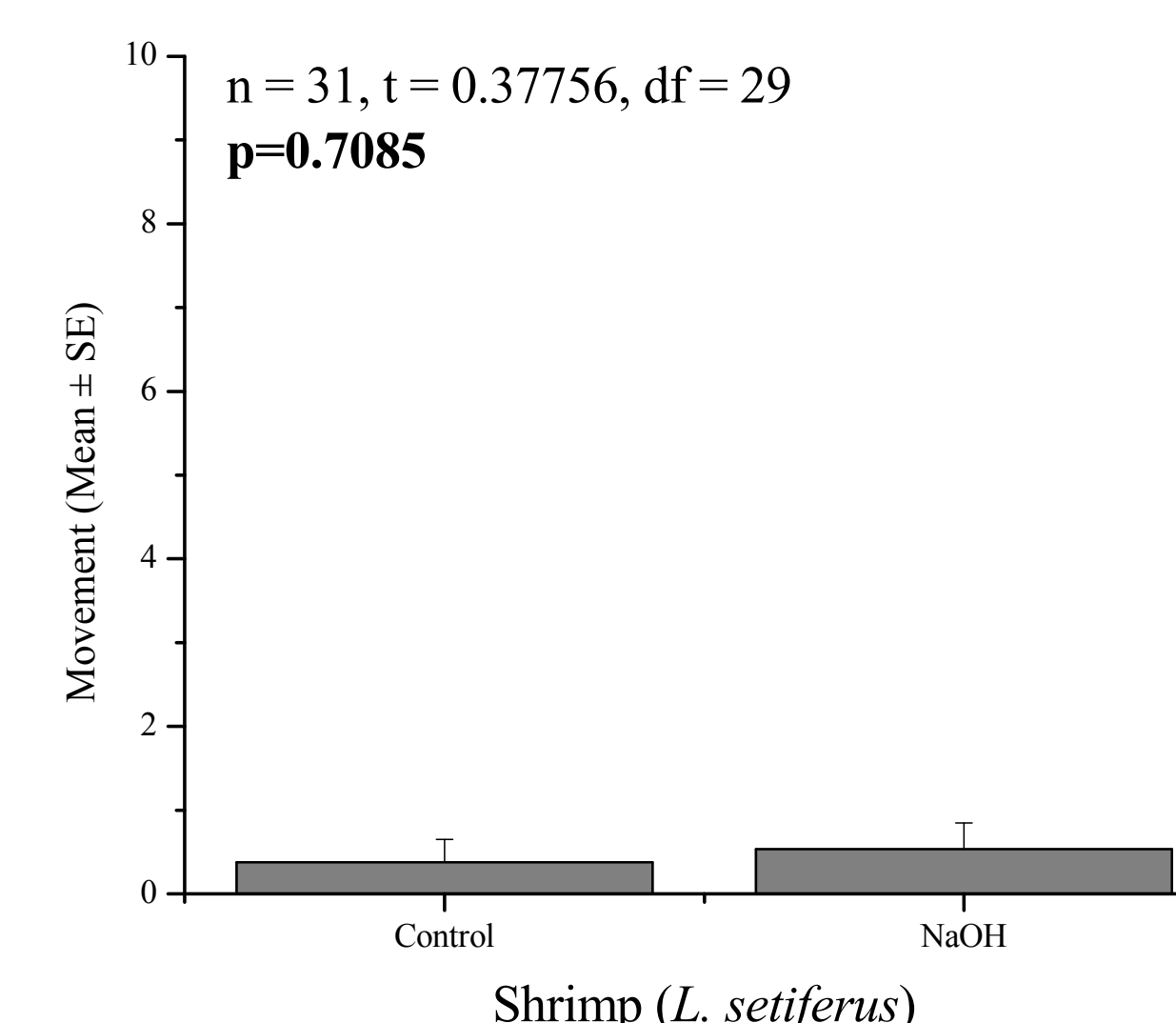


Figure 5b. Shrimp (*L. setiferus*) activity. No significant difference in movement between the control (sea water) and noxious stimuli (6M NaOH). Lower activity compared to Figure 3b (above) is likely due to reduced water level, which limited the shrimps' room to swim.

Discussion

We found no behavioral evidence of nociception in either *L. setiferus* or *P. clarkii*. This outcome contrasts sharply with results that indicated *P. elegans* had nociception (Barr et al. 2007). Some possible reasons for this discrepancy are as follows.

Do *P. elegans* have nociceptors while *L. setiferus* and *P. clarkii* do not?

It seems unlikely that nociception would be confined to specific decapod species. First, the sensory capabilities of decapods are broadly similar. Second, there is no clear ecological reason why nociception should be present in only one of these species; *P. elegans* and *L. setiferus* in particular have a similar morphology and would be expected have live in similar ecological niches.

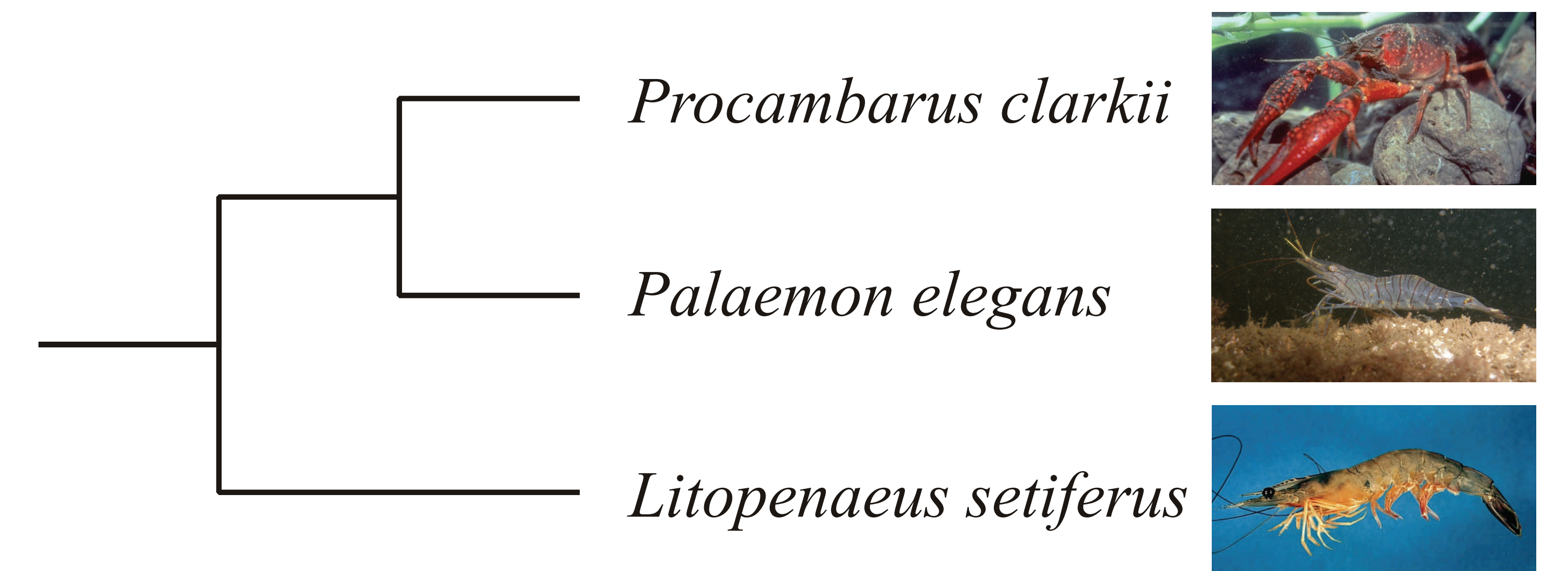


Figure 6. Phylogenetic relationship between species examined in this study and in Barr et al. (2007).

Do *P. elegans* have different antennal grooming behavior than *L. setiferus* and *P. clarkii*?

If *P. elegans* normally groom at high rates than other species, changes to their grooming behavior would be easier to detect than in species that groom at low rates. We have no direct evidence for such differences. Nevertheless, we saw no other behaviors in our experimental animals that seemed to correlate with detection of noxious stimuli.

Are nociception-triggered behaviors robust?

Although we intuitively expect nociception to cause extremely reliable and robust changes in behavior (which we might call "hand on a hot stove" effects), it is possible that such behavioral changes in crustaceans are more sensitive to subtle experimental conditions than expected. Despite our efforts to use experimental protocols in this study that were similar to Barr et al. (2007), it is possible that some subtle factor is gating the behavioral changes triggered by nociception, causing them to be expressed in only some situations.

Future directions

Electrophysiological recordings should be able to uncover any neurons that have the characteristics of nociceptors. In most species, nociceptors respond preferentially to temperatures above 40°C, low pH, and capsaicin.

Molecular techniques have characterized a gene called *painless* in *Drosophila*, which represents the best documented nociceptors in arthropods. We have conducted preliminary experiments to identify a homolog to the *painless* gene in *Drosophila*. We have successfully used species specific primers to recover the *painless* gene in *Drosophila* through polymerase chain reaction (PCR), but have been unable to recover the same gene in *Drosophila* using degenerate primers. We will continue to design new primers to search for a nociception-related gene in crustaceans.

References

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