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Compromised weaponry enhances crayfish tailflipping

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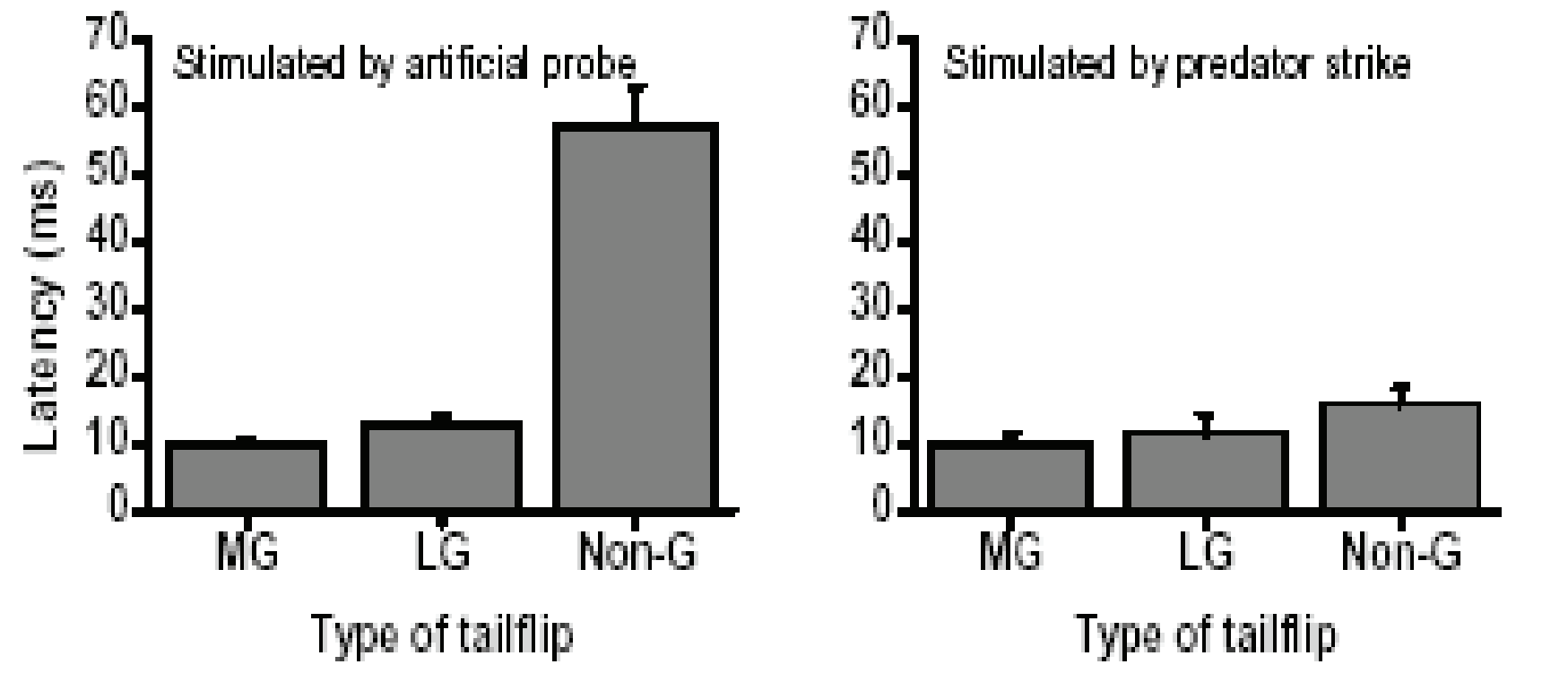
Abstract

As a defense strategy, many crustaceans perform one of two well described stereotyped single fast tailflips (~6 ms in adult crayfish), which are triggered by a neural circuit that includes medial giant interneurons (MG) and lateral giant interneurons (LG). Tailflips can also occur without these giant interneurons firing, however; these non-giant tailflips are variable, repetitive, and significantly longer (~100 ms). Previous work showed that crayfish exposed to natural stimulus respond with a non-giant tailflip significantly faster than when presented with an artificial stimulus and that complete removal of claws lowered tailflipping threshold. We tested whether compromising weapons and vision would influence tailflipping. Claws were deafferented by cutting the leg nerve between the coxa-basis joint. Crayfish were presented with artificial tactile stimuli, and their tailflipping behavior was video recorded in three conditions: intact, deafferentation of one claw, and deafferentation of both claws. Crayfish with either one or both claws deafferented tailflipped greater distances than intact animals when tapped. Charcoal was used as blindfolds to compromise the vision of the crayfish. Crayfish were then presented with artificial tactile stimuli, and video recorded in two conditions: not blindfolded and blindfolded. Blindfolding effects the probability that crayfish will tailflip at all, but if they do tailflip they won't necessarily tailflip further. The orientation that crayfish assumed after tailflipping was not significantly different with claw deafferentation. We plan to conduct similar experiments in species that have no claws and use alternate weapons (e.g., antennae).

Louisiana red swamp crayfish, *Procambarus clarkii* (Girard, 1852).

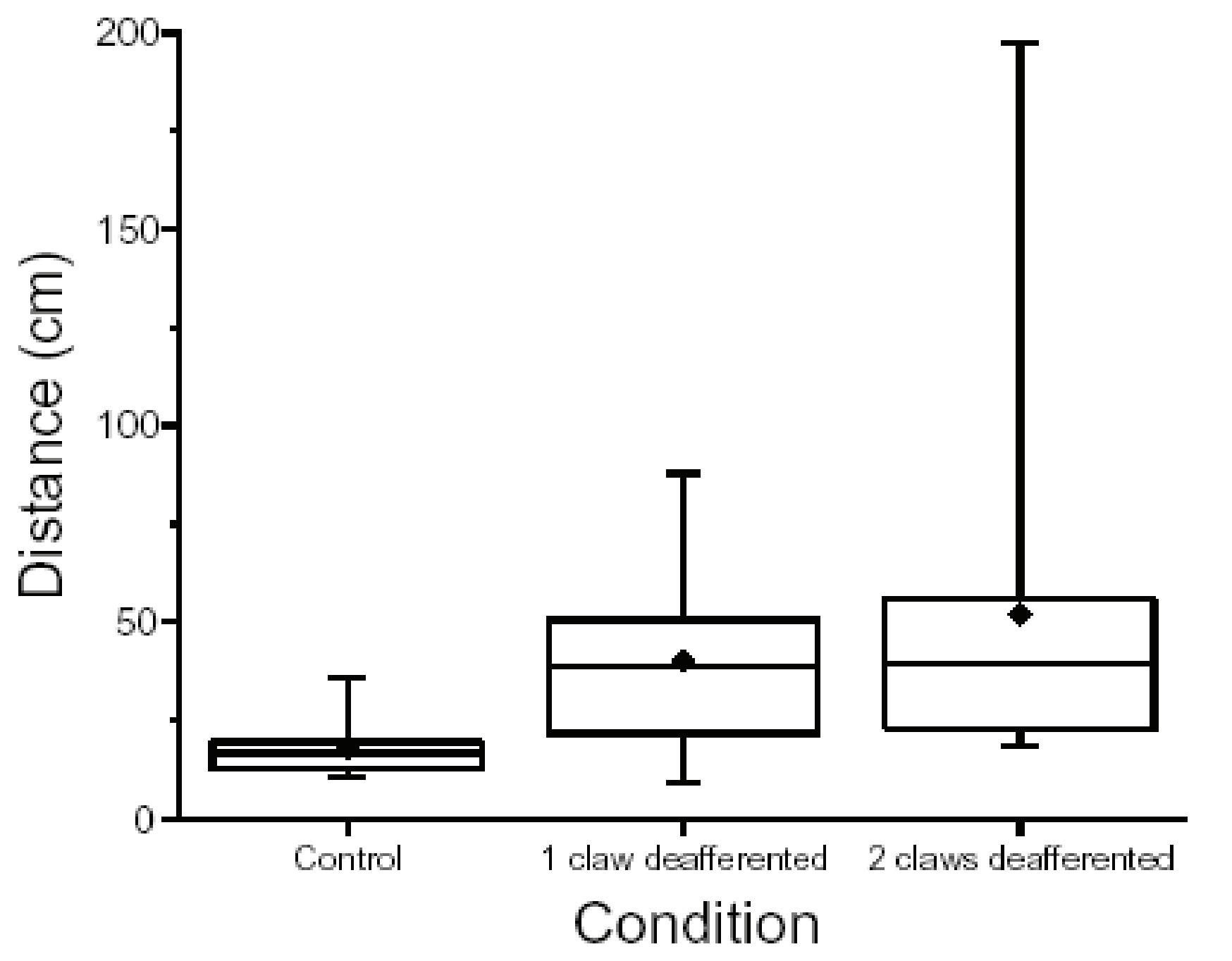
Introduction

Escape responses are found in many organisms (Eaton, 1984; Laura, 2006). The escape response of crayfish and many crustaceans, which are slow walking animals, is a powerful, short-latency tailflip (Wine and Krasne, 1972), which significantly increases the probability of surviving a predator's attack (Herberholz et al., 2004). Escape tailflips and their underlying neural bases have been studied extensively in crayfish, particularly *Procambarus clarkii* (Wine and Krasne, 1972; Edwards et al., 1999). The crayfish ventral nerve cord contains two bilaterally paired giant axons, the medial giant (MG) and lateral giant (LG). Early research concluded that these giant neurons produce escape tailflips (Wiersma, 1947). Later research showed that crayfish tailflipping is three distinct behaviors. Two tailflips are mediated by sets of giant neurons (Wine and Krasne, 1982). These are short latency, stereotyped, single tailflips. The third form of tailflipping is controlled by an as yet undescribed set of non giant neurons, but such tailflips have originally been considered to have significantly longer response latency than giant mediated tailflips (Krasne and Wine, 1984; Herberholz et al., 2004). More recent work has shown that the latency of non-giant tailflips was significantly reduced depending on whether the initiating stimulus was an artificial probe or an actual predator (Herberholz et al., 2004); This may explain earlier reports of spiny lobster short latency tailflips (Newland et al., 1992), which do not have escape-related giant interneurons (Espinoza et al., 2006). It is not known how these non-giant tailflips have a latency nearly as short as those generated by giant neurons. It is presumed that sensory cues modulate the non-giant tailflip circuit.



Latency of crayfish non-giant tailflips changes with type of stimulus. MG = medial giant interneuron mediated tailflips; LG = lateral giant interneurons mediated tailflips; Non-G = non-giant interneurons mediated tailflips. Redrawn from Herberholz et al., (2004).

Claws and vision are important to crayfish and many crustaceans in defense against predators and in aggressive interactions with other crayfish. Removing both claws significantly changes the threshold for tailflipping (Krasne and Wine, 1975; Lang et al., 1977). The removal of claws affects several variables at once: the animal's mass, visual stimuli associated with having claws, tactile and proprioceptive cues. For example, a crayfish with claws that are present but useless might "bluff," and may not tailflip away from a stimuli more than an intact animal. Vision has been shown to have an affect on tailflipping, if an animal knows that the threat is artificial (e.g., experimenters tap) rather then an actual predator it can reduce the latency of non-giant tailflips (Herberholz et al., 2004). Here we further investigate how the presence of weapons and vision affects tailflipping.



Raw (non-transformed) distance data, showing wide variance and skew of data in different experimental conditions. Diamond = 50% of data; horizontal line in box = median; whiskers = minimum and maximum.

P. clarkii with charcoal/Vaseline blindfolds.

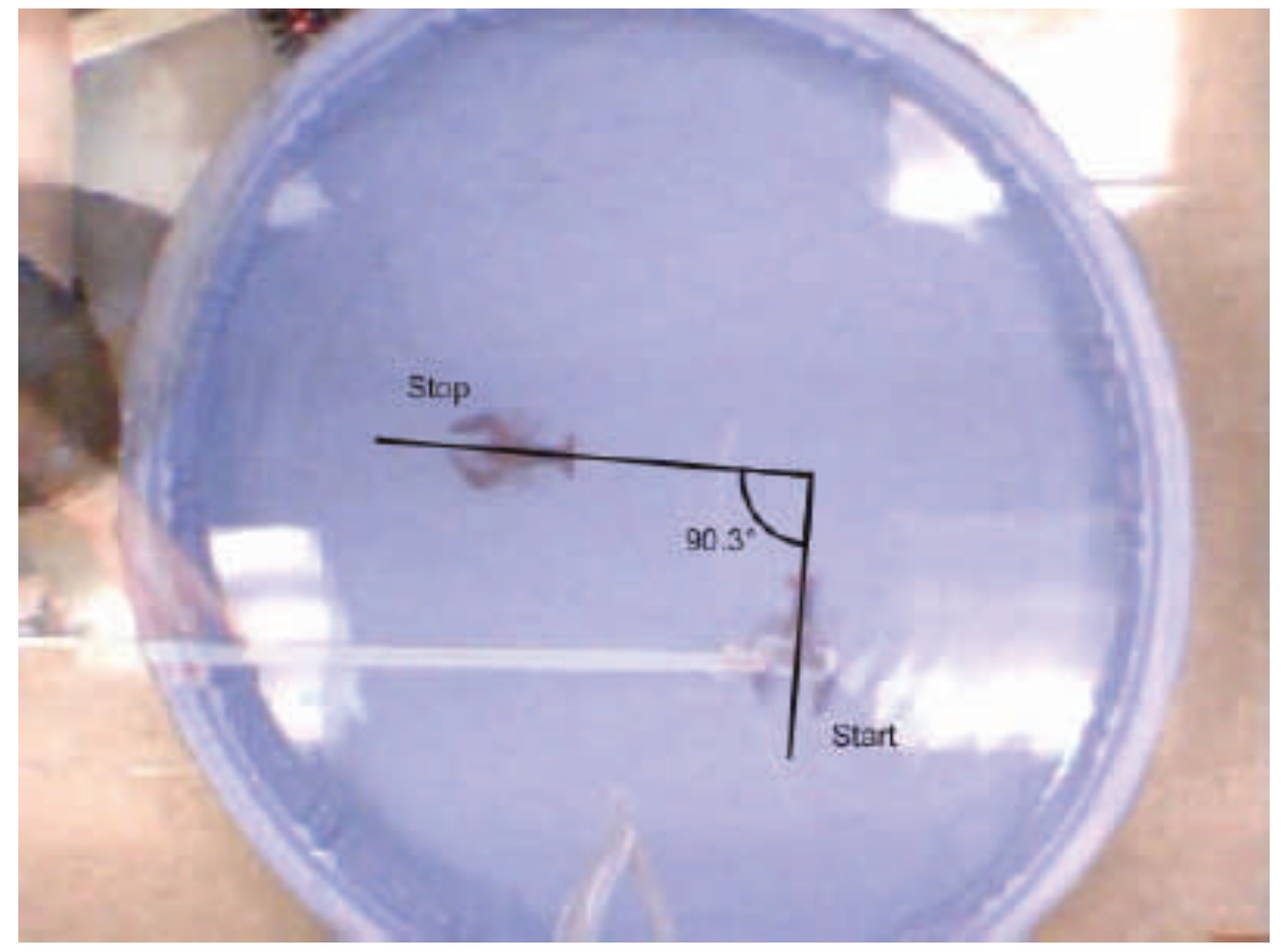
Methods

Crayfish of both sexes, ranging in size from 26-59 mm, were bought from a commercial supplier (Carolina Biological Supply Co.; *Procambarus clarkii*) or collected locally (*Procambarus* spp.) and housed individually in small tanks at The University of Texas-Pan American.

Crayfish were anaesthetized by chilling before surgery. Claws were deafferented by making an incision at the coxa-basis joint and cutting the claw nerve with fine scissors. Surgery was tested by placing an object in the claw and seeing whether the crayfish would grab it. The same animals were measured in all three conditions (i.e., intact control, 1 claw deafferented, 2 claws deafferented).

A mixture of charcoal and Vaseline was used as blindfolds. Vision was tested by placing an object in front of the crayfish and seeing whether the crayfish would react to it.

All behavioral trials were carried out in a wading pool filled with water. Tailflips were initiated by tapping the animal with a "yabby whacker". Behavior was recorded by a web cam (Logitech QuickCam) placed 2.3 m directly over the top of the pool, and video was recorded directly to a PC.



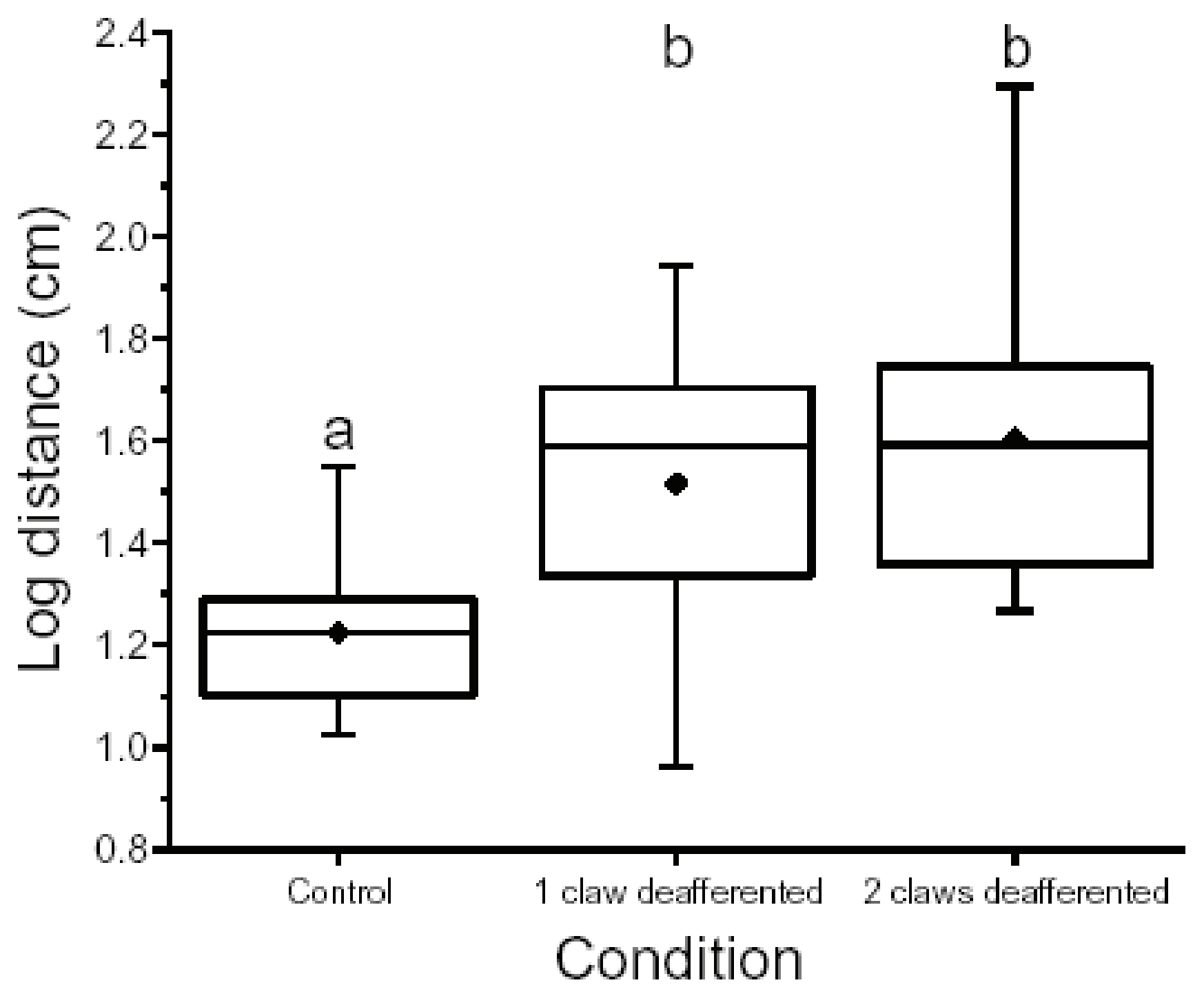
Sample image composed of two merge video frames, showing how the angle of a crayfish's pathway was measured.

Single video frames of various points of the tailflip were grabbed from the file using Classic Media Player version 6.4, and imported into Image-Pro Plus version 4.5 (Media Cybernetics) for change in the angle of body orientation were measured.

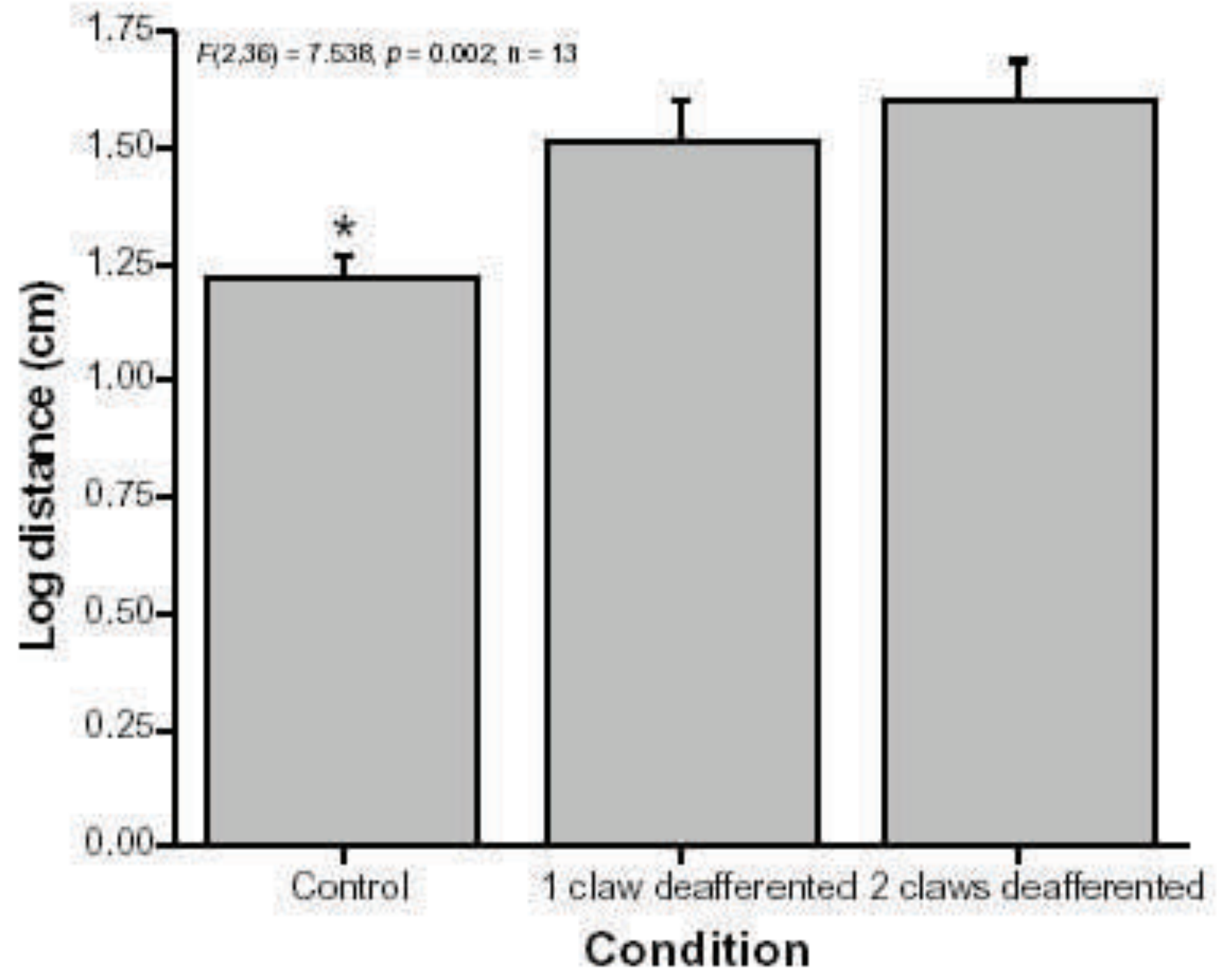
Data were plotted in Origin 7 (OriginLab Corporation). Distance data had skewed distributions in all groups and unequal variances between groups. To solve this, the data were log transformed. Distance data were analyzed using SPSS 12; angular data were analyzed by hand following Batschelet (1981).

Results

Crayfish tailflip further after cutting claw nerves



Log transformed distance data. Treatments sharing a letter (e.g., b) do not differ significantly from each other. Diamond = mean; 50% of data; horizontal line in box = median; whiskers = minimum and maximum.

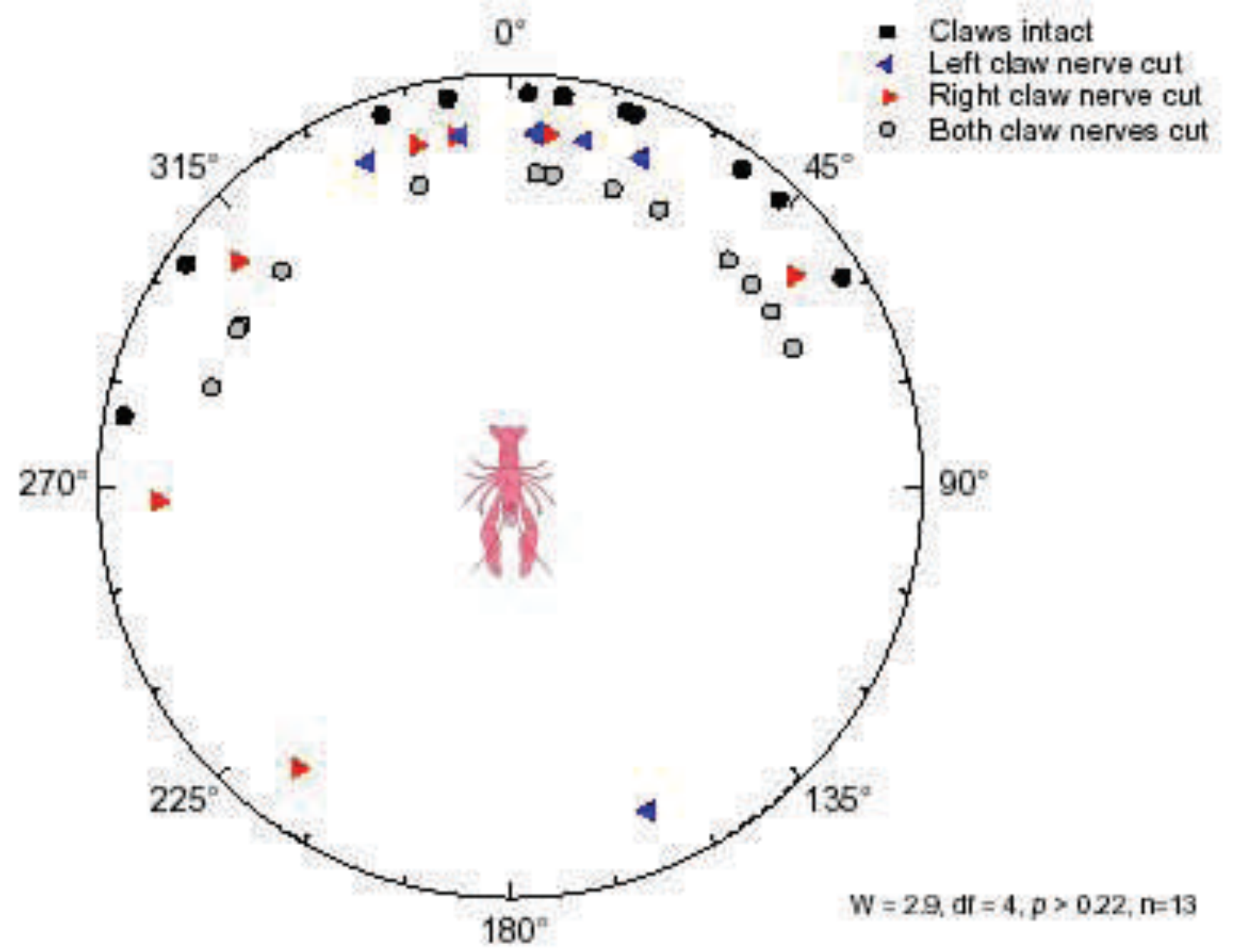


Intact crayfish tailflipped significantly greater distances when tapped after the nerve to one of both claws was cut (one way ANOVA), but there was no difference between one or two nerves being cut (post hoc LSD test).

Conditions contrasted		Mean difference	Standard error	Significance
Control	1 claw deafferented	-.29261(*)	0.10286	0.007
Control	2 claws deafferented	-.38170(*)	0.10286	0.001
1 claw deafferented	2 claws deafferented	-0.08909	0.10286	0.392

Post-hoc comparison of means (LSD test).

Direction of tailflipping does not change after cutting claw nerves



The orientation that crayfish assumed after tailflipping did not differ significantly in mean angle or variation with claw deafferentation (Mardia-Watson-Wheeler test). The orientation that crayfish assumed after tailflipping was not significantly different with claw deafferentation.

Vision affects the probability that crayfish will tailflip, but not distance

Conditions contrasted		N	Mean	Standard deviation	Min	Max
Control	no blindfolds	21	1.5952	.81577	1.00	3.00
Blindfold	both eyes blindfolded	21	1.1905	.51177	1.00	3.00
Control 2	no blindfolds	21	1.6429	.80844	1.00	3.00

Friedman comparison of means (non-parametric test).

Ranks		Mean Rank
Control	no blindfolds	2.12
Blindfold	both eyes blindfolded	1.71
Control 2	no blindfolds	2.17

Friedman comparison of ranks (non-parametric test).

Test Statistics	
N	21
Chi-Square	5.892
Df	2
Asymp. Significance	.053

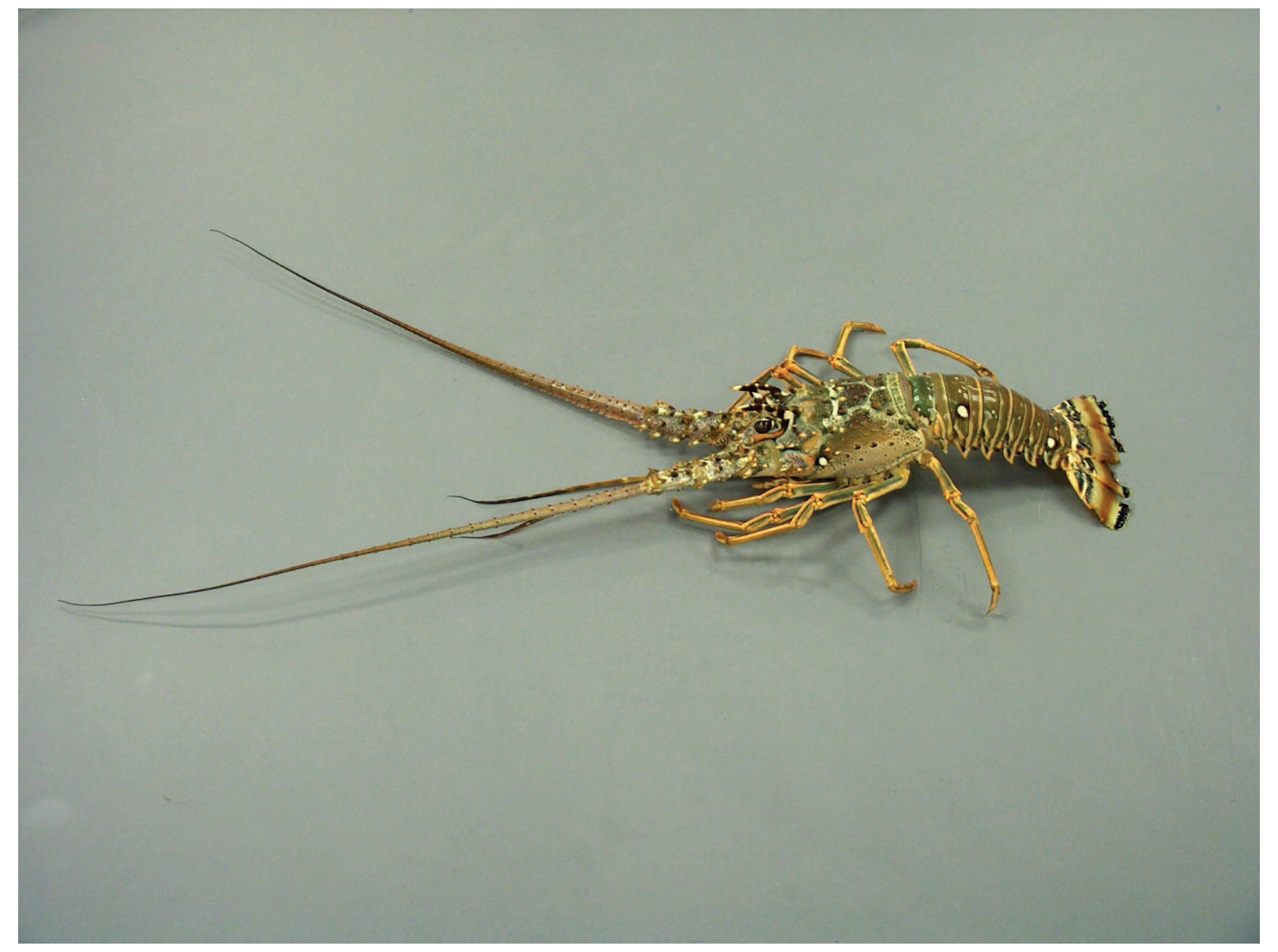
Friedman test statistics (non-parametric test).

Blindfolding affects the probability that crayfish will tailflip at all, but if they do tailflip they won't necessarily tailflip further (Friedman test).

Discussion

Removing sensory input from even one claw significantly increases the distance that a crayfish tailflips when tapped. The results are consistent with previous studies and emphasize the importance of claws in anti-predator behavior and agonistic interactions with conspecifics.

Removing vision input did not have a significant difference in tailflipping distance, but does affect the probability that a crayfish will tailflip. Since the distance is very close to significant, which could have been due to the small number of animals tested; a slightly larger animal size or more animals tailflipping would most likely have significant results. The majority of the animals tested had a pair of large claws; having both claws significantly changes the threshold for tailflipping (Krasne and Wine, 1975; Lang et al., 1977).



Spiny lobster *Panulirus argus*.

Future experiments will be aimed at finding the sensory cues that reduce the latency of non-giant mediated responses. Electromyograms (EMGs) of abdominal muscle activity will be used to measure response latency. This project is also planned as the first step in comparative studies examining modulation of tailflipping in other decapod crustacean species. For example, spiny lobsters use antennae instead of claws as weapons; their sensory input to the non-giant tailflipping pattern generator would be substantially different of the antennae in spiny lobster will also significantly increase the distance they tailflip in response to threats.

Acknowledgements

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