UNIVERSITY OF CALIFORNIA SAN DIEGO

Sharp Shooters: Context Dependent Snap Modulation in Pistol Shrimp

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in

Marine Biology

by

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The Thesis of Noah Nadeau is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

University of California San Diego

2022

DEDICATION

This thesis is dedicated to my parents, Todd and Colleen. Without your help and guidance, I would not have been able to accomplish what I have today. You have helped me keep a good head on my shoulders and taught me how to lead a God centered life.

Love,

Noah

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ABSTRACT OF THE THESIS

Sharpshooters: Context Dependent Snap Modulation in Pistol Shrimp

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Master of Science in Marine Biology

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Professor Jennifer Taylor, Chair

Pistol shrimp have one of the fastest, loudest, and deadliest weapons in the ocean. The rapid snap of their modified claw generates a water jet and cavitation bubble that is used to ward off predators, subdue prey, and settle disputes. It is suggested that pistol shrimp can control the volume of water they let into the socket of their snapping claw, thereby affecting the force of their strikes. The goal of this study was to determine if pistol shrimp exhibit context-dependent snap modulation. We hypothesized that (1) pistol shrimp would snap with different power in response to different stimuli, and (2) that the predator would induce the most powerful snap while the conspecific would induce the least powerful snap. Two species

of pistol shrimp, *Alpheus clamator* and *Synalpheus lockingtoni*, were presented stimuli in random order: predator (shore crab), prey (red rock shrimp), conspecific, and control (paintbrush). Snaps were recorded with a high-speed video camera (25,000 fps) and hydrophone, from which water jet dimensions, velocity, and sound level were calculated. Results show that there is great variability in total water jet velocity, with the prey stimulus inducing a weaker snap than all other stimuli. Yet all other snap characteristics were consistent across stimuli. Pistol shrimp appear to modulate their snap based on context, but to a limited extent, thereby supporting our main hypothesis. This study provides deeper insights into the biomechanics of the pistol shrimp snap, and a better ecological understanding of how they use this potent weapon.

INTRODUCTION

Some of the most powerful weapons in the animal kingdom belong to small marine crustaceans, the mantis shrimp (Stomatopoda) and pistol shrimp (Decapoda: Alpheidae). These distantly related crustaceans have both evolved power-amplified weapons from specialized raptorial appendages and use them in similar contexts, not only for subduing prey, but also for defense against predators, and during aggressive encounters with conspecifics. Despite differing morphologically and mechanically, both mantis shrimp and pistol shrimp raptorial weapons operate at velocities and accelerations sufficient to create cavitation (Green et al., 2019; Versluis et al., 2000), inducing significant damage (Green et al., 2019). Like other animals with powerful weapons (Folkersen et al., 2018; Jennings et al., 2004), mantis shrimp can adjust the kinematics and energetics of their strikes based on context (Green et al., 2019). Modulation can conserve energy and limit damage in situations that do not require maximum power. It is unknown if pistol shrimp exhibit context-dependent snap modulation like mantis shrimp and other weaponized animals.

Why do pistol shrimp snap?

Pistol shrimp snaps are omnipresent in the oceans, where they can be heard as a constant source of crackling noise (Au and Banks, 1998). From tropical, subtropical, and temperate climates (Anker et al., 2006), the snaps of pistol shrimp are heard. The sounds are primarily located in surface waters above 36.6 m, (Knowlton and Moulton, 1963) and to a lesser extent down to 55 m (Everest et al., 1948). Pistol shrimp live in a variety of shallow water marine habitats where they find shelter in kelp holdfasts, rock, reef crevasses, and soft corals (Nakano and Fujii, 2014; Jensen, 2014). Within these kelp holdfasts, pistol shrimp encounter a variety of

animals, including small crabs, shrimp, and conspecifics (pers. observ.). Each interaction is a potential opportunity to put its snapping claw to use, either to capture prey, avoid predation, or fight over resources. The constant snap sounds during day and night are indicative of the high frequency of encounters among populations of pistol shrimp.

Pistol shrimp feed on a range of prey, including mollusks, shrimp (grass shrimp), host sponges, goby fish, and small pearl (Duffy et al., 2002; Herberholz and Schmitz, 1998; Mahadevan and Kurup, 2008). For mobile prey, they use their snapping claw to snap and create a cavitation bubble where unsuspecting prey are lurking (Versluis et al., 2000). Pistol shrimp species do not uniformly use their claw weapon for feeding. Depending on the species, pistol shrimp can primarily be detritus consumers, but may graze on seagrass, sediment organic matter, or mollusks with their pincer claw (Mahadevan and Kurup, 2008). When consuming mollusks, pistol shrimp climb over the shell and insert their snapping claw under the operculum, but then use their pincer claw to tear off part of the foot (Mahadevan and Kurup, 2008).

Common predators of pistol shrimp include eunicid polychaetes, which are occasionally found in *Synalpheus* sponge hosts, but predators seem to be rare (Duffy et al., 2002). While predators of snapping shrimp are not well-documented, there are similar interspecies defense situations that often arise regarding the safety of the holdfasts, sponges, or burrows in which they live (Nakano and Fujii, 2014). For example, large crabs may or may not prey on pistol shrimp, but they consume the habitat in which they live (kelp holdfast, coral colony, etc.), causing pistol shrimp to defend their home (Duffy, 2003).

With pistol shrimp living in high densities, competition for resources such as space, food, and mates is common. Many interactions between conspecifics begin with displays of opening and closing their chela, but don't necessarily result in snapping (Hughes, 1996). Intraspecific

encounters often provide different interactions based on the sex of the opponents (Herberholz and Schmitz, 1998). Male pistol shrimp respond with greater intensity when presented with male chemical signals compared to female chemical signals, when both accompany an open chela as a sign of aggression (Hughes, 1996). The winners of these conspecific interactions is usually determined by who is larger and who is more aggressive with their displays (Schein, 1977).

Snapping mechanism

There are more than 600 species of pistol shrimp that are equipped with powerful snapping weapons. Pistol shrimp possess two claws, a snapping claw and a pincer claw. The snapping claw consists of the propus and dactylus, and four specialized parts (pollex, socket, tip region, and plunger) that are integral to the snap. The propus is the immobile base structure for the claw, and the dactylus is the swinging portion of the claw (Amini et al., 2018; Versluis et al., 2000). On the propus is the pollex, which is the tip of the claw adjacent to the closed dactylus, as well as the socket that holds the water when the claw is opened (Amini et al., 2018; Versluis et al., 2000). On the dactylus, there is the tip region, and the plunger (Amini et al., 2018; Versluis et al., 2000).

In preparation for the snap, pistol shrimp contract antagonistic muscles in the propus to cock the claw in the open position (Versluis et al., 2000). Adhesive discs located on the base of the dactyl get pressed together by the opening of the claw, storing elastic energy (Anker et al., 2006). Muscle tension builds and then upon contraction of a closer muscle, the stored elastic energy is released, causing the plunger to rapidly close into the socket (Amini et al., 2018; Versluis et al., 2000). The dactylus will rotate at speeds of up to 3,500 rad/sec (Versluis et al.,

2000), and as the plunger drives into the socket, it displaces the water volume within it at velocities sufficient to create a cavitation bubble (Amini et al., 2018; Versluis et al., 2000).

The pistol shrimp claw uses an elastically driven mechanism to illicit a water jet that reaches velocities of up to 32 m/s and cavitation bubbles that generate sound pressure levels nearing 215 dB (re 1 µPa peak-to-peak at 1 m; Dinh and Radford, 2021; Versluis et al., 2000). This impressive feat is due to claw morphology and joint structure. A dominant type of joint among pistol shrimp is the cocking slip joint that allows for the dactyl to be latched open and for a torque-reversal mechanism to occur (Patek and Longo, 2018). As a result of the cocking slip joint, the dactyl becomes locked into place behind the fulcrum by part of the closer muscle (Patek and Longo, 2018). To animate this joint, a pair of closer muscles contract; one exerts a positive torque on the dactyl, and the other exerts a negative torque (Patek and Longo, 2018). Thus, torque shifts direction when the dactyl fires from the cocked position, generating high velocities (Patek and Longo, 2018). However, there can be a variety of different snapping mechanisms even among the *Alpheus* genus (Ritzmann, 1974).

It is the cavitation bubble generated by the snap that causes immense damage to those that get too close. The energy released upon cavitation bubble collapse emanates in sound reaching 215 dB, high temperatures that reach up to 5000 K, and even light through a phenomenon called sonoluminescence (Lohse et al., 2001). All of these facets of a cavitation bubble are what allow pistol shrimp to stun or kill prey, and harm more heavily armored animals like small crabs (Herberholz and Schmitz, 1998). Pistol shrimps are not immune to the intense power generated by their own snaps and can incur damage from the shockwaves of cavitation. It was recently discovered that the orbital shield functions like a protective ‘helmet’ to quickly

dissipate the energy from the cavitation bubble without damage to the eyes or other structures (Kingston et al., 2022).

In comparison, mantis shrimp also generate cavitation bubbles with their raptorial strike. They use a similar elastic latch mechanism when striking with either their hammer-like or spear-like raptorial appendages (Patek et al., 2013). Smashing mantis shrimp use their club-tipped, hammer-like appendage to smash prey, whereas spearing mantis shrimp use their spear-like appendage to pierce prey (Patek et al., 2013). With either appendage type, animals prepare for a strike by contracting and compressing a spring (the exoskeletal elastic mechanism), while a latch is engaged that prevents the propodus and dactyl from rotating (Patek et al., 2013). When the latch is released, the strike begins (Patek et al., 2013). Mantis shrimp can modulate this strike so that the kinematics and impact energy differ when sparring and feeding (Patek et al., 2013). During contests with conspecifics, strike velocity and energy correlated with opponent size, but did not scale with prey size when feeding, indicating that mantis shrimp dedicate more energy to striking during contests. The elastic mechanism powering the strike can be tuned by the degree of spring compression (Green et al., 2019). It is possible that pistol shrimp can modulate the latching or elastic components of their snap as well.

Objective

Pistol shrimp constantly encounter situations that require use of their snapping weapon, yet it might be beneficial to conserve energy or limit the potential damage that the snap can cause. The objective of this thesis was to determine if snapping shrimp exhibit context-dependent snap modulation, similar to the mantis shrimp. I tested the general hypothesis that pistol shrimp modulate their snap by exposing two species of snapping shrimp (*Alpheus clamato*r and

Synalpheus lockingtoni) to a series of stimuli (control, prey, predator, and conspecific) and recording and analyzing the snap kinematics. My specific hypotheses were that 1. pistol shrimp would snap with different power (different water jet size and velocity, and sound level) when exposed to a live stimulus compared to the control, and 2. that snaps would vary in power among live stimuli, with predators inducing the most powerful snap, then prey, then conspecifics.

MATERIALS AND METHODS

Animal acquisition and care

A total of 10 *Alpheus clamator* (Fig. 1A) and 9 *Synalpheus lockingtoni* (Fig. 1B) were collected from a kelp holdfast by SCUBA off the San Diego coastline. Shrimp were transported to the Hubbs Experimental Aquarium at Scripps Institution of Oceanography (SIO), University of California, San Diego, where they were placed individually in plastic containers (8 oz). Small holes drilled into the containers allowed for water flow. The containers were then placed in a large tub of flow-through seawater pumped from the Scripps pier at ambient conditions. Shrimp were fed fish flakes *ad libitum* three times a week. Shrimp were held in the aquarium for 13 days prior to the start of the experiment and were starved for four days prior to experimental trials.

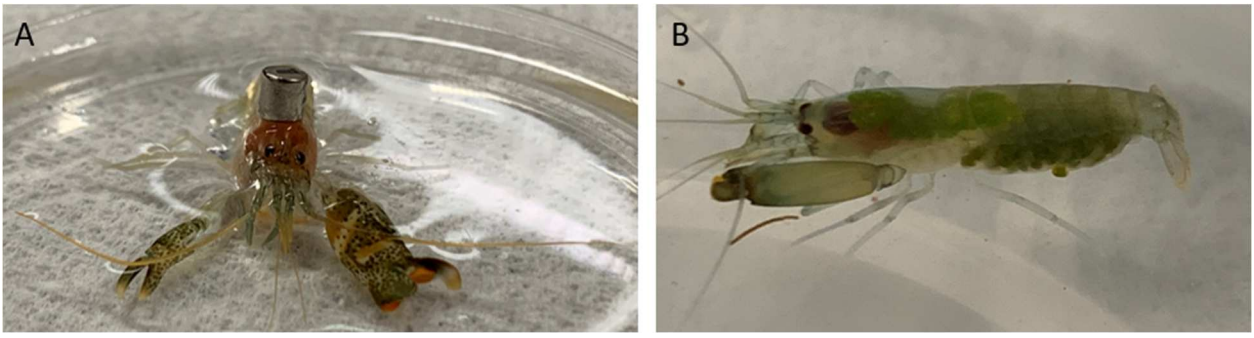

Figure 1: Alpheus clamator on left, Synalpheus lockingtoni on the right (photo credit: Sonya Timko).

Shortly after collection, an extreme heat wave occurred that caused water temperatures to rise to ~23□. In response to the high-water temperature, 7 pistol shrimp died (5 *S. lockingtoni* and 2 *A. clamator)*. This left 6 *A. clamator* and 2 *S. lockingtoni,* however, 2 *A. clamator* and 1 *S. lockingtoni* were missing snapping claws and were not measured until the claws were fully regenerated, approximately 4 weeks later. All shrimp used in this study were estimated to be in the intermolt phase based on hardness of the exoskeleton.

Experimental Setup:

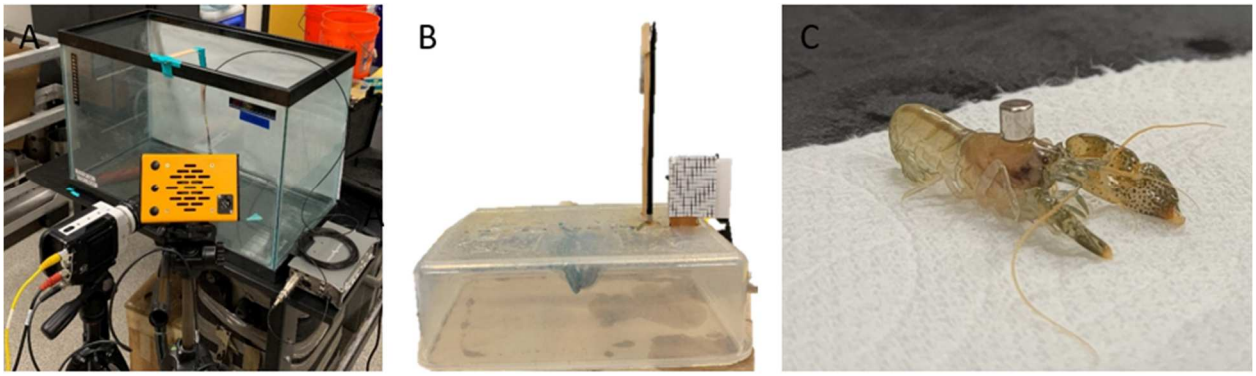

Figure 2: Experimental system for recording pistol shrimp. (A) Aquarium where trials were conducted with HSV and red light in view (B) Apparatus for positioning pistol shrimp in the aquarium. (C) A. clamator with a magnet glued to its carapace.

Individual shrimp were placed in an experimental tank (Fig. 2A) where they were exposed to a series of stimuli and their snaps were recorded. The experimental tank (75.71 liters) was filled halfway with ambient seawater. A custom apparatus was designed to position and hold the pistol shrimp within the experimental aquarium (Fig. 2B).

This apparatus consisted of a craft stick that acted like a crane to position the glued coffee stirrer to hold the shrimp directly below. The craft stick was fixed to a food storage container by cutting a small line in the container. Small magnets (3.18 mm height x 3.16 mm diameter) were attached to the carapace of each shrimp using cyanoacrylate (Fig. 2C) 7 days prior to trials.

Figure 3: Shows the experimental aquarium, Sound Devices 277, and the Teledyne Reson tc-4301 attached to the Sound Devices 277 and hanging down into the experimental aquarium.

However, most of the magnets fell off during positioning of the shrimp in the apparatus and needed to be reattached on the day measurements were taken. This did not seem to affect their snapping behavior. The magnets allowed the shrimp to be connected to the apparatus and held in place during trials. A 2.5 x 2.5 mm square grid was positioned medially to the shrimp and this, along with the magnet on the shrimp's carapace, were used to calibrate the high-speed video for distance and velocity measurements. This apparatus was positioned in the center of the experimental aquarium tank to allow for the sound of snaps to travel and be picked up by an omnidirectional hydrophone (1Hz -170kHz, tc-4301, Teledyne Reson, Thousand Oaks, CA, USA) connected to a digital audio recorder (192 kHz sample rate, maximum 20 kHz frequency response, Sound Devices 772 recorder, Reedsburg, WI, USA). The hydrophone was connected to a craft stick and positioned 10.5 cm from the shrimp apparatus (Fig. 3). A high-speed digital video camera (HSV; 18-55 mm lens, Canon, Melville, NY, USA; Phantom Miro 310 high-speed video camera, Vision Research, Wayne, NJ, USA) was placed in front of the experimental tank (Fig. 3). Light for the HSV was provided by a red L.E.D. light, which minimizes heat and light disturbance.

Procedure

Experimental trials were performed on randomly chosen individuals over the course of one week. Three shrimp (2 *A. clamator* and 1 *S. lockingtoni*) were recorded at a later time due to their missing claws or being too small to attach magnets to. For each trial, individual shrimp were placed in the custom holding apparatus and allowed to adjust to the experimental aquarium for at least 5 minutes. Each shrimp was then exposed to a series of 4 stimuli, in randomized order: control (touching the claw with a paintbrush (Versluis et al., 2000), prey (red rock shrimp, *Lysmata californica*), predator (red rock crab, *Cancer productus*), and a conspecific of similar size.

Each live stimulus (shrimp, crab, and conspecific) had a magnet glued to their carapace with cyanoacrylate, which was then secured to a coffee stirrer so that they could be positioned by hand approximately 5 cm in front of the pistol shrimp. The control paint brush was used to gently contact the claw of the pistol shrimp to illicit a response. All stimuli were quickly pulled away from the shrimp following the snap. Up to 5 snaps were recorded for each stimulus and shrimp were allowed to rest for a minimum of 5 minutes in between stimuli. Water in the experimental aquarium was changed between each shrimp being tested and water temperature was checked and maintained consistently at 18.5 ±1□ for all trials.

Snaps were recorded using the HSV at 25,000 frames s^{-1}, an exposure time of 32.892 μsec, and a pixel resolution of 384 x 288. Snap acoustics were simultaneously recorded with the Sound Devices digital recorder at a sample rate of 192 k, a bit depth of 24, and a gain of 45.1.

Upon completion of all trials, pistol shrimp were anesthetized and euthanized by brief placement in a -20°C freezer. Shrimp were then patted dry and weighed to the nearest 0.01 g and imaged using a stereomicroscope (Leica M165 C, IL, USA) equipped with a digital camera

(Leica DFC290, IL, USA). From these images, carapace length and snapping claw length were each measured 3 times using the Leica microscope software and averaged.

Data analysis

High-speed video sequences of each snap were analyzed using HSV software (PCC, v3.6). Both the width of the magnet (3.16 mm) and the grid (2.5 x 2.5 mm) were used to calibrate the video, but since they provided similar values, the magnet was used for calibration of all videos. From the video sequences, the overall velocity and length of the water jet, which included the cavitation bubble, were measured from the frame in which the jet first became visible to the frame in which it reached its furthest point before the cavitation bubble collapsed. This typically encompassed 4-5 frames. The maximum velocity of the water jet and its length were measured from when the cavitation bubble first became visible to the next frame, encompassing only 2 frames.

Sound level of each snap was analyzed using RavenLite 2.0 Sound Software (Cornell Lab of Ornithology, Ithaca, NY, USA). Due to missing parameters in the acquisition device, calibration of the hydrophone to obtain sound pressure level in dB was not possible at the time of data analysis. Raven Sound Software, however, sets each recording to an arbitrary value of kilo units (kU) that allows it to be used in comparative studies. Thus, these arbitrary units were used to compare sound levels. For each snap, sound level (kU) was measured as the maximum peak from the spectrogram.

Almost all shrimp snapped 1-5 times for each stimulus, with a majority snapping at least 2-3 times. Water jet velocity and length and sound level were measured for each snap for each individual shrimp and stimulus (N = 9 individuals, n = 99 total snaps acquired). Water jet

kinematics (maximum and total velocity, maximum and total length) were averaged across all snaps from a single stimulus for each individual shrimp. Sound level was only calculated as an average of all snaps for a given stimuli and individual shrimp.

Statistical analysis

All snap characteristics (maximum and average jet velocity, maximum and average jet length, and average sound level) were tested for normality using Shapiro-Wilk and homogeneity using Levene's tests. We compared all snap characteristics across the different stimuli using a linear mixed model (LMM; lmer function, lme4 package; Bates et al., 2015) in R (v 4.2.2) due to the non-independence of data (each shrimp snapped for almost all stimuli) and some missing data points (one shrimp did not snap for 2 of the stimuli). Our model included the snap characteristics as the independent variable and stimulus as the dependent variable. Shrimp mass and snapping claw length (CL) could both potentially affect snap characteristics, therefore they were both included as fixed effects, but only CL had an effect on snap characteristics, so the final model excluded mass. Individual shrimp ID was included as a random effect to account for multiple measurements from a single individual. Our resulting model was:

$$\text{Snap characteristic} \sim \text{stimulus} + \text{snapping claw length} + (1|\text{ID})$$

We acquired p-values for our modeled variables using a Satterthwaite approximation for degrees of freedom (lmerTest package; Kuznetsova et al., 2014; Luke, 2017).

Statistical analyses comparing snap characteristics across stimuli were only performed for *A. clamator*, because the sample size for *S. lockingtoni* was too small. Data for *S. lockingtoni*

is provided in Tables 3 and 4. Summary data are presented as mean ± s.d. *N* refers to the number of individuals tested, and *n* refers to the number of trials analyzed.

RESULTS

Relative body and claw size

Individuals of *A. clamator* had a greater size range than those of *S. lockingtoni,* but both species are similar in size (Table 1). Both metrics of body size, mass, and carapace length, were positively correlated for *A. clamator* (linear regression: $R^2 = 0.77$, $F = 16.872$, $p = 0.009$). Snapping claw length was independent of body size (linear regression: mass, $R^2 = 0.57$, $F = 6.592$, $p = 0.05$; CL, $R^2 = 0.42$, $F = 3.593$, $p = 0.117$).

Table 1: Body mass and claw size of Alpheus clamator and Synalpheus lockingtoni used in the study. N = number of individual shrimps. Data presented as mean (s.d.). Asterisk used to note that body and claw measurements were averaged for only two of the three S. lockingtoni due to loss at the end of the study.

Species	N	Body mass (g)	Carapace length (mm)	Snapping Claw length (mm)
Alpheus clamator	7	0.29 (0.16)	5.31 (0.98)	8.27 (1.73)
Synalpheus lockingtoni	3*	0.22 (0.05)	5.17 (0.13)	7.10 (1.82)

Water jet kinematics

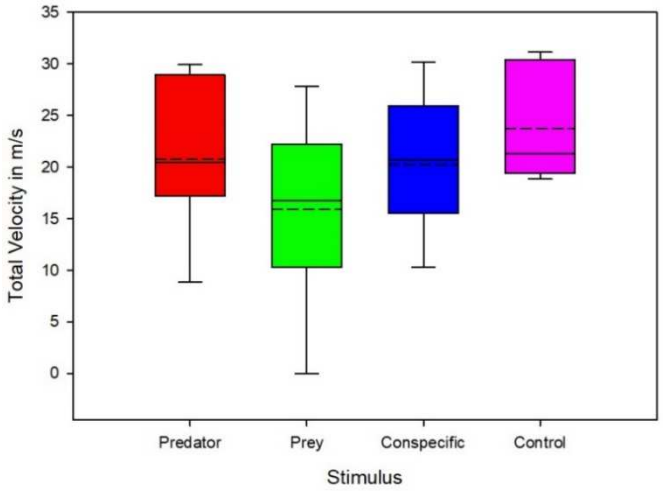

Figure 4: Total water jet velocity of the pistol shrimp A. Clamator snaps in response to predator, prey, conspecific, and control stimuli. Prey stimulus induced the lowest velocity. Box boundaries = the 25th and 75th quartiles, error bars = 1.5 times the interquartile distance, center line = median.

Total velocity of the water jet differed significantly between stimuli in *A. clamator* (LMM, df = 16.996, F = 8.380, p = 0.001), with no effect of mass (p = 0.242), but an effect of CL (p = 0.049). The prey stimulus induced snaps with total velocity lower than all other stimuli (all $p<0.01$) (Fig. 4).

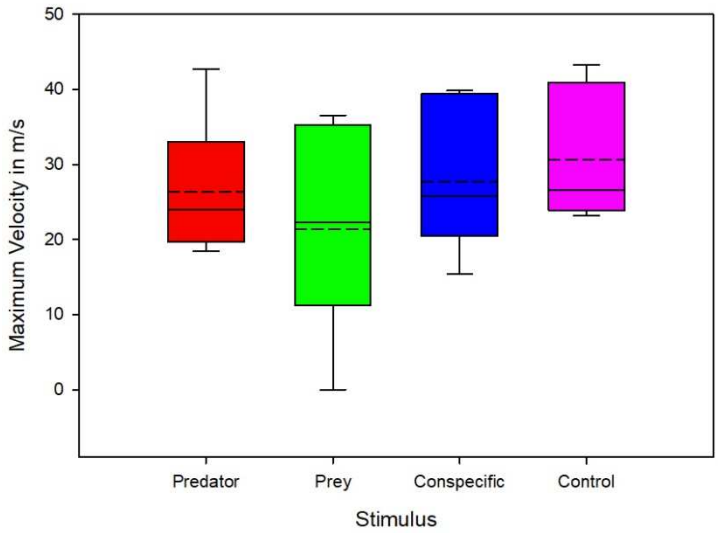

Figure 5: Maximum water jet velocity of the pistol shrimp A. Clamator snaps in response to predator, prey, conspecific, and control stimuli. Maximum velocity was the same for all stimuli. Box boundaries = the 25th and 75th quartiles, error bars = 1.5 times the interquartile distance, center line = median.

Maximum velocity of the water jet was the same for all stimuli in *A. clamator* (LMM, df = 16.976, F = 2.145, p = 0.132), with no effect of mass (p = 0.860), but an effect of CL (p = 0.028) (Fig. 5). Total and maximum velocities of the water jet were consistently higher in *S. lockingtoni* than *A. clamator* and were similar across stimuli, though these patterns were not tested statistically (Table 2).

Table 2: Total and maximum jet velocity of A. Clamator and A. lockingtoni. N = number of individual shrimps. Data presented as mean (s.d.).

		Alpheus clamator			*Synalpheus lockingtoni*		
Variable	Stimulus	N	Mean	st dev	N	Mean	st dev
Total jet velocity (ms^{-1})	Control	6	23.71	5.49	2	43.05	11.23
	Predator	7	20.79	7.23	1	51.23	-
	Prey	6	18.54	8.87	2	42.65	12.89
	Conspecific	7	20.25	6.58	2	34.78	12.65
Max jet velocity (ms^{-1})	Control	6	30.64	8.72	2	54.97	6.03
	Predator	7	26.31	8.74	1	61.86	-
	Prey	6	24.95	9.72	2	56.93	10.44
	Conspecific	7	27.68	9.52	2	52.45	12.69

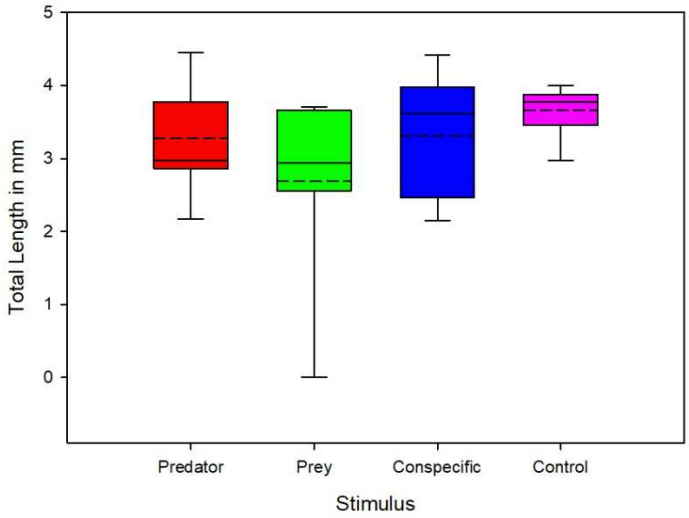

Figure 6: Total water jet length of the pistol shrimp A. Clamator snaps in response to predator, prey, conspecific, and control stimuli. Maximum velocity was the same for all stimuli. Box boundaries = the 25th and 75th quartiles, error bars = 1.5 times the interquartile distance, center line = median.

Total length of the water jet was the same for all stimuli in *A. clamator* (LMM, df = 16.975, F = 2.382, p = 0.105), with no effect of mass (p = 0.233) and no effect of CL (p = 0.820) (Fig. 6).

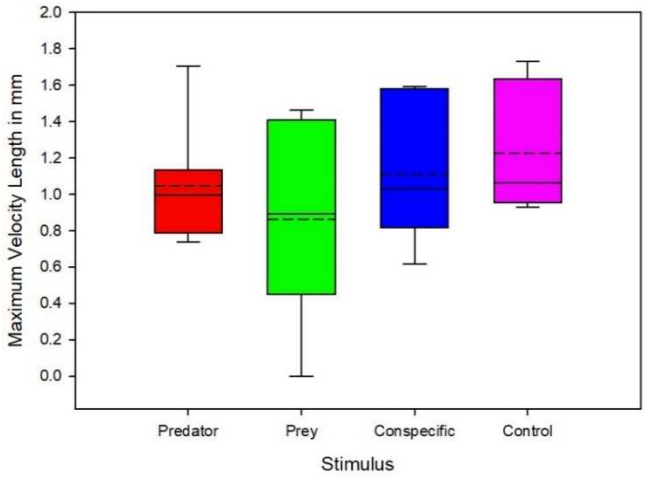

Figure 7: Maximum water jet length of the pistol shrimp A. Clamator snaps in response to predator, prey, conspecific, and control stimuli. Maximum length was the same for all stimuli. Box boundaries = the 25th and 75th quartiles, error bars = 1.5 times the interquartile distance, center line = median.

Maximum length of the water jet was the same for all stimuli in *A. clamator* (LMM, df = 17.004, F = 2.476, p = 0.096), with no effect of mass (p = 0.331), but an effect of CL (p = 0.028) (Fig. 7).

Total length and maximum length of the water jets were consistently higher in *S. lockingtoni* than *A. clamator* and were similar across stimuli, though these patterns were not tested statistically (Table 3).

Table 3: Total and maximum jet length of A. Clamator and A. lockingtoni. N = number of individual shrimps. Data presented as mean a (s.d.)

		Alpheus clamator			*Synalpheus lockingtoni*		
Variable	Stimulus	N	Mean	st dev	N	Mean	st dev
Total jet length (mm)	Control	6	3.66	0.36	2	4.15	0.09
	Predator	7	3.28	0.76	1	4.10	-
	Prey	6	3.14	0.49	2	3.81	0.96
	Conspecific	7	3.31	0.83	2	3.73	0.90
Max jet length (mm)	Control	6	1.23	0.35	2	2.20	0.24
	Predator	7	1.05	0.32	1	2.48	-
	Prey	6	1.01	0.38	2	2.26	0.39
	Conspecific	7	1.11	0.38	2	2.10	0.51

Snap sound level

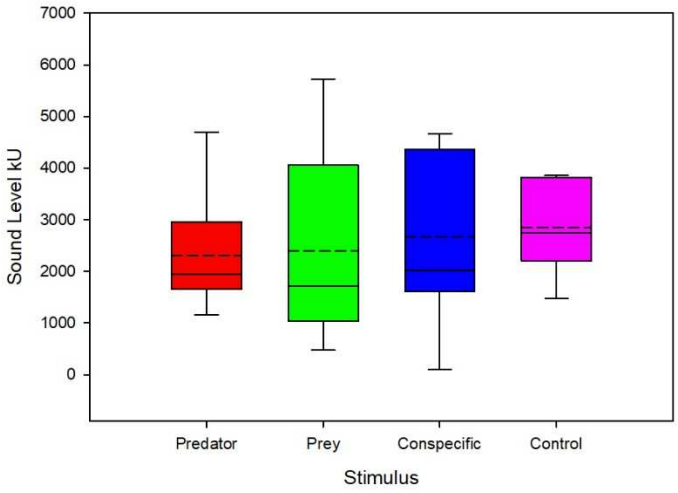

Figure 8: Average sound level of the pistol shrimp A. Clamator snaps in response to predator, prey, conspecific, and control stimuli. Sound level was the same for all stimuli. Box boundaries = the 25th and 75th quartiles, error bars = 1.5 times the interquartile distance, center line = median.

Table 4: Sound level of snaps from A. clamator and S. lockingtoni. N = number of individual shrimps. Data presented as mean (s.d.).

	Alpheus clamator			*Synalpheus lockingtoni*		
Stimulus	N	Sound (kU)	st dev	N	Sound (kU)	st dev
Control	6	2845	901	2	1533	530
Predator	7	2301	1191	3	1049	1200
Prey	6	2389	1917	1	1944	-
Conspecific	7	2665	1718	3	1904	947

Sound levels of cavitation bubbles generated during snaps were highly variable within and among stimuli (Table 4). The magnitude of snap sounds were not statistically different across stimuli in *A. clamator* (LMM, df = 16.106, F = 0.756, p = 0.535), with no effect of mass (p = 0.168) and no effect of CL (p = 0.640) (Fig. 8).

DISCUSSION

In this study I examined whether pistol shrimp exhibit context-dependent snap modulation. It may be beneficial for pistol shrimp to limit the power of their snap to reduce damage and conserve energy in certain situations that may not require as much power, as seen in mantis shrimp (Green et al., 2019). It was hypothesized that pistol shrimp would adjust the snap kinematics to generate different power in response to live stimuli, including prey, predator, and conspecific relative to a non-living control (paintbrush). It was also hypothesized that snap kinematics would vary among the live stimuli, with predators eliciting the most powerful snaps and conspecifics the least. These hypotheses were only partially supported by the data. One aspect of snap kinematics, total water jet velocity, was significantly lower in response to prey than all other stimuli, including the control. All other snap kinematics and sound were the same across stimuli. So, it appears that pistol shrimp can modulate their snap to a limited extent, but not in the way I hypothesized.

Variable water jet kinematics

The power generated by pistol shrimp snaps is reflected in the kinematics of the water jet and cavitation that it creates (Versluis et al., 2000). Most pertinent is velocity, which is set by the mechanics of claw closure. The velocity with which snapping shrimp closes the plunger of the claw into the socket determines the speed and size of the water jet, which in turn determines the magnitude of the cavitation bubble and the energy it releases. Evidence from our data suggests that pistol shrimp can manipulate multiple aspects of the claw snap resulting in highly variable water jet kinematics. Total water jet velocity varied from 10.3 m/s to 31.1 m/s within an individual in response to a single stimulus, which is in line with velocities found in other studies

(Versluis et al., 2000). When averaged over individuals, the ranges in total jet velocity exceeded 20 m/s for each stimulus. This high variability was consistent for all snap kinematics as well as sound level.

Such high variability in snap kinematics and sound suggests that perhaps it is simply inherent in the mechanism of the snap rather than under control of the pistol shrimp. For instance, the slightest incidental differences in how much energy gets stored in the elastic mechanism or even premature unlatching would get magnified through the resulting water jet and cavitation. There were even some instances observed in this study where *A. clamator* snapped, as evident from visible dactyl rotation, yet no water jet was produced, or a bubble was formed, but with no cavitation.

Despite the high variability, the total velocity of the water jet was significantly lower when *A. clamator* were presented with prey compared to other stimuli. This indicates that *A. clamator* snaps differently in response to a specific stimulus, thereby supporting our hypothesis that pistol shrimp can modulate their snaps based on context. Notably, the prey stimulus also generated the greatest variability in jet kinematics. This could potentially be due to individual pistol shrimp perceiving the prey item, a red rock shrimp, differently; some may perceive it as a prey item, whereas others might perceive it as a threat. Red rock shrimp are also found within the same kelp holdfasts that *A. clamator* resides in, so without understanding the nature of their interactions, it is difficult to speculate on why pistol shrimp snap with less power and more variability in their presence.

Other kinematics, including the total length and maximum length of the water jet in *A. clamator* did not have any statistically significant differences between stimuli. This is somewhat surprising given that total jet velocity was lower in response to the prey stimulus, so one might

expect that the other related kinematic variables would follow suit. Either the jet length metrics are simply more variable and subject to measurement error given their small scale, or the snap mechanism is more nuanced than predicted. In this study, the total jet length for *A. clamator* ranged from 2.15 mm to 4.46 mm, which is in line with the 3 mm length found in other studies of pistol shrimp (Versluis et al., 2000). Like total jet velocity, these other snap kinematic measurements also showed more variability in response to the prey stimulus.

Snaps in different contexts

A. clamator was exposed to a potential prey (red rock shrimp), a potential predator (rock crab), a conspecific, and a control, and snapped in response to the prey with the lowest total jet velocity. It was hypothesized that conspecifics would induce the least powerful snaps, reflecting either a preference for less damaging ways to settle disputes, as in mantis shrimp (Green et al., 2019), or a lower assessment of risk to survival. This prediction may not have unfolded for several reasons. First, pistol shrimp exhibit different levels of aggression during agonistic interactions based on sex, but we did not take this into account. In a closely related species of pistol shrimp, *Alpheus armatus*, females are more aggressive than males, as evidenced by their greater likelihood to snap and injure opponents while defending their anemone habitat (Knowlton and Keller, 1982). The greater inclination to snap, however, does not necessarily indicate more powerful snaps, but this was not examined. In the present study, all but one pistol shrimp readily snapped multiple times in response to each stimulus. Another closely related species, *Alpheus heterochaelis*, primarily uses the water jet for communication rather than aggression, but they maintain a sufficient distance from their antagonist (Hughes, 1996)). Second, that *A. clamator* snapped at prey shrimp with less power (jet velocity) than at

conspecifics, and all other stimuli, could be because they might not perceive the shrimp as prey or that they perceive them as prey that require less power to subdue. Other types of prey, such as those with hard shells, might stimulate snaps with greater power.

Interestingly, pistol shrimp snapped with the same power in response to a conspecific, predator, and control paintbrush. This could also be due to several reasons, including a generally heightened aggression induced by artificial experimental conditions. In our experiment, pistol shrimp were assessed in an empty aquarium with no structure to hide within. This was necessary so that shrimp could be carefully positioned in an apparatus for the snaps to be recorded with HSV. In a more realistic setting, pistol shrimp may have responded to the various stimuli differently. Also, if pistol shrimp rely heavily on chemical cues to assess other species during interactions, then their ability to differentiate between the different stimuli might have been diminished because of mixed chemical cues in the experimental tank; water was not exchanged between individual stimuli. Pistol shrimp may rely more on visual cues, however, as their vision is better than previously thought (Hughes, 1996; Kingston et al., 2019). The orbital hood provides protection for their eyes, but is also transparent enough to permit visualization of their environment (Kingston et al., 2019; Kingston et al., 2022). In *Alpheus heterochaelis*, the orbital hood is 80%-90% the transparency of seawater in 400 nm-700 nm range (Kingston et al., 2019). It is believed that pistol shrimp have spatial vision, but if they are able to see their prey, conspecifics, or predators, then they may be able to distinguish between different taxa.

Cavitation and sound levels

The loud sound of a pistol shrimp snap is generated by the collapse of a cavitation bubble, rather than impact of claw closure (Versluis et al., 2000). Sound levels produced by the

snaps of *A. clamator* were highly variable among each stimulus, but not statistically different, and thus similar to the general patterns observed with the water jet kinematics. While water jet velocity contributes to the development and size of the cavitation bubble, it is the collapse of the bubble that produces the energy in the form of sound, light and heat. To better understand how the magnitude of the collapsing cavitation bubble is related to each stimulus, it would be prudent to look at the speed of the cavitation bubble collapsing. It would be the variability in speed of the collapsing cavitation bubble that is driving the variability within the sound levels among each stimulus.

Greater velocities impart greater energy into cavitation bubbles, causing them to become larger, and thus release more energy upon collapse. Cavitation bubble collapse releases energy in multiple forms, light, heat, and sound, so it is possible that energy can be lost to other forms through the conservation of energy. To understand the relationship between the total speed of the cavitation bubble and the magnitude of the collapsing cavitation bubble, video recordings at higher resolution and velocity are necessary.

Interspecific variation in snaps

The snaps of *S. lockingtoni* were not statistically analyzed due to low samples sizes, but the kinematics and sound levels are similar for all stimuli, which is mostly consistent with *A. clamator*. *S. lockingtoni* appears to snap with uniformly greater power than *A. clamator*, as evidenced by the consistently higher values of kinematic variables. This difference in snap power could be a result of distinct claw morphology. *S. lockingtoni* has a relatively longer claw (snapping claw length/body mass) than *A. clamator*, and claw length positively correlates with the size and velocity of water jets (Herberholz and Schmitz, 1999). Detailed morphology of the

claws was not examined and compared in this study, but the shape of the claw affects the volume of water the socket can hold, and the more water held, the more kinetic energy the shrimp needs to move the water out of the socket (Wei et al., 2021). It is known that animals with spring compression strike mechanisms are able to exhibit control over their strikes. Pistol shrimp exhibit a similar variability in their elastically controlled snapping mechanisms as do mantis shrimp, however, the mechanisms of snap production in pistol shrimp differ from those of mantis shrimp, and involve, for example, adhesive disks (Anker et al., 2006) and a different latch mechanism (Kaji et al., 2018). Other animals, like the trap jaw ant, use a latching mechanism to exhibit their fastest movements, and when unlatched they have one of the slowest movements of all the ants (Spagna et al., 2008). Similar to the trap jaw ants, pistol shrimp could exhibit slower movements or lower speeds if the claw is not fully cocked or full of water.

Conclusions

Results from this study demonstrate that pistol shrimp can modulate their snap based on context, but the specific mechanisms and motivation driving this modulation requires further study. Areas of future focus could include analyzing the angular velocity of dactyl rotation and angle of the claw to determine when cavitation bubbles form or to see if the angle the open dactyl plays a role in cavitation speed. Determining if the dactyl rotates the same distance to meet both adhesive discs could provide insight in how prudent they are to making a forceful snap. Analyzing these systems could indicate how dominate the elastic force is in a pistol shrimp snap, and the degree to which other components contribute to making pistol shrimp one of the most powerful animals on the planet. Establishing context-dependent snap modulation in pistol

shrimp strengthens our understanding of pistol shrimp ecology and contributes new information on how elastic mechanisms are used by animals with extremely fast movements.

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