Visual signals of the East Pacific red octopus (*Octopus rubescens*) during conspecific interactions

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Abstract

Multiple species of octopuses have recently demonstrated the use of specific visual signals (such as chromatic, postural, locomotor, and textural signals) to communicate with conspecifics. This study aimed to identify the visual signals of the East Pacific red octopus, *Octopus rubescens*, during interactions with conspecifics. *Octopus rubescens* were collected from Admiralty Bay, WA – a habitat littered with discarded glass bottles which *O. rubescens* opportunistically use as dens. To identify the visual signals of *O. rubescens*, cameras recorded videos of octopuses interacting with conspecifics of the same and opposite gender in an observation tank over the course of 15 mins. *Octopus rubescens* were predominantly aggressive toward conspecifics, but nonetheless displayed visual signals, such as 'upright', 'attack', 'approach', 'ochre' and 'dark ochre', which were recorded in an ethogram. Due to the unique habitat of Admiralty Bay, the observed visual signals of *O. rubescens* may be highly specialized compared to other *O. rubescens* individuals living in different habitats. Consequently, the ethogram produced in this study may be used as a source of comparison for future studies documenting the visual signals of this species in other habitats; this could reveal potential variations in visual signals and may suggest that the visual signals used by *O. rubescens* are influenced by their surroundings.

Introduction

Historically, octopuses have been thought to be solitary, asocial individuals (Barbato et al. 2007; Hanlon and Messenger 2018); however recent studies have suggested that octopuses use a unique and systematic arrangement of visual signals to communicate with conspecifics (Huffard 2007; Caldwell et al. 2015; Scheel et al. 2016). These visual signals include chromatic and textural changes, postures, different forms of locomotion, and inking which can be combined or used consecutively to create specific displays (Hanlon and Messenger 2018). Displays are characterized by being repetitive and discrete, allowing octopuses to portray clear messages to receivers.

Some of the most complex signals octopuses use are chromatic signals. Since octopuses have direct neural control of pigment-containing cells, called chromatophores, octopuses can quickly change chromatic signals, adjust signal strength, and even perform bilateral signaling (Barbato et al. 2007; Hanlon and Messenger 2018). Hanlon and Messenger (2018) have observed that chromatic signals generally include forming line-stimuli consisting of bands (lines, stripes, bars) or spots that are easily detected by other octopuses. Although colorblind, octopuses have excellent vision – consequently, by using highly contrasting chromatic signals, octopuses can clearly display their intent (e.g. to show dominance or submissiveness) toward a conspecific (Tricarico et al. 2011; Hanlon and Messenger 2018). In combination with chromatic signals, textural signals (defined as smooth or papillate skin) can be used to modify the appearance of an octopus. Additionally, postural signals, such as raised arms or flattening of an octopus's body, are often used to adjust an individual's apparent size to demonstrate intimidation or submissiveness (Hanlon and Messenger 2018). Furthermore, specific movements, labelled 'locomotor' visual signals, may include chasing or fleeing (Hanlon and Messenger 2018). All of these signals can be combined in a wide variety of patterns and intensities allowing octopuses to communicate with conspecifics.

Three species of octopuses that have been shown to use visual signals to communicate with conspecifics include the larger Pacific striped octopus (*Octopus* spp., no species name assigned), the Algae octopus (*Abdopus aculeatus*), and the common Sydney octopus (*Octopus tetricus*) (Huffard 2007; Caldwell et al. 2015; Scheel et al. 2016). Each of the studies described specific visual signals used by octopuses during conspecific interactions which were typically agonistic. Both Huffard (2007) and Caldwell et al. (2015) performed observational studies and recorded octopuses' visual signals in ethograms which act as libraries that describe and identify behaviors displayed by animals.

Many visual signals that octopuses utilize are species-specific, therefore characterizing and documenting visual signals of octopuses via ethograms provides useful supplementary information for validating species identification (Barbato et al. 2007, Huffard 2007). Additionally, both Sinn et al. (2001) and Scheel et al. (2016) suggest that ethograms can act as resources for scientists studying how ecological influences, such as conspecific interactions or habitat availability, may affect the evolution of signal development or communication. For example, a population of octopuses living in one type of habitat may utilize a slightly different or more specialized set of visual signals to communicate with each other compared to a population of the same species living in a different type of habitat.

Although nineteen common visual signals were identified for the East Pacific red octopus (*Octopus rubescens)* by Mather and Anderson (1993), these signals were in response to human stimuli during three different situational laboratory tests. No ethogram has been created to describe the visual signals used by *O. rubescens* to communicate with conspecifics. *Octopus* *rubescens* is a subtidal species found along the west coast of North America (from Alaska to California), sheltering in kelp beds and rocky areas, and are commonly found in Admiralty Bay, WA (Cowles 2005). The benthic habitat of this bay is generally barren and flat, characterized by mud, sand, and small rocks with few hiding places for this non-burrowing octopus species. However, the bay is littered with discarded glass bottles which *O. rubescens* opportunistically use as dens (Anderson et al. 1999). *Octopus rubescens* have capitalized on this new habitat source which may have inadvertently concentrated individuals of this species within the bay (Chase and Verde 2011; Verde, conversation 2018, unreferenced). Consequently, *O. rubescens* may interact with conspecifics more frequently within this "artificial" environment and these interactions may be characterized by visual signals used by octopuses to communicate with each other.

Given that octopuses use visual signals to interact, the purpose of this project was to determine the frequency of such signals used by *O. rubescens* individuals to communicate with conspecifics, and to document those visual signals in an ethogram. As such, this study addressed the following questions: 1) what are the visual signals that *O. rubescens* use during interactions with conspecifics; 2) is the frequency of interactions influenced by the gender of octopuses; 3) do the type or frequency of visual signals differ between initiators and reactors of an interaction?

Methods

Overview

To identify the visual signals of *O. rubescens*, cameras recorded videos of octopuses interacting with conspecifics of same and opposite sex in an observation tank. Videos were analyzed for any visual signals used by the octopuses during interactions with conspecifics.

These visual signals were defined and categorized in order to assemble an ethogram for *O. rubescens*.

Octopus collection and care

Octopus rubescens individuals were collected via SCUBA from Admiralty Bay, WA (48°9'43.84" N, 122°38'4.67" W) and housed at the Rosario Beach Marine Laboratory (RBML), Anacortes, WA. Because this species is often found inhabiting bottles in this bay, all bottles found were checked for the presence of *O. rubescens* by scraping away any biofouling on the bottle. If an octopus without any eggs was present, the bottle was collected and placed into a Ziploc® (3.8 L) bag and sealed. Ideally, similar sized octopuses would have been used in this study, but due to time constraints any octopus between 15-70 g was collected. Final octopus mass ranged from 18.8-68.0 g, with the average mass being 42.0 g. Upon completion of each dive, collected octopuses were removed from their resident bottles and transferred to Nalgene© (1 L) bottles. The Nalgene© bottle openings were covered with plastic window screen mesh and secured to the bottle by an elastic rubber band. Bottles were placed in an aerated cooler containing seawater and transported back to RBML; all 'home' glass bottles, or dens, were returned to the ocean prior to leaving the collection site.

Upon arrival to RBML, all octopuses were weighed (g) and their gender determined, as demonstrated to me by Monica Culler (conversation 2018, unreferenced). Weight was measured by placing a tared jar partially filled with seawater onto a Mettler Toledo™ balance (Model: PL601-S; capacity 610 g, readability $\pm$ 0.1 g). Individual octopuses were persuaded into the tared jar from their Nalgene© by emptying all seawater from the Nalgene© and holding it above the jar on the scale until octopuses transferred themselves. Octopus sex was determined by looking for the presence of a hectocotylus, the third right arm on male octopuses modified to carry spermatophores; this arm is enlarged and lacks suckers at its tip (Cowles 2005). Mass, gender, date collected, and the octopus's location in the lab were recorded in a Google spreadsheet.

Individual octopuses were housed in enclosed, opaque plastic containers (36 cm x 23 cm x 28 cm) with constant flowing ambient seawater via a manifold system (Chase and Verde 2011; Fig. 1). Rocks were placed on top of the containers as additional measures to prevent octopuses from escaping. The enclosed containers were maintained in seawater raceways (231 cm x 29 cm x 24 cm) to maintain a constant temperature of 12°C (Perron and Verde 2015). *Octopus rubescens* have been noted to adapt well to captivity and most octopuses are known for being exploratory and responsive to laboratory conditions (Mather 2006). Each octopus was given a minimum of 48 h to acclimate to the containers and sea water system and were fed purple shore crabs (*Hemigrapsus nudus*). Octopuses were fed once per day at night, after all tests were concluded for the day, to avoid the potential influence of increased metabolism (due to specific dynamic action) on social behaviors (Katsanevakis et al. 2005; Hill et al. 2016).

The total sample size for this experiment was 20 octopuses. The seawater system at RBML dedicated for this study could house a maximum of ten octopuses at a time, so the study was divided into halves. One set of 10 octopuses was run through all tests while the second set of 10 octopuses was collected. Upon completing all tests, octopuses were released back into Admiralty Bay; release locations were separate from new octopus collection sites within the bay to prevent recollection. Sets of octopuses were assigned letters, to identify the respective sets that octopuses were from (A = set 1, B = set 2). Each set of octopuses participated in the 'Conspecifics treatments' (see below). The sex ratio for this study was 50/50 female to male octopuses, to represent the typical sex ratio found in the local area for this species (Chase and

Verde 2011). Octopuses were identified by their respective locations in the seawater table (e.g. a female octopus in seawater table “H” in container “2” was identified as “H2”).

Observation tank

A flow-through seawater observation tank (80 cm x 50 cm x 24 cm) was outfitted with three GoPro cameras (Fig. 2) and placed in a closed room to avoid human interaction while tests were conducted. The tank was divided by a piece of plexiglass with holes drilled into it to buffer the rippling effect of the seawater in/outflow (Fig. 2). Consequently, only half of the tank was used as the testing area for the octopuses (46 cm x 50 cm x 24 cm). Fluorescent lights provided maximum light for the cameras, however this may have influenced octopus behavior or activity. To minimize this influence, octopuses were given a minimum of 48 hours to acclimate to the laboratory lighting. This high-lit environment was necessary to capture clear videos of the octopuses and camera settings (see Appendix) were adjusted to accommodate for the lighting. These settings ensured that the highest quality videos were recorded, although coloration and dermal papillation of octopuses may not have been accurately represented in the videos. To reduce glare, some fluorescent light bulbs were removed and white sheets were hung under the lights to filter the light above the tank. The observation tank was white, which provided sufficient contrast between the octopuses and the tank for the cameras to successfully record images. To improve water clarity, two seawater filters were attached to the seawater input lines of the tank. Cotton balls were used as the filtering materials in the seawater filters and were changed as needed, typically every two to three days. The observation tank was cleaned, drained, and refilled every morning before any tests commenced.

Cameras were placed at different locations in the tank (Fig. 2): one directly above and two submerged at the octopuses’ level in opposite corners of the tank. Camera housings (Fig. 3) were made to hold the cameras in place. To eliminate blind spots on the corner cameras in the tank, two plexiglass dividers were cut and angled width-wise along the tank walls to narrow the space (Fig. 2). GoPro cameras were left on and recording independently for the duration of each 15 min trial and these videos were downloaded to a personal computer after each trial. The tank was drained and completely flushed at the end of every trial to ensure chemicals released by octopuses (e.g. ink, pheromones, nitrogenous waste) during interactions did not influence subsequent trials.

Since *O. rubescens* have been noted to display aggressiveness toward conspecifics (Mather and Anderson 1993; Scheel et al. 2016), octopuses were separated in the observation tank space with a piece of plexiglass as a precautionary step while conducting preliminary trials (see 'Ethogram' below). Most octopuses were aggressive toward one another, but not cannibalistic or noticeably harmful, therefore the plexiglass divider was not used for all other trials following the preliminary trials.

Ethogram

To observe and gather baseline visual signals displayed by *O. rubescens*, multiple trial runs of the 'Conspecifics treatment' (see below) were made with octopuses already at RBML. These visual signals observed were compiled into a basic ethogram and used to categorize signals observed during the rest of the study. Visual signals were described utilizing the terminology used in the signal identification table (Table 1) compiled by Hanlon and Messenger (2018). These signals included: chromatic (e.g. banding, spots, darkening) and papillae change (e.g. papillate or smooth), postures (e.g. spreading or flattening of body, raised arms), and forms of locomotion (e.g. chasing, fleeing). Ethogram terminology was also adapted from Huffard (2007), Caldwell et al. (2015), and Scheel et al. (2016). As additional visual signals were observed, they were added to the basic ethogram to create the final ethogram for this species (Table 2). Signal names and descriptions from the final ethogram were summarized and compiled (Table 3).

Conspecifics treatment

Each octopus in a set of ten octopuses was allowed to interact with all other octopuses of the same and opposing sex within each set (Fig. 4). Treatments were as follows: male and male (M/M), male and female (F/M), and female and female (F/F). The order of the octopus combinations was chosen via simple random sampling and random numbers were assigned to each possible octopus combination. To ensure no octopus individual was tested consecutively, selected numbers could be ignored and reentered into the random numbers table. Tests were performed each day (from Sunday to Friday) until all octopus combinations were completed.

Two octopuses were placed in the observation tank as far away from each other as possible. Octopuses were placed in the tank one at a time, therefore the first octopus to enter the tank was always the octopus that was listed first in the written combination name (e.g. combination “H3 and H7”; H3 would be placed in the tank first). Once recording commenced, the octopuses were left to interact for 15 mins. This interaction time was chosen to avoid leaving the octopuses in the observation tank for an extended period of time, as interactions were likely to occur within the first 15 mins, since these organisms are exploratory (Onthank, phone conversation 2018, unreferenced). Octopuses were observed from a distance of at least 2 m to keep track of individuals with no unique characteristics (e.g. unique scars, missing arms) and to intervene if necessary if octopuses became too aggressive.

Collecting and analyzing data

This study only analyzed videos of octopuses from Set A, however Set B will be analyzed in the future and the data will be combined with Set A. VLC Media Player was utilized to observe videos recorded by the GoPro cameras. Snapshots from the videos were added to the basic ethogram created at the beginning of this project and subsequent videos were analyzed using the basic ethogram.

The ethogram was used to describe any octopus interaction that lasted at least 5 s. Any new visual signal observed during video analysis was added to the basic ethogram. The approach of one octopus toward the other marked the beginning of an interaction; the approaching octopus was deemed the ‘Initiator’ and the other octopus the ‘Reactor’ as defined by Scheel et al. (2016). When an interaction began, the time was noted and the visual signals (chromatic, textural, postural, locomotor, inking) of the octopuses were recorded. When there was any change in a given signal during an interaction, the new signal was recorded and the time was noted. A new interaction was recorded only if there was more than a 5 s break between the end of the last interaction (Sinn et al. 2001). The total number and types of signals displayed by the octopuses in the observation tank were recorded and compiled into clustered bar graphs to demonstrate how frequent each of the signals were used by octopuses. Due to the small sample size, statistics could not be correctly performed.

Results

When interactions occurred during the 15-minute trials, *O. rubescens* used a variety of visual signals to communicate with conspecifics. These visual signals included chromatic, locomotor, postural, inking, and textural signals which were catalogued in an ethogram (Table 2). The frequency of interactions differed by 1 interaction per test between the Male/Male octopus combinations (5.2 interactions per test) and Male/Female and Female/Female octopus combinations (4.2 interactions per test).

Interactions were typically characterized by the locomotor signals ‘stationary’, ‘approach’ and ‘flee’ by one or both octopuses for all gender combinations (Male/Male, Male/Female, Female/Female; Fig. 5). The most common chromatic signals included ‘ochre’, ‘dark ochre’, and ‘pale’ (Fig. 5). Octopuses appeared to use ‘ochre’ as a resting camouflage which may have been a result of the white tank walls which the octopuses were likely attempting to camouflage against. The most common postures included ‘upright’ and ‘curled arms’ (Fig. 5). Octopuses rarely inked and textural signals were predominantly ‘smooth’ (Fig. 5).

When interactions between octopuses were divided between the initiator and reactor within each gender combination (Male/Male, Male/Female, Female/Female), locomotor signals used by initiators of an interaction were mostly characterized by ‘approach’ or ‘stationary’, while reactors predominantly expressed the signals ‘stationary’ or ‘flee’ (Fig. 6). Regarding chromatic signals, initiators and reactors were most often ‘ochre’ or ‘dark’ (Fig. 7). The postural signals of both initiators and reactors were characterized by ‘upright’ and/or ‘curled arms’ (Fig. 8). Although, many interactions were characterized by ‘reaching’ – especially by initiators (Fig. 8). Male/Male-paired octopuses grappled the most out of the three gender combinations (Fig. 6 & 8), however grappling made up less than 10 % of all locomotor and postural signals (Fig. 5).

Discussion

A variety of visual signals used by *O. rubescens* during conspecific interactions were identified and catalogued in an ethogram. The number of interactions per test for each gender combination (Male/Male, Male/Female, Female/Female) of octopus were similar which suggests that gender had little influence on the frequency of interactions between octopuses. Both initiators and reactors typically used the same set of visual signals during interactions, however the sequence in which these visual signals were used was not analyzed. Consequently, it cannot be concluded that a certain visual signal was correlated with initiating or ending an interaction. While *O. rubescens* may gather in an area for a specific habitat resource, such as the bottles used as dens in Admiralty Bay, the species demonstrated predominantly aggressive behavior toward conspecifics during this study suggesting that they are not a social species, even if they are not solitary. When interactions did occur, they were characterized by an approach which was either aggressive or exploratory (which often led to aggression) and ended with one or both octopuses attempting to escape. Alternatively, octopuses would simply avoid each other which can be interpreted as another indication that this species is asocial. Nonetheless, *O. rubescens* still demonstrated the utilization of multiple visual signals during interactions which suggests communication was occurring between individuals.

Visual signals are especially important for octopuses to use during aggressive interactions because they can clearly display an octopus's intentions to attack or submit, depending on the likelihood of winning or losing a fight (Barbato et al. 2007; Scheel et al. 2016). Having the ability to display such intent helps octopuses avoid unnecessary harm. *Octopus rubescens* used specific and discrete visual signals to warn a conspecific before attacking: the 'attack' posture and the chromatic signals 'deimatic' and 'dark longitudinal stripes'. The 'attack' posture, although not used as frequently as other postures, such as 'upright' and 'curled arms', is an important example of a warning system that this species used before attacking a conspecific. The chromatic signal 'deimatic' was also used to warn or threaten a conspecific and is a commonly used threatening signal among other cephalopods (Scheel et al. 2016; Hanlon and Messenger

2018). Furthermore, *O. rubescens* displayed ‘dark longitudinal stripes’, similar to *Abdopus aculeatus* (Huffard 2007), prior to or while reaching for a conspecific or before attacking.

Another noteworthy chromatic signal *O. rubescens* used was ‘false frontal white eye spots’ which appeared quite frequently and was interpreted as another warning signal toward conspecifics. This signal, along with ‘dark longitudinal stripes’, was included in Hanlon and Messenger’s (2018) descriptive table of visual signals commonly used by cephalopods. Both signals are examples of how octopuses produce high-contrasting chromatic patterns that are easily visible to an observer. Lastly, *O. rubescens* displayed the posture ‘stand tall’, similar to *Octopus tetricus* and *Abdopus aculeatus* (Huffard 2007; Scheel et al. 2016). While *O. rubescens* did not use this posture as frequently as ‘upright’ or ‘curled arms’, it is an important posture that enables individuals to get a better view of a conspecific or to increase apparent size of an individual (Huffard 2007; Scheel et al. 2016).

One aggressive signal, ‘grappling’, was not as numerous as other postural or locomotor signals, but nonetheless occurred during interactions and most frequently between males. Huffard (2007) observed similar agonistic interactions primarily between males compared to Male/Female interactions; no Female/Female interactions were observed by Huffard (2007) to serve as a comparison with the behaviors documented for *O. rubescens*. Increased aggression between males perhaps could be attributed to their need to compete for females in their natural habitat. Huffard et al. (2010) suggest that Male/Male aggression is influenced by the value of a resource being competed for (a female) and the likelihood that a male can successfully acquire that resource. Therefore, an aggressive interaction between males may determine whether a male copulates with a preferred female or not which may explain why males are more likely to be aggressive toward one another.

Since the sample of octopuses used in this study was from a population in Admiralty Bay, where they congregate to use bottles as dens, these octopuses may use a more complex system of visual signals during interactions to communicate with conspecifics, as opposed to more solitary octopuses. Caldwell et al. (2015) suggest octopus populations with higher local densities interact with conspecifics more frequently and often display more aggression toward conspecifics compared to solitary octopus species. Consequently, the observed agonistic interactions of *O. rubescens* may be due to the denser population of this species in Admiralty Bay. As a result, this population of octopuses may experience increased competition for dens, mates or food which may lead to increased aggression (Huffard et al. 2010).

Using the ethogram produced in this study as a reference, future studies can document the visual signals of this species collected from other habitats and reveal potential variations in visual signals used by this species. This may allow scientists to hypothesize that the visual signals used by *O. rubescens* are influenced by surrounding habitats, like Admiralty Bay, or population density. The basic ethogram created in this study can also act as an additional resource of comparison between octopus species, regardless of the fact that the visual signals identified were during conspecific interactions. Ethograms can be helpful resources that demonstrate evolutionary convergence of signal use (e.g. two distantly related species using similar signals to communicate with conspecifics) or verify a taxonomic similarity between species (Huffard 2007).

While a successful record of a variety of visual signals used by *O. rubescens* to communicate with conspecifics during interactions was created from this study, future studies should consider using higher quality video cameras compared to GoPro Hero 3. Ideally, cameras should be able to connect to a computer while recording in order to deliver a live feed of video during trials. This would allow octopuses to interact free of any potential human influence. One should also consider a method in which both octopuses are released into the observation tank simultaneously to avoid leaving one octopus in the tank for a longer period of time. Additionally, octopuses did not always immediately consume the crabs fed to them in their housing units, therefore some social behaviors observed may have been influenced by either specific dynamic action, if the octopus ate more recently, or hunger. For future analysis of recorded interactions, the sequence of signals used by initiators and reactors should be determined in order to further dissect the question regarding whether there is a difference in the type or frequency of visual signals used by initiators and reactors.

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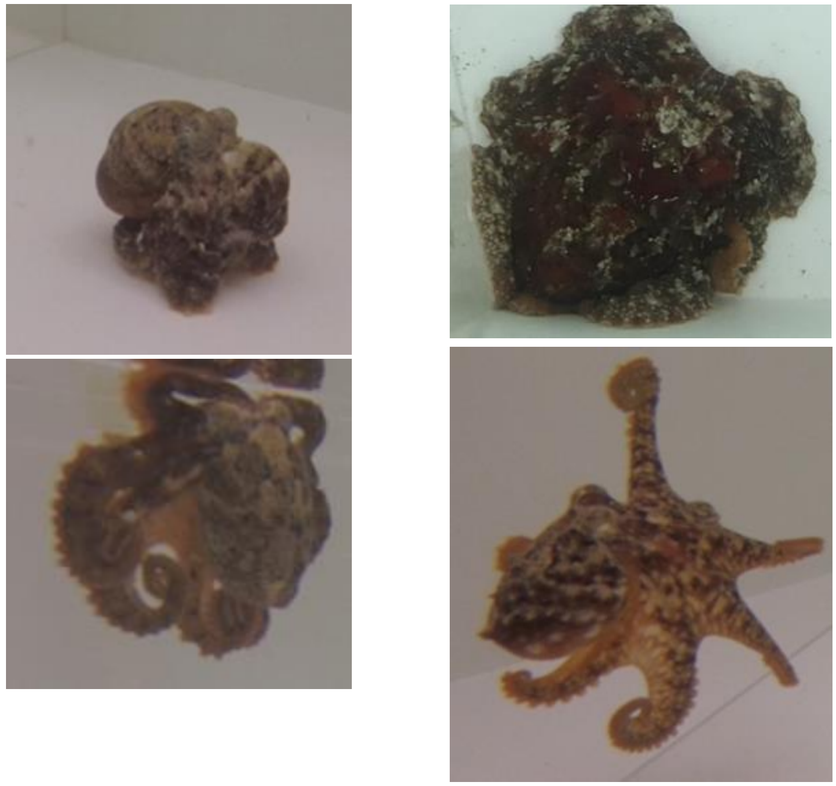
Table 1. A list of visual signals cephalopods may utilize during conspecific interactions. These terms were used to describe recorded visual signals of interacting *Octopus rubescens* conspecifics in an observation tank. Adapted from Hanlon and Messenger (2018).

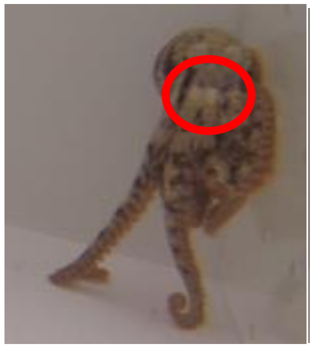
Chromatic Signals	Textural Signals	Locomotor Signals	Postural Signals	Inking
Whole body	Papillate	Chase	***Whole body***	Pseudomorphs
General paling	Smooth	Flee	Orientation to receiver	
Intense whitening		Forward rush	Upward or downward pointing	
General darkening		(Anti)parallel positioning	Spreading	
Flashing (pulsating)			Flattening	
Passing cloud			***Arms only***	
Conflict mottle			Singly	
Part of body only (often unilateral)			In pairs or all together	
Dark stripes or streaks (longitudinal)			Raised or lowered	
Dark bars, bands or rings (transverse)			Splayed	
Dark spots (large or small)			Split	
Bright white spots (large or small)			V-curled	
Dark eye rings			Contorted	
Dilated pupil			Male ligula presentation	
False eyespots				
Dark waving arms				
Suckers (white or dark-edged)				
Zebra bands or flame markings				
Lateral mantle blush				
Fin lines (dark or light)				
Accentuated white gonad				
Red accessory nidamental glands				
Iridescent rings or stripes				
Polarized light from arms				

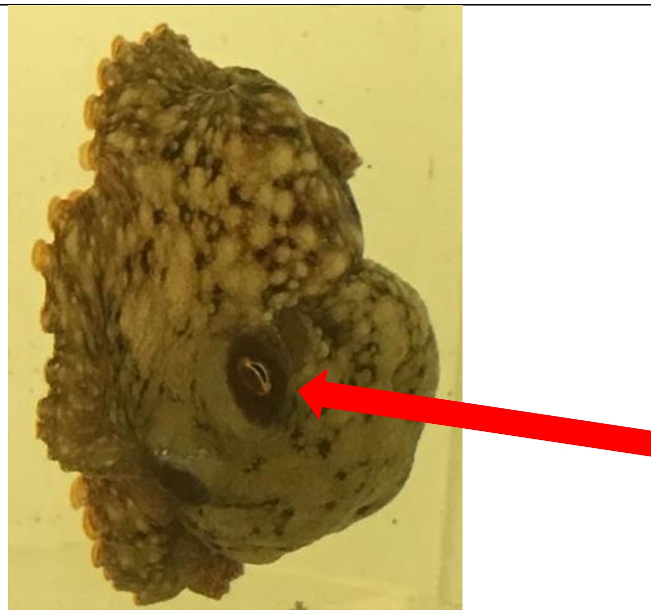
Table 2. Ethogram describing visual signals used by *Octopus rubescens* during conspecific interactions in an observation tank. Includes chromatic, textural, postural, and locomotor signals and inking. Terminology adapted from Huffard (2007), Caldwell et al. (2015), Scheel et al. (2016), and Hanlon and Messenger (2018).

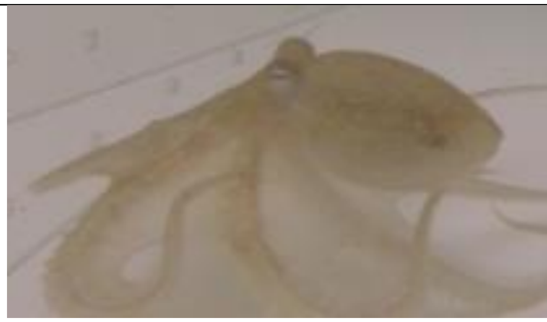
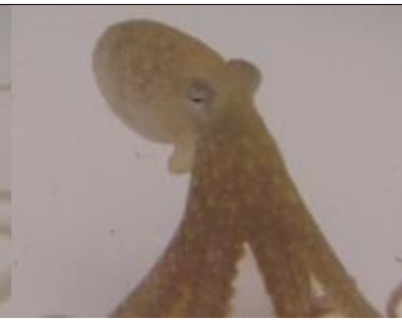
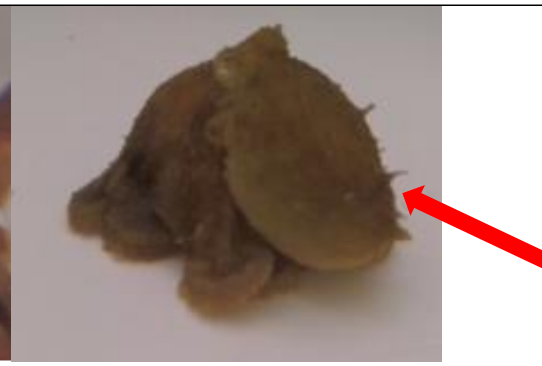
Chromatic Signals	
Signal Name	***Description***
Pale	  Pale body – light ochre to gray or white.
Deimatic	   Dark spots/patches on mantle, pale arms.

Ochre	  Ochre/sand-colored body (some variation in darkness).
Mottled Ochre	    Ochre/sandy-colored body; white and/or brown/black spots (spot density and color varies) across entire body.

Intense Mottle	 High contrast between dark and pale markings on body, bars/bands of dark along arms may be present; often papillate.
Dark	 Completely darkened body, red to brown/dark ochre.

Chromatic: Partial Body (Arms/eyes/etc)	
False frontal white eye spots	 Two adjacent white spots centered below eyes, on front part of octopus body.
Dark longitudinal stripe(s)	 Typically run(s) from eye down first left and/or right arm(s), can be symmetrical on other side of octopus; does not always run length of arm.

Dark eye rings	 Darkened patch encircling eyes.
Darkened Arms	   Typically first left or right (or both) arms of octopus. All arms can be darkened.

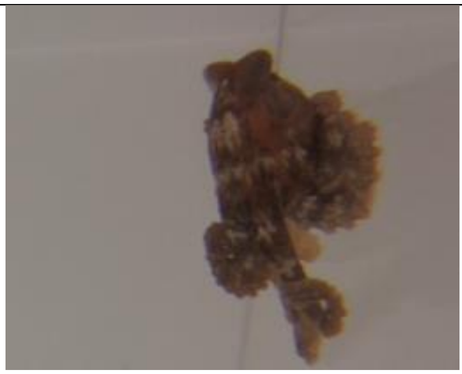
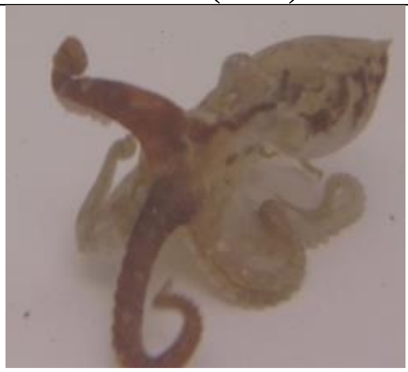
Textural Signals	
Signal Name	***Description***
Smooth	  No papillae.
Papillate	  Papillae visibly raised.

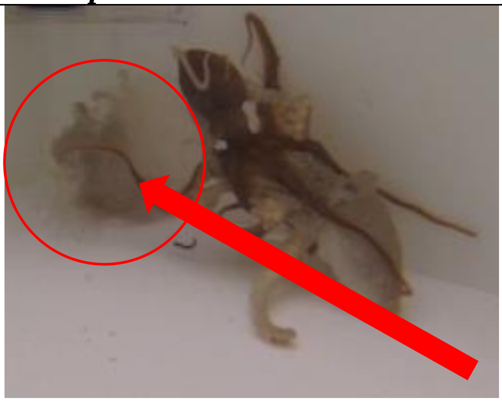
Postural Signals	
Signal Name	***Description***
Spreading arms	 Arms stretched out, feeling bottom.
Flattened	 Octopus low to bottom, mantle lowered.
Beak-to-beak	 Octopuses facing each other, arms spreading around each other. Touching close to beaks.

Reaching	  One or multiple arms reaching for conspecific.
Curled arms	 Arms curled tightly against body.

Loose arms	  Arms hanging loosely around or below body, can be slightly curled.
Upright	 Octopus alert toward conspecific.
Jetting	 Arms together, typically, but can be curled.

Grappling	  Octopuses entangled in each other’s arms, fighting.
Stand tall	  Upright, arms straightened to make self taller/larger.
Crawling	 Arms out, loose or curled, but clearly being used to propel the octopus.

Hanging	 On side of tank (walls). Arms can be curled or loose.
Attack	 Arms poised to attack conspecific (two front arms typically curled and held up toward conspecific). This posture is often combined with the chromatic signal ‘Darkened Arms’.
Raised Arm(s)	 Arms raised, often curled. Typically first front arms, but not exclusively.

Inking	
Signal Name	***Description***
Present/Absent	 Ink.

Locomotor	
Signal Name	***Description***
Stationary	Octopus not moving.
Threaten	Octopus lunges at conspecific but does not attack.
Flee	Octopus moves away from conspecific via crawling or jetting.
Attack	 Octopus launches self at conspecific; forward rush. Jet propulsion commonly utilized.

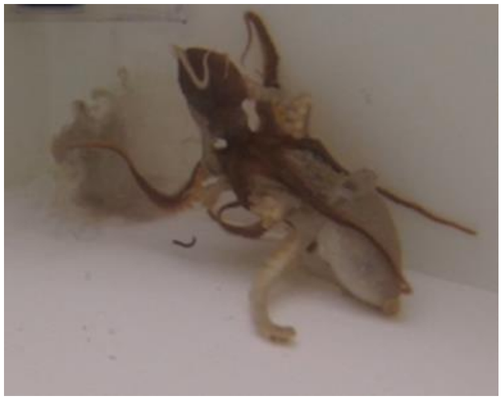
Grappling	  Octopuses entangled in each other's arms, reaching/biting/grabbing.
Chase	Octopus pursues conspecific via crawling or jetting.
Approach	Octopus approaches conspecific via crawling or jetting.

Table 3. Summary of visual signals (chromatic, textural, postural, and locomotor signals and inking) used by *Octopus rubescens* during conspecific interactions observed during 15-min trials in an observation tank. Terminology adapted from Huffard (2007), Caldwell et al. (2015), Scheel et al. (2016), and Hanlon and Messenger (2018).

Visual Signals	
Chromatic	**Description**
Pale	Pale body – light ochre to gray or white.
Deimatic	Dark spots/patches on mantle, pale arms.
Ochre	Ochre/sand-colored body (some variation in darkness).
Mottled Ochre	Ochre/sandy-colored body; white and/or brown/black spots (spot density and color varies) across entire body.
Intense Mottle	High contrast between dark and pale markings on body, bars/bands of dark along arms may be present; often papillate.
Dark	Completely darkened body, red to brown/dark ochre.
Chromatic: Partial Body (Arms/eyes/mantle)	
False frontal white eye spots	Two adjacent white spots centered below eyes, on front part of octopus body.
Dark longitudinal stripe(s)	Typically run(s) from eye down first left and/or right arm(s), can be symmetrical on other side of octopus; does not always run length of arm.
Dark eye rings	Darkened patch encircling eyes.
Darkened Arms	Typically first left or right (or both) arms of octopus. All arms can be darkened.
Textural	
Smooth	No papillae.
Papillate	Papillae visibly raised.
Postural	
Spreading arms	Arms stretched out, feeling bottom.
Flattened	Octopus low to bottom, mantle lowered.
Beak-to-beak	Octopuses facing each other, arms spreading around each other. Touching close to beaks.
Reaching	One or multiple arms reaching for conspecific.
Curled arms	Arms curled tightly against body.
Loose arms	Arms hanging loosely around or below body, can be slightly curled.
Upright	Octopus alert toward conspecific.
Jetting	Arms together, typically, but can be curled.
Grappling	Octopuses entangled in each other's arms, fighting.
Stand tall	Upright, arms straightened to make self taller/larger.
Crawling	Arms out, loose or curled, but clearly being used to propel the octopus.
Hanging	On side of tank (walls). Arms can be curled or loose.
Attack	Arms poised to attack conspecific (two front arms typically curled and raised).
Raised Arm(s)	Arms raised, often curled. Typically first front arms (left and/or right).
Locomotor	
Stationary	Octopus not moving.
Threaten	Octopus lunges at conspecific but does not attack.
Flee	Octopus moves away from conspecific via crawling or jetting.
Attack	Octopus launches self at conspecific, forward rush. Typically jet propulsion.
Grappling	Octopuses entangled in each other's arms, reaching/biting/grabbing.
Chase	Octopus pursues conspecific via crawling or jetting.
Approach	Octopus approaches conspecific via crawling or jetting.
Inking Present/Absent	Ink.

Figure 1. Individual octopuses were housed in enclosed, opaque plastic containers (36 cm x 23 cm x 28 cm) with constant flowing ambient seawater via a manifold system (Chase and Verde 2011). Rocks were placed on top of the containers to prevent octopuses from escaping. Containers were maintained in seawater raceways (231 cm x 29 cm x 24 cm) to maintain a constant temperature of 12°C (Perron and Verde 2015). Photo by Alan Verde.

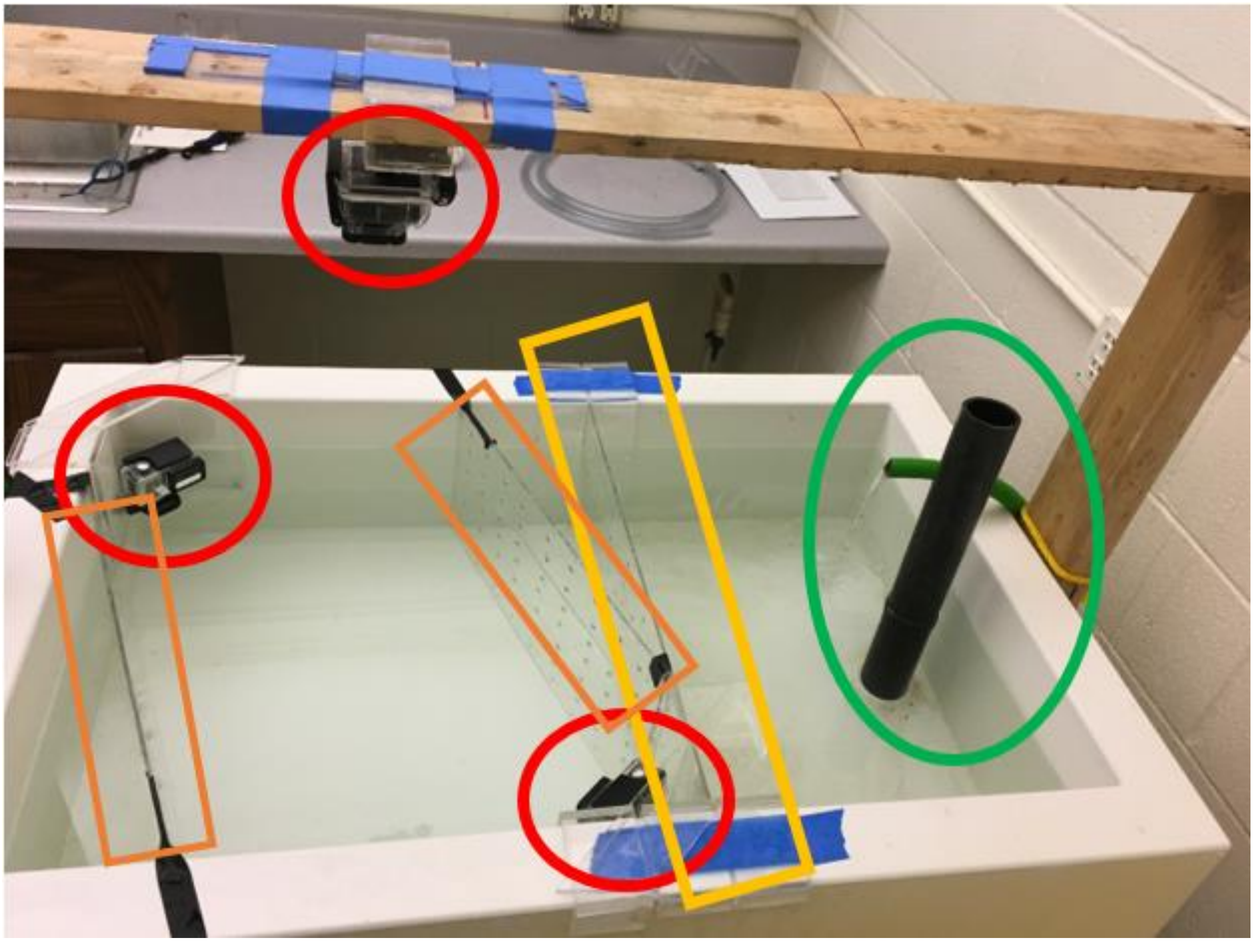


Figure 2. Flow-through seawater observation tank (80 cm x 50 cm x 24 cm) outfitted with three GoPro cameras (circled in red). Tank was divided by a piece of plexiglass (yellow box) with holes drilled in it to buffer the rippling effect of the seawater in/outflow (green circle). Only half of the tank (46 cm x 50 cm x 24 cm) was used as the testing area for the octopuses. To eliminate blind spots on the corner cameras in the tank, two plexiglass dividers were cut and angled width-wise along the tank walls to narrow the space (orange boxes).

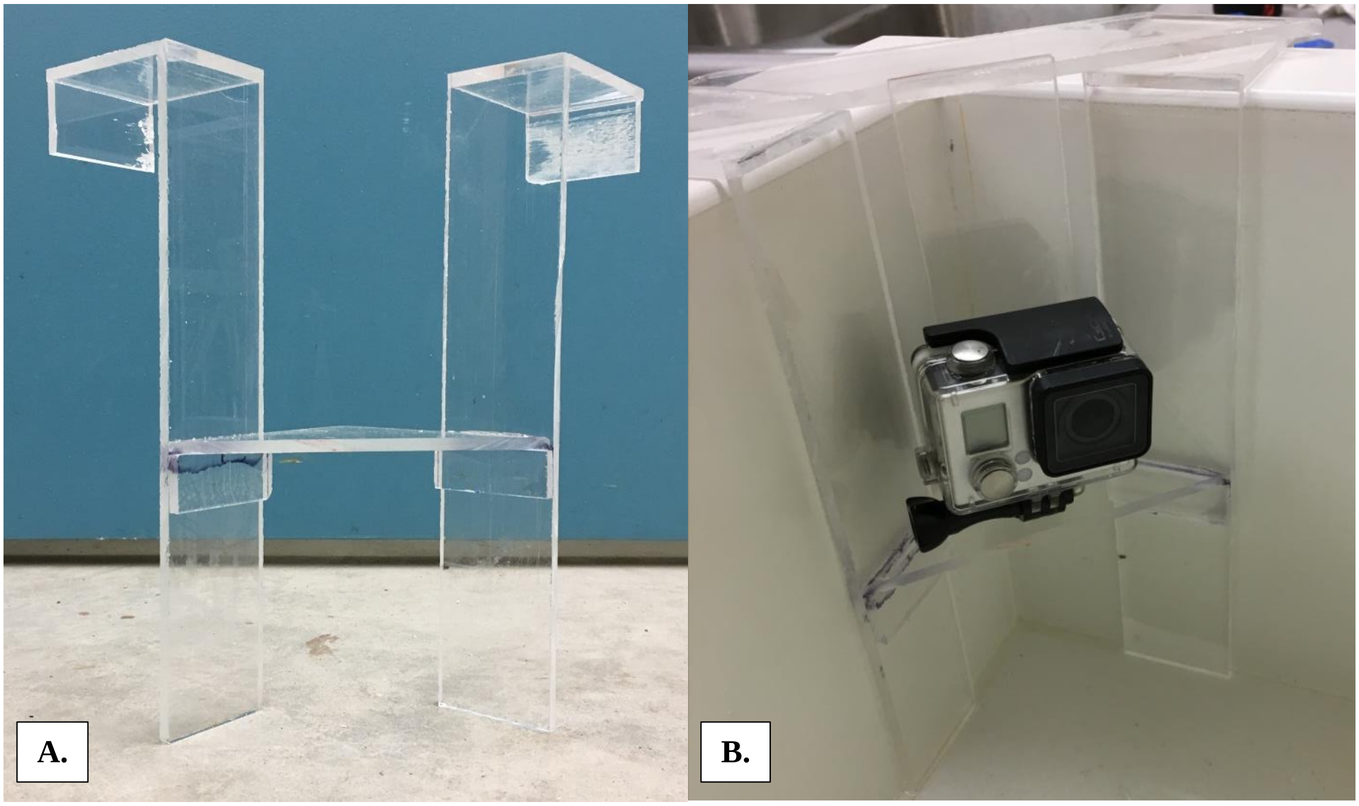


Figure 3. Example of plexiglass frames (A.) that secured cameras (B.) to the corners of observation tank.

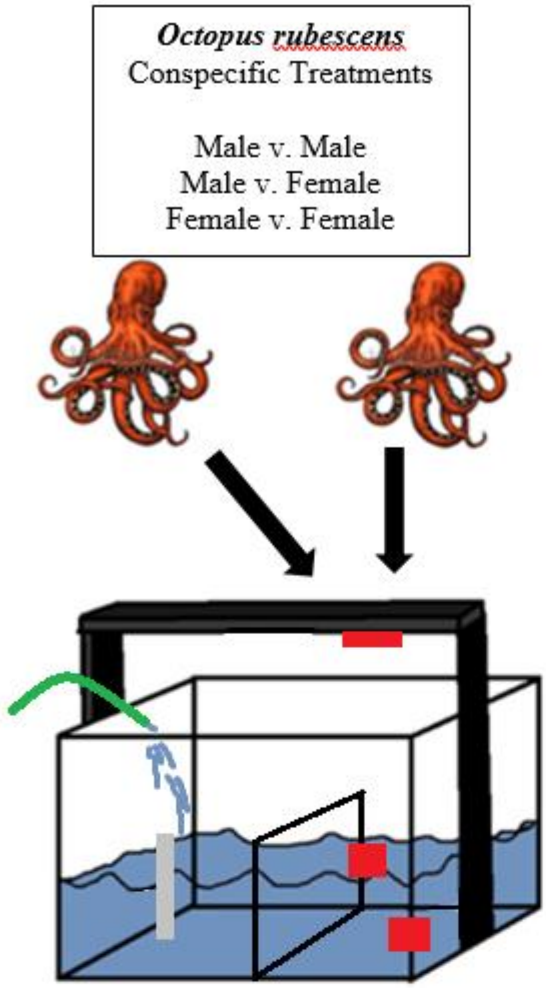


Figure 4. Each octopus (in a set of ten octopuses) was allowed to interact with all other octopuses of the same and opposing sex within each set. Treatments included: Male/Male, Male/Female, and Female/Female. Octopuses were observed via GoPro cameras (red boxes) in an observation tank. The tank was divided by a piece of plexiglass (black box in center of tank) to buffer the rippling effect of the seawater in/outflow (green hose, gray pipe).

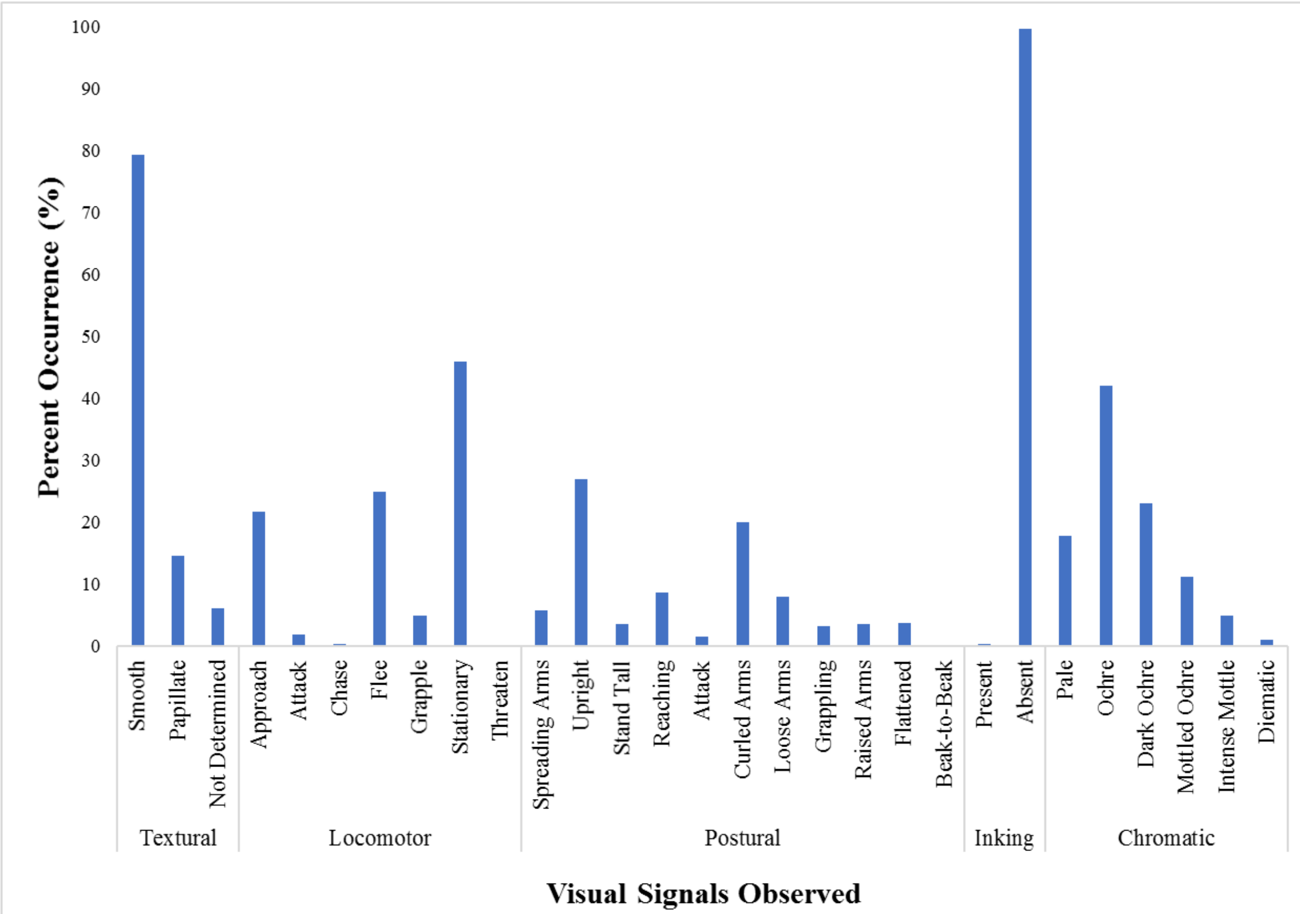


Figure 5. The percent occurrence of the most common visual signals displayed by *Octopus rubescens* during interactions with conspecifics in an observation tank ($n_{male} = n_{female} = 5$). The five categories of signals include textural, locomotor, postural, inking, and chromatic which all have a variety of subcategories.

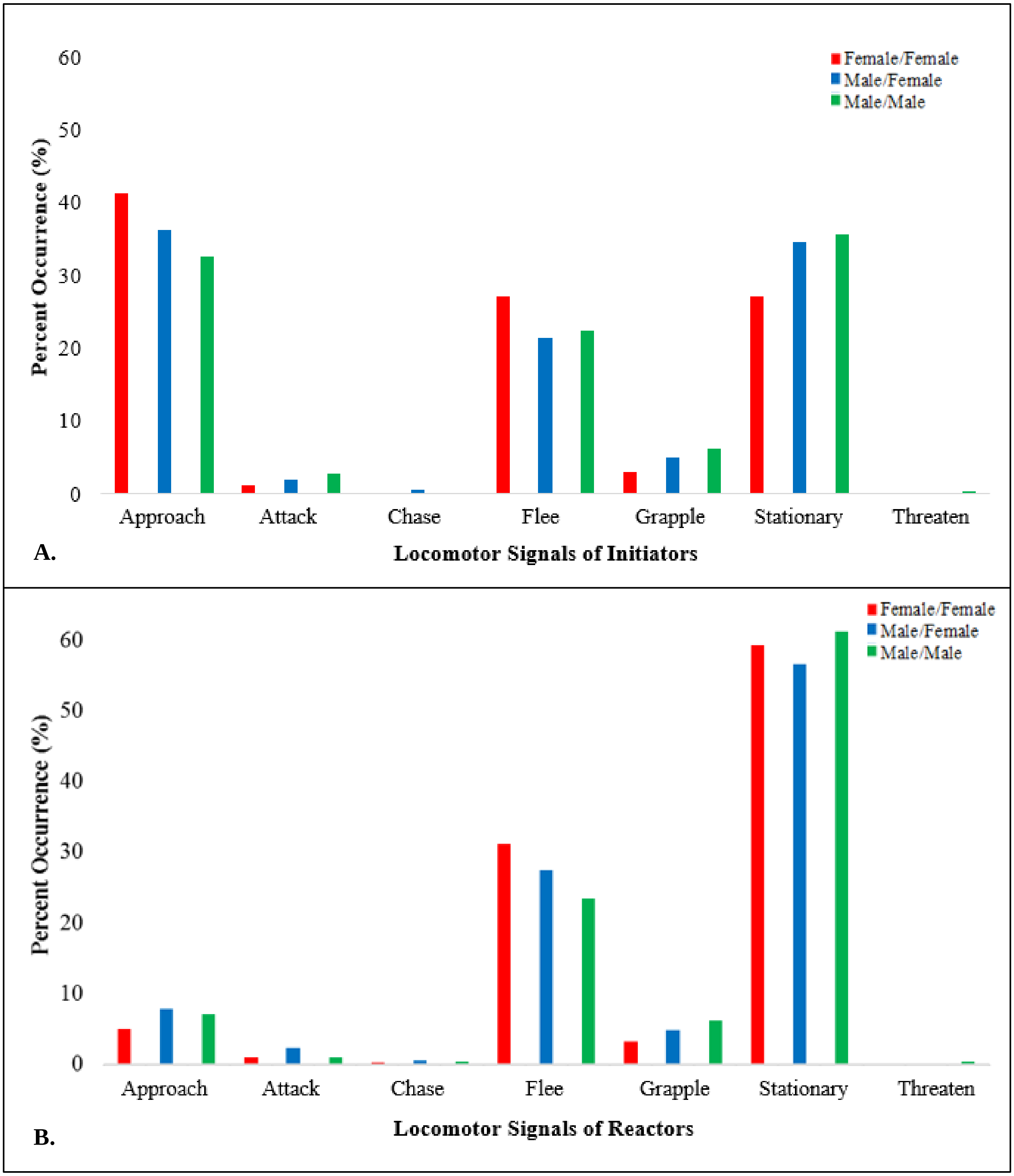


Figure 6. The most common locomotor signals used by initiators (A.) and reactors (B.) of an interaction. *Octopus rubescens* were allowed to interact with conspecifics in Male/Male, Male/Female, and Female/Female pairs in an observation tank ($n_{male} = n_{female} = 5$).

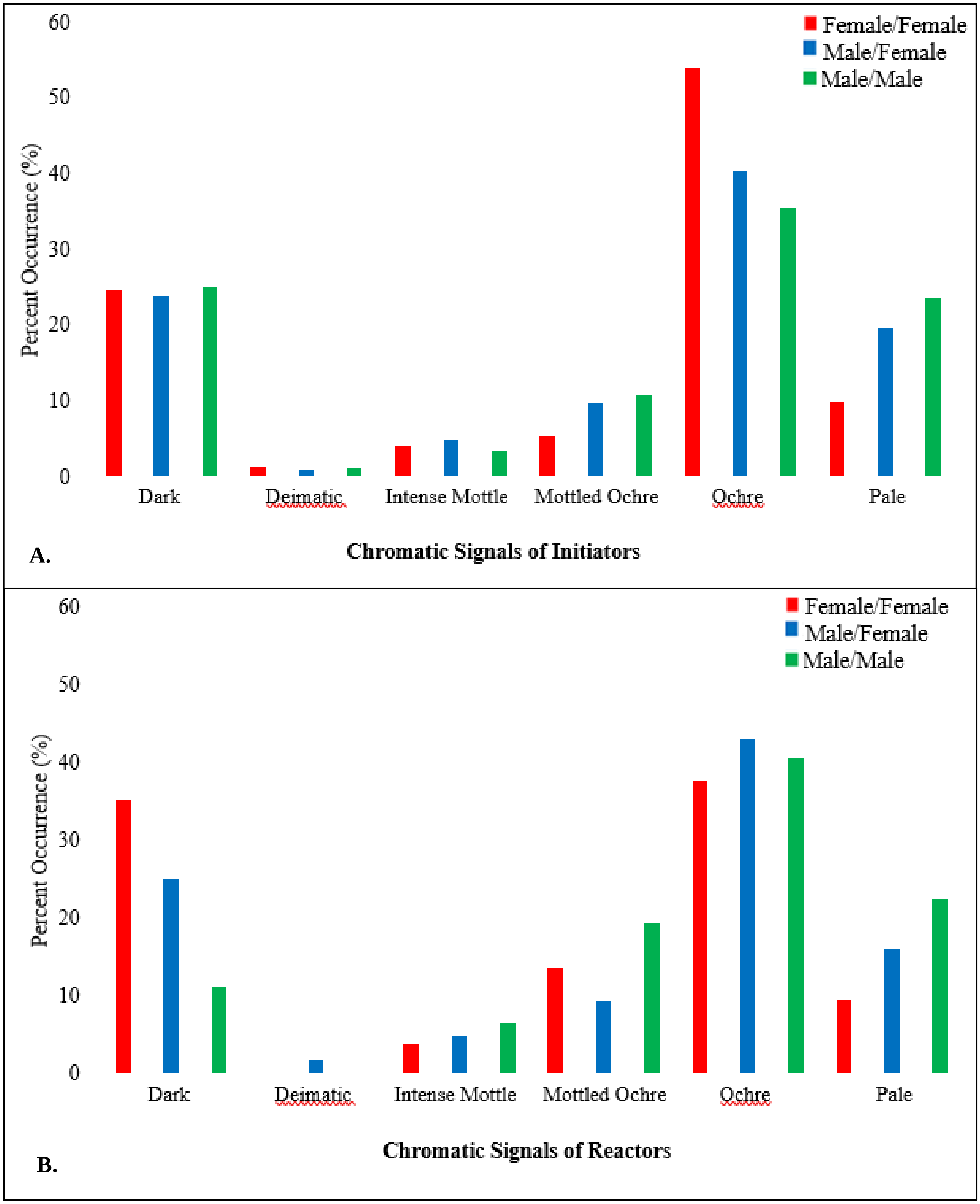


Figure 7. The most common full-body chromatic signals used by initiators (A.) and reactors (B.) of an interaction. *Octopus rubescens* were allowed to interact with conspecifics in Male/Male, Male/Female, and Female/Female pairs in an observation tank ($n_{male} = n_{female} = 5$).

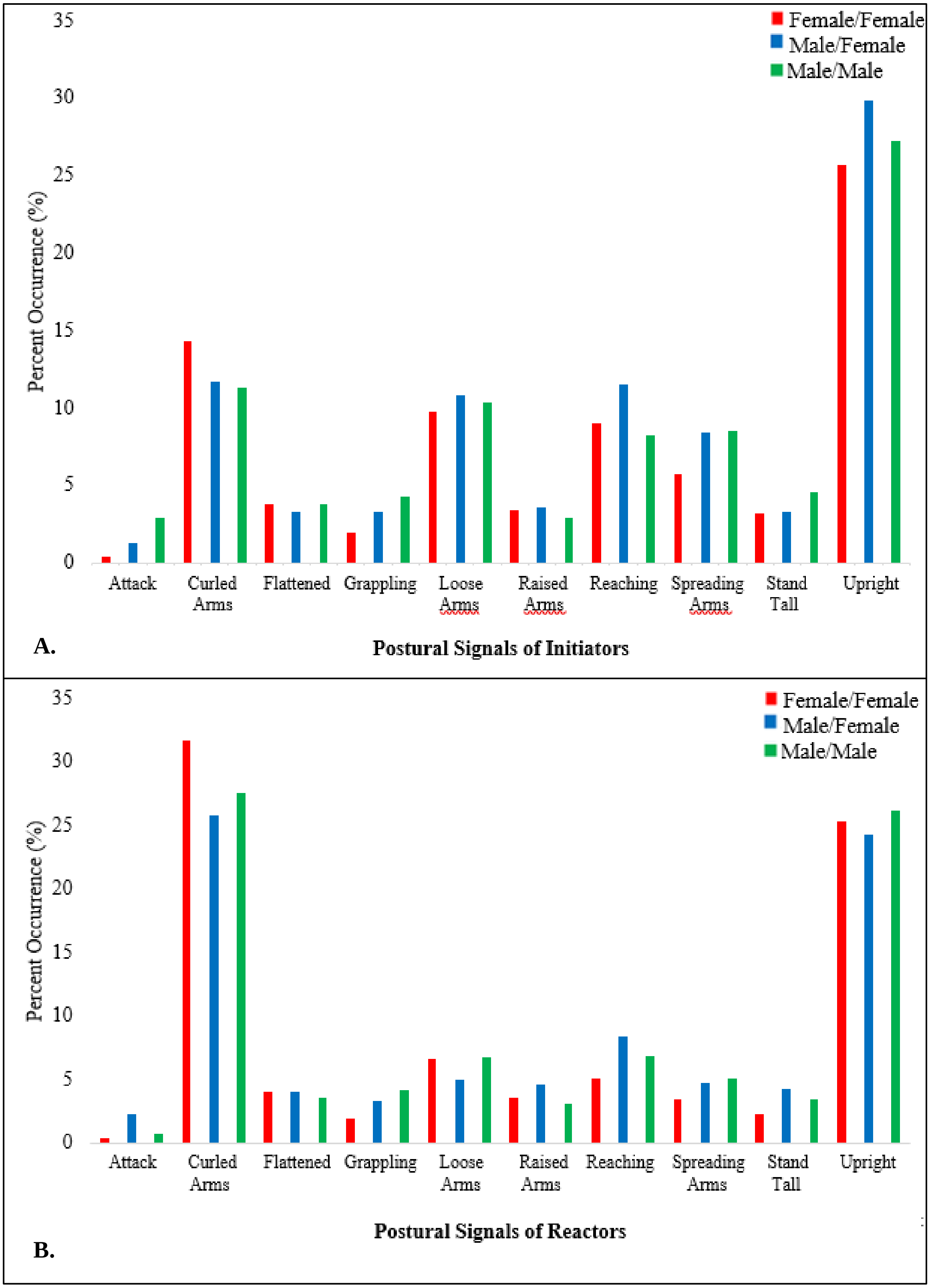


Figure 8. The most common postural signals used by initiators (A.) and reactors (B.) of an interaction. *Octopus rubescens* were allowed to interact with conspecifics in Male/Male, Male/Female, and Female/Female pairs in an observation tank ($n_{male} = n_{female} = 5$).

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Appendix

Table 1. Settings used on the GoPro cameras to capture clear videos of octopuses in high-light situations (GoPro Hero 3 Black Edition User Manual 2018; GoPro Hero 3+ Silver Edition User Manual 2018). Cameras were placed at different locations in an observation tank to record visual signals displayed by octopuses during conspecific interactions.

	GoPro Hero 3	**GoPro Hero 3+**
Pixels	1080	1440
Frames per Second	30	30
Protune	On	On
White Balance	Auto	Auto
Field of View	Wide	Wide

For details about the scale used to weigh octopuses in this study please refer to:

Operating Instructions – Mettler Toledo: Line of Classic Light Balances [Internet]. 2007. Greifensee, Switzerland: Mettler-Toledo AG, Laboratory & Weighing Technologies; [cited 2018 Oct 12]. Available from: https://www.usf.edu/research-innovation/rf/usf-connect/documents/mt-pl652s-balance.pdf