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UNIVERSITY OF CALIFORNIA
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ARMED AND DANGEROUS:
PATTERNS AND DRIVERS OF OCTOPUS ARM LOSS

A dissertation submitted in partial satisfaction
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

ECOLOGY AND EVOLUTIONARY BIOLOGY

by

Kelley Marie Voss

June 2022

The Dissertation of Kelley Marie Voss
is approved:

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Vice Provost and Dean of Graduate Studies

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Negative data values in these graphs are a result of the truncated arm being longer than the predicted (scaling-based) or estimated (in situ) length of the individual arm. High r² values in all eight relationships support the idea that *in situ* estimates of arm length are sufficient to estimate biomass sublethally contributed to the food web by octopuses. While scaling-based volume calculations may be slightly more accurate, they are not necessary to estimate biomass loss for species that are rare or not well-represented in museum collections. Part B shows mostly strong relationships between ISI and in situ volume missing for each species; *O. californicus* and *E. dofleini* had much lower ISI scores than the other six species. This may be due to ecological conditions: *O. californicus* lives at greater depths than the others, and *E. dofleini* is orders of magnitude larger than the rest of the species in this study.

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ABSTRACT

ARMED AND DANGEROUS:

PATTERNS AND DRIVERS OF OCTOPUS ARM LOSS

Kelley M. Voss

Prey organisms have developed a variety of physiological and behavioral

strategies to avoid, or mitigate, predation. Some are capable of regenerating tissue

after sublethal predation, the non-fatal removal and consumption of a body part;

however, the events leading up to arm loss in cryptic organisms such as octopuses are

poorly understood. Octopuses have eight multifunctional, regenerative arms used to

explore, handle objects, and presumably, defend against predators. In this

dissertation, I explored the patterns of arm loss experienced by octopuses across

species and ontogeny, as well as behaviors that put these arms at the highest risk. In

Chapter 1, I observed injured arms, calculating scaling relationships specific to the

arms of three sympatric *Octopus* species to calculate the proportion of arm truncation.

Arm injuries in these species were frequent and asymmetrical, and that when injured,

octopus on average lost about one-third of an arm. These injuries were biased to the

anterior left arms, L1 and L2. However, different measures of injury supported very

different conclusions. Therefore, in Chapter 2, I created and demonstrated the utility

of an Injury Severity Index (ISI) to describe the magnitude of appendage injury in

invertebrates, using counts and proportions of arm losses collected from octopuses in

museums and the field. Museum specimens had most commonly lost part of one arm,

with a mode ISI value of less than 2.00. Wild *O. bimaculatus* had a mean ISI value of

3.13, representing two to three arm injuries. I also calculated volume of lost tissue

based on the shape of a cone to further enhance quantifications of arm loss. In

Chapter 3, I describe the ways in which *O. bimaculatus* used their arms in self-

defense against a common predator (*Gymnothorax mordax*). Octopuses and morays

were observed interacting freely in tanks. Suites of antipredator strategies varied

greatly by sex, leading to greater success for males avoiding an attack. Individual arm

use was biased toward arm L1 and neighboring arms, and predatory bites were

concentrated on L2 and its neighbors. This dissertation revealed octopus arm use and

loss from high-risk contexts, with a bias toward anterior-left arms across species.

DEDICATION

To the aspiring researcher who started this work,

To the aspiring educator she has become,

And to her family and friends.

We made it.

ACKNOWLEDGEMENTS

"A great experiment takes great determination: the will to do the work, and then the wisdom to keep refining, keep tinkering, keep perfecting. The same determination is being realized in America today. I see it in the scientists who are transforming the future. I see it in the parents who are nurturing generations to come; in the innovators and the educators, and everyone everywhere who is building a better life for themselves, their families, and their communities. This, too, is American aspiration."
Vice President Kamala Harris, Inauguration Day 2021

As I said in my defense seminar, it takes a village to raise a doctor. I could have written an entire dissertation document dedicated to describing all of the people who made the research described here possible. This section will be woefully incomprehensive, however, and I've done my best to at least thank most of the VIPs. The primary people who kept me afloat through this process are my family: My spouse, Eryn Strohm-Voss, has been my sun and stars for just about my entire time in Santa Cruz. Thank you for keeping Soft and me fed, clean, clothed, and housed when I was not physically, financially, or mentally able to. I cannot believe I get to watch you become even more "you" for the rest of our lives. Thank you also to my parents, Joan Jackson and Chris Voss, for everything, from combining your genes to make me, to giving me step-parents and siblings, to patiently supporting me through 27th grade (I'm really, really done this time). I also thank my siblings, uncles, aunts, cousins, and others who are practically family for being my cheerleaders from near and far. Thank you also to all my in-laws, especially Leslie and Mike: I am so lucky to be a part of your family. To our nephew, Elliot Blakesley: You can do great things in this world, and Auntie Kelley will always love and help you.

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Here, I would like to remember some important people who were lost during my time at UCSC: my grandmother Joanna Voss, my grandmother-in-law Lelah Crow, and my dear friends Amanda Robles and Glenn Edgar Martin. Having a secret octopus handshake with Barry Sinervo was a highlight of my time in this department, and his wife Jeanie has also been a gift to get to know in his absence. I would like to remember research titans Ron O'Dor for his dedication to cephalopod physiology and behavior, and Nancy Voss for her decades of tireless devotion to building and maintaining cephalopod museum collections (and her great choice in last names).

I am deeply grateful to the invertebrate collections managers who so kindly facilitated days and days of museum specimen data collection, especially Elizabeth Kools and Terry Gosliner at the California Academy of Sciences, Paul Valentich-Scott at the Santa Barbara Museum of Natural History, and Charlotte Seid at the

The text of this dissertation includes reprints of the following previously

published material:

Chapter 1

Voss, K.M., Mehta, R.S. 2021. Asymmetry in the frequency and proportion of arm truncation in three sympatric California *Octopus* species. *Zoology* **147**:125940

For this chapter, I was responsible for the study design, data collection, the majority

of data analysis, statistical analyses, manuscript preparation, and manuscript

submission. The co-author in this publication, R. Mehta, directed and supervised the

research that forms the basis for the dissertation.

INTRODUCTION

Predator-prey interactions are well known to determine population dynamics, shape community structure, and drive species adaptations, including both the lethal and non-lethal effects of predators on prey species in a system (Lima, 1998). Non-lethal effects of predators include intimidating and injuring potential prey, which can further alter the distribution, behavior, and fitness of prey species. Some prey species have evolved ways to overcome injuries that would otherwise be catastrophic such as the loss of limbs or other body tissue from interactions with predators. These incidences, which include amputation and autotomy are known as sublethal predation, trait-mediated effects, or selective predation (Ballengee & Sessions, 2009). An amputation is an externally induced injury that severs a body part from the animal (Bely & Nyberg, 2010), while autotomy is internally induced (Fleming, Muller, & Bateman, 2007). Autotomy is a secondary defense behavior induced by contact with a predator that employs the disembodied limb to distract the predator long enough for the prey to escape (Fleming et al., 2007; Maginnis, 2006). Following limb loss, the capacity for, and manner of, appendage regrowth varies greatly between taxa; some organisms replace a limb from a bud that regenerates the proximal part first (e.g. salamander limbs (Whited & Tabin, 2009), sea star arms (Mladenov, Bisgrove, Asotra, & Burke, 1989), and others regenerate the distal tip first and then lengthen the regrowth from the truncation site (e.g. lizard tails, octopus arms). The tradeoffs between regenerating limbs and the difficulty of making a living during the

regeneration period prompted me to investigate this phenomenon in a taxonomic group that possesses multiple redundant, regenerative limbs.

The variety of predators in marine communities has helped to drive the development of many traits in coleoid cephalopod taxa, including a crown of eight hyper-redundant (Gutfreund et al., 1996), regenerative arms.   The extreme flexibility and dexterity of the cephalopod arm enable octopuses to employ many behaviors related to self-defense, hunting, reproduction, object manipulation, grooming (Packard & Sanders, 1971), locomotion (Huffard, Boneka, & Full, 2005; Levy, Flash, & Hochner, 2015), and exploration (Byrne, Kuba, Meisel, Griebel, Mather, et al., 2006; J. a. Mather & Anderson, 1999), each with some risk of injury. For example, octopuses grappling with a predator or competitor sometimes lose one or more arms, either to amputation or autotomy. The impact of any type of arm loss is an interesting and understudied topic, especially considering sex-related differences, as males have a specialized reproductive arm located at position R3. If R3 is lost, it can take months to regrow (Féral, 1977; Imperadore & Fiorito, 2018), representing a significant portion of the 1-2 year life span common in most octopus species. Thus, the loss of an arm can have a negative effect on reproductive success. In this dissertation, I used octopuses as a model to explore patterns of arm loss due to sublethal predation. I used morphological and behavioral approaches to investigate ontogenetic variations in arm length, the frequency of arm loss, and how octopuses may protect or sacrifice their arms when interacting with a common predator.

In my first chapter, I investigated the relationship between arm scaling patterns and frequency of arm loss in three Southern Californian octopus species: *Octopus bimaculatus*, *Octopus bimaculoides*, and *Octopus rubescens*. The lack of information on octopus arm loss patterns across benthic octopus species motivated me to assess arm truncation using museum collections. Museum specimens allowed me to collect uniform and repeatable data that are otherwise difficult to collect accurately from live octopuses. In addition to counting the frequency of arm injuries, I determined scaling relationships from pristine arms and then used these to approximate intact lengths of the injured arms. In addition, I examined the frequency and proportion of arm truncation, with respect to the location of the arm on the body to look for bias in injury patterns. My data set also allowed me to examine arm loss across ontogeny.

While the number of truncated arms in an individual is the most common metric of appendage loss reported in the literature (Voight, 1992; Wada, 2017), it does not provide a holistic understanding of the severity of injury in an individual octopus. Simple counts of truncated arms may misrepresent the severity of the injury if only the tip of one or more arms is missing; therefore, knowing the proportion of length lost is also critical to more fully understanding arm injuries. While I reported the number and proportion of loss as if they are mutually exclusive in Chapter 1, in Chapter 2, I combine these data into an index that describes the magnitude of arm tissue lost by an individual. This second study takes morphological data from preserved museum specimens of eight species Northeastern Pacific octopuses,

including those from Chapter 1, and presents a case for implementing a

morphological index, which I refer to as the injury severity index (ISI), to holistically

describe arm injury by combining the number of arms that have lost tissue with the

proportion of the length of arm tissue lost. I used ISI to describe the magnitude of arm

loss in the museum specimens, which were indigenous to the Northeastern Pacific

that vary greatly in size and life history. I also used empirical field data collected

from live *O. bimaculatus* to demonstrate how to incorporate tissue loss into long-term

survey data.

Chapter 3 was designed to examine whether the patterns of injury I revealed

in Chapters 1 and 2 are consistent with antipredator behaviors observed in live

octopuses. I brought *O. bimaculatus* into a controlled environment at the USC

Wrigley marine station on Santa Catalina Island to observe their arm use in an

encounter with one of their natural predators, the California moray eel (*Gymnothorax*

mordax). I analyzed data from 37 octopus-moray trials using principal component

analyses to identify suites of antipredator behaviors, accounting for how sex, body

size, and incidences of bites may have affected behavioral responses. I then used

circular analyses to determine whether there is a bias in arm use as well as a bias in

the location of moray bites.

CHAPTER 1

ASYMMETRY IN THE FREQUENCY AND PROPORTION OF ARM

TRUNCATION IN THREE SYMPATRIC CALIFORNIA *OCTOPUS* SPECIES

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Invited Perspective

Asymmetry in the frequency and proportion of arm truncation in three sympatric California *Octopus* species

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A B S T R A C T

Octopuses have eight radially symmetrical arms that surround the base of a bilaterally symmetrical body. These numerous appendages, which explore the environment, handle food, and defend the animal against predators, are highly susceptible to truncation or loss. Here, we used scaling relationships specific to the arms of three sympatric octopus species of the genus *Octopus*, to calculate the proportion of arm truncation. We then compared the frequency and proportion of arm losses between different body locations. Truncated arms were found in 59.8 % of specimens examined, with individuals bearing one to as many as seven injured arms. We found a significant left side bias for greater proportion of arm truncation for all species and sexes except in *O. bimaculatus* males. We also found that sister species *O. bimaculatus* and *O. bimaculoides* had a greater proportion of their anterior arms (pairs 1 and 2) truncated, while in *O. rubescens*, posterior arms (pairs 3 and 4) were more truncated. The mean percent of arm that was truncated was 28.1 % overall but varied between species and by sex and was highest in *O. rubescens* females (56 %). The arms of *O. rubescens* also exhibited the steepest scaling patterns, and showed a positive correlation between body size and number of truncated arms. Overall, we show that arm injuries in our sampling of three intertidal species are frequent and asymmetrical, and that when injured, octopus on average lose a considerable proportion of their arm. Through quantifying the variation in arm truncation, this study provides a new foundation to explore behavioral compensation for arm loss in cephalopods.

1. Introduction

Agonistic interactions with competitors, predators, or prey regularly lead to the catastrophic injury of appendages such as limbs and tails. These events, termed sublethal predation (Lawrence and Vasquez, 1996; Bely and Nyberg, 2010), can induce the body part to separate from the rest of the animal, whether by amputation, the severing of a body part by another organism, or by autotomy, or self-amputation, typically at a predictable breakage plane (Bely and Nyberg, 2010). Unsurprisingly, much interest has been placed on the regeneration of amputated and autotomized body parts in both invertebrate and vertebrate taxa, as well as the ecological and evolutionary factors that shape regeneration (Ballengee and Sessions, 2009; Bely and Nyberg, 2010). Fewer studies have examined the variation in tissue loss resulting from sublethal attacks by other organisms, which has the ability to affect the injured animal's behavior (Alupay, 2013; Emberts et al., 2017), and potentially its reproductive success (Smith, 1992; Wada, 2017). Quantifying variation in the severity of limb loss provides a stronger foundation for studying the effects of agonistic interactions on later activities across functional contexts like foraging, locomotion, defense, and reproduction.

Perhaps the most behaviorally complex marine invertebrates to lose limbs are the cephalopods. Coleoid cephalopods, which include cuttlefish, squid, and octopus, are cryptic marine mesopredators known to lose their eight to ten appendages via sublethal predation or autotomy to escape predation (Bush, 2012; Alupay, 2013; Hanlon and Messenger, 2018). Octopuses are the only coleoid cephalopods without tentacles, meaning that they depend solely on their multifunctional arms to capture and consume prey. Their eight arms are arranged in a radially symmetrical crown at the base of their bilaterally symmetrical bodies. Each arm is a muscular hydrostat appendage, with three muscle layers facilitating an extremely high degree of flexibility (Kier, 2016), and hundreds of strong, individually controlled suckers along the entire oral surface. Octopuses are a fascinating model for investigating arm loss because they have multiple redundant arms that eventually regrow a fully-functional copy after the arm is separated from the body (Imperadore and Fiorito, 2018), which are extremely helpful adaptations to compensate for, and recover from, an arm injury. Losing arms may put

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them at a disadvantage for success across varied functional contexts during the time it takes for the arms to regrow, which can take at least three months to regain full functionality in stage 6 of the regrowth process (Lange, 1920; Féral, 1977, 1978, 1979, 1988; Imperadore and Fiorito, 2018; Zullo and Imperadore, 2019), so the ability to recover behaviorally and physically are crucial to survival. To better understand the behavioral impact of arm loss, it is important to first investigate how patterns of arm loss are related to the morphological characteristics of octopus arms.

There is evidence that octopus do not use their arms equivocally in a given context. Particular arms may be recruited for a variety of behaviors, including but not limited to locomotion (Huffard et al., 2005; Huffard, 2006), object manipulation (Sumbre et al., 2006; Grasso, 2009; Mazzolai et al., 2013), and even chemosensing by bending the arm to bring the surface of the suckers to an object or substrate (Kier, 2002; Grasso and Wells, 2016; Kennedy et al., 2020). Some octopuses have been shown to recruit radially neighboring arms (e.g. L2 and L3) rather than bilaterally opposing arms (e.g. L2 and R2) when multiple arms are necessary to complete tasks (Mather, 1998). Mather (1998) proposed that octopus may have a division of labor between their anterior and posterior arms. A laboratory study using O. vulgaris showed that individuals had a clear preference for using anterior arms to reach out to touch objects (Byrne et al., 2006a). More recently, the distribution of arm movements in O. bimaculoides showed bias towards the anterior arms, which appeared to be preferred for exploration and manipulation (Kennedy et al., 2020), and seven of eight incidences of Octopus cyanea punching at fish while hunting were delivered by anterior arms (Sampaio et al., 2020). Observations in the field suggested that Paroctopus digueti (formerly genus Octopus) are more likely to lose their anterior, or frontal, arms (Voight, 1992). Combined, these studies and observations strongly suggest that octopuses vary in their arm recruitment patterns depending on the functional context, e.g. feeding, exploration, reproduction, and locomotion, and are thereby putting certain arms at greater risk of truncation. Additionally, octopus growth and body size vary inter- and intraspecifically (Domain et al., 2000; Semmens et al., 2004; Villanueva and Norman, 2008); interspecific differences in arm length are apparent between similarly sized individuals (Roper and Voss, 1983). Therefore, information on inter- and intraspecific variation in length across the eight arms of octopuses could shed some interesting insight on differential arm use and loss.

In the wild, octopuses regularly experience agonistic encounters that result in arm amputations. Octopuses are preyed upon by marine mammals (Lowry et al., 1991; Goodman-Lowe, 1998; Santos et al., 2001) as well as conspecifics and sympatric octopus species (Hanlon and Forsythe, 2008; Ibáñez and Keyl, 2010; Hernández-Urcera et al., 2014), but predatory encounters with fish seem most likely to result in arm loss. In Southern California, octopus are preyed upon by a variety of common benthic and reef-associated fish species, including but not limited to multiple species of sculpins, rockfishes, and flatfishes; rubberlip surfperch (Rhacochilus toxotes), barred sand bass (Paralabrax nebulifer), and the sarcastic fringehead (Neoclinus blanchardi) (Feder et al., 1974). The California moray eel (Gymnothorax mordax) is known to pull off octopus arms as a predation strategy (MacGintie and MacGintie, 1949) and recently, a multiyear study showed that whole octopuses and octopus arms were commonly retrieved from their stomachs (Higgins et al., 2018). Therefore, through these instances of sublethal predation, octopuses contribute widely to the food web in marine communities while also continuing their role as mesopredators.

Octopuses almost immediately begin to regenerate the tips of the arms they have lost in predatory encounters (Lange, 1920; Fossati et al., 2013, 2015), but despite regeneration, an individual octopus is presumably at a disadvantage while the arms regrow. While octopuses continue to grow up until the end of their lives (Forsythe and Hanlon, 1988), energy must be diverted from somatic growth and maintenance towards arm regrowth. Arm loss can also have fitness costs beyond energetic demands: Abdopus sp. males possessing fewer intact arms lose

mating opportunities to more intact males (Wada, 2017). The proportion of arm truncated will be a factor in arm regrowth and behavioral success across other functional contexts such as exploration, feeding and self-defense.

The paucity of information on octopus arm loss patterns across benthic octopus species motivated us to assess the frequency and proportion of arm truncation using museum collections. Measuring preserved museum specimens provided an excellent opportunity to collect uniform and repeatable data that are otherwise difficult to collect accurately with live animals. In addition to tallying the frequency of arm injuries, we used scaling relationships from uninjured arms (Table S2) to approximate intact lengths of the injured arms, enabling us to examine the proportion of arm truncation with respect to the location of the arm on the body. We collected data from specimens of Octopus bimaculatus (VERRILL 1883), Octopus bimaculoides (PICKFORD & MCCONNAUGHEY 1949), and Octopus rubescens (BERRY 1953). These three species differ in their maximum body size and inhabit overlapping benthic communities along Southern California and the Baja California peninsula. Comparing arm loss characteristics across ontogeny for ecologically similar species enables us to determine whether body size had an effect on the frequency and proportion of arm loss. We hypothesized that amputation patterns would vary with body size, with larger individuals incurring more injured arms and a higher proportion of arm loss compared to smaller individuals that may be more cryptic. For the males' hectocotylized arm, R3, which they hold close to their body while foraging to prevent potential injury (Imperadore and Fiorito, 2018), we hypothesized that there would be sex-related differences in addition to interspecific differences in arm loss patterns. This is supported by studies showing a variety of cephalopod species had a lower incidence of injury to the hectocotylized arm (Steenstrup, 1857; Bello, 1995). We predicted that males would show a bias for missing arms toward their left side, presuming that males use the left arms while engaging in risky behavior or predatory defense in order to further prevent R3 from being injured. We also hypothesized that for all octopuses, the anterior arms, arm pairs 1 and 2, would incur a higher number of losses, and that the length of an individual arm lost would also be greater in anterior arms compared with posterior arms.

2. Methods

2.1. Study Species

To examine arm injuries, we selected three benthic species of the genus Octopus that partially overlap in range and habitat use. Octopus rubescens inhabits tide pool, rocky reef, and mudflat habitat ranging from British Columbia down into the Baja California peninsula and into the Channel Islands (Jereb et al., 2016). Octopus bimaculatus and O. bimaculoides are sympatric sister species that are found in California south of Point Conception and into Baja California, including the Channel Islands (Pickford and McConnaughey, 1949). Though they may be found in either habitat type, O. bimaculatus often inhabits rocky reef substrate, and O. bimaculoides is often found in mud and sand flats (Lang et al., 1989). All three species have a maximum age range of one to two years (Lang et al., 1989; Jereb et al., 2016). We presume these species could incur a similar risk of arm loss due to encountering similar predator and prey communities in the parts of their ranges where they do overlap.

2.2. Data collection

All specimens were obtained from the California Academy of Sciences (CAS) and the Santa Barbara Museum of Natural History (SBMNH); see Supplementary Table 1, available online, for accession numbers. Preserved specimens were formalin-fixed and then stored in ethanol. Where metadata were available, specimens were collected by hand in the intertidal, by hand on SCUBA, and in research trawls and

seines. We only used specimens that were previously identified to species (Table S1).

We collected external measurements of 199 preserved octopuses as a non-destructive method of studying these specimens. Interocular distance (IO), the distance between eye openings across the top of the head, and mantle length (ML), the distance from the tip of the mantle to the top of the head, were measured with an analog dial caliper to the nearest mm. We used IO instead of ML as a measure of body size for our analyses (Roper and Voss, 1983), due to the high degree of variability we observed in mantle shape and condition from preservation and in specimens that had undergone previous dissections (K. Voss, pers. obs.). The semi-rigid nature of the cephalic cartilage protecting the brain maintains head shape, allowing for a more standardized measurement using IO. We also opted not to use wet weight as a measure of body size in order to standardize this measurement between pristine specimens and those missing arms or those whose internal organs had previously been removed. In addition to measuring body size, arm lengths were each measured once, from the edge of the mouth to the arm tip, using a flexible 150 cm tape measure. The end of the tape measure was held against the edge of the mouth and pressed along the side of the arm bearing suction cups, extending the arm as straight as was possible. Preserved octopus specimens are more rigid than live animals, but generally flexible enough to straighten arms along a tape measure.

We aimed to measure an ontogenetic series of at least 30 male and 30 female specimens of each species that encompassed their respective maximum reported body sizes (Pickford and McConnaughey, 1949; Jereb et al., 2016). The sex of each octopus was determined by observing the presence or absence of a hectocotylus at the tip of the third right arm (R3; see Fig. 1), which is the location of the specialized reproductive arm in males of all three species. R3 becomes morphologically distinct as the males of these species grow, achieving 70-90 % of the length of the other arms at maturity (Pickford and McConnaughey, 1949; Jereb et al., 2016). When R3 was missing in a specimen, if the sex had not been previously noted in the metadata, either by identification at collection or in a previous dissection, the sex of the animal was classified as unknown and not included in this study (n = 2 O. bimaculatus, 13 O. rubescens). We included 62 Octopus bimaculatus (32 females, 30 males; 9-150 mm ML, 5-55 mm IO, mean size 46 mm ML/21 mm IO), 64 Octopus bimaculoides (33 females, 31 males; 7.5-97 mm ML, 5-42 mm IO, mean size 40 mm ML/19 mm IO), and 73 Octopus rubescens (37 females, 36 males; 18-68 mm ML, 10-26 mm IO, mean size 35 mm ML/17 mm IO).

Octopus arms are numbered in pairs along the bilateral axis of symmetry, with arm pair 1 at the anterior end, and pair 4 at the posterior (Fig. 1). The anterior and posterior ends of an octopus have been defined in different ways for taxonomic and behavioral studies. For the purpose of this study, we designate arm pair 1 most anterior, and arm pair 4, located under the mantle, most posterior, as if the octopus is crawling (Norman et al., 1997; Kennedy et al., 2020), as this posture is common for a living animal and can help us understand the connection between

the location of an arm and its associated behaviors (Byrne et al., 2006b; Wells, 1978).

In this study, an arm was considered truncated if any length between the tip and the base had been removed but had healed over and was in some stage of regrowth before the octopus was preserved (Fig. 2). The length of these arms was measured in the same manner as pristine arms, from the oral edge to the truncation point. We did not include any arms that were damaged (e.g. crushed or dried out), or posthumously removed arms, in any of our analyses. It was evident when an arm was damaged or removed postmortem, because sometimes arms were crushed, flattened, or degraded, or there was no evidence of healing (i.e. puckered skin around the wound) or regrowth (Lange, 1920; Shaw et al., 2016).

2.3. Calculating frequency and proportion of truncation using scaling relationships

The two metrics we selected to represent arm injury were frequency and proportion of arm truncation. Frequency data consisted of counts of injured arms in each specimen, which we later grouped by sex and species. To calculate the proportion of arm that had been truncated, we needed to know the original length of the arm prior to truncation. To do this, we determined the scaling relationships of the length of each pristine arm with interocular distance (IO) over an ontogenetic series for each species and sex. We used IO as a measure of body size (Roper and Voss, 1983) after confirming that IO was positively correlated with mass for each of our three species ($R^2 = 0.3$-0.97). We aimed to examine as many pristine individuals, wholly intact and no injuries to any arm, of each species as possible. However, the high proportion of museum specimens with previously injured or posthumously sampled arms required us to include intact arms of individuals with injuries to assemble the necessary range of body sizes for each species. Since no prior studies have compared the effect of arm regeneration on the growth of other arms, we compared the slopes of the relationships between interocular distance and arm length for each intact arm of individuals with one or more injured arms to those of pristine octopuses and found that these groups exhibited arm scaling patterns that were not statistically different. Therefore, we included measurements of intact arms from individuals with injured arms in our scaling analyses to increase our size range in our data set. All data were natural log-transformed for statistical analyses. Standardized major axis (SMA) regressions between arm length and IO were calculated with 'sma' in the R package 'smatr' (Warton et al., 2012) for each sex within each species. The slopes of these scaling relationships are presented in Supplementary Fig. 1 and Supplementary Table 2, available online. Finally, we determined the approximate proportion of each arm that was lost by calculating an original length using the slopes and intercepts of the scaling relationships, subtracting the length of the truncated arm, and dividing the difference by the calculated original length. There were two
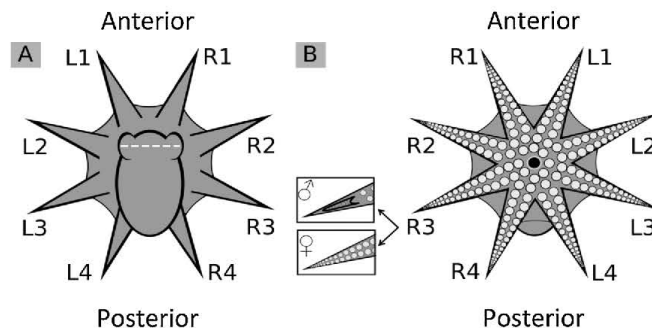
Fig. 1. Dorsal (A) and ventral (B) views of the octopus body. Arm pairs are numbered, 1-4, along the body's axis of bilateral symmetry, with a designation of "left" (L) or "right" (R) in relation to the dorsal view. The anterior and posterior ends discussed in this study are relative to the live animal, *sensu* Norman et al., 1997. In all three species in this study, the male hectocotylized arm is found in position R3 (see inset). Females have suction cups all the way to the distal arm tip, while males have a specialized tissue with a spoon-like groove at the arm tip used to pass sperm packets to females. Interocular distance (IO), the measure of body size in this study, is the distance between the two eyes, located on each side of the top of the head, and is denoted in part A by a white dashed line.

A

B

C
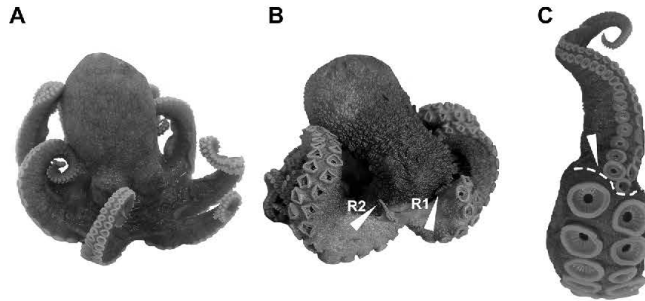
Fig. 2. Examples of uninjured and truncated arms in a museum specimen of *Octopus bimaculatus*. A. depicts an octopus with arms fully intact. White triangles point to the location of regrowth in panels B. and C. Panel B. shows early regrowth in two arms: the arm regrows starting with the tip, so a large size discrepancy is evident. These arms should have regained full functional capacity (Féral, 1977), at this stage. Panel C shows an arm in an advanced state of regrowth: the difference between arm and suction cup diameter on the original arm and the regrowth is evident, but this arm likely had full functionality at the time the animal was preserved.

occasions in *O. bimaculatus* which the original regrowth had been amputated and had regrown again in a stair-step effect; the loss was calculated from the oldest, most proximal truncation. There was one instance of an *O. bimaculoides* with a regrown arm ending in two equally long (3 mm) tips; the loss was calculated from the point where the arm split.

We examined the frequency and proportion of arm truncation for each individual and their location around the body to determine whether arm position corresponded with the severity of loss, and whether this varied by sex or species. Since the log-transformed data were not normally distributed, we used non-parametric Wilcoxon tests to determine differences between the proportion of arm loss between left and right arms, and anterior and posterior arms, within each sex and species. The tally of arms lost at each position (Table 1) were not normally distributed, so we used a Kruskal-Wallis rank sum test to determine interspecific differences in mean number of missing arms, and a Chi-squared test to determine overall differences in the numbers of missing arms across species. We used a Fisher's exact test to compare the number of missing arms between males and females of each species.

3. Results and Discussion

3.1. Frequency of arm loss

We found that 59.8 % (n = 119) of the 199 individuals in this museum study had at least one arm missing or in some state of regrowth before the specimen was euthanized (Table 1). The proportion of injured individuals varied when grouped by sex and species: between 42.4 % (14 out of 33 female *O. bimaculoides*) and 70.3 % (26 out of 37 female *O. rubescens*) of specimens had one or more injured arms (Fig. 3). Injured individuals most commonly exhibited truncation of a single arm, with

the exception of *O. bimaculatus* males, which were more likely to have lost tissue from three or more arms (Fig. 3).

There were no differences in the overall count or mean number of missing arms between the sexes of any of our three species (Table 2). We predicted that the number of truncated arms would depend on sex and arm location. No single arm was lost significantly more often than any other when grouped by species or by sex (Fig. 4). When the arms were divided into anteroposterior sets, our hypothesis of more anterior injuries was supported only by *O. rubescens* females, which had significantly more arms missing in anterior arm pairs 1 and 2 (29 anterior versus 13 posterior observations of missing arms, p < 0.01, Kruskal-Wallis 2 sample exact test). The mean numbers of arms lost were not significantly different across all possible pairs of individual arms, and across bilateral groupings for male *O. rubescens* and either sex of *O. bimaculatus* and *O. bimaculoides* (p > 0.1, Kruskal-Wallis rank sum tests).

We predicted there would be an overall relationship between octopus body size and number of arms injured. The number of injuries increased with increasing interocular distance across all specimens (r-squared = 0.03, p = 0.0084). Body size predicted arm injury in female *O. bimaculatus* and female *O. rubescens*, as we found significant positive correlations between the number of arms injured and log-transformed IO measurements (female *O. bimaculatus*: r-squared = 0.12, p = 0.049; female *O. rubescens*: r-squared = 0.15, p = 0.0161). For males of all three species (*O. bimaculatus*, r-squared = 0.12, p = 0.063; *O. bimaculoides* (r-squared = 0.065, p = 0.17); *O. rubescens*, r-squared = 0.0064, p = 0.64), relationships between IO and number of arms injured trended positive, but were not significant. In female *O. bimaculoides*, the relationship between body size and number of injuries trended negative, but was not significant (r-squared = 0.066, p = 0.15).

The incidence of arm loss is relatively common in these three octopus species, which reflects patterns observed in other natural octopus populations (Voight, 1992; Wada, 2017), in other invertebrates with multiple regenerative limbs such as comatulid crinoids (Mladenov, 1983; Meyer, 1985), crown-of-thorns sea stars (Wilmes et al., 2019), blue and red king crabs (Smith, 1990; Dvoretsky and Dvoretsky, 2009), and even in species with redundant but non-regenerative limbs such as wolf spiders (Brueseke et al., 2001) and harvestmen (Guffey, 1998). These studies, along with a more recent and taxonomically widespread survey of regenerative marine invertebrates, supports the characterization that injury, in redundant body parts such as the arms, limbs, and appendages, are common but also vary considerably by taxon, space, and time (Lindsay, 2010). Our approach, which assessed differences in males and females for each species, enables us to present intra- and interspecific variation in the frequency and patterns of arm loss while also examining a broad size range of animals, addressing the component of ontogenetic time. While arms were lost equivocally for two species in our data set, we observed that the anterior arms of female *O. rubescens* were more

Table 1

Observations of missing arms in each arm position, by sex and species. Arm pair 1 is the most anterior, and arm pair 4 is the most posterior. The mean number of missing arms was not significantly different between species (p 0.2445, Kruskal-Wallis rank sum test). The number of missing arms did not differ between the sexes of a given species (Fisher's exact test).

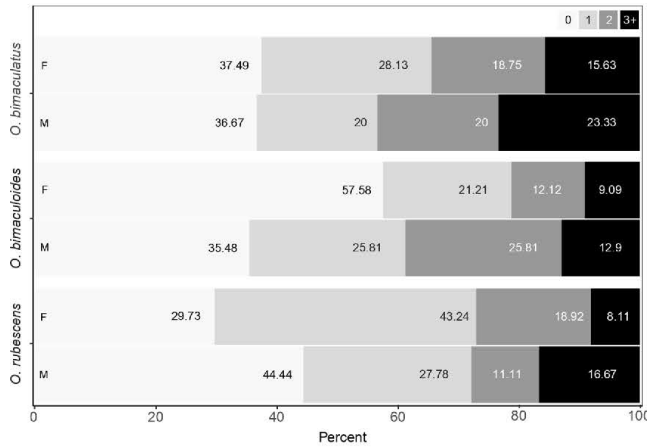|		Number of missing arms, by species					
		O. bimaculatus		*O. bimaculoides*		*O. rubescens*	
Sex	Arm Designation	L	R	L	R	L	R
F	1	5	5	6	3	9	7
	2	10	3	4	3	6	7
	3	7	3	5	2	3	3
	4	6	2	5	1	3	4
M	1	3	7	5	6	5	7
	2	3	3	5	5	10	5
	3	5	2	6	0	5	2
	4	2	6	4	1	6	3

Fig. 3. Prevalence of the number of arm losses found in each specimen, by sex and species. 20 female and 19 male *O. bimaculatus*, 14 female and 20 male *O. bimaculoides*, and 26 female *and 20* male *O. rubescens* were observed to have been injured. In many observations of arm loss, the specimen only had one or two discernible injuries. The palest gray segments on the left end of each bar represent the percentage of uninjured individuals in this study. Light gray segments represent the proportion of octopuses with exactly one total missing arm, dark gray segments represent exactly two, and black segments represent three or more. There were no significant differences between the proportions of the numbers of arm losses between the sexes within each species (Fisher's exact test), or across the three species (Chi-squared test).

Table 2

Comparisons of the number of injured arms in groupings of anterior (A) and posterior (P) arms, and in left (L) and right (R) arms. The numbers of arms lost on opposing sides were compared using non-parametric Wilcoxon tests. The mean number of arms lost did not differ significantly along the anteroposterior or bilateral axes in any group.

Comparison		*O. bimaculatus*		*O. bimaculoides*		*O. rubescens*	
		Female	Male	Female	Male	Female	Male
Anterior-Posterior	Mean # lost A	1.6 ± 0.7	1.7 ± 0.7	1.4 ± 0.9	1.4 ± 0.6	1.4 ± 0.7	1.5 ± 0.6
	Mean # lost P	1.4 ± 0.6	1.6 ± 0.8	1.6 ± 0.7	1.2 ± 0.4	1.2 ± 0.4	1.8 ± 1
	X^2	1.30	0.18	0.66	0.21	0.72	0.33
	df	1	1	1	1	1	1
	N	28	27	21	26	33	27
	p-value	0.25	0.67	0.42	0.65	0.40	0.57
	Bias Direction	N/A	N/A	N/A	N/A	N/A	N/A
Left-Right	Mean # lost L	1.6 ± 0.7	1.6 ± 0.8	1.8 ± 1	1.3 ± 0.6	1.3 ± 0.6	1.6 ± 1
	Mean # lost R	1.4 ± 0.5	1.4 ± 0.8	1.6 ± 0.5	1.1 ± 0.3	1.2 ± 0.7	1.5 ± 0.7
	X^2	0.19	0.92	0.022	1.32	0.93	0.013
	df	1	1	1	1	1	1
	N	28	27	21	26	33	27
	p-value	0.66	0.34	0.88	0.25	0.34	0.91
	Bias Direction	N/A	N/A	N/A	N/A	N/A	N/A

often lost. Anterior arm loss is a frequent pattern shared in many decapod crustaceans (Durkin et al., 1983; Smith, 1990) due to their use in a variety of agonistic interactions (Mariappan et al., 2000). In harvestmen (Guffey, 1998) and some crab species (Spivak and Politis, 1989), the longest arms which are often times the most exposed were more likely to be missing. *O. bimaculoides* have been observed to use anterior arms more frequently (Kennedy et al., 2020); while we lack behavioral data for the other species in this study, the pattern of higher injuries in anterior arms observed for female *O. rubescens* supports the idea that not all arms are used equivocally (Voight, 1992; Byrne et al., 2006a), and that differential use leads to differential exposure and vulnerability. The positive relationship between body size and number of arm injuries for female *O. bimaculatus* and *O. rubescens* also suggest the need for studies into the dynamic nature of predator-prey interactions, arm use, and how these behaviors may coincide with microhabitat shifts over ontogeny. The relationship between size and number of injuries varies across species with redundant limbs (Sekkelsten, 1988; Sheader, 1998; Wilmes et al., 2019). In a size range of newly-settled *Acanther ssp,* smaller individuals incurred more arm injuries compared to larger individuals (Wilmes et al., 2019). Incidence of arm loss increased with body size in male crabs, *Carcinus maenus* (Sekkelsten, 1988) and large male amphipods, *Ampelisca tenuicornis*, were

more frequently injured in natural populations in the Isle of Wight, England (Sheader, 1998). Adult male and female wolf spiders, *Pardosa milvina,* without egg sacs, were more likely to have missing limbs compared to juveniles, which may be attributed to the increase in activity of mature spiders including agonistic interactions between conspecifics (Brautigam and Persons, 2003).

3.2. Proportion of arm truncation

The mean proportion of arm truncation in all species, irrespective of sex, was 36.8 ± 11 % standard deviation (*O. bimaculatus* females: 32.6 ± 3 %, males 37.3 ± 5 %; *O. bimaculoides* females: 36.7 ± 2%, males 20.8 ± 9%; *O. rubescens* females 56.0 ± 4 %, males 33.7 ± 2 %). Log-transformed body size predicted the proportion of arm truncation in *O. bimaculatus* females (r-squared = 0.84, p < 0.0001), both sexes of *O. bimaculoides* (female r-squared = 0.64, male r-squared = 0.42, both p < 0.0001), and *O. rubescens* males (r-squared = 0.26, p = 0.0015) as we observed lower mean proportions of arm truncation as body size increased. Female *O. rubescens* had the opposite pattern, showing higher proportions of arms truncation with an increase in body size (r-squared = 0.37, p < 0.0001). Only male *O. bimaculatus* showed similar mean proportions of arm truncation across their size range (r-
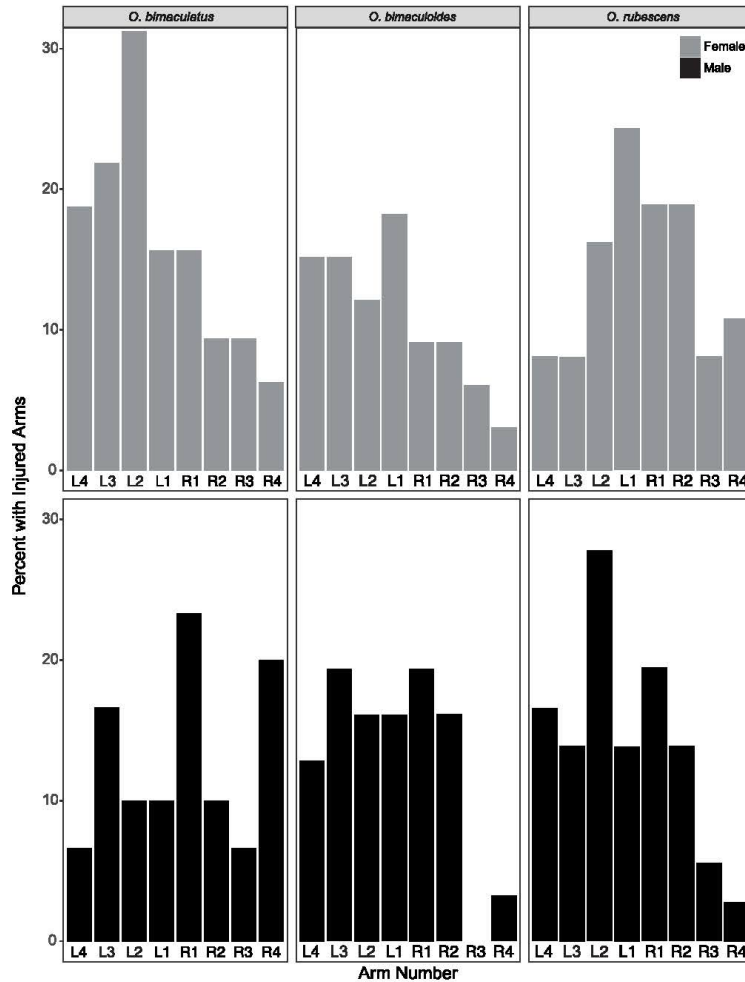
Fig. 4. Percentages of octopuses missing particular arms, by arm location, sex, and species. Arms are labeled L4-L1, then R1-R4, as they are arranged clockwise on the octopus. Gray bars represent females (F), and black bars represent males (M). No single arm was missing significantly more often. When paired bilaterally, either arm pair 1 (male *O. bimaculatus* and *O. bimaculoides*, and female *O. bimaculoides* and *O. rubescens*) or 2 (male *O. rubescens*, and female *O. bimaculatus*) was ranked highest in number of observed arm losses (p > 0.1, Kruskal-Wallis rank sum tests), but mean numbers of missing arms were not significantly different across arm positions.

squared = 0.04, p = 0.2779).

In the anteroposterior axis, male *O. bimaculatus* and both sexes of *O. bimaculoides* had a significantly higher mean arm length missing in arm pairs 1 and 2 (Wilcoxon test, $p = < 0.0001$; see Table 2 for X^2 values). In both sexes of *O. rubescens*, significantly more length was missing from arm pairs 3 and 4 (p < 0.0001). Female *O. bimaculatus* did not show a significant anteroposterior bias in arm truncation (p = 0.0783). When grouped along the bilateral axis, left and right, non-parametric Wilcoxon tests revealed a significantly greater percentage of truncation on left arms versus the right in all but *O. bimaculoides* males (X^2 (df = 1, $N = 124$) = 2.1, $p = 0.1471$). Considering the mean proportion of arm truncation in Table 3 and number of arms lost in Table 2, there is a striking asymmetry in the location and extent of arm injuries. This asymmetry in injury patterns supported our predictions that anterior arms would incur a greater proportion of loss, with an addition bias toward the left side in males. Our predictions were based on the idea that male octopuses protecting their R3 reproductive arm may use the radially opposite arms, L2 and its neighbors, to fend off attacks while

putting as much distance as possible between R3 and potential injury.

The asymmetry in the extent of arm loss we observed supported our prediction that anterior arms, arm pairs 1 and 2, would sustain greater injuries than posterior arm pairs 3 and 4. A strong bias toward left side injuries were apparent in almost all groups in addition to strong species-specific anteroposterior biases. We posit that octopuses turn to protect arm R3, especially males, whose R3 is specialized for reproduction. Other organisms that show directionality in the severity of limb loss appear to be mitigating losses: harvestmen were found to most commonly autotomize anterior legs that are crucial for sensory perception, which is suggested to be evidence of bet-hedging against a total loss of fitness (Guffey, 1998). The octopuses in this study may commonly use the anterior or left arms for risky behaviors for similar reasons. These arms grow positively allometrically in all three species (Table S1), and may regrow faster than the others, mitigating the loss of what may be a preferred arm.

Table 3
Comparisons of the calculated percentage of injured arms in groupings of anterior (A) and posterior (P) arms, and in left (L) and right (R) arms. The calculated percentages of arms on opposing sides were compared using non-parametric Wilcoxon tests. *O. bimaculatus* males and both sexes of *O. bimaculoides* demonstrated greater injuries in anterior arms; *O. bimaculatus* females did not show a significant difference in injuries between anterior and posterior injuries. *O. rubescens* females demonstrated greater injuries in anterior arms, but males on average lost more of their posterior arms. Considering the bilateral axis of symmetry, in all species but *O. bimaculoides*, both females and males demonstrated greater injuries in left arms. *O. bimaculoides* males did not show a significant difference in injuries between injuries on the left and right.

Comparison		*O. bimaculatus*		*O. bimaculoides*		*O. rubescens*	
		Female	Male	Female	Male	Female	Male
Anterior-Posterior	Mean % lost A	32.9 ± 3	40.8 ± 4	37.8 ± 1	28.0 ± 8	55.0 ± 2	32.6 ± 1
	Mean % lost P	32.2 ± 1	33.8 ± 4	35.6 ± 2	13.6 ± 3	57.0 ± 5	34.8 ± 3
	X^2	3.10	100.83	108.52	179.71	36.43	53.81
	df	1	1	1	1	1	1
	N	128	120	132	124	148	144
	p-value	*0.0783*	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	Bias Direction	N/A	A	A	A	P	P
Left-Right	Mean % lost L	33.0 ± 3	40.8 ± 3	38.0 ± 1	19.8 ± 6	58.6 ± 3	34.6 ± 2
	Mean % lost R	32.1 ± 1	33.9 ± 4	35.4 ± 1	21.8 ± 12	53.4 ± 3	32.8 ± 2
	X^2	16.09	100.83	109.64	2.10	71.64	45.92
	df	1	1	1	1	1	1
	N	128	120	132	124	148	144
	p-value	**<0.0001**	**<0.0001**	**<0.0001**	*0.1471*	**<0.0001**	**<0.0001**
	Bias Direction	L	L	L	N/A	L	L

3.3. Patterns of arm loss and implications on behavior

In our analyses, we found a significant relationship between the number of arms injured and the overall severity of arm injuries, represented by mean proportion of arm lost by an individual, in two of the six groups. Female *O. bimaculatus* with fewer injured arms had lost a large proportion of the arms' length, while individuals with a greater number of injured arms incurred less loss of length (r-squared = 0.15, p = 0.0258), indicating a potential tradeoff between incurring these two types of injuries. Female *O. bimaculatus* may recruit uninjured arms for tasks and employ less risky behaviors to mitigate further loss of tissue. Conversely, in the pattern we found in female *O. bimaculoides*, individuals with more arm injuries also lost more of their arm lengths (r-squared = 0.19, p = 0.0119). These opposing patterns may reflect variability in risk-taking behaviors employed by females of these closely related sympatric octopuses. In *O. rubescens* females and in males of all three species, the relationship between the number of arm injuries and mean proportion of arms injured was not significant.

Our two measures of arm injury, frequency of arm loss and proportion of arm length lost, motivated new questions about octopus arm recruitment and use. Female *O. rubescens* in this study had the greatest mean proportion of arm lost (56.0 ± 4 %) and interestingly, a post hoc Tukey test showed that they also had significantly steeper slopes of arm scaling relationships than all other groups. In other words, for a given body size, female *O rubescens* had longer arm lengths compared to the two other species and with male *O. rubescens* at the same body size (Table S2). We also found that as female *O. rubescens* body size increased, they were the only group to have incurred greater proportions of arm truncation. Studying behavioral shifts in arm use patterns over ontogeny for *O. rubescens* would help to determine whether the steeper rates of arm growth correspond with larger individuals using their arms more conspicuously and taking more risks. Moreover, in *O. bimaculatus* males, multiple arm losses were common, with one individual bearing a total of seven injured arms. Although each truncation was on average close to a third of the length of each arm (37.3 ± 5 %), there were more severe truncations in anterior arms than posterior arms. The allometric slopes between body size and arm length of *O. bimaculatus* males were the shallowest, indicating the slowest growth rates in this study. They were also the only group in which body size was not correlated with the proportion of an arm that was truncated. The severity of injury in *O. bimaculatus* males collected in the same geographic location as the other specimens in this data set suggests asymmetrical arm use and bolder risk-taking behaviors throughout their life history.

The results of this study also provide further evidence that males of the three benthic species examined are protective of their reproductive arm, R3. As shown in Fig. 4, arm R3 in males consistently had a relatively low number of losses, especially when compared to their bilaterally opposite arm L3 and their radially opposing arm L2. We hypothesize that male octopuses may turn the left side of their bodies toward an aggressor or preferentially use the arms opposing R3 for risky behaviors. Further behavioral studies are necessary to support these hypotheses. Additionally, the mean percentages of arm loss were lowest in injured R3 for *O. bimaculatus* (29.4 % of length missing), *O. bimaculoides* (no injuries found), and *O. rubescens* (30.4 % of length missing). These observations corroborate previous studies showing that the hectocotylus garners fewer injuries (Steenstrup, 1857; Bello, 1995) and suggests that *O. bimaculatus, O. bimaculoides,* and *O. rubescens* may protect R3 due to its importance in mating and reproduction (Huffard et al., 2008).

The loss and regeneration of body parts occurs in a wide variety of animal taxa (Bely and Nyberg, 2010; Fleming et al., 2007), particularly invertebrates with many redundant limbs such as sea stars (Mladenov et al., 1989; Lawrence and Vasquez, 1996), many arthropods (Wasson and Lyon, 2005; Suzuki et al., 2019) and cephalopods (Bush, 2012; Alupay, 2013). This ability even facilitates autotomy, the self-selected shedding of body parts, to recover from injury (Guffey, 1998; Emberts et al., 2017) and may serve to heal an entire body suffering from a parasitic infection (Mitoh and Yusa, 2021). However, limb loss has both energetic and behavioral consequences. Performance in functional contexts including locomotion (Guffey, 1999; Brueseke et al., 2001) and reproduction (Wada, 2017) are impacted by the number and extent of missing limbs. Our findings support the hypothesis that the complete loss of a single arm, especially an anterior arm, may be more challenging for octopuses to navigate than the partial loss of two or more arms. Considering these patterns in future behavioral studies will be important for understanding an individual's compensatory abilities in a variety of contexts.

4. Conclusions

While museum collections have long facilitated research about the natural world, more recently, they have acted as a repository for understanding environmental change and even global declines of species (Cheng et al., 2011; Primack and Miller-Rushing, 2012; Schmitt et al., 2019). Our study emphasizes the wealth of information museum collections can provide on the changes in morphology throughout the life

history of ecologically important but also highly elusive mesopredators in the marine ecosystem. We quantified the number of arms lost and proportion of arm truncation in 199 museum specimens for three octopus species; arm loss patterns and severity of injury varied across species. We found that the greatest proportions of arm truncation were significantly biased toward the left side for at least one if not both sexes of the three species. Anteroposterior biases for injury were observed in all groups, suggesting asymmetry in arm use during risky behaviors that has been associated with bet-hedging behavior in other taxa (Guffey, 1998). Our results provide a foundation for future inquiries about limb growth and use over ontogeny in species that can regenerate and other potential drivers of limb recruitment in animals with radially symmetrical appendages. This study is also the first of our knowledge to calculate the proportion of arm loss in octopuses by first calculating arm scaling relationships. Our quantification of the relatively high prevalence and severity of limb loss provides more insight into the role of octopuses as prey, and the prevalence of sublethal predation that contributes to the food web of different marine environments.

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Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

Appendix A. Supplementary data

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CHAPTER 2

INJURY SEVERITY INDEX AND *IN SITU* VOLUME: ECOLOGICALLY

INFORMATIVE METRICS FOR QUANTIFYING THE MAGNITUDE OF

INVERTEBRATE TISSUE LOSS

INTRODUCTION

Sublethal predation, the non-fatal loss of a body part to a predator (Bely & Nyberg,

2010; Lawrence & Vasquez, 1996) is commonly experienced by a diverse range of

taxa (Hanlon & Messenger, 2018; Lindsay, 2010; Smith, 1990; Wasson & Lyon,

2005), and is therefore an important process for the transfer of energy in many

ecosystems (Pape-Lindstrom, Feller, Stancyk, & Woodin, 1997). Past studies on

sublethal tissue loss have focused on different aspects of these predatory encounters:

the behaviors and capabilities of the predator (Grüninger, 1997; Higgins, Law, &

Mehta, 2018; MacGintie & MacGintie, 1949; McCleneghan, 1973), the regenerative

capabilities of the prey species (Lange, 1920; Wilmes, Hoey, Messmer, & Pratchett,

2019), and how sublethal predation may ultimately facilitate lethal predation (Berke,

Cruz, & Osman, 2009; Meyer & Byers, 2005). Historically, methods used to quantify

sublethal predation have not been comparable across studies because injury

descriptions vary with respect to the focal taxon. The diverse shapes of tissue

structures and numbers of regenerating appendages in aquatic animals can obscure

the magnitude of tissue loss, resulting in the simplification of injury description,

which often falls along a single axis. Without a unifying approach, our abilities to

incorporate sublethal tissue loss into our understanding of ecological systems; for example, how biomass is transferred across communities, is limited.

Octopuses are an interesting model for studying sublethal limb loss because they are ecologically important marine invertebrates that have eight multifunctional, regenerative arms (Grasso, 2009; Hanlon & Messenger, 2018; Kennedy, Buresch, Boinapally, & Hanlon, 2020). Octopuses are not only active and voracious predators found across marine ecosystems worldwide (Roper et al., 2016); as mesopredators, they are also a protein-rich source of energy for a variety of fishes, marine mammals, and even other cephalopods (Feder, Turner, & Limbaugh, 1974; Goodman-Lowe, 1998; Hernández-Urcera et al., 2014; Higgins et al., 2018). While the drivers of sublethal arm loss in octopuses presumably varies with predator composition across different marine ecosystems, it has been shown that the frequency of arm loss varies widely between species, sex, body size, and location of the arm around the body (Chapter 1). The frequency of arm loss, represented by the number of truncated arms in an individual, is the most common metric of appendage loss reported in the literature (Voight, 1992; Wada, 2017). These data, paired with the location of the injured arm on the body, allows some inference of how sublethal predation may affect subsequent octopus behavior. Octopus behavior (J. A. Mather & Alupay, 2016), especially reproductive success (Wada, 2017), depends on the condition of their arms. For example, loss or truncation of the specialized reproductive arm, R3, in males results in the forfeit of mating if the hectocotylus, the male reproductive structure, is not regrown by the end of the mating season. Arm regeneration rate depends largely

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on caloric intake (Imperadore & Fiorito, 2018), but also on the amount of tissue that was removed. Adopting only simple counts of truncated arms, however, may misrepresent the severity of the injury if only the tip of one or more arms is missing. Therefore, calculating the length or proportion of length lost is critical to our understanding of both the magnitude of tissue lost and the potential loss of function in an individual arm. Up until now, the number and proportion of arm loss is often reported separately (but, see Voss and Mehta, 2021). Therefore, in this study, I tested whether these data combined into a single index value could describe the magnitude of arm tissue lost by an individual. Improving our understanding of the magnitude of arm loss in octopus species has implications for the management of fisheries, bioenergetics of marine ecosystems, and understanding the behaviors of individual octopuses that have experienced sublethal predation.

Here, I demonstrated how I implemented a morphological index, which I refer to as the injury severity index (ISI), to holistically describe arm injury by combining the number of arms that have lost tissue with the proportion of the length of arm tissue lost. ISI may not only be used to describe behavioral, ecological, and physiological implications of appendage loss in octopuses, but may be modified for other invertebrate taxa such as sea stars, brittle stars, anemones, and crustaceans with multiple redundant appendages. I used ISI to describe the magnitude of arm loss in museum specimens of eight different octopus species indigenous to the Northeastern Pacific that vary greatly in size and life history. I also used empirical field data collected for a common southern California species, *Octopus bimaculatus*, to

demonstrate how to incorporate tissue loss into long-term survey data. Through our field effort, I showed how researchers may calculate the volume of arm tissue lost, a critical metric for refining our understanding of marine trophic interactions and for converting sublethal predation to biomass transferred in the community.

METHODS

Study Species

Preserved octopus specimens previously identified to species were obtained from the California Academy of Sciences (CAS), the Santa Barbara Museum of Natural History (SBMNH), and Scripps Institute of Oceanography (SIO); see Supplementary Table S2 for accession numbers. I selected eight species of benthic octopuses that are common in the Northeastern Pacific, specifically, along the West coast of North America between Alaska and Mexico, including the Channel Islands: *Octopus bimaculatus*, *O. bimaculoides*, *O. californicus*, *Paroctopus digueti*, *Enteroctopus dofleini*, *O. fitchi*, *O. hubbsorum*, and *O. rubescens*. Each species was well represented in museum collections except *P. digueti*, with 49 specimens, and *E. dofleini,* with 49 specimens. 61 *O. bimaculatus* were originally measured, but five were excluded from this study. I chose this group of octopus species in order to test whether patterns of arm loss may vary between ecologically diverse species that have comparable morphological characteristics. These species all have eight unspecialized arms, with the exception of a male's hectocotylized arm R3. However, they can vary in many traits including maximum body size, depth range, preferred habitat, and geographical range (Supplementary Table S1). The data collected from these

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specimens included external measurements of interocular distance as a measure of body size; arm lengths (from the edge of the mouth to the tip of the arm; Roper and Voss 1983) following the protocol and rationale laid out in Chapter 1; and the number, location, and length of arms that had been truncated and were in some stage of regrowth at the time the individual was preserved.

Injury Severity Index: A holistic approach to invertebrate injury

Sublethal injury data have the potential to include the number of injured appendages, the location of the injured appendage on the body (Lindsay, 2010; Smith, 1990), and the length, or proportion of length, lost from each injured appendage. Most studies only report the number of appendages injured or the amount of tissue lost. Neither on their own indicates the full extent of an individual's injuries and can mislead the severity of predation which is why some studies have examined both measures independently (Lawrence & Vasquez, 1996). I use octopus as a model to demonstrate the utility of an index which holistically describes sublethal injury. Octopus, similar to many marine invertebrates, are susceptible to sublethal predation events which may lead to a range of arm injures (Voight, 1992; Wada, 2017). An octopus with five injured arms may have five missing arm tips or five completely truncated arms, while an octopus missing an average of 50% of its arm length may have half of its arm missing or several injured arms that when quantified for their loss of lengths, have an average of 50%. When I collected and compared these two measures of injury, number of missing arms and proportion of arm tissue lost, for our dataset on octopus, I did not find a significant relationship between these variables across all museum

specimens. This demonstrated that the two metrics are independent, and therefore could be combined into a single value to describe the extent of arm injury more holistically in an individual.

To holistically describe the extent of arm injury observed in an individual octopus, I propose an Injury Severity Index (ISI; Figure 1) which combines the number of injured arms and the sum of the proportion of each injured arm. The lowest possible ISI value of an injured octopus is 1.01, which would denote one arm with 1% of the expected arm length missing. The highest ISI value is 16, which means that all eight arms have been removed in their entirety. An uninjured, or completely recovered, octopus would have an ISI score of 0.00. A continuum of ISI injuries is shown in Figure 1A. This index allows a researcher to quantify the magnitude of injury an octopus has experienced. For example, a magnitude of 2.10 represents an individual with two injured arms missing 5% of arm length, whereas an octopus with two injured arms where both arms are completely truncated would have an index score of 4.00. In this study, I have calculated the ISI value for previously injured museum specimens of all eight species of octopuses.

Volume calculations

Octopus arms are muscular hydrostats, thereby having a fixed volume despite a high degree of flexibility. I used the scaling relationships of arm lengths and interocular distance (IO), a proxy for body size, for male and female *O. bimaculatus*, to calculate the expected volume of a given arm. Figure 1B is a graphical explanation for how to calculate missing arm lengths and volumes. I tested two methods for calculating

volumes of missing arms. The first method is based on the scaling relationship of arm length and body size while the second method is based on the average length of the uninjured arms of each individual. For visualizing scale-independent organisms of varying body size, I normalized the body size and arm count data with a natural log transformation, and the proportion of arm missing data with an arcsin transformation, since the largest museum specimens were orders of magnitude larger than the smallest.

Field study of live *O. bimaculatus*

I surveyed arm losses *in situ* in live *O. bimaculatus* individuals in the Two Harbors area of Santa Catalina Island during the month of July: nine octopuses in 2017, fifteen in 2018, 27 in 2019, and fifteen in 2021. Our fieldwork was prohibited in 2020 due to the global pandemic. Body measurements were collected on SCUBA in 2017 and 2018, and on shore in 2019 and 2021. These animals were surveyed under California Department of Fish and Wildlife (CDFW) permit # S-190830002-19086-001, and our non-invasive, non-lethal methods were approved by University of Southern California (USC) IACUC Protocol # 20919-CR001, as the fieldwork was conducted out of their marine station, the Wrigley Institute for Environmental Studies. All experiments were performed in accordance with CDFW permitting and USC's IACUC approval. Our home institution, the University of California, Santa Cruz, did not require any IACUC approval for cephalopods at the time this study was conducted.

I recorded interocular distances (IO) for all octopuses and the number, location, and approximate truncation (0, 25%, 50%, 75%, 100%) for all eight arms of

each injured individuals. Arm length measurements were not collected due to difficulty measuring while on SCUBA; IO and the slopes from the scaling relationships from museum data allowed us to calculate arm lengths. Then, I used the proportion of arm length lost to generate ISI values and approximate the amount of biomass lost by octopus to sublethal predation. In one male, all eight arms had been truncated and were in differing states of regrowth, so the mean slope of all eight arms was used to calculate arm lengths, which were then used to calculate missing volume. In five specimens, all but one arm had been injured, and the length of the single pristine arm was used to calculate *in situ* volume.

RESULTS

 Injury Severity Index

Measurements were collected from 467 individuals across eight species. Values for number and proportion of injury and Injury Severity Index (ISI) are summarized in Table 1. The mode ISI score in all eight species was 0.00, but in the specimens with discernible arm injuries, an ISI score of 1.01-2.00, or one arm with some proportion of tissue lost, was most common (Figure 2). ISI significantly increased with larger body size in *O. bimaculatus* ($r^2 = 0.158$), *O. fitchi* ($r^2 = 0.171$), and *O. hubbsorum* ($r^2 = 0.142$) (Figure 2A). ISI did not significantly decrease with increasing body size for any of the remaining five species.

Measures of arm volume lost

To determine whether our two methods for calculating missing arm length generated comparable arm volumes, I directly compared missing volume values calculated with

arm lengths based on scaling relationships, with those based on the measured lengths of the uninjured arms of an individual, which I called *in situ* volume. Formulas for these calculations are shown in Figure 1B. I show that for all eight species, the two volume metrics are positively correlated (Figure 3A). Therefore, biomass loss can be estimated directly from *in situ* measurements in these eight species.

Our two main measures of arm injury, *in* situ volume missing and ISI, were strongly positively correlated in most groups, as demonstrated in Figure 3B. *O. californicus* and *E. dofleini* had lower ISI scores than the other six species, and the low degree of correlation with *in situ* volume indicated that injury may be focused on one to two arms in these species, regardless of the size of the individual.

Field Study of live *O. bimaculatus*

Of 66 wild *O. bimaculatus* individuals, 60.7% of were found with injured arms, and these individuals ranged widely in the number of injured arms and proportion of arm length missing. Arm injuries ranged from 1-8. On average, octopuses had 2.20 ± 2.4 injured arms. A grand mean of $48.9 \pm 27\%$ of arm length had been lost across all individuals. Their mean ISI value was 3.13, which indicates that individuals were missing more than half of their length from two arms or the tips of three arms were missing; the maximum ISI value of 12.5 describes an individual with eight injured arms (Figure 2B). Our two methods of calculating missing arm volume, based on arm lengths derived from the slopes of scaling relationships of museum specimens and on the average lengths of pristine arm measured in each individual *in situ*, were highly correlated overall ($r^2 = 0.985$). Thus, volume calculated *in situ* were sufficient to

describe tissue lost in live octopuses. Mean *in situ* volume of tissue lost calculated in all field specimens across the four years was 6850.0 ± 11303 mm^3. There were no significant differences between mean *in situ* volume lost in a given year. The total amount of biomass lost by these octopuses across all field seasons was 858 g of tissue. The total area of the sites within the Two Harbors area where octopuses were surveyed in those four years was around 83,700 m^2, as estimated from aerial maps of the study sites; therefore, the octopuses I sampled lost about 1 g of biomass per 100 m^2 in a one-month period each year. Each year, the sample population lost an average of 215 g of biomass to predators.

DISCUSSION

Here, I demonstrated the utility of ISI to describe the magnitude of sublethal predation in octopuses that vary greatly in their ecology and life history. ISI combines both the number and proportion of appendage lost, two variables that have been shown to be independent, are readily quantifiable, and comparable across invertebrate taxa. The majority of injured octopuses in museums had an ISI score of 3.00 or less, which reveals that most octopuses had one to two truncated arms. ISI also increased with body size in *O. bimaculatus*, *O. fitchi,* and *O. hubbsorum* which inspires questions about how predator-prey interactions may change across octopus species' longevity. While I demonstrate the utility of ISI for octopuses, ISI may readily be rescaled for any animal with more than a single regenerative appendage such as Asteroids, Annelids, Ophiuroids, and even Crinoids, as they have a characteristically fixed number of limbs or segments that can be measured. ISI may

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be used as a comparative metric across different invertebrate taxa within or across communities by dividing ISI values by the most extreme value possible for the taxon of interest. For example, to compare mean ISI values for brittle stars and octopus inhabiting a single community, I would divide the ISI values for sunflower stars (*Pycnopodia helianthoides*) by 48, and by 16 for octopuses. These new ISI ratio values would then be comparable between sunflower stars and octopuses as they consider the differences in appendage number that would be subject to sublethal predation.

I also implemented two methods for calculating the volume of tissue lost and found that volume can be extrapolated effectively from known scaling relationships of a species and from *in situ* averages of arm lengths, the latter method being more practical for field studies, especially. Calculating the volume of tissue lost and using it to estimate the caloric content of previously lost appendages would complement and strengthen long and short-term field transect data and advance our understanding of biomass, or energy, transfer through sublethal predation. Therefore, ecological applications for understanding the volume of tissue lost are numerous and can enhance our understanding of marine trophic relationships and, potentially, bioenergetic costs of regenerating fully-functioning arms. While not all appendages have the same function, it will be left up to the researcher to understand the division of labor of appendages in order to determine how to calculate *in situ* averages of appendage length to ultimately calculate volume. For example, squid and cuttlefish are cephalopods with two tentacles in their arm crowns that are often much longer

than their eight arms. Therefore, if one tentacle is missing any amount of length, it would be more accurate to use the length of the pristine tentacle in the individual to calculate volume missing rather than average all pristine arms.

Finally, in this study, I effectively calculated both ISI and *in situ* arm tissue volume lost from *O. bimaculatus* in the field. The *in situ* volume missing per unit area for our field specimens of *O. bimaculatus* may seem low at 1 g per 100 m², but this translates to an arm that is approximately 10 cm long, the average arm length for a specimen with an IO of about 20 mm, or a small mature adult. Considering that the population density of *O. bimaculatus* in the area of Two Harbors, Santa Catalina Island ranged between zero and five octopuses per 100 m² in previous studies (Ambrose, 1988; Hofmeister, 2015), and a single *O. bimaculatus* individual can move within an average area of 6143.4±3165.5 m² in a 24-hour period (Hofmeister & Voss, 2017), the chance that a given octopus will lose arm tissue appears high. It is also important to acknowledge that an octopus that experiences sublethal predation early on in its life may contribute more than one octopus' worth of biomass into the food web in its lifetime if its arm or arms completely regenerate before it expires.

Our measurements of octopus arm injuries increase opportunities for future understanding of cryptic trophic contributions of invertebrates to the marine ecosystem and fitness impacts resulting from sublethal predation. Injured octopuses collected in the field, especially those with ISI values over 6.00 (or, 3-5 injured arms) would likely be more vulnerable from a reduced capacity to travel, capture prey, and defend themselves, while many with low ISI values (1.01-3.00) were probably able to

compensate for the loss of one to two arms. Comparisons of ISI values across different populations would increase our understanding of the variability in predation pressures especially when local predators are well-documented. Volume calculations of lost arms would also facilitate our understanding of the amount of energy predators gain from sublethal events. While the biomass contributed by animals in an ecosystem is usually estimated by the number or weight of live or dead individuals, I show how a measure of tissue lost to sublethal predation could be incorporated into models to improve our understanding of the transfer of biomass within marine communities. I also posit that ISI values can be used to enhance descriptions of body condition for a variety of multi-limbed invertebrates in ecological surveys. Invertebrates are not only important predators and prey in marine ecosystems, but they are also ecosystem engineers (Gutiérrez & Jones, 2006; Kristensen, 2008; Scheel, Godfrey-Smith, & Lawrence, 2014) providing nutrients and microhabitats for other organisms. The behavioral and functional implications for tissue loss are not well understood in octopuses. While few studies have incorporated how sublethal predation affects the biology of invertebrates, our holistic approach of quantifying sublethal predation via ISI may help ecologists reveal how a particular magnitude of sublethal predation may have secondary effects on the functional roles of individuals or local diversity within communities.

A

1%

1.01   8.00   16.00

B

w_m w_t

r

h_t

h_m

$$\text{scaling-based } h_m = (m(IO) + b) - h_{truncated}$$

$$\textit{in situ } h_m = \bar{x}\left(\sum h_{each\ intact\ arm}\right)$$

$$V_m = \frac{[\pi(r_m)^2]h_m}{3}$$
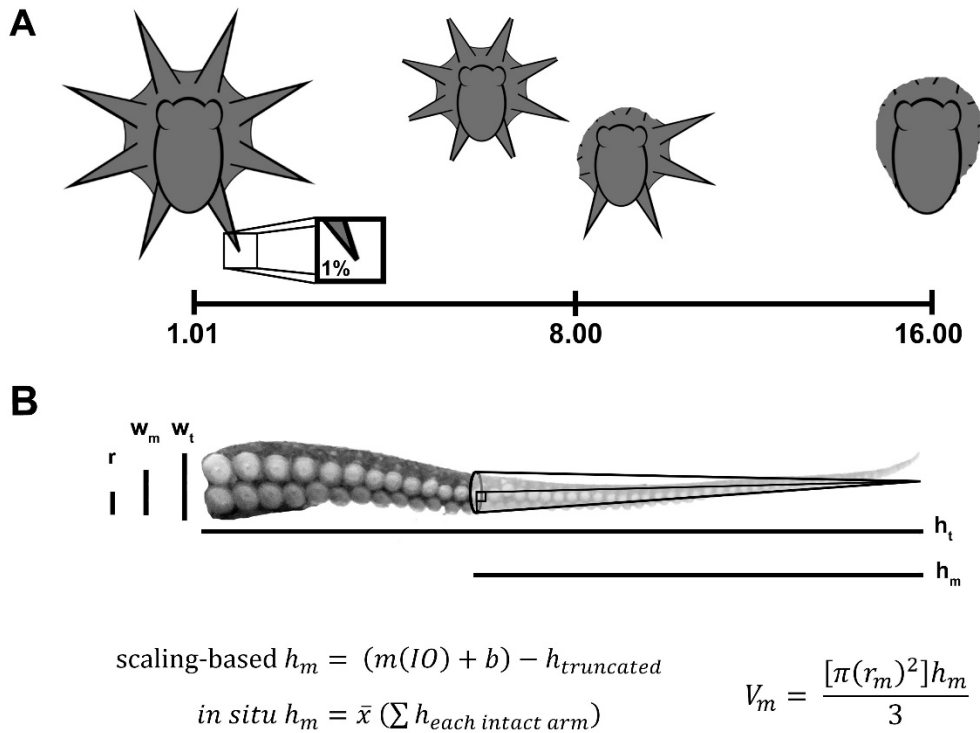
Figure 1. Novel metrics of octopus arm injuries: Injury Severity Index (ISI) and volume calculations. The scale shown in part A demonstrates the arm truncations being described by increasing ISI values. ISI is calculated by adding the number of truncated arms with the proportion (0.0-1.0) of each injured arm found to be missing in order to present a more holistic understanding of the extent of injury sustained by an individual octopus. An ISI value between 1.01-2.00 reflects one truncated arm missing 1% of length; a value around 8.00 represents at least four truncated arms, and a 16.00 represents an octopus that is completely missing all of its arms. Part B shows how we calculated the approximate volume of tissue missing from a truncated octopus arm. Each arm is a roughly conical muscular hydrostat, which has a fixed volume. We used the formula for the volume of a cone to estimate the total volume and volume missing from an arm truncated by an injury. Since it is impossible to know the actual total length of a truncated arm, we extrapolated the length in two ways: based on the ontogenetic scaling relationships of the pristine arms of our museum specimens ("scaling-based"), and based on the mean length of the pristine arms of the individual ("*in situ*").

Figure 2. Number of arms lost, length missing, and Injury Severity Index (ISI) scores over ontogeny in eight species. Part A shows a strong relationship between body size (interocular distance, IO) with the Injury Severity Index (ISI), which combines number of missing arms with the proportion of each truncated arm, in *Octopus bimaculatus*, *O. fitchi*, and *O. hubbsorum*. Part B shows the distribution of Injury Severity Index (ISI) scores in museum specimens and live octopuses. The specimens in this study had ISI scores between 0 and 9.10, with a wide distribution of injury severity both within and across the species. Although a majority of octopus specimens had some arm injury, the largest plurality had zero injuries. The most common incidences of injury in an individual were contained to just one arm, with greater number and severity being increasingly less common. 75 live *O. bimaculatus* were surveyed for arm injuries each summer (July, and sometimes into August) between 2017 and 2021. The distribution of ISI values closely resembles that of the museum specimens: Many octopuses were uninjured (ISI=0), and the plurality of those injured had a single arm injury (ISI=1.01-2.00), with increasingly fewer more severe injuries. The live specimens did have higher ISI values overall than the 56 museum specimens of *O. bimaculatus* in this study (up to 12.5 versus 9.1, respectively). This could be related to the time of year that the specimens were procured, as the live specimens were all surveyed in the summer and the museum specimens were caught across all seasons of the year.

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Figure 3. Correlation between measurements of missing volume. Part A shows how close our two methods of calculating the total volume of arm tissue lost in each specimen were. The x-axis represents a volume calculation based on the mean length of remaining pristine arms on an individual, which we call *in situ* volume. The y-axis volume calculations are based on the ontogenetic scaling relationships of each pristine arm within the museum specimens, which we call scaling-based volume. Negative data values in these graphs are a result of the truncated arm being longer than the predicted (scaling-based) or estimated (in situ) length of the individual arm. High r^2 values in all eight relationships support the idea that *in situ* estimates of arm length are sufficient to estimate biomass sublethally contributed to the food web by octopuses. While scaling-based volume calculations may be slightly more accurate, they are not necessary to estimate biomass loss for species that are rare or not well-represented in museum collections. Part B shows mostly strong relationships between ISI and in situ volume missing for each species; *O. californicus* and *E. dofleini* had much lower ISI scores than the other six species. This may be due to ecological conditions: *O. californicus* lives at greater depths than the others, and *E. dofleini* is orders of magnitude larger than the rest of the species in this study.

TABLES

Table 1. Summary of injury data, arms injured, and proportion of arm length lost in museum specimens. Injury severity index (ISI) combines number of injured arms with proportion of arm length lost and ranges from 1.01-16.00. An ISI value of 0 indicates a completely uninjured arm.

Species	n	Mean ± SD number arms injured (max)	Grand mean ± SD % arm length lost (max)	Mean ISI value (max)	Mean ± SD *in situ* volume missing (mm^3)
O. bimaculatus	56	1.29 ± 1.5 (6)	23.4 ± 24% (70%)	1.64 (8.05)	149.2 ± 253
O. bimaculoides	64	1.01 ± 1.2 (6)	23.2 ± 25% (90%)	1.25 (8.35)	61.7 ± 120
O. californicus	60	0.79 ± 0.9 (3)	18.9 ± 22% (66%)	0.91 (3.21)	38.7 ± 71
P. digueti	49	1.63 ± 1.8 (7)	27.5 ± 23% (83%)	2.09 (9.10)	83.8 ± 152
E. dofleini	33	0.64 ± 1.9 (4)	22.4 ± 28% (59%)	0.74 (3.63)	27.8 ± 202
O. fitchi	60	1.58 ± 1.7 (7)	29.7 ± 23% (61%)	2.06 (9.07)	52.5 ± 78
O. hubbsorum	70	1.27 ± 1.4 (6)	23.7 ± 26% (75%)	1.62 (7.30)	116.1 ± 275
O. rubescens	73	1.18 ± 1.4 (6)	17.8 ± 30% (79%)	1.41 (7.57)	49.8 ± 157

CHAPTER 3

ARMED AND DANGEROUS: HOW *OCTOPUS BIMACULATUS* RESPOND TO A

RESIDENT PREDATOR, THE CALIFORNIA MORAY EEL (*GYMNOTHORAX*

MORDAX)

INTRODUCTION

A major driver of evolution in morphological and behavioral traits is predation (Lima

& Dill, 1990). Prey have developed defense structures and behaviors to repel or

confuse predators. Primary defenses, most notably crypsis, reduce the chance an

organism will interact with a predator. Strategies for secondary defenses range

widely, and provide diverse examples of morphological and behavioral novelty in a

variety of taxa. Weapons such as pincers, horns, claws, and teeth and the emission of

noxious compounds can threaten and or cause pain and encourage a predator to

retreat. Animals may use a combination of primary and secondary defense tactics

depending on the level of perceived threat. The effectiveness of these tactics varies

across individuals, as risk depends on intrinsic (e.g. body size, personality) and

extrinsic factors that the organism must assess and behaviorally modulate (Lima &

Dill, 1990).

Benthic octopuses indigenous to the rocky reefs of Southern California are a

good model to examine antipredator behaviors in multi-limbed organisms, as they are

voracious mesopredators that depend heavily on their arms to make a living.

Octopuses use a combination of morphological traits and cognitive abilities to evade

even sophisticated predators. They are well known to change their skin color and

texture in fractions of a second, expel clouds of ink as a smokescreen and distraction, and squeeze through impressively small holes crevices. Most benthic octopuses are not externally sexually dimorphic aside from arm R3, which is specialized for reproduction in males. The main source of morphological variation in mature adults is body size, as growth is dependent on diet and water temperature (Aguado Giménez & García García, 2002). Therefore, body size, and the characteristics associated with size, may influence survival behaviors, such as resource partitioning (Scheel, Godfrey-Smith, & Lawrence, 2016) and antipredator defense.

We know little about how octopuses use specific arms to defend themselves. The limited body of literature suggests that octopuses have a broad suite of predator avoidance behaviors that they employ (Hanlon, 1999; Hanlon & Messenger, 2018; Packard & Sanders, 1971), specifically using arm pair 2 (arms L2 and R2) in deimatic displays. Although strangling of conspecifics has been documented (Huffard & Bartick, 2015), the differential use of individual arms in an escalated interaction with a predator has never been quantified. Octopuses may be preyed upon by fishes (Feder et al., 1974), marine mammals (Goodman-Lowe, 1998), and other octopuses (Ibáñez & Keyl, 2010). Fishes such as California morays (*Gymnothorax mordax*) are known to commonly eat whole octopuses, or amputate the arms of octopuses for consumption (Higgins et al., 2018; Lane, 1974). Specifically, octopus arms are regularly found in the gut contents of California morays caught around Two Harbors, Santa Catalina Island (Higgins et al., 2018). These arms presumably come from California two-spot octopus (*Octopus bimaculatus*), a populous species in their

habitat. Natural history books describe some details of these predatory interactions in two qualitative experiments: MacGintie and MacGintie showed that *O. bimaculatus* used an ink cloud to dull the olfactory senses of a California moray (MacGintie & MacGintie, 1949). In his book, Lane describes how a moray uses a spinning behavior to amputate the arms of a large octopus to eat it piecemeal (Lane, 1974). Two sets of quantitative experiments have described some antipredator behaviors for tropical octopus. Gruber (1973) concluded that *Octopus vulgaris* and *O. briareus* mostly froze or retreated when confronted with a spotted moray (*Gymnothorax moringa* or *G. vicosa*). Grüninger found that octopuses primarily use vision to detect morays, and that they regularly use a freezing behavior paired with apnea to avoid detection by morays, which appeared to hunt by scent (1997). Octopuses are known to use their arms aggressively against fish partners while cooperatively hunting (Sampaio, Seco, Rosa, & Gingins, 2020), and *Abdopus aculeatus* have been noted to use anterior arms against agonistic fishes (Huffard, 2007).

This study quantifies retaliatory behaviors shown with individual arms, or the reactions of octopuses against predators, in an experimental setting. *O. bimaculatus* were collected *in situ* and introduced to a California moray (*G. mordax*) to examine the behaviors leading up to the incidence of the moray biting the octopus. Here, I present ethograms of the suites of behaviors employed by octopuses that successfully evaded an escalation of physical contact with a moray, and those who experienced escalations leading to one or more bites. I quantify the counts and durations of defensive behaviors in each encounter, and compare these behaviors between the

sexes and across body sizes. I first hypothesized that sex would influence the behavioral repertoire of *O. bimaculatus* in an antipredator context due to males protecting their specialized reproductive arm, R3. I also hypothesized that body size would affect antipredator strategies: larger octopuses would employ more risky behaviors (i.e. reaching toward and grabbing the moray) than smaller ones because they would be more able to fend off a predator. Finally, I predicted that I would find a bias for bites to occur on the anterior arms radially opposing arm R3, also as a result of behaviors meant to protect the reproductive arm.

METHODS

Study species

39 adult *Octopus bimaculatus* were collected by hand on SCUBA from sites around the small boat area of Two Harbors, Santa Catalina Island, CA (33.448123 N, -118.493997 W). Field seasons occurred for six weeks June-July 2019, and four weeks July-August 2021. Using dive lights, octopuses were located in dens along 10 m depth contours in rocky reef habitats outside of marine protected areas. Once they were determined not to be brooding eggs, approximately 50 mL acetic acid diluted with seawater was deployed into the den as a mild irritant, and the octopus that exited was gently collected and placed directly into a mesh bag. To prevent using the same octopus twice in a given year, each octopus was photographed and any prior injuries (i.e. arm truncations, regrowth, and skin abrasions or scars), as well as sex and body size, were noted and compared with individuals previously collected at that site during the field season. Multiple collection sites were searched to further reduce the

chance of repeating octopuses. *O. bimaculatus* only lives 1-2 years, so repeating

octopuses between years was unlikely. The strong seasonality of octopus generations

meant that I was only able to collect adult octopuses. Octopuses were brought back to

the marine station and acclimated in mesh cubes in a holding tank filled with fresh

seawater for a minimum of 24 h prior to trials. After trials, if octopuses were not

visibly injured (no new skin lesions or arm injuries were present), I released them

back into the reef where they were collected. No octopuses incurred new visible

injuries from this experiment. Behavioral data were collected from 37 octopuses (18

males, 23-35 mm IO; 18 females, 23-40 mm IO) and one octopus of undeterminable

sex due to a truncated arm R3 (34 mm IO). Males in this study were not as large as

females, but were not significantly smaller (t-test, $p > |t| = 0.1338$).

To collect California morays (*Gymnothorax mordax*), I used baited, modified

two-chamber traps that had been left to soak for 14 h between dusk and dawn. Each

of the ten captured morays was anaesthetized to obtain its standard length and then

manually palpated to ensure it had an empty stomach. Morays were PIT tagged as

part of a long-term population study, and I was therefore able to determine that no

morays were used more than the intended four trials, and were not repeated between

field seasons. These morays ranged between 462-717 mm standard length. Sex could

not be determined in live morays without sacrificing individuals. Once trapped,

morays were brought back to the USC waterfront and kept in sea water tanks (48 by

30 by 119 cm) with large-diameter PVC joints as shelters. After completing four

trials, morays 1-4 and 7-10 were returned to the approximate trapping location, as this

species exhibits high site fidelity (Higgins & Mehta, 2017). Morays 5 and 6 were transported to the UC Santa Cruz Long Marine Lab in coolers with chilled, oxygenated seawater for separate studies.

Both octopuses and morays were collected under CDFW permit # S-190830002-19086-001. Our samples of octopuses and morays evaluated, and strove to mitigate, sampling bias following the STRANGE framework meant to improve the ethical practices of animal experimentation (Webster & Rutz, 2020).

Experimental setup

Experimental methods were ethically reviewed and approved by University of Southern California (USC) IACUC Protocol # 20919-CR001 with a Memorandum of Understanding from UC Santa Cruz IACUC.

The experimental trials were conducted in flow-through tanks where each moray was housed, and began one hour before sunset due to the morays' crepuscular hunting strategy. Opaque white dividers kept the animals in the front half of the tank for a close lateral view of interactions. Opaque partitions on the ventral and lateral sides of the tank isolated the focal dyad (moray and octopus pairing) from surrounding views. A shelter was intentionally not included, as it would potentially trap octopuses into greater injury, and obscure the view of behaviors, which would render the trials more dangerous and less efficient. A pair of cameras, either two GoPros or two Akasos, were mounted above and in front of the tank to obtain dorsal and lateral views of each trial. Video of the trials was recorded at 60 frames per second.

Behavioral trials

A trial commenced when the octopus was introduced by hand to the experimental tank, at which time the octopus and moray were allowed to interact freely and uninterrupted for 60 minutes. Each octopus only experienced one 60-minute trial, to reduce stress and habituation. A maximum of four octopuses were exposed to the same individual moray to reduce the effect of moray behavior. Observers stood at least three meters away from the clear sides of the tank to avoid distracting the octopus, but remained sufficiently close by to watch the progress of each trial.

Data collection and analysis

Most behavioral data were collected continuously over the 60-minute duration of a trial from the overhead view of the tank in each trial, supplemented by the corresponding lateral view as needed. Recorded octopus behaviors included locomotion (crawl, jet), arm use (reach, recoil, grab), and other antipredator behaviors meant to intimidate (curl, expose suction cups) and evade (ink) potential attackers Table 1 provides descriptions and known interpretation (or function) of these behaviors. The count and duration of each octopus' behaviors leading up to, during, and after an arm loss were also recorded. Additionally, I noted the arm used in each behavior and distinguished the direction associated with the behavior as either toward (in the direction of the moray), neutral (parallel to the moray), or away (in the opposite direction of the moray). The timestamp of each occurrence of a moray biting an octopus was recorded; it was not evident that an octopus ever bit a moray, thus no such data were collected. If ink obscured view of the behaviors, the video between the

time the animals were obscured and the time the first behavior was observable again was left out. The length of this depended on the flow of water through the tank, and whether the octopus was situated close to the lateral camera.

I constructed ethograms for octopus under three scenarios: 1) no engagement by the moray, 2) successful evasion of moray attacks, and 3) escalated interactions resulting in bites. Duration data were natural log transformed to reduce the high variance across behaviors. I then examined the relationship between behavioral duration and the corresponding behavioral counts (e.g. reach duration, and reach frequency). Because I found that durations and counts of behaviors were correlated (Supplementary Table 1), I used a Principal Component Analysis (PCA) on natural log transformed durations only to determine which suites of behaviors explained the most variation in arm use. I did not include the behavioral durations for ink, jet, and remaining still, as these behaviors are not performed primarily with arms. I tested for the effect of body size on each of the significant principal components, designated with an Eigenvalue greater than 1, within each sex by regressing the PC axes against interocular distance, a metric of octopus size (Roper & Voss, 1983).

Based on the behavioral repertoire of *O. bimaculatus*, I hypothesized that reaching toward the moray and grabbing the moray were the highest-risk behaviors in this study. Reaching somewhat parallel to the moray and reaching toward the moray were combined for analysis, as I observed some instances of reaching during the approach of a moray may have been interpreted as redirection or decoy behavior. To determine whether a particular arm was being used more frequently to perform these

high-risk behaviors, I conducted Rayleigh circular analyses and generated circular plots using the R package CircMLE (Fitak & Johnsen, 2017) to determine whether a particular arm was being used more frequently to perform these high-risk behaviors. Circular analyses are useful for identifying directional bias in behaviors of organisms with radially arranged arms, like brittle stars (Sumner-Rooney, Kirwan, Lüter, & Ullrich-Lüter, 2021) and octopuses. I also used Rayleigh circular analyses to test whether octopuses oriented themselves to bias moray bites towards a single or multiple arms. A significant bias is associated with a p-value less than 0.05.

RESULTS

Ethograms

Nine discrete behaviors were recorded across our 37 trials: crawl, curl, expose the oral surface of suckers, grab, ink, jet, reach, recoil, and still. Table 1 shows what these behaviors looked like. Table 1 includes illustrations, counts, and durations of all recorded behaviors. I constructed several ethograms (Supplementary Figure 1) that show behavioral sequences for non-escalating ("No escalation") encounters (n=4), encounters that warranted antipredator behaviors but did not result in a bite ("De-escalation," n = 16), and the behaviors before and after trials with bites ("Escalation," n = 17). I observed little consistency in the sequence of specific behaviors shown in these three scenarios.

The context of the moray and octopus behavior immediately preceding a bite differed between the 33 bites observed in 17 of the trials. Figure 1 shows the direction of the moray and the octopus in each of these encounters, as well as the behaviors

immediately following a bite grouped by the direction of the octopus immediately

preceding the bite. The contexts of interactions ranged between both animals

approaching one another, to one pursuing the other that was behaving neutrally or

retreating. Behaviors preceding the first three bites involved similar proportions of

approaches from the octopus and the moray. The one trial that contained more than

three bites occurred because bites #4, 5, and 6 were solely in retaliation against the

octopus grabbing the moray. Octopuses that were approaching the moray before a

bite, which happened 21 times, were much more likely to get bitten, and exhibited a

greater variety of reactions to a bite, than octopuses that were retreating (n=7), or

neither approaching or retreating, i.e. moving parallel, or were mostly still (n=5).

Octopuses that were already retreating from a moray bite did not expel ink.

Variation in antipredator behaviors

The first three principal components, which had Eigenvalues greater than 1, were

treated as the significant axes of variation due to a lack of a distinct break in the scree

plot. PC axes 1 & 2 cumulatively explained 58.1% of the variation in arm use

duration (Figure 2). Behaviors that loaded strongly and positively on PC1 (31.1%)

were curl and crawl, while grab loaded strongly and negatively. On PC2 (27%)

exposing suckers loaded strongly and positively while reaching, extending the arm

away from the body, loaded strongly and negatively (Figure 2). Males and females

occupied distinct areas of behavioral space with females uniform along PC1 while

males were more disjunct. The behaviors used in the PCA to reveal patterns of arm

use included crawl, curl, expose suction cups, grab, and reach. Recoil was rarely recorded, and therefore was removed from analyses.

There was no overall relationship between body size (IO) of both sexes and PC1, nor did I find a relationship between IO and PC1 for females (p=0.2). Males, however, showed a strong correlation between PC1 and IO, suggesting that larger males were more likely to grab the moray for longer, while smaller males moved around and tried to curl for longer durations. There was a significant positive correlation between body size and PC2 (p<0.0001) for both sexes (Figure 3). In both sexes, smaller octopuses reached for longer, while larger octopuses spent more time with arm tips tucked in and the oral surface of suckers exposed. Behaviors loaded along a spectrum of arm use on PC3: Reach, which was mostly performed with a single arm, loaded the most positively, farthest from crawl, which was regularly, but not always, performed with all eight arms in concert. Body size and PC3 were significantly positively correlated (p=0.0005) for both sexes. This suggested that smaller octopuses were displaying behaviors with individual arms for longer, and larger octopuses were using more of their arms in concert for longer.

Arm use in antipredator behaviors

Because the PCA analysis showed male and female behavioral repertoires differed along the main axis of variation (Figure 2), I compared the durations and counts between the two sexes. Males crawled for significantly longer ($\bar{x} = 588.3 \pm 40.3$ s; t = 3.75, p = 0.0002), and crawled significantly more frequently ($\bar{x} = 33.0 \pm 1.5$; t = 10.00, p < 0.0001). Females stayed still about as frequently as males, and both sexes

jetted approximately the same number of times. Threat display strategies differed

between the sexes. Males employed "curl" more frequently ($\bar{x} = 42.3 \pm 3.4$; t = 6.24,

p < 0.0001) than females ($\bar{x} = 12.5 \pm 18$), and held the display for significantly longer

($\bar{x}_{males} = 1186.7 \pm 698$ s, $\bar{x}_{females} = 686.5 \pm 987$ s; t = 2.95, p = 0.0006). Males and

females exposed their suckers a similar number of times ($\bar{x}_{males} = 7.8 \pm 0.7$, $\bar{x}_{females} =$

7.4 ± 0.8), and females spent slightly more time exposing their suckers ($\bar{x}_{males} = 802.6$

± 1196 s, $\bar{x}_{females} = 866.0 \pm 1027$ s). Male and female octopuses inked to evade the

moray a similar number of times.

The behaviors I perceived as high-risk also differed between males and

females. In general, males used their arms to reach significantly more frequently ($\bar{x} =$

3.0 ± 0.4; t = 2.35 p = 0.02) and for longer ($\bar{x} = 15.3 \pm 2$ s; t = 4.01, p < 0.0001) than

females ($\bar{x} = 1.6 \pm 0.5$ reaches, lasting $\bar{x} = 3.3 \pm 2$ s). Figure 4 shows the

directionality of arm use in these reaching behaviors: Across all individuals, reaches

were significantly biased toward arm L1 (Figure 4), with a Rayleigh's test statistic of

0.236 (p = 0.003). Females had a significant bias for reaching with arm L1

(Rayleigh's = 0.306, p = 0.007), while the slight bias for males fell between L1 and

L2 (Rayleigh's = 0.163, p = 0.145). Both sexes grabbed (engaged their suckers on the

moray or on the outflow pipe) about the same amount of time (except two aggressive

females, L and M, and one female, R, who grabbed at the tank's outflow pipe, away

from the moray, many times). Females grabbed significantly more times ($\bar{x} = 0.26 \pm$

0.04 grabs; p = 0.0045) than males ($\bar{x} = 0.068 \pm 0.04$ grabs). Grabbing the moray was

significantly biased toward arm L1 across all octopuses (Figure 4; Rayleigh's=

0.225; p = 0.018). Males demonstrated a bias for grabbing with arm L1 (Rayleigh's = 0.332; p = 0.077) that fell closer to R1 than L2, while the slight bias for female grabs fell in L1 close to L2 (Rayleigh's = 0.185; p = 0.148). Two of the three bites for the single octopus with an unidentifiable sex also occurred on arm L2, which contributed to the significant result of all grabs.

Bites

A total of 33 bites were observed in 17 of the 37 analyzed trials (Table 3). Octopuses experienced between one and six bites in a 60-minute trial. Females were bitten 25 times, while males were only bitten five times. The octopus of unidentifiable sex, Octopus EE, was bitten three times. None of the morays bit all four of their corresponding octopuses; Morays 2 and 7 did not bite any of their corresponding octopuses.

Bites were concentrated around arm L2 (Figure 5; Rayleigh's = 0.303, p = 0.099). As females were bitten disproportionately more, they drove this pattern; however, males and Octopus EE were also bitten most frequently on arm L2.

DISCUSSION

Interactions between these *O. bimaculatus* and *G. mordax* could be organized into three categories: no escalation, de-escalation, and escalation (Supplementary Figure 3.1). Across these interactions, octopuses in this study demonstrated a wide variety of antipredator tactics, ranging from sitting completely still, to pursuing and grappling with the moray. Despite the variation in strategies by the 37 individuals, I uncovered some general patterns within the three interaction categories. In the four "no

escalation" trials, octopuses simply sat still, or moved away from the moray at opportune moments between long bouts of staying still. These octopuses were all successful in avoiding detection. More active octopuses that did not attempt to engage the moray, but moved away or expelled ink at the beginning of the trial, evaded getting bitten despite being pursued. Inking can be an effective de-escalation tactic, as it obscures both visual and olfactory detection (Hanlon & Messenger, 2018). Inking did not prevent an octopus from being bitten. Octopuses in trials that escalated to a moray bite were crawling toward, exposing suckers toward, reaching toward, or grabbing the moray at the moment they were bitten, regardless of the moray's directionality (Figure 1). Some sampling bias may limit the generalizability of these findings. Only mature octopuses were tested, so one cannot infer how behaviors would vary over ontogeny. These trials occurred in the summer, and therefore, octopus and moray behaviors may vary with season. Additionally, experiments occurred in a tank without substrate or structure to obtaining the clearest view of the interactions and compare behavioral displays between trials that occurred in different tanks; thus, antipredator defenses may be different inside an enclosed crevice, or with another type of predator.

Suites of antipredator behaviors differed by sex and body size. Larger males tended to grab and hold the moray, potentially because they were large enough to successfully fend off the moray. Smaller adult octopuses moved around the tank and curled their arms with the suckers tucked in and web extended to look larger. This posture is known as either a dymantic or deimatic display, and has been described as

an attempt to startle an attacker; it is increasingly employed across ontogeny in

Octopus vulgaris (Packard & Sanders, 1971). In our study, males also reached toward

and grabbed the moray with arms centered around arm L1; these arms radially oppose

the hectocotylized arm R3, which must be intact in order to copulate. The strategies

males used were highly successful in preventing bites. Of the 18 male octopuses, only

5 were bitten (29%), while 11 of the 18 females (61%) were bitten. Females did not

vary as much along PC1, and therefore did not demonstrate the same size-related shift

in antipredator strategy. Females crawled around the test arena for longer amounts of

time, employed fewer threat displays (e.g. curling the arms and exposing suction

cups). Two females also grappled for an extended amount of time with the moray,

leading to one of them being bitten six times (Table 3). It is unclear why females

were bitten much more frequently in these trials, but due to trials occurring during the

reproductive season using mature adults, I posit that the behavioral differences

between the sexes may be associated with preserving the ability to reproduce. The

male imperative is to preserve their reproductive arm for as long as possible, so

employing a mainly avoidant strategy would be important. Females, which don't have

any external reproductive structures or specialized arms in this species, might rely

more on the ability to lose and later regenerate their eight redundant limbs. Their

reproductive investment terminates in a period of defending and aerating their egg

clusters inside a den, which does not presumably require all eight arms to achieve.

Further examination of arm use in egg rearing, and other differences in antipredator

strategies associated with sex, would reveal more about the differences in arm use I observed here.

I discovered that arm L2 was bitten most frequently in females as well as males, despite their disparate antipredator strategies. It has been noted that *O. vulgaris* can produce a deimatic display either by enlarging the web of, or extending, arms L2 and R2, but the sex and directional orientation of these individuals is unknown (Packard & Sanders, 1971). I identified arm L1 as the most frequently used arm in high-risk behaviors. It has been noted that octopuses orient themselves toward an object at a 45 degree angle to improve their visual acuity (Byrne et al., 2006) which would allow them to make use of L1 more often. This orientation puts the arms in pair 2, L2 and R2, at a disproportionate risk. Previous observations of museum specimens of male *O. bimaculatus* revealed a bias toward truncations of arm L2 (Voss & Mehta, 2021). While males are underrepresented in the occurrence of bites in this study, I did find L2 was qualitatively bitten most frequently (2 out of 5 times) in males, supporting our findings from museum data. More detailed analysis of octopus body orientation in an antipredator context is required to determine if orientation influences L2 injuries. The frequency of injury is especially interesting with respect female octopuses that do not have a specialized arm they need to protect.

The behavioral context immediately preceding each of the 33 bites increased our understanding of these predator-prey interactions. Morays and octopuses were most frequently approaching one another preceding the bite (Figure 1). I identified

many of the resultant bites as predatory, because the moray exhibited spinning along its long axis as part of the biting behavior (Diluzio, Baliga, Higgins, & Mehta, 2017; MacGintie & MacGintie, 1949). Morays biting a completely still, non-displaying octopus did so without spinning or opening their jaw wide, as if they were investigating the object to see if it was edible. I observed several retaliatory bites as well. For example, when a retreating moray was grabbed by an aggressive octopus, the moray would turn back around and bite the octopus. Bite duration, regardless of the type of bite, was brief, less than one second long. Interestingly, none of these bites resulted in a visually detectable injury, much less an amputation, despite several observations of spinning behavior meant to separate the octopus arm from the body.

This study is the first to focus on how octopuses use their arms in secondary antipredator strategies. Much work has set out to describe their highly developed and effective primary defenses used to avoid predatory interactions, but our observations of secondary responses show they can be important in determining the result of these predator-prey interactions. Suites of secondary defense behaviors varied between sexes, which I attributed to the differential reproductive behaviors required of the mature adults that comprise our sample. Within each sex, strategies varied with body size. Smaller octopuses were more cautious and employed threat displays while larger octopuses grabbed at the moray. I observed a directional bias toward the anterior left arms, L1 and L2, in both preferential use and the occurrence of strikes from a predator. These results may provide a context for patterns of arm injuries acquired over the life history of octopuses (Chapter 1). Although I reveal antipredator

behaviors across three different contexts, caution is clearly needed when interpreting our laboratory results. Predator-prey interactions in laboratory settings cannot replicate interactions that take place in the wild. However, given the paucity of studies examining antipredator behavior in octopuses, our controlled observations have value and provide further context to how cryptic organisms lose their arms and the nature of sublethal predatory events.
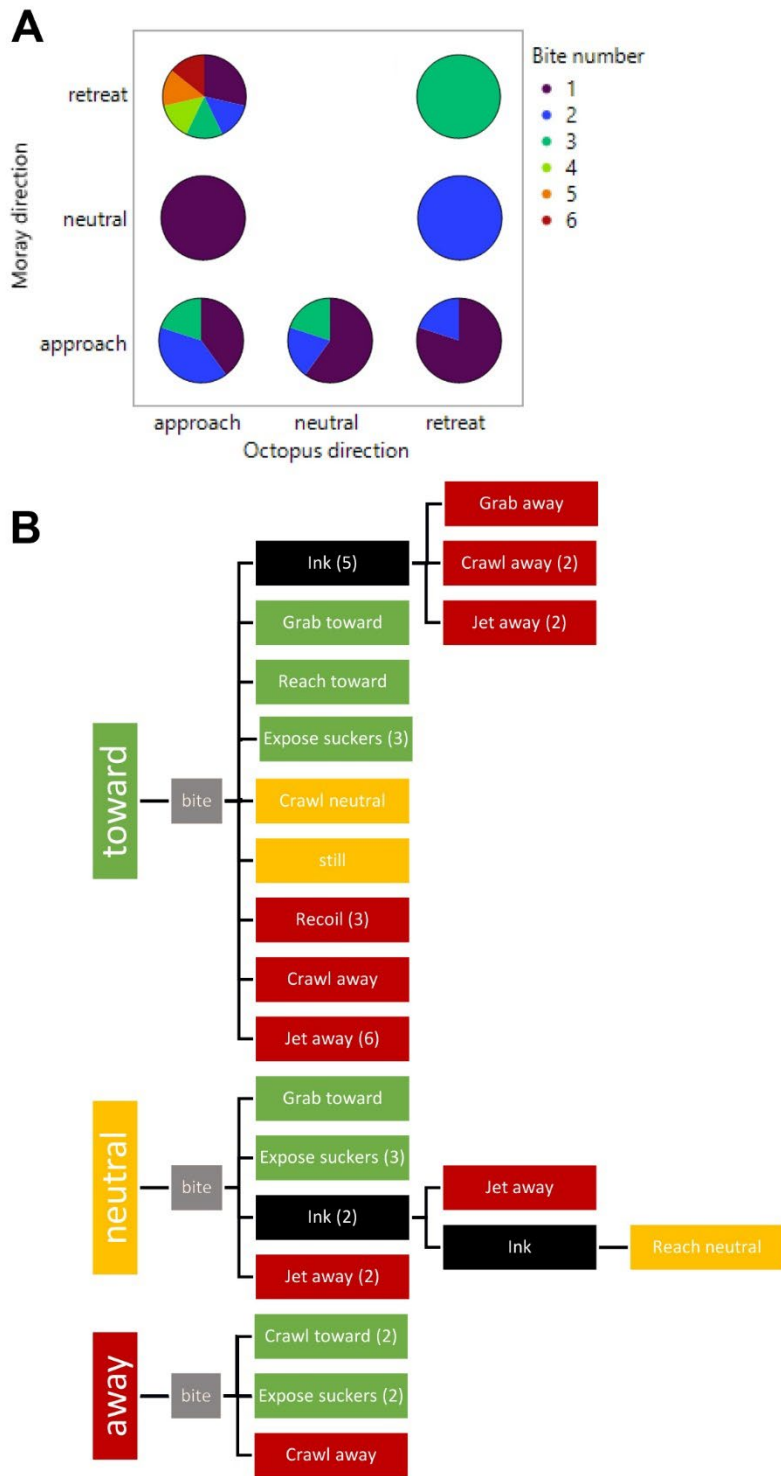
FIGURES

Figure 1. Behavioral contexts of interactions between *O. bimaculatus* and *G. mordax* resulting in bites. A. Direction of moray and octopus immediately before a bite. Octopuses approaching morays moving in any direction were bitten more often than octopuses that remained still, moved parallel to the moray, or were retreating from the moray. The color of each data point denotes the bite number (from one to six) within the trial. Numbers associated with each wedge represent the proportion of total bites that represent a given bite number. B. Ethogram of the behaviors that occurred immediately preceding and following a bite. The rotated words on the left indicate the trajectory of the octopus behavior before a bite. The color of each box indicates the directionality of the behavior: green means toward, yellow is neutral or parallel, red is away, and ink is black. Inking often facilitated a successful short-term evasion of the moray when it was employed, but notably, the octopus did not ink if it was already retreating from the moray. A greater variety of reactions was observed after bites where the octopus was approaching than in the other contexts.
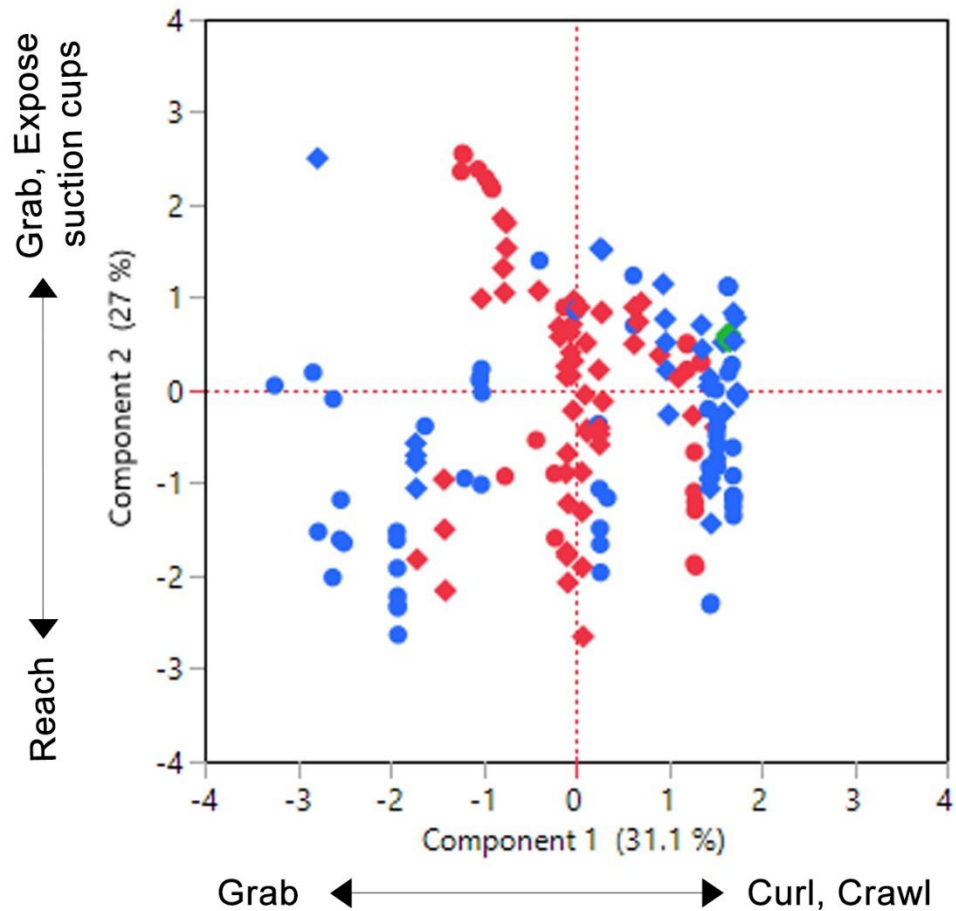
Figure 2. Principal Component Analysis (PCA) of five major arm use behaviors: crawl, curl, expose suckers, grab, and reach. Duration data in seconds were natural log transformed for analysis. Count data and duration data were strongly correlated, so count data were not included in the PCA. Males are shown in blue, females are shown in red, and green represents an octopus of unknown sex (missing tip of arm R3). Circles indicate trials without a bite and diamonds indicate trials that escalated to at least one bite; there were no differences based on escalation shown along these axes of variation.
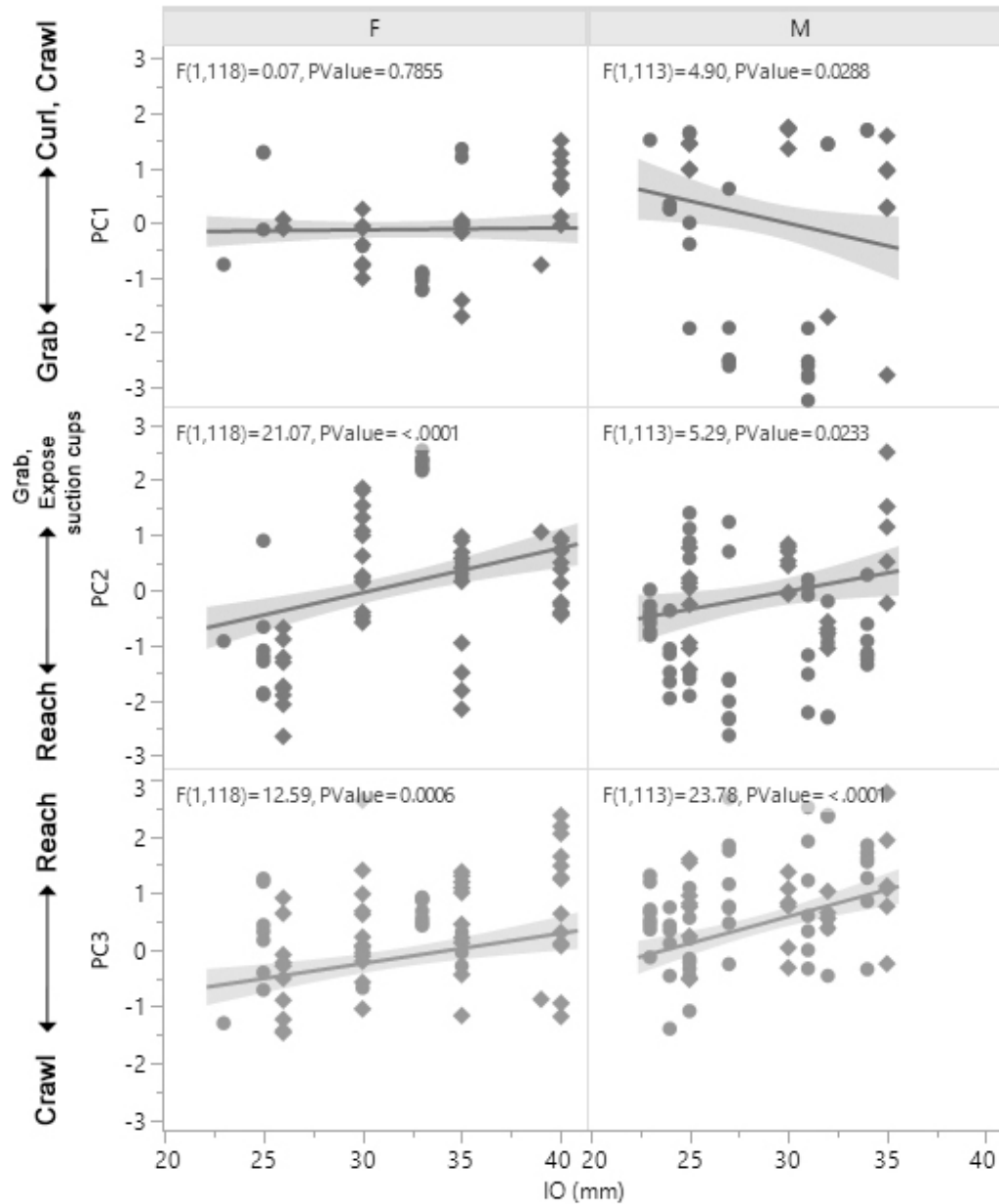
Figure 3. Relationship between body size (interocular distance, IO) and significant principal components. There was a significant relationship between body size and behavioral variation in all comparisons except for females for PC1.
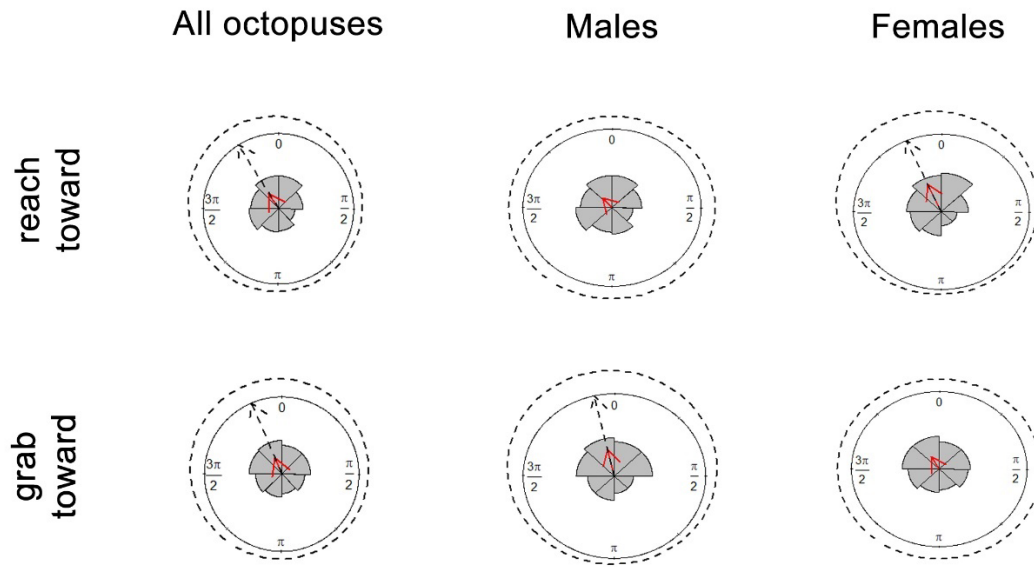
Figure 4. Circle plots of risky arm behaviors, reach parallel/toward and grab moray, for the whole sample and separated by sex. The mean vector direction is indicated by the red arrow. The black dashed arrow shows the predicted mean direction of the bias, and the black dashed line around the outside is the shape of the model density weight. Whole-sample analyses (left) included one octopus of undeterminable sex (Octopus EE) that was bitten three times.
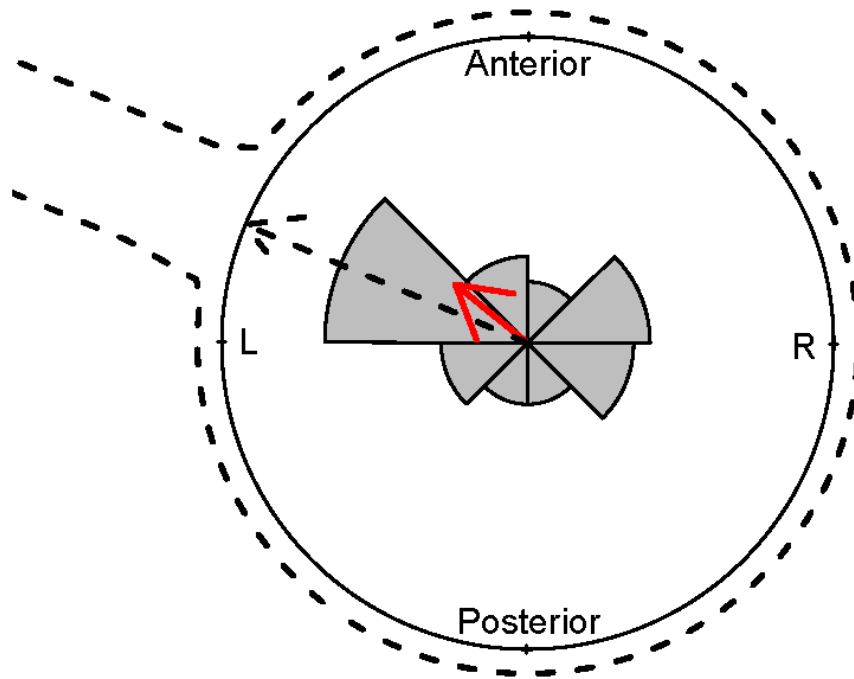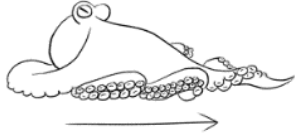
Figure 5. Circle plot of the directionality of bites. The mean vector direction is indicated by the red arrow. The black dashed arrow shows the predicted mean direction of the bias, and the black dashed line around the outside is the shape of the model density weight. Circular analyses demonstrated that bites of octopus arms were concentrated toward arm L2 (Rayleigh's = 0.303, p = 0.099).

Table 1. Descriptions of behaviors and summaries of behavioral data. Counts and durations represent the uses of each individual arm in each trial, except in describing ink, jet, and still.

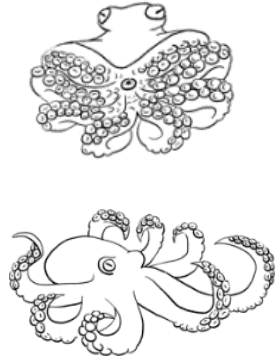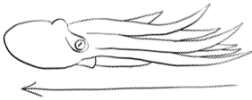| Octopus Behavior (reference)	Function	Description	Count (Mean ± SD)	Duration in s (Mean ± SD)	Example
Crawl ("Multi-armed walking," Huffard, 2007)	Locomotion	Multiple arms walk along the substrate with arms sprawled around the body	491.6 ± 493	9.2 ± 13	
Curl (Fig. 13, Packard & Sanders, 1971)	Deimatic display	Arm curled down with web extended; display to appear larger	240.1 ± 270	32.9 ± 84	
Expose oral surface of suckers (Figs. 9-11, Packard & Sanders, 1971; "retroflex" and "oppose," Mather and Alupay 2016)	Defensive display	Arms curled up with tips in and oral surface of suckers out, as a withdrawal display (top) or defensive display (bottom) following Packard and Sanders.	63.8 ± 59	98.2 ± 208	
Grab	Secondary defense, object manipulation	Uses suckers to manipulate an object	10.8 ± 16	23.0 ± 55	
Ink (Bush & Robison, 2007)	Avoidance, primary defense	Production and expulsion of melanin and mucus from ink sac via siphon	6.6 ± 7	1.0 ± 0.2	
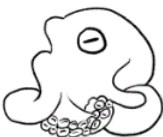
Jet ("Jet (backward swimming)," Huffard, 2007; "Posterior jet," Mather & Alupay, 2016)	Locomotion	Uses funnel for rapid propulsion through water; body elongate, skin smooth; elongate, arm tips curled or straight	9.6 ± 11	3.2 ± 4	
Reach [Recoil] (Kier & Stella, 2007; Packard & Sanders, 1971)	Exploration, potential decoy display?	Extends arm away from body, either via propagating wave or uncurling of arm; suckers not engaged [retracts after reach]	13.1 ± 18 (13.3 ± 17)	6.1 ± 8 (1.6 ± 1)	
Still	Avoidance, primary defense	For the purposes of this study: absence of locomotion, arm use, inking. May incorporate changes in skin color or texture.	13.2 ± 14	123.4 ± 235	

Table 2. Eigenvectors of behavioral durations in all principal components. The number of seconds that each of five behaviors (crawl, curl, expose suction cups, reach, and grab) was recorded for each of the arms of 37 octopuses was totaled and natural log transformed. The proportion of the variation in the data explained by each of five principal component axes is included in parentheses. Since a scree plot did not reveal a clear inflection point in the Eigenvalues of these five components, I could not use the broken stick method to define significant principal components. The first three principal components had Eigenvalues greater than 1, so these were therefore treated as the significant axes of variation, and are designated here with bolded font. Asterisks denote behaviors that loaded significantly along a given principal component axis.

Behavior	PC1	PC2	PC3	PC4	PC5
(% var. explained)	(31.1 %)	(27.0%)	(20.3%)	(12.6%)	(9.0%)
crawl	0.68426*	0.03575	-0.13967	0.33603	0.63094*
curl	0.63145*	0.09685	0.41938*	0.07991	-0.64002*
expose suction cups	0.10853	0.70912*	0.12691	-0.64996*	0.21639
grab	-0.34821*	0.45796*	0.53759*	0.59728*	0.15259
reach	0.00591	-0.52609*	0.70676*	-0.31861	0.34955

Table 3. Summary of bites observed in this study.

Moray	Octopus	Octopus sex	N bites
M1	B	F	1
M1	G	F	3
M3	L	F	6
M3	U	F	1
M4	F	M	1
M4	K	M	1
M4	M	F	2
M5	N	M	1
M6	O	M	1
M6	X	F	2
M8	CC	M	2
M8	EE	U	3
M8	II	F	1
M9	JJ	F	3
M10	LL	F	1
M10	MM	F	1
M10	OO	F	3

CONCLUSION

This body of work reveals how morphology can be an important starting point for investigating topics like ecological interactions and ontogeny in cryptic animals. Marine invertebrates are well known for a variety of adaptations that facilitate self-defense and recovery after sublethal predation. Differential use of morphological structures can lead to selection for size disparities in traits, especially if the trait has a survival benefit (e.g. claw size, tail size). Coleoid cephalopods have eight seemingly identical, regenerative arms that provide a rich model for asking ecological, and behavioral questions. Through my work I found that octopus arm growth is not uniform in all species, and therefore, their recruitment in different behavioral contexts may vary. Researchers have studied how octopuses partition their arms for specific tasks hypothesizing that the anterior arms are used more frequently for exploration (Byrne, Kuba, Meisel, Griebel, & Mather, 2006). Differential arm use has been noted in interspecies interactions like cooperative hunting (Sampaio et al., 2020). However, this dissertation is the first to address arm use in a high-risk antipredator context to bridge our understanding of differential arm use and sublethal injury. Altogether, this body of work used multiple types of observations from 499 museum specimens and 105 live *Octopus bimaculatus* to reveal connections between the structure of a highly cryptic, multi-limbed organism, the patterns of injury they incur, and the behaviors potentially associated with these traits.

In Chapter 1 I explored the possibility that the anterior arms, arm pairs 1 and 2, grew at a steeper rate and attained relatively longer lengths compared to the

posterior arms by examining the ontogenetic trajectory of arm growth in preserved

specimens belonging to three species of the genus *Octopus*. Once I established the

scaling patterns for the eight arms with respect to interocular distance, I determined

how arm growth and truncation patterns differed by sex and species. I found that a

majority of *Octopus bimaculatus*, *O. bimaculoides*, and *O. rubescens* had at least one

arm that was truncated and in some state of regrowth at the time they were preserved.

Using the species-specific scaling patterns, I determined that roughly one third of an

arm was lost on average across all three species, and that a significantly greater

proportion of the arms on the left side had been lost in all except *O. bimaculatus*

males. I also found different anteroposterior patterns of arm loss that varied between

the three species, and a positive correlation between body size and number of

truncated arms in *O. rubescens*. These results highlight how establishing scaling

patterns in octopuses can be used to inform the proportion of arm tissue lost due to

sublethal predation. My study also highlights the importance of museum specimens in

establishing scaling patterns in morphological traits for octopuses as their soft bodies

and highly retractable appendages may confound collecting repeatable

morphological measurements (Semmens et al., 2004). Most importantly, Chapter 1

allowed me to realize that there is an ecological need for a holistic description of arm

injury in octopuses and that quantifying the number of injured arms and the

proportion of tissue lost are distinctly different measures of sublethal predation that

are not necessarily correlated which led to my investigation in Chapter 2.

Chapter 2 allowed me to combine the number of injured arms and the proportion of tissue missing from each of these arms to create an Injury Severity Index (ISI). I then used ISI to test hypotheses about the magnitude of injuries in eight Northeastern Pacific octopus species with varying body sizes and life histories. Indices are useful tools for investigating the relationship between multiple body measurements as a single unitless value, and are frequently used as metrics of octopus morphology (Roper & Voss, 1983). As I showed, ISI is useful to describe injury, as well as demonstrate how the severity of arm injuries changes with respect to aspects of life history, or it can be used in community surveys as a way to quantify body condition. The magnitude of arm loss in octopus species has implications for the management of fisheries, bioenergetics of marine ecosystems, and understanding the behaviors of individual octopuses that have experienced sublethal predation. Beyond octopuses, an ISI can theoretically be re-scaled and used to describe partial limb injuries in any multi-limbed organism, e.g. for sea stars, scores from 1.01-10.00. Further trophic modeling should consider the impact of octopuses and other marine invertebrates being functionally grazed upon by sublethal predators.

In Chapter 3, I observed high-risk interactions between live octopuses (*O. bimaculatus*) and a common benthic predator, the California moray eel (*Gymnothorax mordax*). California morays are common benthic predators that are well-documented to consume octopus arms (Gruber, 1973; Grüninger, 1997; Higgins et al., 2018; MacGintie & MacGintie, 1949). I predicted that arm L2 would be used in self-defense most frequently by males, due to males turning to protect the radially

opposing arm R3, which was injured the least frequently in all three species in

Chapter 1. 37 trial pairings of *O. bimaculatus* with *G. mordax* resulted in 33 bites

that were concentrated on and around arm L2. The context of the bites I recorded

varied by the direction each animal was moving, and the size and sex of the octopus.

While there was a slight bias in the circular analyses toward males recruiting arm L1

to reach toward and grab the moray, this pattern was not significant. Prior studies of

Octopus vulgaris have shown that anterior arms are preferentially recruited for

exploration (Byrne, Kuba, Meisel, Griebel, & Mather, 2006; J. A. Mather, 1998).

While arm recruitment for other tasks of unknown risk like deimatic displays has

been described in *O. vulgaris* (Packard & Sanders, 1971), preferential arm use during

interactions with predators has never been quantified in any species. This study was

limited in that I could not control for the history of the individual octopuses and

morays; however, I was able to elicit a wide range of behavioral strategies that

showed a difference in antipredator strategies between the two sexes. While this

experiment was designed to test arm use, I propose that the way an octopus orients

itself to a potential source of injury (i.e. a predator or another agonistic individual) is

also linked to which arm is bitten and potentially amputated. Further investigations of

bias in arm loss should address body orientation in antipredator contexts and other

potentially risky situations.

Another striking finding in Chapter 3 was that the different suites of behaviors

each sex utilized in self-defense are associated with vastly unequal numbers of bites

between the two groups. Large males were more likely to grapple with the moray,

while smaller males made threat displays and either moved around a lot or sat mostly still to avoid being approached. Smaller females were also more likely to move around, but also reached arms away from their bodies more frequently. Instead of the "curl" threat display, larger females were more likely to expose their suction cups, which can either signal withdrawal or a fighting stance (Packard & Sanders, 1971), depending on the extension of the arms. This led to vastly different patterns of injury: ultimately, over five times more bites were incurred by females than by males, 28 versus five. Interestingly, in Chapter 1, I found that injured male *O. bimaculatus* were most likely to have three or more arms truncated, and injured females were most likely to only have one arm truncated; however, the museum specimens covered an ontogenetic series of octopuses, while Chapter 3 only draws conclusions about adult individuals. The next step in this line of investigation could be to repeat the behavioral trials with individual octopuses that reflect the full ontogenetic trajectory of body sizes for *O. bimaculatus* to determine whether there are ontogenetic shifts in antipredator strategies.

The cryptic nature of octopuses provides a challenge for understanding many aspects of the life history of this taxonomic group. An animal that is difficult to find is inherently difficult to research, so revealing new ways to describe and investigate animals like octopuses is critical to improving our comprehension of marine ecosystem processes and species interactions. This dissertation repeatedly demonstrated the utility in using museum specimens to fill in our gaps in knowledge about octopus injury patterns. Additionally, these findings provide a first

line of inquiry for octopus species that are not well-represented in collections. The power in relating form and function lies in the ability to predict unknown traits to those that are well-understood. While investigating the form and function of muscular hydrostat appendages is challenging, and does come with some degree of variation in measurements due to the lack of hard structure, I was still able to predict behaviors in a specific context from observations of basic characteristics.

Through morphological and behavioral investigations of octopuses, I have revealed more about their role as a mesopredator in the marine community. This body of work advanced our ability to determine ontogenetic patterns in arm growth, use, and injury; holistically describe the magnitude of injury in a multi-limbed organism, and its potential energetic contributions to the food web; and understand predatory interactions between two cryptic species. Continuing research into cryptic nearshore species will provide a more complete picture of the complex communities residing in critical marine habitats.

LIST OF SUPPLEMENTAL FILES

Supplementary materials available for separate download from ProQuest:

Voss_Supplementary Tables.docx Supplementary tables for Chapters 1, 2, and 3

Voss_Supplementary Figures.pdf Supplementary figures for Chapters 1 and 3

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