

Characterization of Arm Autotomy in the Octopus, *Abdopus aculeatus* (d'Orbigny, 1834)

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## ABSTRACT

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Autotomy is the shedding of a body part as a means of secondary defense against a predator that has already made contact with the organism. This defense mechanism has been widely studied in a few model taxa, specifically lizards, a few groups of arthropods, and some echinoderms. All of these model organisms have a hard endo- or exo-skeleton surrounding the autotomized body part. There are several animals that are capable of autotomizing a limb but do not exhibit the same biological trends that these model organisms have in common. As a result, the mechanisms that underlie autotomy in the hard-bodied animals may not apply for soft bodied organisms. A behavioral ecology approach was used to study arm autotomy in the octopus, *Abdopus aculeatus*. Investigations concentrated on understanding the mechanistic underpinnings and adaptive value of autotomy in this soft-bodied animal.

*A. aculeatus* was observed in the field on Mactan Island, Philippines in the dry and wet seasons, and compared with populations previously studied in Indonesia. Frequency of encountered arm loss and habitat characteristics were recorded and compared between sites, seasons, sex, and arm position. The incidence of autotomy was highest in Indonesia; nearly half of the sampled octopuses were found missing or regenerating arms from the base of the body. Most of the octopuses were male. There was no significant difference in the incidence of autotomy between Indonesia and the Philippines or between the wet and dry seasons in the Philippines. Individuals lost between one and five arms, one arm being the most common. The eight arms from each individual were not equally lost. The anterior pairs of arms were more susceptible to loss due to their function as exploratory arms. The male third right arm used for reproduction was lost less frequently.

Attempts to fully describe the behaviors associated with autotomy besides limb detachment have not been made for any organism. Multiple arm autotomy events were observed in *A. aculeatus* to generate an inventory of behaviors that are characteristic of autotomy. Sequential analysis of these behaviors was conducted and found to follow a particular order during autotomy. Some of the classified behaviors include other secondary defense mechanisms, such as jetting and inking.

To understand the mechanism for arm loss at the tissue level, histological sections and force measurements were collected from autotomized arms to characterize the autotomy zone. Individuals were also decerebrated to establish the relative importance of the central nervous system in the occurrence of autotomy. Histology revealed a zone of weakness with no specialized adaptations for loss between proximal suckers four and eight. An average tensile

force of  $0.645 \pm 0.145$  MPa was required for autotomy to occur. Eight out of ten decerebrated individuals did not autotomize an arm post-decerebration suggesting that the central nervous system plays a role in arm autotomy.

Lastly, the costs and benefits of arm autotomy to *A. aculeatus* were assessed by studying the effects of arm loss in whole body and autotomized arm locomotion. Individuals with fewer arms were not found to exhibit any negative impact to their crawling or jetting behaviors. The autotomized arms post detachment performed complex behaviors and suctioned to various surfaces for prolonged periods of time suggesting potential adaptations in the arm to distract a predator while the octopus escapes.

Overall, the octopus provided a good model system for studying the mechanisms and adaptive value of autotomy in a soft-bodied animal. Their multitude of arms were easy to manipulate and induce autotomy in the lab. The relatively high frequency of individuals missing arms in the wild suggest that autotomy plays an important defense role for *A. aculeatus* and needs further characterization to identify the underlying cause. Efforts to study autotomy in other non-model organisms will broaden and enhance our knowledge on what seems like an extreme, but common defense mechanism.

Dedicated to my family and friends –

With special thanks to my aunts Clarita Alupay and Lorenza Ancheta,  
the two most resilient women that ever graced my life with their presence.

You are my rock on a wavy sea,  
the captains of a ship taking me to my next adventure!

## TABLE OF CONTENTS

List of Tables	iv
List of Figures	vi
Acknowledgements	ix
<b>Chapter 1: Introduction and review of autotomy</b>	
Terminology	1
Criteria 1: Autotomy functions as a means of defense	2
Criteria 2: Autotomy is achieved by an intrinsic mechanism	3
Criteria 3: Autotomy is mediated by the nervous system	4
Autotomy: A behavioral approach to a secondary defense strategy	5
A. Mechanism of autotomy	6
B. Ontogeny of autotomy	8
C. Adaptive value of autotomy	10
D. Evolution of autotomy	14
Octopus as a model system to study autotomy	17
Conclusions	18
References	20
Tables	28
Figures	42
<b>Chapter 2: Behavioral ecology of autotomy in the octopus, <i>Abdopus aculeatus</i></b>	
Abstract	44
Introduction	44
Methods	46
Results	47
Discussion and Conclusions	49
References	52
Tables	54
Figures	62
<b>Chapter 3: Mechanism of autotomy – How does <i>Abdopus aculeatus</i> lose its arms?</b>	
Abstract	72
Introduction	72
Methods	74
Results	75
Discussion and Conclusions	77
References	80
Tables	82
Figures	84

<b>Chapter 4: Costs and benefits of autotomy to locomotion in the octopus, <i>Abdopus aculeatus</i></b>	
Abstract	91
Introduction	91
Methods	93
Results	94
Discussion and Conclusions	95
References	98
Tables	101
Figures	106
<b>Chapter 5: Conclusions</b>	<b>111</b>

## LIST OF TABLES

**Table 1.1.** Comparison of mechanisms used for autotomizing limbs with and without an exo-/endo-skeleton. Three features are highlighted: 1) where does autotomy occur, 2) how does it occur with respect to muscle versus passive action, and 3) what happens to the autotomized body part.

**Table 1.2.** Comparison of ontogenetic factors and patterns important for the use of autotomy in organisms with and without an exoskeleton.

**Table 1.3.** Comparison of the adaptive value of autotomy in organisms with and without an exoskeleton. This includes known examples of frequency and factors that influence the occurrence of autotomy as well as associated costs and benefits.

**Table 1.4.** Comparison of historical and adaptive explanations for the evolution of autotomy in organisms with and without an exoskeleton.

**Table 2.1.** Checklist of habitat characteristics and activity comparing *Abdopus aculeatus* in the Philippines and Indonesia (Huffard, 2007).

**Table 2.2.** Field data summary from three sites in Indonesia (Bunaken, Siladen, and Southeast Sulawesi) collected by Christine Huffard. Frequency of autotomy is categorized by total number of individuals sampled according to sex, site, the number of arms lost among autotomized individuals, and the position of the arms that are lost (R1-R4, L1-L4). (n=160).

**Table 2.3.** Field data summary from Mactan Island, Philippines collected July 2011 during the wet monsoon season. Frequency of autotomy is categorized by total number of individuals sampled according to sex, site, the number of arms lost among autotomized individuals, and the position of the arms that are lost (R1-R4, L1-L4). (n = 47).

**Table 2.4.** Field data summary from three sites on Mactan Island, Philippines (Buaya, Marine Station, and Cordova) collected March to April 2013 during the dry season. Frequency of autotomy is categorized by total number of individuals sampled according to sex, site, the number of arms lost among autotomized individuals, and the position of the arms that are lost (R1-R4, L1-L4). (n = 81).

**Table 2.5.** General inventory and description of behaviors exhibited during autotomy. Count and probability are in reference to the occurrence of each behavior in all 26 autotomy trials.

**Table 2.6.** Simple probability matrix for autotomy behaviors described in Table 2.5. Each cell represents the probability that each paired behavior occurs. (a = detach, c = crawl, d = darken, f = forceps contact, I = ink, j = jet, l = lighten, p = flash color change, s = suckers suction, w = wound to mouth, T = total).

**Table 2.7.** Transition probability matrix for autotomy behaviors described in Table 2.5. Each cell represents the probability that each paired behavior occurs in the sequence starting with the row



behavior followed by the behavior designated by the column. (a = detach, c = crawl, d = darken, f = forceps contact, I = ink, j = jet, l = lighten, p = flash color change, s = suckers suction, w = wound to mouth, T = total). \*Probabilities >0.20 considered for transition diagram (Figure 2-6).

**Table 2.8.** p-value matrix for calculated z- scores testing if particular transitions are more likely to occur than expected just by chance. (a = detach, c = crawl, d = darken, f = forceps contact, I = ink, j = jet, l = lighten, p = flash color change, s = suckers suction, w = wound to mouth, T = total). \* $\alpha \leq 0.05$ .

**Table 3.1.** Summary of results for tensile tests using the Instron ® for the arms of 2 *O. macropus* and 6 *A. aculeatus* specimens. ( $\sigma$ =tensile strength, OM = *Octopus macropus*, AA = *Abdopus aculeatus*)

**Table 3.2.** Results from the decerebration test, indicating number of arm injuries prior to decerebration and the results from autotomy post-decerebration.

**Table 4.1:** Summary of crawling and jetting locomotion results before and after autotomy for 8 *A. aculeatus*. Prop trials = proportion of trials spent doing a particular activity, either crawling or jetting, before or after autotomy. Avg speed = Average speed for that individual. Auto arm = Position of the autotomize arm.

**Table 4.2:** Summary of tracked mouth speeds during crawling for 8 *A. aculeatus* before and after autotomy.

**Table 4.3:** Summary of statistical tests results for various locomotor variables.

**Table 4.4:** Summary of movement characteristics of the autotomized arm in 13 *A. aculeatus*.

**Table 4.5:** Summary of threshold tactile sensitivity to von Frey monofilaments in 8 *A. aculeatus*.

## LIST OF FIGURES

**Figure 1.1.** Gradients of autotomy considering Fredericq's three main criteria: its function, the breakage mechanism, and the level of central nervous control. Function is not a gradient as defense is considered the main purpose for autotomy. Important factors involved in the breakage mechanism and central control are listed. Three variants of autotomy: 'economy of autotomy', 'urotomy', and 'attack autotomy' are placed at their respective position on the gradient. Dotted line represents the factors considered in the strict definition of autotomy as it is currently used.

**Figure 1.2.** An integrative approach to animal behavior as described by Tinbergen's questions using autotomy specific as an example.

**Figure 2.1 a-d** Maps of the Philippines showing the following locations: a) Mactan Island and the den locations (numbers) at the three sites; close up of the den locations at b) Buaya, c) the marine station, d) Cordova.

**Figure 2.2 a-c** Pie chart representing the proportion of sampled individuals that exhibit autotomy (either loss of an entire arm or a regenerating arm stub) in a) Indonesia, b) Mactan Island, Philippines during the wet season, c) Mactan Island during the dry season.

**Figure 2.3 a-c** Histogram representing the proportion of males and females that exhibit autotomy (either loss of an entire arm or a regenerating arm stub) in a) Indonesia, b) Mactan Island, Philippines during the wet season, c) Mactan Island during the dry season.

**Figure 2.4 a-c** Histogram representing the proportion of individuals that exhibit autotomy (either loss of an entire arm or a regenerating arm stub) in a) Indonesia, b) Mactan Island, Philippines during the wet season, c) Mactan Island during the dry season.

**Figure 2.5 a-c** Pie chart representing the proportion of individuals found exhibiting arm loss according to the number of autotomized arms (loss or regenerating stub of 1 through 5 arms) in a) Indonesia, b) Mactan Island, Philippines during the wet season, c) Mactan Island during the dry season.

**Figure 2.6 a-c** Histogram representing the arm positions (L1-L4, R1-R4) that are most often autotomized (either loss of an entire arm or a regenerating arm stub) in a) Indonesia, b) Mactan Island, Philippines during the wet season, c) Mactan Island during the dry season.

**Figure 2.7 a-b** Histogram representing the arm positions (L1-L4, R1-R4) that are most often autotomized (either loss of an entire arm or a regenerating arm stub) for each sex in a) Indonesia and b) Mactan Island, Philippines during the dry season.

**Figure 2.8 a-b** Histogram representing proportions of sampled individuals exhibiting autotomy (either loss of an entire arm or a regenerating arm stub) and a) comparing them between Indonesia and the Philippines and b) comparing the dry and wet season in the Philippines.

**Figure 2.9.** Transition diagram for the sequence of behaviors associated with an autotomy event. Transition probabilities appear next to each transition arrow.

**Figure 3.1.** Transverse section of *Octopus mercatoris* arm stained with haematoxylin (black/blue-nuclei) and eosin (pink/red-connective tissue). Brightfield microscopy of 10 $\mu$ m-thick paraffin section. Major components of arm musculature besides three dimensional arrangement of muscle fibers include: an, axial nerve cord; ar, artery; ep, epidermis; in, intramuscular nerve.

**Figure 3.2.** Laboratory set-up for uniaxial tensile test using the Instron ® 5544. Bag containing octopus clamped at the top while the arm is thread through and clamped at the tip.

**Figure 3.3a-f.** Histology of longitudinal sections through arms of different octopus species. (a) Dorsal view of octopus arm position and their designations. 5  $\mu$ m sections of arm tissue stained in haematoxylin and eosin from the following species: (b) *O. bocki*, (c) *O. cyanea*, (d) *H. fasciata*, (e) *O. diguiti*, and (f) *A. aculeatus*.

**Figure 3.4a-f.** Histology of longitudinal sections stained with haematoxylin and eosin. (a-c) and (d-f) represent a series of autotomized ends flanking the autotomy zone from two different arms. (a) autotomized end of the first lost arm, (b) autotomized end in a at 10X, (c) autotomized end of the arm stub on the individual, (d) autotomized end of the arm stub on the individual for the second arm, (e) autotomized end in d at 10X, (f) autotomized end of the second lost arm.

**Figure 3.5a-f.** Histology comparing regenerating arm tissue to original arm stained in haematoxylin and eosin. (a) and (c) longitudinal and transverse sections, respectively of original arm tissue. (b) longitudinal section of a regenerating arm, (d) transverse section of the regenerating arm in b, (e) and (f) longitudinal sections through the regeneration zone.

**Figure 3.6a-b.** Typical autotomy results from extension testing with Instron ®. (a) representative stress-strain curve for octopus arm. Each peak represents breakage of different tissue levels with the maximum peak representing breakage of the axial nerve and surrounding muscles. Arrows point to autotomy of the epidermis. \*represents the autotomy stage clamped to the Instron ® pictured in (b).

**Figure 3.7a-b.** (a) Maximum tensile stress (MPa) averaged for each arm position of *A. aculeatus* (b) Toughness (J/m<sup>3</sup>) averaged for each arm position of *A. aculeatus*.

**Figure 4.1a-b.** Average locomotion speeds do not change with increasing mass. (a) Average crawling speed before and after autotomy for a given mass (linear regression before: slope = 0.0003397, R<sup>2</sup> = 0.37, p-value = 0.11; after: slope = 0.0004382, R<sup>2</sup> = 0.34, p-value = 0.13). (b) Average jetting speed before and after autotomy for a given mass (linear regression before: slope = 0.0020, R<sup>2</sup> = 0.22, p-value = 0.35; after: slope = 0.001495, R<sup>2</sup> = 0.72, p-value = 0.15).

**Figure 4.2a-b.** No significant change in the mode of locomotion used before and after arm autotomy. (a) Proportion of total locomotion trials spent crawling is slightly more after autotomy (Wilcoxon test: p-value= 0.15). (b) Proportion of total locomotion trials spent jetting is slightly more before autotomy (Wilcoxon test: p-value = 0.15).

**Figure 4.3a-b.** Average locomotion speeds do not significantly differ before and after arm autotomy. (a) Average crawling speeds are not significantly different (Wilcoxon test: p-value = 0.46). (b) Average jetting speeds are not significantly different, though it seems higher before autotomy (Mann-Whitney test: p-value = 0.35).

**Figure 4.4a-d.** Maximum and minimum mouth speeds do not significantly differ before and after arm autotomy. (a) Maximum mouth speeds overall and (c) maximum mouth speeds per individual do not significantly differ, except in individual 3 (Mann-Whitney test: p-value = 0.34). (b) Average mouth speeds overall do not significantly differ but some of the (d) individual average mouth speeds exhibit significant differences (Mann-Whitney test: p-value = 0.57).

**Figure 4.5.** Tactile sensitivity thresholds for local response decrease over time. Sensitivity in the base is lower than that in the tip (2-factor ANOVA: p-value = 0.0094).

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## Chapter 1: Introduction and review of autotomy

Most animals rely on defense systems to survive attacks from potential predators. The study of animal defenses such as crypsis and aposematism has a long history in animal behavior (Edmunds, 1974). One anti-predator strategy that is widely exhibited among animals, but is still poorly understood since its first description by Fredericq in 1883 is autotomy. He first presented the term ‘autotomie’ or ‘self-cutting’ as the shedding of a body part, like the limbs of crabs or the tails of reptiles (Fredericq, 1883). In 1887, he specifically defined autotomy based on three criteria: (1) its use for defensive purposes, (2) its occurrence by an intrinsic breaking mechanism at a distinct pre-determined plane and (3) its mediation by the nervous system (Fredericq, 1887). As a result of this definition, we often study and describe autotomy in terms of its form (i.e. the mechanism of breakage and subsequent morphology) and its function (i.e. the adaptive value to survive against predators).

To better understand and assess the current state of knowledge on autotomy, I will review the literature based on Fredericq’s definition. I will clarify terminology that has developed to describe various aspects of autotomy. Because defense systems are an important facet of animal behavior, I will approach this review from Tinbergen’s classic ethological perspective using a wide variety of animal examples. Of particular interest is distinguishing features of autotomy in soft bodied versus hard bodied animals. These terms are in reference to the autotomized body part as either having no hard skeleton (“soft bodied”) or having an internal or external skeleton (“hard bodied”). For instance, scallops have an external shell, but the body part that is able to detach and regenerate is the tentacles which do not have an internal or external skeleton. Thus bivalves are considered soft-bodied for the purposes of this dissertation. Several reviews have been published in recent years (Bateman & Fleming, 2009; Bely & Nyberg, 2010; Clause & Capaldi, 2006; Fleming, Muller, & Bateman, 2007; Juanes & Smith, 1995; Maginnis, 2006; Maruzzo, Bonato, Brena, Fusco, & Minelli, 2005; McVean, 1975; Stasek, 1967; Wilkie, 2001). However they are all nearly taxon specific and focus largely on the mechanism of shedding and the costs and benefits of autotomy. The goal of this review is to identify overall patterns in autotomy among soft and hard bodied animals and to develop ways of predicting when it is likely to be used.

### Terminology

Fleming et al. (2007) evaluated the use of the term “autotomy” in the literature and found only 47% of the 1000 occurrences of the word conformed with the three criteria established by Fredericq (1887). Autotomy has become synonymous with other terms that have widened the range of behaviors associated with what was initially conceived to be a defense strategy. It has been confused with what is commonly thought of as ‘automutilation’, self-inflicted injury in everything from human castration to loss of rat digits from self-biting as a sign of neuronal damage. In an effort to clarify and return to the original definition of ‘autotomy,’ Wilkie et al. (2007) suggested a new term ‘autoblaby’ (‘blabe’ from the Greek word for ‘damage’) to encompass “self-mutilation resulting from a neurological or psychological dysfunction.” The term did not gain popularity and a recent literature search yielded only one instance of use (Pertin, Gosselin, & Decosterd, 2012). ‘Automutilation’ has also been used in the octopus literature to refer to behaviors of self-destruction (Budelmann, 1998). One such example is ‘autophagy’ or ‘self-eating’ which often occurs during senescence in octopuses (Budelmann,



1998). Budelmann (1998) specified ‘autotomy’ to mean voluntary amputation whereby a part of your body is broken off.

In an effort to be more specific, additional terms were developed to address particular situations regarding Fredericq’s three criteria for autotomy. ‘Autopasy’ or ‘autospasy’ involves shedding an appendage at a predetermined plane of weakness by an outside source, i.e. when a predator grabs a hold of a limb (Wood & Wood, 1932). In contrast, ‘autotilly’ involves the animal itself removing the limb at a preformed breakage point, usually by its mouth parts (Wood & Wood, 1932). If the appendage is consumed, as is seen in certain lizards and insects, it is considered autophagy (Maginnis, 2006). A more general term that incorporates all of these modifications to the definition of autotomy is ‘appendotomy’; however, like Wilkie’s ‘autoblaby’ reference, it has been rarely used in the literature (Woodruff, 1937).

Due to the wide application of the term ‘autotomy’ to generally mean loss of a body part, recent literature has turned to individualized definitions that accommodate the situation or scenario for a particular taxon. For example, in the herpetological literature where extensive research has been conducted on the mechanism of shedding, autotomy is often defined as a “reflexive loss of an appendage at a preformed breakage plane” (Maginnis, 2006). Among arthropods, except crustaceans where the mechanism is thoroughly mapped out, autotomy is generally defined as the “loss of an appendage at preformed breakage planes” (Maginnis, 2006). Even more general is the idea of limb loss without a preformed plane of weakness as is seen in vertebrates like fish and amphibians. These deviations have led to notions of treating autotomy as a gradient rather than fitting Fredericq’s original criteria (Fleming et al., 2007; Giard, 1887; Maginnis, 2006; Norman, 1992; Wasson, Lyon, & Knope, 2002). These gradients span everything from how much external versus internal stimulation is required to induce autotomy to how much of the autotomized limb is lost (**Fig. 1.1**). It has also led to the introduction of new terms such as ‘economy of autotomy’ (Daniels, 1985), ‘attack autotomy’ (Robinson, Abele, & Robinson, 1970), and ‘urotomy’ (Dourado, Oliveira, & Prudente, 2013; Stasek, 1967) which will all be discussed in greater detail in following sections.

Given the different conditions in which autotomy has appeared and the variants of definitions that have been added to the lexicon of this field, it is important to address how these definitions coincide with the initial three criteria. Their consideration will also yield more information for the relevant gradients that describe the different phenomena considered as autotomy (**Fig. 1.1**). To show how this applies, consider the position of the term ‘urotomy’ in **Figure 1.1**. ‘Urotomy’ or ‘non-specialized pseudoautotomy’ refers to dropping a portion of the appendage due to an external force (ie. like ‘autospasy’) at no particular breakage point and does not result in regeneration of the lost limb (Dourado et al., 2013; Stasek, 1967). This is exemplified in the snake genera *Dedrophidion* and *Mastigodryas* which have fragile tails that experience high incidences of loss along the entire tail length (Dourado et al., 2013). Another example of urotomy in a terrestrial sea slug is *Limax marginatus* which can discard the last quarter to a third of its own foot with sufficient harassment from a predator (Stasek, 1967). Animals that utilize urotomy would fall somewhere in the in the middle of the gradient describing autotomy.

### **Criteria 1: Autotomy functions as a means of defense.**

Primary defense mechanisms, like crypsis, are an animal’s first line of defense to keep from being detected by a predator. When primary defense strategies fail, they resort to secondary defense mechanisms like flight (physically moving away), startling displays, or expulsion of

substances like ink or venom to avoid being caught (Edmunds, 1974). Autotomy is another example of a secondary defense strategy that has additional benefits beyond escaping the grasps of a predator by shedding a non-essential body part. The autotomized appendage can act as a deterrent if the appendage (1) secretes noxious substances, like the cerata of nudibranchs (Fleming et al., 2007), (2) continues to move apart from the body, like certain lizard tails (Arnold, 1984), (3) serves as a smaller compensatory meal for the predator (Maginnis, 2006), or (4) adheres to the predator, like in the echinoderm *Holothuria scabra* (Wilkie, 2001)

Although autotomy is commonly accepted as a defense mechanism, the term has been used in reference to other potential motivations. Autotomy has been implicated as a way to eliminate a wounded or toxic part of the body. For example, arthropods will shed appendages like the chelae of crabs to escape from their exoskeleton during a fouled molting cycle (Maginnis, 2006) and regenerate the autotomized limb in subsequent molts. Another example of autotomy whereby the animal discards a toxic part of the body is in annelids where oligochaete and polychaete worms are able to sequester their waste and toxins in separate segments of their body to be shed (Fleming et al., 2007; Kaster, 1979). Autotomy has also been used in the context of reproduction. A prime example of this is in cephalopods where the male *Argonauta spp.* detaches its reproductive arm in the female mantle cavity during mating, potentially as a means of ensuring that his sperm will fertilize the females eggs (Stasek, 1967). Detachment as a means of asexual reproduction, such as fissioning in certain groups of echinoderms has also been proposed as a form of “autotomy” (Fleming et al., 2007). Another proposed function for autotomy is ‘parasitic’ whereby an animal discards a part of its body in order to get rid of parasites. This is seen in the sea star *Asterias richardi* which detaches arms infested by myzostomid worms (Stasek, 1967). Stasek (1967) also mentioned ontogenetic autotomy as a means of discarding a once functionally useful part of the body, especially during metamorphosis from larval stage to adulthood. Nudibranchs discarding early shells prior to entering adulthood is an example of this in molluscs (Stasek, 1967)

### **Criteria 2: Autotomy is achieved by an intrinsic mechanism.**

According to Wilkie et al. (2007) animals that are capable of autotomizing a body part have one thing in common, “an anatomically distinct breakage plane” that exhibits specific adaptations to assist the loss, recovery, and regeneration of the appendage. This breakage plane defines a localized, predetermined, and predictable site for autotomy to occur in one of two ways (Edmunds, 1974; Juanes & Smith, 1995; McVean, 1975; Wilkie, 2001). First, it can be a “permanent zone of weakness” where the appendage will always break in response to an external force at a particular location (Wilkie, 2001). One can test this by anesthetizing the animal and still achieve the same breakage zone when autotomy is induced by external stimulation. Alternatively, a breakage plane can be a “potential zone of weakness” that only at the time of autotomy will lose tensile strength and break (Wilkie, 2001). It is considered the area of least resistance and thus the most likely place for breakage to occur. The clean surface of these breakage zones suggests that certain adaptations have stemmed from this defense mechanism in order to minimize tissue damage and stimulate wound healing (Wilkie, 2001). This is exemplified in isopods that regenerate their appendage faster from the breakage plane than detachment from any other joints (Needham, 1961). Similarly, nudibranchs with their toxin filled cerata will only regenerate from the base of the breakage plane (Kress, 1968).

Wilkie’s (2001) explanation for a breakage plane implies one set location for detachment, regardless of where the appendage is grasped by the predator. However, there are a few reptiles,

echinoderms, clams, and even a squid that exhibit ‘economy of autotomy’, the ability to vary the detachment site according to the amount of appendage lost to the predator (Bustard, 1968; Daniels, 1985; Delage & Herouard, 1903; Gilmour, 1963; McVean, 1975). This strategy is thought to mitigate the costs of autotomy including investment in full regeneration of a limb and lipid loss (Cooper, Jr. & Smith, 2009; Copeia, May, & Daniels, 2012). In the gecko, *Phyllodactylus marmoratus*, fracture planes run through each of the gecko’s vertebrae except the ones at the base and very tip of the tail. This makes it possible for the tail to be shed at any of these points, depending on how much of the tail is pulled. This ability, however, may be situation dependent as whole tail autotomy still occurred when certain predators, namely marsupials, attacked. This is similar to Medel’s (1992) findings relating habitat quality and predation levels to the tail detachment site in two species of Iguania lizards, *Liolaemus altissimus* and *Liolaemus fuscus*. *L. altissimus* experienced more variability in the location of the detachment site which is associated with the harsh thermal environment and low predation levels experienced by this species compared to *L. fuscus* (Medel, 1992). The tails of *L. altissimus* contained higher energy content implying the importance of retaining this valuable resource in a harsh environment by breaking off the tail at more distal positions (Medel, 1992).

Prior emphasis on investigating autotomy, particularly in lizards, has been on whole limb loss, thus we do not know with what frequency ‘economy of autotomy’ is present in nature. Recently, Cromie and Chapple (2013) recognized this bias and warned that full understanding of the adaptive significance of this defense strategy will not be achieved without considering the length of the autotomized appendage. They argued that autotomy “should not be viewed as an all or nothing behavior but as a flexible defensive tactic” whose costs and benefits are influenced by how much of the appendage is shed (Cromie & Chapple, 2013). This shift in considering gradients of change can be seen in **Figure 1.1** and has changed how we view this particular criterion of autotomy.

### **Criteria 3: Autotomy is mediated by the nervous system.**

Autotomy can be distinguished from forced removal by the speed and ease with which the appendage is discarded, particularly in a predator-prey situation (McVean, 1975). This inevitably involves control by the nervous system to mediate the loss of an appendage. Debate stemming in the crustacean literature questioned whether nervous control of autotomy was reflexive or involved some degree of voluntary, central nervous control (McVean, 1975; Wood & Wood, 1932). Examples of both mechanisms are present in a wide variety of taxa (all taxa (Fleming et al., 2007), crustaceans (McVean, 1975; Wood & Wood, 1932), echinoderms (Wilkie, 2001; Wilkie, Dolan, Lewis, & Blake, 2007), reptiles (Bateman & Fleming, 2009; Clause & Capaldi, 2006; Elwood, Pelsinski, & Bateman, 2013; Hoogmoed & Avila-Pires, 2011)). More research is required to understand the neural pathways, neurotransmitters, and hormones involved in these mechanisms. Teasing the two apart will be especially difficult given that the assessment of voluntary versus involuntary responses inevitably involve some overlapping pathways. The end result is likely a gradient in autotomy response. For example, Wasson and Lyon (2005) found a wide variety of anti-predatory responses in porcelain crabs (*Petrolisthes spp.*) that were influenced by individual condition. In most predation cases crabs would shed their appendages rapidly, suggesting a more reflex based response. However, there were some instances where the claw would take up to 10 minutes of being pulled before they autotomized, alluding to more voluntary control and influence from the central nervous system (Wasson & Lyon, 2005). Whether autotomy occurs by a reflexive action or involves higher

control centers in the nervous system is not a critical distinction to understand how autotomy occurs mechanistically but may provide insight on how the nervous system has evolved to make use of this defense mechanism (Fleming et al., 2007; McVean, 1975).

Additionally, there seems to be a gradient in how much external stimulation of the nervous system is required for detachment to occur (i.e. “threshold stimuli” (Fleming et al., 2007; Wasson et al., 2002). Some species of crabs (Smith & Hines, 1991; Wasson et al., 2002), arachnids ((Fleming et al., 2007), and lizards (Bateman & Fleming, 2009; Clause & Capaldi, 2006) will shed their respective appendage with only the slightest bit of stimulation while others require a great amount of force, at least equal to their own mass, to evoke detachment (echinoderm (Wilkie, 2010)).

In the extreme case where autotomy occurs without any external stimulation, there is only one known example that has been aptly named ‘attack autotomy.’ In ‘attack autotomy’, the attack and escape response are performed simultaneously. Robinson et al. (1970) first noted freshwater crabs “attacking” their otter predators by firmly attaching their chelae and subsequently autotomizing the claws as they made an escape. In an experiment looking at a suite of terrestrial crabs, he found only two species, *Potamocarcinus richmondi* and *Geocarcinus quadratus* employ this defense mechanism. Unlike *P. richmondi* and *G. quadratus*, the non-attack autotomy species were more gregarious and used their chelipeds for social signals, such as in mating, and foraging in addition to defense (Robinson et al., 1970). Attack autotomy is often referred to as ‘active autotomy’ because it does not rely on the predator applying a force to stimulate autotomy of the appendage. Rather the prey makes a behavioral decision to attach and flee which arguably involves higher centers of nervous control than simple reflexes can provide (Robinson et al., 1970). Aside from crabs, there are few examples of autotomy that employ both an attack and escape strategy to defense. The bioluminescent squid arms of *Octopoteuthis deletron* (Bush, 2012) is a good example and the stinger of social hymenoptera (Fleming et al., 2007) and pedicellariae of echinoderms (Wilkie, 2001) are often cited as other potential cases although with no directed studies specifically on attack autotomy in the latter two. In these examples, the autotomized body part is seen grasping the predator prior to autotomy and stays attached post shedding as the animal escapes. This is similar to crab attack autotomy and falls somewhere in the middle of the gradient (**Fig. 1.1**).

### **Autotomy: A behavioral approach to a secondary defense strategy**

Given what is known about autotomy and the approaches that have been made to study this phenomenon, I define it as the following for my octopus system: autotomy is the shedding of an arm at a particular position, usually at the base, for defensive purposes. More specific criteria regarding the intrinsic mechanism and role of the central nervous system cannot be incorporated until more of the basic neural networks for arm locomotion in octopuses are determined. All of Fredericq’s criteria feed well into studying the various aspects of behavior with regard to autotomy. Four important facets to consider, according to Tinbergen (1963), are the mechanisms, developmental or ontogenetic processes, adaptive value, and evolution of a behavior (Tinbergen, 1963). The questions of interest regarding each behavioral aspect specifically related to autotomy in my focal organism, the octopus, are summarized in **Figure 1.2**. Because of Fredericq’s criteria, most investigations have focused on the mechanism and adaptive value of autotomy. However there are a few example taxa that address all four aspects of behavior. For the remainder of this chapter I will summarize what is known about autotomy for each of these behavioral aspects and conclude with an account of what we know about cephalopod autotomy.

Animals with hard articulated parts have been extensively studied as model organisms for autotomy (Bateman & Fleming, 2009; Clause & Capaldi, 2006; Emson & Wilkie, 1980; Juanes & Smith, 1995; McVean, 1975; Wilkie, 2001; Wilkie, 1978). The major hard bodied organisms I will be discussing are arthropods (Class: Arachnida, Crustacea, and Hexapoda), echinoderms (Class: Asterozoa, Crinozoa, Echinozoa, Holothurozoa, and Ophiurozoa), and chordates (Reptilia: Squamata). In these cases, limbs are predictably lost at joints in animals with exo- or endoskeletons. In contrast, little is known about autotomy in soft bodied animals whose autotomized limb lacks joints. The major phyla covered among soft bodied organisms are Annelida, Cnidaria, and Mollusca (Stasek, 1967). Autotomy has been recognized in 14 families of gastropods, bivalves, and cephalopods and described in at least 4 families of annelids (Fleming et al., 2007). I will survey examples within these aforementioned groups and make salient patterns of behavior between animals whose autotomized body part do or do not have an endo-/exo-skeleton. **Tables 1.1-1.4** summarizes these major examples and lists the relevant review references.

### **A. Mechanism of autotomy**

Notable soft bodied animals that exhibit the ability to autotomize a part of their body often shed parts that are directly exposed and susceptible to predation. These include tentacles in both the polyp and medusa stages of cnidarians, segments of annelid worms, cerata, papillae, and portions of the posterior foot and mantle of gastropod molluscs, mantle tentacles of bivalve molluscs, and arms of cephalopod molluscs (Fleming et al., 2007). In most of these cases, autotomy occurs at a particular region that is either a zone of weakness or a permanent fracture plane of the limb brought about by muscle contractions prompted by an external stimulus, such as the grasp of a predator. These muscles can be oriented transversely, longitudinally, or most often circularly that upon constriction will allow for loss of the body part. Circular muscles in particular result in sphincters that close off the wound, preventing excessive loss of internal fluids, and helping accelerate regeneration (Fleming et al., 2007; McVean, 1975). Regeneration by proliferation of cells distal to the cleavage site often results in a body part nearly identical to the original. There are some cases among gastropods such as *Helicarion spp.* and *Pleurodonte spp.* whereby the tail of the foot autotomizes from activity of the snail itself, “whisking the tail up and down” until it is discarded (Stasek, 1967). Also interesting is the recent finding of scale autotomy in the annelid *Alentia gelatinosa* which breaks away at a weak zone in the scale stalk, during autotomy. Detailed knowledge of neural control over autotomy is sparse. Further specializations of autotomy exhibited particularly among molluscan taxa include the ability to drop the autotomized appendage at the site of external stimulation (“economy of autotomy”) and the capacity of the separated body part to maintain defensive behaviors such as continued motion or adherence to the surroundings including the predator (Stasek, 1967).

Compared to soft bodied organisms, phyla with hard exo- or endo-skeletons experience a greater variety of mechanisms for appendage loss, though the main processes utilized are still very similar as shown in **Table 1.1**. The appendages that are often lost include legs (walking and/or feeding), labial palps, caudal lamellae, and antennae generally in arthropods, stingers (derived from ovipositors) in social hymenoptera, and chelipeds in crustaceans, arms in asteroids and ophiuroids, evisceration of internal systems in holothuroids, cirri in crinoids, and the caudal tail of reptiles (Arnold, 1984; Fleming et al., 2007). Due to their inherent skeletal structure, most autotomy occurs at the joints that attach two hard parts together, whether it’s the chitinous segments of arthropod appendages or the calcareous ossicles of echinoderms. In vertebrates

however, the majority of reptilian groups autotomize their tail at a fracture plane within the vertebra itself (intravertebral autotomy) but still uses the same general principles of separation based on muscle contractions around the fracture plane that are seen in arthropods and echinoderms (Clause & Capaldi, 2006). One recent discovery in reptiles found that intravertebral autotomy in the Tokay gecko, *Gekko gecko*, occurred at a permanent pre-separated site that is held together by adhesion of special microstructures on the surface of the tail. During autotomy, this site undergoes morphological changes that reduce the adhesive capabilities of the structures and allow for separation of the tail (Sanggaard et al., 2012). Only snakes and lizard members of the Agamidae family use intervertebral autotomy which is thought to occur without any special modifications to the vertebrae as their tails look similar to those of reptiles that do not exhibit autotomy (Arnold, 1984). Another mechanistic specialization of autotomy occurs in echinoderms where potential zones of weakness contain mutable collagenous tissues (MCT) that change mechanical properties under nervous control. At the autotomy plane, the MCT undergoes irreversible destiffening, losing tensile strength and making it possible for appendages to detach (Wilkie, 2001). Both echinoderms and reptiles are also able to lose their appendages by passive rupture at non-autotomous regions (Clause & Capaldi, 2006; Wilkie, 2001). Wilkie (2001) called these non-autotomous ruptures “opportunistic self-detachment” which, in echinoderms, occurs in regions with reversible MCT stiffening and destiffening. Distinguishing preformed fracture planes from potential ones that occur only during the time of autotomy is difficult because both mechanisms can be at work on different levels of tissue at the same site (i.e. they are not mutually exclusive mechanisms). For example, brittlestars (ophurioids) have permanent cleavage zones in the skin, but only undergo changes in the ligament that lead to separation during the autotomy event itself (Wilkie, 2001).

Within the arthropods, autotomy of the leg tends to occur at a joint at the base of the appendage. This includes the coxa-trochanter joint in spiders, the basis-ischium joint in decapods and trochanter-femur joint in hexapod insects (Maruzzo et al., 2005). Social hymenoptera, such as bees and harvester ants (*Apis spp.* and *Pogonomyrmex spp.*) have stingers in addition to legs that can autotomize. These stingers have well-developed barbs that, upon insertion in a predator, lodge deep into the tissue. Subsequent contractions in specialized muscles at the abdomen release the stinger as well as venom from the insect providing two means of defense – attack and flight (Shorter & Rueppell, 2011). Other autotomized appendages can also detain the predator post autotomy, such as the chelipeds of crabs in attack autotomy or the adherence of Cuvierian tubules in sea cucumbers (Fleming et al., 2007). The tails of certain reptiles, legs of insects, and arms of brittlestars can also maintain prolonged movement post detachment. In a few cases of reptiles, sea stars, and brittlestars, there are taxa that are able to detach at the point of stimulus and exhibit economy of autotomy much like the molluscan examples.

There are several examples of body parts detaching from the main body that have been referred to as autotomy in past literature that are not included in Table 1. This is because they either don't exhibit some intrinsic mechanism for loss or the purpose for detachment has not been shown to be associated with defense. This includes (1) the loss of pieces of siphons in *Solecurtus* and *Solen* by external means, (2) the detachment of the reproductive arm in *Tremoctopus spp.*, *Ocythoe tuberculata spp.*, and *Argonauta spp.*, and (3) the detachment of the feeding captacular filamentous bundles in scaphopods – a member of the molluscan phylum (Fleming et al., 2007; Stasek, 1967). Additionally, these structures except the reproductive arm of cephalopods do not regenerate but rather regrow by serial replacement. Regeneration occurs

by distal proliferation of cells whereas serial replacement occurs by proximal additions pushing older portions outward, away from the wound site (Stasek, 1967).

One other vertebrate example that is often cited as a form of autotomy is the shedding of skin in mammals. In the Florida mouse, *Peromyscus floridanus*, shedding of the skin sheath surrounding the tail can lead to tail autotomy (Layne, 1972) and for the African spiny mice *Acomys spp.* this results in shedding of their spiny hair (Seifert et al., 2012). No muscular modifications are involved in *Peromyscus floridanus* tail autotomy, detachment is passive, with fewer connective tissue fibers found at weak zones. Excessive movement of a suspended mouse or physical removal by the mouse itself initiates loss of the tail (Layne, 1972). Autotomy in hair follicles of *Acomys spp.* do not exhibit preformed breakage planes, but require very low tension for detachment as evidenced by the ease with which their skin would shed during capture and handling in the wild (Seifert et al., 2012). Wound healing and the highly regenerative properties of the skin and surrounding tissue of *Acomys spp.*, have been the concentration of these studies, specifically from a molecular perspective. Like the previous examples of siphon nipping and reproductive arm detachment, I will not consider these cases in mammals any further as the function of autotomy (i.e. whether it is for defense) is not yet fully known. They can very well be examples of mammalian autotomy given more future studies.

## **B. Ontogeny of autotomy**

Unlike the mechanistic processes involved in shedding a body part, characterizing the ontogeny of autotomy has received far less attention. Studying the development of a behavior, or what Tinbergen (1963) defined as ‘change of behavior machinery during development,’ has in the past largely focused on a debate about whether behaviors were ‘innate’ or ‘learned’. Defense behaviors, like autotomy, are often thought of as being stereotyped (Feder & Arnold, 1982) and the necessary components, such as the breakage plane, presence of a predator, and successful escape of the animal, by definition must all be present in the organism from the start. For example the gastropod *Prophysaon foliolatum* upon hatching is fully equipped with the internal muscular mechanisms to autotomize portions of its tail (Deyrup-Olsen, Martin, & Paine, 1986). In sympatric lacertid lizards experiencing different levels of predation in the lab and in the field, frequency of tail autotomy reflected more of an innate predisposition for tail loss rather than the level of experienced predation pressure (Pafilis, Fofopoulos, Poulakakis, Lymberakis, & Valakos, 2009). These results were contrary to a similar study comparing species of geckos and skinks. In this system, external factors, such as predation and social experience seemed to be more influential on the incidence of tail autotomy (Hare & Miller, 2010). Other examples that involve acquisition or learning in the development of autotomy are based on larger, usually older individuals experiencing more incidences of autotomy and learning in subsequent occurrences to do one of two things. They can avoid predators such as in the wolf spider *Schizocosa avida* avoiding its scorpion predator, *Centruroides vittatus* (Punzo, 1997). Alternatively, they can cope by improving a counterbalancing method such as escape speed in the wall lizard *Podarcis muralis* which runs faster after experiencing multiple tail losses compared to the first loss (Brown, Taylor, & Gist, 1995). An alternate explanation for these results which will be covered in the next behavioral section is that these responses counterbalance the costs and benefits of autotomy which maintain its consistent use and presence in the system (Bateman & Fleming, 2009).

Behaviors are now commonly accepted as being influenced by both genetic and environmentally acquired factors. Studies in ontogeny of behavior have modified their

concentration to identifying those factors and describing their influence on developmental processes themselves. Still few studies of autotomy have directly studied how the machinery involved in limb loss changes during development. One potential reason that complicates such studies is that fully regenerated appendages may not show any evidence of prior autotomy and signs of multiple losses of the same arm may not be present. One exception to this is the focus on studying the machinery (i.e. muscles, connective tissue, and neural networks) involved in regeneration at an autotomy plane. By looking at the developmental process of regeneration, one can better understand how the autotomy plane is first developed. For instance, the previously described study of skin shedding in *Acomys spp.* identified cellular processes involved in regeneration and uncovered the genetic and molecular pathways governing them (Seifert et al., 2012).

Although there are few direct studies on the ontogeny of behavior, there are several that identify and quantify the incidence of autotomy at different life stages. These ontogenetic patterns of autotomy may suggest important factors that influence the use of autotomy as compared to other modes of defense. Most of these studies are reptile focused, but there are some examples of molluscan gastropods that exhibit ontogenetic patterns of autotomy. I broadly characterize these patterns into four categories in **Table 1.2**: (1) frequency of autotomy according to age, (2) frequency of autotomy according to size, (3) capacity to survive post-autotomy based on age, and (4) growth rate based on age. For age and size, two classes are considered: juveniles, who are reproductively immature and often smaller, and adults, which have reached reproductive maturity and are larger. There is an overall tendency to see higher frequencies of autotomy in adults and larger organisms. This is to be expected as adults have, by virtue of age, had more predator encounters and thus accumulate more incidences of autotomy than juveniles (Bateman & Fleming, 2009). For growth rate and energetic investments, it is important to consider both somatic growth and regeneration of the tail, especially for juveniles who undergo various developmental changes during their maturation to adulthood. There are examples for a variety of patterns in energetic investment, some of which result from lab manipulations and others that stem from field observation. This coupling of lab and field studies can shed light on the environmental factors involved in autotomy.

The environmental factors shown to correlate with these aforementioned general patterns of variation include predation intensity, the subject's activity level, conspecific population density, resource availability, differential costs and benefits for different stages, and changing circumstances between juveniles and adults. For example juvenile colubrid snakes *Dendrophidion spp.* and *Mastigodryas spp.* exhibit lower frequencies of tail loss than adults because juveniles are more static and remain hidden from predators (Dourado et al., 2013). In some crustaceans like the blue crab, *Callinectes sapidus*, larger animals experience higher incidence of single cheliped autotomy. This correlates with high conspecific population densities in *C. sapidus* and suggests that intraspecific predation (i.e. cannibalism) may be an influential factor in autotomy for this system (Smith & Hines, 1991). In the metallic skink, *Niveoscincus metallicus*, larger and older individuals follow the general trend of accumulating more instances of tail autotomy and regeneration than juveniles, but particularly with one inefficient predator, the domestic cat (Chapple & Swain, 2004). Lastly, growth rate patterns and energetic investment allocated to a regenerating tail versus somatic growth has been attributed to resource availability (Niewiarowski, Congdon, Dunham, Vitt, & Tinkle, 1997) and differential costs and benefits associated with different species during different life stages (Althoff & Thompson, 1994; Vitt, Congdon, & Dickson, 1977). For example, caudal regeneration in on *Uta stansburiana* results in



a tail that is similar to the original and is high in protein. By contrast, *Coleonyx variegatus* regenerates its tail rapidly by accumulating more lipids. This difference in energetic cost to tail regeneration can explain why we see reduced somatic growth rate of autotomized *U. stansburiana* juveniles and more investment of energy to somatic growth in *C. variegatus*.

One pattern that is less common and contrary to previously described explanations is the observation of select juvenile mollusc, arthropod, and vertebrate species exhibiting higher autotomy rates than adults. An explanation for this anomaly is based on changing circumstances in juveniles that influence alterations in their anti-predator defense mechanisms. Autotomy may be a low cost, high benefit defense mechanism to use in juveniles because they may be faster and conceal their smaller bodies better than adults (Arnold, 1984). In transitioning to adulthood, the general trend exemplified by the few taxa listed in **Table 1.2** is that adults grow larger in size and develop other morphological characters that consequently make it more appropriate to use other methods of defense. The gastropod *Satsuma caliginosa* develops defensive denticles (Hoso, 2012) and iguanids lose fracture planes during ontogeny, but grow to be bigger and more aggressive (Arnold, 1984). In both cases, adults become more prone to fight off predators than flee. An interesting behavior that is a consequence of increasing size occurs with survivorship in the wolf spider, *Gladicosa pulchra*. Older instars experience a lower survival value from scorpion stings due to attacks being better targeted to vital locations in the adult, such as the abdomen and cephalothorax. As juveniles, the abdomen and cephalothorax are not as big or as easy to target, providing more opportunities to use leg autotomy as a means of defense from scorpions who cannot aim (Klawinski & Formanowicz Jr., 1994). Even more interesting is ontogenetic changes in lizard tail morphology that coincide with changes in defense mechanisms. The Be'er Sheva Fringe-fingered lizard *Acanthodactylus beershebensis* produces newly hatched individuals with blue tails that exhibit display behaviors to deflect predator attention and result in subsequent tail autotomy. During growth and development, the lizard loses its tail coloration and patterning and subsequently shifts its defense mechanism from conspicuous tail display to more cryptic and less activity out in the open as an adult (Hawlena, Bochnik, Abramsky, & Bouskila, 2006).

It is important to note that understanding patterns of ontogeny in autotomy should not be confused with 'ontogenetic autotomy' (Emson & Wilkie, 1980; Stasek, 1967) which requires the loss of certain structures for development of the organism. For example, the discovery that autotomy in the crinoid, *Oxycomanthus japonicas*, leads to bifurcation and subsequent increased numbers of arms during a critical period in normal development is an intrinsic program and not an ontogenetic pattern in autotomy (Shibata and Oji, 2003). Even more convincing is evidence that *O. japonicas* will autotomize during fish predation, but only regenerate one arm (Shibata & Oji, 2003).

### **C. Adaptive value of autotomy**

Besides identifying mechanisms of loss, by in large most studies regarding autotomy have concentrated on its adaptive value. There are two things to consider when studying why certain organisms exhibit this particular defense mechanism. First, the frequency of autotomy and its associated factors with its use in natural populations are important to document. Second, there are taxa and situation specific costs and benefits for losing a part of an animal's body that modulate whether an animal will successfully use autotomy or some other defense mechanism. Unlike studying ontogeny or the evolution of autotomy, the environmental conditions, costs, and benefits connected with autotomy are more easily manipulated for experimental purposes. As a

result, several studies covering a wide variety of taxa, though largely concentrating on reptiles, have been conducted and are summarized in **Table 1.3**.

One way to directly study autotomy is to witness an event take place in the natural environment, induced by its predator. Unfortunately, predation events for many of the discussed organisms, let alone sub-lethal autotomy events, are difficult to replicate. As a result, one of the first and easiest observations to make in natural populations that imply the occurrence of autotomy is the number of individuals found with missing limbs and in some cases regenerated limbs. Among soft bodied organisms, example populations of gastropods and cephalopods have been characterized in the wild. Low incidence (less than 1%) of posterior foot regeneration was observed in the figsnail *Ficus ficus* which could be attributed to either efficient lethal predation or a situation where autotomy is not a highly utilized defense strategy given the conditions (Liu & Wang, 1999). Conversely in another snail, *Satsuma caliginosa*, high frequency of foot autotomy is found in areas with larger populations of snakes (Hoso, 2012). In an undescribed octopus species first studied by Ward (1998) in Guam, 20% of octopuses (males and females) were found regenerating arms that resulted from autotomy. These arms are characterized by loss near the base of the arm at a specific set of suckers (Ward, 1998). This species will be referred to as '*Octopus sp.* Ward, 1998' in accordance with the nomenclature designated by Norman and Finn (2001). Difficulty in determining if autotomy has occurred in the many tentacles of bivalves and cnidarians may be a limiting factor in measuring its incidence in natural populations. By comparison, a wealth of information is known about frequency of autotomy in hard bodied organisms. Among arthropods and echinoderms multiple appendage loss has been recorded. In general, it is common for one or two appendages to be lost and among the echinoderms, particularly brittle stars where arms are not functionally differentiated, more than two arm autotomy events is common (Fleming et al., 2007). In crustaceans, the chelipeds, which serve many different functions from defense to foraging, is one of the most commonly lost appendages (Mariappan, Balasundaram, & Schmitz, 2000). Overall, there is a wide range in recorded incidence of autotomy, from 36% in the cricket *Gryllus bimaculatus* (Bateman & Fleming, 2005) to 88% in the common wall lizard *Podarcis muralis* (Bateman & Fleming, 2009) indicating potential wide variation in factors influencing these frequencies.

Many factors that can be measured from the environment modulate the conditions under which autotomy occurs. Some of these, listed in **Table 1.3**, have been identified in hard bodied organisms, but less has been done to identify them in natural populations for soft bodied animals except for some gastropods and one cephalopod. In general, the efficiency of predators and their population in the environment can influence the frequency of individuals we find with missing limbs, such as seen in the brown anole, *Anolis sagrei* (Bateman & Fleming, 2011). Competition with conspecifics and the interaction between predator and prey can also have an influence in the occurrence of autotomy. Typically, the first autotomized appendage tends to be shed faster than subsequent appendages as seen in the cricket *Gryllus bimaculatus* (Bateman & Fleming, 2005). McVean (1975) attributed this to inhibition by the central nervous system of successive autotomy events. In some cases, such as the crustaceans *Carcinus*, *Leander*, and *Mariniogammarus*, there are even asymmetries in which side of the animal multiple appendages tend to be autotomized (McVean, 1975). This is more relevant to bilaterally symmetrical taxa like arthropods and to some degree octopuses, but not for radially symmetrical animals like the pentaradial members of the echinoderm phylum.

All of these factors are interrelated and together influence an organism's likelihood to autotomize. For instance size, sex, and efficiency of escape can affect whether porcelain crabs

will autotomize (“flee”) an appendage or use an alternative mechanism such as fighting. Small females that have low success rates of escaping predators when trying to fight are more likely to use autotomy to escape predation (Wasson & Lyon, 2005). Size is also an important determinant in lizards where bigger individuals are able to fight before autotomizing their tail (Arnold, 1984). The surrounding habitat and their level of interaction with conspecifics also affect their propensity to autotomize. If they can manipulate their habitat to make them look less susceptible during escape, they are more likely to use autotomy. Terrestrial slugs *Deroceras reticulatum* and *Limax tenellus* were better at escaping effectively (i.e. crawled faster and traveled further) and finding the best refuges compared to other members of their groups (Pakarinen, 1994). Except for the special case of eusocial hymenoptera where individuals are highly related and autotomize their stingers to protect themselves and their colony, solitary species tend to use autotomy more often than gregarious species (Robinson et al., 1970; Shorter & Rueppell, 2011).

There are costs and benefits for losing a body part and the success with which an organism uses autotomy as a defense mechanism depends on a balance between the two. The primary benefit of shedding a body part is to escape the grips of a predator, or potentially a conspecific as evidence by cannibalism in some animals like the gastropod *Agaronia propatula* (Rupert & Peters, 2011). For some taxa, the potential predators associated with loss of an appendage have been tested and identified in controlled studies (**Table 1.3** and (Fleming et al., 2007)). These include beetles preying on the terrestrial slugs *Deroceras reticulatum* and *Limax tenellus* (Pakarinen, 1994), cancer crabs (*Cancer oregonensis*) grasping onto sea star *Leptasterias hexactis* arms (Bingham, Burr, & Head, 2000), and kelp crabs (*Pugettia productus*) inducing cerata to autotomize in *Phidiana crassicornis* (Miller & Byrne, 2000). Some organisms get added benefits from the autotomized limbs themselves as they often have adaptations that help benefit the escaping prey. These features include conspicuous coloration and continued movement of the limb even after separation from the main body, a potential substitute meal that must be big enough for the predator, release of toxic or noxious substances during autotomy, and adherence of the appendage to the predator. Movement after severance from the body can be due to a number of things from myogenic activity of tibial extensor muscles in locusts, to neurogenic pacemakers in daddy long leg spiders (Fleming et al., 2007). In gastropods like *Sacoglossa*, distracting tail movement is maintained by longitudinal and transverse muscle activity. Similarly, lizards in the genus *Podarcis* sustain post-autotomy tail movement by anaerobic muscle activity and operate similarly among the different species regardless of varying predation pressures (Pafilis, Valakos, & Fofopoulos, 2005). Leaving behind a substitute meal reduces the risk for both the predator and prey. It is less risky for the predator to stop and eat the autotomized limb than to pursue a prey that it might not be able to catch. However the autotomized portion must be big enough to provide compensation for the predator, as seen in many crabs and lizards (Fleming et al., 2007). In sea cucumbers, the evisceration of their sticky Cuvierian tubules have noxious substances and will stick to a predator and potentially immobilize them while the holothuroid escapes (Fleming et al., 2007). All of these benefits, in addition to the escape of the predator, help maintain the use of this defense mechanism.

The structure that is lost during autotomy likely has some other physiological role in the organism other than to be lost during a predation event. This includes locomotor, foraging, defensive, and reproductive roles. As a result some appendages are more likely to be lost than others. For instance, lizards are more likely to lose their tail than a foot or leg. This variation in loss can be attributed to the many potential costs of autotomy. Costs can generally be categorized in two groups, 1) those incurred from missing a limb and 2) those acquired from regenerating the

appendage. **Table 1.3** lists all potential costs to both soft and hard bodied animals with specific examples of each. An interesting cost to foraging in a soft bodied animal is seen in the gastropod *Agaronia propatula* which holds and transports its food with its foot (Rupert & Peters, 2011). Losing a part of its foot reduces its ability to catch and transport prey which usually consists of other gastropods and burrowing clams (Rupert & Peters, 2011). One of the few examples of locomotor costs to a bivalve is seen in the file shell *Limaria fragilis*. Autotomy of the longest mantle tentacles in *L. fragilis* led to decreased swimming distance produced from clapping the two valves (Donovan, Elias, & Baldwin, 2004). In general, most of the studies that look at costs are conducted in lizards whose loss of the tail could influence how fast they escape, their balance, their food reserves and subsequently their reproductive capacity, particularly in females that invest energy in eggs. Among animals with many legs, particularly arachnids, the “spare legs hypothesis” predicts that the loss of a leg would result in minimal costs, especially in cases where the limbs are all functionally alike (Guffey, 1998). This is not the case for crustaceans who experience the most loss in their specialized chelae which can be used for foraging, defense, and mate attraction. In the sand fiddler crab, *Uca pugilator*, loss of chelae negatively affects male ability to attract females and defend themselves against predators and conspecific competitors (McLain & Pratt, 2011). Like crustaceans, echinoderms also incur high costs when losing arms. Although they have relatively unspecialized arms, albeit fewer than arachnids, the arms are particularly important for stored energetics and can be costly for regeneration as seen in the sea star *Leptasterias hexactis*. *L. hexactis* puts its energy in regenerating autotomized arms such that it reduces egg production by up to 69%, effecting not only short term but also long term reproduction as the effects have been measured to last as long as 19 months post autotomy (Bingham et al., 2000).

Despite the many examples of how costly autotomy can be to individuals, there are some noteworthy examples, primarily among reptiles, of autotomy having no effect or actually being beneficial to certain physiological processes. In the cape dwarf gecko *Lygodactylus capensis* and the wall lizard *Podarcis muralis*, loss of the tail leads to faster running speeds for longer distances because the tails add weight and friction that slow down normal locomotion (Brown et al., 1995; Daniels, 1983; Fleming, Verburt, Scantlebury, Medger, & Bateman, 2009). As a result, *Podarcis muralis* regenerates a tail that weighs less than the original tail making it better and faster to move around. In some other reptile species - *Cordylus melanotus*, *Hemidactylus bowringii*, and *Hemidactylus mabouia* – there seems to be no locomotor effect on autotomized individuals nor do they exhibit differences in the histochemical composition of the regenerated tail compared to the original (Clause & Capaldi, 2006; Lu, Ding, Ding, & Ji, 2010; McConnachie & Whiting, 2003; Meyer, Prest, & Lochetto, 2002; Vervust, Loy, & Damme, 2011). Among invertebrates, one extreme example of beneficial loss of a body part is in the spider genus *Tidarren*. *Tidarren sisypoides* males will remove one of their enlarged reproductive organs (the pedipalps). This loss has been shown to be correlated with increased locomotor performance such as higher maximum speeds and greater endurance (Ramos, Irschick, & Christenson, 2004)

To deal with the negative effects of autotomy, organisms have evolved ways to reduce the costs they incur. In the ground cricket, *Dianemobius nigrofasciatus*, autotomized females during their fourth instar experienced a shorter developmental period and earlier maturation with no cost to the number of eggs they laid (Matsuoka & Ishihara, 2010). So although they were smaller, autotomized females had no effect on their fecundity. In males of this species, autotomized individuals expressed a higher frequency of calling which is preferred by females (Matsuoka, Miyakawa, & Ishihara, 2010). Although it is expected for autotomized males to hide

more often, there was no difference observed in hiding frequency compared to intact individuals. This is presumably so that the females can find these higher calling autotomized males that otherwise might not be found.

Economy of autotomy in *Trachylepis maculilabris* is a particularly good example of reducing the costs of autotomy by only autotomizing the amount of caudal tissue caught in the grasps of a predator (Cooper, Jr. & Smith, 2009). This reduces lipid loss, the amount of tail needed to be regenerated, and moderates many social status costs such as mate attractiveness. The autotomized tail that serves as a distractor still continues to move even at shorter lengths (considered to be one third the total tail length). The duration of twitching and thrashing in the tail did not vary based on its proportional length implying that losing more tissue does not mean more movement in the tail and thus does not equate to more predator distraction (Cooper, Jr. & Smith, 2009). The regenerated tail of *T. maculilabris* was difficult to autotomize and did not exhibit any movement adaptations once detached, indicating that it lost this function of the autotomized limb. Lastly, they found that skinks that lost more of their tail (at least more than two thirds the total length) exhibited a slower running speed that could affect their success at escaping a predator. Although there are still trade-offs to ‘economy of autotomy’ in general the results are in favor of animals shedding the appropriate amount of their body part to offset some of the costs of autotomy (Cooper, Jr. & Smith, 2009).

Lastly, regeneration itself is a way to mitigate costs that are associated with autotomy (Maginnis, 2006). The rate and extent to which regeneration restores the organism to its original state varies across taxa (Arnold, 1984; Bely & Nyberg, 2010). Among lizards, most species regenerate a good portion of their tail quickly, one of the fastest being *Lacerta dugesii* which will regenerate 90% of its original tail size in twelve weeks (Arnold, 1984). On the opposite side of the spectrum, there are some species, particularly among the Agamidae family, that do not regenerate any portion of their tail (Arnold, 1984). The trade-offs between investing energy in regrowth versus other processes, like reproduction, is one potential source for this variation in regenerative ability among different taxa (Maginnis, 2006).

There are a few caveats to keep in mind when looking at the results of these studies. Although they address the adaptive value of autotomy, these studies all looked at the level of the individual and in some cases the population, not necessarily the species level where natural selection and adaptation is usually at work (Juanes & Smith, 1995). Also, in studies that are conducted in a lab environment, the conditions may be too artificial such that expected costs are not detected. For instance, feeding all subjects, autotomized or intact, ad libitum in an experiment may not be representative of what they experience in their natural environment where loss of an appendage may affect their feeding rate and thus be costly to other aspects of their biology (Maginnis, 2006)

#### **D. Evolution of autotomy:**

The aspects of behavior that have been covered thus far consider the use and maintenance of autotomy on an ecological time scale. Behavior, however, is also a product of evolutionary history maintained by multiple processes including natural selection (Cooper, Prez-Mellado, & Vitt, 2004; Maginnis, 2006; Ord & Martins, 2010). Antipredator behaviors, like autotomy, often lend themselves well to being studied as examples of present day adaptations under pressures of natural selection (Ord & Martins, 2010). Yet, studies in lizards have shown that historical factors can affect the costs and benefits that lead to the traits we see in current times (Arnold, 1984). Autotomy as we study it today may have originated in the distant past in a common ancestor and

maintained under current conditions as an adaptation by selection or conserved by phylogenetic inertia.

A complication with studying autotomy is its close association with regeneration and the tendency, though not the rule, for the autotomized body part to regrow. Many phyla, both basal and recent in lineage, have the ability to regenerate a wide range of body parts, tissue types, and cell types. As a result, regeneration is thought to be an ancestral character, evolving as soon as the origin of multicellularity and undergoing multiple losses among many taxa of metazoan (Bely & Nyberg, 2010; Bely, 2010). It is suggested that the trait was initially maintained either by being tightly paired with another trait like asexual reproduction (i.e. pleiotropy in Annelids) or was an ancestral trait that was neither selected for or against (i.e. phylogenetic inertia). Current utility and maintenance of regeneration is thought to be adaptive as a response to sub-lethal predation or competition (Bely & Nyberg, 2010; Bely, 2010). Whether regeneration preceded or proceeded the ability to autotomize is not known for any of the major phyla, but they are often discussed as potential co-evolutionary states.

One of the biggest factors limiting evolutionary studies of autotomy is the incorporation of well-developed phylogenies with known supported evolutionary relationships between major groups. In addition, there is not enough data collected about all represented taxa for more closely related members, depending on the level of analysis (Maruzzo et al., 2005). Information is often incomplete to make species level comparisons and family level investigations are easier to tackle, but often result in more variation in the traits of interest. This makes it difficult to separate whether a trait independently evolved for each species or if it evolved once in a common ancestor and was subsequently lost in some groups (Ord & Martins, 2010). Reptiles are the only example where evolutionary relationships are known and well-supported between major taxa and allow for a comprehensive phylogenetic analysis. **Table 1.4** summarizes these results according to whether the explanations are historical or adaptive. In general, the wide occurrence among very different organisms and autotomizing body parts have led to the idea that autotomy in invertebrates is a result of convergent evolution. In contrast, the wide range in the frequency of occurrence among lizards hints at potential divergent expressions of autotomy in reptiles (Bateman & Fleming, 2009).

Among molluscs, autotomy is thought to be advantageous at the individual as well as at the species level. Autotomy in molluscs is thought to have originated from selection to manage loss of a body part (Stasek, 1967). In snails, Stasek (1967) and Fishelson and Kidron (1968) proposed that autotomy evolved in parallel with the reduction and eventual complete loss of a shell. Without a shell to provide a protective barrier against predators, these gastropods had to rely on a different mode of defense, such as escaping by autotomy. Nudibranchs, which have also lost their hard protective covering, utilize autotomy by shedding their cerata, stinging cells isolated from the nematocysts of their anemone prey. In the gastropods *Agaronia* and *Olivella*, the evolution of autotomy as a secondary defense mechanism to hiding in their shell has been linked to the loss of their operculum and corresponding changes in foot morphology when retreating into the shell (Rupert & Peters, 2011).

Similar to molluscs, autotomy in arthropods is thought to be an adaptation to an environment that favored the loss of an appendage (Glaser, 1904). Some evidence even suggests that the development of the autotomy plane is connected to molting, particularly in the stick insects (Phasmatodea) (Glaser, 1904). The plesiomorphic or ancestral state for arthropods is thought to be the absence of autotomy, arising first within the Pancrustacea and being lost in certain lineages within this group. A key adaptation that seems to correlate with the origins of

autotomy within arthropods is the presence of a diaphragm at the autotomy plane which is used to decrease hemorrhaging during limb loss (Maruzzo et al., 2005).

A particular group of arthropods pose an interesting evolutionary scenario. The eusocial hymenoptera which include *Apis spp.*, *Pogonomyrmex spp.*, and some species of tropical wasps live in colonies where members share a high degree of relatedness with each other. They are known for their stinging defense mechanisms which evolved from a female ovipositor. Upon injection, eusocial hymenoptera will autotomize their stinger and any associated glands that release venom during separation. The released substance may also contain pheromones that will alert other colony members of the nearby threat. This combined defense mechanism of autotomy and venom is thought to have evolved independently three times in each of these groups. It is also considered to be targeted against larger predators that may require stinging from more than one individual (Hermann, 1971; Shorter & Rueppell, 2011).

Echinoderms, much like many arthropods, are known for their high frequency of structural loss as well as high degree of regeneration. Their autotomy planes are specialized for rapid detachment and make regeneration more readily employed (Wilkie, 2001). Whether autotomy is a prelude to regeneration varies depending on the different classes of echinoderms (Wilkie, 2001). Whether autotomy originates from selective pressures or represents a remnant of the past, both autotomy and regeneration are important to echinoderm physiology. One physiological feature common in all echinoderm groups that might hold the key to understanding the origins of autotomy is mutable collagenous tissues (MCT) (Wilkie, 2001). MCT is thought to be an adaptation to the echinoderm's limited ability to move along multiple planes of motion due to a highly calcified skeleton with few muscular connections. MCT sites can be found at locations that are not specifically autotomy zones but undergo cycles of destiffening and restiffening to allow for greater range of flexibility (Wilkie, 2001). Echinoderms can also remove an appendage at these MCT sites as a means of opportunistic self-detachment and serves as an example of potential exaptation for autotomy (Wilkie, 2001).

Crinoidea is the most common class of echinoderm found in the fossil record that shows evidence for regeneration and potentially indicate when autotomy evolved in this group. Crinoid arm articulations are found abundantly with muscular and ligamentary articulations (Oji, 2001). A trend of increasing number of muscular articulations, particularly at proximal locations, with decreasing ligamentary articulations from the Paleozoic to the Mesozoic suggests a possible origin for the ability to autotomize crinoid arms (Oji, 2001). This implies that arm autotomy is a relatively recent trait in crinoids (Gahn & Baumiller, 2010). Autotomy of other parts of the crinoid body, such as their main plate, has a longer standing ability (Gahn & Baumiller, 2010). Donovan (2012) also noted that most fossil crinoids are found with crowns fully preserved and surrounded by disarticulated columns. This hints at potential frequency of autotomy of these organisms in the distant past. Present day crinoids have recent adaptations not seen in Paleozoic crinoids that may have been obtained as a means for faster and more efficient autotomy. These adaptations include more regularly spaced articulations and the ability to seal the axial canal upon detachment (Donovan, 2012).

Lizards and their kin are the best studied examples of autotomy and have fairly resolved phylogenetic trees that make evolutionary history investigations possible. One of the first studies looking at functional morphology of tails suggested that autotomy specific adaptations evolved three times independently in different groups of salamanders (Wake & Dresner, 1967). Among reptiles, intravertebral autotomy is thought to be the ancestral condition with multiple losses occurring in various lineages (Arnold, 1984; Bateman & Fleming, 2009). The second

mechanism, intervertebral autotomy, is thought to be more derived and does not exhibit any particular specializations for autotomy. This is evident in the Agamidae family and in some snakes which re-evolve caudal autotomy by simple mechanisms (Arnold, 1984; Bateman & Fleming, 2009). There are two potential ecomorphotypes among the agamids: they are either climbers or more terrestrial species with long legs and a long tail that give them the ability to bipedally run (Arnold, 1984). These findings are supported by phylogenetic analyses of traits that suggest autotomy is more primitive in Squamata (lizards and snakes). Groups that currently do not autotomize their tail represent recent losses of the ability. Intervertebral autotomy is more derived compared with absence of autotomy and may have arisen as many as eight times in the Agamidae family. Additionally, not all autotomized tails are regenerated. Caudal regeneration is thought to have re-developed up to five times in the agamids. Among the lizards that autotomize intravertebrally, three general situations can explain the loss of autotomy: (1) large species that rely more on fight defenses; (2) slow species or ones with small tails that would render them unable to escape fast enough or gather enough attention for the predators to grasp the tail rather than the body; and (3) lizards that are partially arboreal or live in complex vegetation with slower gaits. These lizards also use more aggressive displays to mediate predation encounters (Arnold, 1984).

Another phylogenetic study that looked at how tail morphology features co-evolved with caudal autotomy surveyed 39 species of lizards expressing a variety of locomotor and foraging behaviors (Zani, 1996). Zani (1996) found positive correlations between the potential autotomy plane, the length of the caudifemoralis longus (CFL) muscle, and the jumping distance, all indicating that these features co-evolved with caudal autotomy.

Lastly, ancestral vertebrates, like the crinoids, leave behind a fossil record that could potentially record features indicative of autotomy. Among Squamata, a few fossil reptiles from the Permian have identifiable fracture planes in the tail as well as partially regenerated tails (Delfino & Sánchez-Villagra, 2010). This leads to the question: what came first, autotomy or regeneration?

### **Octopus as a model system to study autotomy**

Cephalopods are soft-bodied invertebrates observed to lose their arms to varying degrees. Among octopuses, a majority of autotomizing species are benthic: *Ameloctopus litoralis*, *Octopus defillipi*, *O. mutilans*, *O. alecto*, *O. guangdongensis*, *Euaxocephalus panamensis*, *E. pillburyae* (Norman, 1992), *Thaumoctopus mimicus*, *Wunderpus photogenicus*, *Abdopus*, *Octopus horridus*, ‘*Octopus sp.* Ward, 1998’ (Ward, 1998) (Norman & Finn, 2001). However there are some pelagic species with interesting variations, namely the members of the genera *Argonauta*, where the males detach their hectocotylus during copulation and leave the arm inserted in the female, and in female *Tremoctopus sp.* which lose webbing on the first pair of arms along predetermined break line (Nesis, 1987). Among squid, various species lose their tentacles during certain life stages, usually at the end of the larval stage (*Gonatopsis sp.*, *Lepidoteuthis sp.*) or during sexual maturity and spawning (*Histioteuthis*, *Chaunoteuthis*, *Joubiniteuthis*, Cranchiidae). These losses do not undergo subsequent regeneration. Squid tentacle loss and the detachment of the male *Argonauta* hectocotylus do not follow the first criteria of autotomy which states the appendages are lost for defense purposes (Nesis, 1987). However, two species of squid, *Octopoteuthis deletron* (Bush, 2012) and *Mastigoteuthis* have been shown to autotomize their arms as a means of defense (Nesis, 1987).



Among octopuses, little information is known about the different behavioral aspects of autotomy. Mechanistically, some species have been observed to consistently lose their arms at a particular region of suckers, usually at the base, suggesting the presence of a potential breakage plane (Huffard, 2007; Norman & Finn, 2001; Norman, 1992; Ward, 1998). Budelmann (1998) described octopuses losing arms by two ways, either by directly breaking at a pre-determined site below the skin or by the individual biting off the arm by autophagy. With regards to its adaptive value, arm loss in octopuses have been associated with fights and situations where the individual is trapped (i.e. sticking arms into crevices while foraging) and must find a way to escape and survive (Budelmann, 1998). Octopuses use a multitude of other primary and secondary defense mechanisms before resorting to autotomy, suggesting that they experience strong predation pressures in their environment. These defense mechanisms include camouflaging, inking, locomotion, and mimicry (Warnke, Kaiser, & Hasselmann, 2012). The lack of information on octopus autotomy opens up many avenues of research that can be pursued similar to what has been done in other invertebrates and vertebrates.

Octopuses provide a unique system for studying autotomy. The arms can be manipulated such that the position, number, and proportion of arm that is lost can be varied. This leads to differing levels of autotomy that can test the idea of gradients proposed in **Figure 1.1**. The ability of octopus arms to continue moving post-detachment also provides an opportunity to examine mechanisms for arm loss as well as assess the benefits of an autotomized arm as a distractor for predators.

## **Conclusions**

This comprehensive overview of autotomy is the first to summarize what is known from both the invertebrate and vertebrate literature about this secondary defense mechanism. Several different terms were generated as variants to ‘autotomy,’ adding confusion in identifying what kinds of limb loss could be considered true autotomy. A more detailed definition developed by Fredericq has been the basis for most investigations of this defense mechanism. Past studies have taken each of Fredericq’s three criteria to develop a better understanding of autotomy in their model organism. This usually concentrated on crustaceans, echinoderms, and lizards. Fredericq’s criteria can be very specific (i.e. the presence of an intrinsic mechanism and the role of the central nervous system) and approaching it from a narrow view can hinder progress in better understanding the phenomenon. Here I proposed using a broader perspective that still incorporates the three points of Fredericq’s definition for autotomy. Tinbergen’s ethological approach addresses four aspects of a behavior: mechanism, development, function, and evolution. Although he advocated for the study of all four criteria in the same organism to describe a behavior, very few phenomena lend themselves well for being studied in all aspects. We have seen most studies of autotomy biased toward mechanism and function. Recent advancements in genetics and molecular and cell biology technology could allow us to tackle questions about evolution and development.

Although this review shows that lot has been done to characterize autotomy, there are still gaps of knowledge that need to be filled. In particular, broadening our scope to incorporate non- model organisms like the octopus and other soft bodied animals will yield more comprehensive information that can be compared with other organisms. Like many other phenomena, autotomy has been shown to follow a gradient of loss rather than a strict definition. Filling in the gradient with different examples of organisms that exhibit varying degrees of loss will help us identify key causal factors to autotomy. Also finding ways to study the development

and evolution of autotomy using the most appropriate organism will yield more detailed information than we currently have regarding these aspects of behavior. This review reveals that although autotomy is often considered a last resort strategy, it is used frequently among a wide variety of taxa and should appropriately be as well understood as primary defense mechanisms like camouflage.

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**TABLES:**

**Table 1.1.** Comparison of mechanisms used for autotomizing limbs with and without an exo-/endo-skeleton. Three features are highlighted: (1) where does autotomy occur, (2) how does it occur with respect to muscle versus passive action, and (3) what happens to the autotomized body part.

<b>SOFT BODIED</b> (autotomized limb without exo-/endo-skeleton)	<b>HARD BODIED</b> (autotomized limb with exo-/endo- skeleton)
<b>LOCATION OF AUTOTOMY</b>	
<p>1. Clear preformed breakage plane(s)</p> <p>a. <u>Cnidaria</u>: <i>Aglantha digitale</i> (Fleming et al., 2007)</p> <p>b. <u>Mollusca</u>: <u>Gastropoda</u>: <i>Lobiger serradifalci</i>, <i>Caliphylla australis</i>, <i>Zephyrina mucronifera</i>, <i>Prophysaon foliolatum</i>, <i>Agaronia propatula</i>, <u>Bivalvia</u>: <i>Lima dehiscens</i>, <u>Cephalopoda</u>: <i>Ameloctopus litoralis</i>, <i>Octopoteuthis deletron</i> (Bush, 2012; Fleming et al., 2007; Norman, 1992; Rupert &amp; Peters, 2011; Stasek, 1967)</p> <p>2. Zone of weakness breakage plane</p> <p>a. <u>Annelida</u>: <i>Lumbricululus variegatus</i>, <i>Tubifex tubifex</i>, <i>Alentia gelatinosa</i> (Kaster, 1979; Lesiuk &amp; Drewes, 1999; Wilkie, 2011)</p> <p>b. <u>Mollusca</u>: <u>Gastropoda</u>: <i>Harpa ventricosa</i>, <i>Doto coronata</i>, <i>Sakuraeolis enosimensis</i>, <i>Aeolidia poindimiei</i>, <i>Discodoris fragilis</i>, <i>Peltodoris atromaculata</i>, <i>Kentrodoris intramaculata</i>, <u>Bivalvia</u>: <i>Limax marginatus</i>, <i>Lima hians</i>, <i>Tremoctopus violaceus</i>, <i>Deroceras reticulatum</i>, <i>Limax tenellus</i>, <i>Phidiana crassicornis</i>, <i>Melibe leonine</i>, <i>Gena varia</i>, <i>Octopus horridus</i>, <i>Thaumoctopus sp</i>, <i>Wunderopus photogenicus</i></p>	<p>1. Joint (fixed location of breakage plane(s)):</p> <p>a. Between skeletal hard parts: (ligament) Chitinous:</p> <p>i. <u>Arthropoda</u>: <u>Arachnida</u>: <i>Latrodectus variolus</i>, <i>Tegenaria atrica</i> <u>Crustacea</u>: <i>Carcinus maenas</i>, <i>Uca pugilator</i>, <i>Homarus gammarus</i>, <i>Astacus fluviatilis</i>, <i>Pagurus bernhardus</i> <u>Hexapoda</u>: <i>Cuniculina impigra</i>, <i>Gryllus bimaculatus</i> (Maruzzo et al., 2005; Parry, 1957; Randall, 1981) (Fleming et al., 2007; Maruzzo et al., 2005; McVean, 1975; Wood &amp; Wood, 1932) (Bässler, 1984; Fleming et al., 2007; Maruzzo et al., 2005)</p> <p>Ossicles (intervertebral):</p> <p>ii. <u>Echinodermata</u>: <u>Asteroidea</u>: <i>Asterias forbesi</i>, <u>Crinoidea</u>: <i>Antedon spp.</i>, <u>Echinoidea</u>: <i>Paracentrotus lividus</i>, <u>Ophiuroidea</u>: <i>Ophiocomina nigra</i> (Fleming et al., 2007; Wilkie, 2001; Wilkie, 1978)</p> <p>Bone (intervertebral): no planes</p> <p>iii. <u>Chordata</u>: <u>Reptilia</u>: <i>Physignathus</i>, <i>Diporophora</i>, <i>Rhadinaea decorata</i> (Arnold, 1984)</p> <p>b. Within skeletal hard part: Bone (intravertebral): planes</p> <p>i. <u>Chordata</u>: <u>Reptilia</u>: (most common) <i>Niveoscincus metallicus</i>, <i>Hemidactylus flaviviridis</i>, <i>Pygopodidae</i>, <i>Pliocercus</i> (Arnold, 1984; Bateman &amp; Fleming, 2009)</p> <p>2. Zone of weakness breakage plane (loss of tensile strength)</p> <p>i. <u>Chordata</u>: <u>Reptilia</u>: <i>Nephrurus asper</i>, <i>Bolitoglossa</i>, <i>Ensatina</i> (Arnold, 1984; Wake &amp; Dresner, 1967)</p> <p>ii. <u>Echinodermata</u>: <u>Holothuroidea</u>:</p>

<p>(Bickell-Page, 1989; Fishelson &amp; Kidron, 1968; Gilmour, 1967; Liu &amp; Wang, 2002; Miller &amp; Byrne, 2000; Norman &amp; Hochberg, 2005; Norman &amp; Finn, 2001; Norman, 1992; Pakarinen, 1994; Stasek, 1967)</p> <p>3. Economy of autotomy:</p> <p>a. <u>Mollusca</u>: <i>Octopoteuthis deletron</i>, <i>Lima hians</i> (Bush, 2012; Gilmour, 1963)</p>	<p><i>Holothuria glaberrima</i> (Wilkie, 2001)</p> <p>3. Economy of autotomy:</p> <p>i. <u>Chordata: Reptilia</u>: <i>Gehyra variegata</i>, <i>Phyllodactylus marmoratus</i>, <i>Trachylepis maculilabris</i> (Bustard, 1968; Cooper, Jr. &amp; Smith, 2009; Daniels, 1985)</p> <p>ii. <u>Echinodermata: Asteroidea</u>: <i>Luidia ciliaris</i>, <i>Brisinga coronate</i>, <u>Ophiuroidea</u>: <i>Amphiura filiformis</i>, <i>Amphiura chiajei</i> (Delage &amp; Herouard, 1903; McVean, 1975; Wilkie, 2010) (Wilkie, 1978)</p>
<p><b>PROCESS OF BREAKAGE</b></p>	
<p>1. External mechanical stimulation induces active muscle contraction at breakage plane (transverse, longitudinal, or circular sphincter)</p> <p>a. <u>Annelida: Alentia</u> <i>gelatinosa</i>, <i>Tubifex tubifex</i>, <i>Lumbriculus variegatus</i> (Kaster, 1979; Lesiuk &amp; Drewes, 1999; Wilkie, 2011)</p> <p>b. <u>Cnidaria: Aglantha digitale</u> (Bickell-Page &amp; Mackie, 1991)</p> <p>c. <u>Mollusca: Harpa</u> <i>ventricosa</i>, <i>Stomatia</i>, <i>Stomatella mariei</i>, <i>Gena varia</i>, <i>Discodoris</i>, <i>Argus</i>, <i>Peltodoris</i>, <i>Melibe</i>, <i>Tethys leporina</i>, <i>Glaucus</i>, <i>Prophysaon foliolatum</i>, <i>Ficus ficus</i>, <i>Tremoctopus violaceus</i>, <i>Ficus ficus</i>, <i>Phidiana crassicornis</i>, <i>Melibe leonine</i>, <i>Lobiger serradifalci</i>, <i>Sakuraeolis enosimensis</i>, <i>Zephyrina mucronifera</i>, <i>Stiliger vanellus</i>, <i>Dirona albolineata</i>, <i>Lima dehiscens</i> (Bickell-Page, 1989; Fleming et al., 2007; Liu &amp; Wang, 2002; Miller &amp; Byrne, 2000; Stasek, 1967)</p> <p>2. External mechanical stimulation induces physical motion of body part until shed:</p>	<p>1. External mechanical force stimulation induces active muscle contraction at breakage plane</p> <p>a. <u>Arthropoda: Arachnida</u>: <i>Tegenaria atrica</i> <u>Crustacea</u>: <i>Carcinus maenas</i>, <i>Homarus gammarus</i>, <i>Astacus fluviatilis</i>, <i>Pagurus bernhardus</i> <u>Hexapoda</u>: <i>Cuniculina impigra</i>, <i>Apis</i>, <i>Lestes viridis</i> (Parry, 1957), (Fleming et al., 2007; McVean, 1975; Wood &amp; Wood, 1932), (Bässler, 1984; Shorter &amp; Rueppell, 2011; Slos, De Block, &amp; Stoks, 2009)</p> <p>b. <u>Chordata: Reptilia</u>: <i>Lacerta vivipara</i>, <i>Hemidactylus flaviviridis</i> (Arnold, 1984; McVean, 1975)</p> <p>c. <u>Echinodermata: Asteroidea</u>: <i>Asterias rubens</i>, <u>Holothuroidea</u>: <i>Eupentacta quinquesemita</i> (Fleming et al., 2007; Wilkie, 2010) (Wilkie, 2001)</p> <p>2. Passive rupture (no muscle action) by tension</p> <p>a. <u>Echinodermata: Crinoidea</u>: <i>Florometra serratissima</i>, <u>Ophiuroidea</u>: <i>Ophiocomina nigra</i> (Wilkie, 2001) (Wilkie, 2001; Wilkie, 1978)</p> <p>3. Central nervous control</p> <p>a. <u>Arthropoda: Crustacea</u>: <i>Carcinus maenas</i> (McVean, 1974)</p> <p>b. <u>Chordata: Reptilia</u>: <i>Thamnophis sirtalis sirtalis</i>, <i>Teratoscincus bedriagai</i>, (Bateman &amp; Fleming, 2009; Clause &amp; Capaldi, 2006)</p> <p>c. <u>Echinodermata: Echinoidea</u>: <i>Sphaerechinus granularis</i>, <u>Holothuroidea</u>: <i>Thyone briareas</i>, <u>Ophiuroidea</u>: <i>Ophiura nigra</i> (Wilkie, 2001) (McVean, 1975) (Wilkie, 1978)</p> <p>4. Peripheral Reflex</p>

<p>a. <u>Mollusca</u>: <i>Helicarion</i>, <i>Stenopus</i>, <i>Pleurodonte imperator</i>, <i>Pleurodonte apollo</i>, <i>Pleurodonte crassilabris</i>, <i>Deroceras reticulatum</i>, <i>Limax tenellus</i> (Pakarinen, 1994; Stasek, 1967)</p> <p>3. Nervous system control</p> <p>a. <u>Annelida</u>: <i>Lumbriculus variegatus</i> (Lesiuk &amp; Drewes, 1999)</p> <p>b. <u>Mollusca</u>: <i>Ficus ficus</i> (Liu &amp; Wang, 1996)</p> <p>4. Peripheral (localized) reflex:</p> <p>a. <u>Mollusca</u>: <i>Prophysaon foliolatum</i>, <i>Phidiana crassicornis</i>, <i>Melibe leonina</i> (Bickell-Page, 1989; Deyrup-Olsen et al., 1986; Miller &amp; Byrne, 2000)</p>	<p>a. <u>Arthropoda</u>: <u>Arachnida</u>: <i>Leiobunum nigripes</i>, <i>Pholcus phalangioides</i>, <u>Crustacea</u>: <i>Uca pugilator</i>, <u>Hexapoda</u>: <i>Cuniculina impigra</i> (Bässler, 1984; Fleming et al., 2007) (Hopkins, 1993) (Bässler, 1984)</p> <p>5. Microstructure adhesion to surface:</p> <p>a. <u>Chordata</u>: <u>Reptilia</u>: <i>Gekko gecko</i> (Sanggaard et al., 2012)</p>
AUTOTOMIZED BODY PART	
<p>1. Continued motion of detached body part</p> <p>a. <u>Mollusca</u>: <i>Harpa ventricosa</i>, <i>Gena varia</i>, <i>Caliphylla australis</i>, <i>Propemelibe mirifica</i>, <i>Phenacarion</i>, <i>Octopus horridus</i>, <i>Ameloctopus litoralis</i>, <i>Octopoteuthis deletron</i> (Bush, 2012; Fleming et al., 2007; Norman, 1992; Stasek, 1967)</p> <p>2. Adherence of detached body part</p> <p>a. <u>Mollusca</u>: <i>Caliphylla australis</i>, <i>Caliphylla borgnini</i>, <i>Zephyrina mucronifera</i>, <i>Lima dehiscens</i> (Stasek, 1967)</p>	<p>1. Continued motion of detached body part:</p> <p>a. <u>Arthropoda</u>: <u>Arachnida</u>: <i>Leiobunum nigripes</i>, <i>Pholcus phalangioides</i>, <u>Hexapoda</u>: <i>Cuniculina impigra</i> (Bässler, 1984; Fleming et al., 2007), (Bässler, 1984)</p> <p>b. <u>Chordata</u>: <u>Reptilia</u>: <i>Trachylepis maculilabris</i>, <i>Eublepharis macularius</i>, <i>Podarcis hispanica</i> (Clause &amp; Capaldi, 2006; Cooper, Jr. &amp; Smith, 2009; Higham &amp; Russell, 2010)</p> <p>c. <u>Echinodermata</u>: <u>Ophiuroidea</u>: <i>Ophiocomina nigra</i> (Wilkie, 1978)</p> <p>2. Adherence of detached body part:</p> <p>a. <u>Arthropoda</u>: <u>Crustacea</u>: <i>Potamocarcinus richmondi</i>, <i>Geocarcinus quadratus</i>, <u>Hexapoda</u>: <i>Apis</i>, <i>Pogonomyrmex</i>, <i>Polybia rejecta</i> (Robinson et al., 1970) (Shorter &amp; Rueppell, 2011)</p> <p>b. <u>Echinodermata</u>: <u>Holothuroidea</u>: <i>Holothuria scabra</i> (Wilkie, 2001)</p>

**Table 1.2.** Comparison of ontogenetic factors and patterns important for the use of autotomy in organisms with and without an exoskeleton.

<p align="center"><b>SOFT BODIED</b> (autotomized limb without exo- /endo-skeleton)</p>	<p align="center"><b>HARD BODIED</b> (autotomized limb with exo-/endo- skeleton)</p>
<p align="center">AGE STRUCTURE</p> <ul style="list-style-type: none"> <li>• Juveniles with lower frequency of autotomy compared to adults. (Frequency of autotomy increases with age.) <ul style="list-style-type: none"> <li>○ <u>Mollusca: Gastropoda:</u> <i>Prophysaon coeruleum</i> (Stasek, 1967)</li> </ul> </li> <li>• Juveniles with higher frequency of autotomy compared to adults. (Frequency of autotomy decreases with age.) <ul style="list-style-type: none"> <li>○ <u>Mollusca: Gastropoda:</u> <i>Satsuma caliginosa</i> (Hoso, 2012)</li> </ul> </li> <li>• Immediate full development of autotomy mechanism upon hatching. <ul style="list-style-type: none"> <li>○ <u>Mollusca: Gastropoda:</u> <i>Prophysaon foliolatum</i> (Deyrup-Olsen et al., 1986)</li> </ul> </li> </ul>	<p align="center">AGE STRUCTURE</p> <ul style="list-style-type: none"> <li>• Juveniles with lower frequency of autotomy compared to adults. (Frequency of autotomy increases with age.) <ul style="list-style-type: none"> <li>○ <u>Chordata: Reptilia:</u> <i>Dendrophidion dendrophis</i>, <i>Mastigodryas bifossatus</i>, <i>Eremias lugubris</i>, <i>Christinus marmoratus</i>, <i>Niveoscincus metallicus</i> (Arnold, 1984; Bateman &amp; Fleming, 2009; Chapple &amp; Swain, 2004; Dourado, Oliveira, &amp; Prudente, 2013)</li> </ul> </li> <li>• Juveniles with higher frequency of autotomy compared to adults. (Frequency of autotomy decreases with age.) <ul style="list-style-type: none"> <li>○ <u>Arthropoda: Crustacea:</u> <i>Paralithodes camtschatica</i> (Edwards, 1972)</li> <li>○ <u>Chordata: Reptilia:</u> Iguanids, skinks, <i>Tupinambis</i>, <i>Lacerta trilineata</i>, <i>Lacerta dugesii</i> (Arnold, 1984a; P. W. Bateman &amp; Fleming, 2009b; Pafilis &amp; Valakos, 2008)</li> </ul> </li> </ul> <p align="center">SIZE STRUCTURE:</p> <ul style="list-style-type: none"> <li>• Higher frequency of autotomy among larger individuals. <ul style="list-style-type: none"> <li>○ <u>Arthropoda: Crustacea:</u> <i>Callinectes sapidus</i>, <i>Carcinus maenus</i>, <i>Necora puber</i> (McVean &amp; Findlay, 1979; Norman &amp; Jones, 1991; Smith &amp; Hines, 1991)</li> <li>○ <u>Chordata: Reptilia:</u> <i>Niveoscincus metallicus</i> (Chapple &amp; Swain, 2004)</li> </ul> </li> </ul> <p align="center">SURVIVORSHIP:</p> <ul style="list-style-type: none"> <li>• Increasing age, decreasing survivorship with use of autotomy <ul style="list-style-type: none"> <li>○ <u>Arthropoda: Arachnida:</u> <i>Gladicosa pulchra</i> (Klawinski &amp; Formanowicz Jr., 1994)</li> </ul> </li> </ul> <p align="center">GROWTH RATE:</p> <ul style="list-style-type: none"> <li>• Autotomized juveniles exhibit reduced growth rate compared to intact juveniles. <ul style="list-style-type: none"> <li>○ <u>Chordata: Reptilia:</u> <i>Uta stansburiana</i> - field (Niewiarowski et al., 1997)</li> </ul> </li> <li>• Autotomized juveniles do not exhibit a tradeoff</li> </ul>

	<p>between tail regeneration and somatic growth</p> <ul style="list-style-type: none"><li>○ <u>Chordata: Reptilia</u>: <i>Uta stansburiana</i> – lab (Althoff &amp; Thompson, 1994)</li><li>● Autotomized juveniles invest more energy in growth than tail regeneration compared to autotomized adults .<ul style="list-style-type: none"><li>○ <u>Chordata: Reptilia</u>: <i>Coleonyx variegatus</i>, <i>Eumeces skiltonianus</i>, <i>Eumeces gilbert</i> (Congdon, Vitt, &amp; King, 1974; Vitt et al., 1977)</li></ul></li><li>● No change in energy investment and growth across age groups.<ul style="list-style-type: none"><li>○ <u>Chordata: Reptilia</u>: <i>Gerrhonotus multicarinatus</i> (Vitt et al., 1977)</li></ul></li></ul>
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**Table 1.3.** Comparison of the adaptive value of autotomy in organisms with and without an exoskeleton. This includes known examples of frequency and factors that influence the occurrence of autotomy as well as associated costs and benefits.

SOFT BODIED (autotomized limb without exo-/endo-skeleton)	HARD BODIED (autotomized limb with exo-/endo-skeleton)
INCIDENCE AND FACTORS OF AUTOTOMY	
<p style="text-align: center;">INCIDENCE:</p> <ul style="list-style-type: none"> <li>• % population:</li> <li>• # appendages lost (for octopus)</li> <li>• Position of appendages lost (for octopus)               <ul style="list-style-type: none"> <li>○ <u>Mollusca: Gastropoda</u>: <i>Ficus ficus</i>, <i>Satsuma caliginosa</i> (Hoso, 2012; Liu &amp; Wang, 1999)</li> <li>○ <u>Mollusca: Cephalopoda</u>: <i>Octopus brachiotomus</i>, <i>sp. nov.</i> (Guam) (Ward, 1998)</li> </ul> </li> </ul>	<p style="text-align: center;">INCIDENCE:</p> <ul style="list-style-type: none"> <li>• % population</li> <li>• # appendages lost</li> <li>• Position of appendages lost               <ul style="list-style-type: none"> <li>○ <u>Arthropoda: Arachnida</u>: <i>Holocnemus pluchei</i>, <i>Leiobunum nigripes</i>, <i>Leiobunum vittatum</i> <u>Crustacea</u>: <i>Porcellana platycheles</i>, <i>Gammarus pulex</i>, <i>Asellus aquaticus</i>, <i>Uca pugilator</i>, <i>Hemigrapsus oregonensis</i> <u>Hexapoda</u>: <i>Lestes Viridis</i>, <i>Scudderia texensis</i>, <i>Gryllus bimaculatus</i> (Fleming et al., 2007; Guffey, 1998) (Bateman &amp; Fleming, 2005; Fleming et al., 2007) (Fleming et al., 2007; Juanes &amp; Smith, 1995; Mariappan et al., 2000; McLain &amp; Pratt, 2011; McVean, 1975)</li> <li>○ <u>Chordata: Reptilia</u>: <i>Niveoscincus metallicus</i>, <i>Podarcis muralis</i>, <i>Podarcis lilfordi</i>, <i>Anolis sagrei</i>, <i>Desmognathus quadramaculatus</i> (Bateman &amp; Fleming, 2009; Bateman &amp; Fleming, 2011; Chapple &amp; Swain, 2004; Cooper et al., 2004; Marvin, 2013)</li> <li>○ <u>Echinodermata: Asteroidea</u> : <i>Leptasterias hexactis</i>, <i>Heliaster helianthus</i>, <i>Asterias forbesi</i>, <i>Asterias vulgaris</i>, <i>Meyenaster gelatinosus</i>, <i>Stichaster striatus</i> <u>Linckia multifora</u> &amp; <u>Linckia diplax</u>, <i>Asterias tenuispina</i>, <i>Coscinasterias acutispina</i>, <i>Dactylosaster cylindricus pacificus</i>, <i>Pentaceros hawaiiensis</i> <u>Crinoidea</u>: <i>Cenocrinus asterias</i>, <i>Metacrinus rotundus</i>, <u>Comatulida</u> <u>Echinodermata: Ophiuroidea</u>: <i>Amphiura filiformis</i>, <i>Ophicoma echinata</i>, <i>Ophiura albida</i> (Barrios, Gaymer, Vásquez, &amp; Brokordt,</li> </ul> </li> </ul>



<p>FACTORS: (conditional and context-dependent)</p> <ul style="list-style-type: none"> <li>• Propensity for subsequent loss of appendages</li> <li>• Size</li> <li>• Sex</li> <li>• Symmetry in loss</li> <li>• Habitat</li> <li>• Alternative defense strategies</li> <li>• Regeneration ability <ul style="list-style-type: none"> <li>○ <u>Mollusca: Cephalopoda</u>: <i>Octopus brachiotomus</i>, <i>sp. nov.</i> (Guam) (MNorman &amp; Finn, 2001; Ward, 1998)</li> <li>○ <u>Mollusca: Gastropoda</u>: <i>Deroceras reticulatum</i>, <i>Limax tenellus</i> (Pakarinen, 1994)</li> </ul> </li> </ul>	<p>2008; Bingham et al., 2000; Edmondson, 1935; Fleming et al., 2007; Lawrence &amp; Vasquez, 1989)</p> <p>FACTORS: (conditional and context dependent)</p> <ul style="list-style-type: none"> <li>• Cause: Predation (degree of prey exposure, intensity, efficiency, predator types), interspecific competition, intraspecific competition</li> <li>• Propensity for subsequent loss of appendages</li> <li>• Predator-prey behaviors</li> <li>• Alternative defense strategies</li> <li>• Size</li> <li>• Sex</li> <li>• Symmetry in loss</li> <li>• Habitat:</li> <li>• Sociality</li> <li>• Regeneration ability <ul style="list-style-type: none"> <li>○ <u>Arthropoda: Crustacea</u>: <i>Hemigrapsus oregonensis</i>, <i>Petrolisthes</i>, <i>Carcinus</i>, <i>Leander</i>, <i>Marinogammarus</i>, <i>Potamocarcinus richmondi</i>, <i>Gecarcinus quadratus</i> <u>Hexapoda</u>: <i>Gryllus bimaculatus</i>, <i>Apis</i>, <i>Pogonomyrmex</i> (Mariappan et al., 2000) (Easton, 1972; McVean, 1975) (Wasson &amp; Lyon, 2005) (McVean, 1975) (Robinson et al., 1970) (Shorter &amp; Rueppell, 2011)(Bateman &amp; Fleming, 2005)</li> <li>○ <u>Chordata: Reptilia</u>: <i>Niveoscincus metallicus</i>, <i>Podarcis muralis</i>, <i>Podarcis lilfordi</i>, <i>Anolis sagrei</i>, <i>Sceloporus virgatus</i>, <i>Thecadactylus rapicauda</i>, <i>Eublepharis macularius</i> (Bateman &amp; Fleming, 2011; Chapple &amp; Swain, 2004; Cooper et al., 2004; Delorme, Lungu, &amp; Vickaryous, 2012) (Arnold, 1984) (Bateman &amp; Fleming, 2009)</li> <li>○ <u>Echinodermata: Asteroidea</u>: <i>Astropecten indicus</i>, <i>Linckia multifora</i>, <i>Linckia diplax</i>, <i>Asterias tenuispina</i>, <i>Coscinasterias acutispina</i>, <i>Dactylosaster cylindricus pacificus</i>, <i>Pentaceros hawaiiensis</i> (Edmondson, 1935; Fleming et al., 2007; Loh &amp; Todd, 2012)</li> </ul> </li> </ul>
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BENEFITS

<ul style="list-style-type: none"> <li>• Escape/ survival (predation or competition)             <ul style="list-style-type: none"> <li>○ <u>Cnidaria</u>: <i>Aglantha digitale</i> (Bickell-Page &amp; Mackie, 1991; Fleming et al., 2007)</li> <li>○ <u>Mollusca</u>: <i>Phidiana crassicornis</i>, <i>Deroceras reticulatum</i>, <i>Limax tenellus</i>, <i>Agaronia propatula</i> (Miller &amp; Byrne, 2000; Pakarinen, 1994; Rupert &amp; Peters, 2011)</li> </ul> </li> <li>• Continued motion of autotomized limb – decoy             <ul style="list-style-type: none"> <li>○ <u>Mollusca</u>: <i>Octopus horridus</i>, <i>Phidiana crassicornis</i>, <i>Sacoglossa</i> (Miller &amp; Byrne, 2000; Norman &amp; Finn, 2001)</li> </ul> </li> <li>• Continued motion of autotomized limb – substitute meal             <ul style="list-style-type: none"> <li>○ <u>Annelida</u>: <i>Eisenia fetida</i> (Sugiura, 2009)</li> <li>○ <u>Mollusca</u>: <i>Phidiana crassicornis</i>, <i>Prophysaon foliolatum</i> (Deyrup-Olsen et al., 1986; Miller &amp; Byrne, 2000)</li> </ul> </li> <li>• Release of toxic substances with separation             <ul style="list-style-type: none"> <li>○ <u>Annelida</u>: <i>Tubifex tubifex</i> (Fleming et al., 2007)</li> <li>○ <u>Mollusca</u>: <i>Sacoglossa</i>, <i>Lima hians</i>, <i>Prophysaon foliolatum</i>, <i>Phidiana crassicornis</i>, <i>Limaria fragilis</i> (Deyrup-Olsen et al., 1986; Donovan et al., 2004; Fleming et al., 2007; Gilmour, 1963; Miller &amp; Byrne, 2000)</li> </ul> </li> <li>• Adherence of autotomized limb             <ul style="list-style-type: none"> <li>○ <u>Mollusca</u>: <i>Lima</i>, <i>Phidiana crassicornis</i> (Gilmour, 1967; Gilmour, 1963; Miller &amp; Byrne, 2000; Stasek, 1967)</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>• Escape/ survival (predation or competition or self-entrapment)             <ul style="list-style-type: none"> <li>○ <u>Arthropoda</u>: <u>Arachnida</u>: <i>Leiobunum nigripes</i>, <i>Leiobunum vittatum</i>, <i>Pholcus phalangioides</i> <u>Crustacea</u>: <i>Uca</i>, <i>Porcellana platycheles</i>, <u>Hexapoda</u>: <i>Schistocerca Americana</i>, <i>Barytettix humphreysii</i>, <i>Locusta migratoria</i>, <i>Baculum extradentatum</i>, (Fleming et al., 2007)</li> <li>○ <u>Chordata</u>: <u>Reptilia</u>: various spp. (Arnold, 1984; Clause &amp; Capaldi, 2006)</li> <li>○ <u>Echinodermata</u>: <u>Asteroidea</u>: <i>Asterias rubens</i>, <i>Evasterias troscheli</i>, <i>Pycnopodia helianthoides</i>, <i>Leptasterias hexactis</i>, <i>Acanthaster planci</i> <u>Ophiuroidea</u>: <i>Amphipholis squamata</i>, <i>Ophiura albida</i> (Bingham et al., 2000; Lawrence &amp; Vasquez, 1989) (Lawrence &amp; Vasquez, 1989)</li> </ul> </li> <li>• Continued motion of autotomized limb – decoy             <ul style="list-style-type: none"> <li>○ <u>Arthropoda</u>: <u>Arachnida</u>: <i>Leiobunum nigripes</i>, <i>Leiobunum vittatum</i>, <i>Pholcus phalangioides</i>, <i>Holocnemus pluchei</i>, <u>Hexapoda</u>: <i>Cuniculina impigra</i>, <i>Locusta migratoria</i>, Phasmatodea (stick insects) (Bässler, 1984; Fleming et al., 2007) (Bässler, 1984; Fleming et al., 2007)</li> <li>○ <u>Chordata</u>: <u>Reptilia</u>: <i>Trachylepis maculilabris</i>, <i>Eublepharis macularius</i>, <i>Podarcis hispanica</i>, <i>Podarcis lilfordi</i> (Clause &amp; Capaldi, 2006; Cooper, Jr. &amp; Smith, 2009; Higham &amp; Russell, 2010) (Cooper et al., 2004; Pafilis et al., 2005)</li> <li>○ <u>Echinodermata</u>: <u>Ophiuroidea</u>: <i>Ophiocomina nigra</i> (Wilkie, 1978)</li> </ul> </li> <li>• Continued motion of autotomized limb – substitute meal             <ul style="list-style-type: none"> <li>○ <u>Arthropoda</u>: <u>Crustacea</u>: <i>Porcellana platycheles</i> (Fleming et al., 2007)</li> <li>○ <u>Chordata</u>: <u>Reptilia</u>: <i>Trachylepis maculilabris</i>, Various species (Cooper, Jr. &amp; Smith, 2009)</li> <li>○ <u>Echinodermata</u>: <u>Asteroidea</u>: <i>Asterias rubens</i>, <i>Asterias forbesi</i>, <i>Evasterias</i></li> </ul> </li> </ul>
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	<p><i>troscheli</i></p> <ul style="list-style-type: none"> <li>• Release of toxic substances with separation <ul style="list-style-type: none"> <li>○ <u>Arthropoda: Hexapoda: <i>Apis</i>, <i>Pogonomyrmex</i>, <i>Polybia rejecta</i></u> (Shorter &amp; Rueppell, 2011)</li> <li>○ <u>Echinodermata: Holothuroidea: <i>Holothuria scabra</i></u> (Fleming et al., 2007; Wilkie, 2001)</li> </ul> </li> <li>• Adherence of autotomized limb <ul style="list-style-type: none"> <li>○ <u>Arthropoda: Crustacea: <i>Potamocarcinus richmondi</i>, <i>Geocarcinus quadratus</i>, <i>Hexapoda: Apis</i>, <i>Pogonomyrmex</i>, <i>Polybia rejecta</i></u> (Robinson et al., 1970)(Shorter &amp; Rueppell, 2011)</li> <li>○ <u>Echinodermata: Holothuroidea: <i>Holothuria scabra</i></u> (Fleming et al., 2007; Wilkie, 2001)</li> </ul> </li> </ul>
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**COSTS**

<p style="text-align: center;"><b>ABSENCE OF THE APPENDAGE</b></p> <ul style="list-style-type: none"> <li>• Locomotion (decreased speed, endurance, stability, balance, increase escape distance, momentum, changing locomotion type): <ul style="list-style-type: none"> <li>○ <u>Mollusca: Bivalvia: <i>Limaria fragilis</i></u> (Donovan et al., 2004)</li> </ul> </li> <li>• Foraging (decreased feeding rate, decreased prey capture, increased prey handling time, diet change): <ul style="list-style-type: none"> <li>○ <u>Mollusca: Gastropoda: <i>Prophysaon foliolatum</i>, <i>Agaronia propatula</i></u> (Deyrup-Olsen et al., 1986; Rupert &amp; Peters, 2011)</li> </ul> </li> </ul>	<p style="text-align: center;"><b>ABSENCE OF THE APPENDAGE</b></p> <ul style="list-style-type: none"> <li>• Locomotion (decreased speed, endurance, stability, balance, increase escape distance, momentum, changing locomotion type) <ul style="list-style-type: none"> <li>○ <u>Arthropoda: Hexapoda: <i>Gryllus bimaculatus</i>, <i>Sipylodea sipylus</i>, <i>Paroxya atlantica</i></u> (Bateman &amp; Fleming, 2011; Bateman &amp; Fleming, 2005; Tara L Maginnis, 2006)</li> <li>○ <u>Chordata: Reptilia: <i>Trachylepis maculilabris</i>, <i>Niveoscincus metallicus</i>, <i>Desmognathus quadramaculatus</i>, <i>Podarcis lilifordi</i>, <i>Psammodromus algirus</i>, <i>Lygodactylus capensis</i></u> (Bateman &amp; Fleming, 2009; Chapple, Mccoull, &amp; Swain, 2004; Clause &amp; Capaldi, 2006; Cooper et al., 2004; Cooper, Jr. &amp; Smith, 2009; Fleming &amp; Bateman, 2012; Marvin, 2013)</li> </ul> </li> <li>• Foraging (decreased feeding rate, decreased prey capture, increased prey handling time, diet change) <ul style="list-style-type: none"> <li>○ <u>Arthropoda: Hexapoda: <i>Lestes sponsa</i></u> (Stoks, 1999)</li> <li>○ <u>Echinodermata: Asteroidea: <i>Asterias rubens</i>, <i>Stichaster striatus</i>, <i>Heliaster helianthus</i></u> <u>Ophiuroidea: <i>Amphiura filiformis</i></u> (Barrios et al., 2008; Diaz-Guisado, Gaymer, Brokordt, &amp; Lawrence, 2006; Ramsay, Kaiser, &amp;</li> </ul> </li> </ul>
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<p style="text-align: center;">REGENERATION OF APPENDAGE</p> <ul style="list-style-type: none"> <li>• Allocation of energy: <ul style="list-style-type: none"> <li>○ Decreased somatic growth and development <ul style="list-style-type: none"> <li>▪ <u>Annelida: Arenicola marina</u> (Fleming et al.,</li> </ul> </li> </ul> </li> </ul>	<p style="text-align: center;">Richardson, 2001) (Dupont &amp; Thorndyke, 2006)</p> <ul style="list-style-type: none"> <li>• Defense (decrease survivorship of future predation, decreased ability to defend resources – territory for mating and shelter, decrease aggression) <ul style="list-style-type: none"> <li>○ <u>Arthropoda: Hexapoda: Gryllus bimaculatus</u> (Bateman &amp; Fleming, 2005)</li> <li>○ <u>Chordata: Reptilia: Uta stansburiana</u> (Fox &amp; McCoy, 2000)</li> </ul> </li> <li>• Reproduction (less attractive, decreased social status, decrease mating success, decrease mating activity, decreased successful competition for mates) <ul style="list-style-type: none"> <li>○ <u>Arthropoda: Crustacea: Carcinus maenas, Necora puber, Chionoecetes bairdi, Hexapoda: Gryllus bimaculatus</u> (Juanes &amp; Smith, 1995) (Bateman &amp; Fleming, 2005)</li> <li>○ <u>Chordata: Reptilia: Coleonyx brevis, Uta stansburiana</u> (Bernardo &amp; Agosta, 2005; Clause &amp; Capaldi, 2006)</li> </ul> </li> <li>• Spatial use (decreased overall activity, use of more covered, complex space) <ul style="list-style-type: none"> <li>○ <u>Arthropoda: Arachnida: Schizocosa avida Hexapoda: Argia moesta, Ischnura posita, Lestes sponsa</u> (Fleming et al., 2007; Stoks, 1999)</li> <li>○ <u>Chordata: Reptilia: Lampropholis guichenoti</u> (Clause &amp; Capaldi, 2006; Cooper, 2003)</li> </ul> </li> <li>• Reduced immunity <ul style="list-style-type: none"> <li>○ <u>Arthropoda: Hexapoda: Lestes viridis</u> (Slos et al., 2009)</li> <li>○ <u>Chordata: Reptilia: Eutropis multifasciata, Anolis carolinensis</u> (Alibardi, Celeghin, &amp; Dalla Valle, 2012; Kuo et al., 2013)</li> <li>○ <u>Echinodermata: Asteroidea: Asterias</u> (Pinsino, Thorndyke, &amp; Matranga, 2007)</li> </ul> </li> </ul> <p style="text-align: center;">REGENERATION OF APPENDAGE</p> <ul style="list-style-type: none"> <li>• Allocation of energy: <ul style="list-style-type: none"> <li>○ Increased regeneration <ul style="list-style-type: none"> <li>▪ <u>Arthropoda: Crustacea: Uca pugilator, Decapoda</u> (Mariappan et al., 2000; McLain &amp; Pratt,</li> </ul> </li> </ul> </li> </ul>
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<p>2007)</p> <ul style="list-style-type: none"> <li>▪ <u>Mollusca: Gastropoda:</u> <i>Satsuma caliginosa</i> (Hoso, 2012)</li> </ul> <p>○ Decreased reproduction</p> <ul style="list-style-type: none"> <li>▪ <u>Mollusca: Cephalopoda:</u> <i>Octopus</i> (M.D. Norman &amp; Finn, 2001; Mark D. Norman, 1992)</li> </ul>	<p>2011)</p> <ul style="list-style-type: none"> <li>▪ <u>Chordata: Reptilia:</u> <i>Hemidactylus mabouia</i>, <i>Liolaemus belli</i> (Meyer et al., 2002; Naya, Veloso, Muñoz, &amp; Bozinovic, 2007)</li> <li>▪ <u>Echinodermata: Asteroidea:</u> <i>Asterias rubens</i> (Ramsay et al., 2001)</li> </ul> <p>○ Decreased somatic growth and development</p> <ul style="list-style-type: none"> <li>▪ <u>Arthropoda: Hexapoda:</u> <i>Sipylodea sipylus</i>, <u>Crustacea:</u> <i>Uca pugilator</i>, Decapoda (Maginnis, 2006) (Juanes &amp; Smith, 1995; McLain &amp; Pratt, 2011)</li> <li>▪ <u>Chordata: Reptilia:</u> <i>Uta stansburiana</i> (Niewiarowski et al., 1997)</li> <li>▪ <u>Echinodermata: Asteroidea:</u> <i>Heliaster helianthus</i>, <i>Stichaster striatus</i> <u>Ophiuroidea:</u> <i>Amphiura filiformis</i> (Barrios et al., 2008; Diaz-Guisado et al., 2006) (Dupont &amp; Thorndyke, 2006)</li> </ul> <p>○ Decreased reproduction</p> <ul style="list-style-type: none"> <li>▪ <u>Arthropoda: Crustacea:</u> Decapoda (Mariappan et al., 2000)</li> <li>▪ <u>Chordata: Reptilia:</u> <i>Niveoscincus metallicus</i>, <i>Coleonyx brevis</i> (Bateman &amp; Fleming, 2009; Clause &amp; Capaldi, 2006)</li> <li>▪ <u>Echinodermata: Asteroidea:</u> <i>Stichaster striatus</i>, <i>Heliaster helianthus</i>, <i>Leptasterias hexactis</i> (Barrios et al., 2008; Bingham et al., 2000; Lawrence &amp; Larrain, 1994)</li> </ul> <p>○ Energy investment and storage</p> <ul style="list-style-type: none"> <li>▪ <u>Chordata: Reptilia:</u> <i>Niveoscincus metallicus</i>, <i>Coleonyx brevis</i> (Bateman &amp; Fleming, 2009; Clause &amp; Capaldi, 2006)</li> <li>▪ <u>Echinodermata: Asteroidea:</u> <i>Stichaster striatus</i>, <i>Heliaster helianthus</i> (Barrios et al., 2008; Lawrence &amp; Larrain, 1994)</li> </ul> <p>● Performance of regenerated versus original appendage</p>
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	<ul style="list-style-type: none"> <li>○ Structure (less muscle fibers, neural connections, lipids, lactate, or proteins) <ul style="list-style-type: none"> <li>▪ <u>Arthropoda: Crustacea:</u> Decapoda (Mariappan et al., 2000)</li> <li>▪ <u>Chordata: Reptilia:</u> <i>Hemidactylus mabouia</i>, <i>Anolis carolinensis</i> (Fisher et al., 2012; Meyer et al., 2002)</li> </ul> </li> <li>○ Locomotion (slower) <ul style="list-style-type: none"> <li>▪ <u>Arthropoda: Hexapoda:</u> <i>Sipylodea sipylus</i> (Maginnis, 2006)</li> </ul> </li> <li>○ Defense (smaller territory or home range, decreased survivorship from predation, likelihood losing more limbs) <ul style="list-style-type: none"> <li>▪ <u>Arthropoda: Crustacea:</u> <i>Hemigrapsus oregonensis</i>, <i>Asellus</i>, <i>Porcellana</i>, <i>Marinogammarus</i> (Easton, 1972; Juanes &amp; Smith, 1995; McVean, 1975)</li> <li>▪ <u>Chordata: Reptilia:</u> <i>Liolaemus belli</i>, <i>Trachylepis maculilabris</i> (Cooper, Jr. &amp; Smith, 2009; Naya et al., 2007)</li> <li>▪ <u>Echinodermata: Asteroidea:</u> <i>Asterias rubens</i> (Fleming et al., 2007)</li> </ul> </li> </ul>
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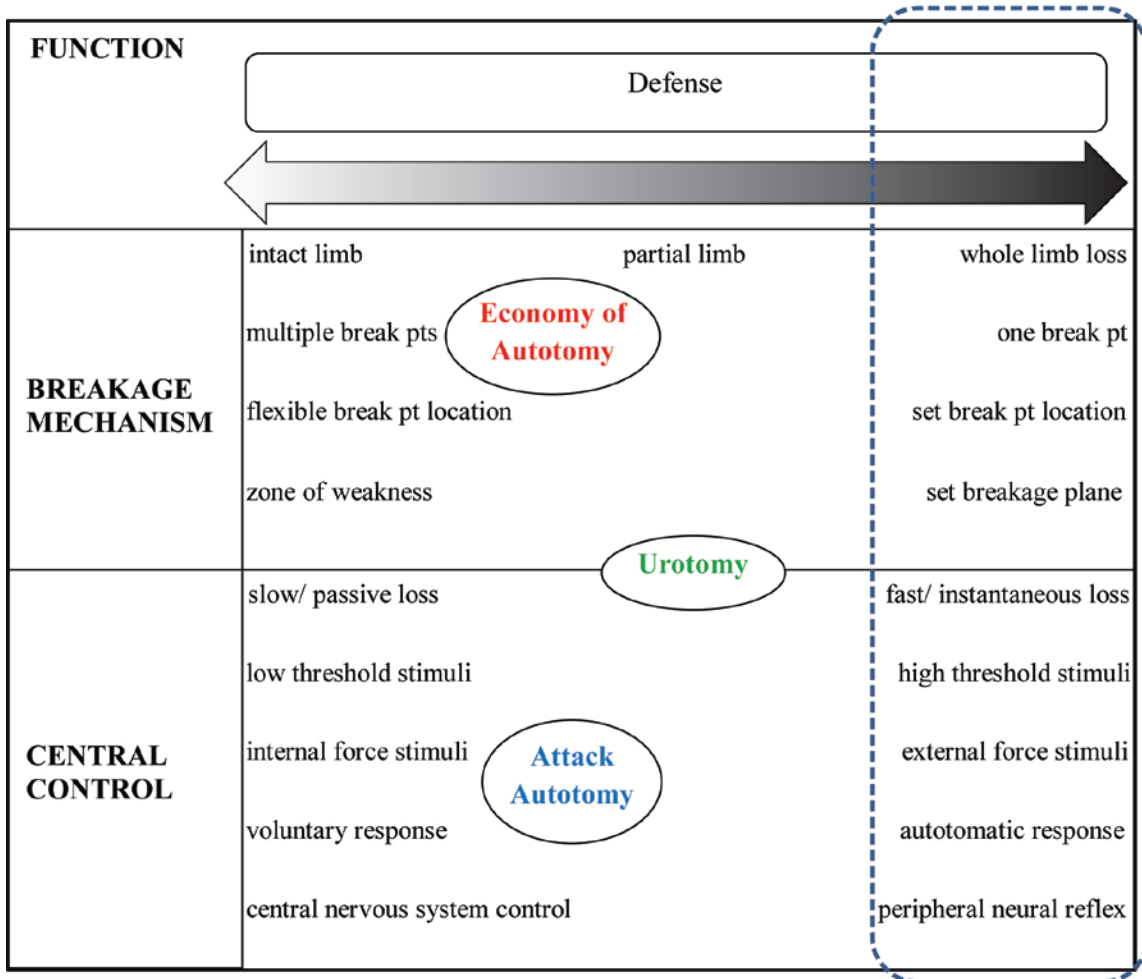
**Table 1.4.** Comparison of historical and adaptive explanations for the evolution of autotomy in organisms with and without an exoskeleton.

<p align="center"><b>SOFT BODIED</b> (autotomized limb without exo-/endo-skeleton)</p>	<p align="center"><b>HARD BODIED</b> (autotomized limb with exo-/endo-skeleton)</p>
<p>SELECTION ON A TRAIT:</p> <ul style="list-style-type: none"> <li>• Reduction of protective cover (shell or operculum)               <ul style="list-style-type: none"> <li>○ <u>Mollusca: Gastropoda</u>: (Fishelson &amp; Kidron, 1968; Rupert &amp; Peters, 2011; Stasek, 1967)</li> </ul> </li> </ul>	<p>SELECTION ON A TRAIT:</p> <ul style="list-style-type: none"> <li>- Diaphragm reducing risk of hemorrhage at breakage point               <ul style="list-style-type: none"> <li>○ <u>Arthropoda</u>: (Maruzzo et al., 2005)</li> </ul> </li> <li>- Female ovipositor evolves into defense weapon with associated glands               <ul style="list-style-type: none"> <li>○ <u>Arthropoda: Hexapoda</u>: <i>Apis</i>, <i>Pogonomyrmex</i>, eusocial tropical wasps (Hermann, 1971; Shorter &amp; Rueppell, 2011)</li> </ul> </li> <li>- Increased # of muscular articulations localization to more proximal positions               <ul style="list-style-type: none"> <li>○ <u>Echinodermata: Crinoid</u> (Oji, 2001)</li> </ul> </li> <li>- Regularly spaced articulations linking short stalks with short ligaments, narrow axial canal that can be sealed               <ul style="list-style-type: none"> <li>○ <u>Echinodermata: Crinoid</u> (Donovan, 2012)</li> </ul> </li> <li>- Mutable collagenous tissue: EXAPTATION               <ul style="list-style-type: none"> <li>○ <u>Echinodermata</u> (Wilkie, 2001)</li> </ul> </li> <li>- Intervertebral Autotomy: re-evolution               <ul style="list-style-type: none"> <li>○ Chordata: Reptilia: **particular families (Arnold, 1984; Bateman &amp; Fleming, 2009)</li> </ul> </li> <li>- Co-evolution of tail morphology (<i>caudifemoralis longus</i> muscle length, tail length, breakage point) and tail autotomy               <ul style="list-style-type: none"> <li>○ <u>Chordata: Reptilia</u> (Zani, 1996)</li> </ul> </li> </ul> <p align="center">AUTOTOMY AS ANCESTRAL WITH LOSSES IN SELECT TAXA:</p>

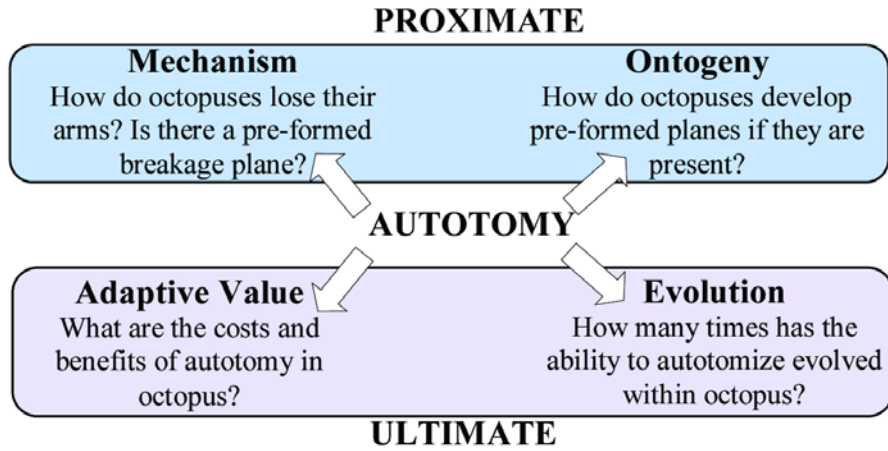
	<ul style="list-style-type: none"><li>- <u>Arthropoda: Pan-crustacea</u> (Maruzzo et al., 2005)</li><li>- Intravertebral Autotomy:<ul style="list-style-type: none"><li>o <u>Chordata: Reptilia</u> (Arnold, 1984; Bateman &amp; Fleming, 2009)</li></ul></li></ul> <p style="text-align: center;">CORRELATION BETWEEN REGENERATION AND AUTOTOMY</p> <ul style="list-style-type: none"><li>- Autotomy closely linked with regeneration<ul style="list-style-type: none"><li>o <u>Echinodermata: Crinoid</u> (Gahn &amp; Baumiller, 2010)</li></ul></li></ul>
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**FIGURES:**



**Figure 1.1.** Gradients of autotomy considering Fredericq’s three main criteria: its function, the breakage mechanism, and the level of central nervous control. Function is not a gradient as defense is considered the main purpose for autotomy. Important factors involved in the breakage mechanism and central control are listed. Three variants of autotomy: ‘economy of autotomy’, ‘urotomy’, and ‘attack autotomy’ are placed at their respective position on the gradient. Dotted line represents the factors considered in the strict definition of autotomy as it is currently used.



**Figure 1.2.** An integrative approach to animal behavior as described by Tinbergen's questions using autotomy specific as an example.

## Chapter 2: Behavioral ecology of autotomy in the octopus *Abdopus aculeatus*

**ABSTRACT:** Autotomy is a secondary defense mechanism that is commonly used by a wide variety of animals, as demonstrated by the frequency of encountering individuals with lost limbs and body parts. The prevalence of autotomy may be an indication of predation levels for a given species population. Individual features, such as sex, body size, and limb size, as well as habitat characteristics, like den availability and protective cover, may also have an effect on how frequent autotomy occurs in a population. Evaluating the incidence of appendage loss in the wild is a first step to better understanding the relative importance of secondary defense mechanisms. Equally essential is being able to identify an autotomy event based on key associated behaviors. Autotomy is typically described based on changes occurring at the level of the autotomized limb and the associated cleavage plane. No published study has created an ethogram specifically for autotomy behaviors of the whole animal, despite its common use. In this study, the benthic intertidal octopus, *Abdopus aculeatus*, was observed in the wild to determine its frequency of autotomy and to distinguish important abiotic and biotic factors in the habitat that may affect this occurrence. Arm autotomy events were also observed in the lab to develop an ethogram of octopus behaviors associated with losing an arm. Populations of *A. aculeatus* at various sites in the Philippines exhibited moderately high frequencies of autotomy (40%) during both the wet and dry seasons. More males than females were encountered in the wild and at some sites, more males were found missing limbs than possessing intact arms. These results were compared with findings from a population of *A. aculeatus* in Indonesia where the incidence of autotomy was even higher (almost 50%). Certain arm positions, usually the anterior pairs, were more likely to be lost. Observations of multiple arm autotomy trials generated in an inventory of classified behaviors that follow a specific sequence of events during arm loss. This is the first analysis of its kind conducted on an octopus to describe autotomy. These observations add to the limited information presently known about this defense mechanism in a soft bodied invertebrate.

### Introduction:

The ability to lose and subsequently regenerate a body part as a secondary anti-predatory defense mechanism is exhibited by a wide variety of organisms. For many animals, direct observation of an autotomy event in nature is rare. As a result, indications of prior sub-lethal encounters with predators such as injuries and missing limbs can be used to assess the intensity of predation and infer a population's propensity for using autotomy (Fleming, Muller, & Bateman, 2007). Several studies have looked at incidence of autotomy in arthropods (Fleming et al., 2007; McVean & Findlay, 1979) and lizards (Bateman & Fleming, 2009). Among marine benthic invertebrates, surveys measuring frequency of general injury, including those resulting from autotomy, have been useful in assessing individual costs and potential community dynamics (Lindsay, 2010). In all of these cases, autotomy has been shown to be a common occurrence that varies according to the taxon, the affected body part, sex, body size, autotomized limb size, and various other factors (Fleming et al., 2007; Lindsay, 2010). Habitat features, both biotic and abiotic, can also have an influence on how frequently autotomy is used by a particular organism. For instance, the level of exposure and general use of a limb can influence the degree of experienced autotomy (Fleming et al., 2007). By evaluating the prevalence of missing limbs among a population of organisms and incorporating observations about the local habitat, one can begin to assess the importance of autotomy for a species and evaluate the relative costs and benefits of this secondary defense mechanism.

To fully understand autotomy, it is important to classify key, recognizable behaviors that describe and distinguish an autotomy event. Laboratory studies can be invaluable in providing a controlled space for determining the elements that distinguish autotomy. No inventory of whole animal behaviors has been generated for this defense mechanism; however descriptions have been made at the mechanistic level involving tissues and limbs. For instance, a “rapid and stereotyped autotomy sequence” has been described in the freshwater oligochaete, *Lumbriculus variegatus* which consists of anatomical changes in tissues at the autotomy zone (Lesiuk & Drewes, 1999). Similarly, mantle autotomy in the gastropod *Ficus ficus* follows a sequence of events starting with swelling of the mantle prior to detachment (Liu & Wang, 1996). Autotomy has been most broadly described as the detachment of the limb or body part from the whole body. More specific behaviors before, after, and during the detachment process may occur in a more stereotyped and sequential manner which necessitates more formal description. Other behaviors, such as anti-predatory defense mechanisms in various species of stalk-eyed flies (Worthington & Swallow, 2011) and aggressive interactions in the fight fruit fly, *Drosophila melanogaster*, (Chen, Lee, Bowens, Huber, & Kravitz, 2002) have been stereotyped, generating an ethogram for subsequent analysis of recurring behavioral patterns. This approach to describing autotomy may not only provide a more specific definition for identifying an autotomy event, but it may also give insight on its relative importance compared to other secondary defense mechanisms.

Octopuses have the ability to detach their eight arms and regenerate them, leaving behind a noticeable stump for assessing frequency of autotomy in the wild. Some species have a higher tendency of losing their arms, such as *Ameloctopus litoralis* (Norman, 1992), ‘*Octopus sp.* Ward, 1998’ (Ward, 1998), and members of the *Octopus horridus* species-group (Norman & Finn, 2001). In addition to possessing anatomically similar features, such as a small mantle size and very long arms (more than three times the mantle length), they live in similar habitats, usually tropical benthic intertidal reef flats (Norman & Finn, 2001). A combination of their habitat and their body morphology is thought to make it easier for individuals to reach into tiny crevices and burrow down into deep holes to forage for food without having to leave their dens or protected location (Norman & Finn, 2001). However, this also makes it more susceptible for potential predators to grab octopus arms, particularly if predators are hiding in the same crevices octopuses are foraging for potential prey (Norman & Finn, 2001).

Octopuses are also known to use a variety of secondary defense mechanisms besides autotomy. These include inking, jetting away, and displaying threatening or startling coloration patterns (Hanlon & Messenger, 1996). These behaviors have been fully described and stereotyped. For example, the startling display, otherwise known as a “dymantic” or “deimatic” behavior, can take many forms but is often characterized by spread arms and webbing displaying a rapid appearance of light and dark color combination elements on the skin (Hanlon & Messenger, 1996). No attempts have been made to fully describe and classify behaviors during autotomy in octopuses, but further investigation and formal descriptions of autotomy behaviors may reveal the relative use of these different secondary defense mechanisms.

In this study, I investigate autotomy in the small benthic shallow water octopus *Abdopus aculeatus*, a member of the *Octopus horridus* species complex. Huffard has done prior work describing the behavioral ecology (Huffard, 2007), locomotion (Huffard, 2006), and mating patterns (Huffard, Caldwell, & Boneka, 2008) of this species in Indonesia. Members of this species have been found missing multiple arms, indicating the potential employment of autotomy as a means of secondary defense against predators (Huffard, 2007). I will quantify observed

frequencies of autotomy and describe surrounding habitat conditions from the Philippines. These will be compared to known observations about the population in Indonesia from Huffard. I will also develop an inventory of behaviors associated with autotomy and determine with sequence analysis if these behaviors follow a recurrent temporal pattern. These descriptive surveys and analyses will provide insight on the factors underpinning autotomy in a soft bodied marine invertebrate.

## **Methods:**

### Field surveys in Mactan Island, Philippines:

*A. aculeatus* were observed in the wild for three weeks during the wet monsoon season (July 2011) and three weeks during the dry season (March – April 2013). Local fishermen, with expertise in identifying and catching octopuses, specifically *A. aculeatus*, in the wild were employed. Searches conducted on snorkel started as early as dawn at low tide and ended during the afternoon high tide. During each search, the following environmental variables were recorded upon encountering an octopus: substrate type, depth, algal coverage, and presence of predatory fish. Additional observations were made for octopuses found in dens including substrate type, objects gathered around the den including middens, and global positioning system (GPS) coordinates for each den. Photos and video were also recorded at each den location. These searches were conducted at three different sites on Mactan Island: Buaya, the Marine Station, and Cordova (**Figure 2-1 a-d**) during the dry season. Fewer surveys were completed during the wet season due to weather limitations. However more locations were surveyed on the island during the wet season resulting overall in fewer surveys per location. Salinity from hydrometers and water temperature from TidbiT® temperature data loggers were recorded at the marine station over a 24 hour period along.

### Frequency of *A. aculeatus* autotomy in the Philippines and Indonesia:

To assess frequency of arm loss and regeneration of *A. aculeatus* on Mactan Island, octopuses encountered in their dens or out on the substrate were collected by fishermen. The following observations were recorded for each collected individual: sex, mantle length (mm), site (Buaya, Marine Station, Cordova, Mactan Island), number of arms missing or regenerating, and position of missing or regenerating arms. Regenerating arms were distinguished by their small size and pale coloration distal to the base of the arm. Statistical analyses were conducted to determine if the frequency of arm loss and regeneration varied according to sex, site, the position of the arm, or season. Arm position is defined according to a dorsal view of the octopus, dividing arms on the left and right side of the body along the medial line and numbering them one through four from the anterior (eyes) to the posterior end.

To better understand the overall occurrence of autotomy in the species *A. aculeatus*, observations made in the Philippines were compared with data collected by Dr. Christine Huffard in Indonesia in 2000-2003. Octopuses were observed on snorkel from three sites in Indonesia: Bunaken, Siladen, and Southeast Sulawesi (Huffard, 2007). The same set of variables were recorded for each encountered octopus and a Chi squared or Fisher's exact test were conducted to determine if location influences the level of arm loss and regeneration experienced by *A. aculeatus*.

#### Laboratory animal care of *A. aculeatus* to classify autotomy behaviors:

Live *A. aculeatus* specimens used for classifying autotomy behaviors were obtained either from (1) a commercial vendor (LiveAquaria, Rhinelander, WI) or by (2) field collection on Mactan Island, Philippines. They were housed in individual 20L artificial sea water (33-34ppt) tanks at 24-26°C and fed grass shrimp (*Crangon spp.* or *Palaemon spp.*) every other day.

#### Laboratory autotomy tests and video recording trials for sequential analysis of autotomy:

To stimulate arm autotomy, modified methods from Ward (1998) were followed. Arms were randomly isolated with forceps a quarter of the length away from the proximal base, between the base and mid-arm. Arms were grasped with forceps and pinned down for maximally 120 seconds to induce animals to autotomize their arm. Each autotomy event was recorded with a Sony HDR-CX580/V high definition camcorder at 30 frames per second for at least two minutes before and up to two minutes after the arm detached. Individuals were acclimated for 10 minutes in an experimental tank prior to the autotomy trial and 10 minutes post trial before being returned to their housing tank.

#### Sequential analysis of arm autotomy in *A. aculeatus*:

To classify the series of behavioral patterns that occur during arm autotomy, video recordings of autotomized arms were analyzed in JWatcher™ (Blumstein & Daniel, 2007). I analyzed each recorded event to identify the key elements of autotomy and generated a subsequent inventory of behaviors used for scoring. A successful trial was initiated when the forceps first contacted an octopus arm and was terminated when the arm detached from the base of the body. Additionally, differences in behavior before and after autotomy were included in the analysis by scoring 30 seconds prior to forceps contact and 30 seconds post arm detachment. I scored all autotomy events at least once according to the generated ethogram.

After scoring each trial, a sequential analysis of events was performed to test for temporal structure in autotomy and to determine the potential sequence patterns of behavior during autotomy (Douglas & Tweed, 1979). This analysis calculates simple and transitional probability matrices for a series of paired events. Each calculated probability gives the likelihood that two events will occur in a sequential order. Adjusted residuals and their associated p-values ( $\alpha = 0.05$ ) were calculated for each transition. A transition diagram incorporating the most likely order of behavioral elements during autotomy was compiled.

### **Results:**

#### Characteristics of *A. aculeatus* habitat in Mactan Island, Philippines:

An abbreviated checklist of *A. aculeatus* habitat characteristics and activity is summarized in **Table 2.1** comparing conditions in the Philippines with those in Indonesia provided by Huffard (2007). In general, conditions at the three study sites in the Philippines were similar to those in Indonesia. Salinity ranged between 32 parts per thousand (ppt) during the monsoon season to 40 ppt during the dry season. Water temperatures in front of the marine station ranged between 29°C and 30°C during the dry season, while air temperature varied between 28°C and 34°C. Tides ranged from -0.2 meters to + 1.82 meters. Octopuses were found either in their dens or moving across seagrass beds and reef flats during snorkel surveys. Octopus dens were distinguishable by holes surrounded by small rocks, pebbles, and pieces of shell and dead coral. Multiple dens were found close together with maximum densities of 7 dens over 100

m<sup>2</sup>. Algae and seagrass include *Ulva reticulata*, *Thalassia hemprichii*, *Padina sp.*, *Halodule pinifolia*, *Bangia sp.*, *Cymodocea serrulata*, *Sargassum sp.*, *Laurencia sp.*, and *Gelidiella sp.*

Den microhabitats were similar between the three sites on Mactan Island, Philippines (**Fig. 2.1a**, **Table 2.1**). Buaya, the most northeastern site, consists of a long expansive reef flat with large patches of seagrass beds (**Fig. 2.1b**). The intertidal reef adjacent to the marine station consisted of a muddy beach and short reef flat extending into a deeper offshore coral reef (**Fig. 2.1c**). Human activity was frequent in the area. Cordova, the southernmost site, is similar to Buaya, but lies adjacent to an extensive mangrove forest (**Fig. 2.1d**). Among the octopus dens that were marked and recorded, 37 were found in Buaya, 10 were found at the marine station, and 11 were found at Cordova.

Predator induced autotomy in the field was not observed. Potential fish predators present at the three sites were recorded and included: Buaya - lizard fish, butterfly fish, wrasse, sergeant major, lion fish, sand perch; Marine Station: damselfish, pufferfish, triggerfish; Cordova: pufferfish, triggerfish, black and white eel, lizardfish, wrasse, fisherman eel, moray eel, cardinal fish, butterfly fish, gobies, goatfish, damselfish, and file fish.

#### High frequency of arm autotomy in the Philippines and Indonesia:

A total of 288 *A. aculeatus* individuals were observed in the wild: 160 from Indonesia observed by Huffard over three years, 47 from Mactan Island, Philippines during the wet season in 2011, and 81 from the three sites on Mactan Island during the dry season in 2013. **Tables 2.2 – 2.4** summarize the general patterns of autotomy for all three datasets, including frequency of occurrence, frequency of loss in males versus females, number of arms lost, and its corresponding arm position. Because an individual can lose multiple arms, the total sample size when looking at arm position will be greater than the actual sample size of octopuses surveyed. Autotomy was defined as missing an arm at the base as well as regenerating an arm stub at the base which could indicate a prior autotomy event. In Indonesia, about half of the individuals exhibited some arm loss or regeneration (**Fig. 2.2a**). Higher proportions of males in particular were found missing or regenerating arms (**Table 2.2**, **Fig. 2.3a**). Autotomy occurred most frequently in Bunaken, Indonesia where over 50% of sampled individuals were missing or regenerating arms (**Fig. 2.4a**). Most instances of autotomy involved the loss of only one arm (48%) and usually in position R1, the right arm directly in front of the eyes (**Fig. 2.6a**). During the wet season on Mactan Island, Philippines, about 40% of encountered individuals exhibited arm loss (**Fig 2.2b**), 45% of which lost only one arm (**Fig. 2.5b**), usually the third left arm (**Fig. 2.6b**) Three quarters of the individuals encountered were male and more individuals of both sexes were found with intact arms (**Fig. 2.3b**). Similar results were seen in the Philippines during the dry season (**Fig. 2.2c**, **Fig. 2.3c**) however a much higher percentage of individuals lost only one arm (70%; **Fig. 2.5c**) and mostly in the second pairs of arms on either side (**Fig. 2.6c**).

Few factors (sex, location, site at each location, and arm position) were significantly correlated with the observed frequencies of autotomy in Indonesia and the Philippines. In Indonesia, the Chi squared test revealed that arms were not equally lost at each position (p-value << 0.01). R1 was most frequently lost while L4 was least frequently missing (**Fig. 2.6a**). There were no statistically significant relationships between sex, site, number of arms lost, and arm position with the frequency of autotomy seen in the Philippines during the monsoon season. However, during the dry season in the Philippines, the Fisher's exact test showed frequency of autotomy was not the same for both sexes (p-value = 0.033). Forty seven percent of males and

only 14% of females exhibited autotomy (**Fig. 2.3c**). Lastly, like Indonesia, individuals on Mactan Island during the dry season did not lose their arms equally at each position (Chi squared test; p-value = 0.023). L2 was most frequently lost while R3 was the least autotomized arm (**Fig. 2-6c**). The overall frequency of autotomy in the Philippines (incorporating both dry and wet seasons) was not significantly different from the frequency in Indonesia (Fisher's exact test; p-value = 0.0951; **Fig. 2-8a**). The frequency of autotomy during the wet and dry season in the Philippines was also not significantly different (Fisher's exact test, p-value = 0.8522; **Fig.2-8b**). Incidence of arm loss and regeneration at the three sites in Indonesia (Bunaken, Siladen, and Southeast Sulawesi) were close to, but not statistically significant (Chi squared; p-value = 0.063, **Fig. 2.4a**). Similarly, the frequency of autotomy at the three sites in the Philippines (Buaya, the marine station, Cordova) were not statistically significant (Chi squared, p-value = 0.568, **Fig. 2.4c**).

#### Octopus arm autotomy follows a predictable series of behaviors:

Twenty six arm autotomy trials were performed in *A. aculeatus* to describe the sequence of behaviors associated with autotomy. **Table 2.5** lists the 10 major behaviors that occur during autotomy (detach, crawl, darken, forceps contact, ink, jet, lighten, flash color change, suckers suction, and wound to mouth) followed by their specific definitions. The probability of each element occurring during autotomy is also listed based on the 26 trials. All elements, except for "forceps contact" (f) and "detach" (a) which mark the start and end of each trial respectively, can be repeated multiple times in a single sequence. **Tables 2.6 – 2.8** report the simple probability, transitional probability, and p-value matrices, respectively, and were used to develop the transition diagram pictured in **Fig. 2-9**. Most all behaviors could occur at any time during the sequence, but at low transitional probabilities (<0.1). Transitions exhibiting the highest probabilities (>> 0.2) were incorporated in the diagram. The resulting figure suggests the following sequence of behaviors takes place during autotomy: (1) The octopus starts off either crawling or showing a light background coloration prior to autotomy. (2) Contact is made between the forceps and an arm, initiating the start of the autotomy trial. (3) There is an overall darkening color response from the octopus. (4) Suckers suction onto the substrate. (5) This leads to either an immediate detachment of the arm or short bursts of color flashes just before detachment. (6) Some form of locomotion, either crawling or jetting away, follows post autotomy to get away from the detached arm. If the resulting motion is crawling, the octopus will either flash short bursts of color or care for the open wound left by autotomy. If the resulting motion is jetting, an array of possibilities may occur, though darkening of the overall skin is the most likely transition (0.4). Inking, crawling, or flashing are the alternatives.

#### **Discussion and Conclusions:**

Field observations of *A. aculeatus* show few differences in habitat and den preference between the Philippines and Indonesia. Octopuses occupy areas with sandy and rocky intertidal reef flats with surrounding seagrass. In both the Philippines and Indonesia, dens are distinguished by pebbles gathered at the entrance of the hole. Many different species of fish are found at both locations suggesting potentially high levels of predation. Among potential octopus predators, some species of fish are the most likely candidates to induce autotomy. They can isolate and target a single arm from the body. The small body size and long arms of *A. aculeatus* allow it to explore and forage around rocks and crevices where fish predators may be hidden and



can grasp an exploratory arm. A highly variable environment with lots of structure coupled with high predation pressures can lead to frequent use of autotomy as a defense mechanism.

Octopus *A. aculeatus* encounters high frequencies of autotomy in their natural environment. Nearly half of the individuals encountered in Indonesia and about 40% in the Philippines were missing or regenerating at least one arm. Because of the similar habitat and environmental conditions found at both locations, a difference in frequency was not expected between the Philippines and Indonesia. Also the sites sampled within the Philippines did not vary significantly in their biotic or abiotic factors thus differences in the incidence of autotomy between Buaya, the marine station, and Cordova were not expected. Higher sample sizes were collected from Buaya compared to the other sites. One potential reason is human activity in the form of transportation, fishing, and leisure activities is higher in Cordova and especially in front of the marine station. This can lead to high levels of disturbance on the environment and potentially lower encounter rates with octopuses in these areas. The high frequency of arm loss and regeneration observed in *A. aculeatus* can be a result of high predation and competition levels. In addition to fish predators, humans targeting octopuses can also induce arm autotomy. I observed *A. aculeatus* autotomizing arms on multiple occasions during collection by fishermen, especially by hand. Collection with a net was less likely to lead to autotomy. *A. aculeatus* also den up in close proximity to one another that intraspecific interactions, including aggressive ones, are likely to occur. Instances of cannibalism in *A. aculeatus* have been observed in Indonesia (Huffard, 2007) as well as in the Philippines. In the Philippines, twice as many males were encountered than females. This could be a result of males more actively foraging and looking for mates while females are protected in their dens, potentially brooding eggs. Reproduction does not appear to be seasonal since fewer females were encountered during both the wet and dry seasons. In general, more individuals were sampled during the dry season than the wet season in the Philippines. This is at least in part a bias of weather conditions limiting the possibility of field collections. Most instances of arm autotomy involved only one arm missing or regenerating. However, individuals were able to lose multiple arms (observed up to five) and still survive. Not all arms exhibited equal probability of being autotomized. This is probably due to the fact that different arms are used for different functions (Byrne, Kuba, Meisel, Griebel, & Mather, 2006; Mather, 1998). For instance the first anterior pairs are often used for exploring the surroundings and looking for food while the back arms are often used for locomotion. Another case of arm specialization is in the third right arm of male octopuses which have a groove, the hectocotylus, through which spermatophore is transferred to the female during mating. Loss of this arm would be detrimental to male fitness and is likely the reason why autotomy of the third right arm was observed to be relatively low in the Philippines. Conversely, anterior arms are often used in exploring the environment and exhibit higher frequencies of arm loss.

This study was the first attempt in an octopus to classify behaviors associated with autotomy. Autotomy is a somewhat stereotyped behavior in that detachment of a limb or body part is an expected result. However, other preparatory and post detachment behaviors are also characteristic of an autotomy event. In octopuses, these behaviors either involved color responses (lightening, darkening, or pulses of color change) or locomotor reactions (crawling, jetting, or suctioning to the wall). While any combination of these behaviors could occur, certain sequences were more likely than others. Observations of arm autotomy events also showed that other secondary defense mechanisms are employed before and after the arm is detached. 'Darkening,' for instance, occurs as soon as an arm is grasped with the forceps. This darkening color includes instances of 'deimatic'/'dymantic' displays which is considered another form of secondary

defense. Jetting and inking also occurred during autotomy, usually right after the arm detached as a means of quick escape and disappearance from the wall of ink. Jetting and inking actions were frequently coupled together; especially as they both involve expulsion of liquid from the funnel. The suction of suckers just prior to detachment may be an important behavior in preparing for detachment. Suctioning to a surface may provide traction and an opposing force for the octopus to work against in order for the arm to separate from the body. Lastly, in some, but not all cases, the arm stub was tended by the mouth as means of helping accelerate wound healing.

Autotomy in the octopus *A. aculeatus* follows trends found in other known arm autotomizing octopus species such as *A. littoralis* and '*Octopus sp.* Ward, 1998'. All three species exhibit long arms and small bodies and live in environments with high structural variability that high frequencies of limb loss is to be expected. Application of the patterned sequence of behavioral events during autotomy to *A. littoralis* and '*Octopus sp.* Ward, 1998' may provide good comparisons for stereotyping arm autotomy in all octopuses in general. Further investigations of the behavioral patterns during autotomy may also provide a way to quantify and directly compare the utility of different secondary defense mechanisms in octopuses. The high frequency of arm loss and regeneration exhibited by *A. aculeatus* in their natural environment suggests that ecological pressures, whether from predation or habitat conditions may be influencing their propensity to use a last resort strategy like autotomy. Future work to directly test and isolate these factors will yield a better understanding of what drives autotomy in this system and why it is an important defense mechanism.

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**TABLES**

**Table 2.1.** Checklist of habitat characteristics and activity comparing *Abdopus aculeatus* in the Philippines and Indonesia (Huffard, 2007).

Functional Group	Behavioral Unit	Character States	
<b>Habitat characteristics</b>		<b>Philippines</b>	<b>Indonesia (Huffard, 2007)</b>
	General habitat	reef flats, sandy and rocky beach, seagrass	reef flat, seagrass, sandy beach
	Depth	intertidal	intertidal
	Energy Level	some areas sheltered lagoon with periodic tidal currents	areas with strong periodic tidal currents
	Den microhabitat	rock crevices and reef flats surrounded by seagrasses and rubble	reef flat tidepool or reef-flat shallows
	Gather objects to den entrance	Yes (rocks, pebbles, dead coral and shell pieces)	Yes (pebbles typically 1-5 cm diameter)
	Small scale density	maximum 7 individuals/ 100m <sup>2</sup>	maximum 10 adults/ 200m <sup>2</sup> (18 total individuals/200m <sup>2</sup> )
	Aggregation	occurs	occurs
<b>Activity</b>	Circadian rhythms	diurnal	diurnal
	Tidal preference	falling and rising tides	Sulawesi: mid-tides (falling, rising)

**Table 2.2.** Field data summary from three sites in Indonesia (Bunaken, Siladen, and Southeast Sulawesi) collected by Christine Huffard. Frequency of autotomy is categorized by total number of individuals sampled according to sex, site, the number of arms lost among autotomized individuals, and the position of the arms that are lost (R1-R4, L1-L4). (n=160)

	<b>Autotomy</b>	<b>No Autotomy</b>
<b>Total # individuals sampled (n=160)</b>	79	81
<b>Sex</b>		
<b>Male (n=96)</b>	50	46
<b>Female (n=53)</b>	25	28
<b>Site</b>		
<b>Bunaken (n=105)</b>	58	47
<b>Siladen (n=6)</b>	1	5
<b>SE Sulawesi (n=47)</b>	19	28
<b># Arms Lost (n=79)</b>		
<b>1</b>	38	
<b>2</b>	29	
<b>3</b>	8	
<b>4</b>	2	
<b>5</b>	2	
<b>Arm Position Lost (n=132 arm samples)</b>		
<b>L1</b>	24	
<b>L2</b>	15	
<b>L3</b>	16	
<b>L4</b>	4	
<b>R1</b>	33	
<b>R2</b>	21	
<b>R3</b>	9	
<b>R4</b>	10	

**Table 2.3.** Field data summary from Mactan Island, Philippines collected July 2011 during the wet monsoon season. Frequency of autotomy is categorized by total number of individuals sampled according to sex, site, the number of arms lost among autotomized individuals, and the position of the arms that are lost (R1-R4, L1-L4). (n = 47).

	<b>Autotomy</b>	<b>No Autotomy</b>
<b>Total # individuals sampled (n=47)</b>	19	28
<b>Sex</b>		
<b>Male (n=40)</b>	17	23
<b>Female (n=4)</b>	1	3
<b>Site</b>		
<b>Mactan Island (n=47)</b>	19	28
<b># Arms Lost (n=20)</b>		
<b>1</b>	9	
<b>2</b>	6	
<b>3</b>	4	
<b>4</b>	1	
<b>Arm Position Lost (n=37 arms sampled)</b>		
<b>L1</b>	5	
<b>L2</b>	5	
<b>L3</b>	8	
<b>L4</b>	4	
<b>R1</b>	4	
<b>R2</b>	4	
<b>R3</b>	2	
<b>R4</b>	5	

**Table 2.4.** Field data summary from three sites on Mactan Island, Philippines (Buaya, Marine Station, and Cordova) collected March to April 2013 during the dry season. Frequency of autotomy is categorized by total number of individuals sampled according to sex, site, the number of arms lost among autotomized individuals, and the position of the arms that are lost (R1-R4, L1-L4). (n = 81).

	<b>Autotomy</b>	<b>No Autotomy</b>
<b>Total # individuals sampled (n=81)</b>	31	50
<b>Sex</b>		
<b>Male (n=53)</b>	25	28
<b>Female (n=14)</b>	2	12
<b>Site</b>		
<b>Buaya (n=49)</b>	19	30
<b>Mactan Island (n=16)</b>	6	10
<b>Cordova (n=6)</b>	1	6
<b># Arms Lost (n=27)</b>		
<b>1</b>	19	
<b>2</b>	3	
<b>3</b>	4	
<b>4</b>	0	
<b>5</b>	1	
<b>Arm Position Lost (n=42 arms sampled)</b>		
<b>L1</b>	7	
<b>L2</b>	11	
<b>L3</b>	5	
<b>L4</b>	3	
<b>R1</b>	2	
<b>R2</b>	9	
<b>R3</b>	1	
<b>R4</b>	4	



**Table 2.5.** General inventory and description of behaviors exhibited during autotomy. Count and probability are in reference to the occurrence of each behavior in all 26 autotomy trials.

<b>Behavior</b>		<b>Description</b>	<b>Count</b>	<b>Probability</b>
<b>detach</b>	<b>a</b>	isolated arm autotomizes or detaches from the base	26	0.11
<b>crawl</b>	<b>c</b>	multiple arms push and pull off substrate to move the overall body	34	0.15
<b>darken</b>	<b>d</b>	overall appearance and background color gets darker by expanding chromatophores for longer than a flash (>1second)	27	0.12
<b>forceps contact</b>	<b>f</b>	first contact isolating an arm with forceps	26	0.11
<b>ink</b>	<b>i</b>	ink, or dark substance, expelled from the mantle through the funnel	14	0.06
<b>jet</b>	<b>j</b>	expulsion of water from the mantle through the funnel to move the overall body	7	0.03
<b>lighten</b>	<b>l</b>	overall appearance and background color gets lighter by contracting chromatophores for longer than a flash (>1second)	27	0.12
<b>flash color change</b>	<b>p</b>	quick pulse of expanding and contracting chromatophores to get a quick color change on the skin (~1 second)	38	0.16
<b>suckers suction</b>	<b>s</b>	suckers active and suction unto vertical surfaces (walls)	23	0.10
<b>wound to mouth</b>	<b>w</b>	tend to arm wound by holding arm stump close to the mouth	9	0.04

**Table 2.6.** Simple probability matrix for autotomy behaviors described in Table 2.5. Each cell represents the probability that each paired behavior occurs. (a = detach, c = crawl, d = darken, f = forceps contact, I = ink, j = jet, l = lighten, p = flash color change, s = suckers suction, w = wound to mouth, T = total).

	<b>a</b>	<b>c</b>	<b>d</b>	<b>f</b>	<b>i</b>	<b>j</b>	<b>l</b>	<b>p</b>	<b>S</b>	<b>w</b>	<b>T</b>
<b>a</b>	0	0.063	0.005	0	0.005	0.029	0.015	0.010	0	0	<b>0.127</b>
<b>c</b>	0.015	0	0.020	0.034	0	0	0.005	0.039	0	0.015	<b>0.127</b>
<b>d</b>	0	0.005	0	0.010	0.015	0	0.010	0.015	0.054	0.005	<b>0.112</b>
<b>f</b>	0.005	0	0.063	0	0.015	0	0.015	0.020	0.010	0	<b>0.127</b>
<b>i</b>	0.024	0	0.010	0	0.005	0.005	0.005	0	0.015	0	<b>0.063</b>
<b>j</b>	0	0.005	0.010	0	0.005	0	0	0.005	0	0	<b>0.024</b>
<b>l</b>	0.020	0.020	0.005	0.039	0.005	0	0	0.015	0.020	0.010	<b>0.132</b>
<b>p</b>	0.029	0.005	0.020	0.005	0.005	0	0.020	0.039	0.015	0.015	<b>0.151</b>
<b>s</b>	0.034	0.015	0	0	0.015	0	0.015	0.034	0	0	<b>0.112</b>
<b>w</b>	0	0.015	0	0	0	0	0	0.010	0	0	<b>0.024</b>
<b>T</b>	<b>0.127</b>	<b>0.127</b>	<b>0.132</b>	<b>0.088</b>	<b>0.068</b>	<b>0.034</b>	<b>0.083</b>	<b>0.185</b>	<b>0.112</b>	<b>0.044</b>	<b>1</b>

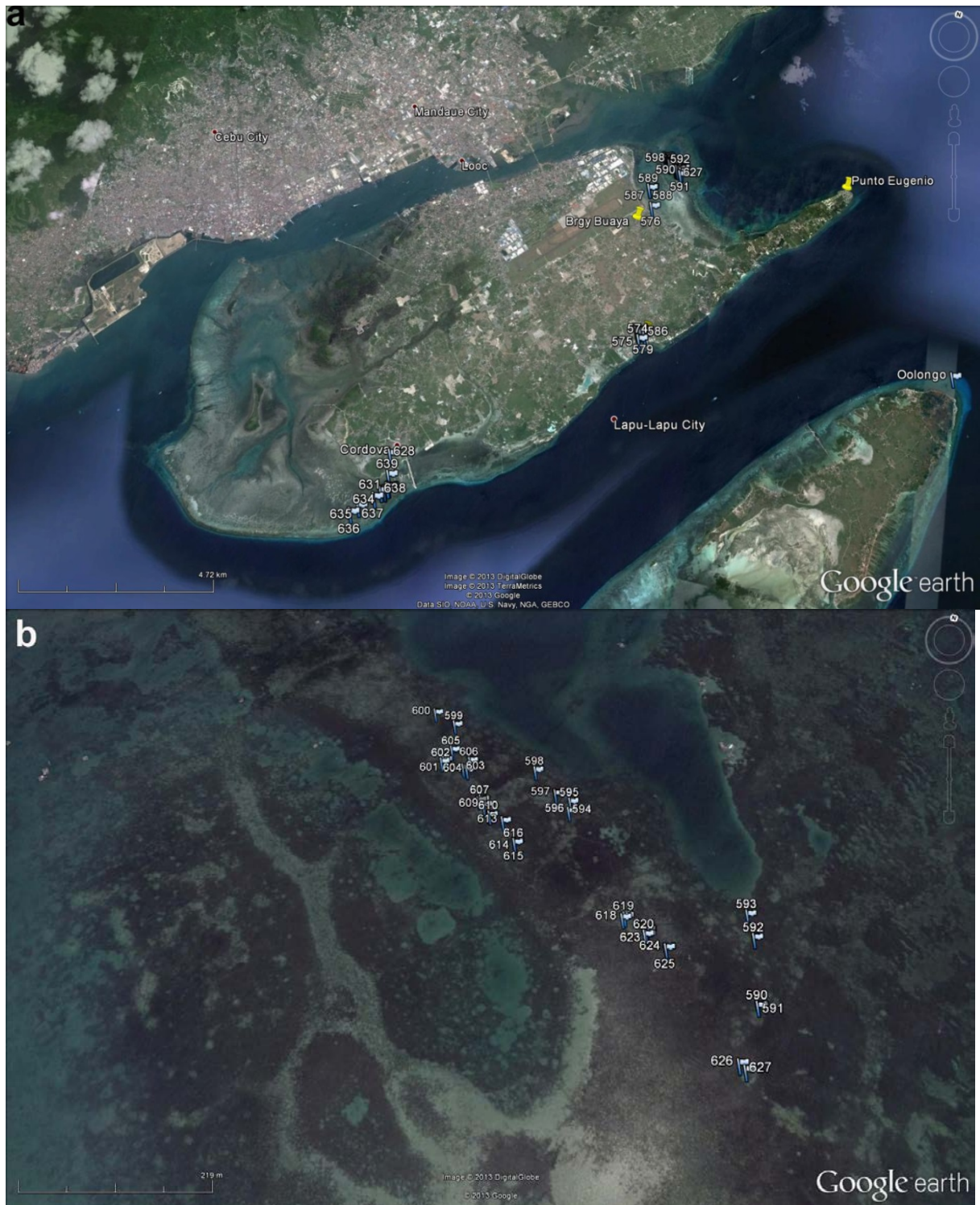
**Table 2.7.** Transition probability matrix for autotomy behaviors described in Table 2.5. Each cell represents the probability that each paired behavior occurs in the sequence starting with the row behavior followed by the behavior designated by the column. (a = detach, c = crawl, d = darken, f = forceps contact, I = ink, j = jet, l = lighten, p = flash color change, s = suckers suction, w = wound to mouth, T = total). \*Probabilities >0.20 considered for transition diagram (Figure 2-6).

	<b>a</b>	<b>c</b>	<b>d</b>	<b>f</b>	<b>i</b>	<b>j</b>	<b>l</b>	<b>p</b>	<b>s</b>	<b>w</b>	<b>T</b>
<b>a</b>	0	0.5*	0.039	0	0.039	0.231*	0.115	0.077	0	0	<b>1</b>
<b>c</b>	0.115	0	0.154	0.269*	0	0	0.039	0.308*	0	0.115	<b>1</b>
<b>d</b>	0	0.044	0	0.087	0.130	0	0.087	0.130	0.478*	0.044	<b>1</b>
<b>f</b>	0.039	0	0.5*	0	0.115	0	0.115	0.154	0.077	0	<b>1</b>
<b>i</b>	0.385*	0	0.154	0	0.077	0.077	0.077	0	0.231*	0	<b>1</b>
<b>j</b>	0	0.2*	0.4*	0	0.2*	0	0	0.2*	0	0	<b>1</b>
<b>l</b>	0.148	0.148	0.037	0.296*	0.037	0	0	0.111	0.148	0.074	<b>1</b>
<b>p</b>	0.194*	0.032	0.129	0.032	0.032	0	0.129	0.258*	0.097	0.097	<b>1</b>
<b>s</b>	0.304*	0.130	0	0	0.130	0	0.130	0.304*	0	0	<b>1</b>
<b>w</b>	0	0.6*	0	0	0	0	0	0.4*	0	0	<b>1</b>

**Table 2.8.** p-value matrix for calculated z- scores testing if particular transitions are more likely to occur than expected just by chance. (a = detach, c = crawl, d = darken, f = forceps contact, I = ink, j = jet, l = lighten, p = flash color change, s = suckers suction, w = wound to mouth, T = total).  $*\alpha \leq 0.05$ .

	<b>a</b>	<b>c</b>	<b>d</b>	<b>f</b>	<b>i</b>	<b>j</b>	<b>l</b>	<b>p</b>	<b>s</b>	<b>w</b>
<b>a</b>	0.038*	0*	0.132	0.090	0.519	0*	0.521	0.128	0.052	0.242
<b>c</b>	0.851	0.038*	0.721	0*	0.140	0.305	0.379	0.086	0.052	0.057
<b>d</b>	0.052	0.2024	0.047*	0.988	0.210	0.338	0.941	0.472	0*	0.992
<b>f</b>	0.147	0.038*	0*	0.090	0.308	0.305	0.521	0.658	0.542	0.242
<b>i</b>	0.004*	0.1556	0.807	0.248	0.899	0.380	0.935	0.076	0.162	0.425
<b>j</b>	0.388	0.6186	0.072	0.482	0.237	0.670	0.496	0.932	0.421	0.628
<b>l</b>	0.721	0.7209	0.118	0*	0.490	0.294	0.094	0.287	0.525	0.412
<b>p</b>	0.226	0.0859	0.962	0.236	0.388	0.256	0.312	0.258	0.768	0.119
<b>s</b>	0.007*	0.9561	0.048*	0.114	0.210	0.338	0.381	0.119	0.070	0.276
<b>w</b>	0.388	0.001*	0.378	0.482	0.540	0.670	0.496	0.211	0.421	0.628

**FIGURES:**

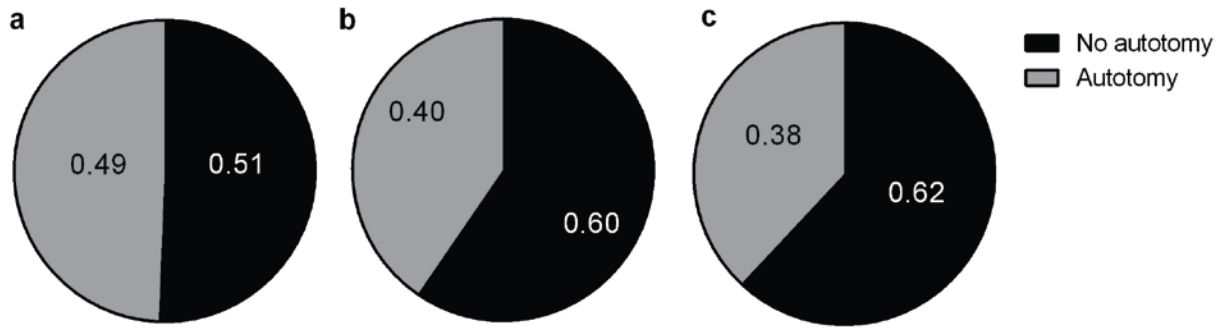


**Figure 2.1 a-d** Maps of the Philippines showing the following locations: a) Mactan Island and the den locations (numbers) at the three sites; close up of the den locations at b) Buaya, c) the marine station, d) Cordova.

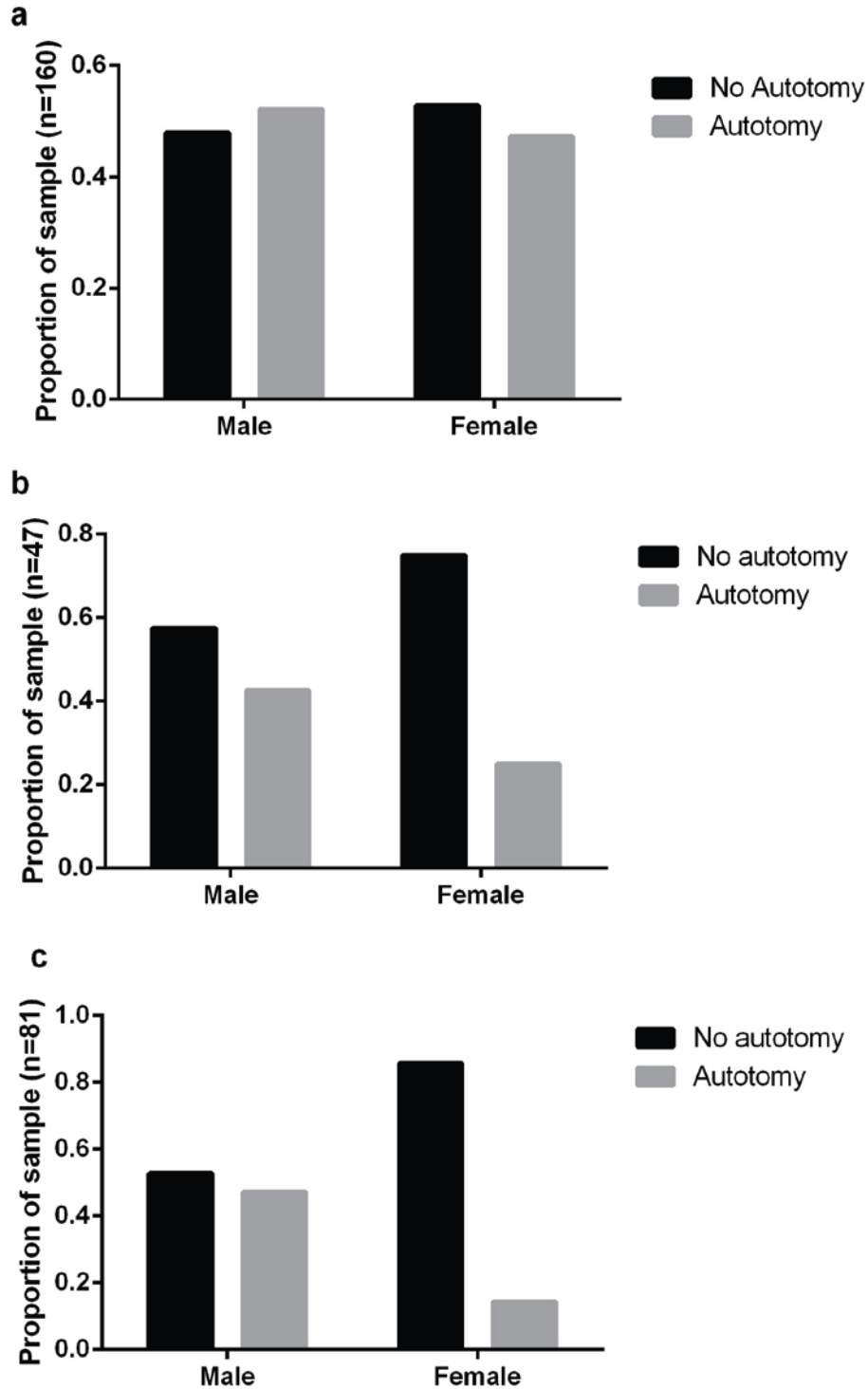




**Figure 2.1 a-d continued** Maps of the Philippines showing the following locations: a) Mactan Island and the den locations (numbers) at the three sites; close up of the den locations at b) Buaya, c) the marine station, d) Cordova.

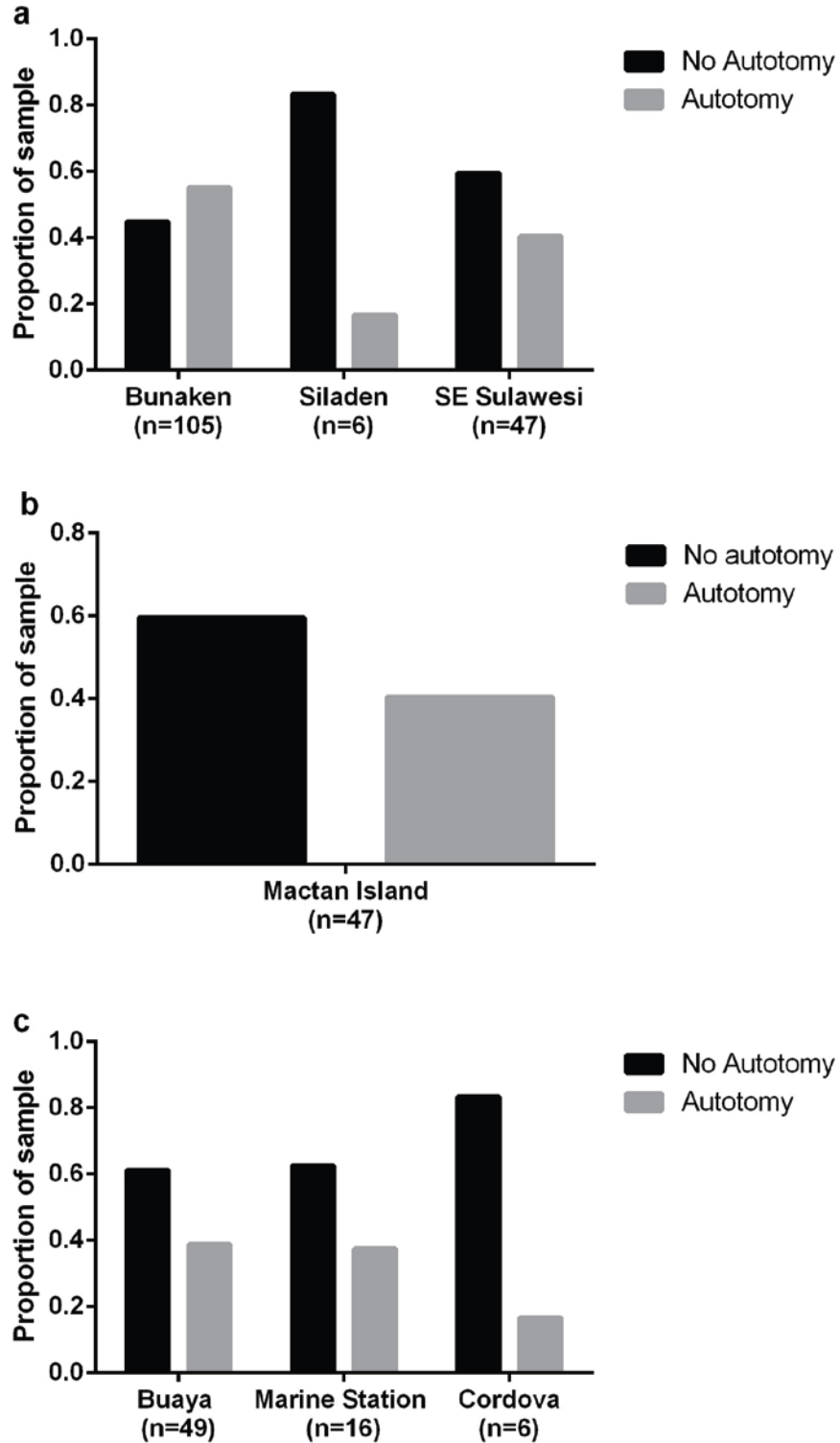


**Figure 2.2 a-c:** Pie chart representing the proportion of sampled individuals that exhibit autotomy (either loss of an entire arm or a regenerating arm stub) in a) Indonesia, b) Mactan Island, Philippines during the wet season, c) Mactan Island, Philippines during the dry season.

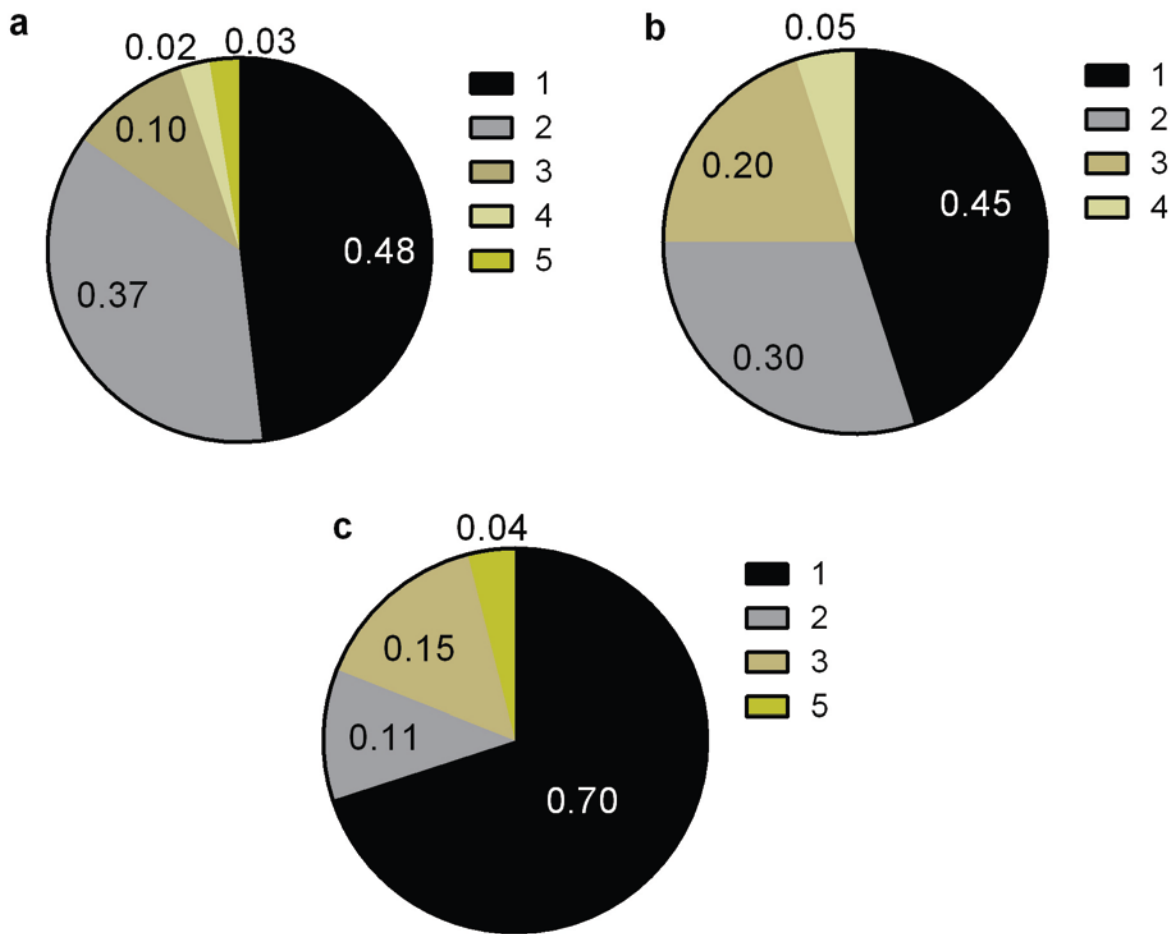


**Figure 2.3 a-c** Histogram representing the proportion of males and females that exhibit autotomy (either loss of an entire arm or a regenerating arm stub) in a) Indonesia, b) Mactan Island, Philippines during the wet season, c) Mactan Island during the dry season.

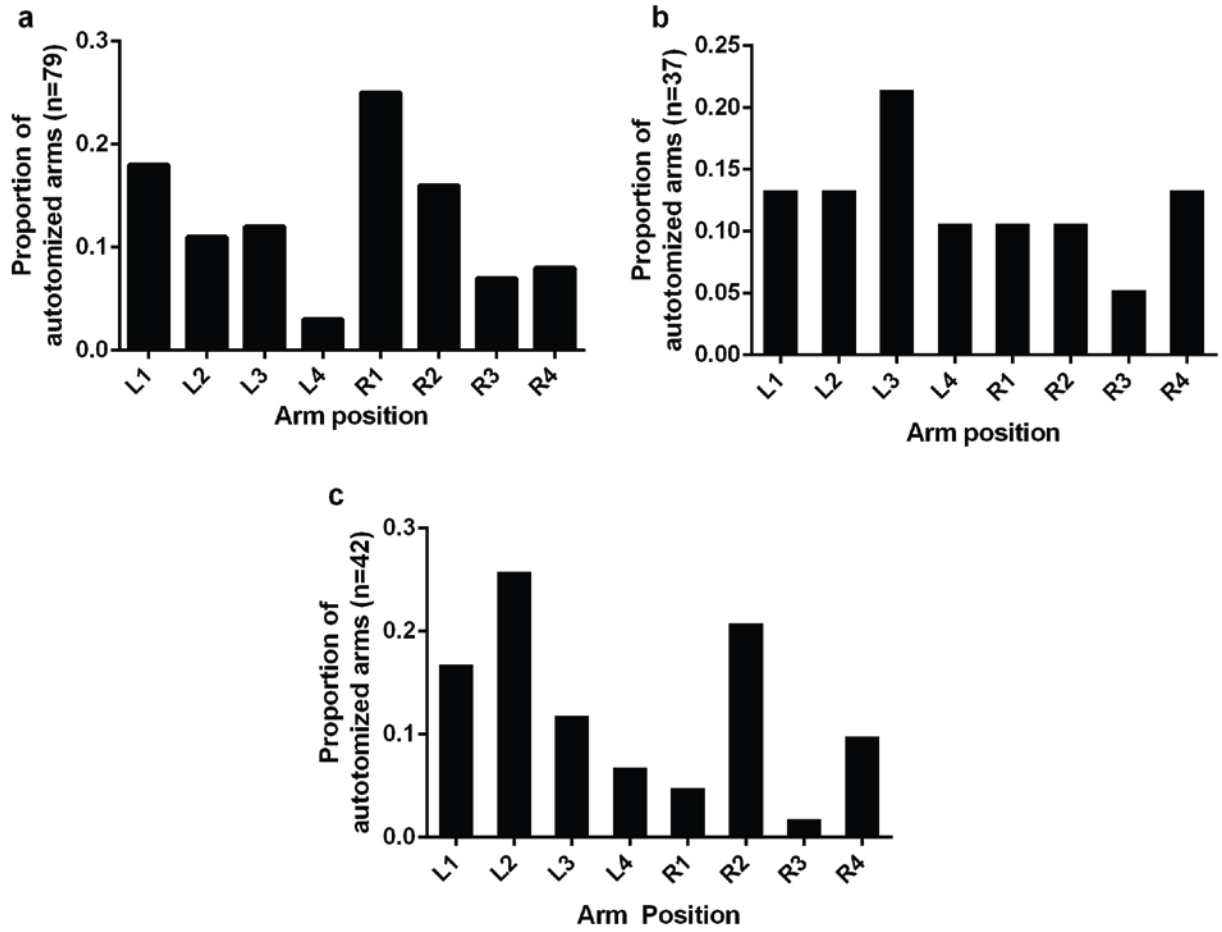




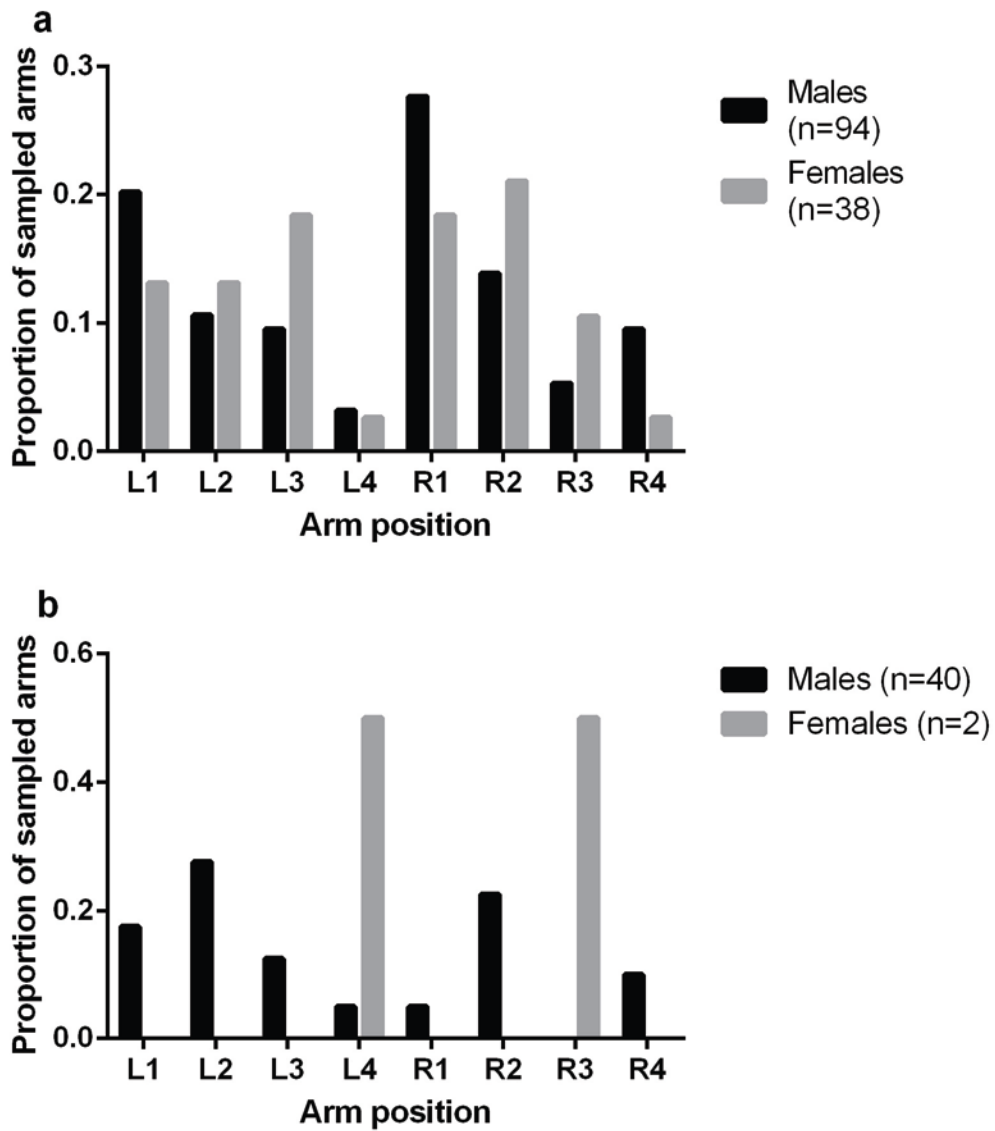
**Figure 2.4 a-c** Histogram representing the proportion of individuals that exhibit autotomy (either loss of an entire arm or a regenerating arm stub) in a) Indonesia, b) Mactan Island, Philippines during the wet season, c) Mactan Island during the dry season.



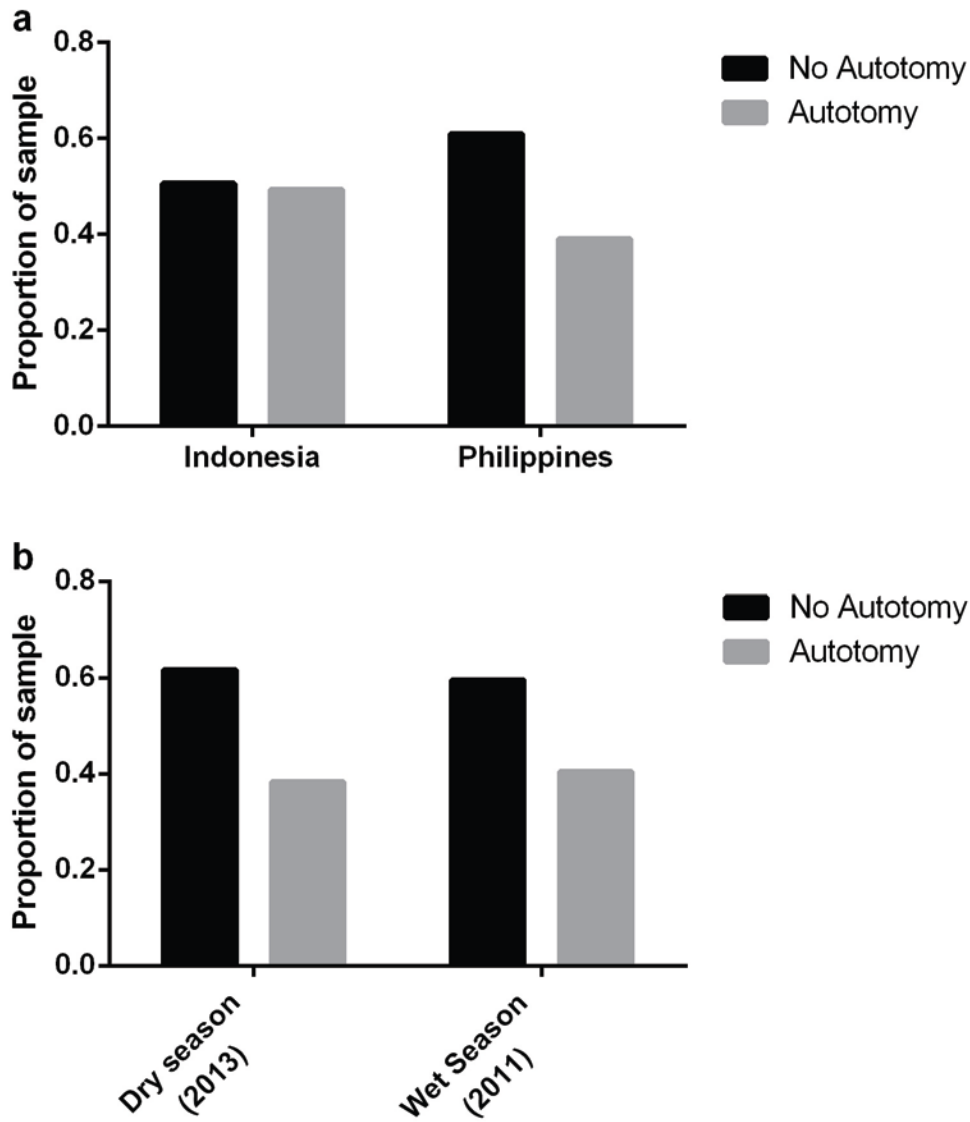
**Figure 2.5 a-c** Pie chart representing the proportion of individuals found exhibiting arm loss according to the number of autotomized arms (loss or regenerating stub of 1 through 5 arms) in a) Indonesia, b) Mactan Island, Philippines during the wet season, c) Mactan Island during the dry season.



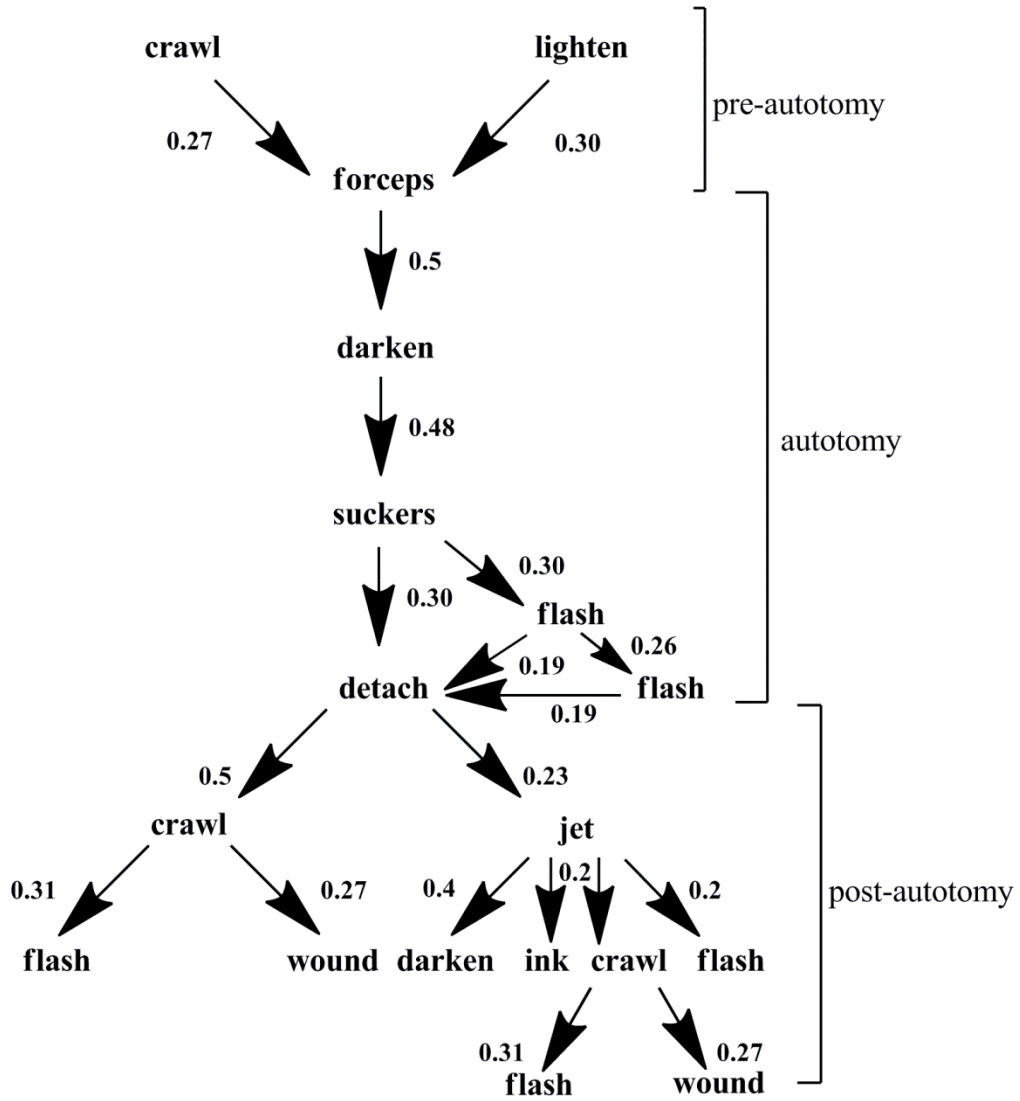
**Figure 2.6 a-c** Histogram representing the arm positions (L1-L4, R1-R4) that are most often autotomized (either loss of an entire arm or a regenerating arm stub) in a) Indonesia, b) Mactan Island, Philippines during the wet season, c) Mactan Island during the dry season.



**Figure 2.7 a-b** Histogram representing the arm positions (L1-L4, R1-R4) that are most often autotomized (either loss of an entire arm or a regenerating arm stub) for each sex in a) Indonesia and b) Mactan Island, Philippines during the dry season.



**Figure 2.8 a-b** Histogram representing proportions of sampled individuals exhibiting autotomy (either loss of an entire arm or a regenerating arm stub) and a) comparing them between Indonesia and the Philippines and b) comparing the dry and wet season in the Philippines



**Figure 2.9.** Transition diagram for the sequence of behaviors associated with an autotomy event. Transition probabilities appear next to each transition arrow.

### Chapter 3: Mechanism of autotomy: How does *A. aculeatus* lose its arms?

**ABSTRACT:** The small intertidal octopus species, *Abdopus aculeatus*, is known to exhibit arm autotomy as a potential defense mechanism against predators. To understand the mechanisms behind this arm loss, two key questions were asked: (1) are pre-formed autotomy planes or zones of weakness present in *A. aculeatus* arms and (2) does the central nervous system play a role in regulating autotomy? To address the presence of pre-formed cleavage planes, histological sections throughout the length of each arm were analyzed with particular attention to the base where arms tend to drop. To determine if autotomy occurs at a zone of weakness, the force required to autotomize octopus arms was measured using an Instron® and the location of breakage was recorded. Autotomy trials following decerebration, the removal of the supraesophageal mass in charge of higher cognitive processes, were performed to isolate the influence of the peripheral from the central nervous system during autotomy. Results from these tests indicate that autotomy in *A. aculeatus* occurs at a zone of weakness between the 4th and 8th proximal suckers. The lack of arm autotomy in a majority of decerebration trials suggests that the central nervous system plays some role in the control of autotomy in this species.

#### Introduction:

Autotomy is an important secondary defense mechanism for a wide variety of animals and often involves adaptations to facilitate the shedding of a limb or body part and subsequent wound healing. This is particularly true of a majority of reptiles that drop their tail using intravertebral autotomy rather than at joints between vertebrae. The autotomy plane crosses the vertebra transversely, coinciding with other layers of the tail including the connective tissue (the myoseptum) between neighboring muscle segments and layers of skin (Bateman & Fleming, 2009). Similar adaptations in multiple limb components are found in echinoderms such as sea stars, brittle stars, and crinoids. For instance, breakage in brittle star arms involves autotomy planes in the skin, the intervertebral ligament, the nervous system, and the water vascular system, each of which can exhibit a pre-formed permanent plane or a zone of weakness (Wilkie, 2001). Among arthropods including crabs, insects, and spiders, cleavage occurs along joints at varying segments for different appendages (Maruzzo, Bonato, Brena, Fusco, & Minelli, 2005; Parry, 1957). Lastly in gastropods, autotomized individuals may often be seen with swollen tails at the point of autotomy (Stasek, 1967). In all of these examples, the nervous system may be involved in facilitating rapid detachment and sphincter muscles may be utilized to close wounds and reduce loss of bodily fluids (Fleming, Muller, & Bateman, 2007).

Among soft bodied animals, cephalopods, particularly shallow water tropical octopuses have been studied for their arm autotomizing ability (Norman & Finn, 2001; Norman, 1992; Ward, 1998). Octopus arms, as well as the arms and tentacles of all cephalopod members, consist of a muscular hydrostat which was first described by Kier and Smith (1985). A muscular hydrostat comprises of a dense three dimensional arrangement of muscle fibers surrounded by connective tissue that provides both structural support as well as a means of producing movements (Kier, 1992; Kier & Smith, 1985). Muscular hydrostats are biomechanically characterized by their ability to change shape while maintaining a constant volume (Kier & Smith, 1985). Kier and Stella (2007) also characterized the internal morphology of octopus arms, identifying the components necessary to rupture during an autotomy event (Kier & Stella, 2007). Besides dense layers of muscle fibers and connective tissue, autotomy must also traverse the epidermis, axial nerve cord, artery, and intramuscular nerves (**Fig. 3.1**) (Kier & Stella, 2007). In

recent years, more experimental tools, particularly *in vivo* techniques, have been developed (Margheri et al., 2010; Margheri, Ponte, Mazzolai, Laschi, & Fiorito, 2011) making it possible to study various morphological, physiological, and mechanical properties of octopus arms and opened avenues for studying autotomy from a mechanistic point of view.

One of the aspects of autotomy that has been addressed in three cephalopod species is the presence of permanent fracture planes in arms. Histology revealed the presence of permanent cleavage planes in the benthic shallow water octopus *Ameloctopus litoralis* (Norman, 1992) and the pelagic deep water squid *Octopoteuthis deletron* (Bush, 2012). These are distinguished by increased concentration of cell nuclei bordering the autotomy zone. However, one benthic octopus species from Guam that was first described and named by Ward (1998) did not reveal the same pattern of morphological specialization for arm autotomy. Instead their arms exhibited a zone of weakness present near the base between proximal suckers four and seven, only undergoing mechanistic changes at the time of the autotomy event itself (Ward, 1998). This species will be referred to as '*Octopus sp.* Ward, 1998' in accordance with the nomenclature designated by Norman and Finn (2001). These studies along with investigations into the octopus genus *Abdopus* and the species complex *Octopus horridus* have revealed that autotomy planes can be either pre-formed or represent a zone of weakness, often occurring at the base of the arm at sucker ranges specific to each species (Norman & Finn, 2001).

An external force, such as that applied from the grasp of a predator, is often required for autotomy to take place (Fleming, Muller, & Bateman, 2007). The threshold resistance to lose an appendage can vary from species to species and even between sexes within the same species depending on external factors. Between the two previously mentioned octopus species, *A. litoralis* autotomizes multiple arms much more readily and with minimal stimulation than *O. sp.* Ward, 1998 (Norman, 1992; Ward, 1998). In the lizard *Sceloporus gadovae*, populations living at lower elevations and experiencing more intense predation pressures detached their tails more easily than populations inhabiting higher elevations with less intense predation (Fox, Perea-Fox, & Franco, 1994). In the lizard *Uta stansburiana*, males required more external force and strongly retained their tails compared to females. This is attributed to the importance of a complete tail to signal social status for mate success in this species (Fox, Conder, & Smith, 1998). In arthropods, this resistance to induce autotomy can also vary from slight pressure, such as in porcelain crabs and daddy-long-leg spiders, to physically shaking the animal to detach an appendage, as observed in the king cricket *Hemideina crassidens* (Fleming et al., 2007).

The speed with which an appendage can be detached from the body and the amount of force required to induce it is often related to how much the central nervous system and peripheral nervous system are in control. In some cases, more processing by the central nervous system is required to stimulate autotomy while in other cases autotomy may be a result of a localized reflex that does not require input from higher centers. Distinguishing between the two can often be difficult to isolate and study, especially given the range of possible responses to stimuli from rapid full limb loss to slow partial loss (Fleming et al., 2007). One method that has been used to isolate the influence of central versus peripheral nervous control in arthropods is decapitation. For example, in the grasshopper, *Schistocerca americana*, chemically induced autotomy still occurred even after decapitation, leading to the conclusion that the metathoracic ganglia is in control of autotomy by reflexive action (Ortego & Bowers, 1996).

*Abdopus aculeatus* is a tropical shallow water octopus species that has been observed to experience high incidences of arm regeneration and arm losses presumably from autotomy (Huffard, 2007; Norman & Finn, 2001). The tendency, according to Norman and Finn (2001) is



to lose arms close to the base between the 4th and 8th proximal suckers. Many of the morphological characteristics of ‘*Octopus sp.* Ward, 1998’ suggest it be a member of the *Abdopus* subgenus (Norman & Finn, 2001; Ward, 1998). I predict *A. aculeatus* will exhibit more features of autotomy in common with ‘*Octopus sp.* Ward, 1998’ than with *A. litoralis*, particularly in the lack of permanent pre-formed cleavage planes. I expect a moderate amount of force is necessary to stimulate autotomy in *A. aculeatus* and that the male third right arm, which is used to transfer spermatophores into the female during mating, takes more force to detach. The speed and threshold force required to stimulate autotomy may hint at a role of either central or peripheral nervous control of arm loss. Previous studies in cephalopod neural networks, particularly related to nociception in squid, have developed successful methods for decerebrating cephalopods to isolate the effects of reflexes from voluntary control (Crook, Hanlon, & Walters, 2013; Rowell, 1963). This involves removal of the supraesophageal mass which has been shown to be involved in learning and memory (Hochner, Shomrat, & Fiorito, 2006). I predict both the peripheral and central nervous systems influence autotomy, manifesting as a more delayed autotomy response in decerebrated octopuses. I will address these aspects of autotomy to better understand the mechanistic underpinnings of octopus autotomy using *A. aculeatus* as a model species and *Octopus macropus* as a control, non-autotomizing species comparison.

## **Methods:**

### Laboratory animal care of *A. aculeatus*:

Live *A. aculeatus* and *O. macropus* specimens used for the following studies were obtained either from 1) a commercial vendor (LiveAquaria, Rhinelander, WI) or by 2) field collection on Mactan Island, Philippines. They were housed in individual 20L artificial sea water (33-34ppt) tanks at 24-26°C and fed grass shrimp (*Crangon spp.* or *Palaemon spp.*) every other day.

### Laboratory autotomy tests for histology:

To study arm tissue morphology at the detachment site, eight individual *A. aculeatus* were stimulated to autotomize their arms. Using modified methods from Ward (1998), arms were randomly isolated with forceps a quarter of the length away from the proximal base, between the base and mid-arm. Arms were grasped with the forceps and pinned down for a maximum of 120 seconds to induce animals to autotomize their arm. Autotomized arms were immediately preserved in 95% ethanol to prepare for histological sections. Trials were repeated on the same individual until all arms were tested. Individuals with multiple arm losses were kept alive in the lab to monitor regeneration until the first signs of senescence which were indicated by reduced to no feeding, pale coloration, lesions on the body, lack of or slow activity and increased uncoordinated movement (Anderson, Wood, & Byrne, 2002). Specimens were slowly anesthetized in cool water and increasing concentration of ethanol before being preserved in 95% ethanol. All autotomy trials were recorded on video.

### Histology of *A. aculeatus* arms:

One to eight autotomized arms from eight *A. aculeatus* individuals were sectioned between 5 to 10  $\mu\text{m}$  to look for breakage planes throughout the length of the arm. Emphasis was given to the 4th through 8th proximal suckers where autotomy has been noted to occur for this species (Norman & Finn, 2001). The remaining stubs of the autotomized arms on the eight individuals were also excised after preservation for sectioning. In addition, sections of one arm

from each of the following preserved specimens were used as controls: *Octopus mercatoris*, *Octopus bocki*, *Octopus cyanea*, *Octopus diguiti*, and *Hapalochlaena fasciata*. None of these species have been observed to autotomize their arms. Longitudinal sections were prepared from paraffin embeddings according to a modified version of Kier's (1992) methods and stained with haematoxylin and eosin.

#### Threshold tensile force of arm autotomy in *A. aculeatus*:

Six live *A. aculeatus* were weighed and mantle lengths were measured prior to testing the threshold force required for arm autotomy. Individuals were sacrificed by anesthetizing it in slowly cooled seawater and 5% ethanol over a period of three hours. Deep ethanol anesthesia (>5% ethanol for more than thirty minutes) was avoided to minimize incidental affects to the viscoelastic properties of arm tissue being tested. Tests were performed using an Instron® 5544 machine with a 1 kN load cell using similar methods to Hou, Bonser and Jeronimidis (2011) as well as Koehl and Wainwright (1986). Tensile stress (force per unit area applied longitudinally) (MPa), tensile strain (relative change in shape and size from longitudinal stress) (mm/mm), and energy at the maximum tensile strain (J) were measured by the Instron® as the arm was extended. Length, width, and thickness of each arm sample tested on the Instron® were measured and used to calculate specimen toughness, the energy required to break a specimen over a given volume (Hou, Bonser, & Jeronimidis, 2011; Koehl & Wainwright, 1986).

In order to measure the force required to autotomize an arm, the arm was tested still attached to the main body of the animal. The freshly dead specimen was secured in a plastic bag with a cut hole to isolate the arm being tested. For uniaxial tensile tests, the bag was clamped at one end and the tip of the exposed arm was secured at the other end along the longitudinal axis (**Fig. 3.2**). Load was applied at a speed of 20 or 30 mm/min. Tests were performed on every arm of the individual. Two *O. macropus* were also tested as controls using the same methodology. This species is not known to autotomize its arms.

#### Decerebration of *A. aculeatus*:

To test if voluntary central control influenced the ability to autotomize an arm, 10 *A. aculeatus* were surgically decerebrated. General anesthesia was used by progressive exposure to ethanol in natural seawater (0.1% to 1.5% over 20 minutes) until animals were grey and unresponsive to light touch. Ventilation rate and responsiveness were monitored during and after the surgery, adjusting the anesthetic mix as needed. The skin and muscle overlying the cranium were injected with 0.1 ml of local anesthetic solution containing 0.5% benzocaine in a mix of 3:1 seawater to ethanol. A dorsal midline incision was made to expose the supraesophageal mass to be excise from surrounding lobes and ganglia. The incision was closed with adhesive and sutures. Recovery in fresh, aerated seawater usually occurred within 5 minutes and were maintained for at least 30 minutes prior to autotomy testing. Decerebrated individuals maintained regular respiration and locomotor activities after recovery as the control of these actions are centered in the suboesophageal brain (Rowell, 1963). Autotomy tests were conducted using the same laboratory methods described in the earlier section.

## **Results:**

### Laboratory Autotomy Tests:

Individual *A. aculeatus* were collected with varying degrees of arm loss prior to testing. Six individuals autotomized between one and four arms, all of which were preserved and

sectioned. Arms are designated according to a dorsal view of the animal, dividing the arms on the left (L) and right (R) side of the body, and numbering each side of arms 1-4 anteriorly to posteriorly (**Fig. 3.3a**). Two individuals, one male and one female, autotomized all arms, except the third right reproductive arm (the hectocotylus) in the male. After preservation in 95% ethanol, R3 was excised from the aforementioned male for sectioning. One intact arm (R1) from a specimen of *A. aculeatus* with prior regeneration mid-way up the arm was excised after preservation and sectioned. Arms at each position autotomized at least once and were sectioned for histology.

#### Histology reveals no permanent fracture planes:

No permanent fracture planes were found at the detachment site of autotomized *A. aculeatus* arms (**Fig. 3.3f**). There were also no permanent fracture planes along the entire length of all eight *A. aculeatus* arms. Control species (*O. cyanea*, *O. diguiti*, *O. bocki*, and *H. fasciata*) also did not exhibit apparent breakage planes throughout the length of their arms (**Fig. 3.3b-e**). The ends of the base of the arm stubs and the ends of the lost autotomized arms exhibited constricted muscle fibers in the longitudinal sections of *A. aculeatus* indicative of wound closing (**Fig. 3.4a-f**). Nuclei, distinguished by dark staining from haematoxylin concentrated at the edges of both ends of arm tissue, consistent with Norman's (1992) observations of autotomy. At the zone of regeneration in arm R1, there were no distinct differences in muscle fiber or their arrangement (**Fig 3.5e-f**). There was also no marked difference between the intrinsic arm musculature in the regenerating arm as compared to an original intact arm (**Fig 3.5a-d**).

#### Arm autotomy occurs at the base of the arm and follows a typical stress-strain curve:

In the six specimens of *A. aculeatus*, all arms autotomized at the proximal base. Not every arm of each individual was measured as many of them were collected with prior arm losses and partial regeneration. Forty trials of arm breakage were conducted. Fifty percent autotomized at the 6<sup>th</sup> proximal sucker, 27.5% at the 5<sup>th</sup> sucker, 1.25% at the 7<sup>th</sup> sucker, and the remaining arms detached at the 4<sup>th</sup> or 8<sup>th</sup> proximal suckers. In the two specimens of *O. macropus* that were tested, 15 arms were broken, but none were indicative of autotomy. All but four arms tore at the tip close to contact with the bottom clamp. The remaining four tore medially, but still close to the tip of the arm.

**Figure 3.6a** shows a typical stress-strain curve for one representative *A. aculeatus* arm. The curve is similar to that expected in tensile tests of octopus skin (Hou et al., 2011). The curve exhibits an initial slow increase in slope with a rapid increase as more of the arm tissue is pulled. Fracture starts at the maximum peak tensile stress with subsequent peaks representing autotomy through various layers of the arm. The first peak marks breakage of the intrinsic musculature, axial nerve cord, and artery. Smaller peaks denote tensile stress required to autotomize the epidermis. **Figure 3.6b** displays a pictorial view of autotomy in between these two small peaks during breakage of the epidermis. Tensile strain at the maximum tensile stress for all forty arm tests averaged  $0.654 \text{ MPa} \pm 0.145$ , thus fracture started when strain reached about 65% of the stretched length.

**Table 3.1** summarizes measurements of maximum tensile stress and calculations of toughness for all 40 arms tested from eight individuals of *A. aculeatus* and *O. macropus*. The last column calculates the average maximum stress and toughness for each arm while the last row gives the average maximum stress for each tested individual incorporating all arms. Average

maximum tensile stress overall for all *A. aculeatus* only trials was  $0.308 \text{ MPa} \pm 0.141$ . Average toughness was  $1.02\text{E-}4 \text{ J/m}^3 \pm 5.16\text{E-}5$ .

**Figure 3.7a-b** looks at differences in maximum tensile stress and toughness between the eight arms of *A. aculeatus*. The arms on the right side appear to exhibit larger stress and energy to break the arm, but this may be an artifact of a disproportionate sample sizes between the left and right sides. All but one specimen of *A. aculeatus* was male allowing for comparison of threshold forces to break the male reproductive arm (R3) to its symmetrical partner, L3. A Mann-Whitney test comparing differences between the third left and third right arms showed no significant difference in maximum tensile stress ( $U=3.00$ ,  $p=0.11$ ) or toughness ( $U=2.00$ ,  $p=0.06$ ). There was no significant difference in the force necessary to break the male reproductive arm.

#### Slow arm breakage post-decerebration in *A. aculeatus*:

Ten *A. aculeatus* with prior arm injuries, including missing and regenerating arms, were decerebrated to test for the role of voluntary control in autotomy (**Table 3.2**). Only two of the ten individuals exhibited autotomy post-decerebration, breaking at the 5<sup>th</sup> proximal sucker in one individual and the 7<sup>th</sup> proximal sucker in the other. On average, it took 15 seconds to autotomize the arms from the time of first contact until the arm was completely detached from the body. Arm breakage occurred in four other individuals, but at the point of contact rather than the typical autotomy zone. One specimen was regenerating five arms, one of which broke but was not an example of true autotomy. Four individuals did not exhibit any arm loss or injury during autotomy trials post-decerebration.

#### **Discussion and Conclusions:**

Two major questions regarding the mechanistic underpinnings of autotomy were addressed in this investigation of arm loss in *A. aculeatus*. The first was whether permanent breakage planes were present in the arms. Histological sections of arm tissue did not reveal permanent breakage points anywhere along the length of the eight arms. However, at the ends of the arm stub and the autotomized arms, there were increased densities of muscle cell nuclei, a characteristic of permanent breakage points in *A. littoralis* (Norman, 1992). This particular adaptation may not be required for *A. aculeatus* just as it is also not present in *O. sp.* Ward, 1998, another presumed member of the *Octopus horridus* group which autotomizes its arms (Norman & Finn, 2001; Norman, 1992). Both abiotic and biotic factors in the environment of *A. littoralis* may impose selective pressures to have a quicker mechanism by which to drop its arms. *A. littoralis* has been shown to exhibit more than one breakage plane close to the base of the arm. *A. littoralis* lives in intertidal reefs and mudflats, inhabiting dens during the day and venturing out to forage at low tide during the night. They extend their arms from their lairs to grab nearby prey during the day, making them susceptible to predation. There are several predators found near their lairs including moray eels, sharks, and other large fish. Unlike *A. aculeatus* and *O. sp.* Ward, 1998, *A. littoralis* has no ink sac, autotomizes its third right reproductive arm in males prior to maturing, and produces benthic direct developing young (Norman, 1992). These differences in life history traits may also indicate separate independent evolutions of arm autotomy in the *O. horridus* species group and *A. littoralis* (Norman & Finn, 2001; Norman, 1992). More specific adaptations for loss may be found in species that require the arm be autotomized more readily. In contrast, *O. sp.* Ward, 1998, like *A. aculeatus* lives in intertidal reefs, foraging both at day and night time during falling low tides and rarely is found with an autotomized third right arm in the

wild (Ward, 1998). Both species rely on inking as an alternative secondary defense mechanism. Thus the need for faster autotomizing arms by way of permanent cleavage planes may not be necessary.

These two sets of arm autotomizing octopuses set up a scenario for studying a gradient in autotomy, whereby some species lose more arms and very quickly where as others do not autotomize any arms or do so very slowly. *A. litoralis* is at one extreme of fast and high frequency of autotomy while *A. aculeatus* falls somewhere in the middle. There are several species of octopus that do not exhibit a tendency to drop any of their arms. A continuum of defense mechanisms utilized by octopuses may also be tested by studying the three aforementioned octopus species. Blue rings, like *A. litoralis* have also lost their ink sac and rely on a different defense mechanism, namely toxins, against predators. Most species of octopus rely on camouflage or other color mediated defense mechanisms rather than specialized secondary defense strategies like autotomy or inking.

The alternative to a permanent cleavage plane is a general zone of weakness where the arm is likely to autotomize (Wilkie, 2001). This is the case for *A. aculeatus* which Norman described autotomizing between the 4<sup>th</sup> and 8<sup>th</sup> proximal suckers (Norman & Finn, 2001). Results from Instron ® extension tests confirm this particular zone as the weakest part of the *A. aculeatus* arm as all arms dropped between the 4<sup>th</sup> through 8<sup>th</sup> proximal suckers. One would expect an object to fracture where the length or diameter required to break is the shortest since stress is inversely proportional to this distance. The tapered shape of the octopus arm suggests more fractures should occur at the tip of the arm where it is clamped which is what occurred with the non-autotomizing species, *O. macropus*. *A. aculeatus* break closer to the base of the arm where the diameter is largest provides further support that arm dropping in this species is an example of true autotomy.

A threshold force is associated with this zone of weakness near the base of the arm in *A. aculeatus* and other autotomizing species of octopus. As a comparison, in the non-autotomizing *Octopus vulgaris*, the mean force to pull bait *in vivo* from a fixed position in arm L2 was 40N (Margheri et al., 2010). This is much higher than the maximum peak tensile stress for arm autotomy recorded for the smaller *A. aculeatus*. Also measurements *in vivo* involve muscle reactions to arm pulling that translate to higher force readings compared to dead specimens. More samples need to be tested to obtain a better estimate of stress and toughness than those presented here, particularly to determine if the breaking forces are statistically different for each of the eight arm positions. Although the stress and toughness of the reproductive arm were not shown to be statistically significant (Mann Whitney test, p-value = 0.0635), the maximum tensile stress and toughness were larger for the reproductive arm for all sampled specimens than L3. This is congruent with the observation in *A. aculeatus* and '*O. sp.*, Ward 1998' of the third right arm being lost less often in the field and in the lab. Lower frequencies of autotomy in R3 could be attributed to a physiological adaptation for larger pulling forces in this arm position or it could be due to behavioral adaptations of octopuses protecting their reproductive arm from the grasps of predators and competitors.

Extension testing on the Instron ® revealed differences in force required to break different layers of tissue in the octopus arm. In ripped samples of *O. macropus*, fracture began at one end of the arm (usually ventral, on the side of the suckers) and spread through a cross-sectional area of the arm prior to breaking. In contrast, arms of *A. aculeatus* broke at the autotomy plane. Intrinsic musculature, the artery, and the axial nerve were the first to break represented by the maximum peak stress. This was followed by breakage of the epidermis, either

at the ventral or dorsal end first. These were represented as separate smaller peak that followed on the stress-strain curve. Similar techniques can be executed on other autotomizing organisms, such as sea stars (Wilkie, 2001) to help distinguish pre-formed planes from zones of weakness in different layers of tissue.

The second question investigated in this study is whether autotomy in *A. aculeatus* is mediated by voluntary control. Absence of autotomy in eight out of ten tested individuals post-decerebration suggests that the supraesophageal mass is important to some degree in. Octopuses have a nervous system with a central brain and two optic lobes that are important for highly organized activity (Hochner, 2012). They also have an extensive peripheral nervous system that innervates the arms and regulates most local arm motor control (Hochner, 2012). Both the peripheral and central nervous systems are likely involved in autotomy, but the degree to which each is in control has yet to be studied. One important component of autotomy that was revealed by the four trials where no autotomy occurred is the role of suckers. In all four cases, the octopus was unable to suction properly to any surface, either due to having fewer arms available to adhere or prolonged exposure to anesthetic. Without the ability to suction, the octopus could not provide any resistance to pulling during autotomy tests post-decerebration. The organisms were easily picked up with the forceps and did not induce autotomy to occur, implying that the force required to detach the arm is greater than that imposed by its own weight.

According to results from this study, autotomy in *A. aculeatus* occurs at a zone of weakness between proximal suckers four and eight at the base of the arm. There are no permanent breakage planes, but when pulled with the certain amount of force, usually that great than their own weight, the arm will break, internally at the muscle fibers first followed by the epidermis. Autotomy involves some degree of central voluntary control as evidenced by the lack of autotomy in most decerebrated individuals as well as a delayed autotomy response in the individuals that lost an arm.

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## TABLES

**Table 3.1.** Summary of results for tensile tests on Instron ® for the arms of 2 *O. macropus* and 6 *A. aculeatus* specimens. ( $\sigma$ =tensile strength, OM = *Octopus macropus*, AA = *Abdopus aculeatus*)

		OM 1	OM 2	AA 1	AA 2	AA 3	AA 4	AA 5	AA 6	MEAN $\pm$ SD (AA)
	Sex	male	female	female	male	male	male	male	male	
	Mass (g)	10.1	21.8	18.3	12.9	27.8	29.3	61.3	66.2	
R 1	Max $\sigma$ (MPa)	0.054	1.081	0.160	0.262	0.236	0.802	0.381	0.373	<b>0.369 <math>\pm</math> 0.228</b>
	Toughness (J/m <sup>3</sup> )	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<b>1.25E-4 <math>\pm</math> 6.05E-5</b>
R 2	Max $\sigma$ (MPa)	0.115	0.840	0.394	0.180	N/A	0.575	0.391	0.409	<b>0.390 <math>\pm</math> 0.140</b>
	Toughness (J/m <sup>3</sup> )	<0.001	<0.001	<0.001	<0.001	N/A	<0.001	<0.001	<0.001	<b>9.24E-5 <math>\pm</math> 2.82E-5</b>
R 3	Max $\sigma$ (MPa)	0.180	0.794	0.308	0.222	0.351	0.520	0.250	0.520	<b>0.362 <math>\pm</math> 0.131</b>
	Toughness (J/m <sup>3</sup> )	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<b>1.23E-4 <math>\pm</math> 3.68E-5</b>
R 4	Max $\sigma$ (MPa)	0.229	0.884	N/A	N/A	0.349	0.237	N/A	0.446	<b>0.344 <math>\pm</math> 0.105</b>
	Toughness (J/m <sup>3</sup> )	<0.001	<0.001	N/A	N/A	<0.001	<0.001	N/A	<0.001	<b>1.66E-4 <math>\pm</math> 1.25E-4</b>
L 1	Max $\sigma$ (MPa)	0.121	1.147	N/A	0.160	0.170	0.257	0.190	0.436	<b>0.242 <math>\pm</math> 0.115</b>
	Toughness (J/m <sup>3</sup> )	<0.001	<0.001	N/A	<0.001	<0.001	<0.001	<0.001	<0.001	<b>8.12E-5 <math>\pm</math> 2.68E-5</b>
L 2	Max $\sigma$ (MPa)	0.160	1.142	N/A	0.208	0.180	0.275	0.164	N/A	<b>0.207 <math>\pm</math> 0.0491</b>
	Toughness (J/m <sup>3</sup> )	<0.001	<0.001	N/A	<0.001	<0.001	<0.001	<0.001	N/A	<b>7.72E-5 <math>\pm</math> 1.55E-5</b>
L 3	Max $\sigma$ (MPa)	0.135	0.772	N/A	0.192	N/A	0.241	0.195	0.334	<b>0.240 <math>\pm</math> 0.0665</b>
	Toughness (J/m <sup>3</sup> )	<0.001	<0.001	N/A	<0.001	N/A	<0.001	<0.001	<0.001	<b>7.82E-5 <math>\pm</math> 1.86E-5</b>
L 4	Max $\sigma$ (MPa)	0.147	N/A	N/A	N/A	0.194	0.249	0.200	0.387	<b>0.257 <math>\pm</math> 0.0901</b>
	Toughness (J/m <sup>3</sup> )	<0.001	N/A	N/A	N/A	<0.001	<0.001	<0.001	<0.001	<b>7.52E-5 <math>\pm</math> 3.31E-5</b>
	MEAN $\pm$ SD Max $\sigma$ (MPa)	<b>0.143 <math>\pm</math> 0.0510</b>	<b>0.951 <math>\pm</math> 0.166</b>	<b>0.287 <math>\pm</math> 0.118</b>	<b>0.204 <math>\pm</math> 0.0360</b>	<b>0.247 <math>\pm</math> 0.0831</b>	<b>0.394 <math>\pm</math> 0.213</b>	<b>0.253 <math>\pm</math> 0.0945</b>	<b>0.415 <math>\pm</math> 0.0600</b>	

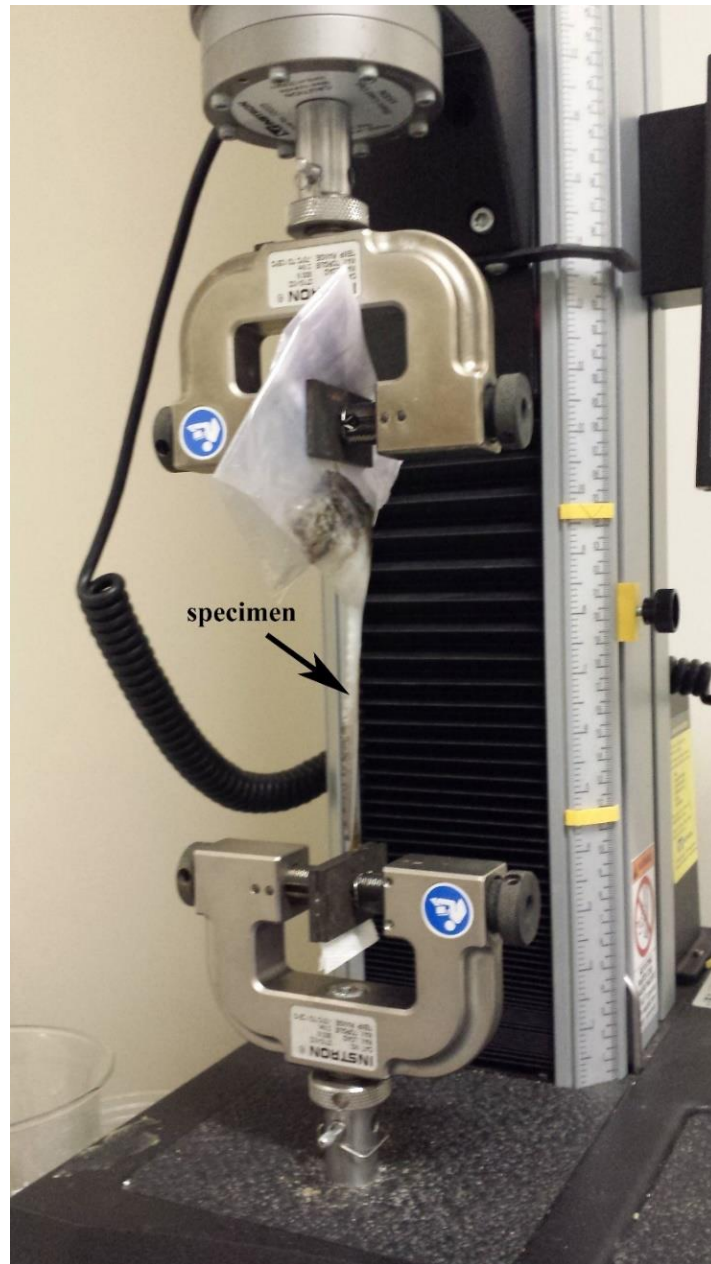
**Table 3.2.** Results from the decerebration test, indicating number of arm injuries prior to decerebration and the results from autotomy post-decerebration.

<b>Individual</b>	<b># Prior Arm Injuries (position)</b>	<b>Autotomy Post-decerebration Y/N? (position)</b>	<b>Time to autotomize (s)</b>	<b>Other Arm Injuries Post-decerebration</b>	<b>Injury location</b>
<i>A. aculeatus</i> 1	2 (R1, R2)	N	-	R4	point of contact
<i>A. aculeatus</i> 2	2 (R1, R2)	N	-	-	-
<i>A. aculeatus</i> 3	2 (R1, R2)	Y (L1, L2)	19, 12	-	7th proximal sucker
<i>A. aculeatus</i> 4	3 (L1, R1, R2)	N	-	-	-
<i>A. aculeatus</i> 5	5 (L1-L4, R4)	N	-	L1, L2	point of contact (tip)
<i>A. aculeatus</i> 6	2 (L4, R4)	N	-	-	-
<i>A. aculeatus</i> 7	2 (L3, R2)	N	-	R1, R4	point of contact
<i>A. aculeatus</i> 8	0	N	-	-	-
<i>A. aculeatus</i> 9	1 (R4)	N	-	L3	point of contact
<i>A. aculeatus</i> 10	0	Y (L2)	14	-	5th proximal sucker

**FIGURES:**

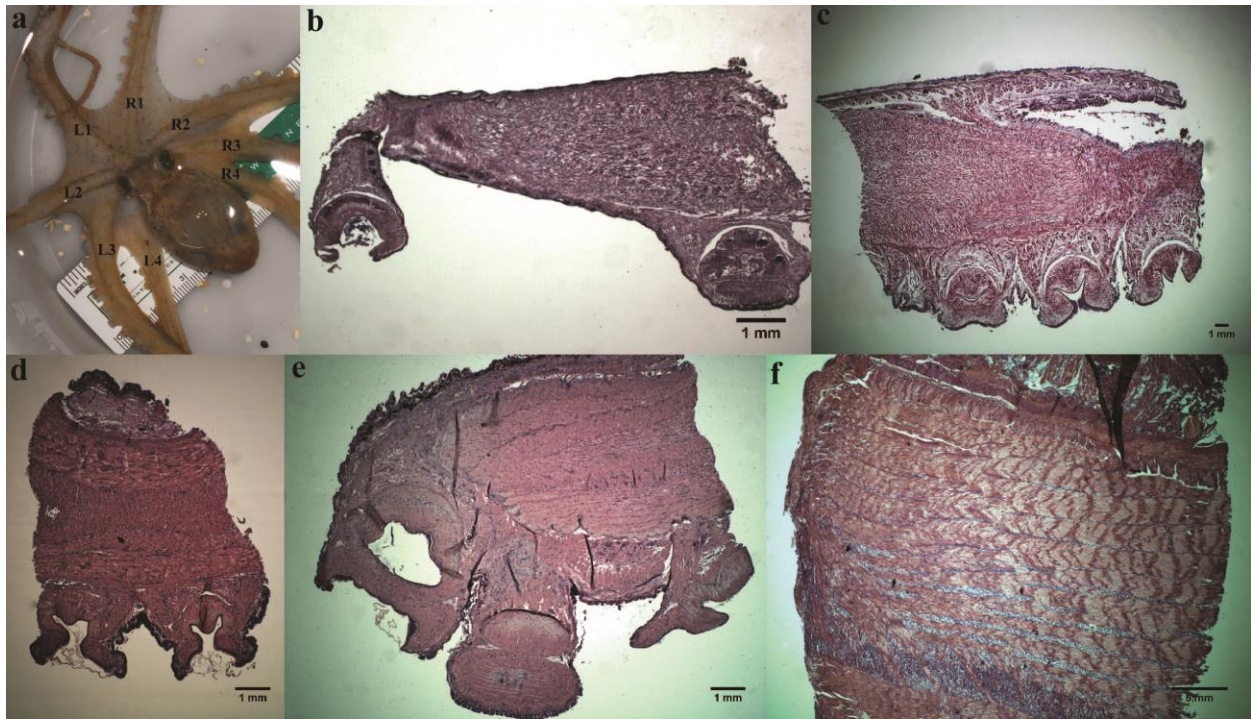


**Figure 3.1.** Transverse section of *Octopus mercatoris* arm stained with haematoxylin (black/blue-nuclei) and eosin (pink/red-connective tissue). Brightfield microscopy of 10 $\mu$ m-thick paraffin section. Major components of arm musculature besides three dimensional arrangement of muscle fibers include: an, axial nerve cord; ar, artery; ep, epidermis; in, intramuscular nerve.

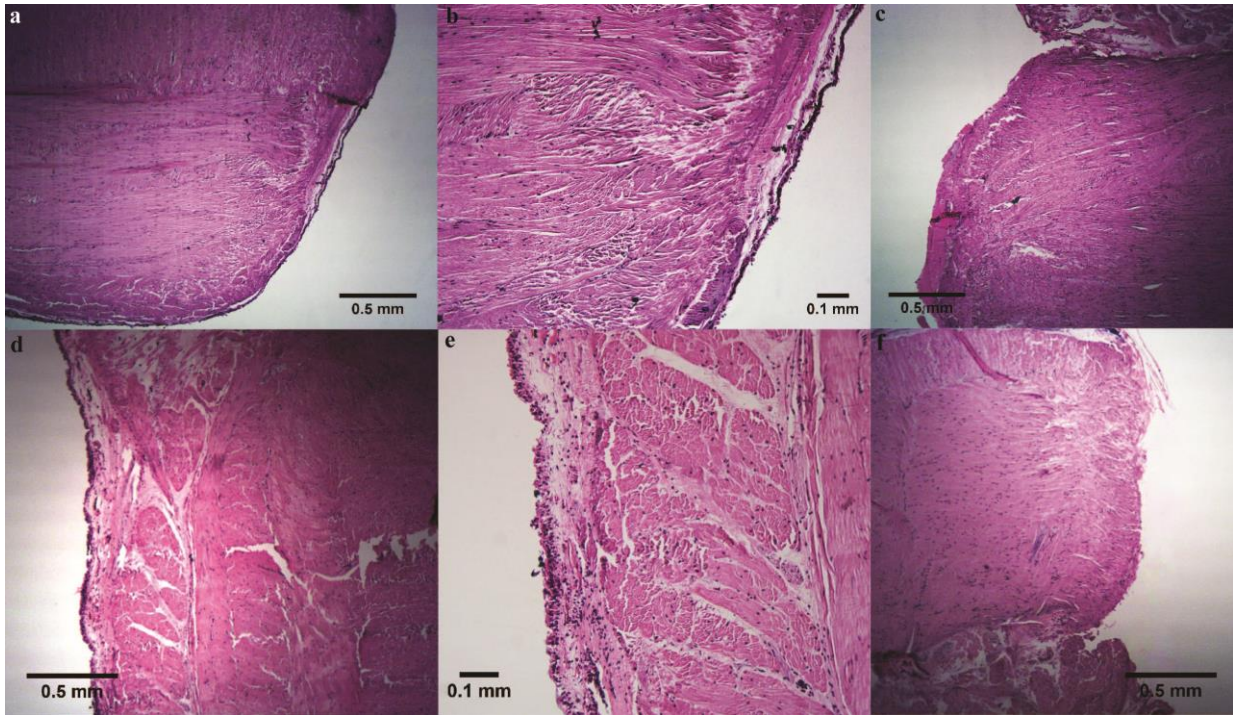


**Figure 3.2.** Laboratory set-up for uniaxial tensile test using the Instron ® 5544. Bag containing octopus clamped at the top while the arm is thread through and clamped at the tip.



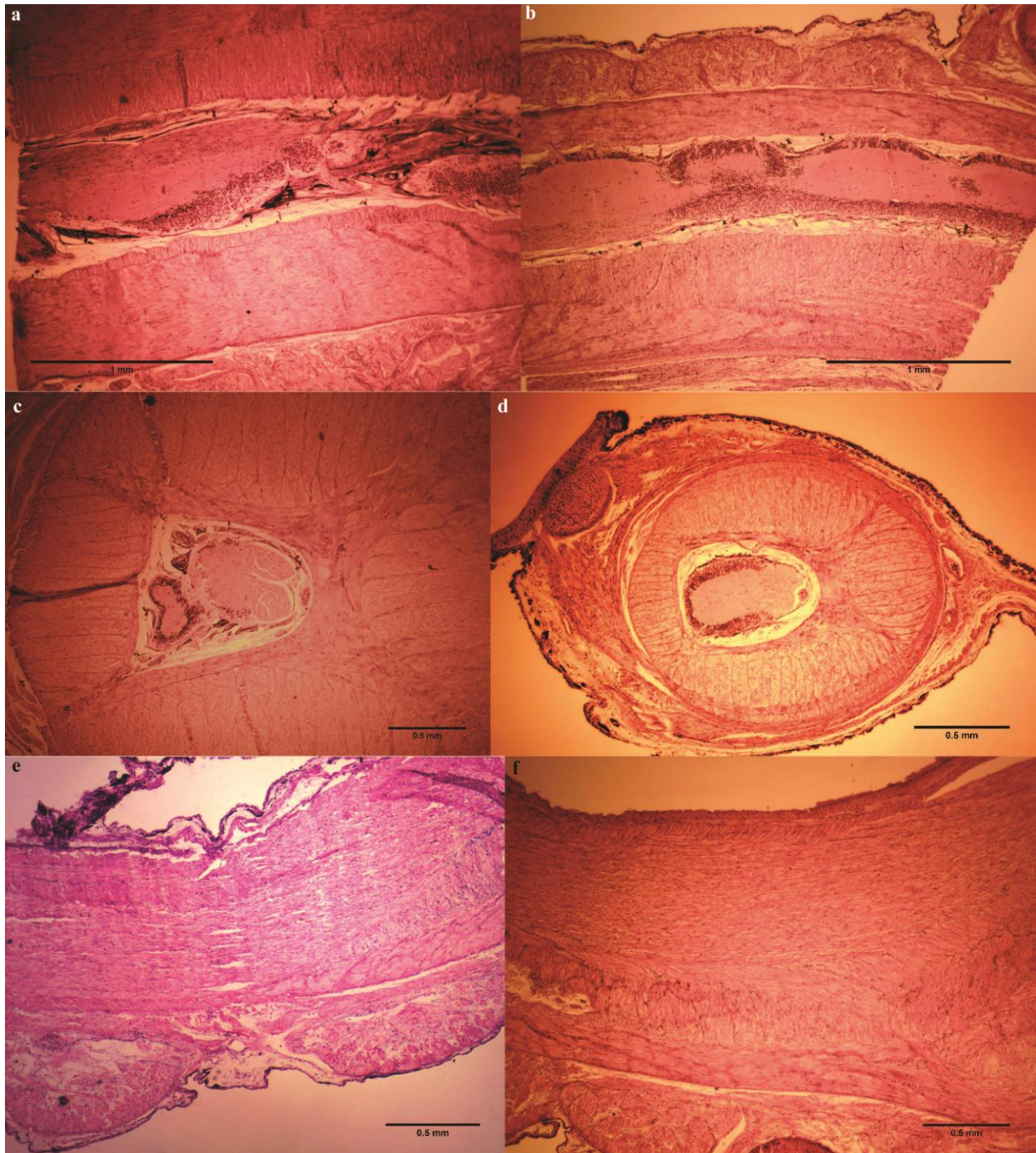


**Figure 3.3a-f.** Histology of longitudinal sections through arms of different octopus species. (a) Dorsal view of octopus arm position and their designations. 5  $\mu$ m sections of arm tissue stained in haematoxylin and eosin from the following species: (b) *O. bocki*, (c) *O. cyanea*, (d) *H. fasciata*, (e) *O. diguiti*, and (f) *A. aculeatus*.

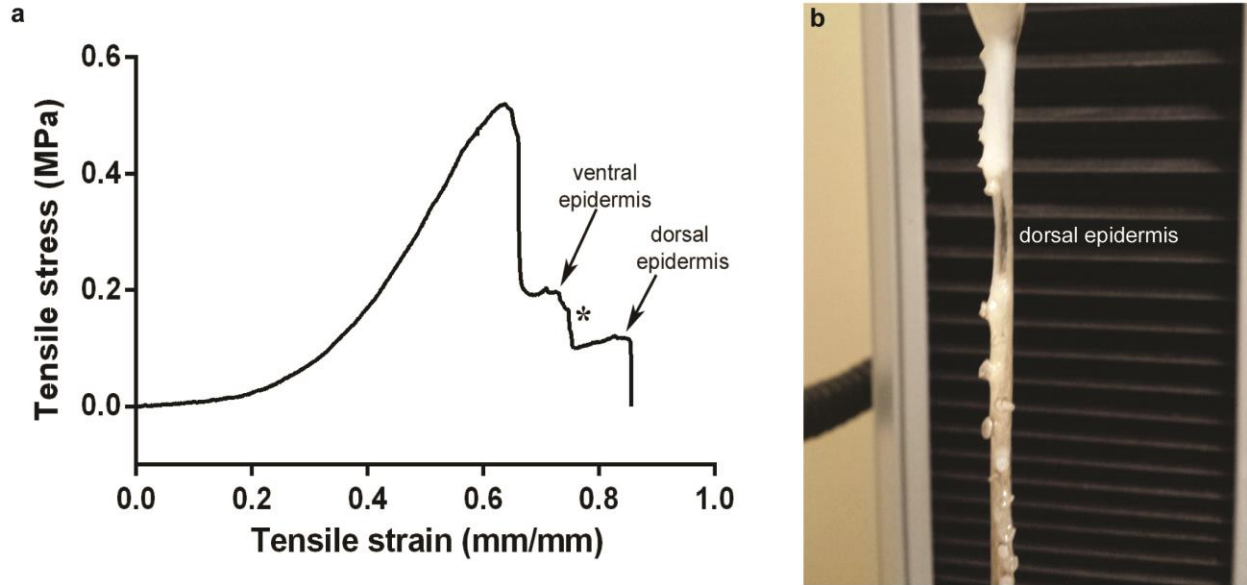


**Figure 3.4a-f.** Histology of longitudinal sections stained with haematoxylin and eosin. (a-c) and (d-f) represent a series of autotomized ends flanking the autotomy zone from two different arms. (a) autotomized end of the first lost arm, (b) autotomized end in a at 10X, (c) autotomized end of the arm stub on the individual, (d) autotomized end of the arm stub on the individual for the second arm, (e) autotomized end in d at 10X, (f) autotomized end of the second lost arm.



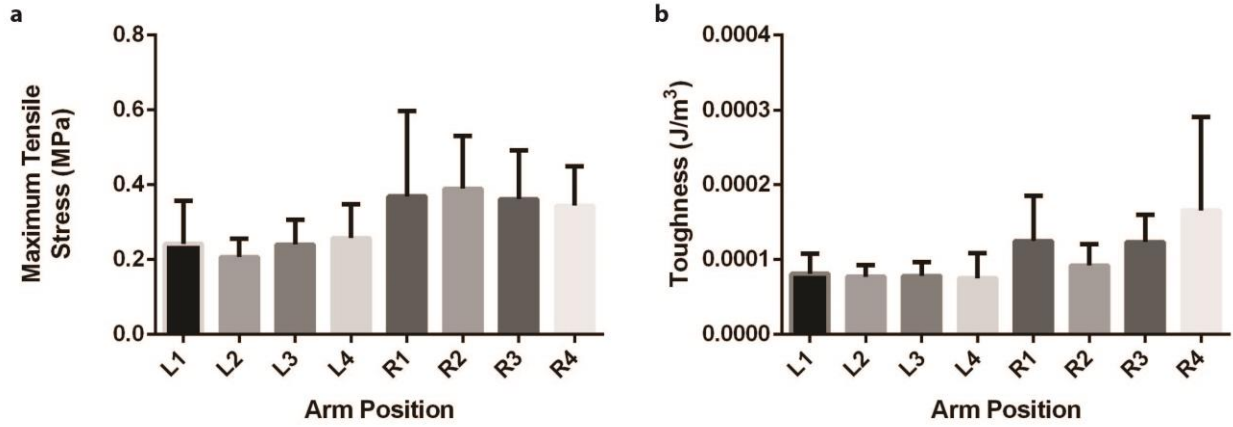


**Figure 3.5a-f.** Histology comparing regenerating arm tissue to original arm stained in haematoxylin and eosin. (a) and (c) longitudinal and transverse sections, respectively of original arm tissue. (b) longitudinal section of a regenerating arm, (d) transverse section of the regenerating arm in b, (e) and (f) longitudinal sections through the regeneration zone.



**Figure 3.6a-b:** Typical autotomy results from extension testing with Instron ®. (a) representative stress-strain curve for octopus arm. Each peak represents breakage of different tissue levels with the maximum peak representing breakage of the axial nerve and surrounding muscles. Arrows point to autotomy of the epidermis. \*represents the autotomy stage clamped to the Instron ® pictured in (b).





**Figure 3.7a-b:** (a) Maximum tensile stress (MPa) averaged for each arm position of *A. aculeatus* (b) Toughness (J/m<sup>3</sup>) averaged for each arm position of *A. aculeatus*.

## Chapter 4: Costs and benefits of autotomy to locomotion in the octopus, *Abdopus aculeatus*

**ABSTRACT:** Autotomy, or the shedding of a limb can be costly to locomotion, foraging activities, and reproduction, but beneficial in the immediate escape and survival of the individual from a predator's grasps. The autotomized limb or body part may have the added benefit of distracting the predator and/or providing an alternative food source while the prey escapes. To assess the costs and benefits of autotomy in a soft bodied animal, I looked at how the loss of an arm affected locomotion in the small intertidal octopus, *Abdopus aculeatus*, and observed behaviors in the autotomized limb to describe its potential distraction to predators. Results from this study indicate that losing one arm does not affect crawling or jetting speeds or change their preferred mode of locomotion. Autotomized arms were observed producing complex movements and suctioning onto surfaces immediately after autotomy and continued to respond to slight touches several minutes after. These results suggest that autotomy is not costly to locomotion in *A. aculeatus* and that this species of octopus may have developed complex movements in the autotomized arm that may distract their predator while they escape.

### Introduction:

Animals utilize secondary defense mechanisms when efforts to go undetected by a predator are no longer possible (Edmunds, 1974). This includes movement away from or deceptive coloration directed at a predator for avoidance. However, when this fails and an animal is in the grasp of a predator, a mechanism like autotomy may be the last resort. The likelihood of losing a body part is likely based on the costs and benefits involved. Losing an appendage has repercussions on all activities involving that body part including locomotion, foraging, defense and reproduction (Maginnis, 2006). Autotomy may also affect daily behaviors and social status of an individual, such as traveling less to forage for food or appearing less desirable to a potential mate (Fleming, Muller, & Bateman, 2007; Maginnis, 2006). Regeneration can also be costly to an individual when energetic investments need to be divided between somatic growth, regeneration, energy storage, and egg production in females (Barrios, Gaymer, Vásquez, & Brokordt, 2008; P. W. Bateman & Fleming, 2009; Clause & Capaldi, 2006; Maginnis, 2006; Mariappan, Balasundaram, & Schmitz, 2000). Despite these costs, the ability to lose a part of one's body is a fairly common occurrence and thus presumably provides a benefit far greater than these costs. The primary benefit of autotomy is survival from a potentially fatal encounter (Arnold, 1984; Clause & Capaldi, 2006; Fleming et al., 2007). In some animals, the autotomized body parts have developed special features that may increase an individual's chances of escape and survival. These include (1) ongoing movement of the autotomized limb post detachment to act as a decoy (Bässler, 1984; Clause & Capaldi, 2006; Cooper, Prez-Mellado, & Vitt, 2004; Cooper, Jr. & Smith, 2009; Fleming et al., 2007; Higham & Russell, 2010; Pafilis, Valakos, & Fofopoulos, 2005; Wilkie, 1978), (2) providing a smaller substitute meal for the predator (Cooper, Jr. & Smith, 2009; Fleming et al., 2007), (3) releasing toxic substances during detachment (Fleming et al., 2007; Shorter & Rueppell, 2011), and (4) adhering to the surroundings, including a predator post autotomy (Gilmour, 1967; Robinson, Abele, & Robinson, 1970; Shorter & Rueppell, 2011).

An individual's ability to move around in its environment after limb loss is one of the most commonly studied adaptive aspects of autotomy. Appendage loss may lead to less efficient modes of locomotion, from slower sprint speeds and low endurance to changes in gait patterns and escape distances (Fleming et al., 2007; Maginnis, 2006). Among arthropods, the field

cricket, *Gryllus bimaculatus*, exhibits slower escape speeds depending on the position and number of legs that are lost (Bateman & Fleming, 2005). However in the wolf spider *Pardosa milvina*, there is no evidence that leg autotomy affects locomotor behaviors, supporting the “spare leg hypothesis” that arachnids have more limbs than necessary and thus bear minimal costs in losing a limb (Brueseke, Rypstra, Walker, & Persons, 2001). Among crustaceans, the blue crab *Callinectes sapidus* Rathbun shows mixed results on escape speed depending on which limbs and how many are autotomized (Smith, 1995). Among molluscs, the file shell, *Limaria fragilis* is able to swim by valve clapping and autotomy of its tentacles results in reduced escape distance, but no change in swimming speed (Donovan, Elias, & Baldwin, 2004). Most studies of locomotor performance have concentrated on tail loss in reptiles and resulted in a wide variety of possibilities from positive to negative and even no effect on performance depending on the species and their environment. In *Uta stansburiana* and *Trachylepis maculilabris*, losing at least two thirds of the tail results in reduced sprint running speeds (Anderson, Cavalieri, Rodríguez-Romero, & Fox, 2012; Cooper, Jr. & Smith, 2009). However the Chinese skink *Eumeces chinensis*, and frog sand gecko *Teratoscincus scincus* do not experience effects to their escape sprint speed or escape distance (Lin, Qu, & Ji, 2006; Lu, Ding, Ding, & Ji, 2010). The extreme end of this spectrum is seen in the sand lizard *Lacerta agilis*, where individuals with autotomized tails run faster than those with their tails present (Ekner-Grzyb et al., 2013). This wide distribution in results show autotomy can have various effects on other aspects of biology and that results are often situation specific.

Despite the large emphasis on studying the costs of autotomy, few investigations have studied the benefits of autotomy, whether direct in terms of survival and fitness or indirect by looking at adaptations in the limb that might be beneficial beyond just promoting escape. Studies that investigate features of the autotomized body part concentrate on adaptations that make the limb look attractive either as a decoy or a potential alternative meal. These focus on tail autotomy in lizards which often involve excessive movement of the detached tail, working as a potential distractor. The autotomized tails of the salamander *Desmognathus ochrophaeus* continue to twitch after detachment and have been shown to be effective food distraction to chicken predators (Labanick, 1984). In different populations of the Balearic lizard *Podarcis lilfordi*, the shed tail continues to thrash post detachment for the same duration independent of the degree of predation influencing each particular population (Pafilis, Pérez-Mellado, & Valakos, 2008). In some species like the leopard gecko, *Eublepharis macularius*, these tail thrashing behaviors exhibit more complex patterns than rhythmic swinging for long periods of time (Higham & Russell, 2010). This excessive movement often leads to the displacement of the autotomized body part which can be measured and is positively correlated with the amount of tail that is shed in the skinks *Lampropholis delicata* and *L. guichenoti* (Cromie & Chapple, 2012). Even with these studies of limb movement, few have directly linked these properties as successful distractors to predators.

Octopuses can autotomize any of their eight arms and are subject to the same potential locomotor costs and distraction benefits exhibited by lizards. Octopuses, particularly the species *Abdopus aculeatus*, can use different modes of locomotion, the two most common being crawling and jetting (Huffard, 2006). Crawling is defined as the use of multiple arms and their suckers to push and pull on the substrate to guide movement. Jetting involves the expulsion of water in the mantle through the funnel resulting in backwards motion. Crawling is slower and the most utilized mode of locomotion (Huffard, 2006). Jetting is more often employed for defense and attack as a means of fast movement (Huffard, 2006). I predict that the loss of an arm by

autotomy will lead to differences in crawling and jetting speed as well as changes in the frequency with which they use each mode of locomotion.

After autotomy, the detached arm will continue to move and suction to surfaces, providing a potential distractor for predators. In the octopus *Abdopus capricornicus*, an arm was observed thrashing for more than an hour after autotomy (Norman & Finn, 2001). Octopuses are able to maintain this level of activity based on an extensive peripheral nervous system consisting of the axial nerve cord and ganglia in each arm (Huffard, 2013; Sumbre, Gutfreund, Fiorito, Flash, & Hochner, 2001). Stereotypical arm extensions, defined as a bend propagation along the length of the arm from the base to the tip, have been shown to be evoked in severed arms (Gutfreund et al., 1996; Sumbre et al., 2001). In this study, I will describe basic characteristics of the autotomized arm such as the duration of unstimulated movement, suction, and displacement of the arm as well as describe complex movements that are observed aside from stereotypical arm extensions. The extensive peripheral nervous system of octopus arms presents a good system for studying evocable responses in autotomized arms that can serve as distractors to predators. I will measure tactile sensitivity in autotomized arms using von Frey monofilaments to understand the duration and intensity of sensory response over time. This evaluation of costs and benefits of autotomy to octopuses will add more information to the reptile biased literature regarding the long- and short-term effects of autotomy

## **Methods:**

### Laboratory animal care of *A. aculeatus*:

Live *A. aculeatus* specimens used for the following studies were obtained either from 1) a commercial vendor (LiveAquaria, Rhinelander, WI) or by 2) field collection on Mactan Island, Philippines. They were housed in individual 20L artificial sea water (33-34ppt) tanks at 24-26°C and fed grass shrimp (*Crangon spp.* or *Palaemon spp.*) every other day.

### Autotomy and video recording trials to test costs to locomotion:

To study the effects of arm loss on octopus locomotion and the movement of the arms themselves, 13 individual *A. aculeatus* were stimulated to autotomize their arms. Using modified methods from Ward (1998), arms were randomly isolated with forceps a quarter of the length away from the proximal base, between the base and mid-arm. Arms were grasped with the forceps and pinned down for a maximum of 120 seconds to induce animals to autotomize their arm before being released. Individuals were allowed to recuperate for five to ten minutes, usually by resting and suctioning onto one place for the duration of the time. Once the individual started to move around the tank without any stimulation, video was recorded for comparison with pre-autotomy locomotion.

Before and after autotomy, each individual was filmed with a Sony HDR-CX580/V high definition camcorder at 30 frames per second in an experimental tank 86 cm long to maximize space for octopus locomotion. Each trial was filmed for a minimum of 10 minutes. Immediately after autotomy, the detached arm was also videotaped for 10 minutes in a smaller 30 cm long experimental tank.

### Whole Animal Locomotion Video Analysis:

All videos were analyzed using the Open Source Physics (OSP) Java framework based-free tracking software Tracker (4.82, OSP) along with MATLAB ® software. Each individual served as its own control for before and after trials. For each, a 10 minute period before and after

trial video was recorded. For each video, the number of times the individual crawled and jetted were counted. A successful crawling and jetting episode were defined as the octopus moving continuously in one direction unstimulated for at least half the length of the tank using typical crawling or jetting gait patterns described by Huffard (2006). Speed was calculated for both crawling and jetting locomotion. In addition, Tracker software was used to determine the speed (m/s) and stride (time it takes to complete a cycle of crawling in reference to the mouth) of the overall body by tracking the mouth as the individual crawled across the front wall of the tank. At least two crawling episodes from each before and after video trial were tracked for their maximum and average speed (m/s). The number of stops each individual made (i.e. no velocity of the mouth) during each crawling episode was recorded to provide information about stride. A Wilcoxon test was applied to determine statistically significant differences in the aforementioned locomotor variables before and after autotomy. Linear regression and Spearman's correlation were also conducted to rule out any effects of mass on the speed of each individual.

#### Autotomized Arm Video Analysis:

All videos of autotomized arms were viewed in Tracker software and the following variables were measured: the distance the arm was displaced (m), the duration of unstimulated motion of the arm (s), and the duration of sucker suction of the arm (s) to the front wall of the tank. Any movements besides stereotypical arm extensions were recorded considering the following factors: suckers, whole arm, local movement, or whole arm movement.

#### Von Frey Monofilaments Sensitivity Tests:

To test for local response thresholds to tactile stimulation in an autotomized octopus arm, an ascending series of von Frey monofilaments (Stoelting hair numbers 1.65, 3.61, 4.08, 4.17, 4.31, 5.07, 5.18, and 6.65 corresponding to the following maximal applied pressures: 0.008, 0.4, 1.0, 1.4, 2.0, 10, 15, and 300g respectively) were applied to the base of the arm and the tip of the arm every 10 minutes for 40 minutes. Monofilaments were positioned perpendicular to the octopus's skin and held down until the filaments were bent for one to two seconds, reaching its maximum tip pressure. Local responses to stimulation consisted of sucker turning or directed arm movement at the point of contact. A 2-factor ANOVA test was applied to determine if statistical differences are present in threshold log bending forces at the two locations of the arm over time.

#### **Results:**

There is no difference in locomotor performance before and after autotomy.

Eight of 13 *A. aculeatus* individuals were filmed moving before and after the arm was autotomized. Average crawling and jetting speeds do not exhibit a significant change or trend with increasing mass (**Figure 4.1 a-b, Table 4.1**) (crawling before:  $r = 0.40$ ,  $p\text{-value} = 0.33$ ; crawling after:  $r = 0.52$ ,  $p\text{-value} = 0.19$ ) (jetting before:  $r = 0.086$ ,  $p\text{-value} = 0.92$ ; jetting after:  $r = 0.40$ ,  $p\text{-value} = 0.75$ ). All of the following locomotor variables (**Table 4.1** and **Table 4.2**) showed no statistically significant difference ( $p < 0.05$ ) when compared before and after autotomizing an arm: proportion of trials spent jetting and crawling (**Figure 4.2 a-b**), average crawling and jetting speed (**Figure 4.3a-b**), maximum and average mouth speeds (**Figure 4.4 a-d**), and the number of stops made by the mouth during each crawling trial (**Table 4.2**). **Table 4.3** summarizes all conducted statistical tests and gives their respective  $p$ -values.

### Autotomized arms exhibit excessive and complex movements.

Thirteen autotomized arms were filmed moving immediately after detachment on the front wall of the small experimental tank (**Table 4.4**). Arms continuously moved between 19 and 176 seconds with an average time of 70 seconds. The proximal suckers (the base) either suctioned immediately to the front wall and stayed suctioned or they suctioned to the wall and subsequently moved to the bottom of the tank a maximum displacement distance of 0.23 m and a mean displacement distance of 0.05 m. In the cases where the proximal suckers did not stay suctioned, the minimum duration of sucker attachment to the front wall was 8 seconds and the average suction time was 45.92 seconds.

The autotomized arms displayed stereotypical arm extensions as well as other complex types of movement described below.

1. The whole arm can move a distance by extending and contracting the arm tube while the suckers walk by coordinated attachment and detachment on a substrate.
2. The whole arm can pivot 360° around a sucker attachment, usually consisting of one or two proximal suckers.
3. The whole arm can curl around a central sucker attachment, usually proximal suckers, and attach all suckers to the substrate.
4. The whole arm can undulate side to side, detaching and reattaching to the surface and allowing for fast displacement across a substrate.
5. The whole arm can twist around its central axis.
6. Smaller sections of the arm can undergo localized twisting, especially at the arm tip, to direct suckers towards a stimulus.

These movements were observed in nearly every trial for a few seconds.

### Thresholds for tactile sensitivity decrease over time and differentially at the tip and base of the arm.

Five of the 13 arm autotomy trials were tested with von Frey monofilaments for tactile sensitivity. A 2-factor ANOVA yielded significantly different responses to the monofilaments depending on the location of stimulus – either the tip or the base of the arm (p-value = 0.0094). The base of the arm exhibited lower threshold bending forces than the tip. A decreasing trend in threshold sensitivity over time is seen at both locations, but the values were not significantly different between time points (p-value = 0.48) (**Figure 4.5, Table 4.5**).

### **Discussion and Conclusions:**

Autotomy in the octopus *A. aculeatus* does not incur cost to their locomotion with respect to speed or the mode of locomotion used. This is in line with results seen in another eight limbed organism, the spider. According to Guffey (1998), the “spare-leg hypothesis” predicts that minimal to no costs should be experienced by spiders if they lost one or a few legs because they have more legs than are necessary for daily activities. He predicted this to be the case in Opiliones harvestmen based on the high frequency of autotomy observed in the field (Guffey, 1998). More direct support for this hypothesis is seen in the wolf spider *Pardosa milvina* which does not incur any costs to locomotor performance or foraging behavior (Brueseke et al., 2001). Octopuses, with their multiple highly maneuverable arms which are unconstrained by any exoskeleton, may exhibit the same degree of robustness to limb loss seen in spiders. During crawling, octopuses can use any combination and number of arms to push and pull off the substrate to move. One to four arms are involved in active crawling (Calisti et al., 2011) so the

loss of one or a few arms would not be expected to influence the speed of the animal. However, which arms are lost, depending on what side, may affect the direction they move as their body does not rotate during crawling. They change which arm positions to engage in crawling to guide their direction. Looking at the effects of autotomy on crawling gait itself, specifically how they use their different arms in combination to power directed motion is a future avenue to pursue to look at the effects of autotomy on locomotor performance.

Costs of autotomy on octopus crawling may also be hidden by their tendency to use intermittent locomotion. Intermittent locomotion occurs when “the force an animal exerts to move itself through space is applied discontinuously and the pauses last longer than the non-power phase of a single cycle of limb movement” (Kramer & McLaughlin, 2001). This has been described in *Octopus cyanea* which will move continuously for 13.5 seconds, but takes pauses that last 7.9 seconds (Forsythe & Hanlon, 1997; Kramer & McLaughlin, 2001). In the frog-eyed sand gecko, *T. scincus*, loss of the tail did not impose any costs to locomotor performance and behavior which was partly attributed to their use of intermittent locomotion (Lu et al., 2010). They never ran the entire length of the tank of the experimental procedure, but moved in multiple interrupted bouts with pauses. The same occurred during crawling locomotion of *A. aculeatus*, but not during jetting locomotion. As a result, using an average speed to represent locomotor performance may not be the best proxy for detecting differences before and after autotomy. Kramer and McLaughlin (2001) point out other potential factors to study the costs and benefits of utilizing frequent pauses during locomotion. These include acceleration and deceleration and its effects on energetics for each bout as well as the time it takes to cover a given distance with many pauses. These are factors to consider in future studies of crawling specific locomotor costs of autotomy to octopuses.

One study design improvement for the future is filming multiple post-autotomy locomotion trials for an individual at regular intervals. Although the octopuses were allowed to recuperate for five to ten minutes prior to the locomotion trial, filming so soon after an autotomy event may not capture the effects of arm loss on movement and may instead be representing a response to stress. Recording locomotion videos an hour later or a day later may provide a better post-autotomy comparison than directly after the event. Measures to do this were attempted with some of the tested individuals and were initially found unsuccessful as the octopuses habituated to the trials and would not move from the corner of the experimental tank for long periods of time.

Observation of autotomized arm movements indicate that a single detached arm can do more than a single arm extension, the most studied and simplest octopus arm movement pattern (Gutfreund et al., 1996). More complex arm behaviors observed in this study indicate that the peripheral nervous system in the arm can produce patterned movements even without input from a central nervous system. The autotomized tail of the leopard gecko, *E. macularis*, exhibits complex jumps and flips in addition to rhythmic swings, all of which have been quantified with respect to their neuronal motor control patterns and movement characteristics (Higham & Russell, 2010). These different movement patterns of the tail are controlled by separate and independent neural circuits that can be modulated by external sensory information (Higham & Russell, 2012). A similar system may occur in single octopus arms where movement is mostly controlled by the peripheral nervous system even when intact (Sumbre et al., 2001). This system of localized control allows for a hierarchical organization of the nervous system in coordinating multiple command centers to produce overall animal locomotion. Within the arm itself, movement must be coordinated between the suckers, localized regions and the whole arm to

produce these complex movements that may act as a distractor to the predator after autotomy. Quantification of the motor control and movement patterns, similar to that seen in the leopard gecko tail, is the next step in understanding the behaviors and potential adaptations developed by the autotomized limb.

This study did not directly test whether movements of the autotomized arm are distractors to predators. The tactile sensitivity test showed that the arm becomes less responsive overtime. However even 10 minutes after autotomy, slight touches with a low force monofilament evoked whole arm reflexes and sucker responses towards the stimulant. Suckers even suctioned firmly onto the monofilaments themselves for long periods of time. This suggests that interactions of the autotomized arms with predators may provide a distraction in detaining the predator with its suckers which is potentially an added benefit not seen in the distracting tails of lizards or arthropods. The complex movement patterns alone can be maintained for several seconds without interaction with the predator, providing a distraction from the start.

The duration of movement of the autotomized arm exhibited a wide range of time. One potential factor is the amount of time it took to autotomize the arm. Prolonged grasp of the arm with forceps may have caused damage to some of the nerves relaying information to the rest of the arm. Related to this is the decreased sensitivity exhibited by the base of the autotomized arm compared to the tip. Detachment occurs at the base of the arm, damaging more of the peripheral nervous system at the base than at the tip. The base of the arms in all 13 trials were also suctioned to a surface for a period of time, preventing it from responding with other localized complex behaviors upon stimulation.

The results of this study found no costs of autotomy to locomotor performance and suggest that the movements made by the detached arm can be a potential distractor and even detain the predator due to their powerful suckers. Future studies that incorporate temporal changes in gait (hours and weeks after autotomy) may yield significant differences in locomotor performance before and after autotomy. Also more direct observations of predator and autotomized arm interactions will elucidate the benefits for having arms move and suction excessively after separation from the body.



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**TABLES:**

**Table 4.1:** Summary of crawling and jetting locomotion results before and after autotomy for 8 *A. aculeatus*. Prop trials = proportion of trials spent doing a particular activity, either crawling or jetting, before or after autotomy. Avg speed = Average speed for that individual. Auto arm = Position of the autotomize arm.

#	Mass (g)	Auto arm	Crawling				Jetting			
			Before Autotomy		After Autotomy		Before Autotomy		After Autotomy	
			Avg speed (m/s)	Prop trials	Avg speed (m/s)	Prop trials	Avg speed (m/s)	Prop trials	Avg speed (m/s)	Prop trials
1	44.9	R1	0.031	1	0.039	1	-	0	-	0
2	46.4	L1	0.024	0.727	0.027	0.833	0.124	0.272	0.147	0.166
3	20	L3	0.027	0.625	0.025	0.8	0.118	0.375	0.100	0.2
4	18.8	R2	0.024	0.8	0.041	0.571	0.125	0.2	0.093	0.428
5	14.7	R2	0.027	0.416	0.013	1	0.182	0.583	-	0
6	15.7	R1	0.013	1	0.015	1	-	0	-	0
7	45.3	L1	0.043	0.750	0.040	1	0.278	0.25	-	0
8	17.3	L1	0.025	0.260	0.027	0.75	0.118	0.739	0.120	0.25

**Table 4.2:** Summary of tracked mouth speeds during crawling for 8 *A. aculeatus* before and after autotomy.

Individual	Trial	Mass (g)	Before Autotomy		After Autotomy	
			Maximum speed (m/s)	Average speed (m/s)	Maximum speed (m/s)	Average speed (m/s)
1	1	44.9	0.130	0.037	0.117	0.043
	2	44.9	-	-	0.118	0.045
	3	44.9	0.082	0.039	0.136	0.051
2	1	46.4	0.069	0.032	0.106	0.037
	2	46.4	0.066	0.021	0.084	0.039
	3	46.4	-	-	0.074	0.025
3	1	20	0.199	0.049	0.12	0.038
	2	20	0.254	0.033	0.066	0.025
	3	20	-	-	0.110	0.030
4	1	18.8	0.095	0.026	0.098	0.049
	2	18.8	0.171	0.024	0.147	0.034
	3	18.8	0.085	0.026	0.104	0.039
5	1	14.7	0.117	0.034	0.041	0.013
	2	14.7	0.163	0.028	0.039	0.017
	3	14.7	0.108	0.031	0.039	0.016
6	1	15.7	0.041	0.013	0.036	0.017
	2	15.7	0.039	0.0167	0.055	0.028
	3	15.7	0.039	0.016	0.048	0.011
7	1	45.3	0.098	0.034	0.152	0.046
	2	45.3	0.111	0.043	0.162	0.046
	3	45.3	0.180	0.041	0.149	0.055
8	1	17.3	0.077	0.031	0.099	0.031
	2	17.3	0.124	0.037	0.067	0.026
	3	17.3	0.106	0.027	0.090	0.024

**Table 4.3:** Summary of statistical tests results for various locomotor variables.

<b>Factors</b>	<b>Test</b>	<b>Test statistic</b>	<b>p-value</b>
mass vs average crawl velocity before autotomy	Spearman correlation	$r = 0.40$	0.33
mass vs average crawl velocity after autotomy	Spearman correlation	$r = 0.52$	0.20
mass vs average jet velocity before autotomy	Spearman correlation	$r = 0.08$	0.92
mass vs average jet velocity after autotomy	Spearman correlation	$r = 0.40$	0.75
proportion crawling before and after autotomy	Wilcoxon test		0.16
proportion jetting before and after autotomy	Wilcoxon test		0.16
average crawling speed before and after autotomy	Wilcoxon test		0.46
average jetting speed before and after autotomy	Mann-Whitney test		0.25
max mouth speed before and after autotomy	Mann-Whitney test		0.34
average mouth speed before and after autotomy	Mann-Whitney test		0.57

**Table 4.4:** Summary of movement characteristics of the autotomized arm in 13 *A. aculeatus*.

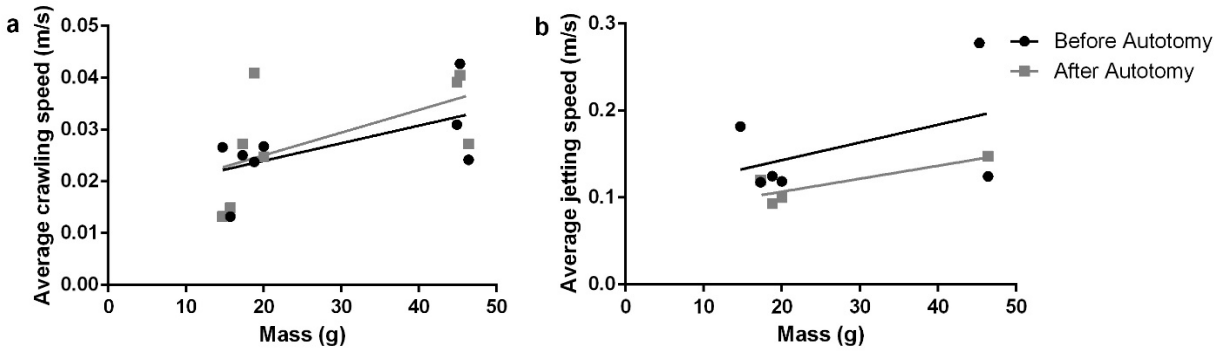
<b>Trial</b>	<b>Auto arm</b>	<b>Displacement (m)</b>	<b>Duration of continuous motion (s)</b>	<b>Duration of suction (s)</b>	<b>Suckers</b>
1	R1	0.227	94	56	all
2	L1	0.118	176	123	base
3	L3	0.085	48	32	base/ medial
4	R2	0.164	110	15	few
5	R1	0.006	34	34	base
6	L1	0.080	95	27	
7	L1	0.003	19	19	base
8	R1	0	26	26	base
9	R1	0	20	20	base/ medial
10	R2	0	109	109	base
11	R2	0	23	23	base
12	R2	0	105	105	base
13	L1	0.030	51	8	all

**Table 4.5:** Summary of threshold tactile sensitivity to von Frey monofilaments in 8 *A. aculeatus*.

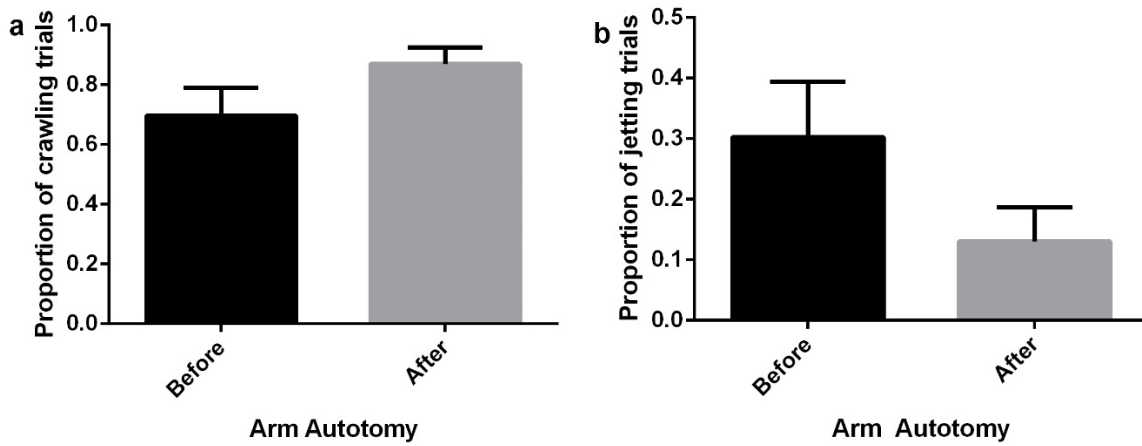
Trial	Auto arm	Before auto	Tip				Base			
			10 min	20 min	30 min	40 min	10 min	20 min	30 min	40 min
1	L1	-	1.65	4.08	5.18	-	-	4.17	5.18	6.65
2	R1	3.61	-	1.65	-	3.61	-	3.61	1.65	3.61
3	R2	-	-	1.65	1.65	1.65	1.65	1.65	-	1.65
4	R2	3.61	1.65	1.65	1.65	1.65	3.61	4.31	4.31	5.07
5	R2	3.61	1.65	1.65	1.65	1.65	3.61	3.61	3.61	3.61



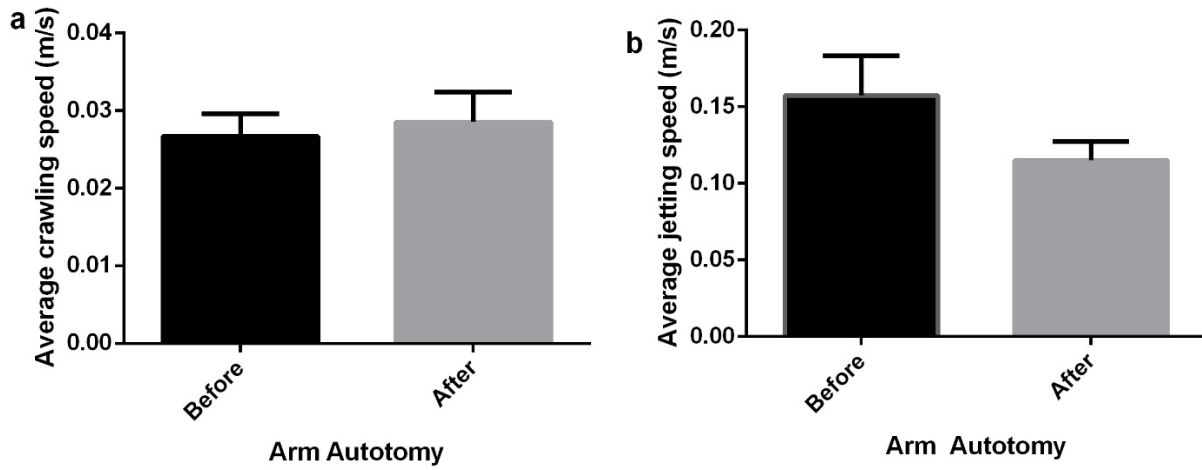
## FIGURES



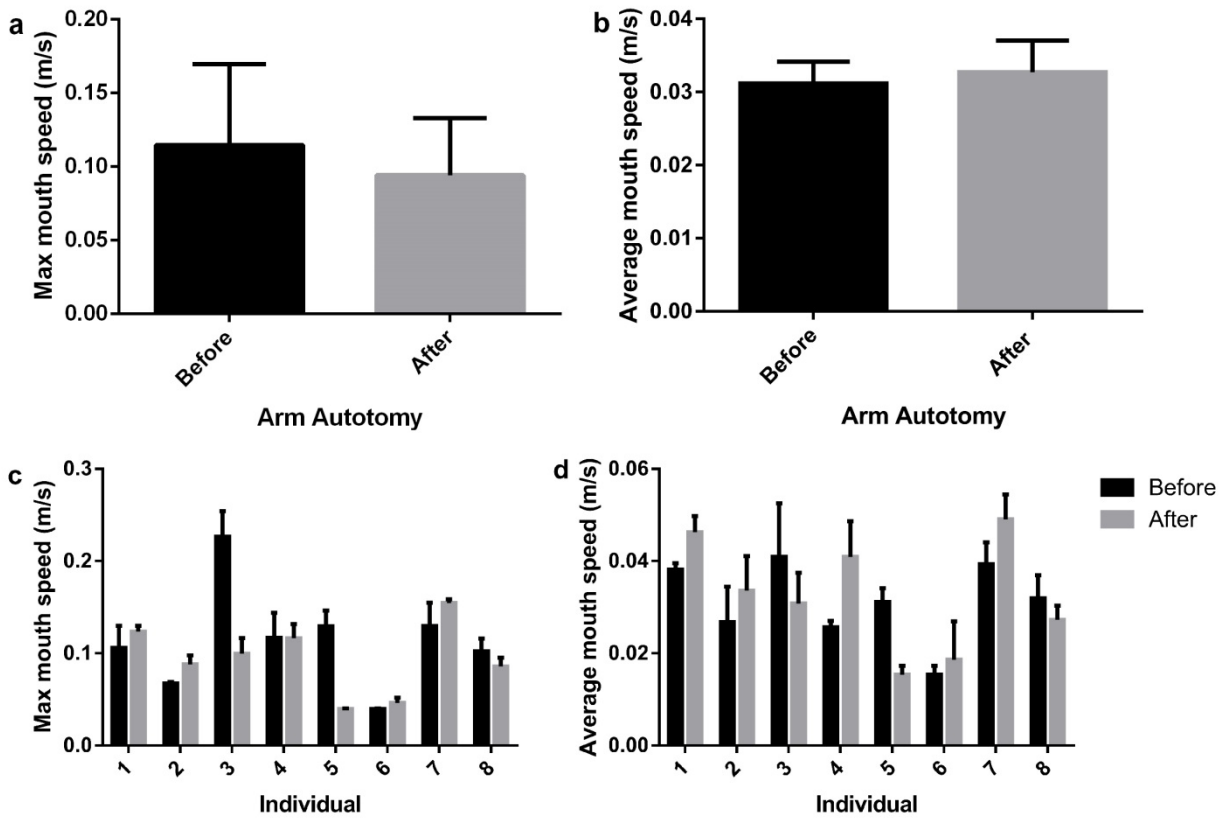
**Figure 4.1a-b.** Average locomotion speeds do not change with increasing mass. (a) Average crawling speed before and after autotomy for a given mass (linear regression before: slope = 0.0003397,  $R^2 = 0.37$ , p-value = 0.11; after: slope = 0.0004382,  $R^2 = 0.34$ , p-value = 0.13). (b) Average jetting speed before and after autotomy for a given mass (linear regression before: slope = 0.0020,  $R^2 = 0.22$ , p-value = 0.35; after: slope = 0.001495,  $R^2 = 0.72$ , p-value = 0.15).



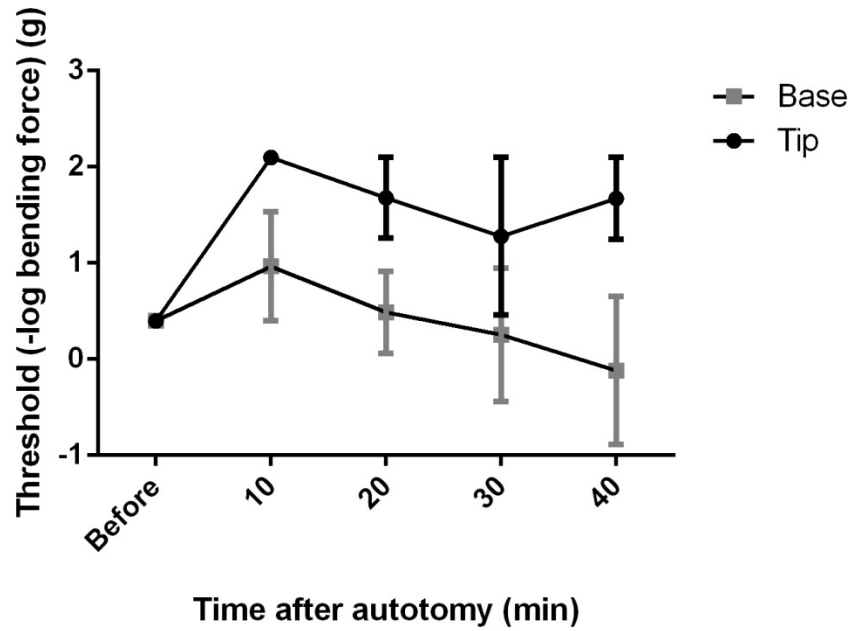
**Figure 4.2a-b.** No significant change in the mode of locomotion used before and after arm autotomy. (a) Proportion of total locomotion trials spent crawling is slightly more after autotomy (Wilcoxon test: p-value= 0.15). (b) Proportion of total locomotion trials spent jetting is slightly more before autotomy (Wilcoxon test: p-value = 0.15).



**Figure 4.3a-b.** Average locomotion speeds do not significantly differ before and after arm autotomy. (a) Average crawling speeds are not significantly different (Wilcoxon test: p-value = 0.46). (b) Average jetting speeds are not significantly different, though it seems higher before autotomy (Mann-Whitney test: p-value = 0.35).



**Figure 4.4a-d.** Maximum and minimum mouth speeds do not significantly differ before and after arm autotomy. (a) Maximum mouth speeds overall and (c) maximum mouth speeds per individual do not significantly differ, except in individual 3 (Mann-Whitney test:  $p$ -value = 0.34). (b) Average mouth speeds overall do not significantly differ but some of the (d) individual average mouth speeds exhibit significant differences (Mann-Whitney test:  $p$ -value = 0.57).



**Figure 4.5.** Tactile sensitivity thresholds for local response decrease over time. Sensitivity in the base is lower than that in the tip (2-factor ANOVA: p-value = 0.0094).

## Chapter 5: Conclusions

This investigation of autotomy in octopus widens our view of its use in soft-bodied animals and provides detailed knowledge about how a limb or body part detaches. Our currently narrow perspective that autotomy is a secondary defense mechanism only important to lizards, arthropods, and echinoderms has led to specific definitions of autotomy that only apply to these organisms. Autotomy has been defined as the shedding of a body part for 1) defensive purposes, 2) at a pre-determined plane, and 3) controlled by the central nervous system. A more comprehensive behavioral ecology approach was taken to investigate arm loss in the octopus, *Abdopus aculeatus*. This approach involves descriptive observations of 1) the mechanism of autotomy, 2) how the octopus develops the ability to lose arms, 3) the costs and benefits associated with limb loss, and 4) how autotomy evolves. Concentration on the mechanism and costs and benefits of autotomy were made in this study.

Autotomy in *A. aculeatus* can be characterized by a few key behaviors which were shown to follow a specific sequence during an autotomy event. Some of these behavioral elements were other types of secondary defense mechanisms such as inking and jetting. Field surveys of *A. aculeatus* in the Philippines and Indonesia also showed that octopuses frequently encounter arm loss in the wild. Most of these arm losses occurred in males as they were more frequently sampled than females. The number of arms found missing on individual octopuses ranged from one to five arms, one arm loss being the most common. Each arm position was not equally lost. Arms directly in front of the eyes are specialized for exploring in octopuses and were more susceptible to loss in *A. aculeatus*. The third right reproductive arm in males is often protected and thus less susceptible to loss.

Mechanistically, arm loss in *A. aculeatus* occurs at a zone of weakness between proximal suckers four and eight. No specialized structures were present at the zone of weakness, but a measured average force of 0.654 MPa was required to stimulate autotomy. Some degree of central voluntary control was necessary for autotomy to occur. The high frequency of observed arm loss was not significantly costly to octopus locomotion by either crawling or jetting. The immediate response of the arm to continuously move after detachment showed that the arm exhibited complex movements and suctioning power. These potential arm adaptations could provide the added benefit of distracting a predator while the octopus escapes after autotomy.

These findings provide a basis for understanding how autotomy works in octopuses and can be used for comparative studies with other soft-bodied organisms in the future. There is still a lot to learn about autotomy that is important to describe. Even as a secondary defense mechanism, high frequencies of arm loss indicate that autotomy can be used fairly commonly, even in organisms that do not seem to need this mode of defense. Other important aspects of behavioral ecology that were not addressed in this study, namely how the behavior developed and evolved over time, should be described in the future to fully grasp the importance of autotomy in octopuses. Another important corollary to studying autotomy is looking at regeneration. Although not all animals that can autotomize a body part have the ability to regenerate, those that do can provide insight on the short and long-term costs and benefits to fitness in having a limb that may or may not function like the original.