Title

Examining the effects of prey type and water temperature on the feeding behavior of a benthic generalist mesopredator, the California two-spot octopus (Octopus bimaculoides)

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EXAMINING THE EFFECTS OF PREY TYPE AND WATER TEMPERATURE ON THE FEEDING BEHAVIOR OF A BENTHIC GENERALIST MESOPREDATOR, THE CALIFORNIA TWO-SPOT OCTOPUS (*Octopus bimaculoides*)

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by

Andrew J. Lankow

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EXAMINING THE EFFECTS OF PREY TYPE AND WATER TEMPERATURE ON THE FEEDING BEHAVIOR OF A BENTHIC GENERALIST MESOPREDATOR, THE CALIFORNIA TWO-SPOT OCTOPUS (*Octopus bimaculoides*)

Andrew J. Lankow

Coleoid cephalopods (squids, octopuses, and cuttlefish) have been widely studied for a diversity of scientific inquiries. Despite their relevance as model organisms to fields such as cognitive psychology, neuroethology and physiology, there is still much to be learned about their own biology and how they interact with their environment. One group in particular, nearshore benthic octopuses, occupy an important ecological role as mid-level predators who are also a food resource to a myriad of other predators. Octopuses are generalist carnivores consuming a wide range of prey. They are also poikilotherms and are therefore physiologically sensitive to changes in their physical environment. However, little is known about how biotic and abiotic factors—such as prey type and water temperature—influence their complex suite of feeding behaviors. To investigate the effects of prey type and acute changes in water temperature on prey search, capture, and handling, I conducted controlled feeding trials with the California two-spot octopus (*Octopus bimaculoides)* and analyzed the videos to extract an integrative combination of behavioral measures and attack kinematics. In Chapter 1, I presented each octopus with either a live crab or live clam, to assess the behavioral variation associated with the characteristics of two distinct prey types. In clam trials, octopuses were less hesitant to approach or touch the prey, but conducted slower attacks, whereas while octopuses were more initially hesitant in crab trials their attacks on crab prey were more dynamic and deliberate. Prey type did not affect octopuses body orientation, arm usage, or eye choice. I also describe an interesting locomotion pattern for these octopuses where they move in a sinusoidal path along the bottom of the tank immediately prior to the attack of a crab, in an apparent corralling of the more mobile prey. For Chapter 2, I studied the effects of acute temperature change at three different treatment levels (baseline (14-17 °C), 20 °C, and 23 °C) on the feeding behavior of *O. bimaculoides* on live crab prey. I found that temperature had a significant negative effect on the probability that an octopus would attack their prey occurring in the feeding trial, and that warmer water produced longer latency to make an initial movement during the commencement of a trial, slower attack velocity and acceleration, and longer prey handling time. Finally, I discuss the ecological implications of my findings, and how these kinds of changes to octopus predator-prey dynamics could scale up to have system-level impacts.

Dedication

"Of course it is happening inside your head . . . but why on Earth should that mean that it is not real?" – Albus Dumbledore

"A person's work is nothing but this slow trek to rediscover, through detours of art, those two or three great and simple images in whose presence their heart first opened." – Albert Camus

There's simply no way to acknowledge all the beings (both human and non-human) who helped me get to this point and contributed to this thesis. To be in this setting, finishing up a graduate degree focused on octopus behavior, with an "office" right next to a giant blue whale skeleton, just steps from the ocean, surrounded by octopuses and moray eels… I have to pinch myself every day. Solomon, Huey, Zorba, Ollie, Frodo, Samwise, Merry, Pippin, Hank, Huey, Hercules, Wallace, and Plop have changed my life forever. Getting the opportunity care for them, observe their behavior, look into their eyes, and simply be in their presence was a journey that few people on Earth have ever been lucky enough to experience. To the countless gastropods, bivalves, and crustaceans who gave their lives in order to provide energy and sustenance to the octopuses, this work quite literally could not have been possible without your sacrifice.

To my family, thank you for giving me all the love and support anyone could ever ask for, and for always encouraging me to follow my heart. To my advisors, thank you for taking me in to your labs, putting up with me, and guiding me through the past two (and first real two) years of what I know will be a lifelong scientific journey. You are both exemplary role models, I am constantly blown away by you, I admire and respect you to the utmost degree, and I couldn’t be more grateful for having the opportunity to learn how to be a scientist from both of you. To my lab mates and fellow EEB grad students, thank you from the bottom of my heart for all of the wonderful conversations, help, support, and—most of all—for making me feel welcome in this community and for making me feel like I belong as a biologist; it is a feeling that I will never forget. Finally, to my lovely wife (!!!) and our two fur-children, words could never express my gratitude for your unconditional love and support throughout this whole process. Thank you for your patience and understanding and energy, for the countless meals and snacks and coffees, and for stoking and sharing that child-like sense of wonder about our world. Although I know that this has all been real, it certainly has felt like a dream every day.

I dedicate this thesis to everyone mentioned here, and countless others, for helping me truly understand the type of work, and the feeling it produces, that I will continue seeking for the rest of my life.

Overall Introduction

The very concept of animal behavior seems to defy definition (Levitis et al., 2009), but perhaps the most succinct way to describe it is “what an animal does” (Davis, 1966). The ways in which an organism interacts with others, and its physical environment, are shaped by natural selection, and molded by the habitat in which it lives. Sea otters (*Enhydra lutris*) are a good example of these forces at play. A streamlined body, enlarged hind paws for propulsion, and the ability to swim while submerged all result in increased speed, acceleration, and maneuverability with significantly reduced transport costs (Williams, 1989).

While energy conservation is an important component of certain behaviors, energy acquisition and utilization are critical physiological and behavioral requirements that unite all organisms, and animals have developed diverse strategies for finding and obtaining food (Curio, 2012; Krebs, 1973). The particular strategies employed by a species, population, or individual in terms of foraging and feeding are affected by both abiotic factors and biotic factors alike. The feeding behavior of the California moray eel (*Gymnothorax mordax*) for example, has been shown to be sensitive to acute increases in temperature (Moretto et al., 2022) and also affected by prey characteristics, which are both products of its habitat and its elongate, limbless morphology (Diluzio et al., 2017; Mehta et al., 2020).

Although certainly not limbless, coleoid cephalopods (the clade of ten- and eight-limbed cephalopods without shells, comprised of squids, cuttlefishes, and octopuses) have been increasingly important “model organisms” in a wide range of scientific inquiries, from psychology and neurobiology to cognition and biochemistry (Di Cosmo et al., 2021; O’Brien et al., 2018). Of the coleoid cephalopods, octopuses are widely seen as one of the most successful groups of marine organisms and, therefore, can have an outsized impact on local ecological processes (Doubleday et al., 2016). With more than 300 species worldwide, octopuses are voracious mesopredators and have garnered much attention in terms of their diet, physiology, ecology, and behavior (Di Cosmo et al., 2021; Jereb et al., 2014). Shallow-water and nearshore species are a key part of their coastal marine food webs and have adapted to living in a dynamic environment that is constantly changing and contains and abundance of prey types.

As generalist predators, they have the ability to prey on a wide variety of diverse food items in order to satisfy their nutritional needs (Mather et al., 2012) and, as poikilothermic ectotherms, they are particularly affected by their environment in terms of temperature (Brett, 1970). These particular characteristics raise some interesting questions regarding octopus feeding behavior. Will their prey search, capture, and handling methods vary by prey type? Will their feeding behavior be impacted by temperature differences caused by regional variation and by daily fluctuations that characterize the nearshore and intertidal environment (Bates et al., 2018; Helmuth et al., 2016), as are other invertebrate predators (Pincebourde et al., 2008; Sanford, 1999)? Understanding and describing these kinds of behavioral asymmetries is of great importance, due to how individual behavior can scale up to have population-, community-, and system-level consequences (Kroeker & Sanford, 2022).

The California two-spot octopus (*Octopus bimaculoides*) represents an ideal model to investigate these questions about the effects of prey characteristics and temperature on feeding behavior. *Octopus bimaculoides* consumes a wide variety of prey, is more active during the day, and has a geographic range in the Northeast Pacific along the coast from San Simeon, California to Guerrero Negro, Baja California (Jereb et al., 2014; D. L. Sinn, 2008). This species inhabits various environments, such as rocky reefs, mud flats, sand substrates, and kelp forests, from the intertidal zone to depths of 20 meters. While octopuses spend most of their time safe in their dens, in the course of foraging, these generalist predators likely encounter a variety of potential prey items and experience a range of temperatures. To examine the effects of each of these different contexts, I conducted controlled feeding trials with the California two-spot octopus feeding on two different prey types (the littleneck clam and the striped shore crab) and at three different temperatures (15 °C, 20 °C, and 23 °C). Although experimentation in a laboratory does not perfectly simulate the natural environment in which these predators live and hunt, controlled feeding trials allowed me to isolate the effects of my variables of interest (i.e., prey type and temperature) on various aspects of feeding behavior and control for potential confounds like octopus satiation, prey activity level, and prey size.

This thesis is separated into two chapters: one examining the variation in *O. bimaculoides'* feeding behavior when faced with different prey types, and the second evaluating the effect of water temperature on behaviors associated with prey search, capture, and handling times. By focusing on these two general areas, I broadly cover a range of biotic and abiotic factors that may be influencing important octopus predator-prey dynamics like how they may vary their strategy based on prey availability, which behaviors or kinematic variables are conserved across contexts, and how sensitive they are to foraging in different areas of the water column.

The findings of chapter one showed that *O. bimaculoides* waits significantly longer to approach and make contact with a live crab than a live clam. After initial contact, attack decision and prey handling time are shorter for a crab as prey versus a clam. Attacks on crabs were much more dynamic, exhibiting a greater maximum speed and acceleration for both the approximate center of mass and the attacking arm. The proportion of observed attack types was significantly different between the two prey types, with the mobile crabs eliciting a webover attack 25% of the time, whereas the sessile clams elicited a typical grabbing arm attack for 100% of successful feeding trials. In terms of body orientation, arm choice, and eye usage, *O. bimaculoides* showed no significant differences between trials of different prey types. However, within-group comparisons revealed a bias, for clam trials only, towards a lateral body orientation, anterior arms, right-side arms (R2, specifically), and the right eye being used for the attack. All biases (with the exception of a bias towards an anterior body orientation) were not maintained when faced with the unpredictable mobility of a live crab. Finally, an intriguing locomotor behavior was observed, during which *O. bimaculoides* exhibits a distinct sinusoidal movement pattern immediately preceding the attack. This pattern occurred significantly more often during crab trials and appears to guide a visual attack and help to corral a more mobile prey type.

In chapter two, I employed a combination of multi-level models to determine the effects of temperature on five behavioral response variables and four kinematic measurements of the attack, while controlling for the effects of three covariates: crab activity level, relative prey mass, and trial number. To account for the variation introduced by the individual octopuses, I included random intercepts in the model for octopus, cohort, or octopus nested within cohort (depending on the response variable being modeled), and a random slope for treatment (when my model selection process confirmed it would improve the model's fit). The findings show that temperature had a significant effect on four of the response variables: latency, body velocity, body acceleration, and handling time. In addition, for total time to attack and attack decision time (the time between first touch and attack), the effects of temperature were moderated by one or more covariates. Finally, temperature had no effect on time to first touch or arm velocity, as those two response variables were only impacted by trial number, and none of the predictors in the model had a significant effect on arm acceleration. Together, these findings reveal that temperature influences the feeding behavior of *O. bimaculoides.* As a result, *Octopus bimaculoides* may alter its behavior accordingly or seek a more thermally preferable area to feed more efficiently. My study suggests that acute temperature change not only influences the behavior of an individual or a population of octopuses, but may have widespread impacts on their broader community structure and predator-prey relationships.

Chapter 1: Prey-dependent feeding behavior in a kelp-forest mesopredator, the California two-spot octopus (*Octopus bimaculoides)*

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Abstract

Predators have evolved a diverse behavioral repertoire to search for, capture, and handle prey. Within coleoid cephalopods, octopuses have developed considerable behavioral complexity. As generalist mesopredators contributing to coastal marine food webs, many species of octopuses are predominantly generalists, feeding on a wide variety of invertebrate and vertebrate prey. Despite their broad diets, few studies have examined the behavioral repertoire of octopus with respect to prey type. I compare how prey type may affect octopus feeding behavior by conducting controlled feeding trials during which I recorded California two-spot octopuses (*Octopus bimaculoides*) predating on a sessile prey (*Protothaca staminea)* and an active prey with the potential for retaliatory behaviors (*Pachygraspus crassipes*). I found that prey type had no effect on octopus body orientation, arm choice, nor eye use, but did have a significant influence on the timing events within a feeding sequence, the type of attack used and attack kinematics. During clam trials, individuals exhibited a shorter latency and time to first touch, longer attack decision time and handling time, and slower attack speed and acceleration; during crab trials, they exhibited a longer latency and time to first touch, shorter attack decision time and handling time, and faster attack speed and acceleration. I also describe the relationship between octopus body orientation, arm choice, and eye use, and discuss any bias or lateralization during feeding. Finally, I report a previously undescribed behavior in the California two-spot octopus: a sinusoidal locomotory pattern that precedes the attack of a prey.

Introduction

Finding and acquiring food are critical survival behaviors, and animals have developed an arsenal of strategies to meet their energetic needs (Curio, 2012; Krebs, 1973). The strategies employed by a species, population, or individual are shaped by factors such as the traits of their prey and the resources available (Kalinoski & DeLong, 2016; Klecka & Boukal, 2013). Within a species, predators may exhibit variable strategies to obtain a single prey, as exemplified by different populations of West African chimpanzees (*Pan troglodytes)* preying on army ants. They either use their hands to directly extract ants from nests or use a freshly manufactured stick tool to dip in nests and ant trails, collect a mass of ants on the tool, and then either pull the length of the tool through their fingers, sweep the tool sideways through their mouth, or insert the tip of the tool in their mouth to consume the ants (Möbius et al., 2008). Alternatively, killer whales (*Orcinus orca*) implement the same sequence of predatory techniques (i.e., approaching from behind, diving, and surfacing from underneath) to hunt prey as diverse as penguins, harbor seals, sea lions, and walruses (Martinez & Klinghammer, 1970).

Depending on local predator-prey dynamics such as prey abundance and the presence of competition, predators make decisions that may result in tradeoffs in order to maximize the opportunity cost and value of energy spent foraging, capturing, and handling prey (Anholt & Werner, 1998; Lima & Dill, 2011; Sih, 1982; Werner & Anholt, 1993). Tradeoffs may also result in predators employing variable tactics for different prey types; for example, southern sea otters vary their dive behavior depending on whether they are preying on large invertebrates, small and intermediate-sized bivalve mollusks, or kelp-dwelling marine snails, as many of these preferred otter prey also vary in typical capture depth and relative abundance (Tinker et al., 2007).

Coleoid cephalopods (i.e., squid, cuttlefish, and octopuses) exhibit considerable behavioral flexibility (Villanueva et al., 2017), especially during the prey acquisition stage of feeding. For example, squid (*Illex illecebrosus*) adopt rapid, head-first attacks for capturing small prey, and slow, stalking attacks from behind on large prey (Foyle & O'dor, 1988). While once thought to be relatively fixed, the behavioral repertoire of cuttlefish during prey capture is quite variable, as observed through the various hunting strategies of ambushing, luring, and pouncing (Hanlon & Messenger, 2018; Villanueva et al., 2017; Zoratto et al., 2018).

Extensive work has examined the individual aspects of octopuses' sensory, cognitive, and physical abilities, including those used during prey search and capture (Fiorito et al., 1990; Kier, 2016; Kier & Smith, 1985; Levy et al., 2015; Maselli et al., 2020; Sumbre et al., 2005; Walderon et al., 2011). With their laterally-placed eyes, octopuses exhibit monocular vision to orient themselves with objects and during attack (Muntz, 1963), and *Octopus vulgaris* are reported to have a lateral bias for the left or right eye in certain contexts (Byrne et al., 2002, 2004; Frasnelli et al., 2019). Once octopus locate their prey, they use their highly maneuverable body and eight exceptionally flexible arms, each lined with hundreds of powerful, neuron-rich suckers, to capture and handle the prey (Bagheri et al., 2020; Grasso, 2008; Graziadei, 1962; Kennedy et al., 2020; Nödl et al., 2015; A. M. Smith, 1991). These radially-symmetrically arranged arms are used for locomotion (Huffard, 2006) and prey attack (Maldonado, 1964), and may be recruited unevenly for different functions (Byrne et al., 2006a; Mather, 1998; Voss & Mehta, 2021).

Once prey is captured, octopuses can employ a diverse repertoire of prey handling techniques based on prey species morphology (Fiorito & Gherardi, 1999; Perron & Verde, 2015). For example, octopuses change their prey extraction technique (i.e., pulling open versus penetration by drilling) depending on bivalve species (R. C. Anderson & Mather, 2007; Steer & Semmens, 2003), and vary the location of their bore hole depending on whether they are feeding on a crab versus a snail (Runham et al., 1997). Clams and crabs, both encased in hard shells, present similar immediate challenges to a prospective predator, to which cephalopods have responded with flexible feeding habits (Grisley et al., 1996; Villanueva et al., 2017). Despite our broad understanding of octopus feeding behaviors, relatively few studies have incorporated a more wholistic view of predation strategies and techniques, especially as a comparison between different prey types.

The California two-spot octopus (*Octopus bimaculoides*) is a generalist shallow-water benthic mesopredator that inhabits rocky reefs, kelp forests, and mud flats from the intertidal zone to shallow subtidal waters on the Southern California coast, from approximately Santa Barbara, CA south to San Quintin, Mexico on the upper Baja peninsula (Forsythe & Hanlon, 1988). I chose *O. bimaculoides* as my behavioral model for two reasons: 1) it is active both day and night but appears to be more active during the day helping to facilitate an experimentation schedule that aligns with natural behavior; and 2) it is a generalist mesopredator, feeding on a wide range of prey including crustaceans, gastropods, and bivalves (Jereb et al., 2014). This broad prey depth may require *O. bimaculoides* to employ a diverse behavioral repertoire in order to effectively take advantage of local prey abundance (Maselli, 2020).

Therefore, I assessed whether *O. bimaculoides* employed different behaviors during prey search, acquisition, and handling when faced with a sessile, harmless prey and a highly active, potentially harmful prey. I conducted separate controlled feeding trials with two different live prey types: littleneck clams (*Protothaca staminea*) and striped shore crabs **(***Pachygrapsus crassipes)*. Specifically, I sought to answer the following questions: (a) How does prey type affect the timing of the feeding sequence (assessment, exploration, approach, attack, and handling) for California two-spot octopuses, (b) how does prey type affect attack kinematics (speed and acceleration of the body and the attacking arm), and (c) how does prey type affect body orientation, arm choice, and eye use during exploration and attack? Given the relatively broad behavioral repertoire of octopuses (Borrelli et al., 2020; Mather & Alupay, 2016), and the wide range of prey items found in their diet (R. F. Ambrose, 1982, 1984; Greenwell et al., 2019; Grubert et al., 1999; Hartwick et al., 1978; Mather, 1991; Scheel & Anderson, 2012; C. D. Smith, 2003; Vincent et al., 1998), I predict that *O. bimaculoides* will exhibit variable and context-dependent feeding behaviors.

Methods

Eight wild-caught *Octopus bimaculoides* were maintained at the Long Marine Laboratory (LML) in Santa Cruz, CA (Table 1.1). Octopuses were housed individually in transparent plastic enclosures (25 x 18 x 17 cm) with vents, which allowed for minimal disturbance and easy observation. Enclosures were kept inside larger tanks with flow-through seawater pumped in from Monterey Bay. Ambient water temperature ranged from 14 to 17°C and tanks were exposed to natural light conditions according to the seasonal light/dark cycle. Upon arrival to the lab, octopuses were inspected for health and welfare according to Fiorito *et al.* (2015) and were monitored daily thereafter using the same guidelines. They were allowed 14 days to acclimate to the laboratory environment, and were fed either a crab, clam, mussel, or fish daily (each octopus was fed the same prey on any particular day). Each individual was cared for according to the enrichment considerations consistent with the 3Rs principles (Replacement, Refinement, and Reduction) formulated by Russel and Burch (1959) and accepted ethical and welfare guidelines (Beigel & Boal, 2006; Browning, 2019; Cooke & Tonkins, 2015; De Sio et al., 2020; Drinkwater et al., 2019; Fiorito et al., 2014, 2015; Moltschaniwskyj et al., 2007; Yasumuro & Ikeda, 2018).

Although the protocols of this non-invasive study conformed with the statutes of the Animal Welfare Act and guidelines of the Public Health Service, the study did not require approval from the Institutional Animal Care and Use Committee (IACUC) of University of California, Santa Cruz. As IACUC applies only to vertebrate animals, and at this time there are no federal, state, or local regulations governing the study of cephalopods, the IACUC committee was consulted but institutional approval was not required. In light of Webster & Rutz's (2020) framework to help researchers navigate potential sources of sample bias, I have identified several aspects of my octopuses' STRANGEness—their Social Background, Trappability and self-selection, Rearing history, Acclimation and habituation, Natural changes in responsiveness, Genetic makeup and Experience—that should be acknowledged. First, although all octopuses were housed in standardized environments and exposed to the same levels of enrichment, they were collected from the wild and, therefore, prior experiences and opportunity with food for each individual are unknown and are suspected to vary. Despite differences in experience, all animals contributed data to the study, and no specific behavior traits or personality types were excluded. Second, each study animal was collected using the same method, and all animals came from the same location (i.e., via hand-take in a 500-meter collection area), which could select for individuals with certain behavioral characteristics and potentially similar genotype; therefore, generalizations beyond this population should be made with caution (see Discussion).

Experimental Design

Controlled feeding trials were conducted between April and October 2021. The experimental arena was a 75.7-liter aquarium (77.5 x 33.0 x 33.3 cm) with ambient seawater (average temperature 15.7°C) filled up to 15 cm. A clear, removeable plexiglass barrier was installed 15 cm from one end of the tank to maintain separation between the study subject and the prey at the start of the trial. Two Osmo Action cameras (DJI, China) were positioned overhead and to the side of the tank to acquire both dorsal and lateral views. During acclimation, individuals were initially fed a mixed diet of crabs, clams, mussels, and fish, both wild-caught and procured from local farmers' markets and supermarkets, to determine which prey elicited a motivated feeding response. I recognized a motivated feeding response as one where the subject successfully captured the prey and began to feed in under 30 minutes. Preliminary trials revealed that crabs (success rate of 75%) and clams (success rate of 67%), were readily consumed while octopuses were less likely to consume mussels (success rate 25%) and fish (success rate 0%). Crabs and clams not only exhibit striking differences in their movement, but the energy gained from consuming clams necessitate extraction typically in the forms of drilling or prying. Drilling to open bivalve prey was reported to take up to 137 minutes for *O. vulgaris* and up to 90 minutes for *O. dierythraeus*, while pulling open the bivalve can take as little as one minute (Fiorito & Gherardi, 1999). As my research was focused on how octopuses approach prey and how they access the flesh, clams were cut open by severing both adductor muscles, but not pried open any further, in order to eliminate variability associated with the decision of pulling open the bivalve versus needing to drill into the shell (R. C. Anderson & Mather, 2007; Perron & Verde, 2015; Steer & Semmens, 2003).

Experimental Procedure

Octopus were fed a mixed diet of clams, crabs, *Mytilus californianus*, and *Tegula atra* on non-trial days, and were fasted for 48 hours before a trial. To ensure that octopuses were adequately stimulated during feeding, the last meal before a trial was never the same prey as offered in the upcoming trial. For each feeding trial, octopuses were transferred from their individual enclosures to the experimental tank and habituated behind the barrier for three minutes (Figure 1.1). For clam trials, after three minutes, a clam (mean percentage of subject mass ± standard deviation = 42 ± 11%) was placed 30 cm from the opposite side of the barrier. After another three minutes, the barrier was lifted, and the trial continued until ten minutes post-attack. Preliminary trials showed that this relatively short duration of time was sufficient for observing prey handling behavior and feeding. At ten minutes post-attack, the subject was removed from the experimental tank and transferred back to its individual enclosure. For crab trials, after the initial three minutes with the prey in the tank, the crab (mean percentage of subject mass ± standard deviation = 4.9 ± 1.7%) was corralled by the experimenter to the middle of the wall opposite the barrier, then the barrier was lifted. The trial continued until *O. bimaculoides* had conducted a successful attack (i.e., captured the prey during a hunting sequence), subdued the prey, and started to feed.

For all trials regardless of prey type, if the subject had not successfully attacked the prey and begun to feed in 30 minutes, the trial was terminated and both the prey and subject were removed from the experimental tank. In the case of a terminated trial, the subject was transferred back to its individual enclosure and fed a small portion (~10%) of its prey item. To prevent detection of chemical cues from previous prey and conspecifics, I changed the water in the experimental tank after each trial.

Behaviors Extracted from Video Analyses

I analyzed video recordings of each trial to extract the following data (recorded in seconds unless otherwise noted): i) latency: time elapsed between the lifting of the barrier and the subject crossing the starting line in the direction of the prey, ii) time to first touch (TFT): time elapsed between the subject crossing the starting line and the first contact made with the prey, iii) attack decision time (ADT): time elapsed between the first touch of the prey and when subject took the prey under its buccal web and iv) prey handling time (HT): time elapsed between *O. bimaculoides* placing the prey under its buccal web and when the feeding commenced (Fiorito & Gherardi, 1999). Therefore, prey handling time included predatory behaviors such as getting the prey under the mouth region via manipulation by the arms and suckers, orienting the prey, and (for clam trials only) pulling at the prey by the proximate part of the arms, opening the shell, and inserting the arms and mouth inside the shell (Fiorito & Gherardi, 1999).

I also measured the kinematics of the attack, which was a dynamic sequence of events involving the body and arms: i) peak body velocity (ms^{-1}): maximum velocity attained by subject's approximate center of mass, from movement towards prey until prey is placed under the buccal web, ii) peak arm velocity (ms^{-1}): maximum velocity attained by an arm during the initiation of attack or capture of the prey, iii) peak body acceleration (ms^{-2}): maximum acceleration attained by subject's approximate center of mass, iv) peak arm acceleration (ms^{-2}): maximum acceleration attained by an arm. I used Tracker Video Analysis and Modeling Tool, version 6.0.1, copyright © 2021 to calculate behavioral durations, velocities, and accelerations.

Lastly, I recorded the type of attack (Figure 1.2; Supplemental Videos S1-S4), how the subject oriented its body with respect to the prey, which arm was used, and which eye appeared to be dominant at the initiation of the attack. I determined if a subject's eye use was influencing its arm choice during attack by developing a framework in which each of the three possible body orientations corresponded with the two arms on that side of the body that would likely form a line between the target and the subject's eye in that particular orientation (Figure 1.7a). I then recorded whether each trial fell within this framework (i.e., an anterior body orientation for attack was paired with arms 1 or 2, lateral with 2 and 3, and posterior with 3 and 4).

During analysis, I observed a novel movement pattern that I designated sinusoidal locomotion (Supplemental Videos S5 and S6). I quantified whether sinusoidal locomotion immediately preceded the attack. I also calculated total duration (seconds), amplitude (meters), and period (seconds) for the sinusoidal path traced in each locomotor bout (Figure 1.6).

Statistical Analysis

Statistical analyses were performed using RStudio software, version 1.3.1093, © 2009-2020 RStudio, PBC. For all statistical tests, significance was defined as $p < 0.05$ and all continuous responses (timing and kinematic variables) were log-transformed before any statistical analyses. The difference between the mean for each prey type was assessed with a t-test using Satterthwaite's method via linear mixed model (fit by restricted maximum likelihood), with individual octopus included as a random effect. The same method was used to determine the prey-specific differences regarding the three dimensions of the sinusoidal locomotion path in trials where such behavior was exhibited. The differences between prey types for categorical variables such as body orientation, arm choice, eye use, and attack behavior were assessed with chi-squared tests via log-linear mixed models with individual octopus included as a random effect. To measure the within-group differences (e.g., whether a certain body orientation or specific arm was used more often than expected by chance), I conducted binomial tests with expected probabilities of 0.33 for body orientation (classified as one of three possibilities), 0.125 for arm used (given the choice to use one of eight arms), 0.5 for arm and eye laterality (with the choice between left or right), 0.5 for the anteroposterior axis, and 0.5 for attack behavior (having observed only two variations of attack).

Finally, to determine what underlying factors could contribute to explaining each of the four timing variables and four attack kinematic variables, I used linear mixed models fit by restricted maximum likelihood with t-tests via Satterthwaite's method. The following variables were included as predictors: octopus mass, initial prey mass, trial number, log of latency, log of TFT, log of ADT, and log of handling time. Predictors were excluded from the initial model if they occurred chronologically after the particular dependent variable being modeled in the sequence of a feeding trial (e.g., ADT was not included in the model for TFT, since the attack occurs after the first touch and, therefore, was assumed to play no role in its explanation).

Results

I analyzed 40 trials for clams and 40 trials for crabs, with all 8 octopuses having 5 trials for each prey type. Octopuses exhibited variability in prey search, acquisition, and handling behaviors when feeding on clams versus crab prey (Tables 1.2 and 1.3).

Timing Response Variables

Latency, TFT, ADT, and HT differed significantly between clam and crab trials (Figure 1.3). Latency and TFT were lower for clams compared to crabs while ADT was much higher for trials with clams than crabs (Table 1.2). Octopuses implemented different attack behaviors for clam and crab ($p = 0.0012$; Table 1.3): arm attacks were implemented in 100% of clam trials while, in crab trials, an arm attack strategy was still predominantly used ($p = 0.003$) but a webover attack was also observed. Finally, prey handling time was significantly longer for clams (84.6 ± 6.5 seconds) compared to crabs (47.1 ± 5.8 seconds; $p < 0.0001$).

The linear mixed model analyses identified few significant predictors in the explanatory models for each timing variable (Table 1.4). In clam trials, I found that octopus mass, prey mass, and trial number had a significant effect on latency. In crab trials, I found that prey mass had a significant effect on TFT, and TFT had a significant effect on HT. However, I found no other significant relationships in the models for the other timing variables during trials of either prey type. Of note, octopus mass and trial number only had a significant effect on decreased latency patterns during clam trials, but the lack of significance in any other model indicated that growth and learning had minimal effect on the overall feeding patterns observed.

Body Orientation, Arm Choice, and Eye Use

Body orientation, arm choice, and eye use did not differ between prey types (Table 1.3). I found no relationship between prey type and the octopuses' body orientation relative to the prey (Figure 1.4a) for first contact ($p = 0.27$), nor for attack ($p = 0.29$). They used a lateral body orientation more often than by chance during clam trials for first touch (23/40, $p = 0.0014$) and attack (19/40, $p = 0.044$), while an anterior orientation was chosen significantly more often than by chance during the attack for crab trials (26/40, $p < 0.0001$). For both clam and crab trials, octopus chose a posterior orientation for first touch and attack significantly lower than by chance (first touch, clam: 4/40, $p = 0.00065$; first touch, crab: 7/40, $p = 0.021$; attack, clam: 3/40, $p = 0.00013$; attack, crab: 3/40, $p = 0.00013$). When examining across all trials, body orientation for first touch was the same as that which was used for the attack ($p < 0.001$), regardless of prey type ($p = 0.63$).

The proportions of specific arms used did not differ significantly between prey types for first touch ($p = 0.80$) nor for attack ($p = 0.92$; Figure 1.4b). Binomial tests revealed that L4 and L3 were used the least during all trials with both prey types (Table 1.5). R2 was used significantly more often than by chance for first touch in clam (11/40, $p = 0.0083$) and crab (8/33, $p = 0.047$) trials, as well as for attack in clam (10/40, $p = 0.023$) and crab trials (11/40, $p = 0.0083$), while L2 and R2 were used in similar proportions for attacking clams (10/40, $p = 0.023$). The arm used for first touch was the same arm used for attack during a significant percentage of feeding trials ($p < 0.001$), irrespective of prey type ($p = 1.00$).

I found no relationship between prey type and whether the octopuses used a left or right arm (Figure 1.4c) for neither first touch ($p = 0.26$) nor the attack ($p = 0.14$). There was, however, a right-side bias for clam trials, for both first touch (27/40, $p = 0.019$) and attack (27/40, $p = 0.019$). Also, when looking across all trials, I found 100% of the time the same side was used for both the first touch and attack ($p<0.001$), with prey type having no effect ($p = 1.00$).

There was no significant relationship between prey type and whether the subject used an anterior or posterior arm for first touch ($p = 0.43$) nor for attack ($p = 0.89$; Figure 1.4c). When looking within each prey type, an anterior arm was used for the majority of trials for first touch and attack, but only at a proportion significantly greater than predicted by chance for the attack in clam trials (26/40, $p = 0.040$). With no effect from prey type ($p = 0.61$), anteroposterior arm choice for first touch matched that which was used for the attack during a significant proportion of trials ($p < 0.001$).

Finally, prey type had no effect on whether the octopuses appeared to favor the left or right eye (Figure 1.4c) for neither the first touch ($p = 0.49$) nor the attack ($p = 0.07$). Within prey types, octopuses tended to orient their right eye towards the prey for both first touch and attack during clam trials ($p = 0.0083$), but during crab trials neither the left nor right eye was favored. Across all trials, octopuses used the same eye for both first touch and attack during a significant proportion of trials ($p < 0.001$), which held true regardless of prey type ($p = 0.34$). According to my framework described above, eye use appeared to influence arm choice in a significant proportion of trials within each prey type (binomial test; clam trials, 38/40, p < 0.001; crab trials, 27/30, p < 0.001), and there was no significant difference between prey types (Table 1.3; $p = 0.13$).

Attack Kinematics

Octopuses exhibited more extreme kinematics in crab trials relative to clam trials (Figure 1.5). Mean peak body velocity during attack was 0.351 ± 0.023 ms^{-1} for crab trials, which was significantly greater ($p < 0.0001$) than during clam trials (0.128 ± 0.011 ms^{-1}). Similarly, peak velocity of the attacking arm was significantly greater ($p < 0.0001$) for crab trials (0.539 ± 0.028 ms^{-1}) than for clam trials (0.155 ± 0.0087 ms^{-1}). During trials with both prey types, the attacking arm reached a greater peak velocity than the body during both crab trials (crab: $p < 0.0001$, clam: $p = 0.0053$).

Octopuses exhibited greater peak accelerations during the attack in crab trials than in clam trials ($p < 0.001$; Table 1.2). Mean peak body acceleration was 2.92 ± 0.29 ms^{-2} for crab trials and 0.826 ± 0.085 ms^{-2} for clam trials. Mean peak arm acceleration during the attack for crab trials (5.33 ± 0.39 ms^{-2}) was significantly greater ($p < 0.0001$) than for clam trials (1.16 ± 0.079 ms^{-2}). Finally, peak acceleration for the attacking arm was significantly greater than the body acceleration for both crab trials ($p < 0.0001$) and clam trials ($p = 0.0020$).

The modeling analyses produced mixed results for the four kinematic attack variables in trials of each prey type (Table 1.5). During clam trials, the following five predictors contributed to a model of body velocity: octopus mass, prey mass, trial number, TFT, and ADT; however, I found no significant explanatory variables for body velocity during crab trials. For body acceleration, I found one significant predictor each for clam trials (ADT) and crab trials (prey mass). Arm velocity had no significant predictors for either prey type. Arm acceleration had no significant predictors in clam trials, while latency had a significant explanatory effect in crab trials.

Sinusoidal Locomotion

I observed sinusoidal locomotion in 14 feeding trials (Figure 1.6). A chi-squared test via log-linear mixed model with octopus included as a random effect showed that the rate of occurrence of sinusoidal locomotion was no different in clam trials than crab trials ($p = 0.087$; Table 1.3). T-tests using Satterthwaite’s method via linear mixed model revealed that average duration (*p* = 0.19), average amplitude (*p* = 0.31) and average period (*p* = 0.061) of sinusoidal locomotion did not differ between prey types. I found that sinusoidal locomotion preceded the attack in crab trials at a rate significantly higher than predicted by chance (8/10, binomial test, *p* = 0.055), but not for clam trials (0/4, binomial test, *p* = 0.063), representing a significant difference in behaviors leading to the attack between prey types (*p* = 0.033).

Discussion

Here I reveal striking differences in the behavioral repertoire of *O. bimaculoides* when feeding on clams versus crabs. Previous studies have found that the feeding behavior pattern of *O. vulgaris* does not vary bivalve prey species (Fiorito & Gherardi, 1999), and that crabs are preferred as prey over bivalves in *O. bimaculatus* (R. F. Ambrose, 1984) and *O. rubescens* (Onthank & Cowles, 2011). However, my study shows how the California two-spot octopus varies its whole suite of feeding behaviors when predating on a sessile, harmless prey versus a mobile, potentially harmful prey.

Most evident were differences in the timing of how *O. bimaculoides* assessed, approached, attacked, and handled clams versus crabs. Latency and TFT were significantly lower for clam trials, while ADT and HT were significantly higher for clam trials. Given that octopuses can detect chemical cues via contact or distant chemoreception (Maselli et al., 2020), and are also visually oriented to newly introduced objects (Kuba et al., 2003; Kuba et al., 2006; Mather & Anderson, 1993), they were presumably aware of the prey item’s presence during the habituation period. Thus, for the clam trials, they may have observed that the sessile clam could be approached with less risk, resulting in lower latency. In crab trials, prey movement likely attracted the predator’s attention (Boycott & Young, 1950; Wodinsky, 1971) but may have also induced caution, resulting in octopuses exhibiting both increased latency and TFT. The linear mixed model supported this idea, with a significant negative correlation between crab mass and TFT (Table 1.4) indicating that larger crabs induced longer periods of assessment for the octopuses before making first contact. Since vision is not dominant in octopus foraging or decision making (J. Forsythe & Hanlon, 1997; Maselli et al., 2020), the shorter TFT in clam trials facilitated obtaining tactile and chemical information via their suckers (Chase & Wells, 1986; Graziadei, 1962) which presumably guided a future attack. Therefore, a longer ADT for clam trials may be attributed to the octopuses often touching the prey early in the trial, likely recognizing it as an immobile bivalve that presents no risk of fighting or fleeing, and subsequently exploring the rest of the tank until deciding to finally attack. Crab trials motivated direct attacks, which indicates that the longer latency period for crabs was spent assessing the highly mobile and potentially harmful prey item.

HT was significantly longer for clam trials than crab trials, which contradicts previous findings (Onthank, 2008; Onthank & Cowles, 2011). This discrepancy may be attributed to how I define HT in this study: the time elapsed between *O. bimaculoides* taking the prey under its buccal web and when the actual feeding commenced (Fiorito & Gherardi, 1999). Previous studies have included feeding on the prey until the last prey remains were relinquished by the predator (Onthank, 2008; Onthank & Cowles, 2011). In the present study, despite severing the clam adductor muscle, octopuses exhibited longer handling times for clams, which still required opening the shell, inserting their arms and mouth, and pulling at the flesh before actually beginning to feed. For crab trials, HT encompassed orienting the prey under the subject's mouth which took place within seconds, at which point all movement by the crab usually ceased. The quick dispatch of the crab may be attributed to the octopuses use of cephalotoxin that can be effective within minutes (Boyle, 1990; Grisley et al., 1999). Under these constraints, HT in the present study was much shorter than if measured until the subject had finished feeding and dropped all remains, which could last more than two hours (Onthank & Cowles, 2011). Therefore, *O. bimaculoides* feeding on clams exhibits a shorter latency before moving towards the prey, a shorter TFT, a longer ADT, and a longer HT to begin feeding; for a highly mobile and active prey item, *O. bimaculoides* exhibits a longer latency before moving towards the prey, a longer TFT while presumably assessing the prey's attributes, a more decisive attack, and a quicker HT to begin feeding.

Although speed and acceleration have been measured in squid attacking small versus large fish (Foyle & O'dor, 1988), planktonic octopus paralarvae attacking live prey (Villanueva et al., 1997; Villanueva & Norman, 2008) and the movement of octopus arms, there is still a considerable lack of data on the attack kinematics of adult coleoid cephalopods during feeding. Here, though, I show that *O. bimaculoides* achieves higher magnitudes of body velocity, body acceleration, arm velocity, and arm acceleration when capturing a crab versus a clam. My findings are consistent with observations on *O. vulgaris* preying on live crabs (Maldonado, 1964). While my findings for variation between speed of attack for different prey types may not be surprising, they help to illuminate the context-dependent behavioral flexibility and diverse physiological tools that enable octopuses to be active mesopredators. My study also suggests that peak velocities were not correlated with body size (Table 1.4). Therefore, a smaller octopus may also not experience decreased hunting success in environments where highly mobile prey are the most abundant resource.

While evidence of asymmetric use and division of labor between octopus arms has previously been reported in a variety of species in different ecological and behavioral contexts (Grasso, 2008; Huffard, 2006; Kennedy et al., 2020; Levy et al., 2015; Mather, 1998; Mather & Alupay, 2016; Mazzolai et al., 2013; Nesher et al., 2014; Voight, 1992; Voss & Mehta, 2021), asymmetric use was not observed in my study. I found no differences in body orientation, arm choice, nor eye usage between prey types for neither first touch nor attack (Table 1.3). Further, the proportions of possible body orientations, specific arms used, arm laterality, and anteroposterior classifications for first touch significantly matched those that were used for attack, suggesting that octopuses do not reorient their bodies or arms between tactile, speculative foraging and actual prey capture.

Examining body orientation, arm and eye use revealed a more wholistic understanding of predatory behavior in the two-spot octopus. During clam trials, octopuses engaged the prey for first touch and attack with a lateral body orientation significantly more often than by chance. Given their lateral eye placement this observation supports the assertion that octopus almost always use monocular vision to look at objects and hunt (Muntz, 1963). During crab trials, octopuses attacked the prey with an anterior orientation at a rate significantly higher than by chance, which I attributed to prey activity level. Thus, with an immobile prey item, a lateral position may be adopted in order to align with a specific side preference or eye preference (Byrne et al., 2004; Frasnelli et al., 2019; Schnell et al., 2016). However, with a prey item that is actively trying to flee or defend itself, an anterior body orientation may act as a postural hedging of bets in order to recruit the most arms for the job (Byrne et al., 2006a; Kennedy et al., 2020) and increase chance of capture, given the unpredictable movement of the crab.

Even with an anterior orientation, though, octopuses always approached the prey for first touch and attack with one eye more oriented towards the prey than the other, which is consistent with previous work on *O. vulgaris* (Byrne et al., 2002, 2004; Levy et al., 2015). Although there was no relationship between prey type and eye lateralization (Figure 1.4), my finding that the right eye was used significantly more often than chance in clam trials (but not crab trials) aligns with my finding of a significant right arm bias for first touch and attack in clam trials. Together, these results suggest that the preferred side of the body may be more easily employed in a prey-capture context that is less urgent, as opposed to a context when a highly mobile prey may move unexpectedly and cause the subject to use whichever side gives it the best chance of success, resulting in a more symmetrical anteroposterior, arm laterality, and eye laterality distribution (as I report in the present study).

My finding that R2 was used significantly more often than chance aligns with the right-side and anterior arm bias and is consistent with previous findings that anterior arms appear to be preferred for exploring objects and collecting food in *O. vulgaris* (Byrne et al., 2006a) and *O. bimaculoides* (Kennedy et al., 2020). Further, Byrne *et al.* (2006b) demonstrated that arm choice is strongly influenced by eye use in *O. vulgaris*, which I also assessed in the present study by combining various measurements to adopt a more wholistic view of body use. My findings of lateral and anterior body orientation biases (for clam and crab trials, respectively), anterior arm bias, and right arm and eye biases successfully predicted a significantly high usage of R2 for attacks. Furthermore, I observed that L3 and L4 were used the least often, which would also be expected under this framework with a posterior body orientation being used at a rate significantly less often than chance for first touch and attack during both clam and crab trials. This finding aligns with previous work by Mather (1998) and Byrne *et al.* (2006a) showing that the two posterior arm pairs may be reserved for locomotion in a variety of octopus species. My results support the assertion that *O. vulgaris* uses visual information to determine the location of its arm (Gutnick et *al*., 2011) and extends the findings of Byrne *et al.* (2006b) to show that the arm chosen by *O*. *bimaculoides* to use in prey capture is strongly influenced by eye use. This idea is further supported by the lack of bias and more even distribution of anteroposterior arm choice, arm laterality, and eye laterality in crab trials- where the prey was evasive and individual octopuses had to change their attack strategy at the last minute, with the target's new location.

O. bimaculoides body orientation and arm use suggest the possible presence of lateralization at the population level. It has been suggested that population-level lateralization may make each individual more predictable to predators and prey (Ghirlanda and Vallortigara, 2004). For example, if *O. bimaculoides* predominately uses its right side for attacking prey, its left eye is available to scan for predators. Schnell and colleagues (2016) found similar lateralization in cuttlefish, demonstrating an eye-use bias in opposite directions for predatory and antipredator behaviors. My results suggest that the left side is more vulnerable to predators, which is consistent with recent observations that the males of intertidal species such as *O. rubescens*, *O. bimaculatus*, and *O. bimaculoides* had significantly greater arm truncation on the left side of their body (Voss & Mehta, 2021). My finding of population-level lateralization does not align with previous work in *O. vulgaris* (Byrne et al., 2004). This incongruence, is curious given that, even though octopuses are popularly described as asocial, one explanation for the origin of lateralization in populations is the social pressures that require individuals to coordinate their behavior with the behavior of other individuals of the same or different species (Vallortigara & Rogers, 2005). However, as suggested by other studies, both population- and individual-level lateralization may be present in the same species, depending on ecological context (Frasnelli and Vallortigara,2018 ;Frasnelli *et al.*, 2019). Future research addressing potential fitness advantages of lateralization at the individual level and its benefits to perhaps multi-tasking and better long-term memory in *O. bimaculoides* and other octopus species are necessary (Dadda & Bisazza, 2006; Pascual et al., 2004).

Diverse foraging methods, attack behaviors, and feeding habits have been described in numerous octopus species (J. Forsythe & Hanlon, 1997; Maldonado, 1963, 1964; Villanueva et al., 2017). Speculative hunting, characterized by chemotactile exploration and saltatory searching, is most common in shallow-water benthic octopuses (Hanlon & Messenger, 2018; John O'Brien et al., 1989; Villanueva et al., 2017). My observations on *O. bimaculoides* feeding behaviors were consistent with those already reported in *O. cyanea* (Forsythe & Hanlon, 1997), *O. insularis* (Leite et al., 2009), and *O. vulgaris* (Mather, 1991). During clam trials, octopuses exhibited the type of speculative foraging described above, crawling slowly around the bottom of the tank while their arm tips appeared to be exploring the space independently (Carls-Diamante, 2019). Often, the subject appeared to be crawling past the clam towards the end of the tank when an arm tip would make contact with the clam and the subject would change direction and initiate an attack by pulling the clam towards itself while also recruiting other arms and moving towards the clam (Byrne et al., 2004). The nature of such attacks is indicative of visual opportunism, strengthening the idea that vision may not be the dominant sense during foraging and feeding (Maselli et al., 2020). This type of arm attack (Figure 1.2, Supplemental Videos S1 and S2) whether appearing directed or opportunistic, was the only type of prey capture method or attack behavior observed in clam trials.

In contrast to clam trials, crab trials elicited significantly more webover attacks (Figure 1.2, Supplemental Videos S3 and S4) than clam trials. These attacks rarely appeared opportunistic and were often preceded by jetting or swimming rapidly towards the crab, suggesting that the subject recognized the prey from a distance, selected a particular prey capture strategy, then executed a direct attack guided by vision. In addition to a webover attack, the arm attacks employed for crabs appeared to be more goal-directed and visually guided. While previous studies have reported the lack of direct visual hunting (Forsythe & Hanlon, 1997; Mather, 1991), I observed a range of behaviors (opportunistic visual, opportunistic tactile, and direct visual) dependent on prey type, lending further evidence to the octopus's expansive behavioral flexibility. Given its range of habitats, and importance as both predator and prey, future research could examine whether foraging techniques and prey capture methods for *O. bimaculoides* vary with abiotic environmental factors such as substrate and season (Leite *et al.* 2009).

In addition to the behaviors above, I describe a previously unreported behavior which I refer to as sinusoidal locomotion, during which the subject traced a sinusoidal path along the bottom of the tank. I hypothesize that sinusoidal locomotion guides the octopus during a visual attack. This behavior occurred immediately preceding the attack at a significantly higher rate in crab than in clam trials and, as discussed above, my findings suggest that *O. bimaculoides* uses direct visual attacks on crabs. Because of their lateral eye placement, octopuses are known to use motion parallax, a behavior characterized by moving the head up and down to achieve sequential views of a target and as a means of focusing and gauging distance to an object (Hanlon & Messenger, 2018; Kral, 2003; Maldonado, 1964; Mather & Alupay, 2016; Sinn, 2000). Mather (2021) argues that, since octopuses are not usually visual hunters, these head bobs are performed in a wide number of contexts besides just foraging or prey capture. However, my observations suggest that the octopuses may be using sinusoidal locomotion in order to sequentially orient each eye towards the prey and achieve a kind of lateral motion parallax. Levy *et al.* (2015) found that crawling octopuses are guided by one eye and move with a body orientation that is most often 45° off of the direction of movement in order to avoid placing the target at the borderline of the visual field of each eye; however, they do not report a sequential shifting of that particular orientation from side-to-side as crawling progresses. Huffard (2006) provides a comprehensive list of body positions and methods of locomotion, with in-depth descriptions of different body posture and patterns of movement while crawling, with no description of a sinusoidal behavior. Therefore, I report observations of sinusoidal locomotion as a behavior used for foraging and prey capture.

As outlined earlier, the potential exists for bias in my sample according to the STRANGE categories of trappability, genetic make-up, and prior experience (Webster & Rutz, 2020). Due to those factors, the findings reported in the present study may be limited to *O. bimaculoides* in the geographic area of Southern California from which my study animals were collected. Future studies could build upon my findings by employing variable collection methods, using octopuses from different populations throughout the species' geographic range, or using lab-reared octopuses in order to examine any behavioral differences that may have been due to potential sampling biases. Despite the possibility of initial sampling bias, considerable efforts were made to adopt the STRANGE framework to increase the generalizability and reproducibility of my results. Octopuses were housed singly in standardized environmental conditions and were not able to observe each other's feeding trials. Individuals experienced the same levels of enrichment and human observation, had sufficient time to acclimate to captivity, did not participate in any earlier experiments, and feeding trials were conducted during the same time of day to account for natural changes in responsiveness.

Quantifying the behavior of captive *O. bimaculoides* when feeding on clams versus crabs revealed interesting patterns about how a generalist predator exhibits variable and context-dependent strategies to search, capture, and handle diverse prey items. Their behavioral flexibility undoubtedly serves to allow octopuses to quickly adapt and succeed in myriad dynamic environments. I hope that my work encourages future studies to assess how this feeding behavior may be influenced by various abiotic environmental factors, how such behavioral variation may translate to increased success for wild individuals, and how that success may scale up to affect community interactions and local food web dynamics.

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CHAPTER 1: TABLES

Table 1.1 Size and sex data for each individual octopus observed in this study.

Octopus	Mass at arrival (g)	Final mass (g)	Sex	Dates tested
Solomon	43.25	156.5	F	06/21/21 – 08/31/21
Huey	31.32	78.84	M	06/04/21 – 07/11/21
Zorba	41.23	119.87	M	05/28/21 – 07/02/21
Ollie	35.31	98.32	M	05/25/21 – 07/11/21
Frodo	90.82	116.7	M	08/27/27 – 10/07/21
Merry	50.85	64.52	F	08/30/21 – 10/07/21
Samwise	69.14	87.83	M	08/28/21 – 10/08/21
Pippin	38.10	70.28	F	08/28/21 – 10/26/21

Table 1.2. Summary of continuous response variables measured during feeding trials. Direction of arrow for clam-crab comparison represents relative magnitudes of measurement for each variable for clam trials in relation to crab trials (e.g., latency was less for clam trials than crab trials, as shown by the downward arrow, while ADT was higher for clam trials than crab trials). Significance levels shown are for a comparison of means via linear mixed model with individual octopus included as a random effect: $p < 0.05$: *, $p < 0.01$: **, and $p < 0.001$: ***.

Variable	**Clam Trials** (mean ± s.e.)	**Crab Trials** (mean ± s.e.)	**Model *p*-value**	**Direction** (Clam-Crab)
Latency	4.1 ± 0.74 (s)	7.8 ±1.6	0.010*	↓
TFT	19.8 ± 5.8 (s)	31.7 ± 9.0	0.027*	↓
ADT	171 ± 63.1 (s)	6.4 ± 3.3	0.0076**	↑
HT	84.6 ± 6.5 (s)	47.1 ± 5.8	0.0035**	↑
Body Attack Velo	0.128 ± 0.011 (ms^{-1})	0.351 ± 0.023	< 0.001***	↓
Body Attack Accel	0.826 ± 0.085 (ms^{-2})	2.92 ± 0.29	< 0.001***	↓
Arm Attack Velo	0.155 ± 0.009 (ms^{-1})	0.539 ± 0.028	< 0.001***	↓
Arm Attack Accel	1.16 ± 0.079 (ms^{-2})	5.33 ± 0.39	< 0.001***	↓

Table 1.3. Summary of categorical variables measured during feeding trials. χ^2 values represent the comparison between the observed frequencies of possible outcomes for crab trials vs. clam trials, as measured via log-linear mixed model with individual octopus included as a random effect. Significance levels are $p < 0.05$: *, $p < 0.01$: **, and $p < 0.001$: ***.

Variable	**Possible Outcomes**	χ^2	**df**	***p*-value**
Body orientation for first touch (BOFT)	Anterior, lateral, posterior	2.57	2	0.27
Body orientation for attack (BOA)	Anterior, lateral, posterior	2.48	2	0.29
BOFT $\overset{?}{=}$ BOA	Yes, no	0.23	1	0.63
Arm used for first touch (AFT)	L1-L4, R1-R4	3.80	7	0.80
Arm used for attack (AA)	L1-L4, R1-R4	2.54	7	0.92
AFT $\overset{?}{=}$ AA	Yes, no	0	1	1.00
AFT $\overset{?}{=}$ BOFT	Yes, no	0.44	1	0.51
AA $\overset{?}{=}$ BOA	Yes, no	2.19	1	0.13
Anteroposterior axis for first touch (APFT)	Anterior, posterior	0.61	1	0.43
Anteroposterior axis for attack (APA)	Anterior, posterior	0.021	1	0.89
APFT $\overset{?}{=}$ APA	Yes, no	0.27	1	0.61
Arm laterality for first touch (LFT)	Left, right	1.27	1	0.26
Arm laterality for attack (LA)	Left, right	2.16	1	0.14
LFT $\overset{?}{=}$ LA	Yes, no	0	1	1.00
Eye used for first touch (EFT)	Left, right	0.49	1	0.49
Eye used for attack (EA)	Left, right	3.28	1	0.070
EFT $\overset{?}{=}$ EA	Yes, no	0.93	1	0.34
Attack behavior	Arm attack, webover	27.5	1	<0.001***
Sinusoidal locomotion present	Yes, no	2.94	1	0.087
Sinusoidal locomotion precedes attack	Yes, no	4.55	1	0.033*

Table 1.4. Coefficients for linear mixed models. Octopus was included as a random effect, and a model was fit for each of the four timing response variables and four kinematic attack variables. Cells with "n/a" indicate that a predictor was not included in the regression analysis due to it occurring after the particular response variable being modeled. Significance levels are $p < 0.05$: *, $p < 0.01$: **, and $p < 0.001$: ***.

	Intercept	Mass	PreyMass	Trial	Latency	TFT	ADT
Latency							
Clam	0.224	-0.0103**	0.0182*	0.143**	n/a	n/a	n/a
Crab	0.999**	-5.23e-03	2.34e-03	3.57e-03	n/a	n/a	n/a
TFT							
Clam	1.02*	1.21e-03	-6.55e-03	-0.0399	0.265	n/a	n/a
Crab	1.95***	-2.99e-03	-0.103*	8.57e-03	-0.203	n/a	n/a
ADT							
Clam	1.05	-2.29e-03	0.0186	-0.172	0.369	0.0349	n/a
Crab	1.14	-3.17e-03	-0.0657	-9.08e-03	-0.241	-0.0642	n/a
HT							
Clam	1.97***	-3.19e-03	4.91e-03	2.09e-03	-0.144	0.0181	0.0274
Crab	0.860*	-4.19e-03	0.0356	-3.24e-03	0.177	0.345*	0.178
Body Velo							
Clam	-0.667***	4.76e-03*	-9.66e-03*	-0.0465*	0.0834	-0.150**	-0.0956**
Crab	-0.911***	-3.54e-04	0.0462	0.0333	0.0839	0.0719	0.0852
Body Accel							
Clam	-0.473	4.35e-03	2.95e-03	0.0172	7.62e-03	-0.0551	-0.168*
Crab	-0.393	-7.68e-04	0.0836*	0.0689	0.187	0.102	0.143
Arm Velo							
Clam	-0.937***	-4.82e-04	0.00413	0.0161	-0.0550	0.0257	-0.0384
Crab	-0.415*	1.59e-03	1.43e-03	2.91e-03	-0.0995	0.0190	0.0455
Arm Accel							
Clam	-0.138	2.15e-03	-1.80e-04	0.0263	-5.74e-04	-6.23e-03	-0.0801
Crab	0.590**	2.31e-03	-0.0138	0.0424	-0.243**	-0.0301	0.0101

Table 1.5. Results from binomial tests. Tests were performed to assess the within-group differences, relative to chance, for each variable. Crab-first touch and crab-attack trials do not add up to 40 because trials where the web was used were removed from arm use analysis (7 for first touch and 10 for attack). Significance levels are $p < 0.05$: *, $p < 0.01$: **, and $p < 0.001$: ***.

	Observed Count	Possible Count	Relative to Chance	*p*-value
Body Orientation				
First Touch: Clam				
Ant	13	40	↓	n.s.
Lat	23	40	↑	0.0014**
Post	4	40	↓	6.5e-04***
Crab				
Ant, Lat	17, 16	40	↑	n.s.
Post	7	40	↓	0.021*
Attack: Clam				
Ant	18	40	↑	n.s.
Lat	19	40	↑	0.044*
Post	3	40	↓	1.3e-04***
Crab				
Ant	26	40	↑	<0.001***
Lat	11	40	↓	n.s.
Post	3	40	↓	1.3e-04***
Arm Used				
First Touch: Clam				
L4	1	40	↓	0.032*
R2	11	40	↑	0.0083**
L3, L2, L1, R1, R3, R4	2, 7, 3, 2, 9, 5	40	↓, ↑, ↓, ↓, ↑, =	n.s.
Crab				
L4	0	33	↓	0.012*
R2	8	33	↑	0.047*
L3, L2, L1, R1, R3, R4	6, 5, 4, 1, 6, 3	33	↑, ↓, ↓, ↓, ↑, ↓	n.s.
Attack: Clam				
L4	0	40	↓	0.0048**
L3	0	40	↓	0.0048**
L2	10	40	↑	0.023*
R2	10	40	↑	0.023*
L1, R1, R2, R3, R4	3, 3, 9, 9, 5	40	↓, ↓, ↑, ↑, =	n.s.
Crab				
R2	8	30	↑	0.028*

L4, L3, L2, L1, R1, R3, R4	1, 5, 5, 4, 2, 4, 1	30	↓, ↑, ↑, ↑, ↓, ↑, ↓	n.s.
Anteroposterior Axis				
First Touch: Clam				
Ant, Post	23, 17	40	↑, ↓	n.s.
Crab				
Ant, Post	18, 15	33	↑, ↓	n.s.
Attack: Clam				
Ant	26	40	↑	0.040*
Post	14	40	↓	0.040*
Crab				
Ant, Post	19, 11	30	↑, ↓	n.s.
Arm Laterality				
First Touch: Clam				
L	13	40	↓	0.019*
R	27	40	↑	0.019*
Crab				
L, R	15, 18	33	↓, ↑	n.s.
Attack: Clam				
L	13	40	↓	0.019*
R	27	40	↑	0.019*
Crab				
L, R	15, 15	30	↓, ↑	n.s.
Eye Laterality				
First Touch: Clam				
L	13	40	↓	0.019*
R	27	40	↑	0.019*
Crab				
L, R	16, 24	40	↓, ↑	n.s.
Attack: Clam				
L	12	40	↓	0.0083**
R	28	40	↑	0.0083**
Crab				
L, R	20, 20	40	=	n.s.

CHAPTER 1: FIGURES

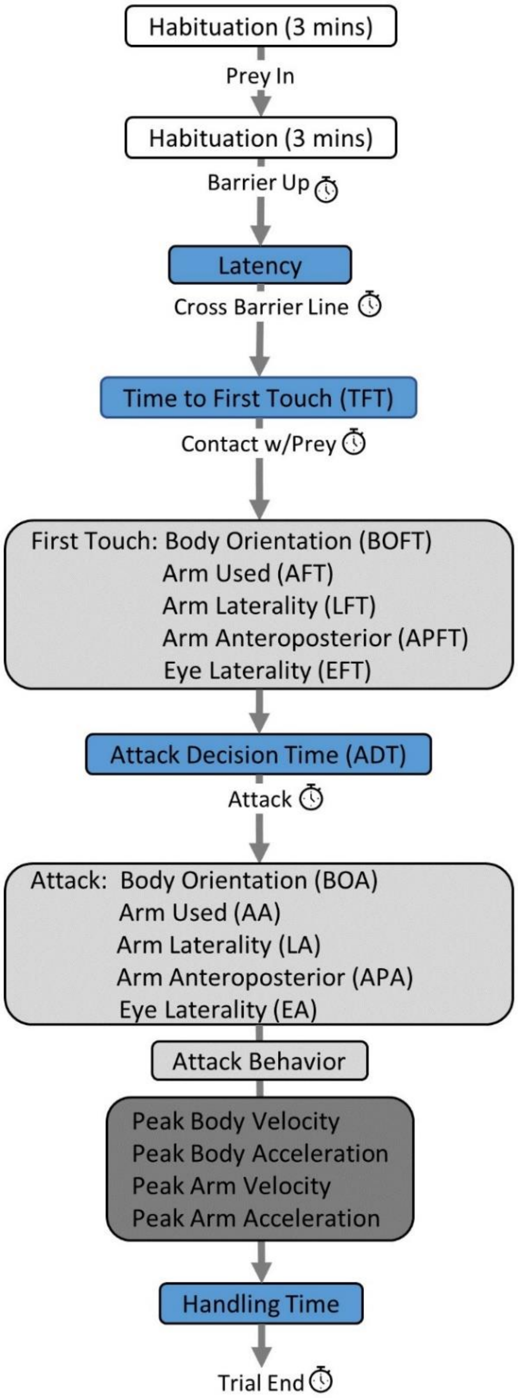

Figure 1.1. Schematic of a *O. bimaculoides* feeding trial, starting with the habituation period (white boxes), followed by the timing response variables (blue boxes), categorical body usage variables (light gray boxes), and kinematic attack variables (dark gray boxes). Stopwatch icons indicate where clock started/stopped for each trial (e.g., clock started at barrier up, latency was measured until subject

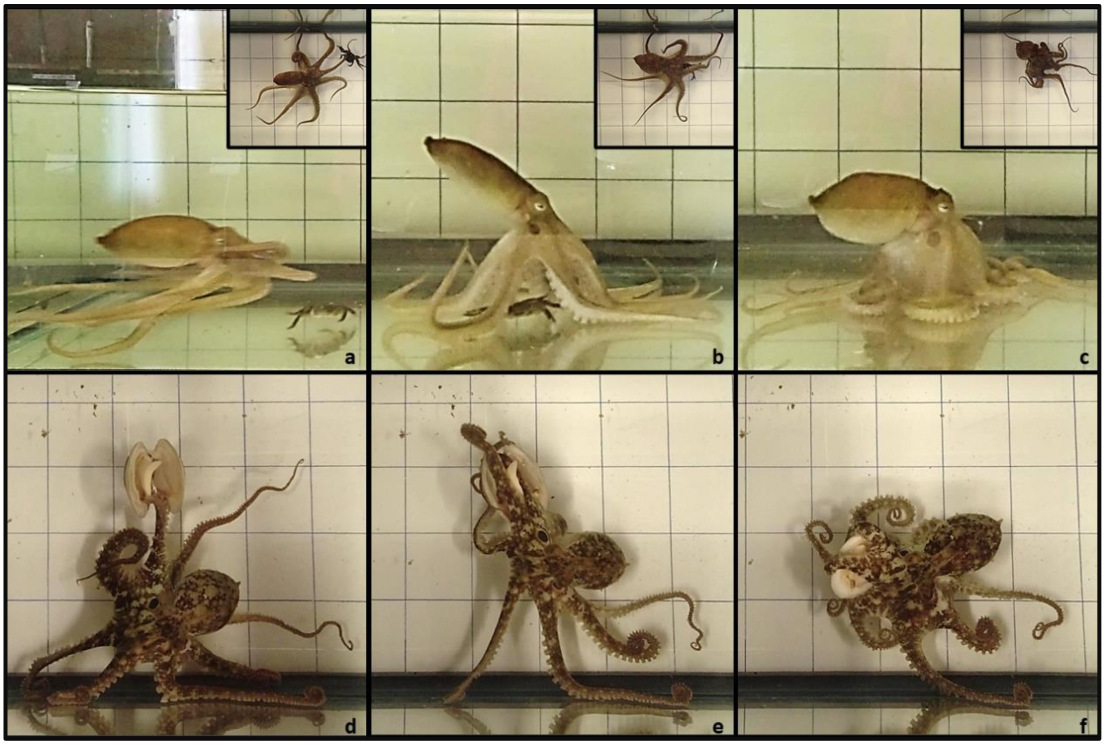

Figure 1.2. Screenshots from feeding trial recordings. Pictures show two different types of attack methods observed in *O. bimaculoides* (attacks progress chronologically from left to right). Top row: web-over attack (only observed in crab trials), where subject approached prey on a higher plane (a), descended from above with arms surrounding the prey (b), and then deployed the web to secure the prey (c). Bottom row: arm attack (observed in trials with both prey types; see inset on top row for arm attack on crab prey), where subject typically approached prey on the same plane, extended one arm out to initiate contact with the prey (d), brought neighboring arms forward to help grasp and secure prey (e), and brought prey under web (f).

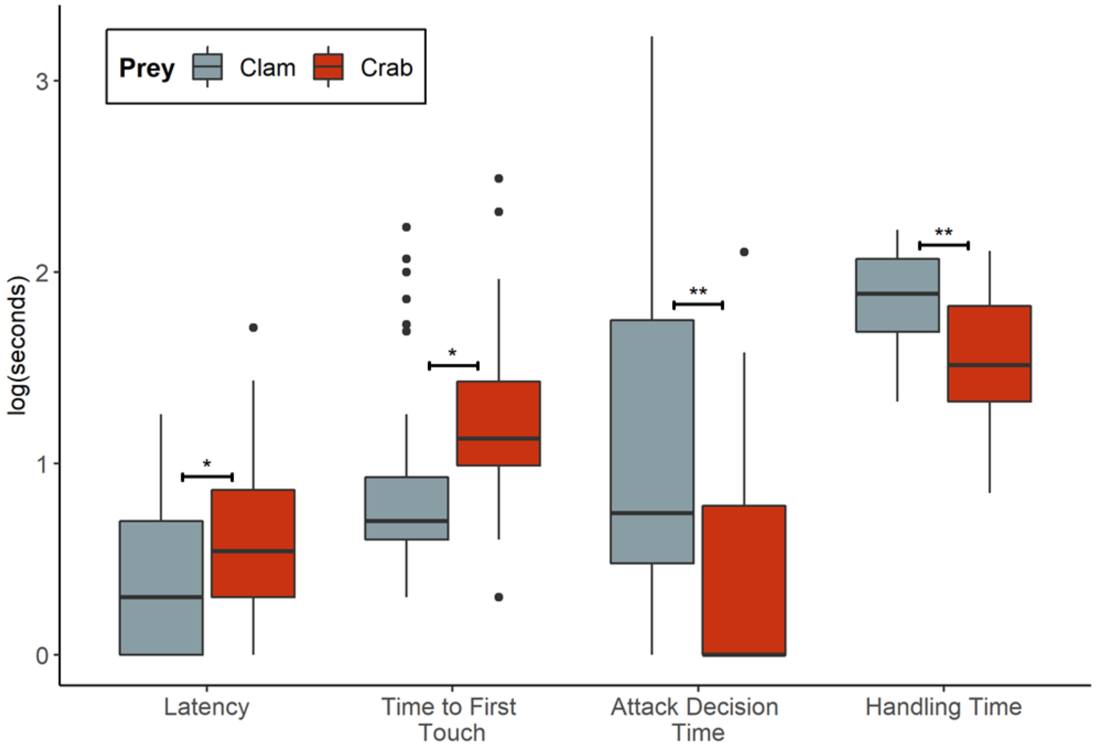

Figure 1.3. Timing response variables for feeding trials with live crabs (*n* = 40) and live clams (*n* = 40) by *O. bimaculoides*. Comparisons between prey types for each response were performed with linear mixed models where individual octopus was included as a random effect. The significance levels are shown above each pair of clam-crab boxplots ($p < 0.05$: *, $p < 0.01$: **).

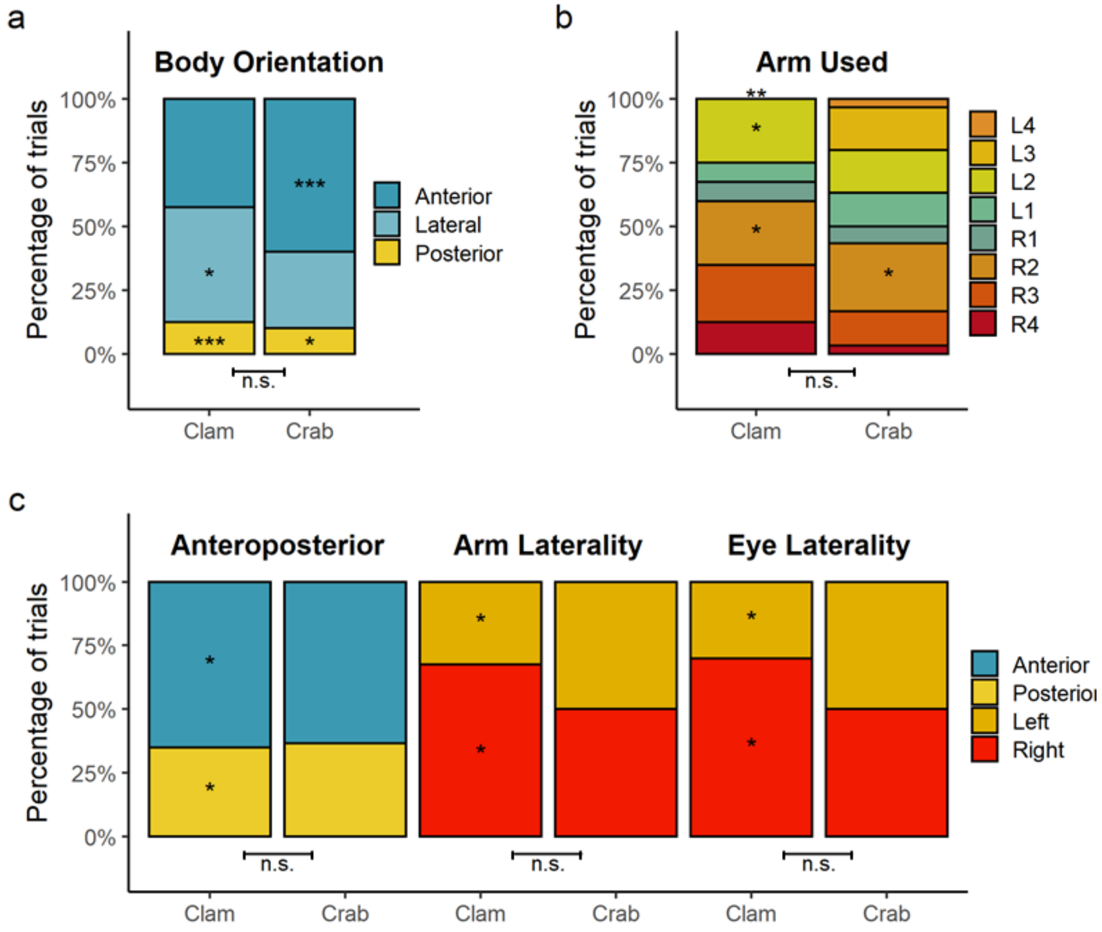

Figure 1.4. Body orientation, arm choice, and eye use for *O. bimaculoides* during feeding trials with live clams (n = 40) and live crabs (n = 40). (a) Body orientation comparisons between prey types for attack (chi-square test results shown beneath bars) and within each group (significance levels corresponding to binomial tests shown inside bars). (b) Arm used to initiate attack, showing comparisons between prey types and within each group (note: n = 30 for crab trials due to 10 trials including a web-over attack and, thus, no specific arm used). (c) Analysis of anteroposterior arm choice, arm laterality, and eye laterality for attack during feeding trials, showing comparisons between prey types and within group (note, n = 30 for anteroposterior and arm laterality for crab trials, for the same reason as above). Given that the proportions of possible outcomes for each of these variables were no different between first touch and attack (Table 3), data for first touch were omitted here for clarity. Significance levels are $p < 0.05$: *, $p < 0.01$: **, $p < 0.001$: ***.

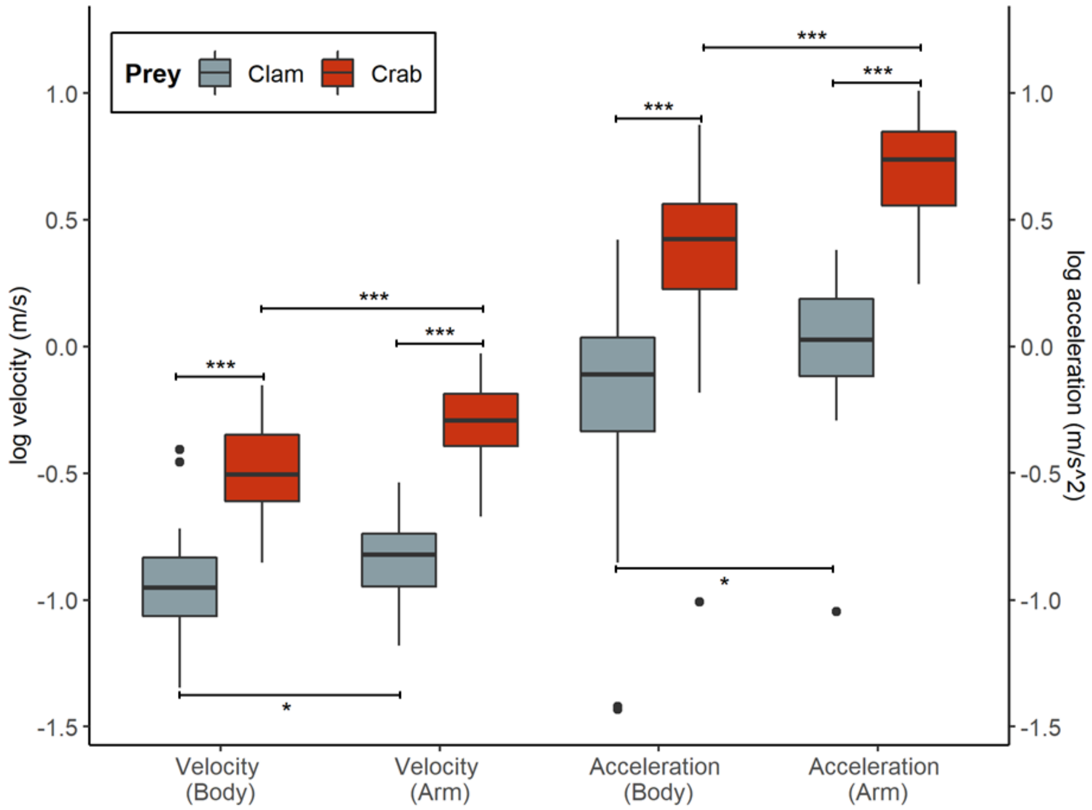

Figure 1.5. Comparisons of attack kinematics for O. bimaculoides feeding on crabs (*n* = 40) and clams (*n* = 40), assessed via linear mixed models with individual octopus included as a random effect. Significance levels are shown for comparisons between crab and clam trials for each attack variable, and for comparisons between arm and body velocity and acceleration for each prey type ($p < 0.05$: *, $p < 0.01$: **, $p < 0.001$: ***).

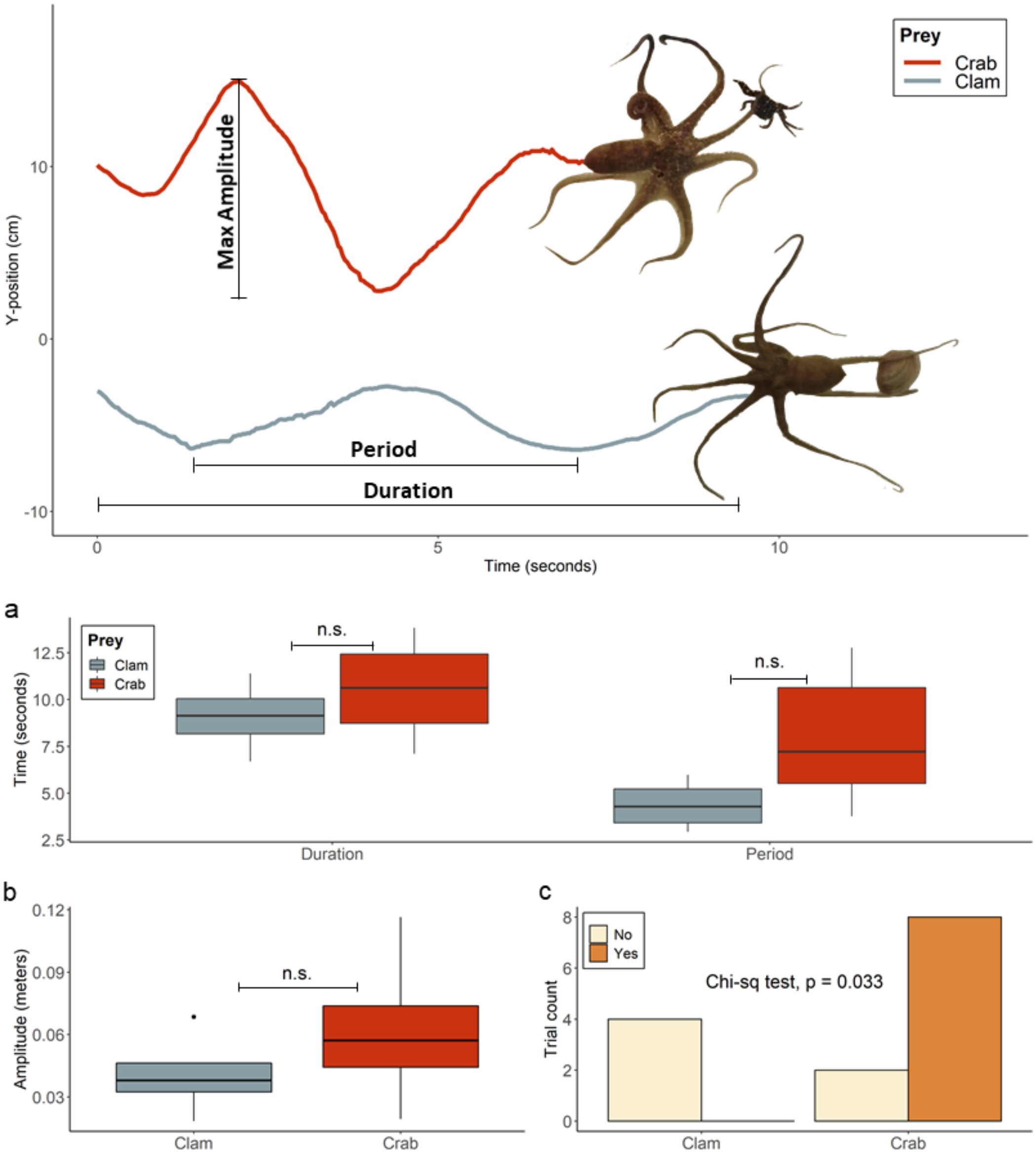

Figure 1.6. Characteristics of sinusoidal locomotion observed in *O. bimaculoides* while feeding on clams (*n* = 4) and crabs (*n* = 10). Top: two examples of the serpentine-like paths taken, from the dorsal view camera looking down into the experimental tank, along with illustrations showing the three parameters measured for each sinusoidal path. Bottom: (a) boxplots of the duration (seconds) of behavior and period (seconds) of the sinusoidal wave, shown with comparisons of means via linear mixed model (not significant); (b) peak amplitude (meters) of sinusoidal (serpentine-like) movement pattern, shown with a comparison of means via linear mixed model (not significant); (c) trials in which the serpentine-like motion immediately preceded the attack, shown with a chi-squared test via log-linear mixed model (occurs at a significantly higher rate in crab trials than clam trials).

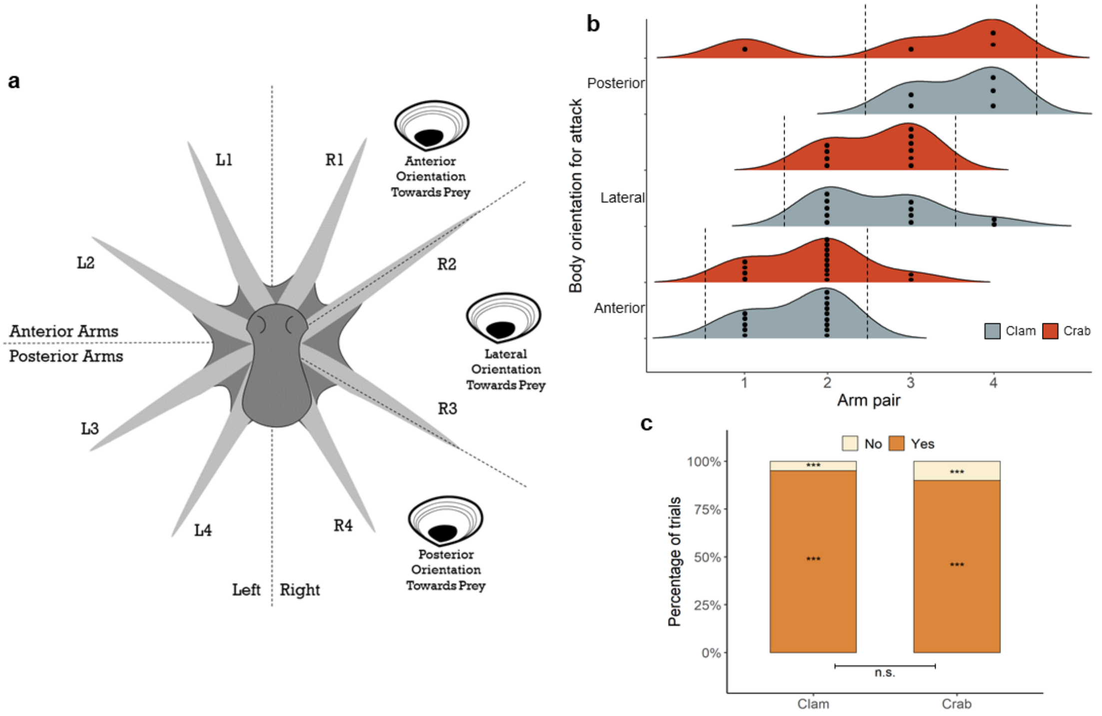

Figure 1.7. Assessment of whether arm choice was influenced by eye use during *O. bimaculoides* feeding trials (note: *n* = 40 for clam trials and *n* = 30 for crab trials because this analysis excluded the 10 crab trials where a webover attack was utilized, i.e., no specific arm was used). (a) Dorsal-view diagram showing the various ways that each first touch and attack were categorized: body orientation towards prey, eye laterality, specific arm chosen, and which side of the anteroposterior and lateral axes that arm was on. (b) Density distribution showing the arm used for attack for each possible prey type and body orientation. The data that fall in between each set of parallel dotted lines show where arm usage was consistent with one of the two arm pairs that I assigned to each body orientation (arms 1 and 2 for an anterior orientation, 2 and 3 for lateral, and 3 and 4 for posterior), indicating the connection between eye use and arm choice. (c) Percentage of trials for each prey type where the arm used for attack falls into the framework previously described. For each prey type, binomial tests against a probability of 0.5 showed that arm choice was connected to eye use significantly more often than chance would predict, indicating a strong influence: clam trials, 38/40, $p < 0.001$; crab trials, 27/30, $p < 0.001$. As shown beneath the bars, prey type had no significant effect on this result (assessed via log-linear mixed model).

Chapter 2: Effects of acute temperature change on the feeding behavior of the California two-spot octopus (*Octopus bimaculoides)*

Acknowledgments:

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Abstract

Octopuses are marine invertebrates whose physiology is affected by changes in their surrounding environment. In particular, water temperature has been shown to have pronounced effects on octopod metabolism, growth, reproduction, and life span. However, the majority of research to date has been on the thermal sensitivity of octopuses when experiencing in a gradual or chronic elevation (range?) in temperature, and none of it has examined the effects of temperature on feeding behavior. Despite their ecological importance as mesopredators, little is known about how temperature influences octopods' complex suite of predatory behaviors that consists of identifying, locating, capturing, and handling prey. Moreover, nearshore benthic species like the California two-spot octopus (*Octopus bimaculoides*) are subject to widely variable temperatures, with habitats in the intertidal zone that can experience daily temperature fluctuations that are far more extreme than predictions of future ocean warming conditions. To assess the effects of acute temperature change on feeding behavior, I conducted controlled feeding trials during which *O. bimaculoides* was presented with a live striped shore crab (*Pachygraspus crassipes*) and analyzed the videos to extract 11 behavioral responses. The three temperature treatments were designed to represent an ecologically relevant thermal gradient: 14-17 °C (baseline), 20 °C, and 23 °C. Using an integrative statistical approach that encompassed behavioral and kinematic attack variables, I used a combination of multi-level models to test for the main effect of temperature on each of the response variables, with random effects accounting for individual variation and the following covariates: crab activity level, relative prey mass,

and trial number. Elevated acute temperature was shown to affect the likelihood of a successful attack, latency to approach, body velocity and acceleration, and prey handling time, but not the likelihood of a successful foraging attempt, arm acceleration, or the time until first contact. I show that differential thermal responses are present even at temperatures within the species' thermal tolerance range and discuss the implication of how those sensitivities could lead to changes in predator-prey dynamics that are known to scale up to have system-level impacts.

Introduction

Marine environments are often described as thermally stable and predictable compared to terrestrial environments, due to the oceans' ability to absorb heat and shield against short term fluctuations in temperature (Steele, 1985; Steele et al., 2019; Vasseur & Yodzis, 2004). However, at finer temporal scales (hours, days, months) oceanographic processes cause large fluctuations in water temperature. Phenomena such as upwelling, sea surface temperature anomalies, and extreme warming events known as marine heat waves, which have increased in intensity and duration over the last century, can raise water temperatures 2 or 3 °C in a matter of hours (Barth et al., 2007; Booth et al., 2012). These occurrences certainly have widespread impacts (Rogers-Bennett & Catton, 2019; Smale et al., 2019), but perhaps even more so on environments like the intertidal zone, with shallow or fragmented water habitat that will be much more susceptible to rapid change (Menge et al., 2022).

On a finer spatial scale, instability and unpredictability are the norm to organisms living in nearshore habitats or the intertidal zone. For example, a single tidal cycle can change the water temperature by as much as 10 °C (Bates et al., 2018) and, therefore, can expose certain populations or individuals to drastically different thermal gradients on a daily, cyclical basis. Furthermore, a worldwide dataset spanning two decades showed that intertidal mussel beds experience daily temperature fluctuations up to 15-20 °C (Helmuth et al., 2016). To add to this intertidal variability, fluctuations in tide pool temperature differ greatly depending on location. For example, a 2011 study of the heat-shock response in tidepool sculpins in

British Columbia found that, in addition to having a significantly higher mean temperature, that mean temperature of pools in the high tidal zone can vary up to two times more than pools in the low tidal zone (Fangue et al., 2011). Therefore, two conspecific populations of organisms living in different tidal locations could have completely different thermal experiences. In general, how an individual or population experiences and reacts to temperature variability will depend on its thermal sensitivity. Although marine poikilotherms are unable to physiologically thermoregulate, mobile members of the intertidal community may be able to behaviorally thermoregulate by relocating to a region where there is the possibility to submerge into more thermally preferable water (Angilletta et al., 2002).

One example of such a marine poikilotherm are benthic octopuses, whose life histories are greatly affected by environmental temperatures and may be further susceptible to changes that have already been shown to influence search times, prey handling, and satiation in other coastal mollusks, such as oysters, mussels, and snails (Kroeker et al., 2014). Given octopuses' important role in marine ecosystems as both predator and prey, fluctuations in temperature, at any spatial or temporal scale, may have an outsized impact on octopus predator-prey dynamics, resource utilization, and interspecific competition (Villanueva et al., 2017).

Previous work has shown that temperature has a substantial impact on octopus physiology, growth, life span, and reproduction, but those experiments have largely focused on chronic or gradual temperature change, wherein the octopus has a substantial acclimation period (Culler-Juarez & Onthank, 2021; J. Forsythe &

Hanlon, 1988; García-Fernández et al., 2019; Logose, 2017; Noyola et al., 2013; Rigby, 2004; Uriarte et al., 2016; Zúñiga et al., 2013). In terms of more acute changes, the effect of temperature stress has been tested in *Octopus vulgaris* paralarvae growth (Logose, 2017), reproduction in *O. maya* (Domínguez-Estrada et al., 2022), and heat shock protein 70 (HSP70) expression in *O. tankahkeei* (Long et al., 2015). The effects of acute temperature change, especially with regards to critical behaviors related to energy acquisition, have remained largely unstudied. Thermal sensitivity not only varies between species, but within a species as well, especially one like the California two-spot octopus (*O. bimaculoides*) with a large geographic range, and more than likely high site fidelity (Cigliano, 1993).

Acute temperature changes have been shown to affect muscle performance in terms of prey capture kinematics (Moran et al., 2021), swimming speed in various fishes (Deslauriers & Kieffer, 2012; Jones et al., 2008; Yu et al., 2018), and the non-ballistic portions of prey capture (tongue retraction and mouth closing) in frogs, salamanders, and chameleons (Anderson & Deban, 2012; Deban & Lappin, 2012; Deban & Scales, 2016; Sandusky & Deban, 201). Much like those amphibian and reptilian tongues, octopuses are largely muscular hydrostats composed mainly of incompressible muscle tissue, and the production of movement and force has long been thought to be dictated by this constant volume constraint (Kier & Smith, 1985; Wilson et al., 1991). Given that an octopus's arms are critical to its ability to find and capture prey (Buresch et al., 2022; Forsythe & Hanlon, 1997), examining how temperature may influence muscle activation speed in the arms used when feeding will be important for determining feeding success in the context of acute temperature variability. However, performance, preference, and tolerance must not be conflated. For example, the thermal preference of *O. bimaculoides* has been measured at 16.6-17.8 °C (Ontiveros, 2014), which may have ecological implications in terms of influencing thermoregulatory behavior, but kinematic performance and critical thermal limits should be considered separately.

To determine the effects of acute temperature changes on octopus feeding behavior, I conducted controlled feeding trials in the lab with *O. bimaculoides* feeding on the striped shore crab (*Pachygraspus crassipes*), a common prey item in the intertidal zones of California. I chose *O. bimaculoides* as my behavioral model for several reasons: (a) its diurnal activity facilitates a day time experimentation schedule that aligns with natural behavior (J. Forsythe & Hanlon, 1988; Jereb et al., 2014), (b) it is a generalist predator, feeding on a wide range of prey including crustaceans, bivalves, and gastropods (Jereb et al., 2014), and (c) it establishes itself in the benthos early in its life history and experiences acute temperature changes associated with traveling up and down the water column during the critical early period when foraging behaviors are learned and prey preferences are established, as with other cephalopods (Darmaillacq et al., 2006).

While the extent of temperature variability that an octopus experiences will depend on its habitat, *O. bimaculoides* is a good model to answer the following questions: (a) do acute changes in water temperature affect feeding motivation? (b) how does acute temperature elevation affect the various timing components of a feeding trial (e.g., latency to approach, handling time, etc.)? and (c) how do acute temperature changes affect attack kinematics? Understanding how acute temperature change influences the feeding behavior of *O. bimaculoides*, an important mesopredator along the California coast, will help to elucidate the physiological and ecological variation found at temporal and spatial scales that are often overlooked and have consequences on predator-prey interactions and community structure in the marine environment.

Methods

Animals

Eight *O. bimaculoides* were collected from the wild (four in August 2021, four in February 2022) in Long Beach, CA and shipped overnight to the Joseph Long Marne Lab, University of California, Santa Cruz (36° 56' N, -122° 3' W). All animals were collected from the same location and varied in size and sex (Figure 2.1, Table 2.1). Upon arrival, and every day thereafter, specimens were evaluated consistent with the accepted guidelines for cephalopod care (Beigel & Boal, 2006; Browning, 2019; Cooke & Tonkins, 2015; De Sio et al., 2020; Drinkwater et al., 2019; Fiorito et al., 2015; Moltschaniwskyj et al., 2007; Russell & Burch, 1959; Yasumuro & Ikeda, 2018). Octopuses were housed in individual transparent plastic containers (25 x 18 x 17 cm) with ventilated lids. PVC dens, rocks of assorted of sizes, and various other aquaria décor and enrichment items were provided for each individual. Individual tanks were maintained inside larger transparent fiberglass tanks receiving constant flow-through sea water of ambient-temperature (13° to 17°C) and all animals were exposed to natural light-dark cycles. After arrival in the lab, an acclimation period of 14 days was initiated before any experimentation began, during which octopuses were fed daily with a rotation of live littleneck clams (*Protothaca staminea*), mussels (*Mytilus californianus*), snails (*Tegula altra*), and striped shore crabs.

Experimental Design

Controlled feeding trials were conducted between August 2021 and May 2022. Trials took place in a separate experimental arena, which consisted of a clear fiberglass tank (75 liters, 77.5 x 33.0 x 33.3 cm) with sea water filled up to 15 cm. A removable, transparent, permeable barrier was installed 15 cm from one end of the experimental tank. Two DJI Osmo Action cameras (DJI, China) were used to film overhead and lateral views of each trial. To determine the effects of temperature on feeding behavior feeding trials were conducted with only one prey type, the striped shore crab. While the mass consumed or feeding rate may have been more easily measured with bivalve prey, I chose the striped shore crab as the experimental prey item as I previously observed more dynamic attack behaviors by *O. bimaculoides* with this prey (Lankow & Mehta, in review). The three temperatures (baseline: ambient inflow sea water at 13 to 17 °C; T2: 20 °C; T3: 23 °C) represent an ecologically relevant temperature gradient consistent with what individual California two-spot octopuses may experience in the course of a day or during a single foraging trip throughout the water column or in different tide pools (Fangue et al., 2011; Helmuth et al., 2016). These temperature treatments are also consistent with NOAA recorded sea surface temperature anomalies at ± 3 °C as well as previous laboratory studies with *O. bimaculoides* (Forsythe & Hanlon, 1988; Ontiveros, 2014).

After completing five successful trials at the baseline temperature, each individual completed a pseudo-randomized schedule of trials at 20 °C and 23 °C until five successful trials were completed at each of the two treatments. In the course of experimentation, one individual laid eggs in her home tank, thus ending her participation in the trials due to self-inflicted extended starvation after egg-laying (Wodinsky, 1978). Her remaining six trials (four at 20 °C and two at 23 °C) were distributed evenly among the other three individuals in her cohort leading to an unbalanced design.

Experimental Procedure

Prior to each feeding trial, an individual was fasted for 48 hours to increase feeding motivation. The last meal prior to fasting was never a crab, but rather one of two sedentary prey, *P. staminea* or *T. altra*. For each feeding trial, an individual was transferred to the experimental tank filled with water at either T1, T2, or T3, and placed behind the barrier. After three minutes of habituation, the crab was added to the tank in the center of the area on the other side of the barrier. After an additional three minutes the crab was corralled to the center of the far wall opposite the barrier, and the barrier was lifted. In the case of a successful attack (i.e., the octopus captured the crab during a hunting sequence), the time was marked and at ten minutes post-attack the octopus was transferred back to its home tank and the crab was weighed. If there was no successful attack within 30 minutes of the barrier being lifted, the trial was stopped, and the octopus was transferred back to its home tank and given a small piece of frozen crab ten minutes later. The water in the experimental tank was changed before each new trial, and an individual was never tested more than once per day.

Video Data Extraction

Videos from all successful trials were analyzed from both camera angles in order to extract the following continuous variables in seconds and counts: i) total time to attack (TTA): time elapsed between the barrier being lifted and a successful attack (comprised of the following three measurements), ii) Latency: time elapsed between the barrier being lifted and the octopus crossing the line where the barrier had previously rested (henceforth known as the “starting line”), iii) time to first touch (TFT): time elapsed between the octopus crossing the starting line and making first contact with the crab, iv) attack decision time (ADT): time elapsed between first touch and when the octopus brought the prey under its buccal web for a successful attack, v) attempts to capture: the number of times prey capture was unsuccessful, and vi) handling time (HT): time elapsed between initially taking the prey under its mouth and when the octopus stopped moving after a characteristic series of actions that included subduing the prey, manipulating they prey with its arms, and orienting it in a specific way under the mouth. Behaviors that constituted handling time were typically accomplished in one continuous series until no further movement could be seen under the octopus’s web; however, in some cases further manipulation was visible at later periods of the ten-minute post-attack observation phase, in which cases those durations were added to the handling time.

In addition to the timing response variables previously described, the kinematics of the actual attack sequence were calculated using Tracker Video Analysis and Modeling Tool, version 6.0.1, copyright © 2021. Given that the attack is typically a dynamic serious of events involving the body and the arms, the following kinematic variables were measured: i) peak body velocity (ms^{-1}): maximum velocity attained by subject's approximate center of mass, from movement towards prey until prey is placed under the buccal web, ii) peak arm velocity (ms^{-1}): maximum velocity attained by an arm during the initiation of attack or capture of the prey, iii) peak body acceleration (ms^{-2}): maximum acceleration attained by subject's approximate center of mass, iv) peak arm acceleration (ms^{-2}): maximum acceleration attained by an arm. See Table 2.2 for a summary of all variables aggregated across the full sample of feeding trials.

Statistical Analyses

I first assessed how well each response variable conformed to the assumptions of parametric statistics along four dimensions: 1) independence of observations, 2) presence of outliers, 3) homogeneity of variance between groups, and 4) whether the data could reasonably be assumed to have come from a Gaussian distribution. While observations were conducted independently, multiple observations were collected from each octopus at each treatment level, which was accounted for in the statistical model type chosen. I tested for homoskedasticity by assessing whether the group with the highest variance had no more than three times that of the group with the lowest. Finally, the distribution of each response variable was tested with a Shapiro-Wilk's test. Following all assessments, transformations for the four timing variables—TTA, Latency, TFT, and ADT—did not result in normal distributions and, therefore, were analyzed with nonparametric statistical methods (Figure 2.2). Five variables were suitable for parametric tests after a logarithmic transformation: body velocity, body acceleration, arm velocity, arm acceleration, and HT (Figure 2.3).

To test for the effects of water temperature on each of the feeding response variables, I used a combination of general linear models (GLM), linear mixed models (LMM), and generalized linear mixed models (GLMM). Mixed models, also known as multi-level models (MLM), excel at controlling Type I error and are therefore more generalizable at a population level (Barr et al., 2013; Bolker et al., 2009; Judd et al., 2012). The present study is explanatory in nature, rather than predictive, which informed decisions at every step of the modeling process: study design and data collection, data preparation, choice of variables, choice of methods, validation, model evaluation, model selection, and model reporting (Shmueli, 2010). MLMs exist in their most general form as $Y_i = X_i\beta + Z_i\gamma_i + \varepsilon_i$ where X_i and Z_i are the fixed and random design matrices, β represents the fixed effects, γ_i is the random effects, and ε_i is the unknown random (residual) error (Kincaid, 2005). In this structure, the γ_i parameters have the flexibility to vary across individuals, which is why MLMs were appropriate given the individual variation in feeding and prey handling behavior shown by octopuses (Arnold & Arnold, 1969; Grisley et al., 1996; Young, 1956) and the need to account for it (Hertel et al., 2020).

While testing for the effects of temperature treatment, I chose to include three covariates in every model: crab activity level (CAL), the percentage of time that the prey was moving, after being added to the tank and before being captured; relative prey mass (RPM) the ratio of prey mass to octopus mass at the time of the trial; and Trial, the number of successful trial that was completed by a particular octopus at a particular treatment level. I controlled for size effects by feeding the smallest crabs to the smallest octopuses and largest crabs to largest octopuses; however, due to the difficulty in finding crabs of a uniform size during each collecting trip, a range of relative masses was inevitable and I still included RPM as a predictor because it is known that prey size has an impact on octopus prey handling (Grisley et al., 1999; Steer & Semmens, 2003). CAL was excluded from the model for HT, since the activity level of the crab was only measured prior to capture and HT, by definition, takes place exclusively post-capture.

In the case of each model, the variable Treatment was of main interest, and the other variables are covariates whose function is to control for differences between the groups and to help explain the variance observed in the response variable. Due to the characteristics of the data, the nine measured response variables were fit into three categories of models. The four kinematic variables and HT were log-transformed and fit with linear mixed models (LMM). Meanwhile, TTA, Latency, TFT, and ADT, were fit with generalized linear mixed models (GLMM), which are a flexible extension of linear mixed models that allow for response variables from different distributions and are particularly suited for data sets in ecology and evolutionary biology (Bolker et al., 2009). To model my four timing variables, I chose to use the Gamma family of distributions, which are characterized by continuous, zero-bounded data and a logical choice for temporal data in behavioral research (Schmettow, 2021). The purpose of the link function is to transform a non-linear relationship to linear form and, with a dependent variable mean of μ_i, the canonical link for a Gamma distribution is the "inverse" $g(\mu_i) = 1/\mu_i$. Instead, however, I chose the log link function as a commonly-used alternative to produce a multiplicative model on the original scale that is easier to interpret (Ng & Cribbie, 2017).

For the final category of models, I used a general linear model (GLM) with a binomial family distribution of errors, or a logistic regression model, to analyze two different binary response variables: Trial Success and Forage Success. Trial Success modeled the probability of whether a particular feeding trial ended with a successful attack or was declared a 'no-trial' after the 30-minute time period elapsed with no attack. For Forage Success, I created a binary dummy variable from the number of attempts it took an octopus to successfully capture its prey, with exactly one attempt receiving a score of 1 and anything more than one attempt receiving a score of 0. Although these feeding trials were conducted in a controlled, closed environment where an unsuccessful attack had no impact on whether the prey item would be available for another attempt, the rocky reefs and subtidal areas in which *O. bimaculoides* live naturally would likely not afford the same level of opportunity and the crab would likely escape after an unsuccessful attempt (Cigliano, 1994; Jereb et al., 2014). For both logistic regressions, the same covariates were included as for the LMMs and GLMMs, with one exception: CAL was only available for trials that ended with a successful attack, and was therefore excluded from the model for Trial Success (since that analysis also includes unsuccessful trials).

With all three categories of models, I followed the same process of model fitting, using Bolker et al. (2009) as a guide. First, a null model (with only the response variable and intercept) was fit via restricted maximum likelihood (REML). Next, a nested random intercept (with individual octopus nested within cohort) was added to estimate the intraclass correlation coefficient (ICC) and the impact of the random effect, using the standard variance components covariance structure. After that, a saturated model was fit with maximum likelihood (ML) in order to assess the inclusion of candidate fixed effects (Barr et al., 2013), and multicollinearity was assessed via variance inflation factor scores. Predictors that did not show a statistically significant impact on the outcome of the variable were retained in the model due to the explanatory, rather than predictive, nature of this analysis (Shmueli, 2010). Next, a random slope was fitted for Treatment to allow for the variance of effect, as well as intercept, at the individual octopus or cohort level. The interaction terms were then added between Treatment and all other covariates, the significance of each term was assessed via Wald Type III chi-sq test, and non-significant interactions were removed to limit potential multicollinearity and improve model fit. Finally, a final model was fit for each response variable: for GLMs and GLMMs, the final models were fit via maximum likelihood and Laplace approximation; for LMMS, the final models were fit via restricted maximum likelihood with Satterwaithe's method for denominator degrees of freedom.

At each stage of model fitting, depending on the model type, a deviance or Likelihood Ratio Test was performed to assess whether the more complex model added enough explanatory power in order to make up for the loss of degrees of freedom with additional terms and judged via Bayesian Information Criteria. For GLMMs, convergence and singularity errors were addressed by varying the optimizer used for fitting the model (Nelder Mead, "bobyqa", and "nloptwrap"), or (as a last resort), reducing the complexity of the random effect structure (i.e., removing the random slope or the nested random intercept). The final, best form of each model (Table 2.3) was run through a series of diagnostic tests examining various aspects of model fit: the normality and constant variance of residuals, the linearity of each predictor vs. the residuals, and the sensitivity of the data to high-leverage observations and outliers. Because of the lack of consensus regarding how to calculate standard effect sizes for individual model terms (Rights & Sterba, 2019), I refrained from reporting standardized effect sizes when possible, with the following exceptions: any predictors involved in interactions were standardized in order to reduce multicollinearity caused by higher-level terms, and predictors were centered in final models in order to facilitate interpretation and biological relevance (e.g., mass ratio was mean-centered because interpreting the model intercept when a crab is zero percent of the octopus mass is meaningless, as it is for trial number zero, but it makes biological sense to evaluate a model's intercept when a crab is completely motionless).

The significance (defined as $p < 0.05$ for all tests) of each predictor's effect on the response variable for all models was assessed via analysis of variance using Type II sums of squares when interactions were present and Type III sums of squares when no interactions were present. Additionally, the intra-class correlation coefficient (ICC) and marginal and conditional R^2 (Nakagawa & Schielzeth, 2013) were calculated for each final model, along with the predictive accuracy of the logistic regression models when assessed with a 20% validation set. All analyses were performed using R Statistical Software (v4.0.3; R Core Team 2020), model fitting was performed with the lme4 R package (v1.1.30; Bates et al., 2015), tables were produced with the stargazer R package (v5.2.3; Hlavac, 2022), and reproducible model outputs were created with the *report* R package (v5.1.3; Makowski et al., 2020).

Results

I analyzed video recordings of 40 trials at each of the three temperature treatments, conducted by 8 octopuses. For each of the eleven response variables and behavioral measures, I used a combination of multi-level models (Table 2.3) to examine the temperature effects while accounting for the random variation inherent in individual animal behavior and controlling for three covariates: Crab Activity Level (CAL), Relative Prey Mass (RPM), and Trial Number. Octopuses exhibited temperature dependent variability in the probability of Trial Success, Latency, Body Velocity, Body Acceleration, and Handling Time, while the effect of temperature was moderated by one or more covariates for Total Time to Attack (TTA), Attack Decision Time (ADT), and Arm Velocity. Probability of Foraging Success, Time to First Touch (TFT) and Arm Acceleration did not vary significantly with temperature. For the outputs of each model and to see the specific effect sizes of each predictor variable, see Tables 2.4-2.6.

Trial Success and Foraging Success

In T1, 77% of trials and, in T2, 89% of trials resulted in successful predation events, while only 49% of trials at T3, the warmest treatment, were successful (Figure 2.4). While controlling for the effects of RPM and Trial, I confirmed that temperature treatment had a significant effect on the probability of a successful trial (X^2=25.5, df=2, p<0.001). Specifically, using pairwise comparisons of the estimated marginal means, or the equally weighted means of the model's predictions at specified margins, I estimated that the odds of a successful trial at T1 are no different than for T2 ($p = 0.34$). However, I found that the probability of a no-trial at T3 was 3.84 times ($p = 0.0036$, 95% CI = {1.67, 8.80}) more likely than at T1 and, and 9.61 times ($p < 0.001$, 95% CI = {3.23, 28.6}) more likely than at T2. When measured on a 20% validation set, this model displayed a moderate predictive accuracy of 54%.

In terms of Foraging Success, octopuses completed a successful attack with their first attempt for 80% of the trials at T1, 75% of the trials at T2, and 65% of the trials at T3. Neither Treatment (X^2 = 1.67, df = 2, p = 0.434), Trial (X^2 = 1.21, df = 1, p = 0.270), nor RPM (X^2 = 1.23, df = 1, p = 268) were shown to have a significant effect. When measured on a 20% validation set, this model displayed a 75% predictive accuracy.

Total Time to Attack (TTA), Latency, Time to First Touch (TFT), and Attack Decision Time (ADT)

In modeling the response variable TTA, I found temperature to have a significant interaction with CAL (X^2 = 8.48, df = 2, p = 0.014) and RPM (X^2= 89.19, df = 2, p = 0.010). For example, an increase in CAL (measured as a percentage of time moving prior to attack) at T2 had no impact on TTA compared with T1, while at T3 it had a significant negative effect. Alternately, the opposite was true for the interaction between treatment and RPM, with the effects being positive and the relative effect at T2 being significant (t = 3.032, p = 0.00243) and much greater than the effect at T3. Therefore, after accounting for Trial Number, the effect of temperature on TTA was moderated by CAL and RPM.

Once the barrier was lifted, octopuses waited an average of 51 seconds before moving towards the prey; however, there was considerable variation between treatments. After accounting for the covariates, Treatment was shown to have a statistically significant effect on Latency (X^2 = 47.54, df = 2, p < 0.001). With predicted results from the model averaged over the range of each covariate, I show that Latency is no different at T1 than at T2. However, T3 was found to be significantly greater than both T1 (z-ratio = -6.08, p < 0.0001) and T2 (z-ratio = -5.30,

$p < 0.0001$). Due to the nature of the relationship between Latency and Treatment, I used a contrast based on average temperature within each Treatment level to test for a linear trend in the differences between estimated marginal means at each Treatment and confirmed that Latency increases linearly with water temperature (z-ratio = 5.808, $p < 0.0001$; Figure 2.5).

After crossing the starting line, the time to first touch (TFT) varied little between Treatments. Trial Number was the only significant predictor in the model ($X^2 = 9.64$, df = 1, $p = 0.0019$), and produced a negative but small effect on TFT (log-effect = -0.22, 95% CI = {0.35, -0.08}).

After touching the prey for the first time, the time elapsed until the successful attack (ADT) ranged from zero seconds (i.e., a simultaneous first touch and attack) to 28 minutes. The main effect of Treatment on ADT had a significant interaction with CAL: estimated marginal means of ADT decrease with CAL at each Treatment, but at significantly different rates. Compared with T1, the effect of CAL on T2 is not statistically significant ($t = -0.96$, $p = 0.0337$), but the effect of CAL on T3 is 2.4 times (log-scale) that of T2 ($t = -2.42$, $p = 0.017$). Therefore, after accounting for Trial Number and the Relative Mass of the prey compared to the octopus, I found that the effect of temperature on Attack Decision Time was moderated by CAL at the highest temperature.

Attack Kinematics

The peak Body Velocity during attack, measured at the approximate center of mass of the octopus, showed considerable variation between temperature treatments, with slower attacks occurring at warmer temperatures. The overall effect of temperature on log-transformed Body Velocity was significant (X^2 = 16.9, df = 1, $p <$ 0.001) and the pairwise contrasts of estimated marginal means at each treatment (averaged over the levels of the covariates) show that the velocity at T3 is significantly slower than T1 (Kenward-Roger df = 109, *t*-ratio=4.92, $p <$ 0.0001) and significantly slower than T2 (Kenward-Roger df = 109, *t*-ratio=3.26, $p =$ 0.0042). Because of this relationship, I used a specific contrast based on average temperature at each treatment, and determined that Body Velocity decreases linearly with water temperature (Kenward-Roger df = 109, *t*-ratio=-4.856, $p <$ 0.0001), within this range of temperatures.

The effect of temperature on *O. bimaculoides*' ability to accelerate to attack a crab was modeled with the log-transformed acceleration data as the response. The main effect of temperature was statistically significant ($\mathrm{X}^2 = 10.1, df = 2, p = 0.0063$) and, after controlling for the covariates, body acceleration decreased by 34% in T3 (*t* = -2.90, p = 0.004) compared to T1 (*t*-ratio = 2.90, *p* = 0.013) and decreased by 31% compared to T2 (*t*-ratio = 2.61, *p* = 0.028). A specific contrast showed that body acceleration also decreases linearly with temperature (Kenward-Roger *df* = 108, *t*-ratio = -2.81, *p* = 0.0059).

O. bimaculoides typically used one or more arms during prey capture. In modeling the log-transformed data, we found that the main effect of temperature alone was not a statistically significant contributor to Arm Velocity. However, the model showed a significant interaction with Trial (X^2 = 7.13, df = 2, p = 0.028). The effects of Trial on Arm Velocity at T2 and T3 were positive (indicating faster arm speeds at warmer temperatures), but only the effect on T3 was statistically significant (t = 2.66, p = 0.0009). After controlling for CAL and RPM, I found that the effect of temperature on Arm Velocity was moderated by Trial Number, suggesting that octopuses' arms moved faster during later trials.

In modeling the most dynamic part of an octopus attack, I used the log-transformed Arm Acceleration data and did not detect a significant effect of temperature (X^2 = 1.97, df = 2, p = 0.37), nor any of the other terms in the model.

Handling Time (HT)

After the attack, the octopus performs a characteristic set of handling behaviors to subdue the prey, bring it under the buccal web, and orient it for feeding. Using the log-transformed data, I found that the main effect of temperature on HT was significant (X^2 = 53.2, df = 2, p < 0.001). The fixed effects at T2 (β = 0.18, 95% CI = {0.08, 0.28}, t = 3.50, p < 0.001) and T3 (β = 0.38, 95% CI = {0.28, 0.49}, t = 7.29, p < 0.001) were both positive and significant, representing a 52% increase in HT from T1 to T2 and a 142% increase from T1 to T3. Going from T2 to T3 also represented a significant increase (β = 0.21, 95% CI = {0.099, 0.31}, t = 3.89, p =

0.005); because of this relationship, I performed a specific contrast and found that HT increased linearly with water temperature (z-ratio = 6.85, $p < 0.001$) within this range.

Discussion

I found that, after accounting for the Relative Prey Mass and Trial Number, temperature had a significant negative effect on the probability of a successful feeding event happening within the allotted time limit. Feeding trials were least likely to be successful at the warmest treatment. This was not entirely unexpected, given that the thermal preference of *O. bimaculoides* has been measured at 16.6 – 17.8 °C (Ontiveros, 2014). However, preference, tolerance, and performance must not be conflated. There is reason to predict, from a theoretical perspective, that this particular species would have a wide thermal tolerance, given the amount of variability in temperature it experiences (Brett, 1970; Sunday et al., 2011). From a species distribution standpoint, at the northern edge of its range *O. bimaculoides* encounters temperatures that average 12-15 °C in the colder months; further south, at Santa Catalina Island, temperatures range up to 20 °C, and at the southern portion of its range, at San Quintin, Mexico, nearshore water temperatures are even warmer (R. F. Ambrose, 1988; J. W. Forsythe & Hanlon, 1988). Therefore, adaptation at the regional level to local conditions could result in variable intraspecies thermal tolerance, which has already been shown in *O. maya* (Noyola et al., 2013). Further, entire populations of *O. bimaculoides* have been observed living above the mean low tide line (J. Forsythe & Hanlon, 1988), where daily fluctuations in temperature at a smaller spatial scale can regularly exceed even the most extreme marine heat waves exacerbated by global warming (Frölicher et al., 2018; Frölicher & Laufkötter, 2018; Helmuth et al., 2016).

Through a combination of multi-level models, I found support for the effect of increased temperature on three of the four measured timing responses leading up to an attack: TTA, Latency, and ADT. The effect of temperature on TTA was moderated by CAL (which had a significantly negative effect at T3) and RPM (which had a significantly positive effect on TTA only at T2). These results are perhaps more informative given that CAL and RPM are not correlated (Appendix A; Pearson's correlation coefficient, $r = 0.09$; Anova, $p = 0.34$)*;* however, the asymmetry in the magnitude of these effects at different temperatures is striking. The main effect of temperature on Latency was significant and positive, meaning once the barrier was lifted, octopuses waited much longer to cross the starting line during a trial at T3 than they did at cooler treatments. This might be explained by the six-minute period (three minutes before the prey was added to the tank, and three minutes after) not being enough for the octopuses to acclimate to the acute elevation in water temperature from their home tank to the experimental tank. Although beyond the scope of this study to analyze quantitatively, when placed octopuses in the experimental tank for a trial at T3, I observed some noticeable signs of acute thermal stress (e.g., globular mantle and increased respiration), but never to the level of studies that have deliberately been testing for CTMax (Noyola et al., 2013) where they witnessed sudden mantle spasm, expulsion of ink, and uncoordinated chromatophore flashes.

Here I do not interpret increased Latency as a decrease in performance, given that octopuses can employ a variety of hunting strategies, including ambush (Villanueva et al., 2017). Rather, increased Latency may indicate that octopuses were detecting the mobile prey from a distance (Maselli et al., 2020), determining how to allocate their energy in a more thermally demanding environment, and waiting to see if the prey would stumble into their arms as witnessed in situ by (Forsythe & Hanlon, 1988).

After touching the prey for the first time, the ADT was affected by an interaction between Crab Activity Level and temperature: the direction of the effect was the same (more active crabs resulting in shorter ADT), but the strength of the affect was much greater at T3. This relationship, clearly seen in Figure 2.6, is driven by the fact that ADT at T1 was nearly always close to zero (indicating that the successful attack was also the first time the octopus had contacted the prey). At T2, and even more distinctly at T3, I show that extended ADTs occurred when the crab was less active but decreased as the crab became more active and increased the possibility of a chance encounter with the octopus that resulted in an opportunistic attack. Therefore, a longer Latency (or TTA, TFT, or ADT*)* may not necessarily translate into decreased food consumption; rather, it may indicate that, at some temperatures, an octopus is more likely to employ one method of food procurement over another and can vary its strategy based on the situation and context. Longer ADT was also often characterized by constant movement around the edges of the tank, possibly searching for thermal refugia. Although substantial site duration has been observed in populations of wild *O. bimaculoides* (Cigliano, 1994), the possibility of behavioral thermoregulation (Angilletta et al., 2002) in terms of seeking a thermally preferable area could have implications on how their daily activity patterns are understood (D. L. Sinn, 2008).

Even though temperature did have significant effect on the probability of whether a particular feeding trial was successful, it is difficult to translate that into a direct measure of feeding performance in the wild, due to the experimental time constraint placed on each trial. Perhaps this species octopus may avoid a highly active prey such as a crab in order to target a more sessile prey item (such as an immobile bivalve) or perhaps they would decrease their feeding rates. Regardless, the octopuses foraging choices would have an impact on the intertidal community's composition and structure (Ambrose, 1982). To evaluate foraging success, I modeled a binary variable coded for whether the octopus successfully captured the crab on its first attempt, implying that an unsuccessful attempt in the wild would have allowed the crab to escape into the rocks. I found that temperature had no effect on Foraging Success, nor did any of the other predictors included in the model despite the fact that Body Velocity and Body Acceleration were both significantly slower at higher temperatures. Although CAL had no relationship with temperature (Appendix A; Pearson correlation coefficient, r = -0.14; Anova, p = 0.29), it is possible that the crabs' ability to escape predation was negatively impacted by the acute temperature change more so than the octopuses' ability to capture them, which has been demonstrated in other marine systems (Allan et al., 2015). Crab prey were acclimated to the same T1 water temperature as the octopuses.

There was no main effect of temperature on Arm Velocity or Acceleration, which were of the same magnitude as previous studies (Gutfreund et al., 1998; Maldonado, 1964). This implies that the neuromuscular activation producing the kinematics of their hydrostat arms (Matzner et al., 2000; Rokni & Hochner, 2002; Sumbre et al., 2005) exhibits less thermal dependence, at least within the thermal range of my experiment. This effect is similar to the lack of thermal dependence in the ballistic mouth opening and tongue extension during prey capture in various reptiles and amphibians (Anderson & Deban, 2012; Deban & Lappin, 2011; Deban & Scales, 2015; Sandusky & Deban, 2012), which are reliant on elastic recoil mechanisms. Even though body kinematics were reduced, the octopuses were able to overcome that impediment and still use their dynamic arms to capture the prey at the same rate regardless of temperature; however, this may not hold true for prey items that exhibit more mobility than crabs. Given that octopuses are generalist predators capable of switching prey items based on abundance (R. C. Anderson et al., 2008; Mather et al., 2012; Murdoch, 1969), there are many factors that contribute to hunting strategies and prey choice such as optimizing energy uptake and minimizing time spent foraging (J. Forsythe & Hanlon, 1997; Hanlon et al., 2007; Hofmeister, 2015; John O’Brien et al., 1989; Stephens & Krebs, 1986). I offer this as further evidence that the thermal profile of an individual’s home range and foraging territory may be included as part of this calculation, as has been shown in other marine predators (Sims et al., 2006).

After the octopus successfully captured a crab, I measured the duration of a series of characteristic actions (pulling the crab under the buccal web, immobilizing it, orienting it towards the mouth) that comprised HT. This flurry of activity typically ended with one obvious large expulsion of water through the mantle cavity, indicating that feeding had begun. In the present study, I show that water temperature had a significant effect on HT, with warmer temperatures resulting in longer handling times (after accounting for the variation in CAL, RPM, and Trial Number). Most of *O. bimaculoides*' other typical prey items (e.g., gastropods, bivalves, and other crustaceans) also have hard chitinous or calcified exteriors that make for difficult access; however, crabs have ten sharp, flailing appendages that may require more time and energy to handle at higher temperatures, especially immediately after capture and before returning to safety. With this in mind, optimal foraging theory would predict that a generalist predator like an octopus may specifically target prey that would minimize this handling time, which could have community structure impacts when scaled up to the population level (R. Ambrose, 1986; Leite et al., 2009; Stephens & Krebs, 1986).

The consideration of individual variation in animal behavior studies is increasingly becoming recognized as vital to understanding the underlying mechanism of the phenomenon being observed, and there is more impetus on researchers, and more tools with which, to examine this variation (Bolker et al., 2009;

Hertel et al., 2020). Individual variability was considered in the present study through the incorporation of random effects in the model for each measured outcome. Depending on the model, I incorporated a random intercept for either cohort, octopus, or octopus within cohort, in order to depict what portion of the overall variance in the response variable could be attributed to among-group differences (also known as intraclass correlation). During model selection, the inclusion of a random slope for Latency and TFT was also supported by examining the Bayesian Information Criteria of the null model vs. the saturated model. ICCs ranged from 13% (arm acceleration) to 68% (ADT), and two of the three response variables showing no effect of temperature (TFT and arm velocity) had considerable ICCs (46% and 31% respectively). Multi-level models, and GLMMs in particular, are a highly flexible class of models that are well-suited for ecological research and the analysis of repeated measures designs in animal studies (Schielzeth et al., 2020; Wang & L.A, 2004). While any further analysis of my particular individual octopuses and how they performed with respect to temperature is beyond the scope of this study, the statistical tools I employed allowed me to account for individual variation but still generalize my results to a larger population (see Appendix B for visualizations of the distribution of random effects with respect to each response variable).

My results showed a main effect of temperature, with no interacting covariates, on four measured response variables: Latency, Body Velocity, Body Acceleration, and HT. With all four variables, I found that there was a linear trend describing the relationship between that particular response and water temperature.

No measure of performance changed directions from T2 to T3 (i.e., I never detected a significant positive effect at T2 and negative effect at T3, or vice versa, for the same response variable); therefore, I may conclude that my experimental temperatures—20 °C and 23 °C—are all well within the thermal tolerance of *O. bimaculoides* acclimated to a seasonal range of 14-17 °C. At both temperature treatments, body velocity was significantly slower and handling time was significantly longer, but latency and body acceleration were both only significantly impacted at T3; therefore, I may also conclude that the thermal optimum of *O. bimaculoides* with regard to feeding behavior is between T1 (14-17 °C) and T2 (20 °C).

While I found that acute temperature change does have a significant effect on various aspects of octopus feeding behavior, my findings do have some limitations that are intrinsic to the animals used in my study. First, due to resource constraints, my octopuses were not reared in the laboratory, but were collected from the wild. Therefore, I cannot control for the prior experiences that they had before arriving at the lab. Once under my care, I made every effort to standardize their experiences in terms of diet, daylight, enrichment, and shielding from conspecifics and other researchers. The two cohorts were collected from the same 500-meter stretch of beach, but cohort 1 was collected in August and cohort 2 was collected in February, meaning they may have been acclimated to slightly different temperatures. However, the two cohorts were kept in the exact same conditions in the lab throughout the course of experimentation, and the inclusion of a random effect for cohort in my models only increased the model fit in one case (TTA).

While I have established that acute thermal elevation impacts octopus Latency, Attack Velocity (body and arm), and HT, the effects of other abiotic environmental factors (e.g., pH, pCO2, and dissolved oxygen) on octopus foraging and prey handling behaviors need to be assessed. This work would also benefit from an examination of the impacts of temperature on prey choice, the physiological aspects of feeding (e.g., energy conversion efficiency and metabolism), and the accumulated effects over time of repeated exposure to sub-lethal thermal stress (as these and other intertidal residents would experience in situ). Further understanding of these kinds of thermal effects will elucidate the impact of the thermal landscape on predator-prey dynamics and community structure in a dynamic, variable environment.

The implications of my study suggest that the different components of *O. bimaculoides* feeding behavior show varying levels of acute thermal dependence and resiliency. While the timelines of global change predictions are far beyond the lifespan of any one octopus, intertidal octopuses will surely experience marked temperature changes in the course of its life (e.g., sea surface temperature anomalies or marine heat waves), or even in a single day while foraging through temporally and spatially variable thermal gradients that are far steeper than any global prediction (Bates et al., 2018; Helmuth et al., 2016). Continuing to study the effects of temperature changes that occur on a more acute level will help paint a more complete picture of intertidal ecosystems and their important predator-prey dynamics.

Conclusions

I have shown that the California two-spot octopus exhibits variable behavior in the contexts of varying biotic and abiotic factors. Given that they are voracious generalist predators, consuming a wide range of vertebrate and invertebrate prey, in Chapter 1, I conducted controlled feeding trials with littleneck clams and striped shore crabs and found that prey type influences the feeding behavior of *O. bimaculoides*. When presented with a sessile prey not capable of retaliation or escape, octopuses exhibited less hesitancy to approach and touch the prey but waited longer to attack and attacked with less velocity and acceleration. On the contrary, when presented with a more mobile prey, octopuses waited longer to assess the situation and approach the prey, but then conducted a more dynamic and decisive attack. I found no differences in the body orientation, arm choice, nor eye usage based on prey type, although within clam trials there was a significant bias towards a lateral body orientation, the anterior arm pairs, and the right side (both eye and arm). Due to the unpredictability of the crab's movement, my data showed that those same biases did not hold during crab trials, when the crab would often move at the last second and the octopus would execute an opportunistic attack based on the crab's new position. I also described a new pattern of octopus locomotion, during which a sinusoidal path is traced along the bottom of the tank. This behavior occurred significantly more often in crab trials than clam trials, and was almost exclusively observed immediately preceding an attack, leading to the conclusion that this behavior was helping to guide a visual attack and corral the more mobile prey.

For Chapter 2, I focused on one prey type and sought to investigate the effects of acute temperature change on feeding behavior. Through controlled feeding trials and a combination of multi-level models, I determined that an acute increase of water temperature does have significant impacts on various aspects of how *O. bimaculoides* searches for, captures, and handles its prey. After controlling for the effects of Crab Activity Level, Relative Prey Mass, and Trial Number, I showed that warmer water significantly impacted the chances of a particular trial ending with a successful predation event within the allotted time, with the warmest temperature resulting in the smallest chance of a successful (or even attempted) attack. In the trials that were successful, warmer water resulted in increased Latency, slower Attack Velocity and Acceleration, and increased HT.

My findings could be enhanced by further considering the effects of individual octopus variation on the different behaviors described herein. Previous work has reported on the distinct personalities and temperaments in a wide range of cephalopods (Mather & Anderson, 1993; Pronk et al., 2010; Sinn et al., 2001; Sinn & Moltschaniwskyj, 2005; Voss, 2016). Therefore, each individual might employ different hunting tactics and prey handling procedures based on its own cognitive abilities, spatial memory, and behavioral preferences (Mather et al., 2012). While beyond the scope of the present study, the individual preferences and strategies of each individual should be considered (Bolnick et al., 2003; Estes et al., 2003) in future work to understand how they contribute to the octopus's complex behavioral suite employed to find, capture, and handle a wide range of diverse prey in highly variable conditions.

Octopuses are inherently fascinating to humans, and have captivated our imaginations for centuries (Nakajima et al., 2018). Beyond fascination, though, with the explosion of octopus research in recent decades (Di Cosmo et al., 2021) we are now using them as models to help answer questions about cognition, neuroscience, evolution, ecology, and ethology. Occupying an ecologically important role as predators and prey, a better understanding of octopus predatory behavior—and how it is affected, both directly and indirectly, by prey characteristics and water temperature—will add a small piece of clarity to the ecological puzzle of a highly variable environment being subject to dynamic future conditions (Anderson et al., 2001; Kroeker & Sanford, 2022). At the very least, the entirety of these findings brings finer resolution to the complex and flexible behavioral repertoire of a voracious and charismatic marine predator.

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CHAPTER 2: TABLES

Table 2.1. Size and sex data for each individual octopus observed in my study.

Octopus	Mass at arrival (g)	Final mass (g)	Sex	Dates tested
Frodo	90.82	116.7	M	08/27/27 – 10/07/21
Merry	50.85	64.52	F	08/30/21 – 10/07/21
Samwise	69.14	87.83	M	08/28/21 – 10/08/21
Pippin	38.10	70.28	F	08/28/21 – 10/26/21
Hank	45.00	70.10	M	02/17/22 – 05/17/22
Harold	20.40	34.10	M	02/17/22 – 05/24/22
Wallace	68.36	114.05	M	02/17/22 – 05/17/22
Plop	97.52	112.1	F	02/17/22 – 04/26/22

Table 2.2. Summary of descriptive statistics aggregated over entire sample.

		Treatment		
		1	**2**	**3**
TTA	Min/Max	7.0 / 846.0	7.0 / 1466.0	7.0 / 1679.0
	Med [IQR]	31.5 [19.5;87.0]	169.5 [18.0;408.2]	224.0 [38.2;702.5]
	Mean (sd)	94.6 (167.2)	288.5 (344.4)	472.2 (531.1)
Latency	Min/Max	1.0 / 51.0	1.0 / 296.0	1.0 / 1030.0
	Med [IQR]	2.0 [1.0;8.0]	2.0 [1.0;11.2]	7.5 [1.0;85.2]
	Mean (sd)	6.8 (9.8)	24.9 (63.3)	121.5 (245.5)
TFT	Min/Max	2.0 / 655.0	5.0 / 860.0	5.0 / 680.0
	Med [IQR]	19.0 [11.0;35.2]	13.0 [9.0;76.0]	18.5 [9.8;28.0]
	Mean (sd)	66.8 (131.5)	100.0 (196.5)	59.1 (130.0)
ADT	Min/Max	0 / 339.0	0 / 1450.0	0 / 1654.0
	Med [IQR]	1.0 [0;9.0]	2.0 [0;160.5]	13.0 [0;305.0]
	Mean (sd)	21.0 (61.0)	163.6 (319.7)	291.6 (509.2)
HT	Min/Max	10.0 / 195.0	11.0 / 179.0	26.0 / 279.0
	Med [IQR]	35.0 [27.0;53.8]	54.5 [36.8;77.0]	85.5 [53.8;129.8]
	Mean (sd)	43.2 (31.5)	64.6 (41.0)	97.4 (55.0)
Body Velo	Min/Max	0.1 / 0.6	0.03 / 0.5	0.04 / 0.5
	Med [IQR]	0.3 [0.2;0.3]	0.2 [0.1;0.3]	0.1 [0.1;0.2]
	Mean (sd)	0.3 (0.1)	0.2 (0.1)	0.2 (0.1)
Arm Velo	Min/Max	0.1 / 0.8	0.2 / 1.0	0.1 / 0.8
	Med [IQR]	0.5 [0.3;0.6]	0.5 [0.4;0.6]	0.4 [0.3;0.6]
	Mean (sd)	0.5 (0.2)	0.5 (0.2)	0.5 (0.2)
Body Accel	Min/Max	0.1 / 7.0	0.4 / 4.4	0.1 / 3.6
	Med [IQR]	1.6 [0.9;2.4]	1.3 [0.8;2.1]	0.9 [0.5;1.7]
	Mean (sd)	1.8 (1.4)	1.5 (1.0)	1.2 (0.9)
Arm Accel	Min/Max	0.4 / 9.9	0.4 / 8.8	0.7 / 9.9
	Med [IQR]	4.1 [2.6;5.8]	5.3 [3.3;6.3]	4.8 [3.2;5.7]
	Mean (sd)	4.4 (2.2)	4.9 (2.2)	4.8 (2.1)
Forage Success	No	8 (25.00%)	10 (31.25%)	14 (43.75%)
	Yes	32 (36.36%)	30 (34.09%)	26 (29.55%)

Table 2.3. Summary of final model characteristics/parameters. Dashes indicate cells that were not applicable to that model.

	Model Characteristics						
Response Variable	**Model Type**	**Family (“link”)**	**Estimation Method**	**Optimizer**	**Covariates**	**Random Effect**	**BIC**
Trial Success	GLM	Binomial (“identity”)	ML	--	RPM, Trial	--	226.06
Forage Success	GLM	Binomial (“identity”)	ML	--	CAL, RPM, Trial	--	161.45
TTA	GLMM	Gamma (“log”)	ML	Nelder-Mead	CAL, RPM, Trial	(1|Cohort)	1548.0
Latency	GLMM	Gamma (“log”)	ML	BOBYQA	CAL, RPM, Trial	(Treatment|Octopus)	965.64
TFT	GLMM	Gamma (“log”)	ML	Nelder-Mead	CAL, RPM, Trial	(Treatment|Octopus)	1268.73
ADT	GLMM	Gamma (“log”)	ML	BOBYQA	CAL, RPM, Trial	(1|Octopus:Cohort)	1169.96
Body Velo.	LMM	--	REML	nloptwrap	CAL, RPM, Trial	(1|Octopus:Cohort)	42.91
Body Accel	LMM	--	REML	nloptwrap	CAL, RPM, Trial	(1|Octopus:Cohort)	96.50
Arm Velo.	LMM	--	REML	nloptwrap	CAL, RPM, Trial	(1|Octopus)	-14.36
Arm Accel	LMM	--	REML	nloptwrap	CAL, RPM, Trial	(1|Octopus)	68.88
HT	LMM	--	REML	nloptwrap	RPM, Trial	(1|Octopus)	48.35

Note: Model fitting was performed with the lme4 R package (v1.1.30; Bates et al., 2015). (1|Octopus) indicates a random intercept for Octopus, (1|Cohort/Octopus) represents a random intercept for Octopus nested within Cohort, and (Treatment|Octopus) is a random slope for Treatment. Acronyms: GLM = General Linear Model, GLMM = Generalized Linear Mixed Model, LMM = Linear Mixed Model, ML = Maximum Likelihood, REML = Restricted Maximum Likelihood, BOBYQA = Bound Optimization BY Quadratic Approximation, CAL = Crab Activity Leve, RPM = Relative Prey Mass.

Table 2.4. Summary of logistic regression model outputs. For each predictor variable, estimate from the model is shown with 95% CI in parentheses. For each level of treatment, comparison was performed against the reference level of Treatment 1. Trial Success was a binary outcome representing whether predation occurred within the 30-minute time allotment, and Forage Success was a binary variable coded from whether an octopus successfully captured the crab with its first attempt. CAL was not included in the model for Trial Success.

	Dependent variable:	
	Trial Success	**Forage Success**
Treatment 2	0.918	-0.320
	(-0.217, 2.053)	(-1.410, 0.770)
Treatment 3	**-1.345*****	-0.419
	(-2.158, -0.532)	(-1.506, 0.667)
CAL		0.313
		(-0.138, 0.764)
Relative Prey Mass	0.073	0.220
	(-0.267, 0.412)	(-0.220, 0.661)
Trial Number	0.050	-0.096
	(-0.056, 0.157)	(-0.238, 0.046)
Constant	**1.198*****	**1.387*****
	(0.551, 1.844)	(0.594, 2.180)
Observations	179	120
Log Likelihood	-100.062	-66.362
Akaike Inf. Crit.	210.124	144.725

Note: See Table 2.3 for information about model characteristics. $^{*}p < 0.05$ $^{**}p < 0.01$ $^{***}p < 0.001$

Table 2.5. Summaries of mixed model outputs for the timing response variables measured before an attack.

	Dependent variable:			
	TTA	**Latency**	**TFT**	**ADT**
Treatment 2	**1.185*****	0.112	0.388	**1.659*****
	(0.721, 1.649)	(-0.829, 1.052)	(-0.580, 1.357)	(0.824, 2.495)
Treatment 3	**1.624*****	**2.484*****	0.177	**1.916*****
	(1.158, 2.090)	(1.683, 3.285)	(-0.970, 1.325)	(1.024, 2.808)
CAL	-0.099	-0.144	0.078	-0.185
	(-0.523, 0.325)	(-0.465, 0.178)	(-0.227, 0.382)	(-0.756, 0.387)
RPM	-0.256	**-0.245***	0.129	0.196
	(-0.602, 0.090)	(-0.532, 0.041)	(-0.133, 0.391)	(-0.122, 0.515)
Trial Number	**-0.113***	0.116	**-0.217*****	-0.055
	(-0.238, 0.012)	(-0.051, 0.282)	(-0.353, -0.080)	(-0.262, 0.153)
T2: CAL	-0.381			-0.393
	(-0.914, 0.153)			(-1.192, 0.406)
T3: CAL	**-0.820*****			**-0.963****
	(-1.385, -0.256)			(-1.741, -0.184)
T2: RPM	**0.821*****			
	(0.293, 1.350)			
T3: RPM	0.342			
	(-0.128, 0.813)			
Intercept	**4.206*****	**1.795*****	**3.652*****	**2.654*****
	(3.214, 5.197)	(1.268, 2.322)	(2.877, 4.427)	(0.798, 4.510)
Random Effects				
σ^2	1.11	1.68	1.60	3.29
τ_{00}	0.50_{Cohort}	0.38_{Octopus}	1.7_{Octopus}	$1.9_{\text{Octopus:Cohort}}$ 4.91_{Cohort}
τ_{11}		$1.9_{\text{Octopus:T2}}$ $0.9_{\text{Octopus:T3}}$	$2.4_{\text{Octopus:T2}}$ $3.5_{\text{Octopus:T3}}$	
ρ_{01}		0.66 -0.29	-0.60 -0.92	
ICC	0.31	0.18	0.46	0.68
Marginal R^2 / Conditional R^2	0.411 / 0.595	0.483 / NA	0.047 / 0.483	0.124 / 0.717

Note: See Table 2.3 for information about model characteristics. $^{*}p < 0.05$ $^{**}p < 0.01$ $^{***}p < 0.001$

Table 2.6. Summaries of mixed model outputs for the four kinematic attack variables and Handling Time. Colons indicate interactions between terms, and blank cells indicate a predictor or effect that was not included in the final model.

	Dependent variable:				
	Body Velocity	**Body Accel**	**Arm Velocity**	**Arm Accel**	**HT**
T2	**-0.087***	-0.022	0.021	0.066	**0.182*****
	(-0.182, 0.008)	(-0.142, 0.097)	(-0.048, 0.090)	(-0.044, 0.177)	(0.080, 0.284)
T3	**-0.246*****	**-0.181*****	0.0003	0.073	**0.385*****
	(-0.343, -0.148)	(-0.304, -0.059)	(-0.071, 0.071)	(-0.040, 0.186)	(0.281, 0.488)
CAL	-0.005	-0.014	0.013	0.027	
	(-0.049, 0.038)	(-0.068, 0.041)	(-0.019, 0.045)	(-0.022, 0.076)	
RPM	0.009	0.013	-0.010	-0.009	-0.003
	(-0.032, 0.049)	(-0.038, 0.063)	(-0.040, 0.019)	(-0.056, 0.037)	(-0.046, 0.040)
Trial	-0.008	-0.002	**-0.038****	-0.005	0.021
	(-0.034, 0.017)	(-0.033, 0.030)	(-0.073, -0.003)	(-0.034, 0.025)	(-0.006, 0.048)
T2: Trial			0.032		
			(-0.016, 0.080)		
T3: Trial.			**0.061*****		
			(0.016, 0.106)		
Intercept	-0.639***	0.132	-0.363***	0.564***	1.560***
	(-0.928, -0.349)	(-0.150, 0.414)	(-0.450, -0.276)	(0.461, 0.667)	(1.445, 1.676)
Random Effects					
σ^2	0.05	0.07	0.02	0.06	0.05
τ_{00}	0.01 $_{\text{Oct:Cohort}}$	0.03 $_{\text{Oct:Cohort}}$	0.01 $_{\text{Octopus}}$	0.01 $_{\text{Octopus}}$	0.02 $_{\text{Octopus}}$
	0.04 $_{\text{Cohort}}$	0.03 $_{\text{Cohort}}$			
ICC	0.51	0.44	0.31	0.13	0.24
Marg. R^2 / Cond. R^2	0.104 / 0.561	0.051 / 0.467	0.052 / 0.346	0.023 / 0.149	0.275 / 0.450

Note: See Table 2.3 for information about model characteristics. $^{*}p < 0.05$ $^{**}p < 0.01$ $^{***}p < 0.001$

CHAPTER 2: FIGURES

Figure 2.1. The eight participants in my study, clockwise from top left: Frodo, Sam, Merry, Pippin, Hank, Harold, Wallace, and Plop.

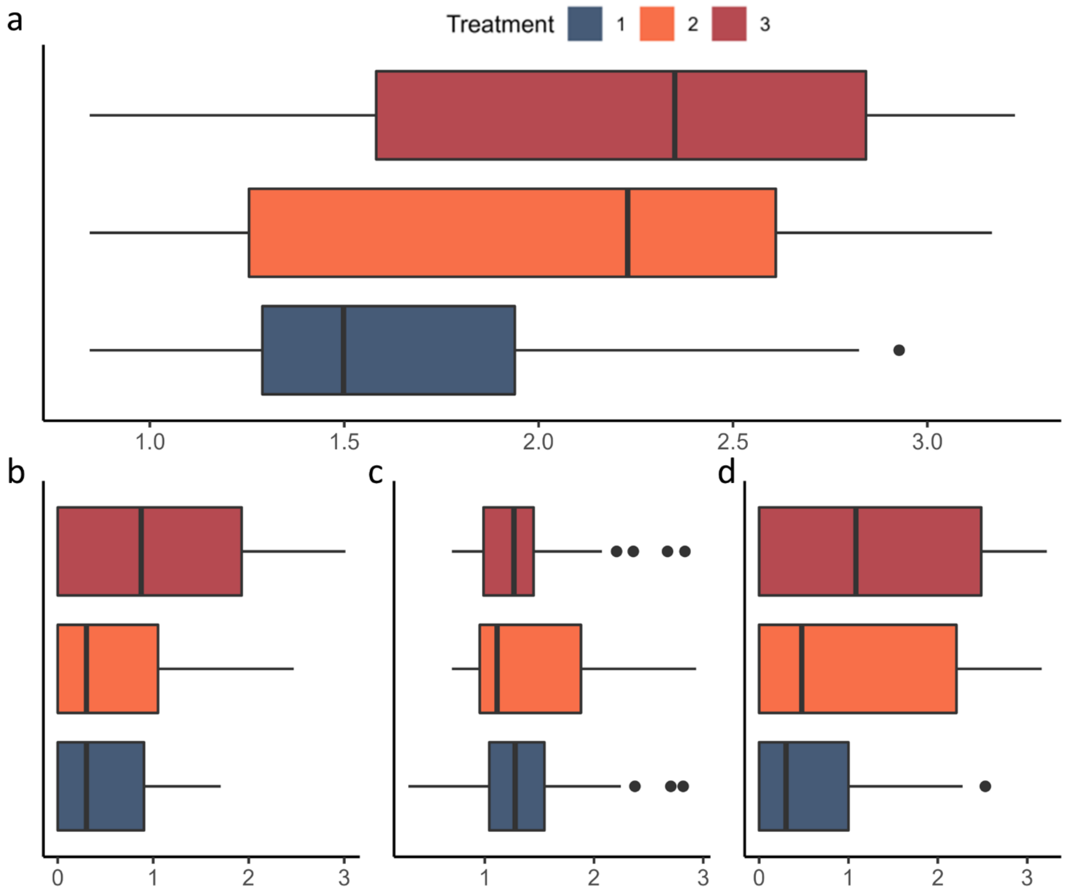


Figure 2.2. Raw data visualization of the four timing variables leading up to an attack: (a) total time to attack (TTA), which is comprised of the other three components, (b) latency, (c) time to first touch (TFT), and (d) attack decision time (ADT). Treatments correspond to a baseline of 14-17 °C, 20 °C, and 23 °C and, for easier visualization, the x-axis is showing the log-transformation of seconds measured for each variable. All temporal measurements of behavior, all four responses seen here are zero-bounded and positively skewed, and could not be transformed to a normal distribution, so non-parametric methods of analysis were used.

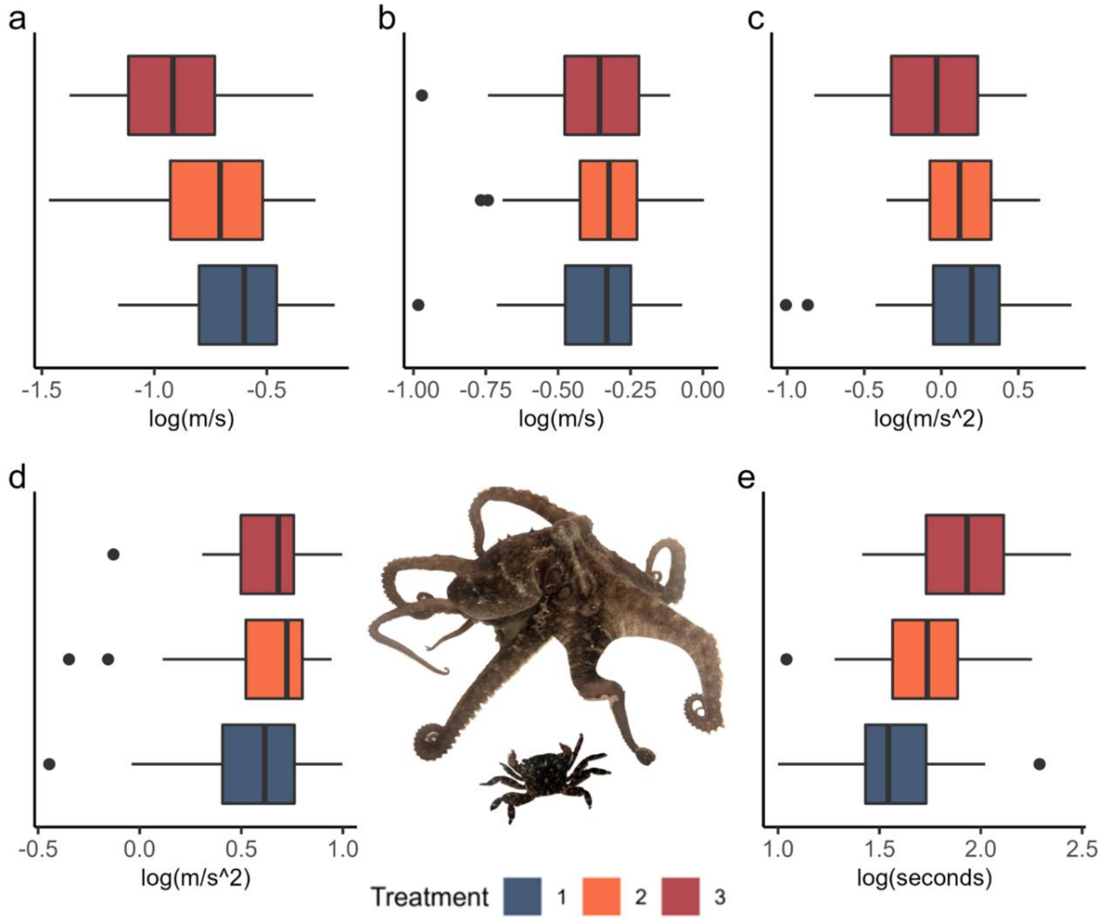


Figure 2.3. Raw data visualization of the four kinematic attack variables and handling time: (a) body velocity, (b) arm velocity, (c) body acceleration, (d) arm acceleration, and (e) handling time. Treatments correspond to a baseline of 14-17 °C, 20 °C, and 23 °C and, for easier visualization, the x-axis is showing the log-transformation of each measured response variable.

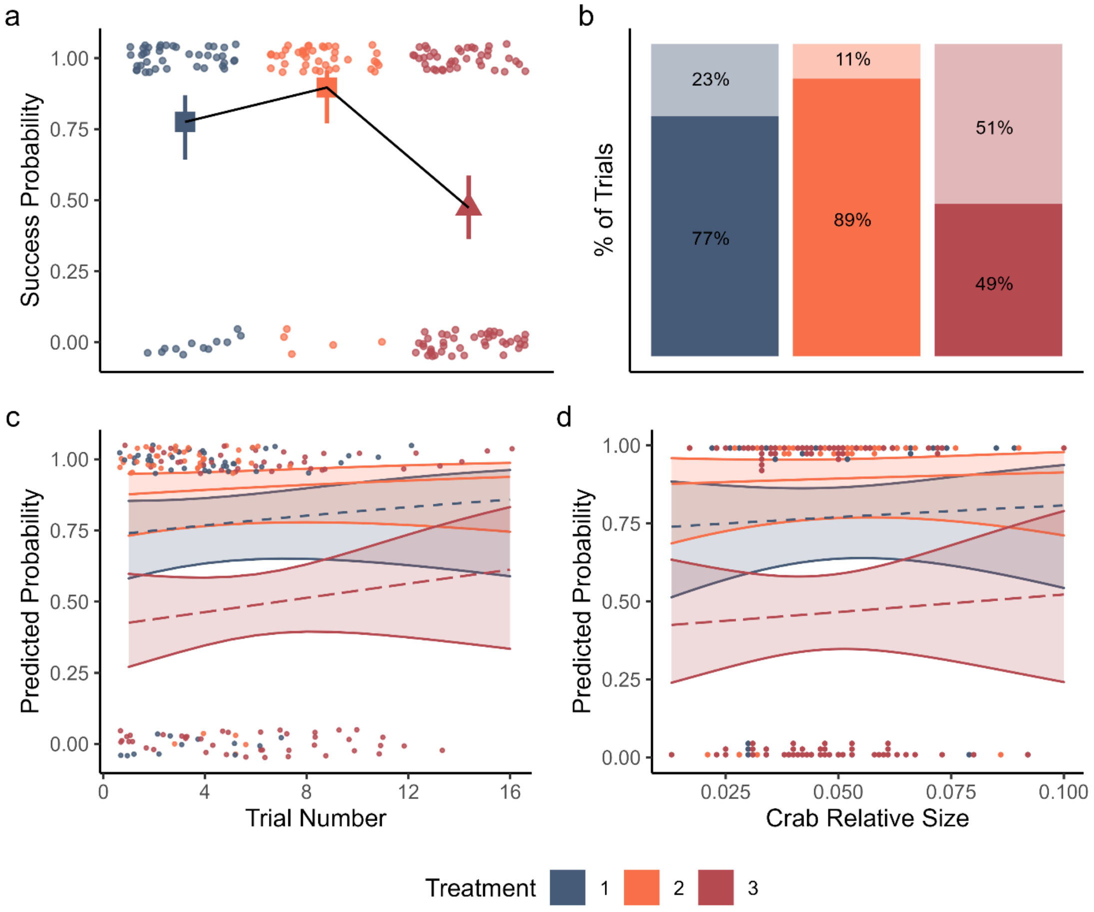


Figure 2.4. Probability of a successful feeding trial, modeled by treatment level, trial number, and relative prey mass, where treatments correspond to a baseline of 14-17 °C, 20 °C, and 23 °C. (a) Estimated marginal probability of success for each treatment level, controlling for all other predictors, where temperature was shown to have a significant effect of trial success. Treatment means represented by different shapes are significantly different at the $p < 0.05$ level. (b) Percentages of successful and unsuccessful trials for each treatment level. (c) Models of predicted probability of success by trial number, controlling for other predictors. (d) Models of predicted probability of success by relative prey size (ratio of crab mass to octopus mass), controlling for other predictors. In parts (a), (c), and (d), actual data points are shown. In parts (c) and (d), parallel lines for each treatment indicate the main effect of treatment with no interactions (Table 3).

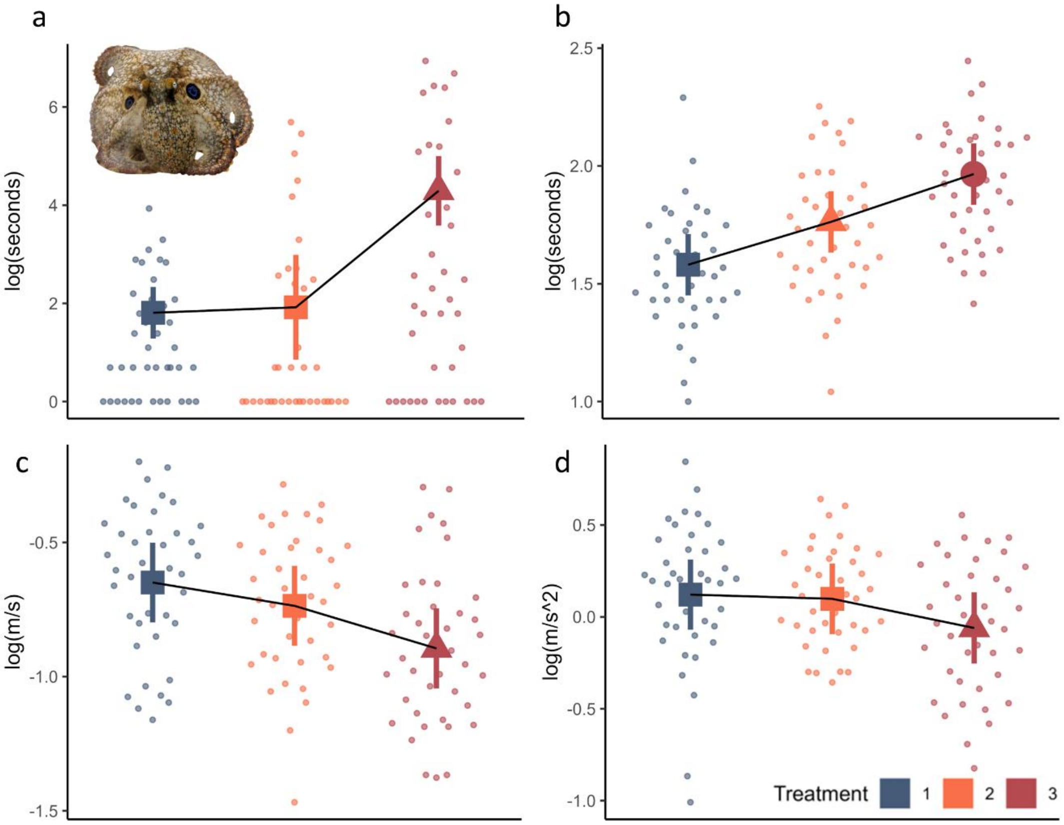


Figure 2.5. Predicted marginal means for the four models that showed a main effect of treatment with no interactions: (a) latency, (b) handling time, (c) body velocity, (d) body acceleration. Points represent the actual data, and treatment means represented by different shapes are significant at the $p < 0.05$ level. Latency was fit with a GLMM with a log-link function, and therefore the data shown here are the predictions from the model, not inverse-transformed. Handling time, body velocity, and body acceleration were log-transformed prior to model fitting, and therefore the transformed data is shown here as well. Despite the appearance to the contrary (caused by the transformations), all four response variables in this figure were found to vary linearly with temperature. Treatments correspond to a baseline of 14-17 °C, 20 °C, and 23 °C.

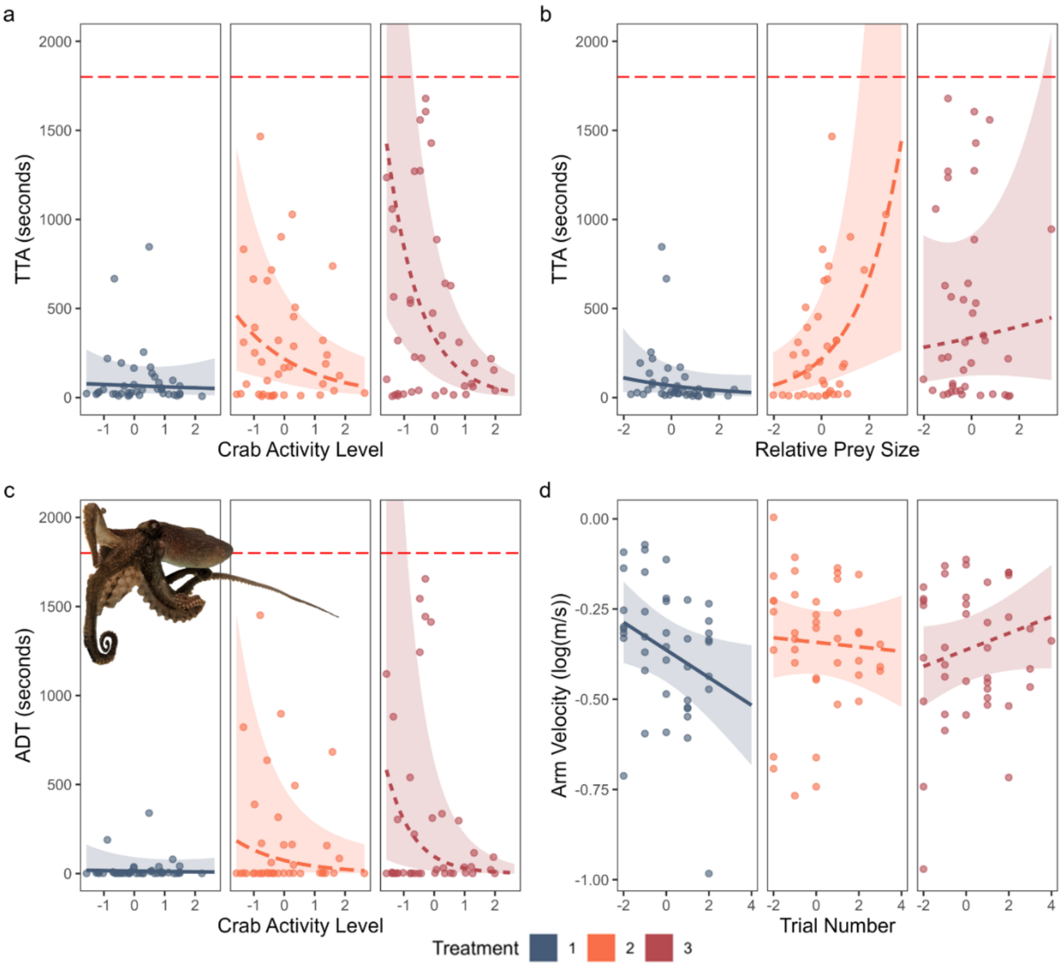


Figure 2.6. Outputs from the three models that showed interactions with treatment. For TTA, temperature was found to have a significant interaction with (a) crab activity level (CAL) and (b) relative prey mass (RPM). CAL had a significant negative effect on TTA at treatment 3, while RPM had a significant positive effect on TTA at treatment 2. (c) CAL had a significantly negative effect on ADT at treatment 3 but not treatment 2. (d) The relationship between trial number and arm velocity was significant and positive at treatment 3, but not treatment 2. Treatments correspond to a baseline of 14-17 °C, 20 °C, and 23°C, and the red dashed line in (a), (b), and (c) indicates 1800 seconds, where a trial was stopped if there had not yet been a successful predation event.

Appendix A – Additional Figures

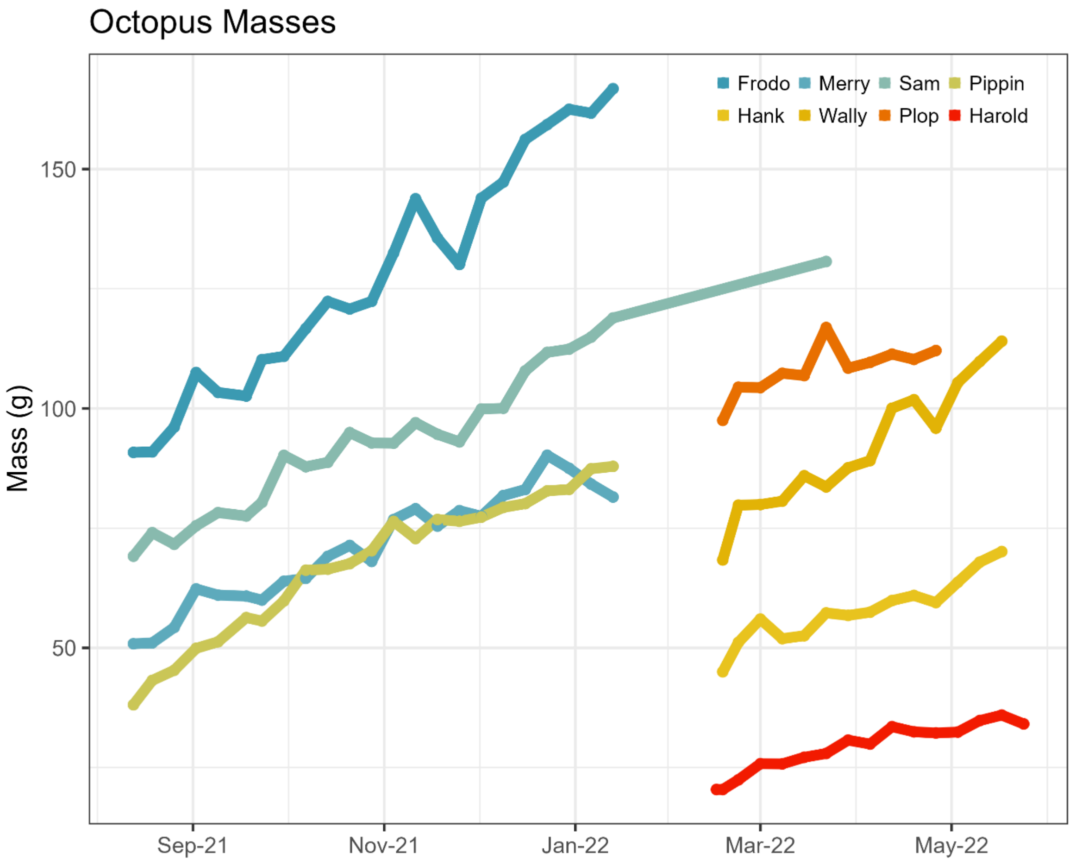


Figure A1. Octopus masses over the course of experimentation.

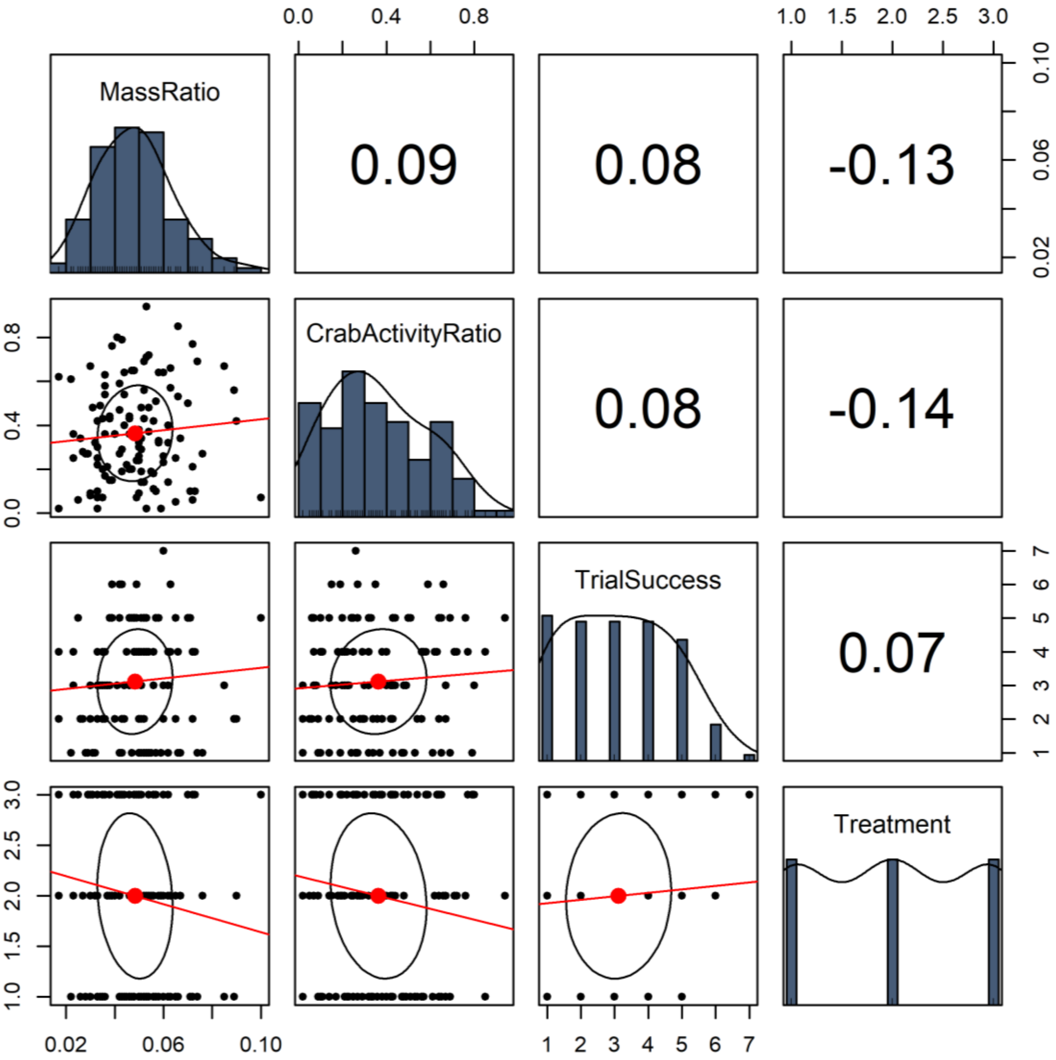
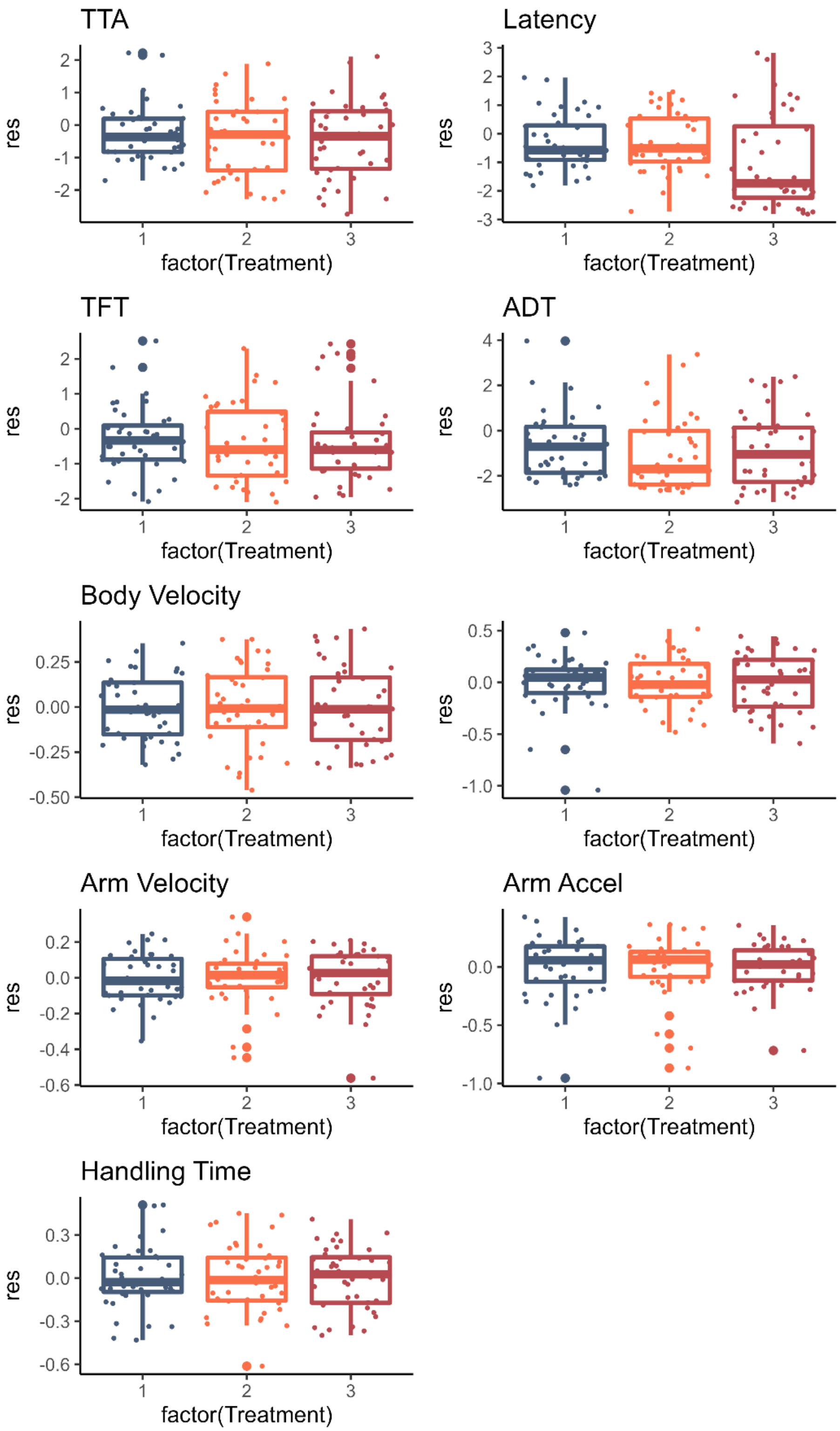


Figure A2. Correlation plot of covariates included in models for Chapter 2.



TTA

Latency

TFT

ADT

Body Velocity

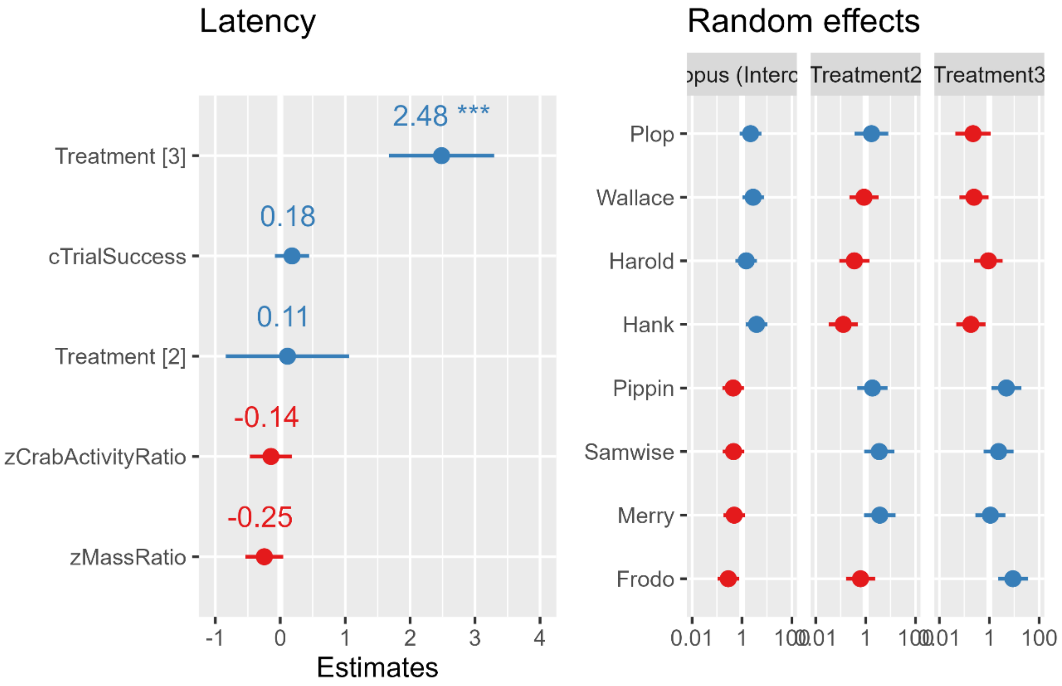
Arm Velocity

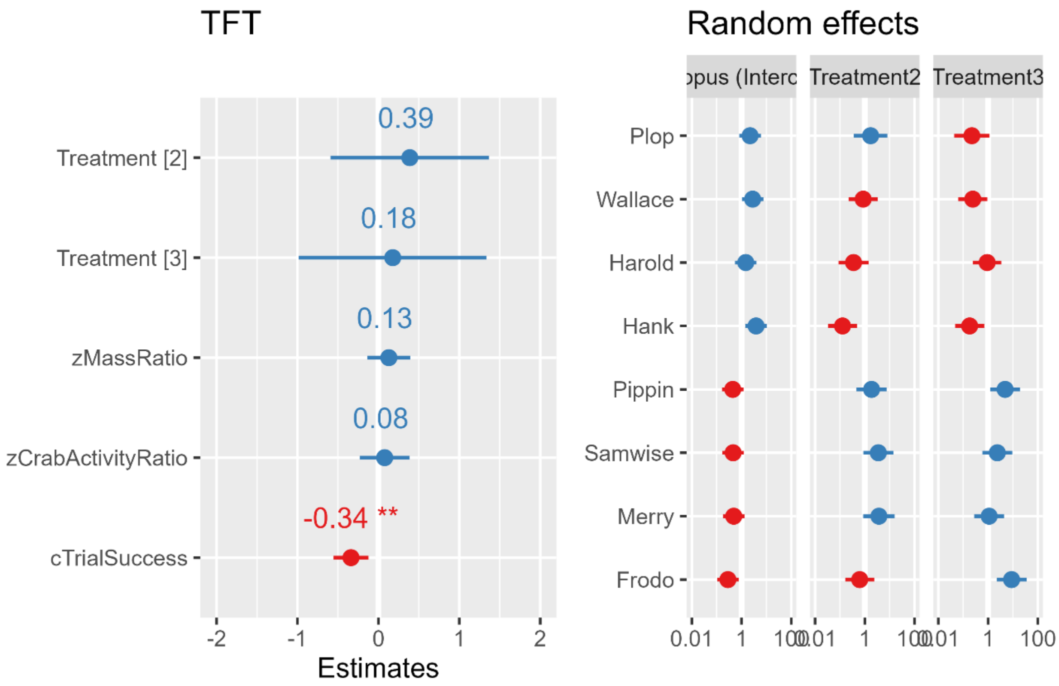
Arm Accel

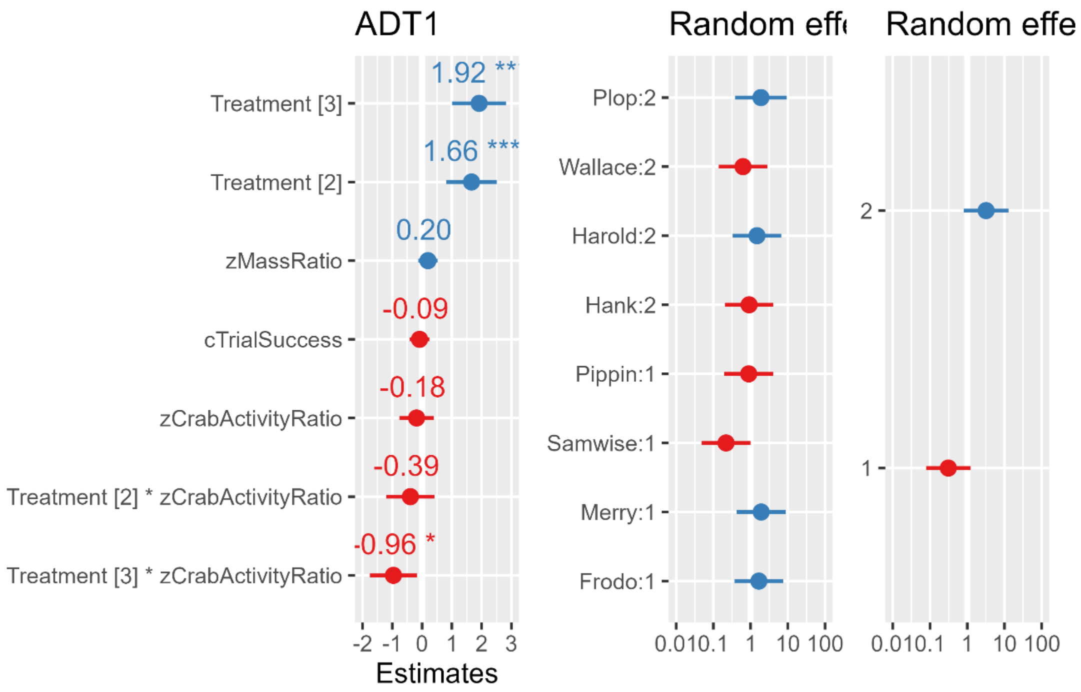
Handling Time

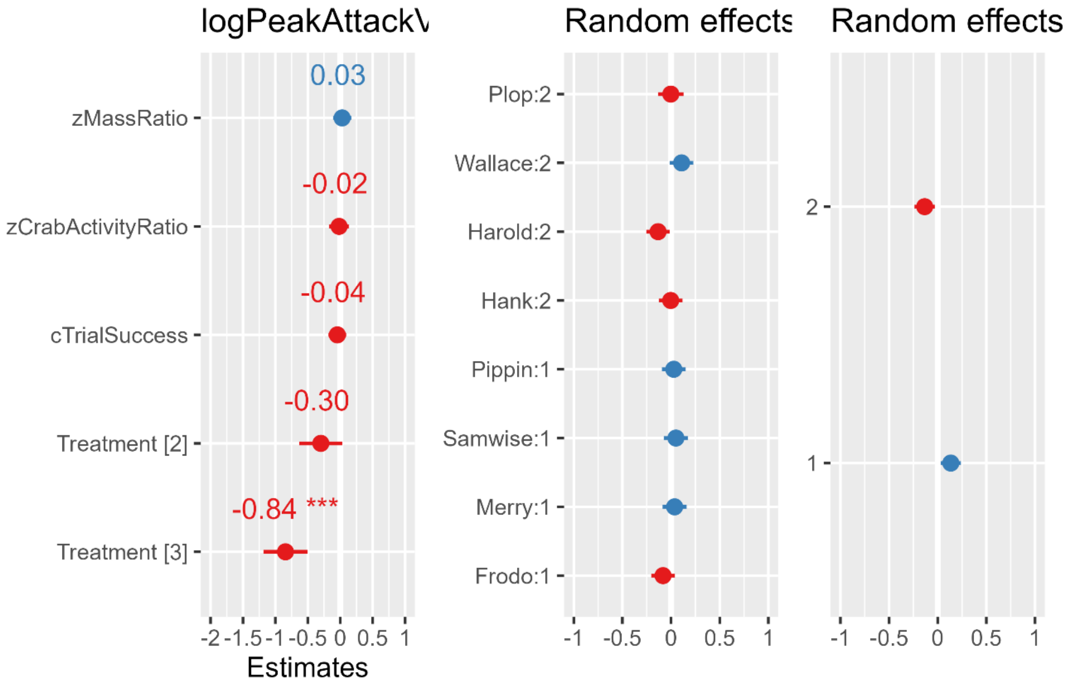
Figure A3. Boxplots of residuals from the mixed models employed in Chapter 2.

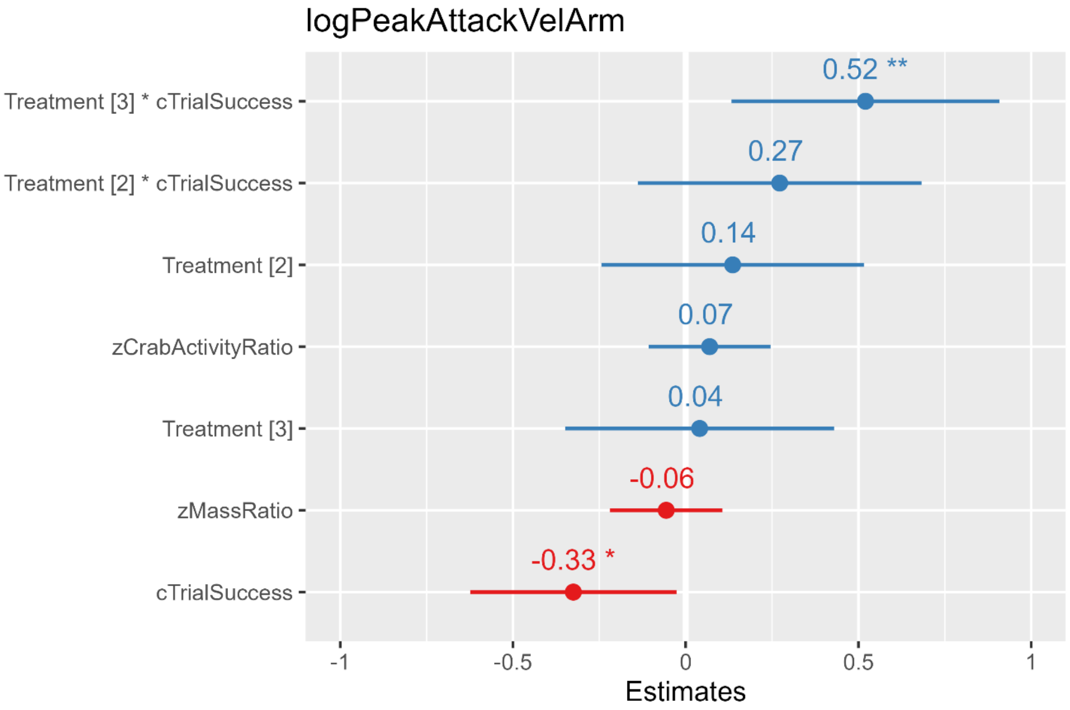
Appendix B – Visualizations of Fixed and Random Effects

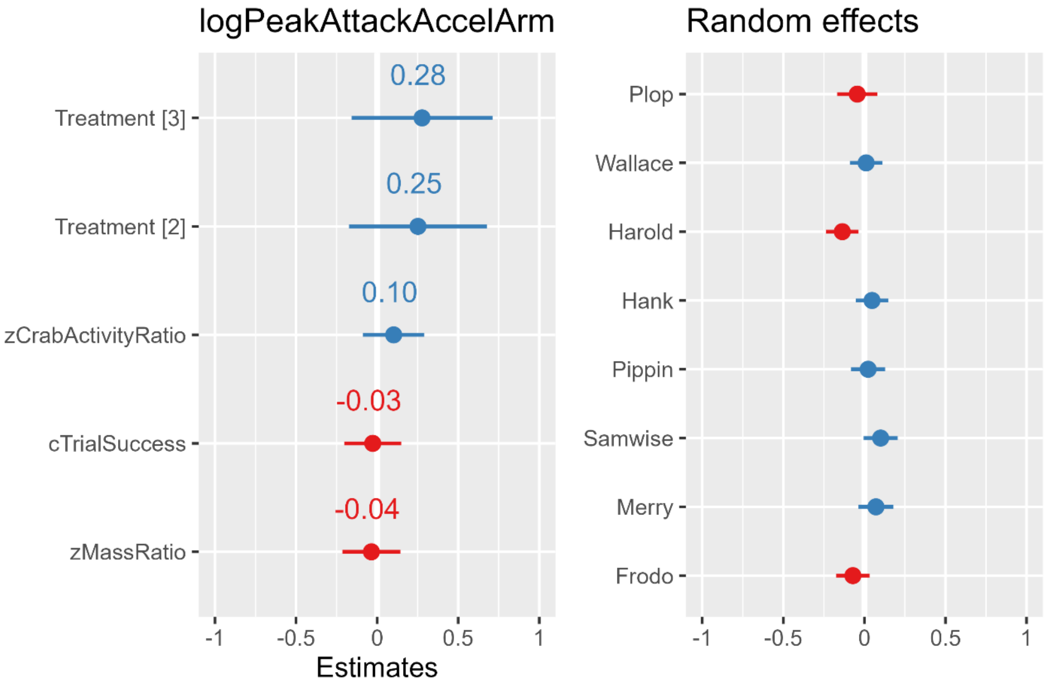


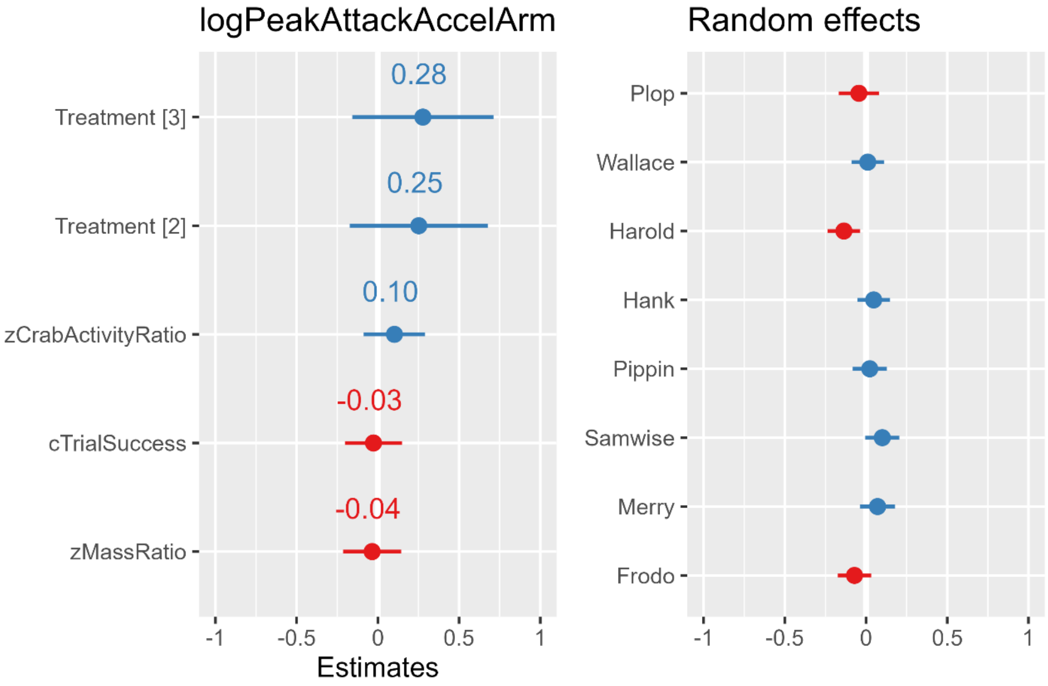

TFT
Random effects
opus (Interc
Treatment2
Treatment3
Treatment [2]
Treatment [3]
zMassRatio
zCrabActivityRatio
cTrialSuccess
0.39
0.18
0.13
0.08
-0.34 **
-2
-1
0
1
2
Estimates
Plop
Wallace
Harold
Hank
Pippin
Samwise
Merry
Frodo
0.01
1
100
0.01
1
100
0.01
1
100

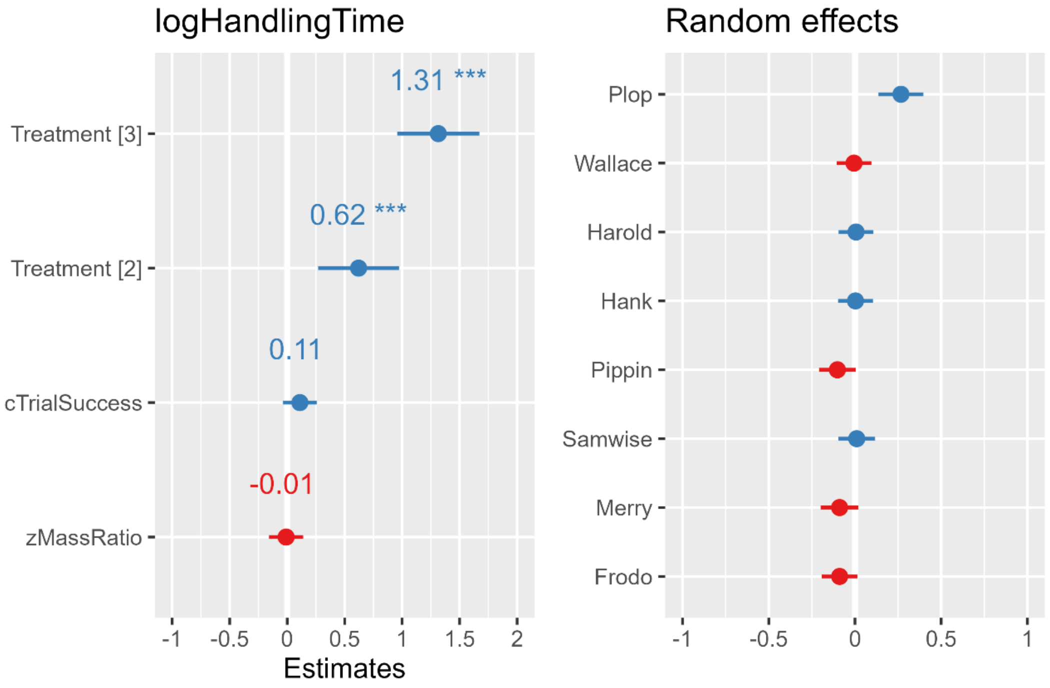

ADT1
Random eff
Random effe
Treatment [3]
Treatment [2]
zMassRatio
cTrialSuccess
zCrabActivityRatio
Treatment [2] * zCrabActivityRatio
Treatment [3] * zCrabActivityRatio
1.92 **
1.66 ***
0.20
-0.09
-0.18
-0.39
-0.96 *
-2
-1
0
1
2
3
Estimates
Plop:2
Wallace:2
Harold:2
Hank:2
Pippin:1
Samwise:1
Merry:1
Frodo:1
0.01
0.1
1
10
100
2
1
0.01
0.1
1
10
100


logPeakAttackV\
Random effects
Random effects
0.03
zMassRatio
-0.02
zCrabActivityRatio
-0.04
cTrialSuccess
-0.30
Treatment [2]
-0.84 ***
Treatment [3]
-2 -1.5 -1 -0.5 0 0.5 1
Estimates
Plop:2
Wallace:2
Harold:2
Hank:2
Pippin:1
Samwise:1
Merry:1
Frodo:1
-1 -0.5 0 0.5 1
2
1
-1 -0.5 0 0.5 1


logPeakAttackVelArm
0.52 **
Treatment [3] * cTrialSuccess
0.27
Treatment [2] * cTrialSuccess
0.14
Treatment [2]
0.07
zCrabActivityRatio
0.04
Treatment [3]
-0.06
zMassRatio
-0.33 *
cTrialSuccess
-1 -0.5 0 0.5 1
Estimates


logPeakAttackAccelArm
Treatment [3]
Treatment [2]
zCrabActivityRatio
cTrialSuccess
zMassRatio
0.28
0.25
0.10
-0.03
-0.04
-1
-0.5
0
0.5
1
Estimates
Random effects
Plop
Wallace
Harold
Hank
Pippin
Samwise
Merry
Frodo
-1
-0.5
0
0.5
1


logPeakAttackAccelArm
Treatment [3]
Treatment [2]
zCrabActivityRatio
cTrialSuccess
zMassRatio
0.28
0.25
0.10
-0.03
-0.04
-1
-0.5
0
0.5
1
Estimates
Random effects
Plop
Wallace
Harold
Hank
Pippin
Samwise
Merry
Frodo
-1
-0.5
0
0.5
1


logHandlingTime
Random effects
1.31 ***
0.62 ***
0.11
-0.01
Treatment [3]
Treatment [2]
cTrialSuccess
zMassRatio
Plop
Wallace
Harold
Hank
Pippin
Samwise
Merry
Frodo
-1
-0.5
0
0.5
1
1.5
2
Estimates
-1
-0.5
0
0.5
1