

DIGGING IN: AN INVESTIGATION OF BURROWING BEHAVIOR IN
MUUSOCTOPUS LEIODERMA

by

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ABSTRACT

Burrowing, or movement through soft sediment to form semi-permanent structures, is a common but understudied behavior with large impacts on marine ecosystems. Octopus are a charismatic and intelligent invertebrate group that are expanding in both range and numbers. This thesis focused on *Muusoctopus leioderma*, a typically deep-water and inaccessible burrowing octopus with a shallow-water population in Burrows Bay, WA. This study aimed to explore octopus burrowing by examining *M. leioderma*'s burrowing behavior, comparing burrowing performance across taxa, and exploring certain morphological traits linked with burrowing. Comparison of burrowing rate indices between *M. leioderma* and *Octopus kaurua* showed similar burrowing rates in the only two recorded burrowing octopus species, despite differences in temperature and sediment size. Study of *M. leioderma* in the lab corroborated field observations of nocturnal activity, with individuals burrowing at consistent times. Finally, I found phylogenetic evidence of an evolutionary link between known burrowing behavior in benthic octopus species and the keel, a morphological trait colloquially connected to burrowing octopus species. This connection between the keel and burrowing was also supported in an examination of *M. leioderma*, with non-burrowing individuals losing their keel more rapidly and consistently than burrowing individuals.

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INTRODUCTION

Marine sediments are the most expansive and pervasive environments on Earth.¹ These seemingly barren underwater habitats make up roughly 70% of Earth's surface, yet their depths and temperatures make them the least accessible environments possible for humans.¹ These habitats host a diverse range of marine life, and are largely dominated by burrowing organisms, organisms that live below the surface of the sediment bed. Little is known about the behavior and ecology of these marine infauna, as the immense difficulty of examining these habitats has stymied their research.¹

Burrowing organisms utilize diverse strategies to burrow in marine sediments, and modes of excavation depend largely on the strata the organism needs to penetrate.² Muddy sediments are characterized by adhesive (attractive) and cohesive (resisting separation) forces holding grains together with a degree of elasticity.¹ In less pliable sediments such as dense mud, polychaetes burrow by fracture, entering cracks in mud and widening these into larger burrows by exerting force from its body.¹ However, softer, more porous muds require less forceful methods, such as plastic granular rearrangement, where organisms physically displace or move sediment out of their way,² or fluidization, where sediment granules are separated and dispersed in water.²

Octopuses are a diverse and prevalent group of invertebrates found in most marine environments. Charismatic, highly intelligent, and displaying a wide range of novel adaptations for invertebrates, this group plays an important role, both in ecological systems, and in facilitating public engagement and interest in science.³ Octopuses act as both generalist predators and prey, playing crucial roles in trophic webs and ecological health.⁴⁻⁶ Cephalopods, including octopuses, are also expanding in range and population.⁶⁻⁹ This expansion suggests that their biological significance will continue to grow in the future, and sparks questions about octopus' environmental resilience and adaptation to anthropogenic disruption.

The smooth-skinned octopus (*Muusoctopus leioderma*) is a deep-water species and most commonly found between 450-650 meters¹⁰ throughout the northern Pacific ocean from California up to the Sea of Okhotsk off Siberia, and until recently had never been recorded at depths shallower than 70 meters.^{11,12} Yet a population of *M. leioderma* resides in Burrows Bay, WA, an accessible area only 15 meters deep.¹³ This exciting discovery allows for collection and study of live and minimally disturbed benthic octopuses via SCUBA that would normally be inaccessible.

Observation of *M. leioderma* revealed a novel behavioral trait among octopuses. This species is an ecosystem engineer that actively modifies its environment by burrowing, an adaptation allowing organisms to move through and compact soft substrata to form semi-permanent structures.² Burrowing organisms have an unusual level of influence on their environment by directly altering marine sediment biochemistry and structure,¹⁴ and indirectly by impacting co-occurring bacteria, microalgae, macrofauna, seagrasses, and other secondary consumers affected by sediment nutrition.¹⁴ The bioturbation of sediments from burrowing can result in microbiomes ideal for certain microbes and meiofauna that drive nutrient cycling processes.¹⁴ Burrows can also create problems for grazers, filter feeders, and large invertebrates reliant on undisturbed sediments¹, and *M. leioderma*'s presence may exert similar levels of influence.³

Burrowing is a common behavior in both marine and terrestrial organisms, and is used as a strategy for concealment, predation, energetic conservation, and social dynamics.¹⁵⁻²⁴ Octopuses are known to burrow as a means to avoid predation,²⁵ but the function of burrowing for *M. leioderma* is still unclear.

These revelations form the basis of several questions circling this species. How and why does this octopus burrow? Why does this octopus burrow when other species in the area do not? How does this octopus compare with other marine burrowers? This

species has only been spotted at night, and the highly silty marine sediment bed it occupies makes observation difficult. This thesis represents the first in-depth investigation of the burrowing behavior and performance of *Muusoctopus leioderma*, the first exploration of the keel as a burrowing trait, and one of only two in-depth studies of burrowing in any octopus species.²⁵ I hope that this research may improve our understanding of the biology of octopuses as a crucial group of benthic organisms, as well as the ecological impact of the understudied marine infauna that occupy so much of the global ocean.

Factors Influencing Burrowing

Burrowing in marine organisms is influenced by a range of both biotic and abiotic factors. Burrowing can be quantified and analyzed using measures such as burrow size and shape, the time it takes for a species to burrow, energetic expenditure while burrowing, and ecological impact of the burrows.^{2,26-29} The variables influencing these measurements have different effects on burrowing performance. Burrowing rate is one of the most widely used metrics for evaluating burrowing performance, as it is easily standardized to account for other factors that impact burrowing rate, like body size, making it ideal for interspecific comparison.³⁰

The effect of species has the strongest influence on burrowing rate. For example, worms and crabs have vastly different strategies, morphologies, and abilities allowing those organisms to successfully descend into the sediment layer. Octopuses are cephalopods, a group known for their biological eccentricities. The lack of hard bodily structures (other than the beak), their siphons, their unique skin texture and environmental resilience could potentially give octopuses an edge in developing a successful burrowing strategy. However, these features also make them starkly distinct

from other burrowers, making interspecific comparisons of burrowing performance more of a challenge.

Temperature is another factor known to influence virtually all biological activity and performance, and burrowing is no exception.^{31,32} Low temperatures tends to slow aerobic activity and reduce metabolic work capacity, and consequently there are slower burrowers at higher latitudes.³³

Probably the most significant abiotic factor is the sediment the organism burrows in. Sediments largely dictate the strategies and mechanisms of burrowing, as different sediments will require different solutions. Hard-packed sediments require more forceful tactics such as fracture and crack propagation, while looser sediments like granular sands can be fluidized and/or manipulated by body movement and excavation.^{1,2} Such variety in burrowing sediments means that every burrower expends different amounts of time and energy during the burrowing process, resulting in a wide range of interspecific burrowing rates. *Muusoctopus leioderma* occupies a silty, muddy sediment bed. These soft muds are highly adhesive and cohesive granular solids, and require a strategy of plastic granular rearrangement, or the displacement of sediment grains relative to each other, as crack propagation in such a fine mud would be unfeasible.^{2,26} While another burrowing octopus, *Octopus kaurna*, uses its siphon to fluidize sediment in granular sands, the specific mechanics of *M. leioderma*'s burrowing remains unknown²⁵.

Burrowing rates are also impacted by body size. Larger organisms are forced to displace a correspondingly larger amount of sediment for their burrows, as burrow size is normally proportional to body size.^{34,35} Additionally, the energetic cost of burrowing is proportional to the amount of sediment the organism removes,³⁵ meaning smaller species expend less energy while burrowing. Finally, the inverse relationship between burrowing speeds and energetic expenditure³⁵ translates to a trend of smaller burrowers displacing less sediment, using less energy, and burrowing more quickly compared with

larger species. This trend between burrowing rates and body size is not unique to marine burrowers, with a review of over 50 terrestrial mammals showing slower excavation rates (volume of soil removed per unit land per unit time) in larger species.³⁴ Meanwhile, smaller terrestrial burrowing species remove more soil per unit time and area, meaning smaller species not only burrow faster, but also create more burrows.³⁴ It is important to note that because interspecific burrowing performance is variable and subject to so many factors, there are many exceptions to this basic trend between body size, burrowing energetics, and burrowing rates.³⁵

The variability in burrowing performance makes interspecific comparison a challenging prospect. However, by focusing on how some of these factors influence burrowing, I may reveal performance trends across taxa.

Circadian Rhythms and Burrowing

The time of day an organism is active is an important factor to consider when examining its ecological impact, as chronobiology can dictate strategies for predation, navigation, reproduction, and migration.³⁶ While biological clocks of marine organisms can be influenced by tides, wave splash, and seasonal environmental changes, most research into daily activity cues focus on light.^{36,37}

Research into chronobiological influences on cephalopods is well established. Humboldt squid (*Dosidicus gigas*) and the veined squid (*Loligo forbesii*) will drop low into hypoxic zones of the water column, allowing them to avoid predation and target prey at ideal times.^{38,39} This daily migration pattern seems to largely be set by light, allowing the squid to avoid visually-dependent predators, then move up in the water column to feed during darker hours.³⁹

Octopuses' natural activity patterns vary depending on species and environment. For example, *Callistoctopus macropus* is a nocturnal species, and its activity cycle is

highly light-dependent.³⁷ Although current research is still limited, light appears to be a primary environmental cue of circadian rhythms in octopuses occupying the photic zone, with some evidence suggesting deep-water species are influenced by light as well.^{37,40} However, it is unclear how light could impact biological clocks in aphotic environments. *Muusoctopus leioderma*'s typical depth range (450-650 meters) is within the aphotic zone^{10,41}, so it is possible the species' nocturnal activity is triggered by other cues.

Typical adaptive values of temporal rhythms revolve around interspecific interactions. Benthopelagic coupling is a dynamic in which there are daily movements of organic material by animal movement back and forth between pelagic and benthic environments, and this phenomenon can influence biological clocks of the organisms that consume them.⁴² For larger organisms, the avoidance of predators, controphic competitors, and the targeting of prey at ideal hours can dictate activity cycles.⁴² Octopus diurnal patterns have been hypothesized to be influenced by temporal spacing, with one study finding three sympatric Hawaiian species were utilizing different active times to coexist.⁴³ *Octopus vulgaris* has been a species of chronobiological controversy, with early studies reporting the species as nocturnal, but later studies showing diurnal activity under laboratory investigation.³⁷ This difference is due to *O. vulgaris*'s temporal plasticity, with the octopus modifying its temporal cycle in response to both researchers in the lab, and predators in nature.^{37,44}

The Burrows Bay population of *M. leioderma* has only been spotted at night, but there is virtually no published data on the temporal behavior of this species in a lab setting, and only one published study on the chronobiology of any deep-water octopus.⁴⁰ It is uncertain, as a shallow-water population, whether light will influence this population differentially from deep-water individuals, or if light will act as a daily environmental cue at all. However, a light-controlled study of this octopus may reveal hidden details, such as temporal plasticity or further confirmation of nocturnal behavior. Whether this behavior

is driven by engrained circadian rhythms, or is merely an avoidance of light is unclear. Regardless, by examining the temporal patterns of behavior in this species, we may determine the ecological impact of this species' burrowing.

Functions, Adaptations & Burrowing

There are several hypotheses explaining why an organism might burrow, and these vary based on the species and environment. The first suggests that burrowing is a concealment strategy that organisms use to avoid predation.¹⁵ Norway lobsters (*Nephrops norvegicus*) are assumed to use burrows for this reason¹⁶ as does the hard shell clam (*Mercenaria mercenaria*), which burrows to greater depths when confronted by the predacious sea star *Asterias forbesi*.¹⁷ Another hypothesis explaining burrowing function posits concealment as a predation strategy, allowing organisms to ambush unsuspecting prey.¹⁵ Mantis shrimp (*Stomatopoda*) "spearers" have been observed hiding in sandy burrows to surprise prey in this fashion.¹⁸ A third hypothesis suggests burrowing is a strategy for energetic conservation, allowing organisms to spend long periods of time immobile and reduce metabolic rate.¹⁵ This hypothesis is especially interesting when considering the interplay between metabolism, ventilation, and hypoxia. Burrows are generally hypoxic environments, and organisms that reside in burrows tend to exhibit hypoxia tolerance.¹⁹⁻²¹ Hypoxia would normally stress organisms and impair aerobic metabolism,²² but burrowing organisms tolerance to hypoxia may enable burrowing as an effective energetic budgeting strategy. Alternatively, burrows may be constructed to allow adequate ventilation of oxygen, allowing for hypoxia intolerant organisms to oxygenate their burrows and effectively conserve energy.²³ Finally, burrows are also sometimes utilized in intraspecific social dynamics, such as the courtship behavior of male fiddler crabs (*Uca beebei*).²⁴

It is speculated that octopuses burrow to avoid predation⁴⁵ but this idea has not been explored in any detail, and *M. leioderma*'s burrowing function in Burrows Bay is unknown. All that is certain is that this octopus species consistently burrows in response to divers in the field, that it burrows in the absence of predators in the laboratory (personal observation), and that it preys on burrowing organisms like polychaete worms.⁴⁶ While this thesis will not directly address the question of burrowing function for *M. leioderma*, it is helpful to keep the various possibilities in mind to contextualize the results of this study.

This thesis is the first study of cephalopod burrowing in muddy sediments. This is important because as mentioned earlier, the modes of excavation depend largely on the type of strata the organism needs to penetrate.² These different methods of burrowing are usually associated with some type of morphological trait that facilitate this behavior. One of the most simplistic examples is the strategy of polychaetes, which burrow in the dense muds they occupy by fracture, entering cracks and widening them into larger burrows by exerting force from its body.¹ More complex organisms have adapted more specialized traits that facilitate effective excavation and movement through sediment. Burrowing requires an organism to be able to initially penetrate the strata, then to be able to extend and expand the burrow without it collapsing. Initial penetration can be achieved by digging up the sediment. Many crustaceans use shovel-like appendages to slowly move sediment particles out of the way.⁴⁷ Spanner crabs (*Ranina ranina*) do this by either digging forward and pushing sediment underneath them as they burrow, or else moving sediment up while driving them downwards posterior-end first.⁴⁷ Other organisms use appendages with quick movements to fluidize sediment.^{15,25}

More porous muds exhibit less adhesion and more fluidity, and burrowers in softer muds use locomotion more akin to swimming.²⁶ Fluidization refers to propelling fluid upward, bringing sediment up with it and temporarily suspending it in the water.⁴⁸

Fluidization is distinct from liquefaction, where sediment settles downward through fluid from gravity, displacing the liquid upwards.⁴⁸ For example, *Octopus kaurna* exhibits fluidization of sandy sediment in its burrowing by blasting the sand with water jetted from its mantle through the siphon, then using its arms to expand and extend the burrow once below the surface.²⁵ This hydraulic method of burrowing by expelling water from the mantle is also seen in bivalves.⁴⁹

Another notable method of burrowing is a system known as cavity expansion, which involves penetrating and burrowing into wet sands by the contraction and expansion of the body.⁵⁰ This method is seen in the Atlantic razor clam (*Ensis directus*). The clam opens internal valves, allowing water to flow in, and then penetrates the sediment with its foot as an anchor point. Then it closes its valves, driving fluid to the foot and expanding it, before immediately retracting it and pulling the valves back towards the anchor, constricting the foot and creating a space that the clam can move through. These novel adaptations allow for the rhythmic expansion and contraction system of burrowing in this organism.⁵⁰

Muusoctopus leioderma does not appear to burrow hydraulically (personal observation) and as an octopus, it lacks the traditional hard burrowing appendages found in other marine and terrestrial burrowers. In addition, the silty sediment found in Burrows Bay makes burrow by fracture an unsuitable burrowing strategy.²⁶ The species' most notable defining characteristic is the presence of a keel, a flap of skin that protrudes from the lateral margin of the mantle.¹³ This trait, while not unique, is fairly novel among octopuses (Fig 6) and has been anecdotally linked to burrowing in octopuses.⁵¹ The keel is essentially just an expandable flap of skin, meaning it lacks muscle tone or rigidity, and is therefore an unlikely candidate as an appendage used directly for excavation. However, the keel's apparent connection with known burrowing octopus species makes it an intriguing point of study as a possible burrowing trait for

benthic octopuses, and may lend clues to the burrowing strategy of *M. leioderma* and other soft-sediment octopuses.

As a final note, burrow maintenance is essential to prevent collapse and increase the longevity of burrows. Marine organisms largely stabilize by simple compaction of substrate, or by secreting a substance that strengthens and/or lubricates the burrow walls, such as with *O. kaurna*.^{15,25} *Muusoctopus leioderma* lacks the mucous secretion capabilities of *O. kaurna*, and its burrowing mechanism appears to be quite distinct from the latter's completely hydraulic method of burrowing, so *M. leioderma*'s mechanism of burrow maintenance is still unknown.

As cephalopods expand their range, understanding how the ways this versatile group can influence habitats, such as burrowing mechanics and the adaptations that enable burrowing in specific substrates, will be essential in understanding the scope of their ecological influence.

METHODS

Overview

The goal of this project was to determine how *Muusoctopus leioderma* burrows in the wider context of marine organisms, and this was accomplished by tackling three key concepts. First, I examined this species' relative burrowing performance by measuring the rate of burrowing *in situ* via SCUBA, and comparing these rates with other burrowing organisms to see what abiotic factors may influence burrowing performance. Next I wanted to better understand this octopus's general burrowing behavior by exploring what time of day this octopus tends to burrow and when they enter and exit their burrows in the lab using motion detecting cameras. Finally, I investigated what role the keel plays in burrowing by 1) examining how keel morphology of captured *M. leioderma* corresponds with burrowing in the lab and 2) performing a phylogenetic correlation analysis of keels in known burrowing and non-burrowing octopuses to determine if this trait is associated with burrowing in octopuses.

Burrowing Performance

In order to determine the burrowing performance of *M. leioderma*, I measured how quickly they burrowed *in situ*. Octopus burrowing events were recorded at night by SCUBA divers using GoPro Hero 7 and Hero 8 cameras. The GoPros were equipped with parallel-oriented lasers to provide a known scale to accurately measure octopus mantle length from video using ImageJ.

In situ Burrow Recording

The burrowing rate index (BRI) was determined for each instance of *M. leioderma* burrowing that was recorded. BRI has been previously used in burrowing octopuses and many other burrowing organisms.^{25,30} Dives measuring BRI data *in situ*

were separate from collection dives because BRI calculations required collecting burrowing times, and octopuses are exceptionally difficult to collect after they burrow. When an octopus was spotted, divers followed and recorded it until it burrowed and throughout the burrowing process. After the octopus finished burrowing, divers moved on to find other octopuses, as individuals don't typically reemerge from their burrows quickly. The start of burrowing was marked by when the octopuses pressed their arms into the mud to initiate burrowing, and ended when the octopus fully submerged below the surface of the sediment bed. Roughly half of recorded burrowing events were actually octopuses retreating into pre-made burrows, as opposed to making their own, and these pre-made burrows were marked during data entry into Google Sheets, and excluded from the final calculation of *M. leioderma*'s mean BRI.

Burrowing Rate Indices (BRIs) are calculated using the equation:

$$BRI = \frac{\sqrt[3]{Wet\ Weight}}{Burrowing\ Time} \times 10^2 .^{30}$$

ImageJ software was used to measure mantle lengths of the burrowing octopuses using laser dots as a known size reference. Mantle lengths were then used to estimate octopus wet weight using the relationship between measured lengths and weights of 8 octopuses (2 were excluded for missing lengths or weights) from the mud tanks using linear regression in R. Then, the predict function was used to estimate the wet weight of recorded burrowing octopuses based on their mantle lengths using the linear regression determined from captive octopuses. Burrowing times in seconds were calculated using the formula: *Burrowing Time = Burrow End Time – Burrow Start Time*.

Sediment coarseness is also a factor in burrowing performance^{52,53} which necessitated a measurement of sediment found in Burrows Bay. Sediment was collected from the surface of the sediment bed in Burrows Bay, and was measured using standard

wet-sieving by lightly running water over sediment and shaking sediment on scientific grade sieves, until water ran clear.

These data were then used to get a mean BRI for *M. leioderma*. To contextualize this data to understand its significance, *M. leioderma*'s BRI was then compared with other burrowing organisms, including the only other burrowing octopus with published data on its burrowing behavior, *Octopus kurna*²⁵.

Temperature was also compared between *O. kurna* and *M. leioderma*. Temperatures in Burrows Bay were recorded using an Oceanic Proplus 4 dive computer, and these readings were validated using the temperature probe from a Barrow YSZY-01 water chiller.

Literature Review and BRI Comparison

BRI from 26 burrowing organisms (not including *M. leioderma*) in different sediment types were collected from published literature.^{33,52-57} BRI of *O. kurna* were collected by extracting data points of raw BRI in three sediment grades from a figure²⁵ using G3Data software, then calculating the mean in Google Sheets.

BRI of the crustaceans *Emerita analoga*, *Lepidopa californica*, and *Blepharipoda occidentalis*⁵⁷ were calculated from the published linear regressions of BRI vs. carapace length of each species using the middle point of the carapace length range using the equation $\frac{\text{Highest Range} - \text{Lowest Range}}{2}$. For ranges given as greater than or less than (i.e. carapace length greater than 10 cm) the lower or upper limit provided was used (i.e. 10 cm).

Laboratory Burrowing Behavior

Next, I studied *M. leioderma*'s burrowing behavior in more depth by examining them in a laboratory setting.

Octopus Collection and Processing

Octopuses were collected from Burrows Bay, Skagit County, WA at 48°28'12" N, 122°40'53" W by SCUBA. Once found, they were captured and returned to Rosario Beach Marine Laboratory (RBML) in re-sealable plastic storage bags filled with saltwater. Upon arrival, octopuses were sexed, weighed, and assigned a name. Lengths were also obtained in the laboratory using the same laser dot camera method used to measure lengths during BRI recordings *in situ*. These data, along with species, date caught, depth, the tank number the octopus were to be placed in, and any notes were recorded digitally on a Google Sheet entitled "Octopus Collection Record 2022". Then octopuses were immediately transferred to a 61 cm x 33 cm x 41 cm acrylic aquarium, or "mud tank", filled approximately halfway with sediment from Burrows Bay for their 24-hour laboratory acclimation period. The time that octopuses begin this acclimation period, along with the date, sex, name, and tank were recorded in Sheets.

Octopus Holding

Aquaria or "mud tanks" were connected to RBML's seawater system to provide fresh saltwater. Tanks were modified for a flow-through system, with an inflow and passive outflow (Fig 2a). Tanks outflow lines ran to a single plastic tub with a sump pump connected to a float switch, allowing all tanks to have short lines and consistent outflow to a single drain. Light levels in Burrows Bay and tanks were measured using a LI-1400 data logger and light probe. For *in situ* measurements, the probe was dropped to the bottom of Burrows Bay where researchers normally search for octopuses in about 15 meters of water during peak daylight hours on a sunny day. The tanks' individual light arrays were measured by dropping the probe midway into the sediment tanks, above the

sediment layer. These lights were diffused green LEDs, controlled by a Raspberry Pi Pico microcontroller, and powered by a DC wall socket. Each mud tank was equipped with a light array programmed to brighten and dim closely following a diurnal cycle approximating what occurs naturally (Fig 2b). Peak brightness hours and ramp down periods were calculated using “sunriseset” function from the maptools R package,⁸² using July 31, 2022, a date roughly halfway through our data collection period, as a set date, and Burrows Bay, Skagit County, Washington, USA as our set location. Peak brightness for the light arrays was chosen by selecting an intermediate light level for this species' known brightness range, with *in situ* measurements in Burrows Bay representing the maximum, and complete darkness as the minimum. There were also red light sources that allowed nighttime photography of octopuses when the green lights were off without disturbing them. *Muusoctopus leioderma*'s eyes are non-reactive (no discernable pupillary response) to red light (Onthank, personal communication).

Mud for the tanks was collected directly from Burrows Bay either by SCUBA or using box core sampler or Van Veen grab samplers. Octopuses were held for at least 12 days, then periodically released by divers while new individuals were collected.

Mud tanks had motion-activated cameras utilizing custom software run on Raspberry Pi-type single-board computers based off of work by Onthank Lab graduate student, Jesse Humbert.⁵⁸ Cameras captured high resolution images triggered by octopus movement which provided temporal information on when the octopuses are moving above the surface of the mud and actively burrowing. Active times, the times of day when *M. leioderma* were triggering the motion cameras above the sediment, was the primary measure of activity. Details on image analysis are documented in the Data Analysis section.

Keel and Burrowing

Finally, I wanted to understand morphological adaptations in this species associated with burrowing. Specifically, I investigated the role of the lateral mantle skin ridge, or “keel”. This was done on two fronts: first on the species level with captured *M. leioderma* to determine the persistence of keels in burrowing versus non-burrowing octopuses in captivity, and second on the order level, with a phylogenetic analysis of multiple octopus species.

Muusoctopus leioderma Keel

After their initial acclimation period, captured individuals were periodically evaluated for keel prominence. The data collection involved taking images of the octopus’s keel with an iPhone 12 mini. Target keel days were days 2, 6, and 12 in captivity. However, keels could not be photographed when the octopus was in its burrow, so occasionally octopuses would have to be photographed later, as soon as the octopus was visible. Images were normally taken either in the early morning, or late at night. Octopuses were kept in treatment for a target of 3 keel collection dates in the mud tanks, although time restraints forced treatment to end early in some cases. After completing their treatment in the mud tanks, individuals were transferred to non-light controlled plastic containers, or “Octocondos,” connected to RBML’s saltwater system to await return and release to Burrows Bay via SCUBA, and allowing new octopuses to begin treatment. In some cases, the octopuses never emerged from their burrow after their initial burrowing event. These octopuses were dug out of their burrows after 10-20 days. Occasionally, such as when octopuses needed to be dug out from their burrows, images for keel evaluations were collected after octopuses were transferred to the Octocondos, and this was noted in the data. Later, octopuses were categorized into two groups; burrowers, or octopuses that consistently burrowed below the sediment layer, and non-burrowers, or octopuses that did not burrow in captivity.

Keels were evaluated later using a key I created for this purpose. Initial attempts to measure the keel quantitatively using ImageJ were unsuccessful due to the fine degree of difference in such a small trait. Therefore, I evaluated keels qualitatively based on 5 levels of prominence, with a 0 or “Missing” being a completely absent keel, and a 4 or “Very Prominent” being the strongest level of prominence (Fig 4).

The key provided descriptions of distinctions between each of the levels of prominence, as well as multiple images highlighting those differences. Keels were evaluated independently by myself and 3 undergraduate volunteers, and the consistency of the evaluations was examined (see Data Analysis). These were blind evaluations, meaning evaluators did not know if images were of burrowing or non-burrowing octopuses, and they lacked access to other evaluators’ results. Details of the comparison between burrowers and non-burrowers can be found in the Data Analysis section.

Octopoda Keel

Phylogenetic analysis of the occurrence of keels across the order Octopoda in relation to burrowing was accomplished using visual identification of keels as “Present” or “Absent” by examining images from literature, species descriptions and reaching out to species-specific experts. These species were classified as “known to bury or burrow” or “not known to bury or burrow.” The octopus species selected for analysis were all benthic species with known genetic sequencing of the cytochrome oxidase subunit 1 (COI), cytochrome oxidase subunit 3 (COIII), 12S, and/or 16S genes obtained from GenBank. These genes were chosen because they provided the best coverage of the most number of species, and each of these species had sequences of one or more of these genes available, sufficient for an alignment. Species lacking genetic sequencing, or that lacked data on both keels and burrowing behavior, were mostly deep water

species with very little research focus, and these were omitted from the analysis. The final tree included 110 total species. Octopuses in which only keel presence was unknown, the keel was considered missing. Octopuses in which only burrowing behavior was unknown were considered “not known to bury or burrow”. Evolutionary correlation between mantle keel and burrowing or burying behavior were tested using Pagel's test in R.

Data Analysis

These methods produced datasets that required statistical analysis to calculate the significance of their results. All statistical tests were performed using R.⁵⁹ All data entered into the “Muus Data Master” sheet were pulled into R using the googlesheets4 package.⁶⁰

BRI and Sediment Coarseness

After obtaining burrowing rate indices for *Muusoctopus leioderma* from video recordings, and the other organisms from literature, I examined how burrowing rates changed in different sediments by plotting results with log burrowing rate index on the y-axis and sediment granule size in micrometers on the x-axis. All organisms cited from the literature had BRIs measured in multiple sediments of various granule sizes, while *M. leioderma* was only measured in its native sediment. Sediment coarseness values were translated from phi to micrometers using the EMMAgeo package⁶¹.

Burrowing Activity

Ex situ mud tanks provided data on what time of day these animals are active and out of their burrows, and when they were burrowed below the strata surface. Field observations of Burrows Bay *M. leioderma* are largely at night, and we expected the data

to corroborate this. These images also provided temporal data on more specific behavior, such as times of day when octopuses exited and entered their burrows.

A team of undergraduates and I analyzed 61,164 images collected by the cameras. Observers would open a folder with the octopus's name, and find folders labeled with the date the images contained in those folders were taken. Each image was labeled according to the date and time it was taken. For example, "00110__TE_D20220727_T201459_pi_triggercam_001" is the 110th image in that folder, taken on July 27 2022 at 20:14:59." Observers would copy and paste these file names into the Muus Data Master sheet under "Tank Activity" for every image containing an octopus in frame, along with their name, the octopus name, its tank number, and any comments. The octopuses' mantle lengths and weights were recorded in a separate sheet in the Muus Data Master sheet under ML/W, and burrower/non-burrower statuses were recorded in the Keel Prominence sheets.

The cameras were designed to only collect data of active octopuses, octopuses moving above the sediment layer. However, the cameras were occasionally triggered by other things, like small invertebrates moving in the tanks, or lighting changes, and these images had to be filtered out of the final analysis. Images were filtered by assigning the designation of "People" to images in which someone was in the lab and possibly disturbing the animals, or "Idle" for images triggered by small movements by the octopus or its surroundings during times when the octopus was immobile and inactive. Those images were ignored when pulling data into R.

Images in which someone was in the lab and possibly disturbing the animals were also removed. For non-burrowing octopuses, many times these individuals would spend long periods of time immobile and inactive, but small movements by the octopus or its surroundings would trigger images. These periods of inactivity were also filtered out, leaving only images of active octopuses for analysis. Activity time data were plotted

as radial histograms using the CircularTimeHistogram R package.⁶² The differences in activity between nighttime and daylight hours were tested for statistical significance using a chi-squared test, and were examined for Entering/Exiting events, and for burrowing and non-burrowing individuals.⁵⁹ Differences in nocturnal activity between burrowers and non-burrowers were tested using a test of equal or given proportions using the stats package in R.⁵⁹

Keel Evaluations

Each observer had their own private sheet to perform evaluations independently from other observers. These results were averaged to get keel prominence values for each keel measurement of all individual octopuses. Individual octopuses were split into two groups based on burrowing status as burrowers and non-burrowers. Keel evaluations were categorized as either mud tank or Octocondo evaluations. Individuals that had one or fewer keel measurements were excluded because keel loss could not be determined. Octopuses' keel prominence was compared with how many days it had been held in captivity, which was calculated using the dates of octopus collections and keel evaluations.

To test consistency between evaluators, we calculated an intraclass correlation coefficient (ICC) as an index of interrater reliability. ICC was run as a two way consistency model using the irr package in R.⁶³

Statistically significant differences in keel prominence between burrowing and non-burrowing octopuses were analyzed using a Linear Mixed Effect (LME) model from the nlme package in R.⁶⁴ This is because these data are hierarchical, meaning I have multiple observations nested under a single octopus, and non-independent, since I took multiple keel evaluations of the same octopuses. The LME model also accounted for two

different keel measurement scenarios; mud tanks or Octocondos. This model was analyzed using an Wald Chi-Squared test from the car R package.⁶⁵

Keel Phylogenetics

Genetic sequences were obtained from GenBank using the read.GenBank function from the ape R package using accession numbers⁶⁶ for benthic octopus species with known sequencing of COI, COIII, and 16S, as these 3 genes provided the best genetic coverage of benthic octopuses. Sequences were aligned using the AlignSeqs function from the DECIPHER package.⁶⁷ This allowed for the creation of a NEXUS file that could then be used in creating the final tree. For each of the three genes, a model test was conducted with the phangorn package for each codon position independently, so the dataset could be partitioned into each of the three codon positions.⁶⁸ All of the above, the pulling of sequences, alignment, NEXUS file creation, was all performed for each of the target genes.

The resulting files were combined into a single nexus file and a MrBayes command block generated using a custom BASH script. This file was then used for the final analysis and production of the multi-gene tree using MrBayes.⁶⁹

To test for a statistically significant evolutionary relationship between keel presence/absence and known burrowing behavior, the multi-gene tree was analyzed using Pagel's Test. Pagel's test examines the independent evolution of 2 binary characters by comparing a ratio of likelihoods of two models.⁷⁰ One model assumes the rate of change in each trait we are measuring evolved independent of the state of the other, while the other assumes the rate of change in one trait is dependent on the other. Pagel's test was run using the phytools R package.⁷¹

Data Availability

In situ burrow recording videos and keel evaluation photos were stored redundantly on my personal laptop and an external hard drive. Temporal burrow behavior data from the mud tanks were stored both on sd cards housed in the Raspberry Pi cameras, as well as on the external hard drive.

Data collected from these images and videos during analysis was recorded and stored using Google Sheets. Google sheets automatically keeps infinite records of every modification made to a datasheet, and every previous version is restorable in case of accidental data deletion. All these data, including keel evaluations, burrow times, mantle lengths and weights, burrowing status, tank activity, and interspecific BRI data were stored in the Muus Data Master datasheet. Surveys of octopus taxa with keels and known burrowing behavior were stored in the “Octopus burrowing” datasheet.

All R code used in data analysis was continually updated/uploaded online via GitHub. Finalized code for interspecific burrowing rates can be found at <https://doi.org/10.5281/zenodo.8021581>. Finalized code for *Muusoctopus leioderma* temporal tank activity can be found at <https://doi.org/10.5281/zenodo.8021836>. Finalized code for *Muusoctopus leioderma* keel evaluations can be found at <https://doi.org/10.5281/zenodo.8021840>. Finalized code for octopus keel phylogenetic analysis can be found at <https://doi.org/10.5281/zenodo.8021847>.

The image data will be available on request. High resolution photos and video data are too large for online data repositories, but will eventually be stored in the Walla Walla University Biology Department.

RESULTS

In situ Burrowing Performance

Muusoctopus leioderma recorded a mean BRI of 14.3 based on 8 individuals. Burrowing times of those individuals were varied (9.73-39.09 seconds). There were also 20 individuals recorded going into pre-made burrows not included in the BRI calculation. This BRI was slower than *O. kaurna*'s fastest BRI in 500 um sediment, but faster than BRI's recorded in 750 um and 1500 um (17.0, 11.5, 5.1). These BRI's were measured in similar water temperatures (*M. leioderma* 10 C, *O. kaurna* 14 C) and used similarly sized octopuses (3-57 g, 12-53 g).

Literary review of BRI's across marine taxa revealed a wide range of BRI's (0.001-128.24) with most species burrowing fastest at an intermediate sediment grain size, and gradually becoming less efficient in both coarser and finer sediments, and demonstrating a non-linear relationship between BRI and sediment granule size. Ideal burrowing sediments varied by species, but most species followed a similar non-linear trend.

Laboratory Burrowing Behavior

Octopuses followed consistent clear patterns of activity while held in captivity. All 10 octopuses were more active during the night than day, which is consistent with field observations (Chi-squared test for given probabilities, $X^2 = 9981$, $df = 1$, $p\text{-value} < 0.001$, $n = 43649$). This pattern became even more apparent when focusing on octopuses that were actively burrowing ($X^2 = 6509$, $df = 1$, $p\text{-value} < 2.2e-16$, $n = 10617$). Six out of ten individuals burrowed in captivity, with at least one octopus burrowing in each of the five tanks. A comparison of burrowing vs non-burrowing activity patterns showed burrowing individuals followed a stronger pattern of nocturnal activity compared with

non-burrowers, with the proportion of activity during the day being significantly different from non-burrowers (Two-sample test for equality of proportions with continuity correction, $X^2 = 1279$, $df = 1$, $p\text{-value} < 0.001$, $n = 43649$). It appears that octopuses may also enter (X^2 test for given probabilities, $X^2 = 10.09$, $df = 1$, $p\text{-value} = 0.001491$, $n = 27$) and exit (Chi-squared test for given probabilities, $X^2 = 9.0133$, $df = 1$, $p\text{-value} = 0.00268$, $n = 24$) their burrows more frequently at night than during the day (Fig 7).

In situ peak light conditions were recorded a maximum level of 65.54 umol photons/second/square meter (65.01-65.54). Mean tank light measurements were recorded at 1.3463 umol photons/sec/sq m (0.8048-1.931).

Keel and Burrowing

Keel Evaluations

Keel evaluations of captive octopuses showed a clear effect of burrowing on keel prominence. Keel evaluations were conducted blindly and independently by four observers, and results were consistent (ICC = 0.913, $p\text{-value} < 0.001$, 95%-Confidence Interval: $0.85 < ICC < 0.953$).

Average keel prominence values of the linear mixed effects model with an Wald Chi-Squared analysis showed a statistically significant interaction between days and burrowers ($X^2 = 4.6648$, $df = 1$, $p\text{-value} = 0.03079$). There was also a significant effect of day on keel prominence ($X^2 = 4.3657$, $df = 1$, $p\text{-value} = 0.03667$). Non-burrowing octopuses lost their keel more rapidly than burrowing octopuses (Fig 4, $X^2 = 4.6648$, $df = 1$, $p\text{-value} = 0.03079$). In addition, the analysis found that whether the image was taken in the mud tank or in the Octocondo after removal from the mud tank did not have a significant effect on these results (linear mixed-effect model, $X^2 = 0.8436$, $p\text{-value} = 0.35837$).

Keel Evolution & Pagel's Test

I found evidence of an interdependent evolutionary relationship between the presence of a keel and known burrowing behavior in benthic octopus species (Fig 6, Pagel's test, AIC = 188.4336, likelihood ratio = 20.5514, p-value = 0.000388571).

DISCUSSION

Burrowing Rates & Sediment

Muusoctopus leioderma had a comparable burrowing rate when compared with *Octopus kurna*, with *M. leioderma* burrowing faster in some sediments, but slower in others. These two species offered an interesting comparison, as they were essentially the same body size, one of the key factors in relative burrowing performance³⁴. The main differences between the two were environmental. Temperature is a key factor in metabolic processes and energy expenditure. Metabolic rates, and the functions and behaviors they drive have been shown to be slower in populations occupying colder waters than warmer ones³¹. *Octopus kurna*'s BRI was measured in similar, but slightly warmer temperatures than *M. leioderma*²⁵. However, the impact of temperature on burrowing performance in marine organisms is highly variable. Many burrowing organisms have shown to be significantly slower in polar environments compared with biologically similar species occupying more temperate and tropical waters.³³ Others demonstrated interspecific burrowing performance unaffected by changes in temperature.⁷² *Muusoctopus leioderma* and *O. kurna* occupy very similar marine climates,⁷³ yet *O. kurna*'s slightly warmer water temperatures could explain a performance difference in ideal sediments. It may also be possible that *M. leioderma* could be compensating for colder temperatures, or that octopus burrowing performance is not directly reliant on temperature-dependent physiological reactions, as is the case in some bivalves⁷². However, I have yet to measure *M. leioderma* in varying sediment grades, so all I can say for now is that the two species burrow at similar rates in somewhat similar temperatures.

One of the biggest factors influencing burrowing performance is sediment coarseness, as a borrower's sediment granule size and adhesive properties largely

dictate burrowing mechanics and strategies². These variable burrowing strategies inevitably produce variable burrowing rates between species, while sediment granule size also impacts intraspecific performance (Fig 8).^{33,52-57,72} Review of existing literature shows a non-linear relationship between sediment size and BRI among marine burrowers, with organisms burrowing fastest in intermediate sediment coarseness, then getting slower in both finer and coarser sediments. The performance loss in coarser sediments is logical, as coarser sediments will require more effort to displace. Meanwhile, silty sediments like in Burrows Bay are highly adhesive and cohesive, and this added resistance to the organism may explain the drop in BRIs in finer sediments. *Muusoctopus leioderma* BRIs were only measured in its native sediment (<220 μm). However, BRIs of other marine organisms tested in variable sediments form a nonlinear pattern or curve. When compared to the similarly sized *O. kaurna*'s BRIs (measured in 500 μm , 1000 μm , and 2000 μm sediment), *M. leioderma*'s measured BRI is consistent with this curve (Fig 8). This suggests that the relative influence of sediment on burrowing performance may be consistent across marine taxa, with differences in ideal sediments being largely species-dependent. Future studies should involve testing *M. leioderma* in varying sediment grades and temperature ranges.

Laboratory Tank Activity and Nocturnal Patterns

My results support field observations that *M. leioderma* is a nocturnal species. Nocturnal behavior is common among octopuses⁷⁴, but recent studies have suggested that some previously identified nocturnal species may in fact be diurnal,^{37,58} and that other factors, such as constant laboratory lighting and stress from researchers during the day, and rest and reprieve at night, caused octopuses to be misclassified as nocturnal. My tanks were light-controlled, and set at a peak daytime brightness well within the range of that experienced by *M. leioderma* in the wild, which ranges from 65 μmol

photons/sec/sq m at about 15 meters in Burrows Bay, the shallowest and presumably brightest known depth for this species,^{11,75} all the way down to approximately 0 umol photons/sec/sq m at 1400m, it's maximum known depth,¹⁰ and well within the aphotic zone.⁴¹

M. leioderma in the laboratory showed octopuses being most active at peak darkness hours (Fig 7). This pattern of inactivity during the day was consistent for both burrowing and non-burrowing octopuses, with non-burrowers remaining stationary and inactive in the corners of the tank during daylight hours. However, this pattern was noticeably stronger in burrowing octopuses. This may simply be due to non-burrowing individuals being more susceptible to disruption from people entering the lab during the day. However, non-burrowing octopuses also demonstrated a weaker, but still significant pattern of nocturnal activity, suggesting that this pattern is driven both by the effect of burrowing and some other circadian system. Entering and exit events were also non-random, occurring mostly at night. Octopuses would move in and out of their burrows throughout the night, although this activity was most concentrated around the beginning and end of the nighttime cycles.

This consistent cycling of activity at set times brings up questions about the chronobiology and circadian rhythms in this species. This focus of this study was burrowing activity, so we did not directly search for evidence of an endogenous biological clock. However, the fact that this species followed temporal patterns consistent with field observations tells us that captive individuals were responding to light because of one of three possibilities. One, this octopus has endogenous circadian rhythms that were cued daily by the mud tank lights. Two, the octopus is merely adverse to light, so it burrows and/or reduces activity in response to light as a stressor. Three, captives were insensitive to our lighting system and merely continued to follow their temporal patterns that were entrained by another cue before collection. Further research would be

necessary to parse out the answer. However, the reason for the response is beyond the scope of this study. The octopus is still following a pattern of burrowing consistent with field observations, and this pattern is stronger with burrowing individuals. The pattern is consistent, and this at shows that *M. leioderma* is temporally regimented, unlike the temporal plasticity shown in *Octopus vulgaris*.⁴⁴

Adaptive advantages of nocturnal activity and daytime burrowing behavior for *M. leioderma* in Burrows Bay are still not clear. There appears to be a generally higher amount of organismal activity in Burrows Bay above the sediment layer at night, with crabs, shrimps, and other benthic organisms more abundant during darker hours (personal observation). Night may therefore be a more advantageous period for predation. Controphic competition could also be a factor. *Octopus rubescens* is another common octopus in the Puget Sound, and this species is known to follow diurnal activity patterns.⁵⁸ This observer noted and recorded an individual *O. rubescens* in Burrows Bay, which raises questions about possible interactions between these two species. However, this is the first record of *O. rubescens* being spotted in Burrows Bay, and the octopus was found in the early evening, in an area closer to shore than where we normally search for *M. leioderma*. In addition, we did not observe any *M. leioderma* in the area during that dive. It is therefore extremely premature to hypothesize any kind of dynamic, such as temporal spacing, between the two species. However, future research in the area should remain vigilant of any co-occurrences between these two species. Regardless, these results will help serve as a baseline to better understand how this octopus interacts with other organisms in Burrows Bay, and facilitate conclusions about its ecological impact as a bioturbating organism in future studies.

Finally, observers also noticed that octopuses would periodically move up and down the burrow shaft in their tanks, with arms or tops of the mantles visible at the burrow entrance, but not fully out (Fig 9). This could be compacting behavior involved in

strengthening the burrow to avoid collapse. Maintenance is important for burrow stability, and *M. leioderma* lacks the mucus secretion utilized by *Octopus kaurma* to maintain its burrows.²⁵ Compaction is a common method of maintenance for burrowing organisms,^{15,76,77} so this may represent a potential mechanism for this species.

The Keel & Octopus Burrowing

Perhaps the most interesting aspect of the study was the exploration of the keel as a possible morphological trait associated with burrowing. Tackling the question on multiple fronts allowed for independent methods in evaluating the question, both narrowly on the species level, and broadly on the group level.

First, in burrowing captive *Muusoctopus leioderma*, I saw a significant interaction between the effect of octopus burrower/non-burrower status and number of days in the mud tank on keel prominence, meaning that the condition of an octopus being a burrower or non-burrower impacted how keel prominence changed over time. Specifically, I saw a stronger negative relationship between keel prominence and time in captivity for non-burrowers than for burrowers (Fig 5). This suggests that the keel is better maintained when octopuses continue burrowing in captivity. The mechanism behind this is still unclear, as the precise function of the keel is still unknown. It is possible that the keel is a “use it or lose it” type of trait, and that the keel disappears when the octopus does not continue the behavior. The keel loss could also be a result of stress from captivity, and that burrowing individuals were less prone to stress in their tanks, despite the minimal disturbance and light-controlled nature of the experiment.

Next, I broadened my focus to benthic octopuses as a whole to explore the possibility of an evolutionary relationship between known burrowing behavior and keel presence. Pagel’s test is a statistical test that compares models of evolutionary relationships between binary traits, and this test revealed that the model of best fit for the

phylogenetic tree was one of interdependence, suggesting the two traits co-evolved. While keels have been anecdotal linked to burrowing in octopuses⁵¹, this is the first study to provide phylogenetic evidence of a link between the two traits. This alone does not prove that the keel is a burrowing trait, but combined with the examination of keel regression in *M. leioderma*, these data together point towards a connection. As cephalopod research continues to grow and we learn more about benthic species, this survey can be expanded to improve our understanding of this relationship.

The next step is to think critically about the mechanisms at play in burrowing octopuses. If we are to assume that the keel is in fact a trait connected with burrowing, then what exactly is this trait doing? Burrowing takes place in sediment and it is extremely difficult to see what's happening below the sediment layer in an octopus burrow. It is therefore unclear what exact function the keel may perform in the burrowing process.

It was originally speculated that the keel may act as a kind of rudder, allowing the octopus to orient and guide itself into and/or through the sediment,⁵¹ hence the name "keel." Octopus keels are essentially a flap of skin with very little rigidity or structure, so it is less likely that it functions directly as an excavating appendage to displace sediment. However, the extra skin is the only external textural feature in this species, as *M. leioderma* lacks any papillae or other prominent skin texture. This unusually smooth and streamlined profile is ideal for burrowing, as it may help to reduce resistance from marine sediment. The keel is the only thing that disrupts this low resistance body shape, but the notion that this skin is being used to displace sediment is unlikely.

Instead, the keel may be involved in improving the efficiency of burrowing. In general, there are advantages for burrowing organisms with smaller body sizes. Smaller animals are faster and expend less energy in making their burrows compared to larger organisms.^{35,78} However, there are also advantages to constructing a larger burrow. For

example, a larger burrow would allow for improved water flow and could reduce the risk of anoxia in such a confined environment. The width of a burrow is usually proportional to the size of the organism,⁷⁹ but larger individuals burrow more slowly⁵² and expend more energy than smaller individuals.^{35,78} A keel may help to offset these issues while allowing the octopus to build a larger burrow.

To accomplish this, the keel may act as an extra reservoir of skin that allows the octopus to expand its mantle during the burrowing process. Octopuses expand and retract their mantles as part of the normal respiration process, and octopus skin is very flexible, normally retracting into a highly textured skinscape of papillae or folds.⁸⁰ *M. leioderma*'s skin texture is concentrated at the lateral margin of the mantle, but the mantle still expands and retracts during respiration. It's possible that the extra skin allows the octopus to expand its body size while burrowing beyond what would be possible otherwise, allowing for a disproportionately large burrow, and an overall less resistive skinscape for burrowing. Additionally, mantle expansion may also be a part of this octopus's overall burrowing strategy. The razor clam (*Ensis directus*) utilizes a strategy of bodily expansion and retraction in its burrowing, and it is possible that *M. leioderma* could be burrowing in a similar way.⁵⁰

The elastic nature of octopus skin may also explain the loss of keels in captive non-burrowers. Individuals no longer expanding their mantles during burrowing could show reduced keels as the extra skin is pulled back into the mantle from disuse. There is currently no research on the impacts of inactivity on octopus skin, but inactivity in humans results in reduced skin elasticity, muscle mass, tone, and alteration of skin and muscle fiber composition.⁸¹

Alternatively, the keel may just be an adaptation connected with *M. leioderma*'s smooth skin to improve sediment resistance. Next steps would involve trying to get *M. leioderma* to burrow in a transparent medium in the laboratory to document the keel

below the sediment, and to investigate interspecific variation in mantle plasticity and skin texture to explore connections between octopus skin and burrowing behavior.

FIGURES

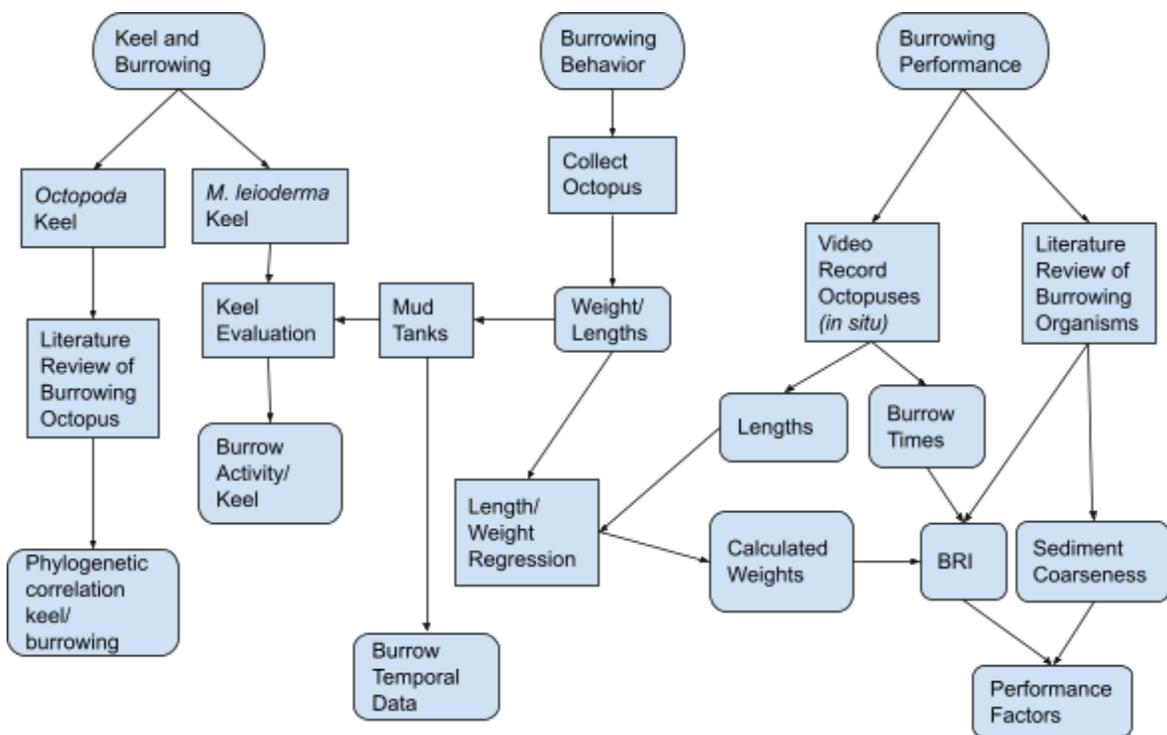
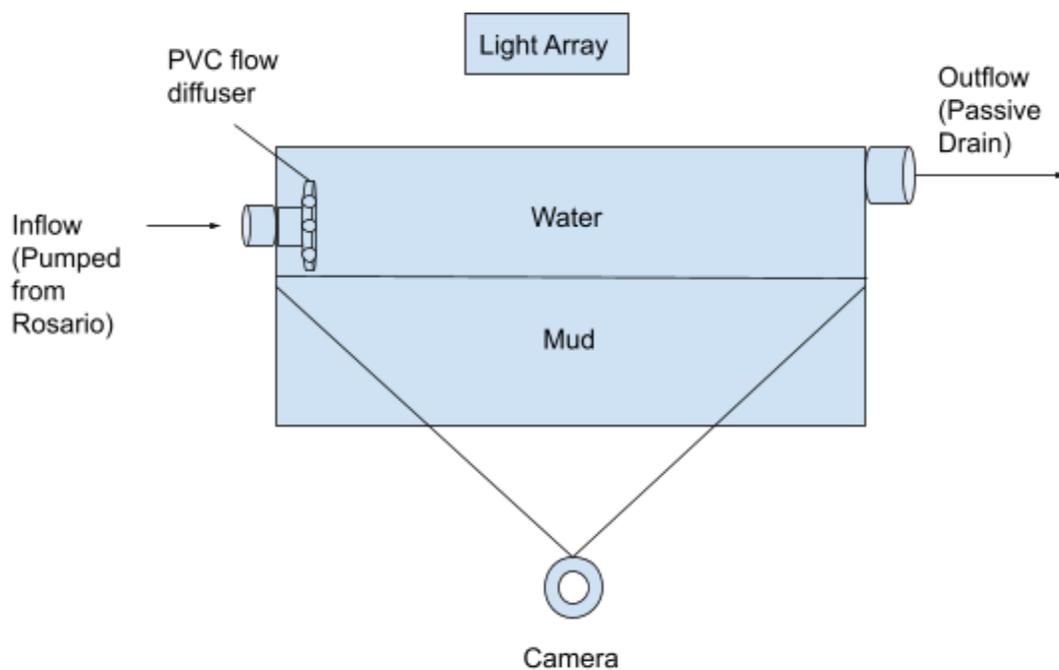


Fig 1. A flow chart of general methods.

a)



b)

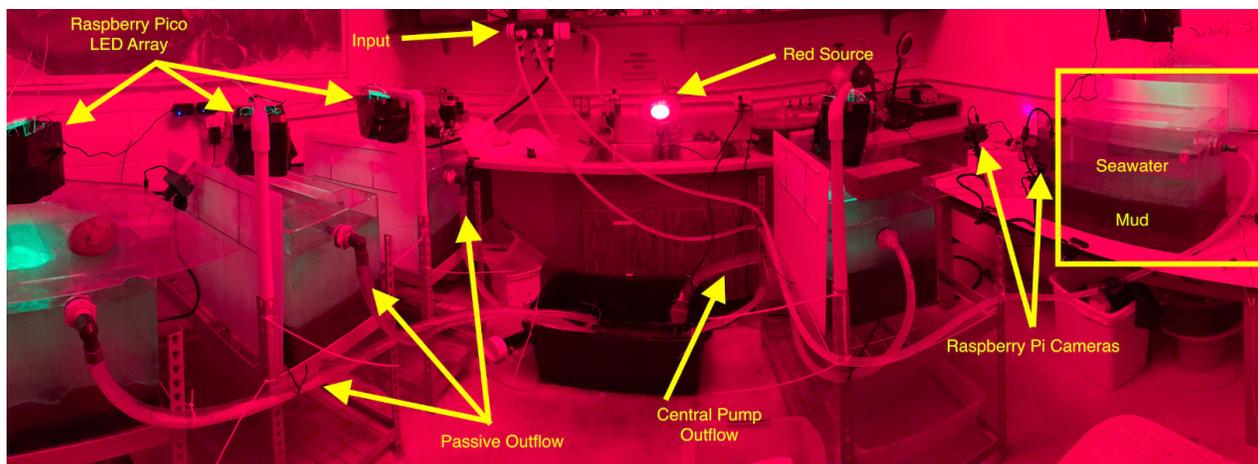


Fig 2. Laboratory setup showing a) Individual mud tank schematic and b) labeled photograph of the entire lab's water, camera, and lighting system, with all 5 experimental mud tanks shown.

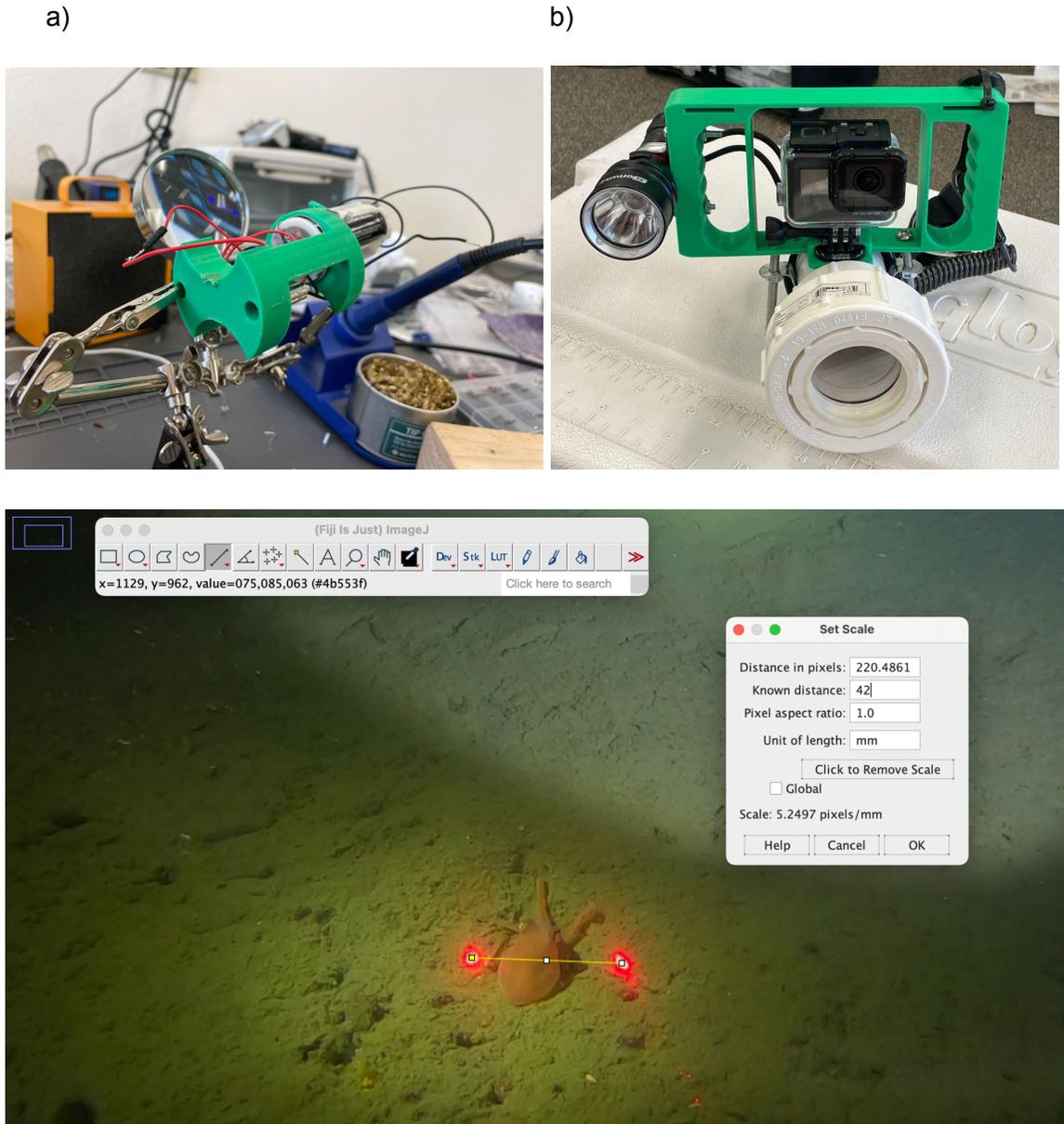


Fig 3. Tools to record octopuses *in situ*. a) I measured mantle lengths with lasers (black holes on edges of housing) with known distances, set in 3D printed housings, connected to batteries. Lasers were accurate to 10 ft and tested before dives. b) Lasers, camera, and light were attached to a 3D printed mount designed by Alnavasa at Thingiverse. Lasers and batteries were housed in PVC housing with acrylic lenses. c) Mantle lengths were measured using ImageJ, finding the center of the laser dots and setting the scale with a line tool, then using the scale to measure distances between the center of eyes and the apex of the mantle.

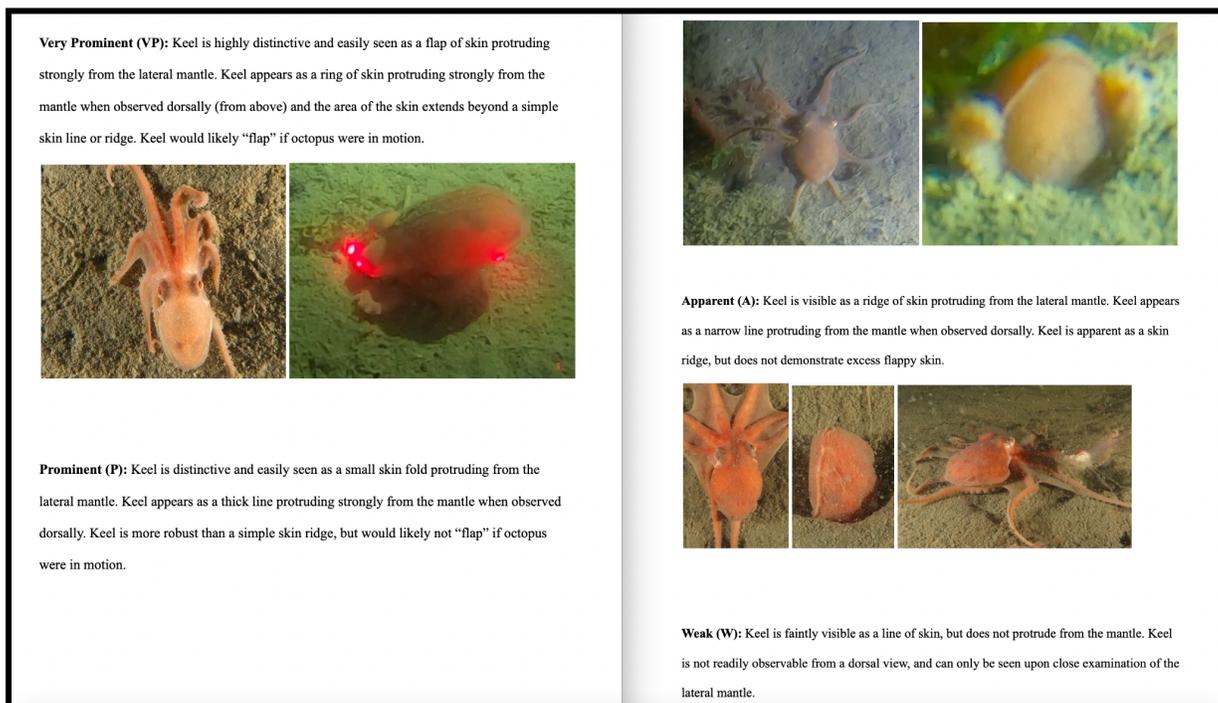


Fig 4. Keel evaluation key. Keels were rated as Very Prominent (4), Prominent (3), Apparent (2), Weak (1), or Missing (0, not shown). Pictures and descriptions were used in differentiating levels of prominence, and keels were evaluated blindly and independently with low inter-observer error (ICC two-way, consistency model, type = average, ICC=0.913, p-value = 2.55e-20). The full key is shown in the Appendix.

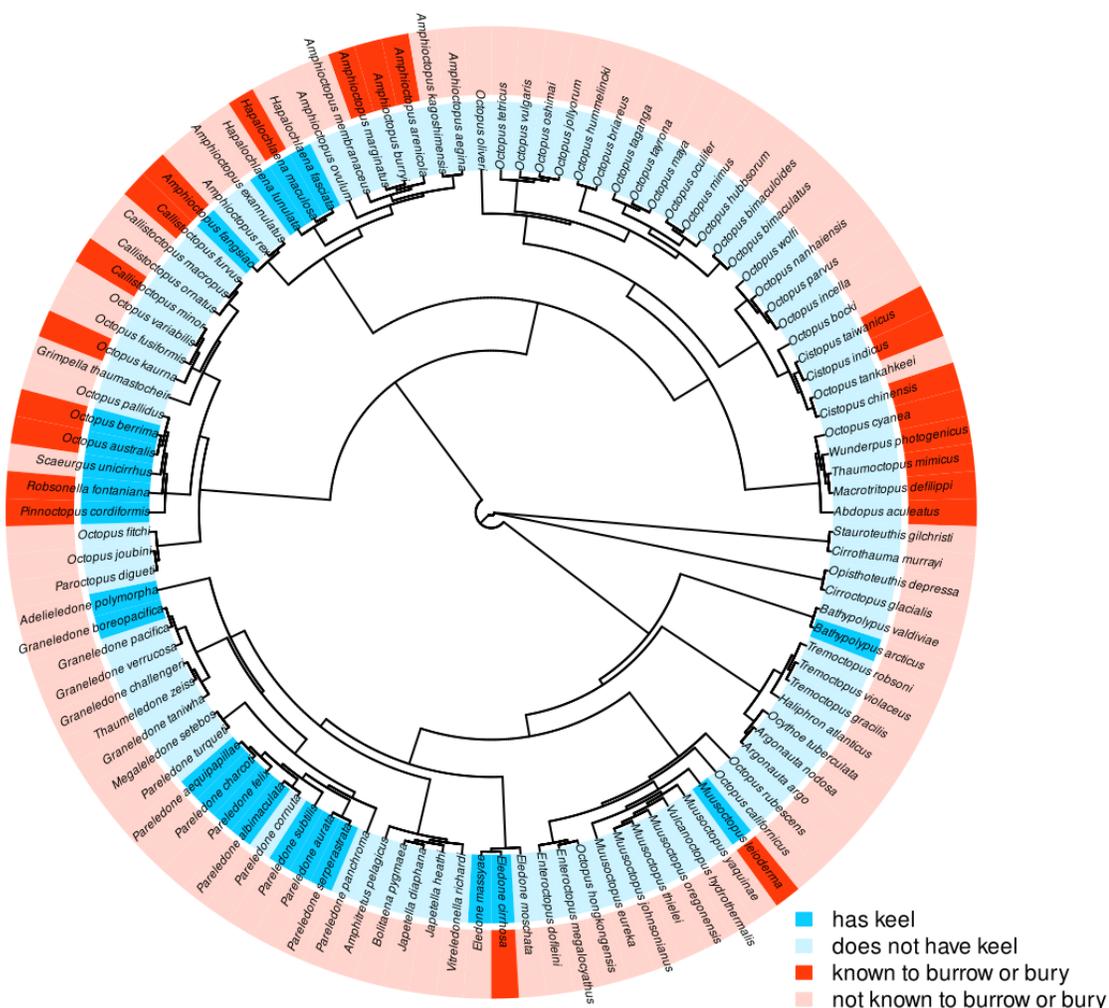


Fig 6. Multi-species phylogenetic tree showing results of Pagel's binary character correlation test between keel presence and known burrowing/burying behavior. ARD substitution model used between dependent and independent model rate matrices of 4 possibilities; burrowing, non-burrowing, keel, no keel. Results show interdependence to be the model of best fit (AIC=188.4336, likelihood-ratio = 20.5514, p-value = 0.000388571).

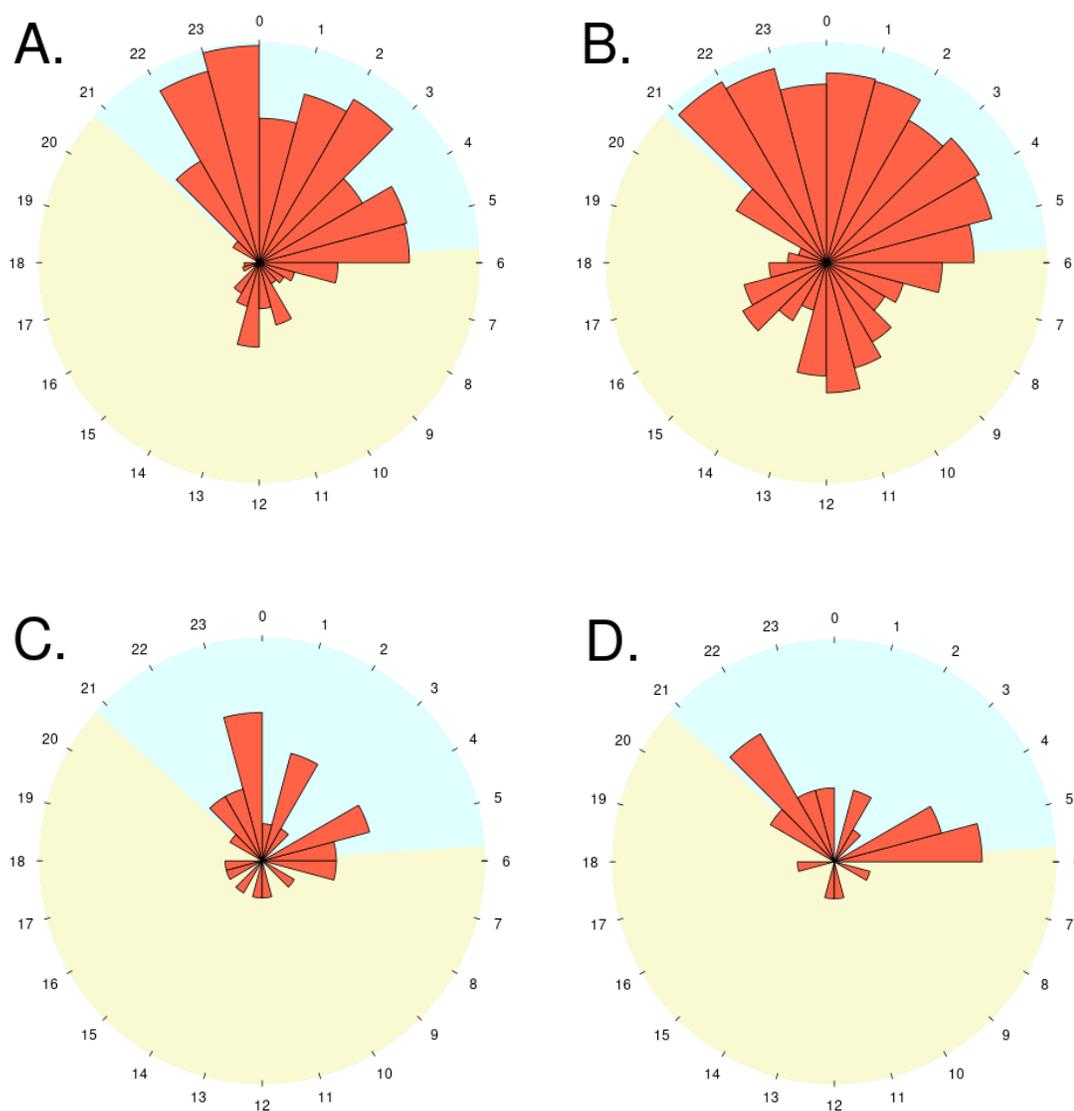


Fig 7. Radial time histograms showing frequency of photos triggered for tank activity for A) burrowing octopuses ($X^2 = 6509$, $df = 1$, $p\text{-value} < 2.2e-16$, $n = 10617$), B) all tank activity for non-burrowing octopuses ($X^2 = 4.775$, $df = 1$, $p\text{-value} < 2.2e-16$, $n = 33032$), C) octopuses entering burrows ($X^2 = 10.09$, $df = 1$, $p\text{-value} = 0.001491$, $n = 27$), and D) octopuses exiting burrows ($X^2 = 9.01$, $df = 1$, $p\text{-value} = 0.00268$, $n = 24$). Yellow shows daylight hours, blue shows nighttime hours, and red number of pictures taken at each hour. Burrowers demonstrated a stronger pattern of nocturnal behavior compared with non-burrowers, with a significantly different proportion of activity in daylight/nighttime hours between the two groups (Two-sample test for equality of proportions with continuity correction, $X\text{-squared} = 1279$, $df = 1$, $p\text{-value} < 0.001$, $n = 43649$).

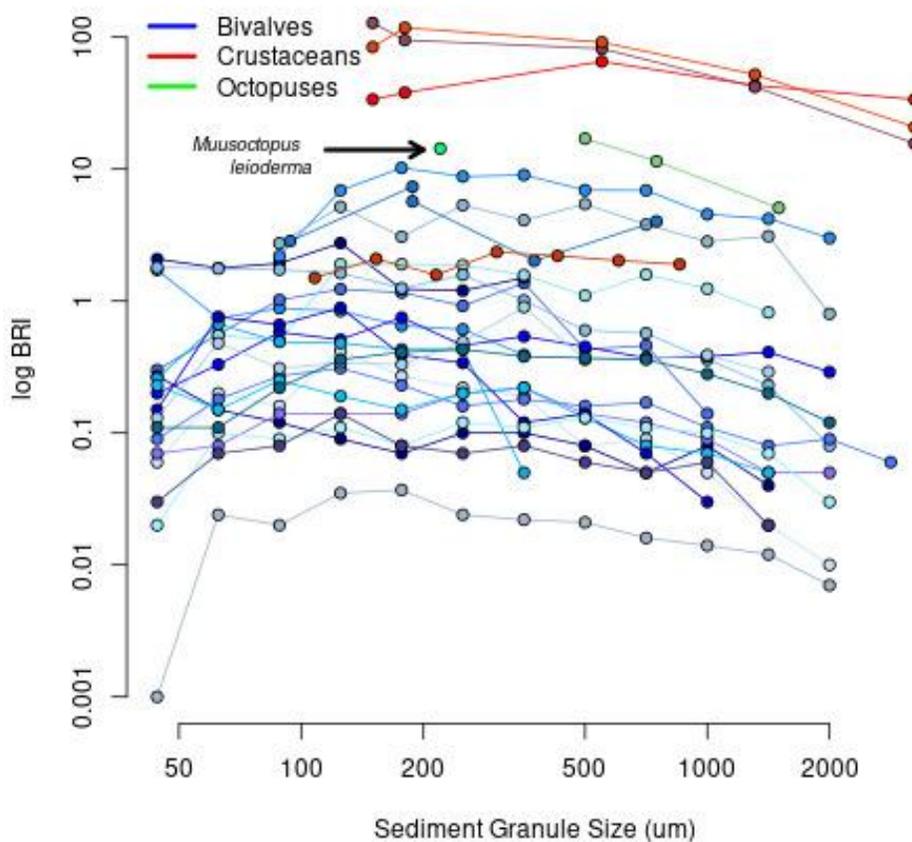


Fig 8. Burrowing Rate Indices vs sediment size in 27 marine burrowing species, including two octopus species shown in shades of green, bivalves in blues, and crustaceans in reds, with each color showing a different species. Burrowing efficiency follows a non-linear pattern across taxa, with burrowing rates peaking at an ideal sediment, then dropping in coarser and finer sediments. *M. leioderma* appears to follow this pattern, showing a slower BRI (14.32243) in fine mud (< 220 um) when compared with *O. kaurna*'s fastest BRI (17.03979978) in coarser sediment (500um) . Tested *M. leioderma* and *O. kaurna* were similar sized (3-57 grams, 12-53 grams) in similar water temperatures (10 C, 14 C).

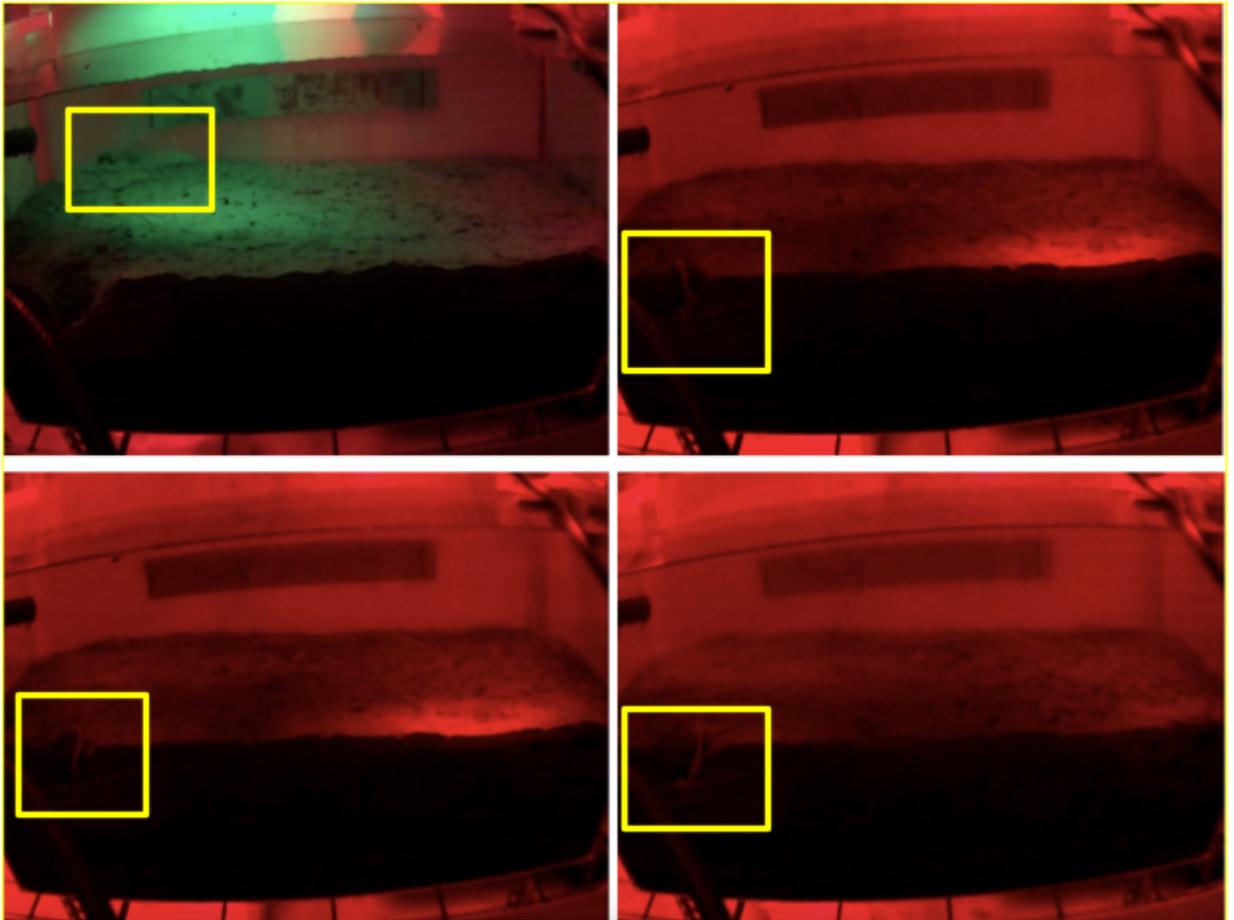


Fig 9. Octopus repeatedly moving up and down the burrow shaft. Octopus arms and/or activity partway out of burrows is visible in yellow boxes.

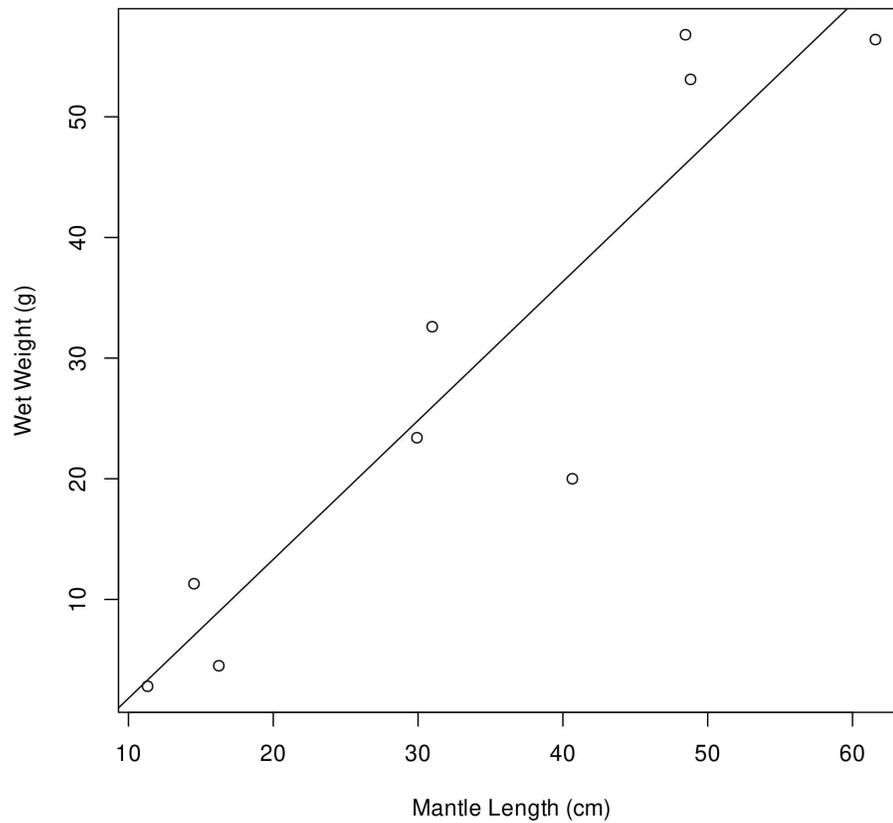
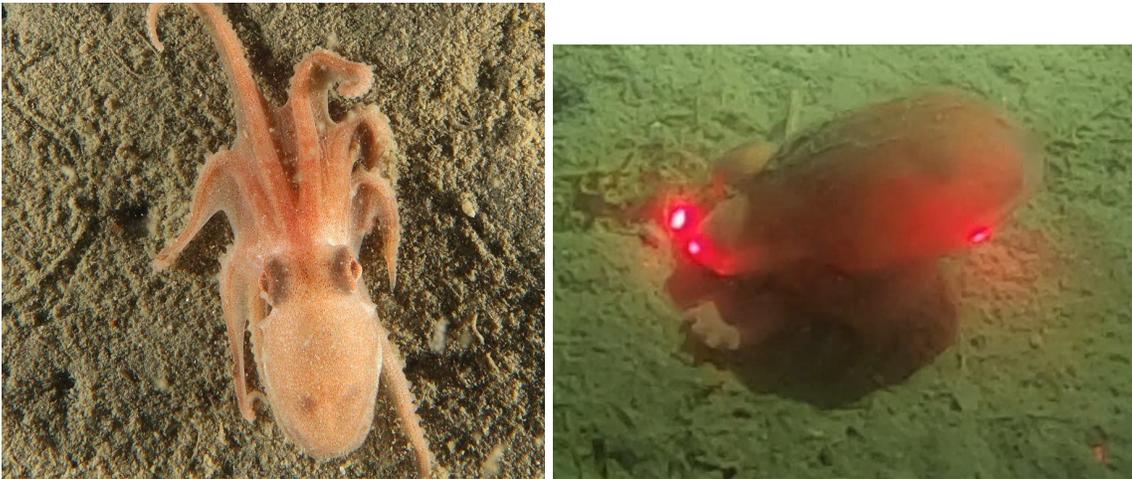


Fig 10. Length (in centimeters)/weight(in grams) relationship for 8 collected *Muusoctopus leioderma*.
Residual standard error: 8.931 on 7 degrees of freedom, multiple R-squared: 0.8542, adjusted R-squared:
0.8334, F-statistic: 41.01 on 1 and 7 DF, p-value: 0.0003658.

APPENDIX

Keel Evaluation Key

Very Prominent (VP): Keel is highly distinctive and easily seen as a flap of skin protruding strongly from the lateral mantle. Keel appears as a ring of skin protruding strongly from the mantle when observed dorsally (from above) and the area of the skin extends beyond a simple skin line or ridge. Keel would likely “flap” if octopus were in motion.



Prominent (P): Keel is distinctive and easily seen as a small skin fold protruding from the lateral mantle. Keel appears as a thick line protruding strongly from the mantle when observed dorsally. Keel is more robust than a simple skin ridge, but would likely not “flap” if octopus were in motion.



Apparent (A): Keel is visible as a ridge of skin protruding from the lateral mantle. Keel appears as a narrow line protruding from the mantle when observed dorsally. Keel is apparent as a skin ridge, but does not demonstrate excess flappy skin.



Weak (W): Keel is faintly visible as a line of skin, but does not protrude from the mantle. Keel is not readily observable from a dorsal view, and can only be seen upon close examination of the lateral mantle.

Missing (M): No distinctive lateral mantle ridge line is visible. Keel is completely missing from the octopus and no line can not be readily seen from any view.

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