DEN ASSOCIATED BEHAVIOR OF *OCTOPUS RUBESCENS* REVEALED BY A MOTION-ACTIVATED CAMERA TRAP SYSTEM

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For my parents,

who have only shown me love and support,

who have praised me in triumph and supported me in failure,

who instilled in me a deep love of nature.

Abstract

Dens are a crucial component of the life history of most shallow water octopuses. However, den usage dynamics have only been explored in a few species over relatively short durations, and *Octopus rubescens* denning behavior has never been explored *in situ*. We built four underwater camera traps to observe the behavior of *Octopus rubescens* in and around their dens. To distinguish individuals, octopuses were captured and given a unique identifiable visible implant elastomer tag on the dorsal side of their mantle. After being tagged and photographed, each octopus was released back to its original capture site within its original den bottle. The site is unique in that octopuses reside almost exclusively in discarded bottles, therefore aiding in locating and monitoring dens. Motion-activated cameras were suspended in a metal field of view above bottle dens of released octopuses to observe den associated behaviors. Cameras were regularly retrieved and replaced to allow continuous monitoring of den locations in 71-hour intervals for over a month. We found that *O. rubescens* was primarily active during the day and had frequent interactions with conspecifics (other members within the species). We also found that rockfish and red rock crabs tended to frequent den locations more often when octopuses were not present, while kelp greenling both visited dens more frequently and stayed longer when octopuses were present. Our results demonstrate the utility of motion-activated camera traps for behavioral and ecological studies of nearshore mobile organisms.

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1. Introduction

Octopuses' soft, unarmored bodies and limited swimming ability means that small octopuses must rely upon crypsis and dens for protection from predators, such as large fish, sharks, pinnipeds, and cetaceans (Dorsey 1976; Oxman 1995; Clarke 1996). Due to the security provided by a denning site, many octopuses spend the majority of their time within their dens, revealing the importance of shelter in their ecology and life history (Kayes 1973; Mather 1988). *Octopus vulgaris* has been shown to spend up to 88% of daylight hours in a den (Mather and O'Dor 1991), with only 7.3% of *O. vulgaris* encountered outside dens (Katsanevakis and Verriopoulos 2004). This necessity for a den often leads to octopuses occupying any available den types, such as discarded shells (Mather 1982), rocky dens/outcroppings (Anderson 1997), and human refuse e.g., discarded bottles (Anderson et al. 1999; Katsanevakis and Verriopoulos 2004; Freitas et al. 2022). Den availability has been found to constrain population sizes in *Octopus briareus* and *Octopus Joubini.* (Mather 1982; Aronson 1986; Katsanevakis and Verriopoulos 2004). Since refuge from predation is so important for survival, it is no surprise that octopuses compete for dens (Dorsey 1976; Cigliano 1993; Edsinger et al. 2020). Limited denning sites also cause lower localized population densities, to minimize competition and reduce interactions (Kayes 1973; Aronson 1986; Scheel et al. 2016; O'Brien et al. 2021). These observations of reduced population densities helped form the long-held concept of the "asocial octopus", as suggested by Mather (1982) when describing the absence of territorial ranges and the rarity of conspecific interactions in *O. joubini* , limiting interactions to those necessary for procreation (Huffard et al. 2008, 2010). In recent years, the concept that octopuses are asocial has been contradicted by the discovery of social aggregations and non-aggressive interactions in several species, although many of these aggregations could be influenced by population density, feeding success, protection from predators, mate accessibility or localized resources (Hunt 1996; Huffard 2007; Godfrey-Smith and Lawrence 2012; Caldwell et al. 2015; Scheel et al. 2016; Scheel et al. 2018; O'Brien et al. 2021). While these types of social behaviors are not widespread among octopuses, they indicate species-specific behavioral plasticity, which would be expected from such an intelligent animal (Mather and Dickel 2017).

Due to the difficulty of observing wild octopus behavior the majority of social behavior in cephalopods has been observed *ex situ*, with very few studies examining *in situ* denning behavior or ecology in octopuses (Aronson 1986; Voight 1992; Katsanevakis and Verriopoulos 2004; Mereu et al. 2018), leading to biased data on cephalopod social behavior due to laboratory confinement (Dorsey 1976; Tricarico et al. 2011). Confinement in an aquarium has been found to cause profound changes in social behavior. Aggression level has been related to aquarium size in cichlid fish (Oldfield 2011), zebrafish (Granquist and Berges 2013), and common cuttlefish (Geary 1999). In Atlantic salmon (*Salmo salar*) social behavior is significantly different between fish that have been raised in the wild and those raised in hatcheries (Fenderson and Carpenter 1971).

When space is limited, such as in an aquarium, interactions with other inhabitants of that space become more common and dominance hierarchies or territories may be formed, even within species otherwise considered solitary or asocial (Yarnall 1969; Van Heukelem 1977; Boyle 1980; Mather 1980). These types of social interactions can be a strategy to reduce aggression between conspecifics, such as through the "dear enemy effect" (Fisher 1954), which posits that neighboring animals will become less aggressive towards each other when territorial borders have become established. In a previous study with laboratory-confined *O. rubescens*, 47% of octopus interactions were found to result in den evictions, possibly due to territorial constraints (Dorsey 1976).

Due to the impacts of aquarium confinement on the behavior of aquarium animals, observing the den usage and behavior of wild animals is preferred. Much of the previous work on octopus den-associated behavior has relied on direct observation (Ambrose 1982; Forsythe and Hanlon 1997; Huffard 2007) or tagging studies (Hartwick et al 1984; Hofmeister and Voss 2017). Direct observation suffers from the limited time a human diver or snorkeler can continuously remain with the den and the disturbance caused by the observer. Tagging studies in octopuses have been impeded by poor tag retention and health impacts to the octopuses (Barry et al 2011). More recently, use of visible implant elastomer (VIE) tags have shown promise in octopus studies, with long retention times and minimal injury to the animal (Barry et al 2011; Brewer and Norcross 2012). To date, VIE tagging has only been employed in physical recapture studies in octopuses, and not used to study den-associated behavior. The ruby octopus (*Octopus rubescens* Berry 1953) occurs from Southeastern Alaska to Northern Mexico from intertidal regions to 200m subtidally in kelp beds, sandy mud buttons, and rocky areas (Hochberg 1998). Ruby octopuses in the study region do not appear to have a distinct breeding season, as egg clutches can be found throughout the year (pers obs). Within its range *O. rubescens* is one of the smaller octopus species, growing to an adult mass of up to 500 g (Hochberg 1997). *Octopus rubescens* is thought to forage at night, feeding on a variety of gastropods, crustaceans, euphausiids, bivalves and even fish, although they prefer small crabs and hermit crabs (Dorsey 1976; Hochberg and Fields 1980; Laidig et al. 1995; Onthank and Cowles 2011). *Octopus rubescens* has been observed living in clumped dens (~1 m- apart) in California (Hanlon and Messenger 1996), while the occurrence of glass bottles in Puget Sound have allowed *O.*

rubescens to utilize habitat where naturally occurring dens are rare (Anderson et al. 1999). Additionally juvenile *O. rubescens* have been found exhibiting schooling behavior as they move through the water column (Hunt 1996). In addition to observations of wild conspecific interactions, several examples of interspecific associations have also been found. Octopuses have been found engaging in cooperative interactions with non-octopus species, such as hunting with fish (Kayes 1973; Bayley and Rose 2020; Sampaio et al. 2021), utilization of cleaning stations (Johnson and Chase 1982; Sazima et al. 2004), and attraction of scavengers to midden piles (Hartwick and Thorarinsson 1978). Our research investigates the individual, interspecific, and conspecific social behaviors of *O. rubescens.*

To understand the wild denning behavior of *O. rubescens* this study attempts to answer the following questions: (1) What is the general diel activity pattern of *O. rubescens*? (2) What interspecific interactions can be observed surrounding *O. rubescens* den locations? (3) What trends of conspecific social interactions can be observed among *O. rubescens* at denning locations?

2. Methods

2.1 Octopus capture and transport

Twenty-seven octopuses over 15 g (140g ± 101.5g, mean ± SD) (52% female: 48% male) were collected from 15-18 m depth using SCUBA from Driftwood Park, in Island County, Washington state (48.16397, -122.63746). All octopuses were gathered opportunistically from June to August and housed for the shortest duration possible before release (typically 3-5 days). The majority of octopus collected were found inhabiting discarded glass bottles. Our study location represents a unique opportunity for the observation of conspecific and interspecific interactions among octopuses due to the substantial number of discarded glass bottles at the site, lack of alternative denning locations on the shell-hash/sediment bottom, and high octopus density of at least 1 octopus per 26.3 m^2 (about the size of a parking space), (Chase and Verde 2011). On shore, openings of collected bottles containing an octopus were covered with flexible nylon mesh and secured with rubber bands to allow water flow while preventing octopus escape. Bottles containing octopuses were transported to Rosario Beach Marine Laboratory (RBML) inside a ~130-liter (about 34 gal) cooler filled with fresh seawater and aerated with a battery-powered aquarium air pump. At RBML octopus were housed in individual 11-liter flow-through aquaria with their original bottle dens. Octopuses were fed a diet of purple shore crab (*Hemigrapsus nudus*) and a variety of small commercially available clams *ad libitum*.

2.2 Octopus tagging

Weight, sex, health and identifying characteristics (missing arms, etc.) were recorded for each octopus, before assigning each octopus a unique color pattern (Green, Yellow, Orange, Blue and/or Red) that would be injected into the dorsal side of the mantle using Visible Implant Elastomer (VIE) tags produced by Northwest Marine Technology, Inc. (NMT). To apply the VIE tags, octopus were anesthetized by submersion in an aerated 2.5% ethanol seawater mixture (Estefanell et al. 2011). Once an octopus was sedated, VIE was injected below the chromatophore layer using a 1mL syringe and 27-gauge needle. After tagging, octopuses were returned to their individual saltwater tanks to recover. Tagging had a negligible impact on octopus health, no necrosis or mortalities were observed throughout the experiment and tags were still unchanged at the termination of the study (~3 months later).

2.3 Open-Source Motion-Activated Camera System

Camera trap systems were designed using a Raspberry Pi 3B+; and a Raspberry Pi HQ camera (Figure 1B, C) outfitted with a 6 mm lens. A custom hat-type PCB (an expansion board that connects to the Raspberry Pi GPIO pins) was used to additionally connect the Raspberry Pi to far-red (FR) and ultra-violet (UV) LED strobes (Figure 1B, C, 2), a microcontroller board to trigger the LED strobes, a real-time clock, batteries and associated power management systems, and a small LED screen to monitor the system. The camera system is powered by six 12V 4500mAh Ni-Mh batteries wired in parallel to produce a total charge of ~27 Ah, yielding a ~71 hr. camera runtime (Figure 1B). Cameras were housed in a 1m section of schedule 80, 3 inch (7.62 cm) PVC pipe with three clear ports, two covered by 1.27 cm thick acrylic for the camera and loading port and one covered by 0.635 cm glass for the strobes (to allow UV transmittance) (Figure 1B, 2). The camera trap system detects motion by capturing low resolution (320 x 240) monochrome evaluation images at one second intervals while illuminating with the FR strobe. These evaluation images are saved and analyzed using a Mixture-of-Gaussian foreground detection algorithm (Aslam and Sharma 2017) from the python version of the OpenCV library version 3.4.1. (Bradski 2000). If a test image shows sufficient change in foreground pixels, indicating motion in the image, a 2040 x 1520 pixel color image is taken using combined illumination of FR and UV lights to illuminate the subject and any VIE fluorescent tags present. Once a full resolution image was triggered, the system would wait 5 seconds before restarting motion detection. To optimize camera motion detection settings for field deployments in both daytime and nighttime, variable underwater current strengths and expected animal motion characteristics, cameras were tested over several weeks on captive, tagged *O. rubescens* in a large outdoor 700-gallon (about 2650 L) flow-through aquarium in addition to several short field deployments at RBML. During these evaluations we were able to determine that we could successfully read VIE octopus tags in 87% of photos with visible octopus present. System construction files and software have been banked at the Zenodo repository (DOI: 10.5281/zenodo.6543944).

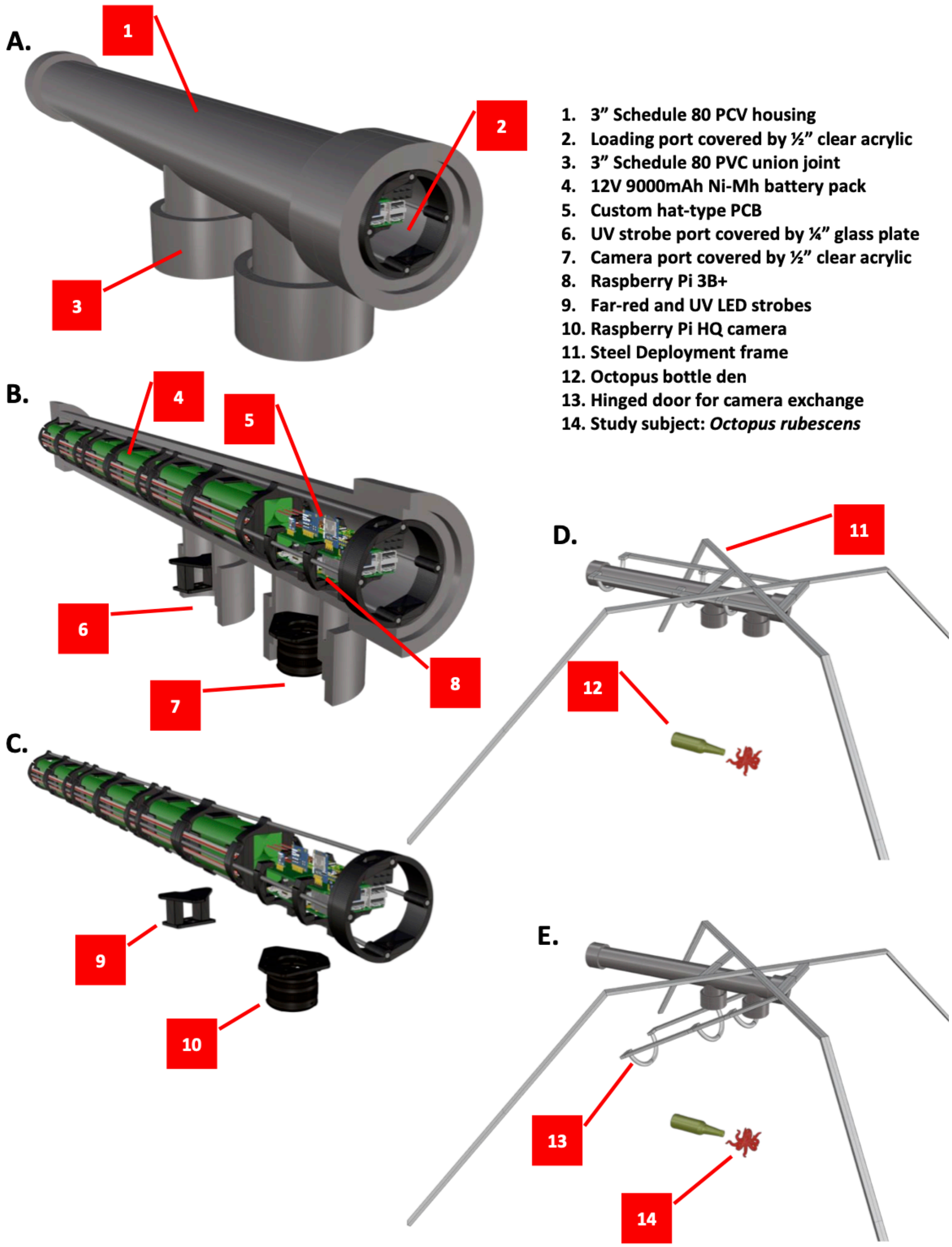

Figure 1: Camera trap assembly and field deployment system. (A) Camera PVC housing with installed system and union joints demonstrating port locations. (B) Camera schematics showing port cover locations, battery packs and attached components. (C) Lighting array and camera placement. (D) Deployment frame with installed camera, ready for deployment over den site. (E) Deployment frame open and ready for camera exchange

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2.4 Camera deployment and octopus release

Cameras were mounted in deployment frames 61 cm above the sea floor, facing down upon bottle entrances (Figure 1D, E). This setup resulted in a field of view of approximately 0.5 X 0.35 meters. Deployments varied from 10.5 to 70.7-hour durations (49 hours ± 20.0, mean ± SD), some running short of the maximum ~71 hr. due to battery difficulties. After at least 71 hrs. of deployment, cameras were collected and immediately replaced with a fully charged camera system.

Two cameras with frames were deployed at depths between 15-18 m and octopuses released on the same dives at the Driftwood Park site. Octopuses were released in their original collected denning bottle. One octopus, chosen at random, was placed beneath each deployment frame and camera. On occasion 1-4 additional octopuses would be released haphazardly over 8 m away from the deployment frames, to minimize captivity durations. To maintain octopus densities similar to those encountered at the start of the study, octopus were only collected and released within the study area.During subsequent deployments, the camera systems were swapped with another system with charged batteries, but deployment frames were not relocated. Deployment frames were built to include a hinged door, allowing cameras to be exchanged quickly. After exchanging the camera, divers would evaluate the bottles within the camera's field of view to determine if an octopus were present. If no octopuses were found within these bottles, a newly tagged octopus within its original denning bottle would be placed beneath the camera and an unoccupied bottle removed. If non-tagged octopuses were encountered, they were collected for VIE tagging and identification.

2.5 Data Analysis

All triggered photos were examined and when any animal, (except shrimp and small crabs which were exceptionally abundant) was captured in the photos the event was recorded into a spreadsheet (hereafter referred to as the "event log"). For each event date and time, the deployment number, animal species observed, octopus ID for tagged octopuses, behavior observed, and event type were recorded into the event log. Because of the high rate at which we could read tags during early tests (~87% of tagged octopus photos), coupled with the slow approach of octopuses to bottles ensuring that >10 photos were taken of each octopus, we have high confidence that we did not miss any tags on octopuses. In addition, in any case in which a tag is visible on an octopus, there was always at least one image in which the tag was readable. Behaviors were assigned for each photo based on a set of pre-defined behaviors that could be readily discerned from still photos (Supplemental Table 1). Event types were categorized into those involving a resident octopus, defined as an octopus that entered a bottle in the camera field-of-view during the deployment, a non-resident octopus, or a combination thereof., We also noted events involving an interaction between octopuses or between an octopus and interspecific animal. Over 4,200 events were extracted from the triggered photos and this event log was used for all further statistical analyses. All analyses were performed using R (R Development Core Team 2021). Frequencies of common octopus behaviors during day and night were compared using chi-squared tests. Day was defined as the period between sunrise and sunset, while night was defined as the period between sunset and sunrise. Changing sunset and sunrise times were determined for the specific location of the Driftwood Park site on the date the behavior occurred using the sunriset() function in the maptools package in R (Bivand et al. 2022). Expected probabilities for the chi-squared analysis were generated by calculating the proportion of day and night for each 24h-period when the behavior in question occurred.

Frequencies of observation for the four most observed species were compared by chi-squared between periods when an octopus was present versus when no octopus was present. Octopuses were judged to be "present" from the time the octopus entered the camera field-of-view until it left the field-of-view, even if it was inside a bottle or obscured by algae and not directly visible. To generate expected probabilities for used in the chi-squared analysis, for each interspecific animal observation the probability of the observation occurring when an octopus was present was calculated based on the proportion of time when octopuses were present versus absent in the day or night, depending on if the observation was during the day or night. For example, for an observation of a kelp greenling at night the probability of that event occurring when an octopus was present was calculated by dividing the total amount of time cameras were deployed at night when octopuses were present by the total amount of time that cameras were deployed at night. The mean of the probabilities for each individual observation of a species to occur when an octopus was present was used as the probability for the whole analysis for that species. The probability of that event occurring when an octopus was not present was calculated by subtracting the probability of it occurring when octopuses were present from 1. The distinction between day and night observation was made to avoid biases that could be introduced by diurnal or nocturnal animals. The visitation duration, defined as the time difference between the first and final photos in a continuous set of photos containing an individual animal, of the four most observed species, was also compared between periods when an octopus was present versus when no octopus was present using a two-sample permutation test. Similarly, the frequency of observations, and visitation duration were compared for non-resident octopuses when resident octopuses were present or were not present by chi-square and two-sample permutation test, respectively. The frequency of non-resident octopuses reaching into bottles versus not reaching into bottles when a resident octopus was present were compared by chi-squared with expected values set by the relative proportion of those behaviors performed by non-resident octopuses when resident octopuses were absent.

The frequencies of occurrence for two common octopus behaviors, fortify (pulling material over the bottle entrance) and periscope (placing eyes out of the bottle entrance while leaving arms and mantle inside the bottle) were compared when octopuses were alone (no other octopuses) in the field of view to frequency of occurrence when multiple octopuses were in the field of view by chi-squared. The expected probabilities for these chi-squared tests were generated using the proportion of time when one octopus versus multiple octopuses were present in the camera field of view, weighted by the number of octopuses present (for instance, a particular behavior is twice as likely to be observed when two octopuses are present versus one octopus).

3. Results

3.1 Collected Data

Cameras produced a total of 249,163 motion-activated photos with ~2.8 million additional low resolution evaluation photos. Motion-activated cameras recorded activity of octopuses and other common animals near octopus bottle dens for over 785 total hours (33 days).

Individual octopuses were never observed leaving or arriving twice in succession, which demonstrates the cameras were not missing arrivals or departures of octopuses. Thus, we conclude the system is effective at capturing octopus arrivals and departures without missing important data. In total 31 non-resident octopuses and 14 resident octopuses were observed. Non-resident octopuses were observed for a mean of 2.9 ± 3.5 min (mean ± SD) per octopus, while resident octopuses were observed for a mean of 1309 ± 1384 min (mean ± SD) per octopus.

3.2 Daily Activity patterns

Octopus activity was higher during daylight hours than at night. Departures, the behavior defined as an octopus leaving the camera field-of-view, washigh significantly greater during the day (Figure 2B, chi-squared, X^2=6.04, df=1, p=0.014). The frequency of octopuses entering and exiting bottles was also significantly higher during the day (Figure 2D, chi-squared, X^2=4.61, df=1, p=0.032). A high rate of fortification, the behavior defined as an octopus pulling in material to cover the den entrance, was observed within the first two hours post-sunset, followed by reduced fortification events throughout the remainder of the night. While fortification was one of the most frequently observed octopus behaviors, it was almost never observed during daylight hours, occurring significantly more often at night (Figure 2A, chi-squared, X^2=148, df=1, p<0.0001).

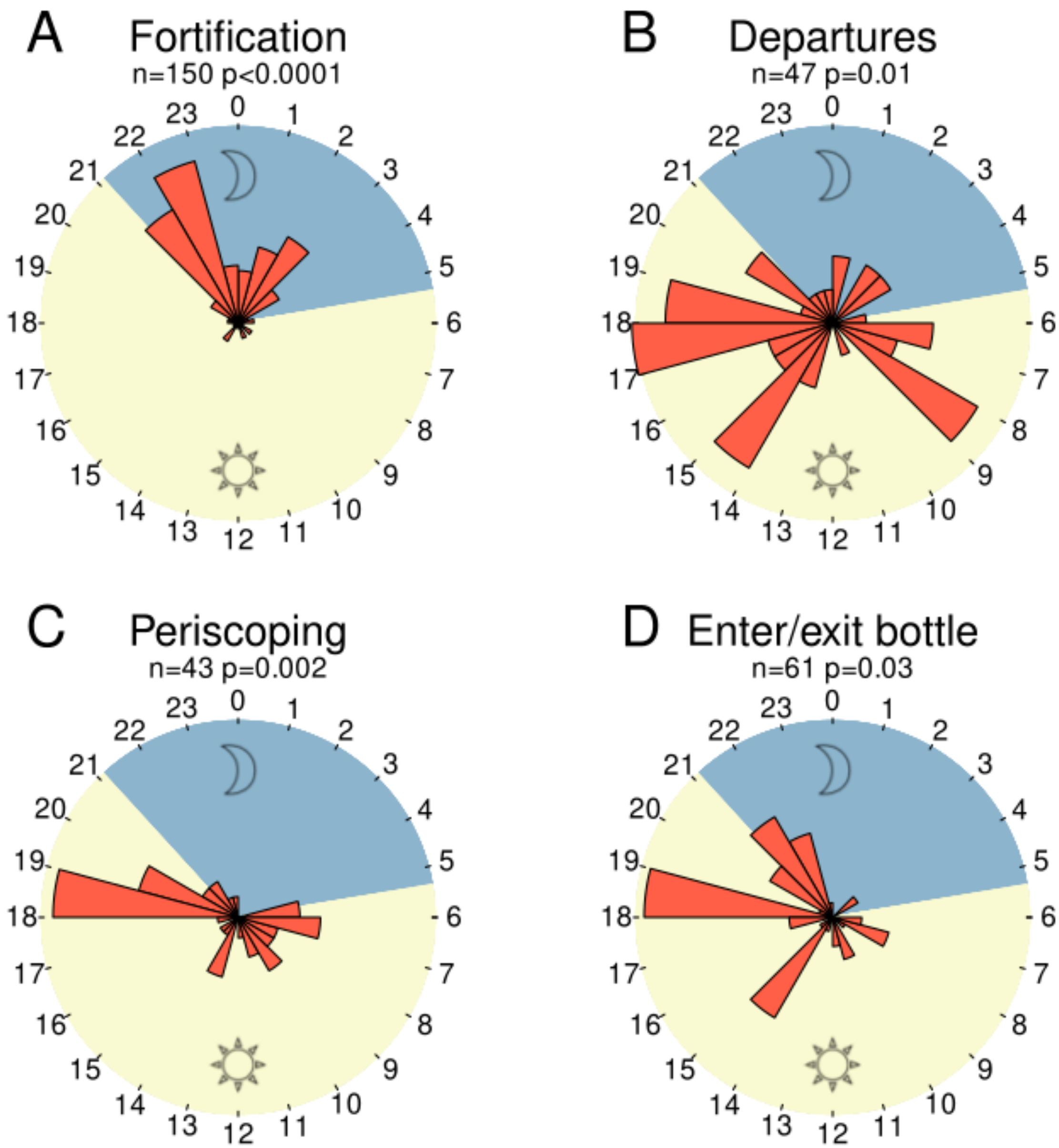

Figure 2: Radial histograms representing the absolute frequencies of hourly daily activities for O. rubescens recorded by marine camera traps throughout the day. Categorical behaviors were graphed individually, A, represents observed fortification events, n=150, Chi-squared test; X-squared = 148, df = 1, p-value < 0.0001, B, all observed field of view departures, n=47, Chi-squared test; X-squared = 6.04, df = 1, p-value = 0.014, C, periscoping behaviors observed, n=43, Chi-squared test; X-squared = 9.24, df = 1, p-value = 0.002, D, entering and exiting of bottles, n=61, Chi-squared test; X-squared = 4.61, df = 1, p-value = 0.032.

3.3 Interspecific interactions

Direct interactions between octopuses and other animals were rarely observed, however behavior of some animals appeared to change when octopuses were present or absent at the den (Figure 3). When an octopus was present in the camera field-of-view kelp greenling (*Hexagrammos decagrammus)* spent significantly more time per visit (n=291, permutation two-sample t-test, p-value=0.0002), and also visited at higher frequency (although not significant) when octopuses were present (n=291, X^2=3.2, df=1, p=0.072). In addition to increased visitation frequency and duration when octopus are present, *H. decagrammus* were regularly observed in close proximity with *O. rubescens* (Figure 4). Octopuses were repeatedly observed entering and exiting the camera field-of-view while closely accompanied by *H. decagrammus* (Supplemental Figures 1,2). *Sebastes sp.* rockfish and *Cancer productus* crabs were observed significantly less frequently when octopuses were present (*Sebastes* sp.: n=612, X^2=43.21, df=1, p=<0.001, *Cancer productus*: n=368, X^2=13.56, df=1, p<0.001).

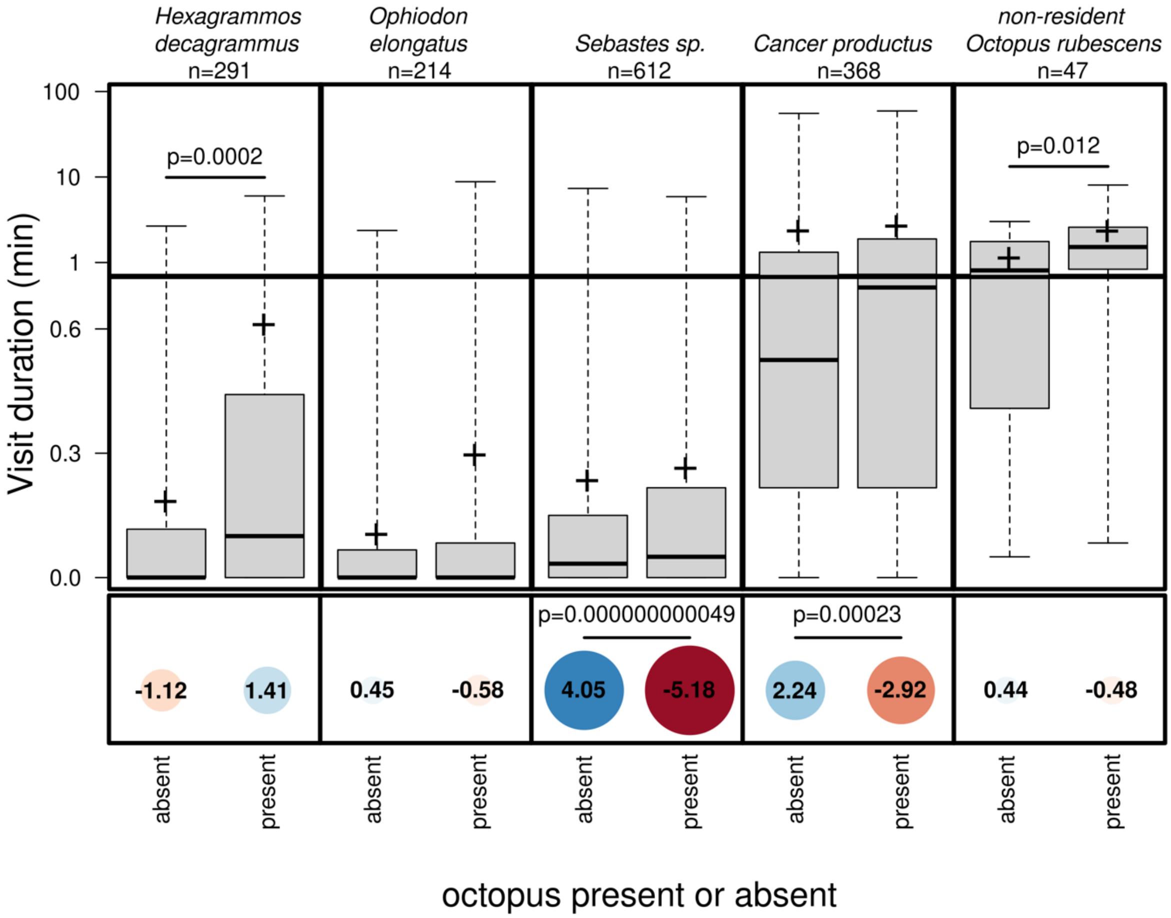

Figure 3: Commonly encountered species were evaluated for visitation duration and frequency, with and without a resident octopus present. Four non-octopus species were examined with a fifth comparison of non-resident octopus visitations. The top panel displays boxplot of visitation durations for each species when an octopus was not present or present in the camera field of view. Plusses (+) indicate average visitation durations. Y-axis is broken at 0.7 mins, above which is logarithmic to display long tails. Bottom panel displays Pearson residuals of chi-squared analysis of visitation frequency when octopuses were not present or present in the camera field of view.

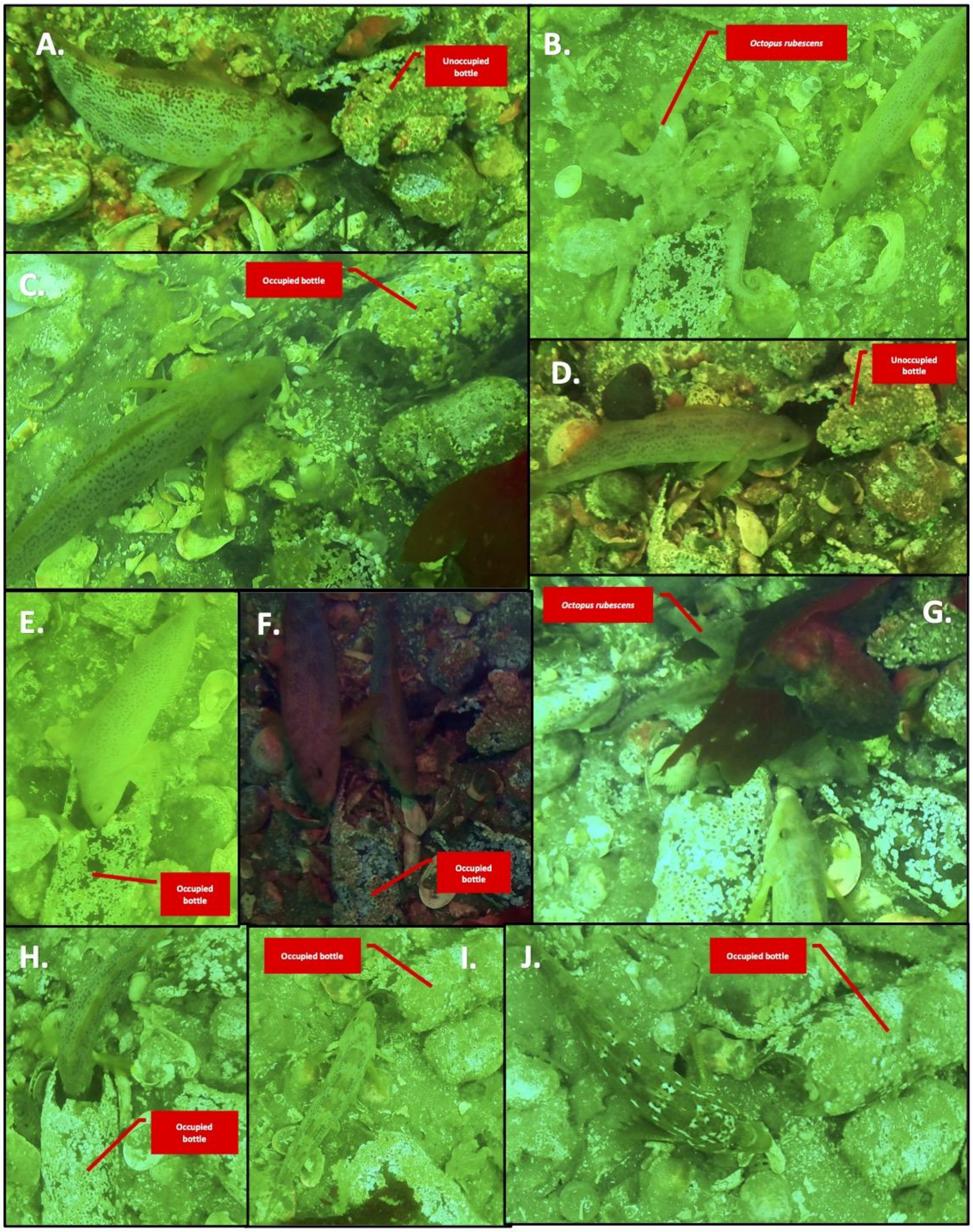

Figure 4: Photo data demonstrating regularly encountered, interspecific interactions between Hexagrammus decagrammus and Octopus rubescens

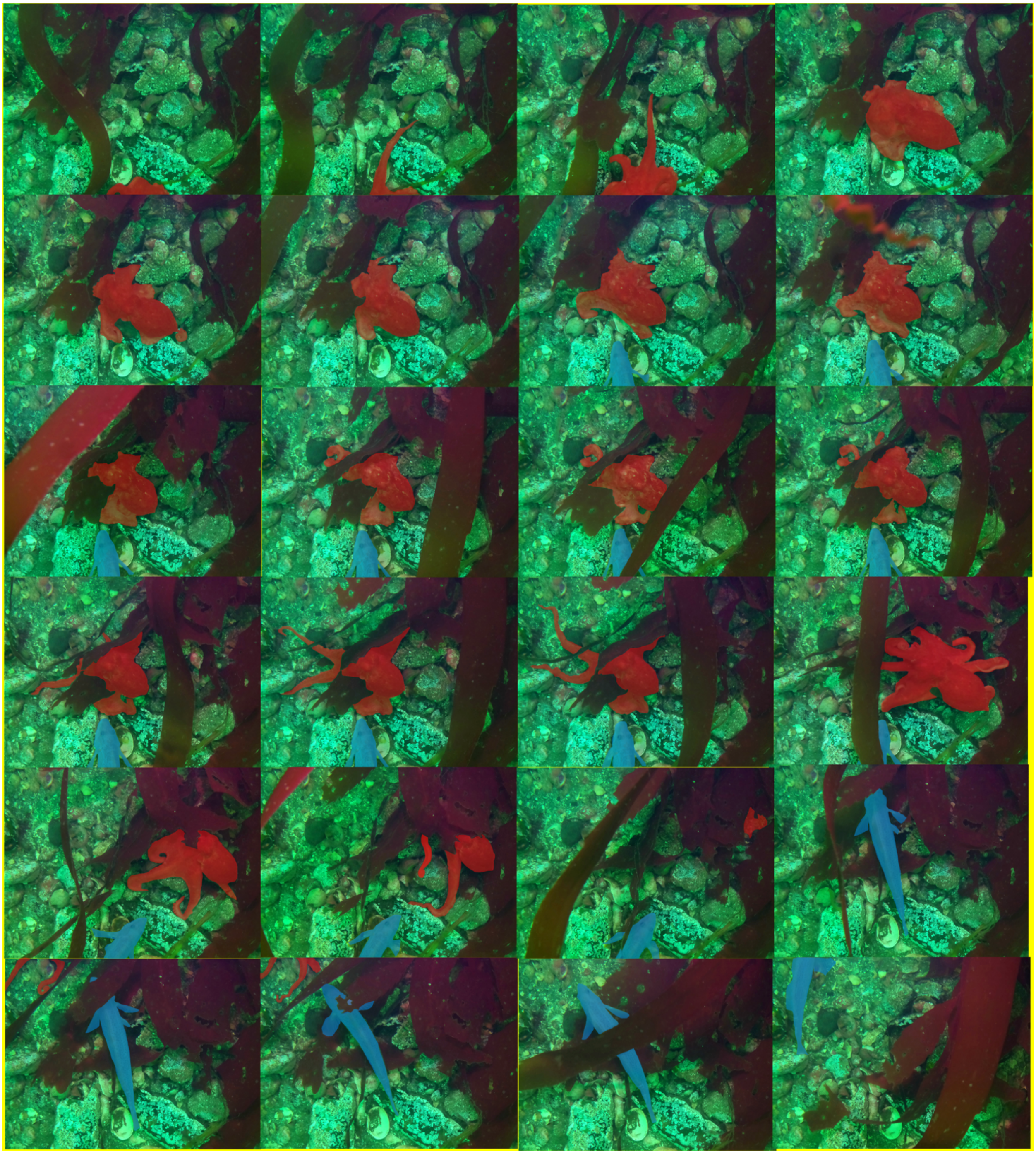

Figure 5: Photo data set of Hexagrammus decagrammus (highlighted in blue) and Octopus rubescens (highlighten in red) interactions. Photos are sequential, taken at 6 second intervals (between evaluation photos) on 2021/08/17 at 6:53AM.

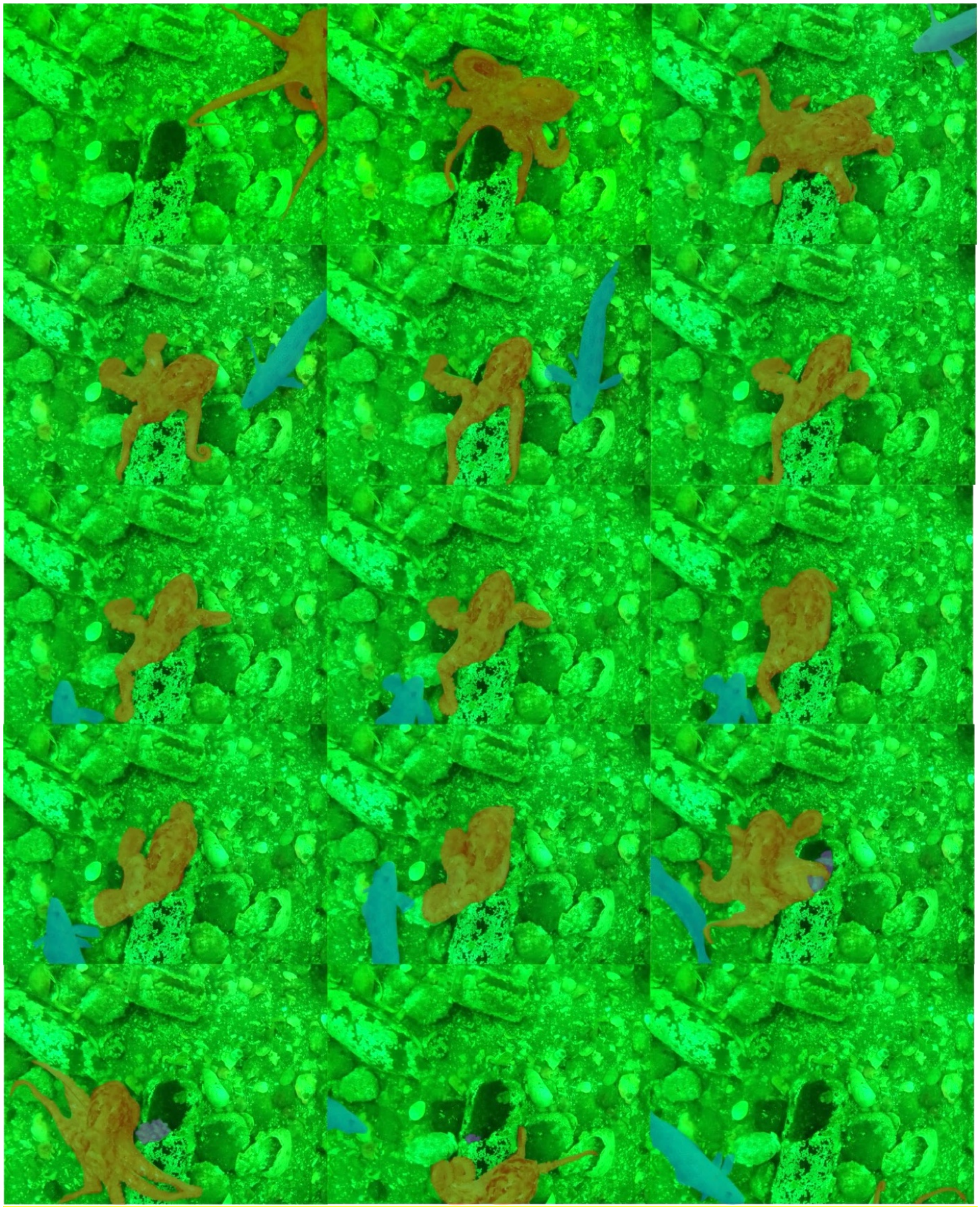

Figure 6: Photo data set of Hexagrammus decagrammus (highlighted in blue) and Octopus rubescens (highlighten in red) interactions. Photos are sequential, taken at 6 second intervals (between evaluation photos) on 2021/07/24 at 7:10AM.

3.4 Conspecific Social Interactions and Social Tolerance

Conspecific interactions were frequently observed, with multiple octopuses present over 40% of the time that octopus were present within the camera field-of-view (6,535 minutes with multiple octopuses out of 15,972 total minutes of octopuses within the camera field-of-view). Despite the substantial amount of time with octopuses near each other, these interactions never resulted in den evictions or any apparent aggressive behaviors. Non-resident octopus visitation duration and frequency was compared when a resident octopus was present versus absent to understand social tolerance. Among the 42 visitations by non-resident octopus, 28 occurred while no resident octopus were denning within the camera field-of-view, a non-significant difference (Figure 3, chi-squared, X^2=1.72, p=0.189, df=1), but non-resident octopus visitation durations were significantly longer when a resident octopus was present (138 s ± 121, mean ± SD) ., more than double the average when a resident octopus was not present (64 s ± 48, mean ± SD) (Figure 3, two-sample permutation test, p=0.012). Octopuses also alter their behavior when in close proximity to other octopuses, with periscoping behavior (an octopus placing its eyes outside the den, while its arms and mantle remain inside) occurring at a higher frequency when octopuses were alone than when multiple octopuses were present (Table 1, chi-squared, X^2=16.2, df=1, p<0.0001) although fortification behavior (an octopus pulling in material to cover the den entrance) appears to favor periods when only one octopus is in the field of view (Table 1, chi-squared, X^2=0.754, df=1, p=0.39*)*. To determine if visiting non-resident octopus were interacting with resident octopus the number of times each visiting octopus reached inside a bottle was recorded, in addition to the bottle's occupancy (with or without a resident octopus). When passing through the camera field of view, non-resident octopus were found to reach inside bottles significantly more frequently (68% of visits) if a resident octopus were present, however if no resident octopus were present, visiting octopus would reach inside bottles less frequently (32%) (Table 1, chi-square, X^2=2.58, df=1, p=0.024).

Table 1: The influence of conspecific presence on octopus behaviors.

	Bottle interaction by non-resident octopuses when a resident octopus is present	
	Do not reach	Reach into bottle
Observed	6	13
Expected	10.9	8.1
Person's residuals	-1.47	1.70
χ^2	5.07	
p-value	**0.024***	
	Fortification behavior	
	Octopus alone	Octopus not alone
Observed	68	82
Expected	62.8	87.2
Person's residuals	0.66	-0.56
χ^2	0.75	
p-value	0.39	
	Periscoping behavior	
	Octopus alone	Octopus not alone
Observed	31	12
Expected	18.0	25.0
Person's residuals	3.07	-2.60
χ^2	16.17	
p-value	**<0.001***	

4. Discussion

This study represents the first examination of wild *Octopus rubescens in situ* denning behavior, with observations of conspecific and interspecific interactions occurring at den locations. This study observed octopus dens for 33 days over a 44-day period. Any organisms and behaviors encountered within the camera field of view were recorded for analysis. *Octopus rubescens* is believed to be nocturnal (Hochberg and Fields 1980). We found that *O. rubescens* departs from the camera field of view andenters and exits their bottle den significantly more frequently during the day. In contrast *O. rubescens* engaged in closing their den entrances with material (fortifying behavior), the most common octopus behavior observed, occurred virtually exclusively at night. These findings indicate that *O. rubescens* at the Driftwood Park site are diurnal. This is surprising as this species has been exclusively reported as being active primarily at night(Dorsey 1976; Anderson 1987; Hochberg 1997, 1998). However, these have relied on data from the behavior of *O. rubescens* in captivity or anecdotal encounter rates by SCUBA divers. The larger, sympatric giant Pacific octopus (*Enteroctopus dofleini)* is considered nocturnal, through the use of sonic tagging telemetry in multiple studies (Mather et al. 1985; Scheel and Bisson 2012). Diurnal activity in *O. rubescens* could lessen competition between this species and similar sized *E. dofleini,* which have remarkably similar diets (Anderson et al. 1999; Onthank 2008; Scheel and Anderson 2012).

Throughout the study *O. rubescens* regularly interacted with conspecifics with virtually no antagonistic behavior observed. In a previous study of captive *O. rubescens*, 47.8% of 69 conspecific interactions resulted in den evictions, in which one octopus successfully removed another octopus from abottle den (Dorsey 1976). However, among the 43 wild conspecific interactions observed throughout this study lasting a total of 6,535 minutes, no evictions were observed. Octopus interactions are not rare in this population, and there is not a strong avoidance of conspecifics. Nearly half (40.9%) of the time at least one octopus was within the camera's field of view (a total of 15,972 minutes, about 1 and a half weeks), it would be accompanied by at least one more octopus. Further, during periods when a resident octopus is present, a significant increase in non-resident octopus visitation duration was seen, although no significant difference in visitation frequency was observed. This seems to indicate a preference for interaction over avoidance of conspecifics. This conflicts with the long-held belief that octopuses avoid interacting with conspecifics (Mather 1982). These results could indicate a level of social tolerance and behavioral plasticity previously unobserved within this species. This behavioral change may also be influenced by an abundance of denning locations which could reduce aggressive behaviors by removing the need to compete for a key resource.

During conspecific interactions, non-resident octopus were found to reach inside the bottle den significantly more when octopus were inhabiting the bottle, despite rarely seeing the bottle interior successfully prior to an interaction. interactions, Although octopuses are highly visual, they may struggle to see within the bottle interior (due to biofouling of the bottle's surfaces, fortification material blocking the bottle entrance or approaching the bottle from the rear). The use of sucker-to-sucker contact as an effective means of interaction may indicate the importance of chemotactile reception within octopus social behavior, or simply an alternative when visual recognition fails (Polese et al. 2016).

In some instances, the behavior of fish and crabs was influenced by octopus presence. At times when octopuses were present both *Sebastes sp.* and *Cancer productus* visited significantly less frequently, indicating active avoidance of areas where octopuses occur. Repantian crustaceans, such as crabs and lobsters, are common prey items of virtually all shallow water octopuses including *O. rubescens* (Anderson et al. 1999; Onthank 2008) and the other common octopus species occurring in the area, *E.dofleini* (Vincent et al. 1998; Scheel and Anderson 2012). Crabs are in fact preferred by *O. rubescens* over other prey taxa (Onthank and Cowles 2011). It is unsurprising, therefore, that several species of crustaceans have been shown to avoid octopus chemical cues (Brooks 1991; Berger and Butler IV 2001). Juvenile *C. productus* specifically have been found to be consumed by *O. rubescens* (Onthank 2008), and *C. productus* of all sizes are consumed by *E. dofleini* and are likely the most common prey item of this octopus species in the Puget Sound area (Scheel and Anderson 2012). The avoidance of octopus chemical cues would, therefore, be quite adaptive in this species and consistent with our data.

It is less easy to explain why rockfish (*Sebates* sp.) avoid octopus dens when an octopus is present. Rockfish are not major predators of octopuses, nor are octopuses predators of rockfish. Despite intensive investigation of rockfish diet in this area, only *Sebastes caurinus* has been found to rarely prey on *O. rubescens* (Dorsey 1976; Palsson et al. 2009). Rockfish are not eaten by the larger sympatric octopus *E. dofleini* (Cosgrove 2002), and to the contrary *Sebastes caurinus* and *Sebastes nebulosus* have both been observed sharing dens with *E. dofleini* (Love 1996; NOAA 2004). Competition may be a more likely explanation for this interesting interaction between *O. rubescens* and *Sebates* sp. fishes. Crustaceans make a substantial component of the diet of many species of rockfish found in the Puget Sound area (Palsson et al. 2009). If crustaceans avoid the areas near bottle dens when octopuses are present, as we found with *C. productus*, it is understandable that rockfish seeking out those crustaceans would also be less likely to visit the area.

Instead of avoidance, kelp greenling (*Hexagrammos decagrammus)*, appeared to be attracted to *O. rubescens*. Kelp greenlings were observed significantly more frequently when octopuses were present (11.4 observations per 24 hrs.) than when octopuses were absent (8.7

observations per 24 hrs.), and mean visit duration more than tripled when octopuses were present (mean visit duration of 36.4 s ± 70.0 s) than when octopuses were absent (mean visit duration of 10.9 s ± 25.6 s). During these visits when octopuses were present *H. decagrammus* was regularly observed in close proximity (within 10 cm) of *O. rubescens* without noticeable indications of aggression or predatory behavior from either species (Figure 4,). On several occasions *H. decagrammus* was observed closely following an octopus. In each instance, as the octopus entered the camera field of view it was closely followed by an individual *H. decagrammus,* which remained within arms-reach of the octopus throughout the entire observation, before leaving the camera field of view still accompanying the visiting octopus (Supplemental Figure 1,2). This data suggests some form of non-aggressive interspecific relationship between *H. decagrammus* and *O. rubescens*. Examples of cooperative hunting between octopus and fish have been documented (Kayes 1973; Bayley and Rose 2020; Sampaio et al. 2021); this relationship may be a similar example of such behavior. Our data, however, is not able to shed further light on this interaction and future studies should explore the relationship between *H. decagrammus* and *O. rubescens.*

The use of motion-detecting camera systems for this study proved essential to its success, providing a comprehensive look at octopus conspecific and interspecific social interactions while allowing daily behavioral trends to be quantified. The use of motion activation over a time lapse system reduced the number of images to be reviewed by ~10X, in addition to reducing storage space and download times. By combining the cameras UV lighting and VIE tagging, subjects too small for other forms of tracking could be identified, allowing the examination of organisms whose biology is otherwise only known from captivity or brief encounters in the wild.

The behavior and daily den use of *O. rubescens* shows a significant preference for out-of-den activity during daylight. This is the first evidence contradicting the long-standing belief based on SCUBA and captive observations that *O. rubescens* is primarily nocturnal. Additionally, *O. rubescens* were found to interact with *H. decagrammus* in a way perhaps similar to previously reported fish-octopus interactions. Finally, we show that conspecific interactions between individual *O. rubescens* are common *in-situ*, adding further evidence that octopuses are not as asocial as once believed. Among all the direct conspecific interactions found throughout this study no behaviors were observed that could be categorized as aggressive.

Conflict of Interest
The author declares no conflict of interest.

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Availability of Data and Materials
All datasets and code used for this study are available on Zenodo.
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Statistical analysis code DOI:10.5281/zenodo.6407342
Camera construction and system files DOI: 10.5281/zenodo.6543944

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Appendix A: Camera build instructions

Specifications Table 1

Hardware name	The Open Underwater Trigger Camera
Subject area	● Biological Sciences ● Environmental Sciences ● Open Source Alternatives to Existing Infrastructure
Hardware type	● Field measurements and sensors ● Imaging tools ● Electrical engineering and computer science
Closest commercial analog	https://www.spotx.com.au/underwater-camera-trap/underwater-camera-trap
Open Source License	Creative Commons Attribution-ShareAlike license
Cost of Hardware	~$900-1000
Source File Repository	Software, Housing and PCB: Zenodo
OSHWA Certification UID	US002113

1. Hardware in context

The general deficiency of affordable long-term marine monitoring systems has become a limiting factor in our understanding of aquatic environments. Systems capable of non-invasive aquatic ecosystem surveillance provide valuable data capable of expanding our understanding of ecology, biodiversity, and conservation in marine and freshwater environments. Within terrestrial systems, camera traps have become established as a key tool in research, leading to determination of species richness, distribution and abundance (Rowcliffe, 2017). Terrestrial mammals have been estimated to account for 95% of camera trapping research (Burton, 2015), indicating a severe lack of application in aquatic systems. This divide between terrestrial and aquatic camera trapping is primarily due to the many limitations faced by underwater camera trap systems. Likely the biggest constraint in aquatic use is the attenuation of infrared light underwater since infrared sensors struggle to detect organisms through any substantial depth of water, thus limiting the use of terrestrial camera trapping systems in waterproof housings. Often easily affordable and attainable action cameras such as GoPro's are placed in waterproof housings and used for underwater visual studies, however these systems face limitations in battery capacity and often results in partial or missing data since they rely on a timed trigger instead of a motion trigger. Many different vision-based systems have been developed in prior years but are limited by one or more constraints. The proposed system attempts to address as many limitations to these systems as possible, while remaining affordable and open-source. This system automates data collection using a trigger function which detects motion and captures the organism or disturbance. A trigger system extends battery life and storage capacity by only capturing useful data, instead of running continuously. During data analysis a trigger system further reduces workload by reducing the number of "empty" photos captured which still require analysis. This system operates off a Raspberry Pi which runs Far-red and UV strobes and a camera, three large and easily upgraded battery packs allows this system to run continuously for ~72 hour durations. The low-cost Schedule 80 PVC housing used for this system can withstand pressures up to 370 psi, allowing deployment depths in excess of 800 ft. This system was designed to monitor octopus den locations and associated behaviors but has a variety of applications within benthic ecology and marine population ecology. Requirements for the camera system were (1) affordable (~1000USD), (2) motion-detecting and autonomous, (3) long duration deployments (~3 days)), (4) capable of detecting UV florescent markers on organisms for distinguishing individuals, (5) capable of observing organisms during day and night, and (6) be easily constructed using open source components.

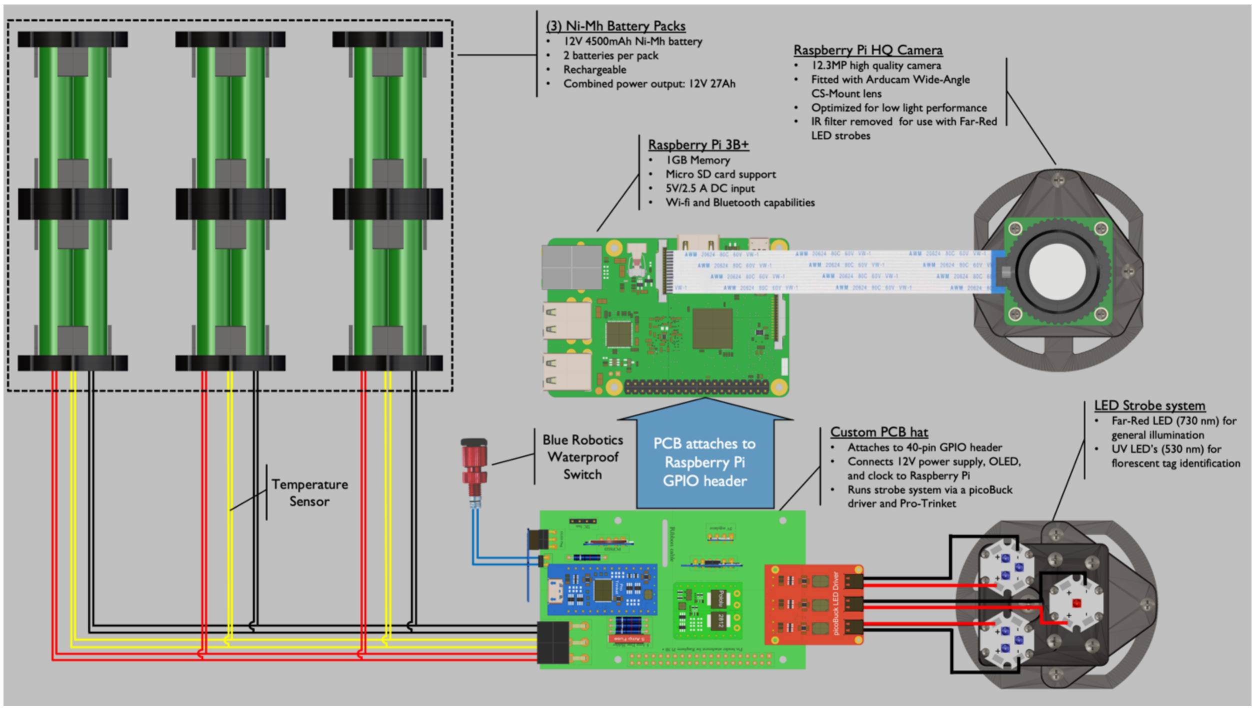
2. Hardware description

2.1 Electronics

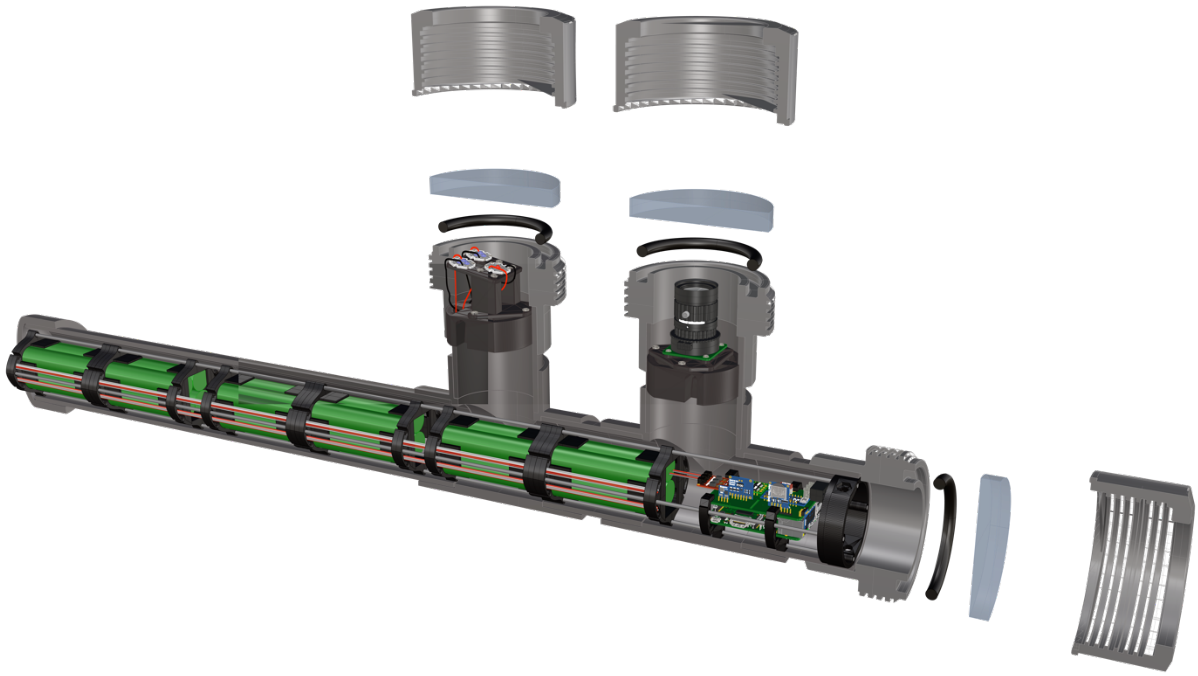
This system relies on a Raspberry Pi 3B+ which controls a variety of low-cost electronics attached to a custom PCB hat (**Appx. Fig. 13**). The Raspberry Pi 3B+ (RPI) contains an impressive 64-bit quad core processor capable of running wireless LAN and Bluetooth. An extended 40-pin GPIO header can be used for connection to a custom PCB, while an HDMI connection allows a monitor, keyboard and mouse to be connected, for easy access to the files, code and settings. A 64 GB microSD card holds the operating system and stores all acquired images. The PCB hat houses a Adafruit Pro Trinket microcontroller (3V version) which operates at 12 MHz (PTM), a Adafruit PCF8523 Real Time Clock integrated circuit (RTC), a INA 219 Voltage and Current Sensor (VCS), a Pololu Electronic power switch (EPS), a 5V power voltage regulator for powering the RPI, a Adafruit PiOLED - 128x32 Monochrome OLED (OLED), and a SparkFun PicoBuck LED Driver (PLD).

2.2 Basic system operation

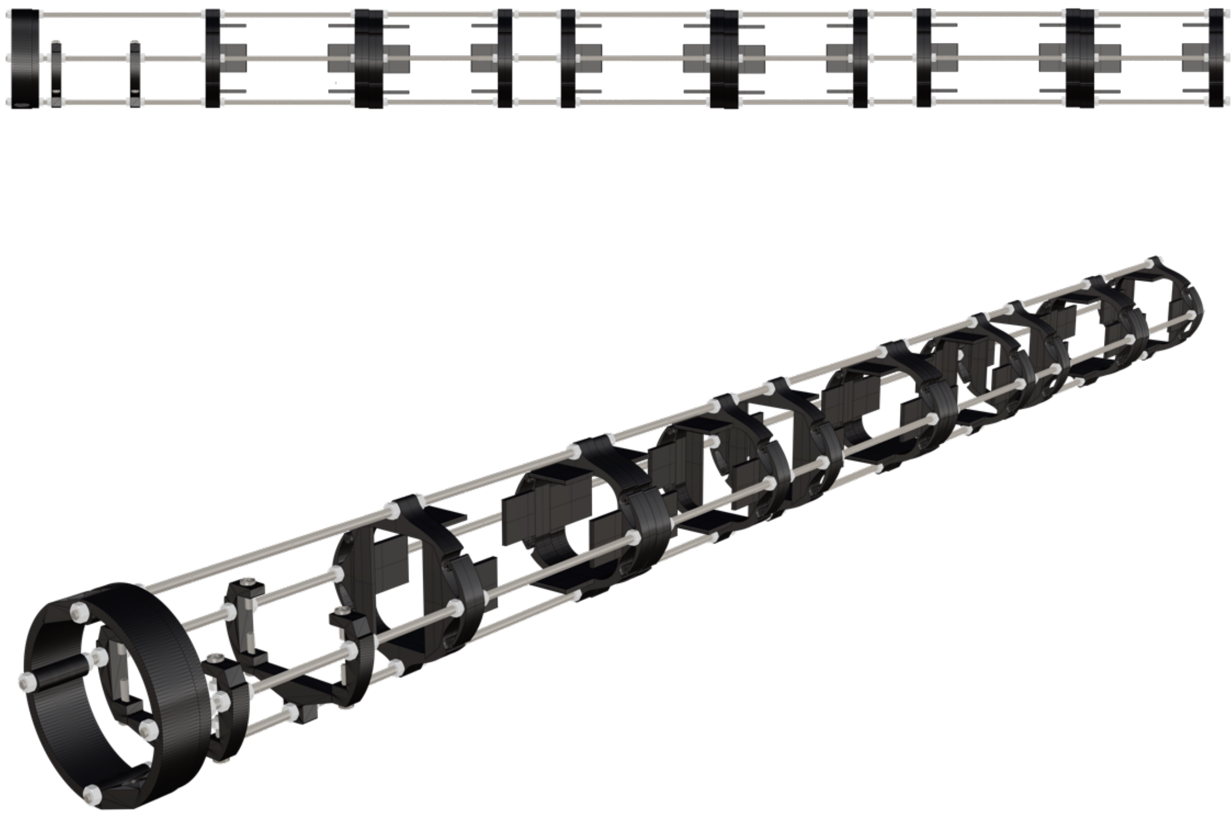
The battery bank is plugged into the PCB and is routed through a fuse and connected to the EPS on the VIN and ground pins. The battery power is also connected directly to the PTM, which is powered at all times when the battery is connected to the PCB. The PTM is connected to the Blue Robotics underwater switch. When the switch is activated, the PTM sends a logic signal to the EPS to provide power to the RPI and PLD. The RPI then boots up and activates the OLED, starting the image acquisition process. The PTM also serves as the strobe channel selector as the strobe output pin from the RPI is separate from the strobe channel pin. The OLED, RTC, and VCS) are all connected to the RPI i2c interface for communication.



Appendix Figure 1: Overview of camera system wiring diagram and key functional components within housing.



Appendix Figure 2**:** Overview of key functional components within housing.



Appendix Figure 3: Side view and 3D overview of key structural components with battery and PCB mounts.

3. Design files

Appendix Table 1: Summary of 3D printed design files, PCB hat files and software necessary for project manufacture.

Design file ID	Design file name	File type	Open source license	Location of the file
DF1	port_mount_spreader.stl	3D mesh	CC BY-SA 4.0	Zenodo
DF2	port_mount.stl	3D mesh	CC BY-SA 4.0	Zenodo
DF3	camera_mount.stl	3D mesh	CC BY-SA 4.0	Zenodo
DF4	led_holder_base.stl	3D mesh	CC BY-SA 4.0	Zenodo
DF5	led_holder.stl	3D mesh	CC BY-SA 4.0	Zenodo
DF6	battery_holder_cap.stl	3D mesh	CC BY-SA 4.0	Zenodo
DF7	battery_holder_middle.stl	3D mesh	CC BY-SA 4.0	Zenodo
DF8	pi_bulkhead.stl	3D mesh	CC BY-SA 4.0	Zenodo
DF9	front_bulkhead.stl	3D mesh	CC BY-SA 4.0	Zenodo
DF10	trigger_camera_disk.img	Software source code	CC BY-SA 4.0	Zenodo
DF11	Pro_trinket_code.ino	Software source code	CC BY-SA 4.0	Zenodo
DF12	PCB_hat_final	Kicad file	CC BY-SA 4.0	Zenodo

4. Bill of Materials

Appendix Table 2: Necessary components for project with purchasing specifics and location.

Part #	Component	Mfr. Model #	Number Used	Cost per Unit (US$)	Total Cost	Component Source
P1	Custom PCB hat	NA	1	~20.00	~20.00	JLCPCB
P2	10K ohm resistors (0.5 Watt)	611355173112	4	5.99 (100)	5.99 (100)	Amazon
P3	5 Amp Fuse	0297005.WXNV	1	0.29	0.29	Digi-Key
P4	Fuse clip	3544-2	1	0.95	0.95	Mouser
P5	I2C bus (4 pin male header)	B09MYF8XPC	1	7.99	7.99	Amazon
P6	Preci dip 2 pin angle header (female)	801-83-002-20-001101	1	0.52	0.52	Mouser
P7	6 pin angle header (male)	90122-0123	1	1.94	1.94	Mouser
P8	Dual Row Tin 6 pin Header	76825-0006	1	2.73	2.73	Mouser
P9	Pololu Electronic power switch	2812	1	5.95	5.95	Pololu
P10	Picobuck LED driver	COM-13705	1	17.50	17.50	Sparkfun
P11	Screw Terminals 3.5 mm Pitch (2 pin)	1729128	3	1.05		Sparkfun
P12	Adafruit Pro Trinket 3V 12MHz	2010	1	9.95	9.95	Adafruit
P13	INA 219 I2C Current Sensor	B01ICN5OAM	1	6.99	6.99	Amazon
P14	5V 3A Output Voltage Regulator	B0823QLMWC	1	12.99	12.99	Amazon
P15	Adafruit PCF8523 Real Time Clock	3295	1	6.95	6.95	Adafruit
P16	LiCB 3V Clock Battery	B0797NRXZY	1	5.00	5.00	Amazon
P17	Adafruit PiOLED 128x32 Monochrome OLED	3527	1	7.99	7.99	Adafruit
P18	40 pin female header connector	PRT-16764	1	1.95	1.95	Digi-Key
P19	Raspberry Pi 3B+	5060214370165	1	35.00	35.00	PiShop.us
P20	10cm Female to Female jumper	B07S2RH6Q4	1	5.49	5.49	Amazon
P21	Arducam Wide-Angle CS-Mount lens	B088BLZKRG	1	15.99	15.99	Amazon
P22	Flex Cable for Raspberry Pi Camera	A1 FFCs	1	13.99	13.99	Amazon
P23	Raspberry Pi HQ Camera	0633696492738	1	50.00	50.00	PiShop.us
P24	Molex 6 Circuit Wire Connector	39121400	1	10.99	10.99	Amazon
P25	Wiring 20 AWG (100ft of red and black)	B07K9JKXM9	1	23.98	23.98	Amazon

P26	Machine screw M5-.8 nut	B07CDZMXYR	2	8.27	8.27	Amazon
P27	Machine screw pan head Philips M5-.8 x 50mm	B00918KNBI	2	8.27	8.27	Amazon
P28	Sheet metal screws #6 x ¾”	B08SJ11HG7	1	8.49	8.49	Amazon
P29	#2-56 UNC Machine Screws	NA	1	11.99	11.99	Amazon
P30	Sheet metal screws #4 x ½”	B08P2J19WM	1	6.98	6.98	Amazon
P31	530nm Starboard UV LED	XPEBGR-L1-0000-00F03-SB01	2	6.65	13.3	Digi-Key
P32	FR LED’s (INDUS STAR A008)	A008-CE20FAR27	1	9.99	9.99	Digi-Key
P33	12V 4500mAh Ni-Mh Battery (Pack of 2)	945-0129	3	49.99	149.97	Amazon
P34	Molex Female freehang Mega-Fit plug	1716920106	3	1.11	3.33	Digi-Key
P35	Molex Female plug tin crimp pin	0768230321	100	0.1611	16.11	Digi-Key
P36	#6-32 x 36in threaded rod	52102	4	4.99	19.96	ACE
P37	#6-32 Machine Hex Nut	B07ZWDDGPS	1	9.40	9.40	Amazon
P38	Molex Male freehang Mega-Fit plug	1054110106	4	1.19	4.76	Digi-Key
P39	Molex Male plug tin crimp pins	1054170334	100	0.187	18.71	Digi-Key
P40	Schedule 80 PVC 3 inch Pipe 10ft	H0800300PG1000	1	91.82	91.82	Grainger
P41	Schedule 80 PVC Pipe cap	847-030	1	24.39	24.39	Grainger
P42	Schedule 80 PVC T-joint	801-030	2	28.90	57.8	Grainger
P43	Schedule 80 PVC Union joint	897-030	3	40.96	122.88	Grainger
P44	½ Plexiglass	SL-AS13-12x12	1	22.95	22.95	Amazon
P45	¼ in Glass plate					
P46	BlueRobotics High Pressure Switch	SWITCH-M10-5A-R1-RP	1	20.00	20.00	Blue Robotics
P47	Charger for 9.6V-18V NiMh/NiCd Battery Packs	H02400918-US-1	2	24.95	49.9	BatterySpace.com
P48	Schedule 80 PVC cement grey	20603	1	8.01	8.01	Grainger
P49	Silicone-Based vacuum grease	013161037532	1	32.95	32.95	Amazon
P50	Rechargeable desiccant packets	634301317538	1	10.98	10.98	Amazon
P51	SanDisk 64 GB MicroSD card	B08GYBBBBH	1	11.99	11.99	Amazon
P52	0.6 mm Solder Wire	B07PBD71V2	1	15.99	15.99	Amazon
P53	Heat shrink tubing	B089D82FLG	1	13.99	13.99	Amazon

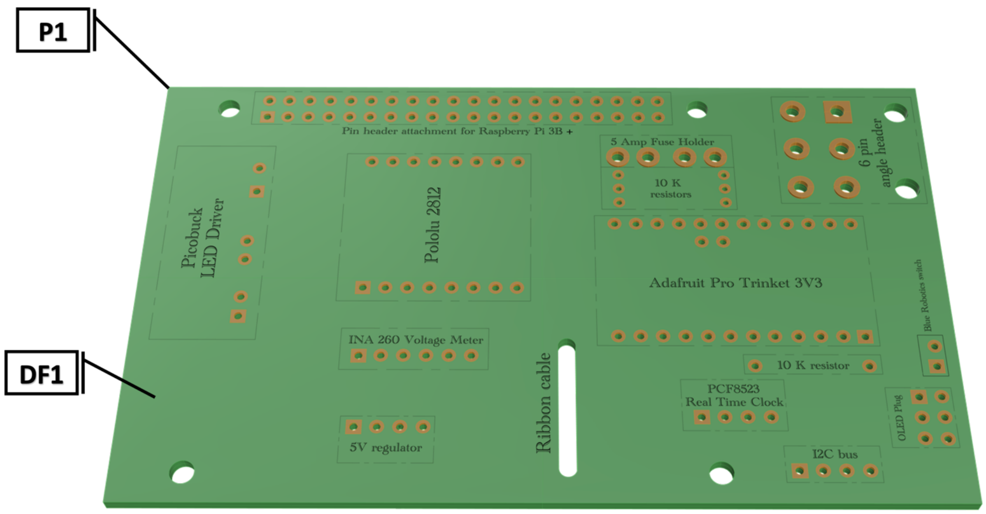
5. Build Instructions

Step-by-step instructions to assemble the PCB hat, batteries, wiring and 3d-printed structural components. Refer to parts list (**Appx. Table 2**), design files (**Appx. Table 1**), and visual aids (**Appx. Fig. 1-55**) for necessary components and assembly process.

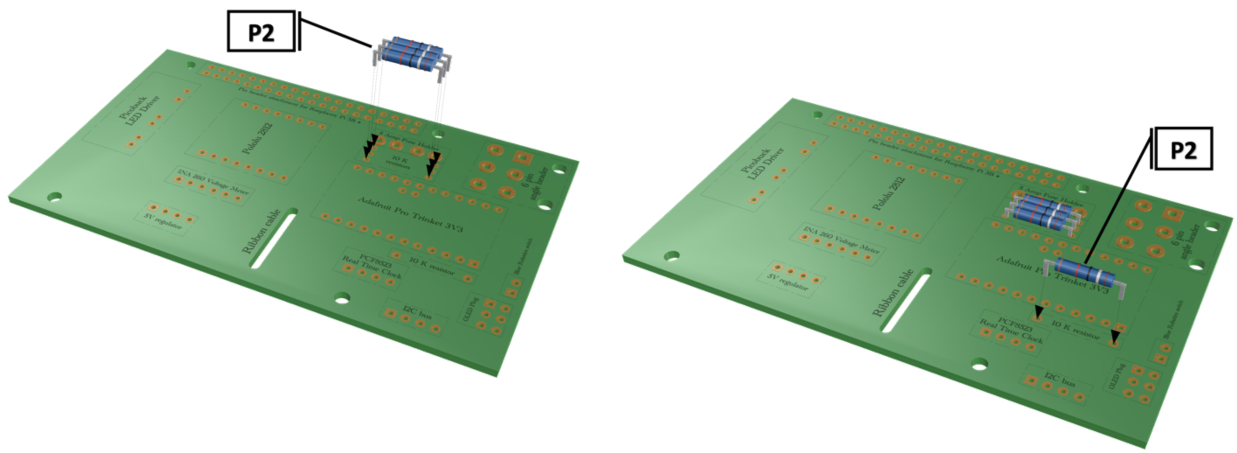
5.1 PCB Assembly Instructions

1. Insert 10K resistors (**P2**) into through hole on PCB and solder to board (**Appx. Fig. 5.**), trim any excess resistor leads after soldering. All soldering done during construction was performed using 0.6 mm solder wire (**P52**).
2. Solder fuse clip (**P4**) pins to the board before inserting the 5 Amp fuse (**P3**), **Appx. Fig. 6.**
3. Solder 4 pin male header (**P5**) to board for use as I2C bus **Appx. Fig. 6**.
4. Solder two pin header (**P6**) to board for attachment of Blue Robotics switch terminals (**P46**) **Appx. Fig. 7.**
5. Solder 6 pin angled header to board (**P7**) for PiOLED (**P17**) attachment **Appx. Fig. 7.**
6. Solder female 6 pin plug header (**P8**) to board for power input from wiring harness (**Appx. Fig. 8**).
7. Solder Pololu power switch (**P9)** directly to board **Appx. Fig. 8**.
8. Solder screw terminals (**P11**) to picobuck driver (**P10**) (if not previously installed). Solder the complete picobuck to board, **Appx. Fig. 9.**
9. Solder Pro Trinket (**P12**) to board (**Appx. Fig. 9**) and upload Arduino code (**DF11**) to Trinket.
10. Solder current sensor (**P13, Appx. Fig. 10),** 5V regulator (**P14, Appx. Fig. 10**), and real time clock (**P15, Fig. 11**) to board. Insert LiCB 3V battery (**P16**) into the real time clock (**Appx. Fig. 11**).

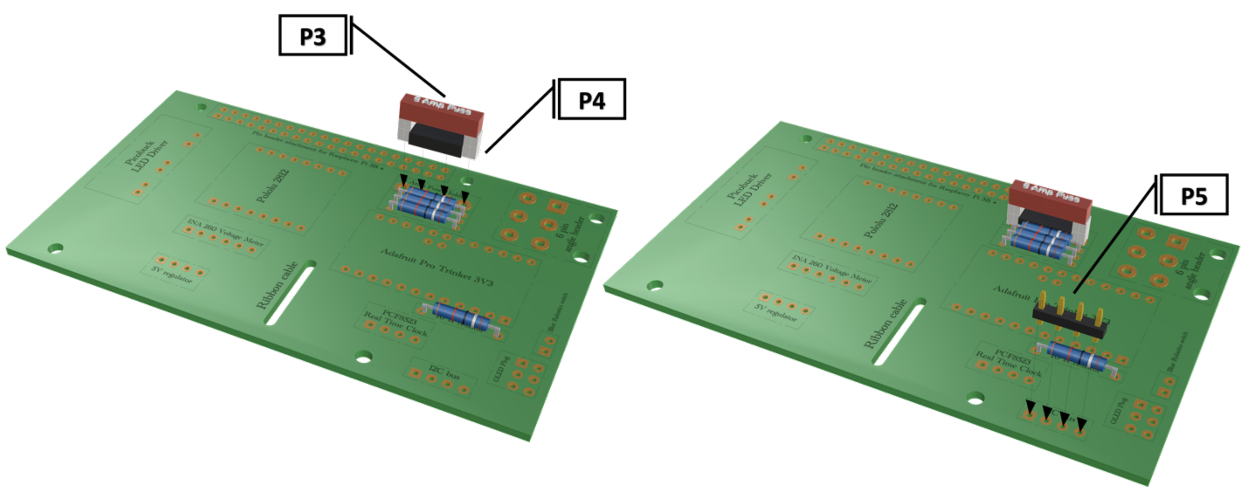
11. Insert the PiOLED (**P17**) into 6 pin header (**P7**), **Appx. Fig. 12**.
12. Solder 40 pin female connector (**P18**) to PCB header **Appx. Fig. 13**, followed by insertion of Raspberry Pi 3B+ (**P19**) to base of attached 40 pin female header **Appx. Fig. 13**.
13. Attach a Female to Female jumper (**P20**) from I2C bus (**P5**) to GPIO2 (Pin# 3) and GPIO3 (Pin# 5) on Raspberry Pi 3B+ (**P19**) (**Appx. Fig. 15**).
    . If the female to female jumper is too long or obstructing board access, a small zip tie can be used to secure the jumper to the top of the real time clock (**P15**) and/or current sensor (**P13**) using the attachment points at the top corner of each board.
14. Camera and attached ribbon cable (**P22**) can be inserted into Raspberry Pi CSI port **Appx. Fig. 16,** following assembly of camera components and removal of FR filter (See sections 2.6.2 and 2.6.3.)
15. To operate the strobe system (assembly instructions in 2.6.4) a wiring harness must be connected to picoBuck LED driver (**P10**) to allow LED control. Using screw mount terminals (**P11**), connect 10-12 inches of wire (**P25**) to each terminal following the colored diagram shown in **Appx. Fig. 17**. Attach wires to tin crimp pins and insert into 6 circuit Molex plug (**P24**) in accordance with the diagram found in **Appx. Fig. 18.**
16. After uploading trigger_camera_disk.img (**DF10**) onto a 64 GB MicroSD card (**P51**), insert the card into the receptacle at the base of the Raspberry Pi.



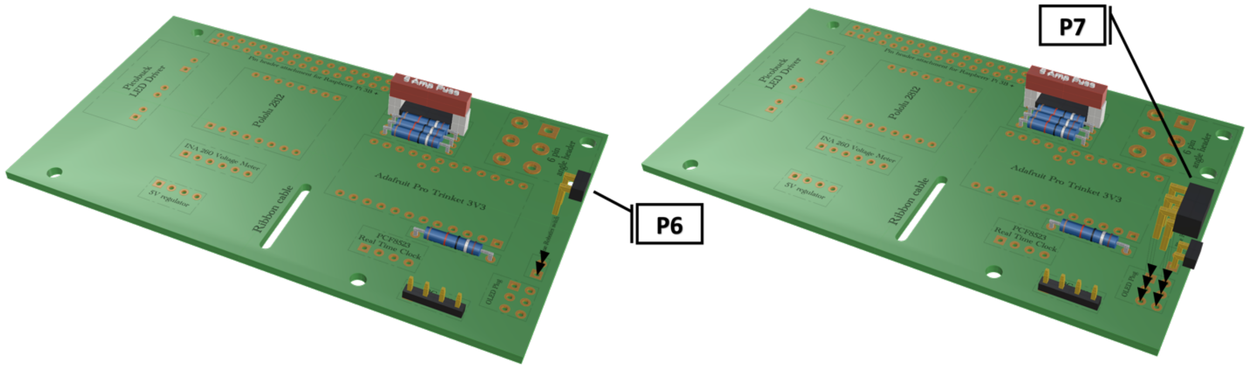
Appendix Figure 4**:** Bare PCB with labeled component placement locations.



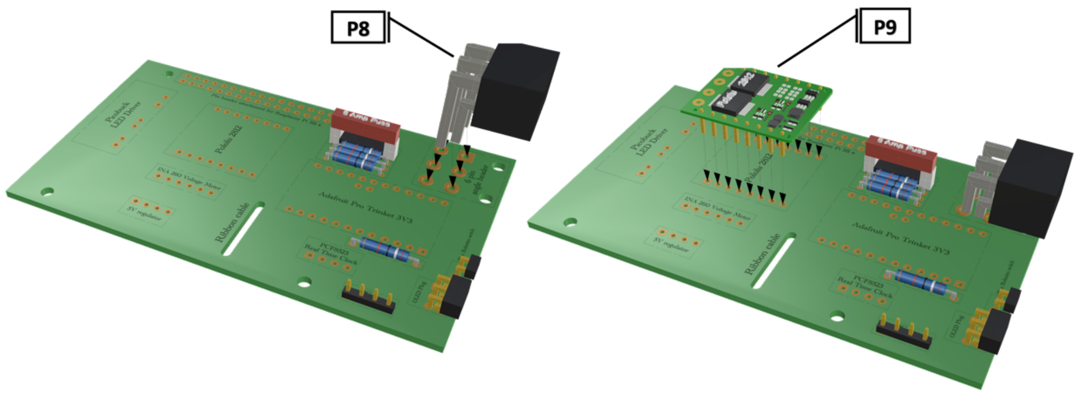
Appendix Figure 5**:** Installation of 10K resistors



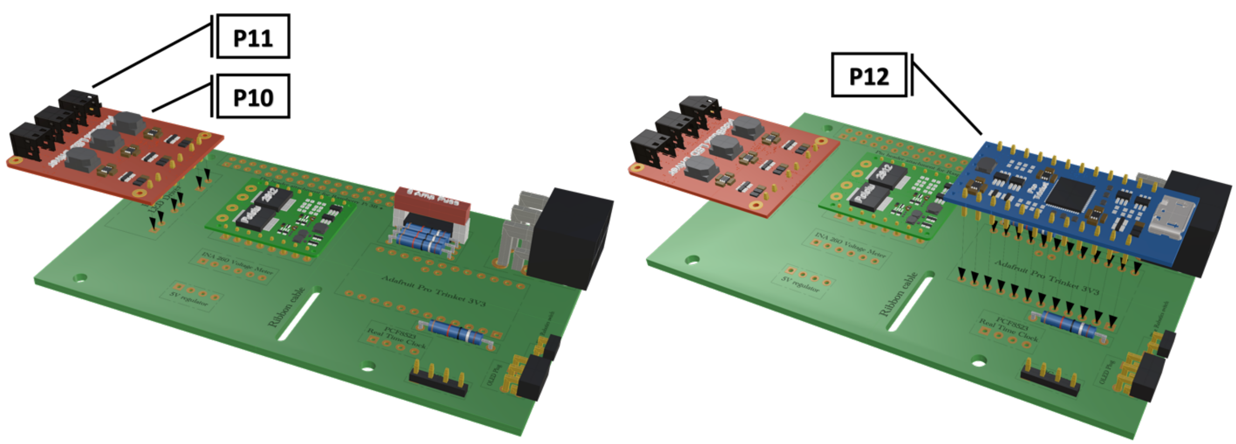
Appendix Figure 6: Installation of 5Amp fuse and fuse clip, installation of I2C bus for later connection to Raspberry Pi pins via a female-to-female 10cm jumper wire (**P20**).



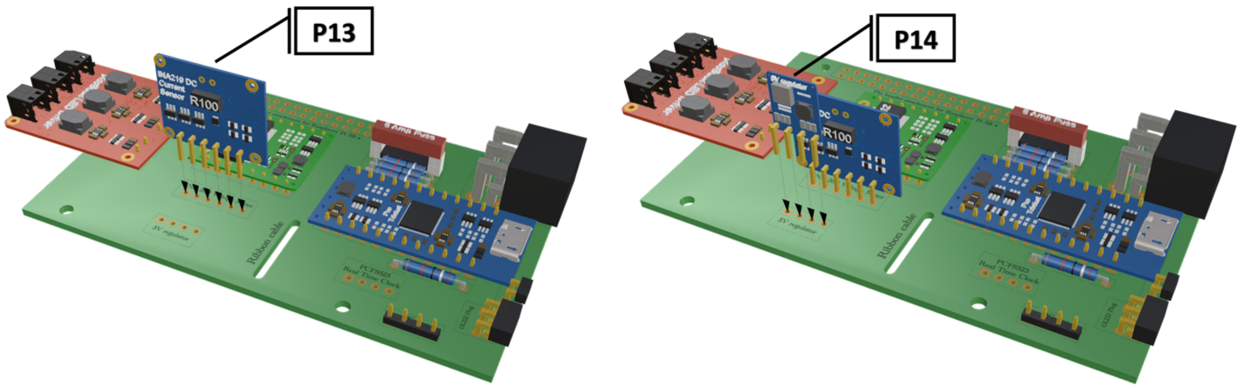
Appendix Figure 7: Installation of two pin header for connection to Blue Robotics switch (**P46**), followed by installation of 6 pin angled header for later attachment of PiOLED (**P17**).



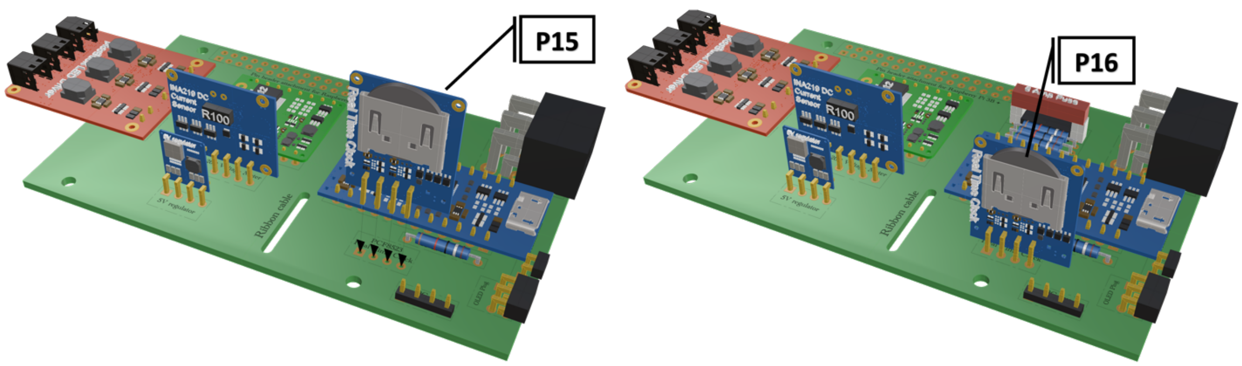
Appendix Figure 8: Installation of 6 pin header, power input from battery pack wiring harness, and installation of Pololu power switch.



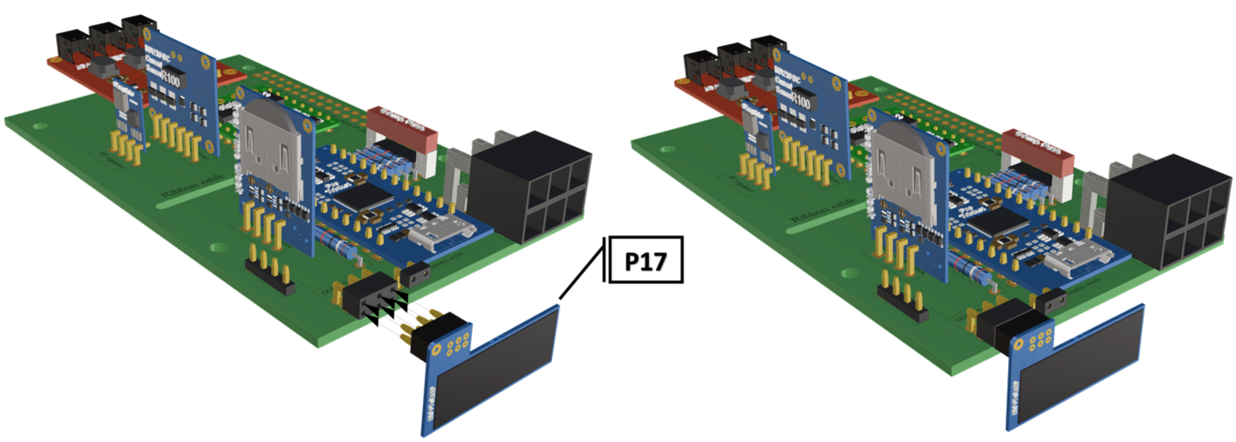
Appendix Figure 9: Installation of Picobuck LED driver with attached screw terminals, and Pro Trinket.



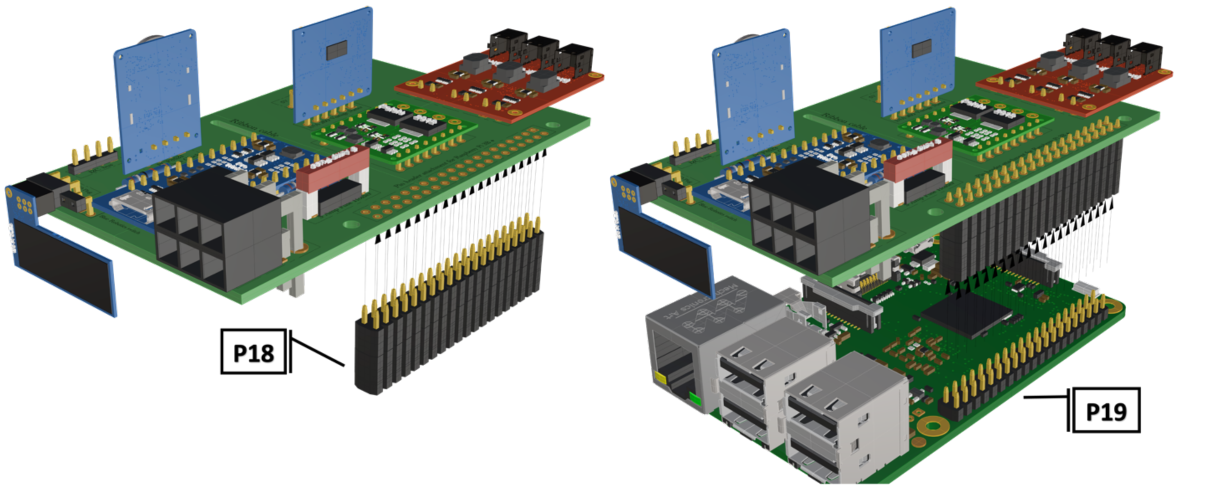
Appendix Figure 10: Installation of current sensor and 5V regulator.\



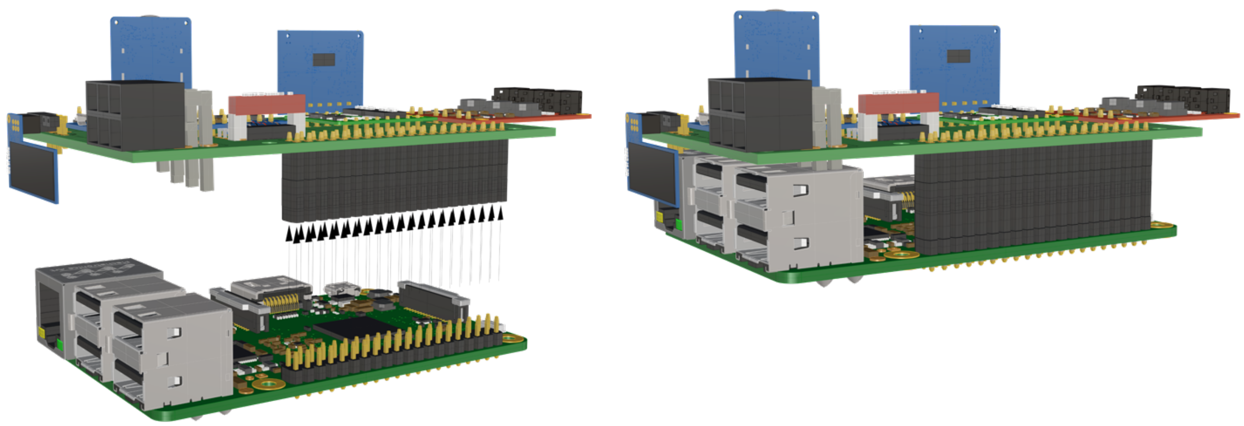
Appendix Figure 11: Installation of real time clock with inserted LiCB 3V clock battery .



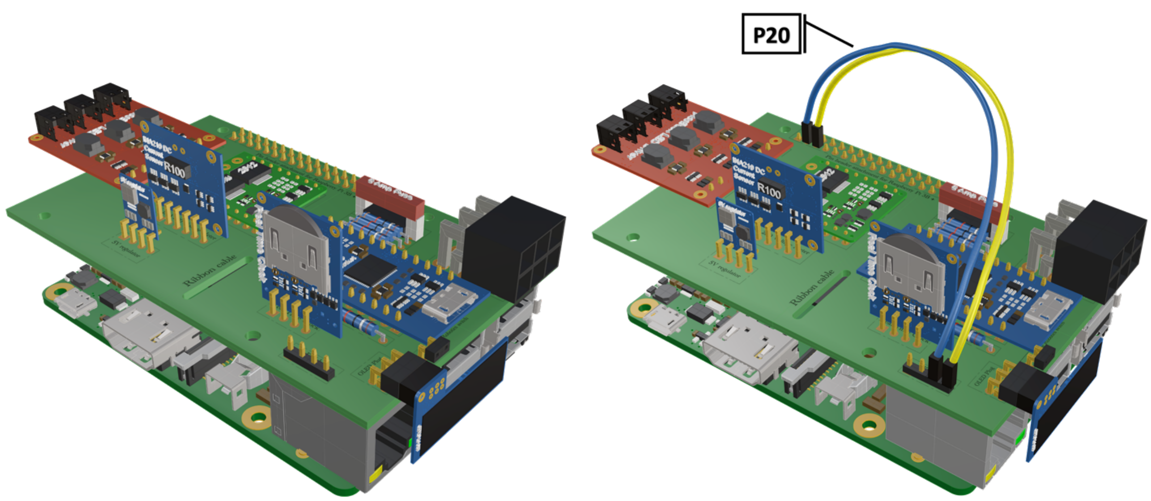
Appendix Figure 12: Insertion of PiOLED into 6 pin header.



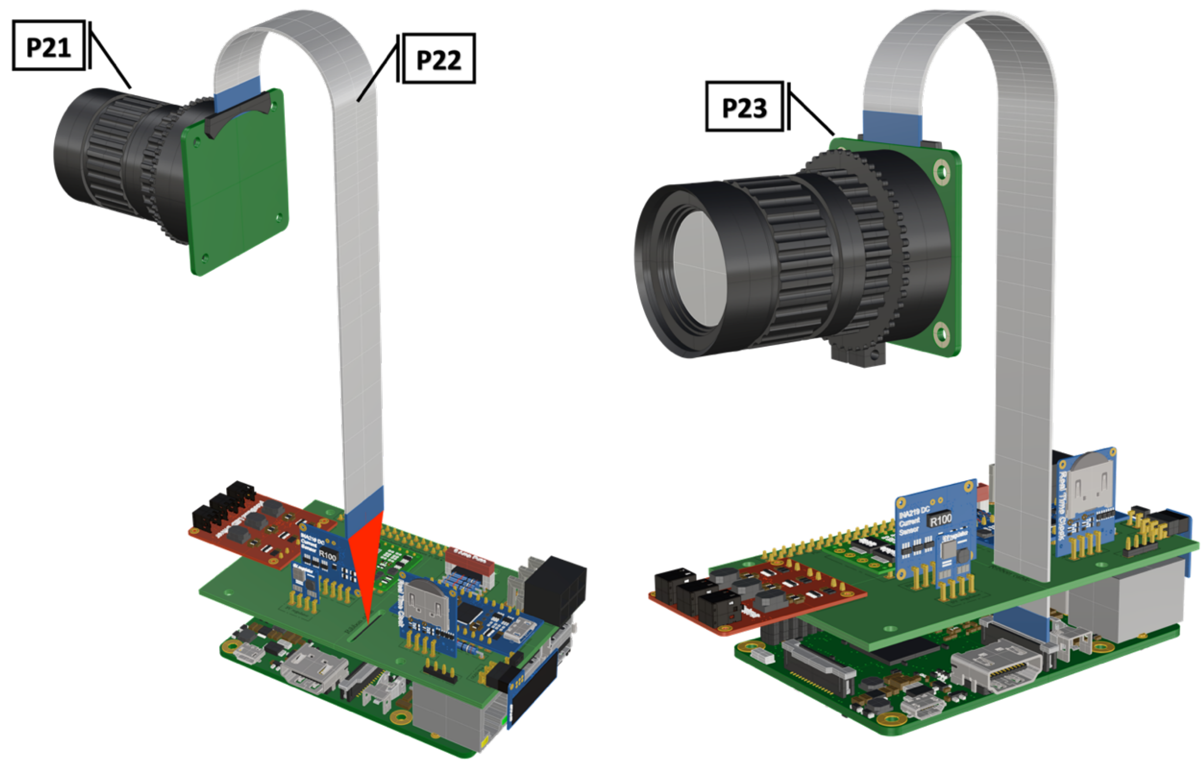
Appendix Figure 13: 40 pin female header connector soldered to PCB header, Raspberry Pi 3B+ inserted into base of 40 pin female header.



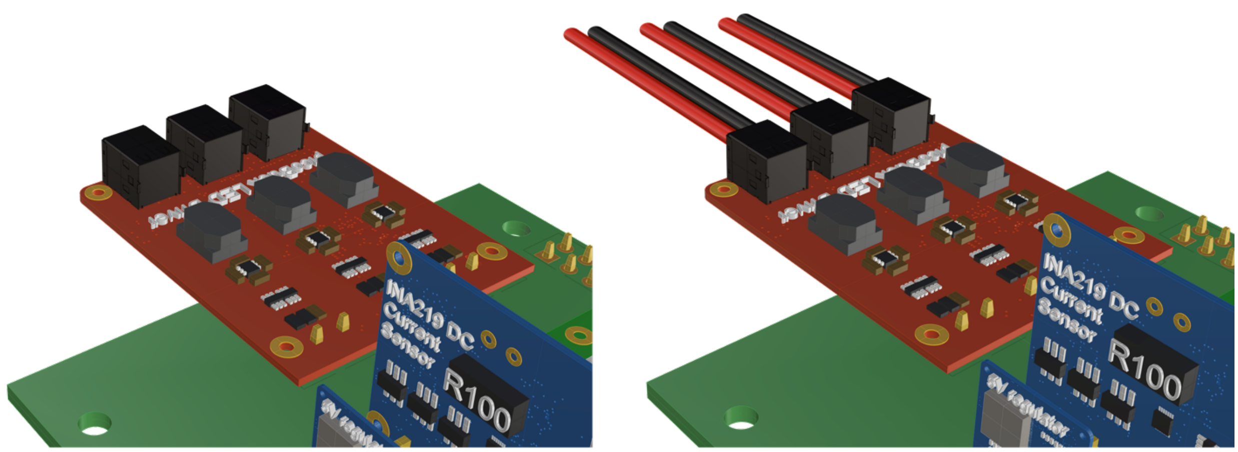
Appendix Figure 14: Alternate view of completed PCB ready for attachment, with side view of connected components.



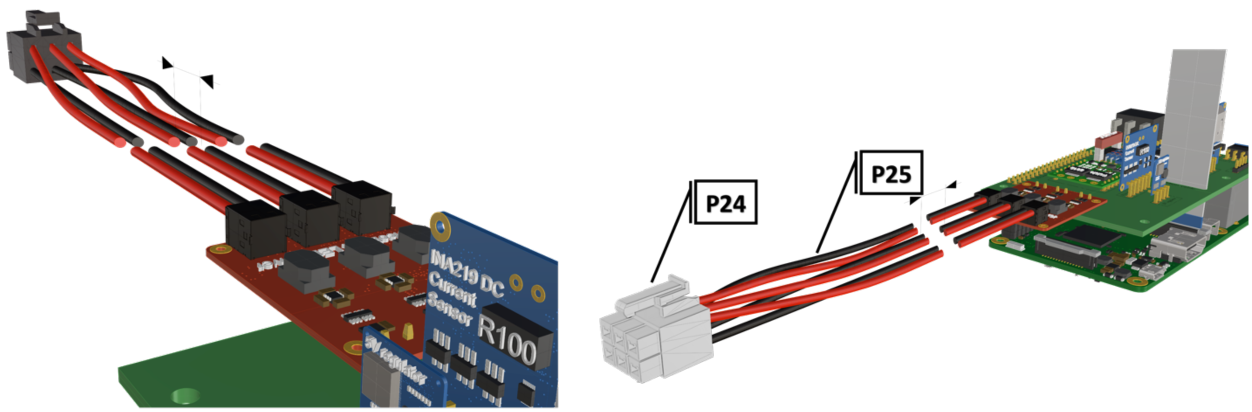
Appendix Figure 15: Close up of PCB with attached Raspberry Pi 3B+, female to female jumpers connecting PiOLED with Rasbperry Pi pins GPIO2 & 3.



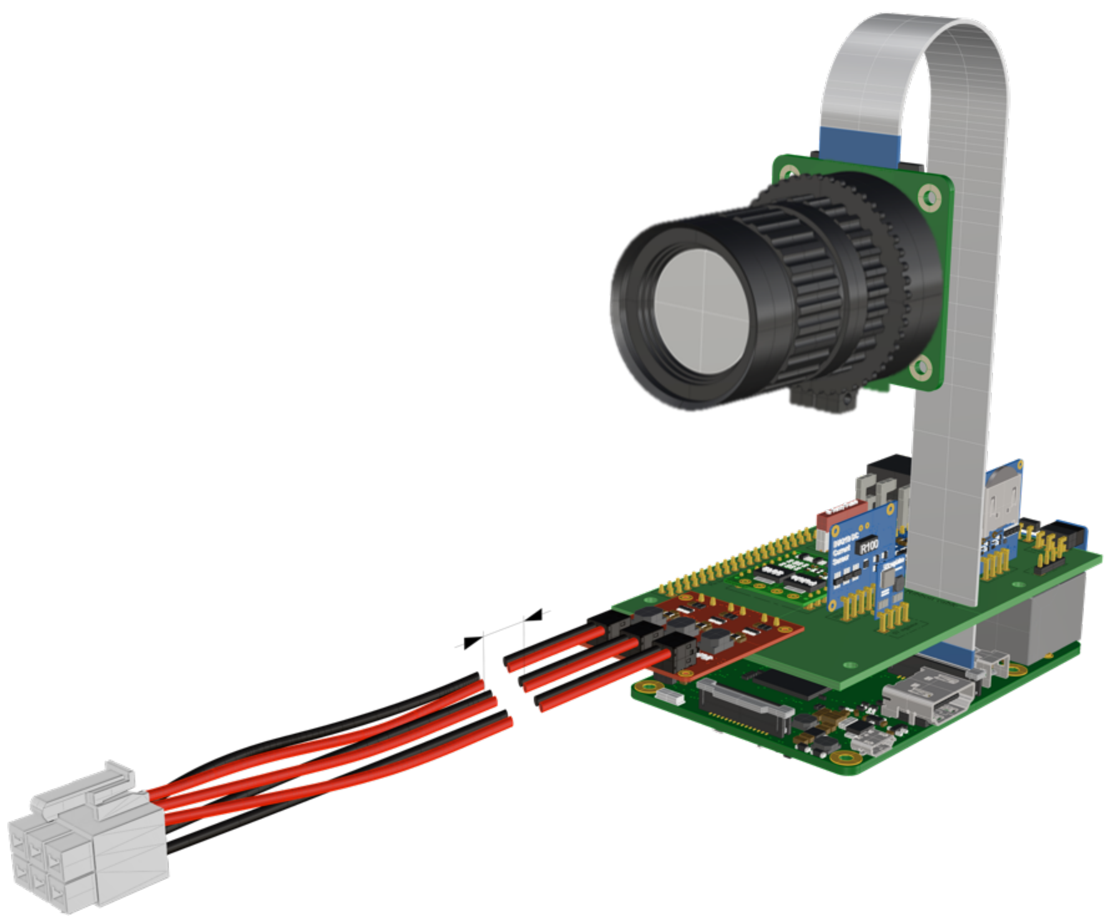
Appendix Figure 16: Attachment of Arducam ribbon cable to Raspberry Pi CSI port.



Appendix Figure 17: Wiring connected to picoBuck LED driver allowing LED control during deployment.



Appendix Figure 18: Wiring connected to male Molex 6 pin connector, allowing the disconnection of strobe system during maintenance and charging. Break in wiring represents 10-12 inches depending on desired length of LED connection.

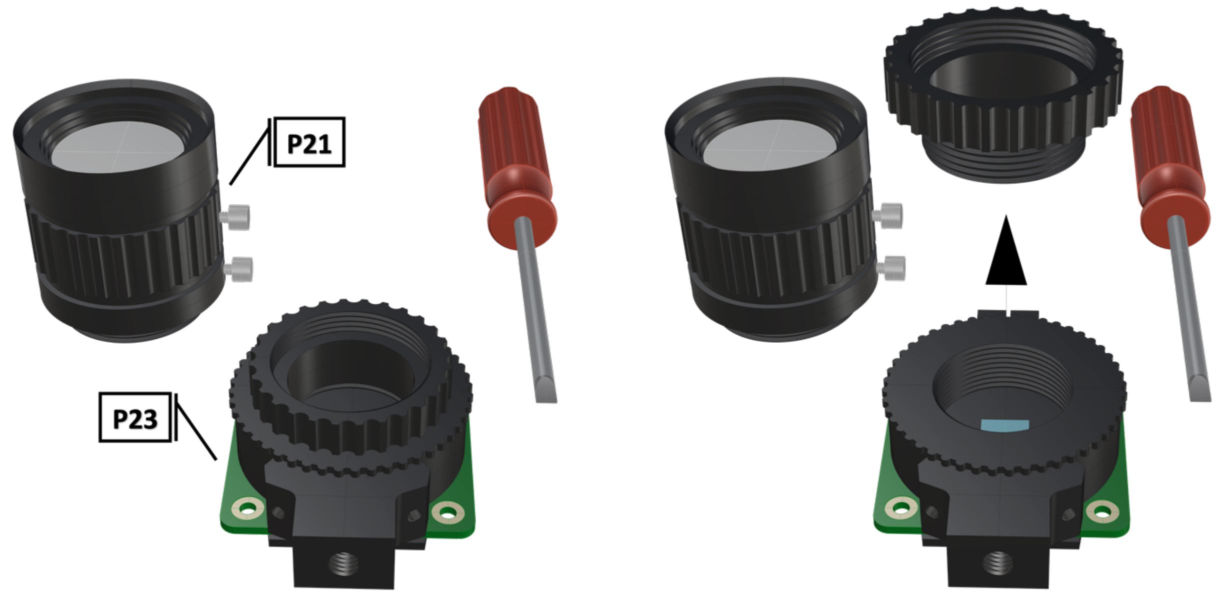


Appendix Figure 19:Fully assembled computer components with attachments of strobe and camera use.

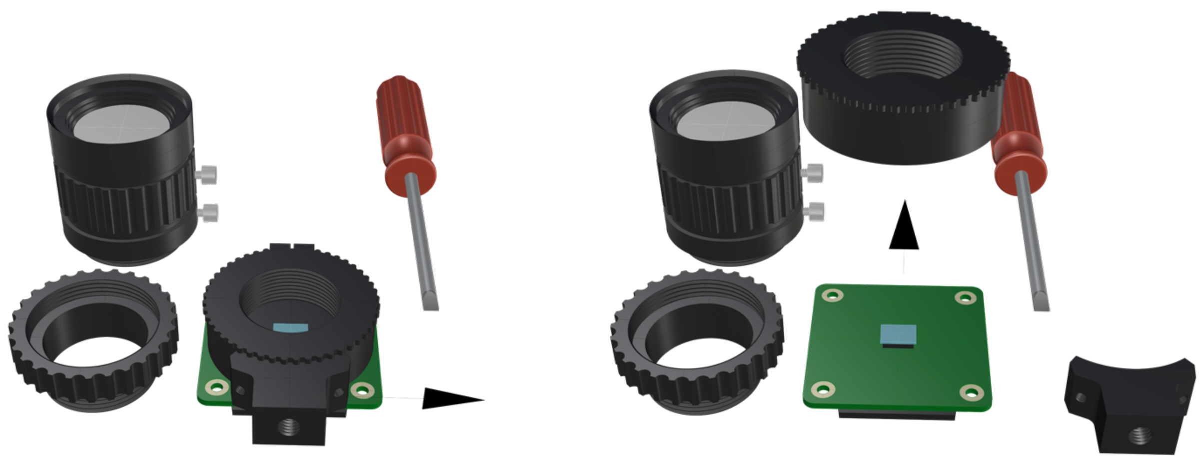
5.2 Camera FR Filter Removal

Before the camera components can be used in tandem with an FR illumination system, the factory installed FR filter must be removed, for removal instructions beyond this manual refer to HQ camera filter removal.

1. Begin by cleaning the project area in an effort to minimize any particulates which may fall in the exposed camera sensor during filter removal.
2. Remove the CS-mount adapter attachment ring **Appx. Fig. 20,** followed by the ¼" tripod mount (requiring hex lock keys) which is unnecessary and will be permanently removed (**Appx. Fig. 21**).
3. Remove the two 1.5 mm hex lock keys from the base of the main circuit board and gently lift the lens mount to expose the FR filter (**Appx. Fig. 21**).
4. Using a sharp blade or fine tipped flathead screwdriver, carefully loosen the edges of the filter from the top of the Sony IMX477 sensor and remove the FR filter without breaking it (**Appx. Fig. 22**).
5. Reinstall the lens mount and replace the CS-mount adapter ring before attaching the wide angle lens (**P21**), refer to **Appx. Fig. 23, 24**.



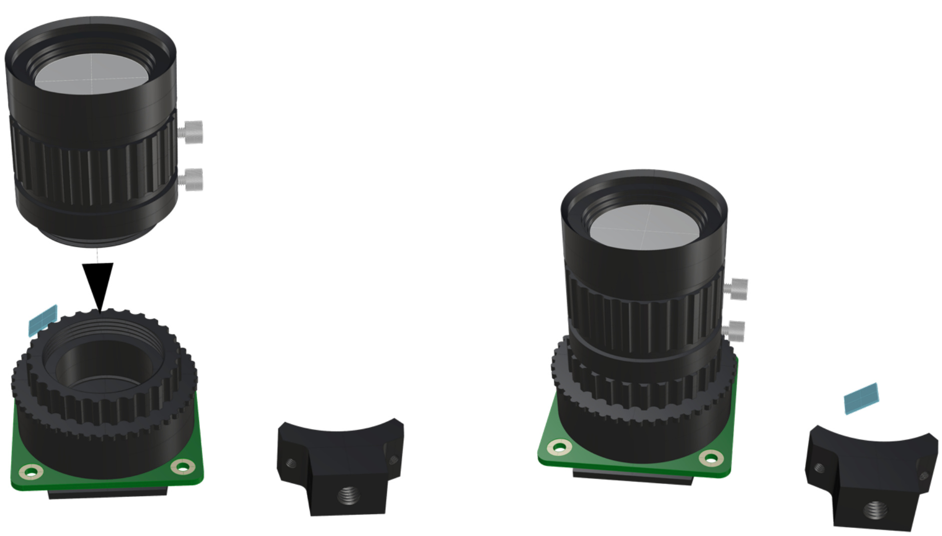
Appendix Figure 20: Unaltered camera components, followed by removal of CS-mount adapter attachment ring.



Appendix Figure 21: Permanent removal of integrated 1/4"-20 tripod mount followed by removal of the two 1.5 mm hex lock keys on the underside of the main circuit board to remove the lens mount and expose the FR filter.

Appendix Figure 22: A flathead screwdriver can be used to remove the FR filter from the Sony IMX477 sensor, removal of the FR filter exposing the sensor.

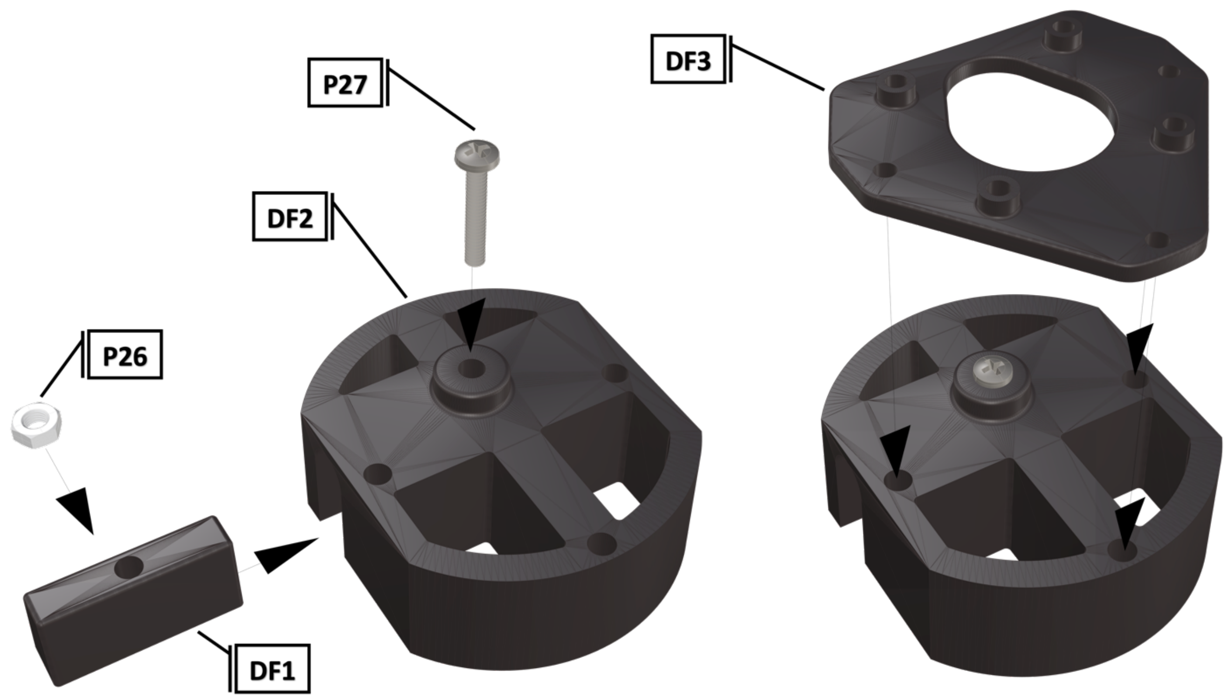
Appendix Figure 23: Reinstallation of lens mount by reattaching hex lock keys to underside of the main circuit board followed by replacement of CS-mount adapter ring for use with wide angle lens (**P21**).



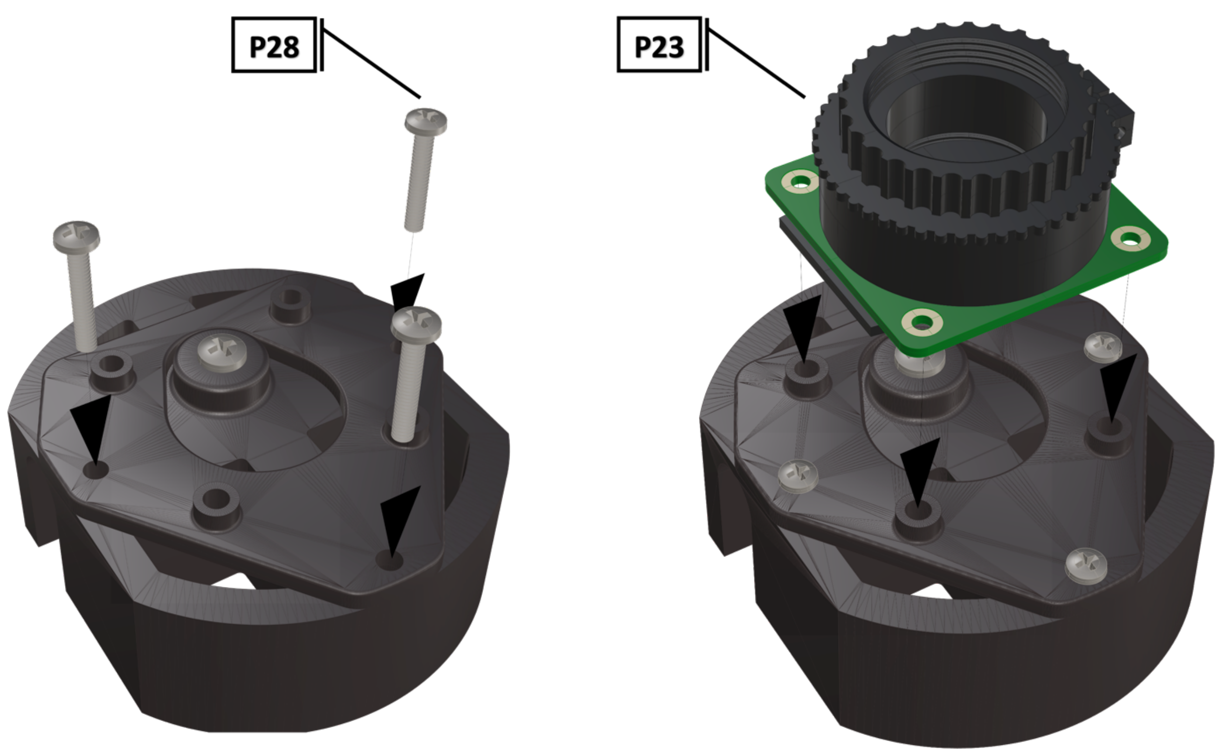
Appendix Figure 24: Installation of camera lens (**P21**) and fully assembled camera with removed components.

5.3 Camera Assembly

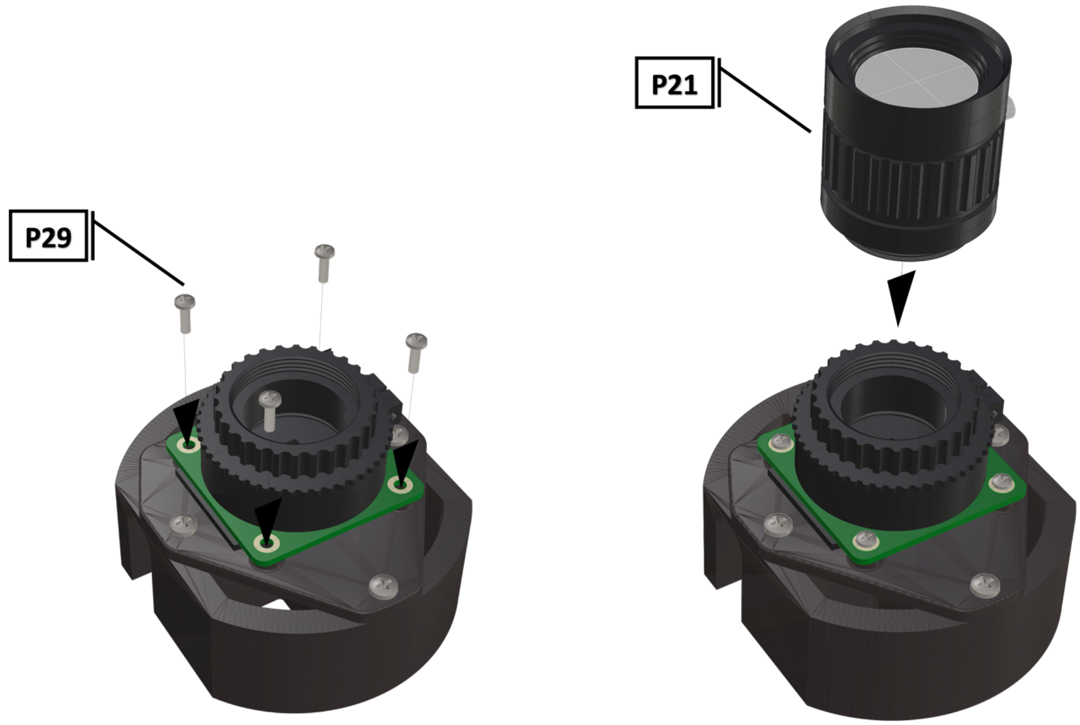
1. To create the port mount assembly for camera and LED attachment, insert a nut (**P26**) into the fitted hole in the center of **DF1.** Slide the resulting part beneath **DF2** and thread the bolt (**P27**) into its corresponding nut **Appx. Fig. 25**. By tightening this bolt (**P27**) the **DF1** wedge will spread the footprint of **DF2** allowing it to mount firmly within a 3" pipe.
2. The camera mount attachment (**DF3**) will be mounted on top of the port mount and secured with bolts (**P28**), **Appx. Fig. 26**. Next the camera circuit board **(P23)** is installed above the camera mount (**DF3**) using bolts (**P29**), **Appx. Fig. 26, 27**.
3. Screw the CS-mount lens (**P21**) onto the arducam base and insert the ribbon cable (**P22**) into the CSI/DSI connector, **Appx. Fig. 27, 28**.



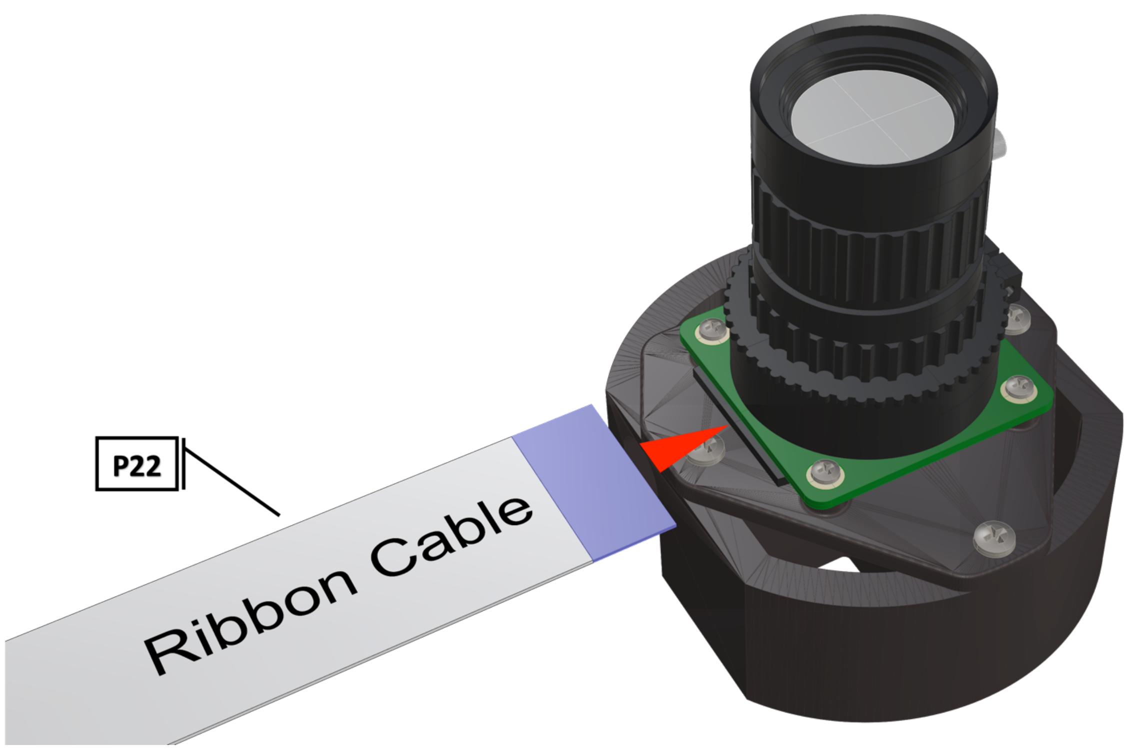
Appendix Figure 25: Port mount assembly and placement of camera mount attachment.



Appendix Figure 26: Bolts installed to hold camera mount in place, camera circuit board installation on manufactured camera mount.



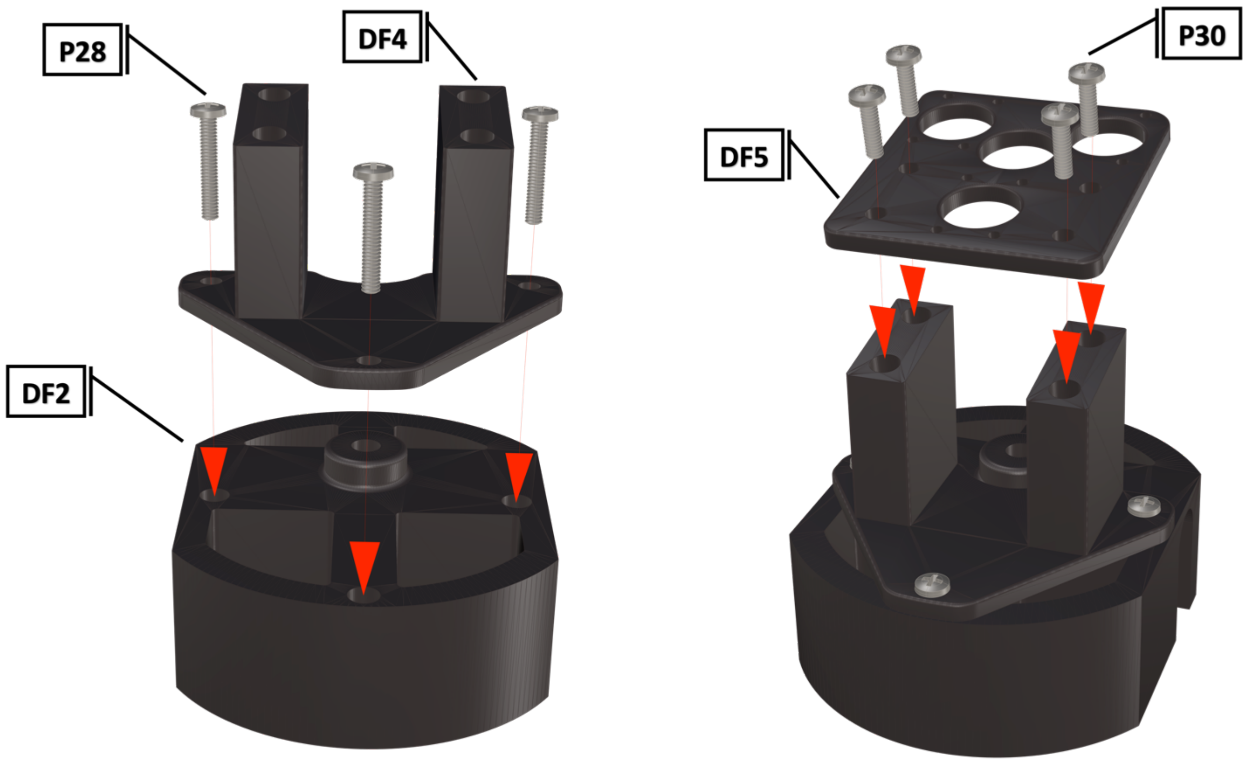
Appendix Figure 27: Bolts used to hold camera circuit board in place, wide angle CS-mount lens attached to Arducam base.



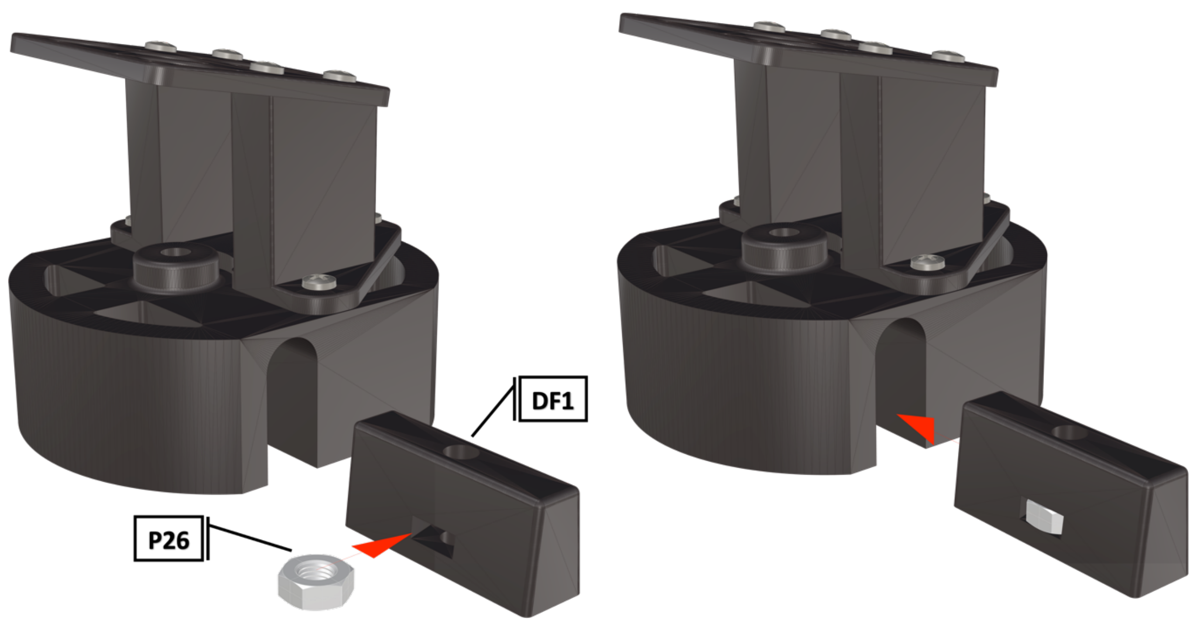
Appendix Figure 28: Installation of ribbon cable to CSI/DSI connector.

5.4 LED Strobe Assembly

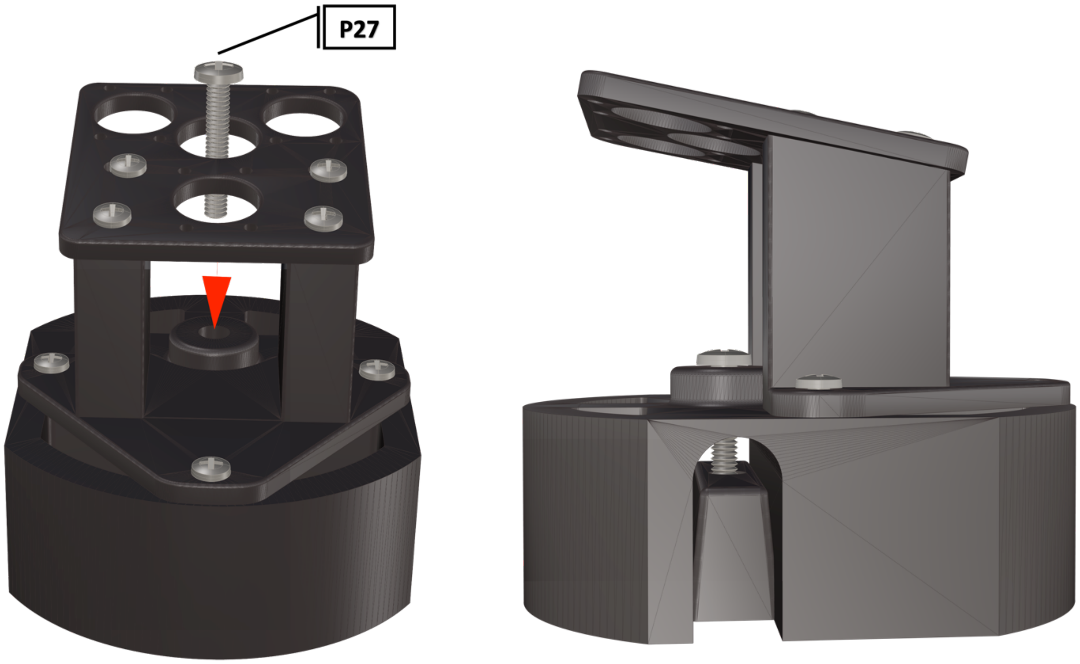
1. Attach the LED support base (**DF4**) to the port mount (**DF2**) and secure with bolts (**P28**) (**Appx. Fig. 29**).
2. Secure the LED mounting plate (**DF5**) to the LED assembly base and secure with bolts (**P30**) (**Appx. Fig. 29**).
3. Install the wedge (**DF1**) used for expanding the port mount by inserting a nut (**P26**) into the wedge and threading the bolt (**P27**) through the port mount and into the nut (**Appx. Fig. 30, 31**).
4. Mount LED starboards (**P31, P32**) onto LED mounting plate (**DF5**) using screws (**P29**).
5. Solder 12-16 inch sections of wiring (**P25**) onto LED starboard terminals. Run wire through port mount and out, to prevent wires from getting pinched during LED port mounting (**Appx. Fig. 33**). Install wire terminals in a female Molex connector (**P24**) (**Appx. Fig. 33, 34**), for later connection to the male Molex connector, attached to the strobe system seen in **Appx. Fig. 19**.



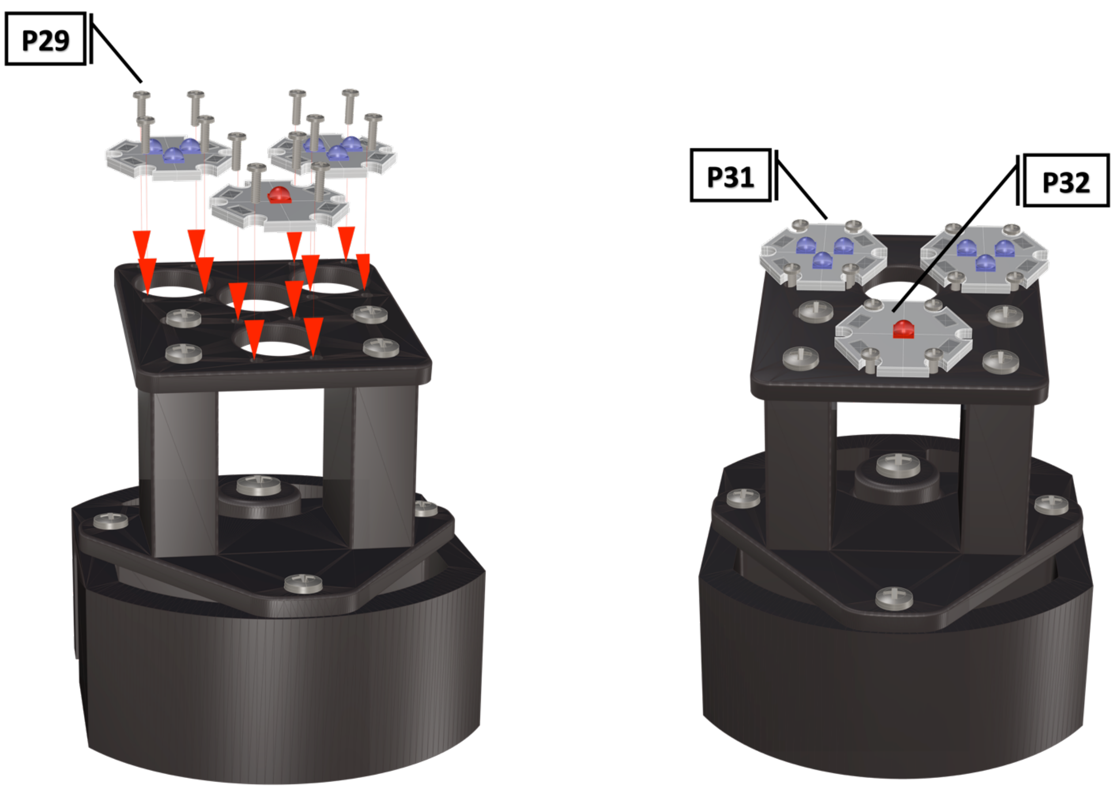
Appendix Figure 29: Installation of LED support frame on port mount, and LED starboard mounting plate.



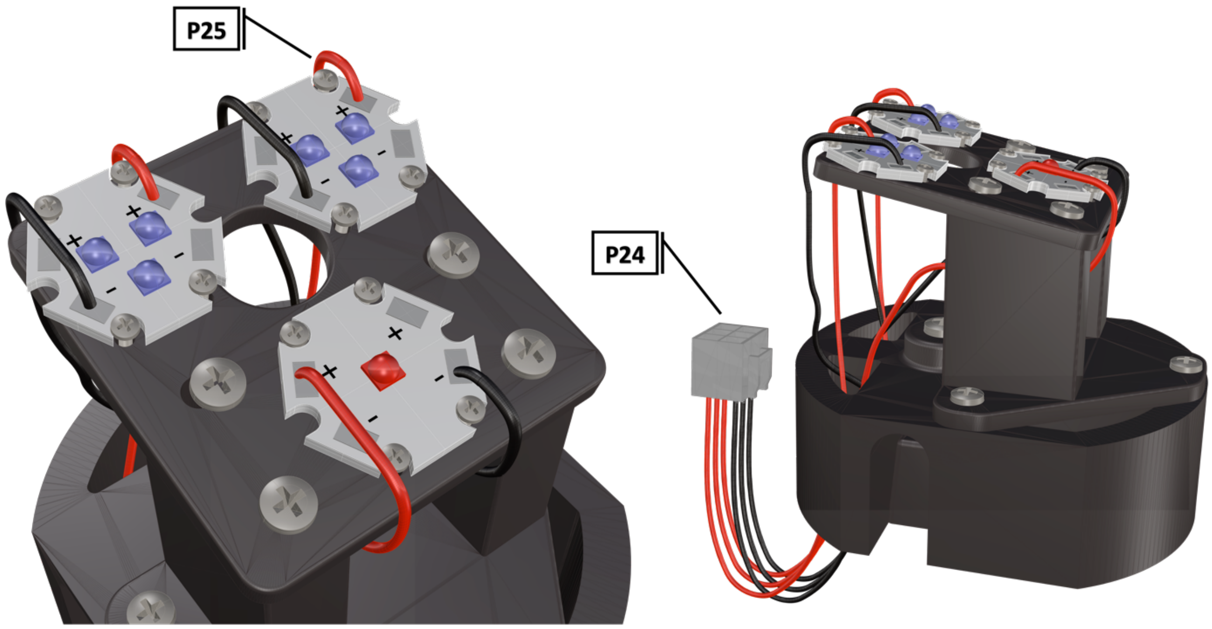
Appendix Figure 30: Insertion of nut into spreading wedge, spreading wedge inserted inside port mount.



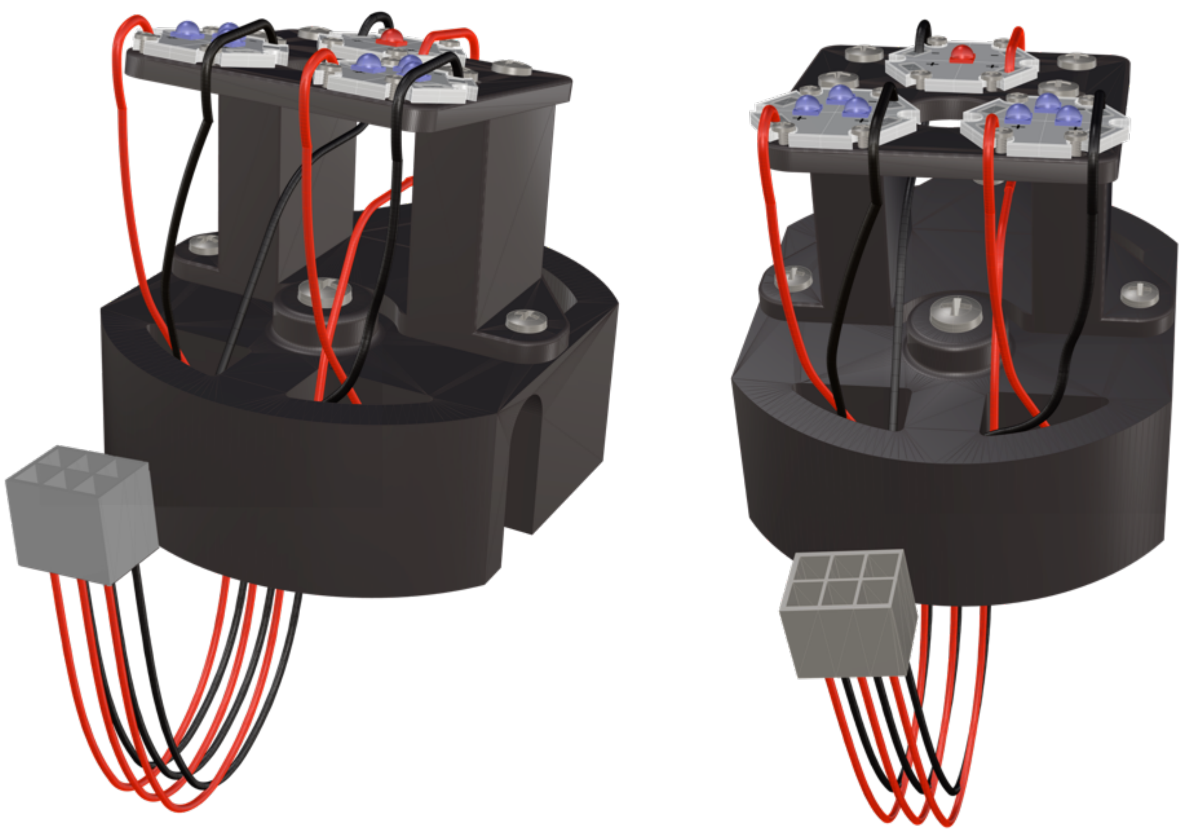
Appendix Figure 31: Bolt is threaded into spreading wedge, tightening of bolt draws wedge into opening and expands port mount diameter, creating a tight fit within a 3” pipe.



Appendix Figure 32: LED starboards secured with bolts and final placement of LED starboards



Appendix Figure 33: Wiring diagram for LED starboards, wires are soldered in place. Wiring is installed in a female Molex connector for direct attachment to camera strobe system (**Fig. 18**).

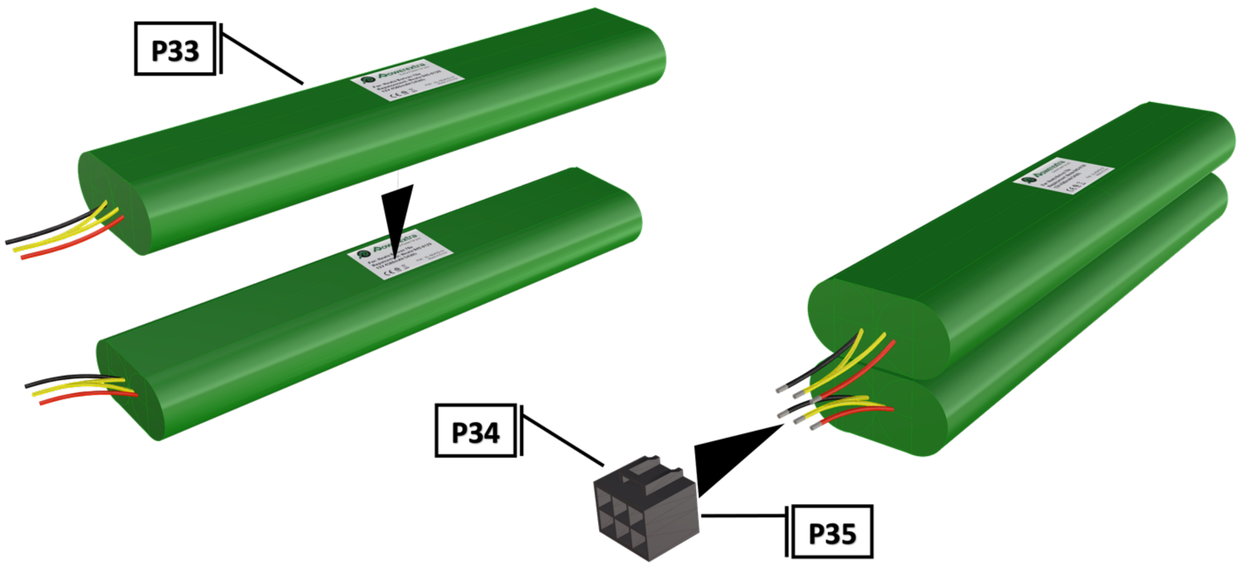


Appendix Figure 34: Completed LED system with wiring harness and attached LED starboards.

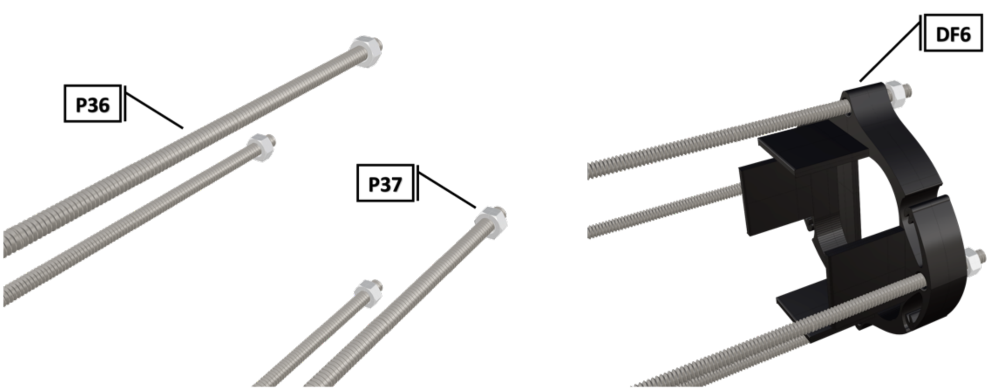
5.5 Battery and structural assembly

1. Original stock battery pack (**P33**) plugs are exchanged with a female 6 pin Molex plug (**P34**) using tin crimp pins (**P35**) (**Appx. Fig. 35**). Two yellow wires from each battery pack are combined into one crimp pin.
2. Threaded rods (**P36**) are fitted with nuts (**P37**) to mount battery holders in place along the length of the rods (**Appx. Fig. 36**). After installing all the components along the length of the rods the tightening of the nuts will form a rigid structural design.
    a) To efficiently move nuts along the threaded rod a dremel with a soft polishing tip was used to spin nuts quickly along the length of the rods.
    b) Depending on preference, battery packs may also be assembled as individual units without attachment rods and then fit together after construction.
3. Two battery holders are mounted back-to-back (**DF7**), to support the middle of the battery (**Appx. Fig. 37, 38**).
4. Before installing the final battery mount end cap, the wired battery pack is placed inside the battery holders before being fixed in place with the last mount (**Appx. Fig. 38, 39**), two more battery packs are installed in a similar fashion (**Appx. Fig. 39**).
5. Install mounts for the computer components (**DF8**), **Appx. Fig. 40**.
6. Mount the components using bolts and nuts (**P29**) placed according to the diagram in **Appx. Fig. 41, 42**.
7. Install the front bulkhead (**DF9**) by loosening all the nuts along the length of each threaded rod and use a powered drill to turn the rod through the bulkhead by tightening the drill head on the far end of the rod and slowly spinning it as it feeds into the mounting holes in the bulkhead (**Appx. Fig. 43, 44**).

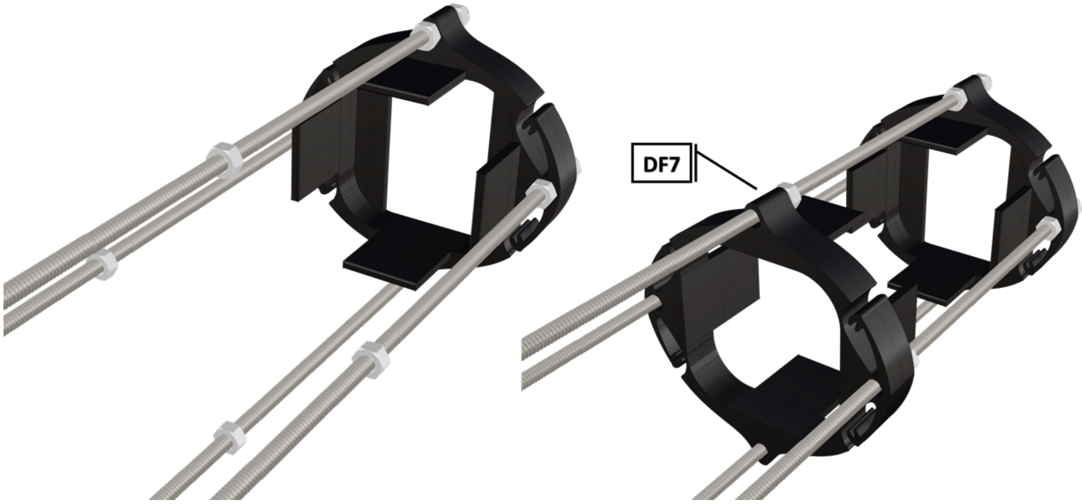
8. A wiring harness to connect all three battery packs and provide power input (**P8**), can be fabricated by wiring (**P25**) 4 male Molex connectors (**P38**) in parallel (**Appx. Fig. 45, 46**). The wiring harness clips to the sides of the battery holders (**DF6,7**) to prevent damage during loading and unloading from the housing. At each wire intersection along the wiring harness heat shrink tubing (**P53**) was used to maintain a strong waterproof connection.
9. After all components are in place and securely fastened, trim off any excess threaded rod using an angle grinder.
10. Battery packs can be charged by wiring two battery chargers (**P47**) to a male Molex connector to allow individual charging of each battery.



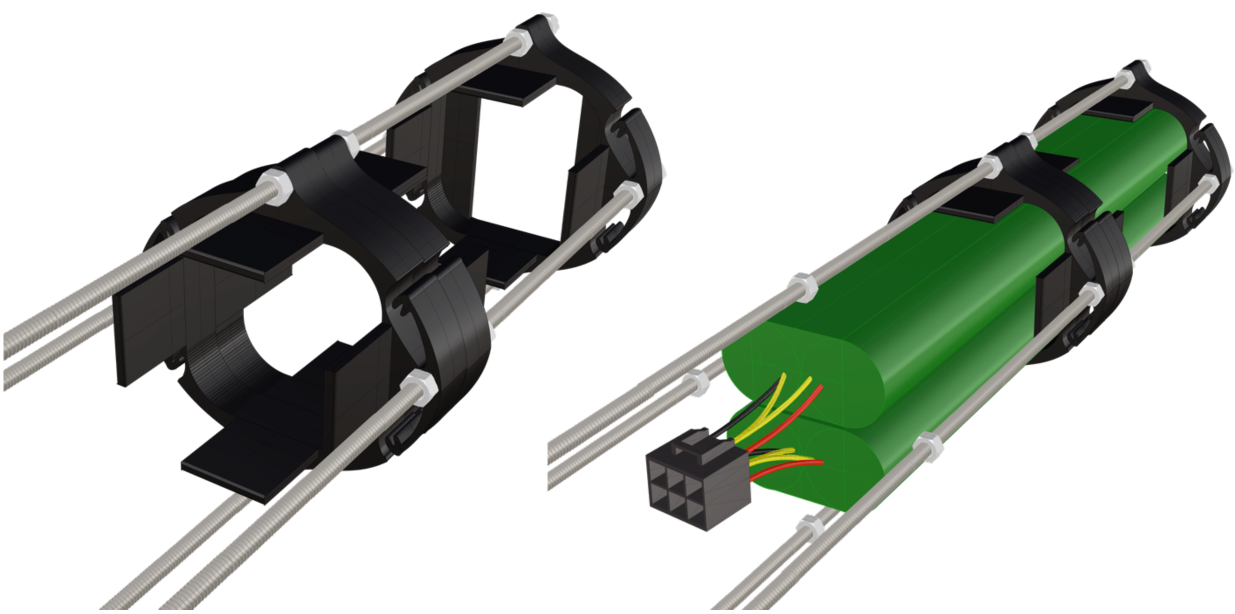
Appendix Figure 35: Battery packs oriented above each other, battery array is wired together into a female Molex connector.

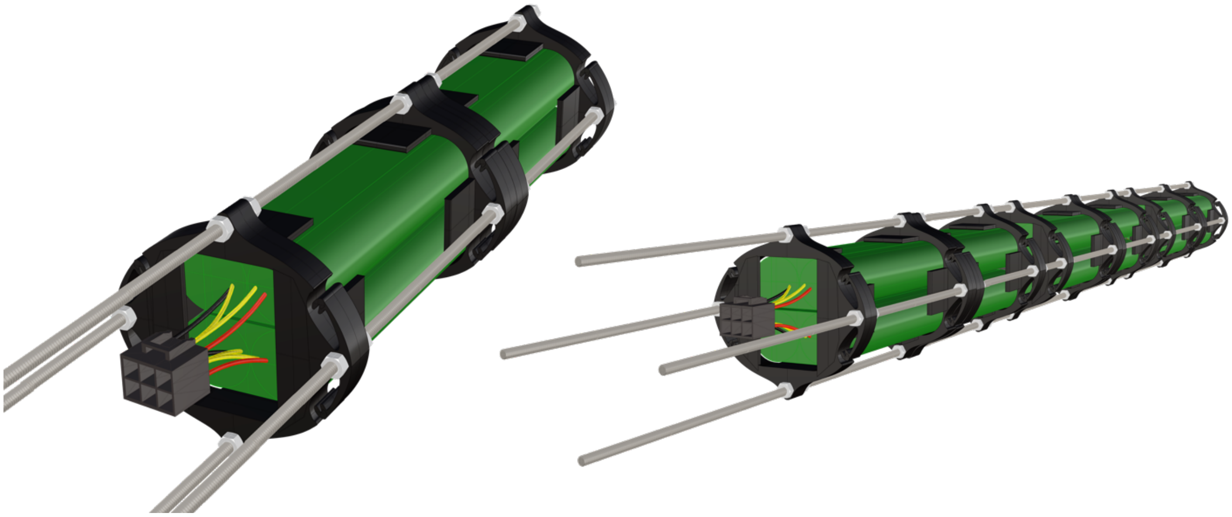


Appendix Figure 36: Threaded rods with attached nuts, battery holder end cap placed against bolt nuts.

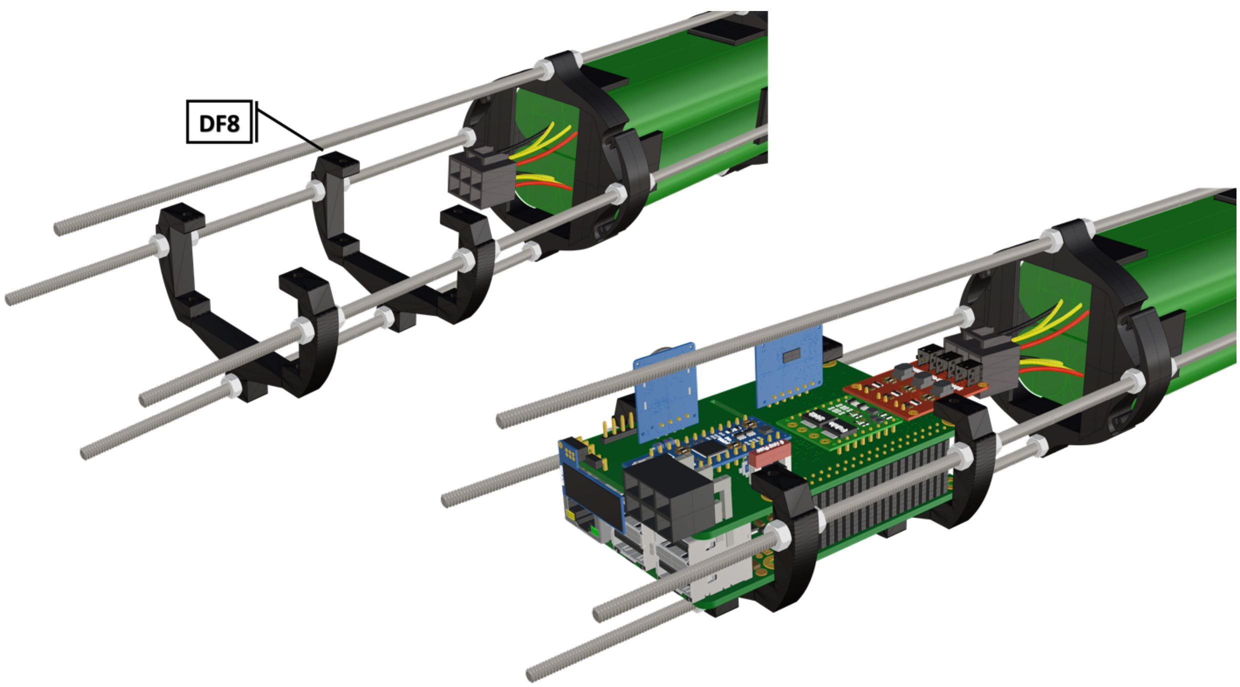


Appendix Figure 37: Attached nuts are used to hold a battery support in place.

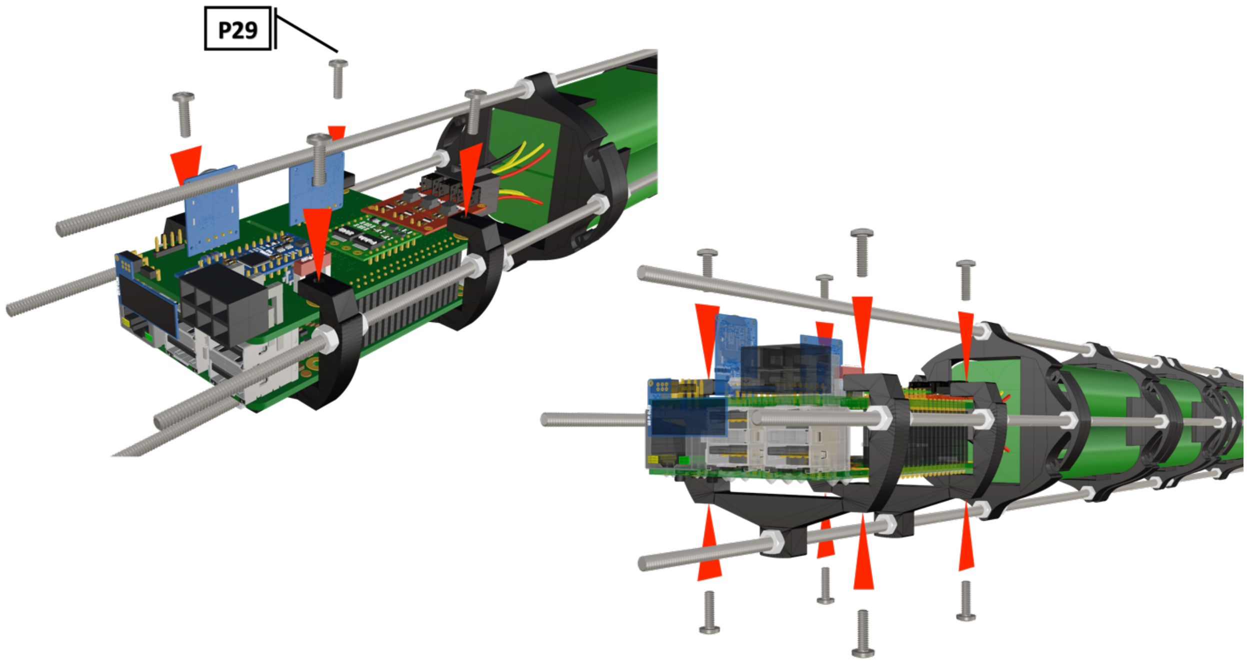

Appendix Figure 38: The addition of a second opposing battery holder adds additional support, followed by the tightening of attached nuts.



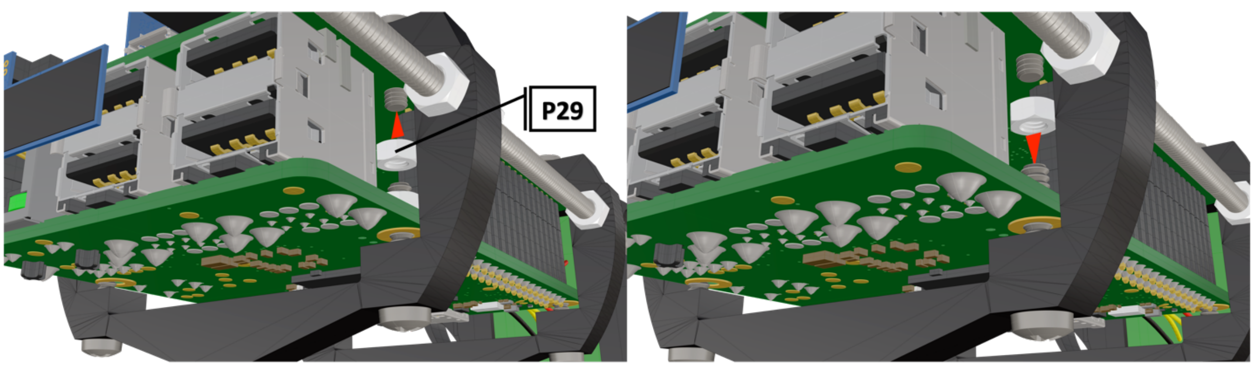
Appendix Figure 39: Complete assembly of one battery unit, followed by 2 more identical battery packs following the same assembly instructions and attached together on threaded metal rods (**P36**).



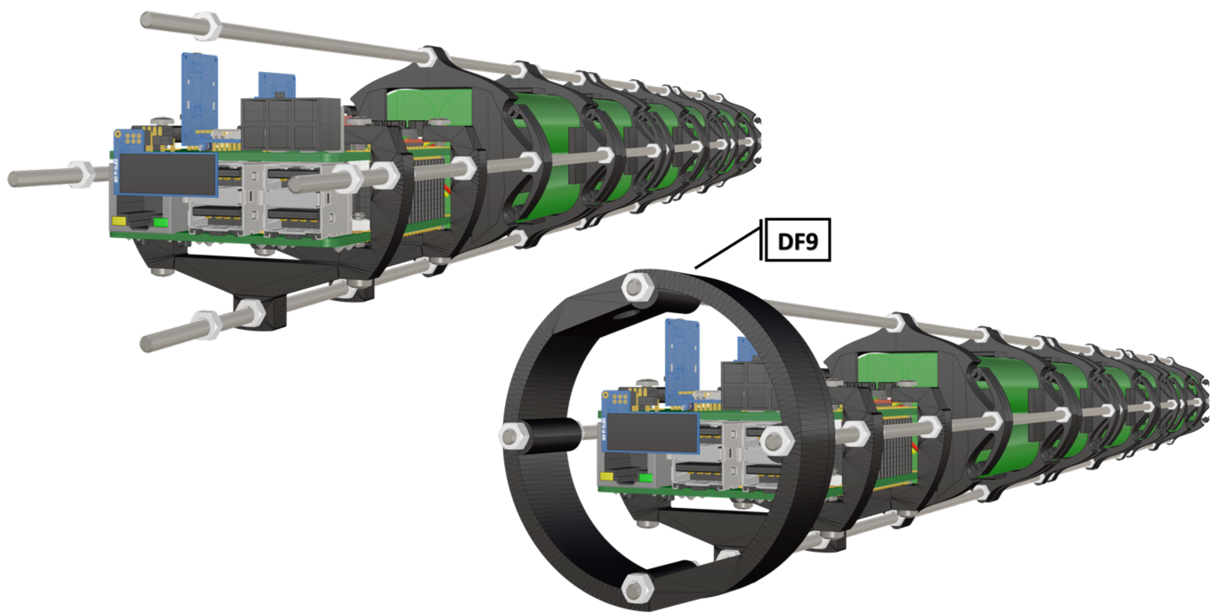
Appendix Figure 40: PCB mounts attached in the same manner as battery holders.



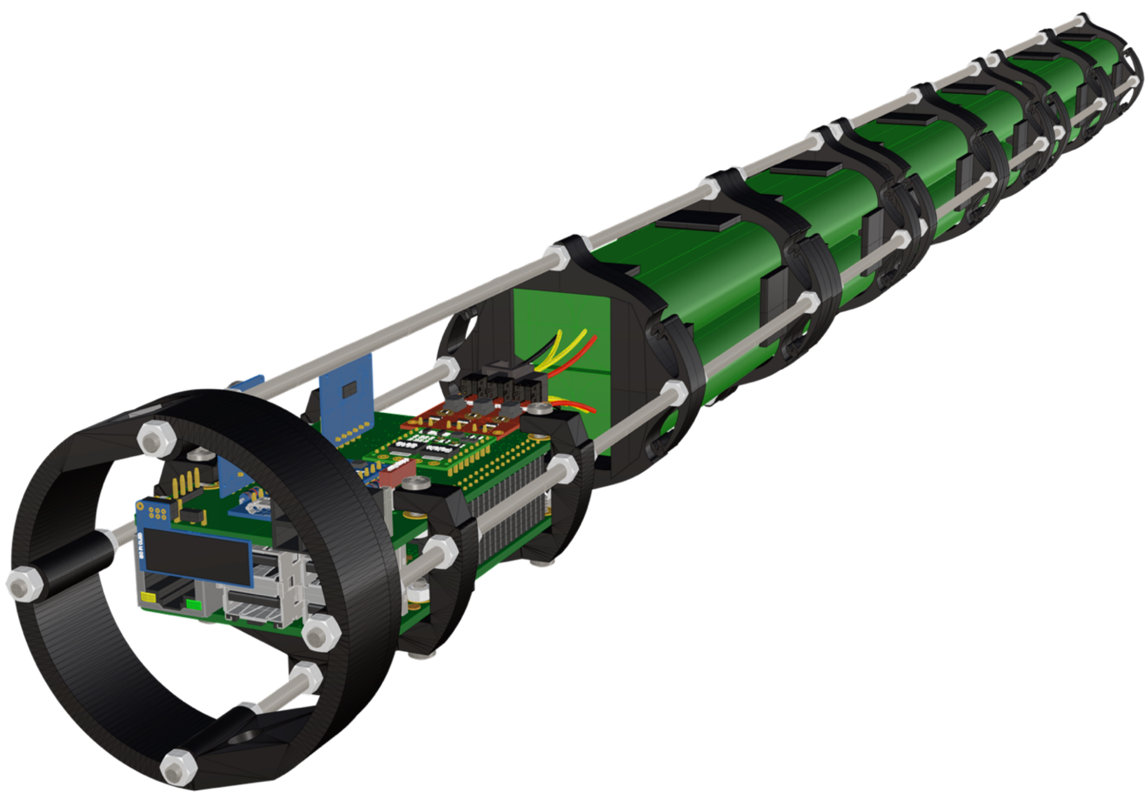
Appendix Figure 41: Bolts are inserted through attachment points to keep PCB in position.



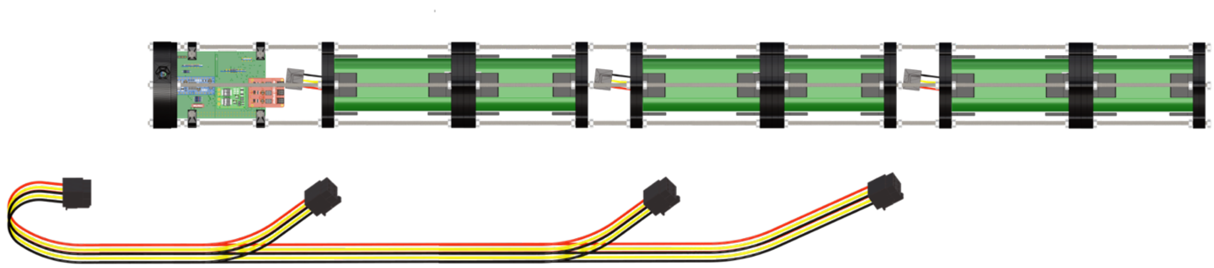
Appendix Figure 42: Nuts are attached to all 8 bolts inserted into PCB mount (**Fig. 41**) and tightened to secure PCB hat and Raspberry Pi3B+ in place.



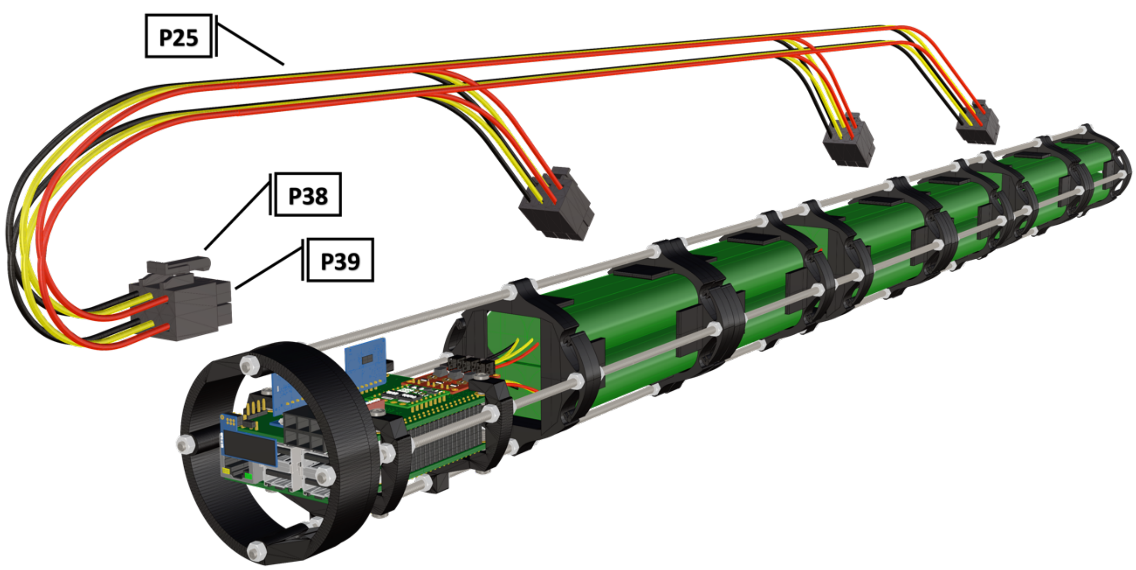
Appendix Figure 43: After computer components and battery packs are assembled, a front bulkhead is attached to aid in structural support.



Appendix Figure 44: Completed structural assembly.



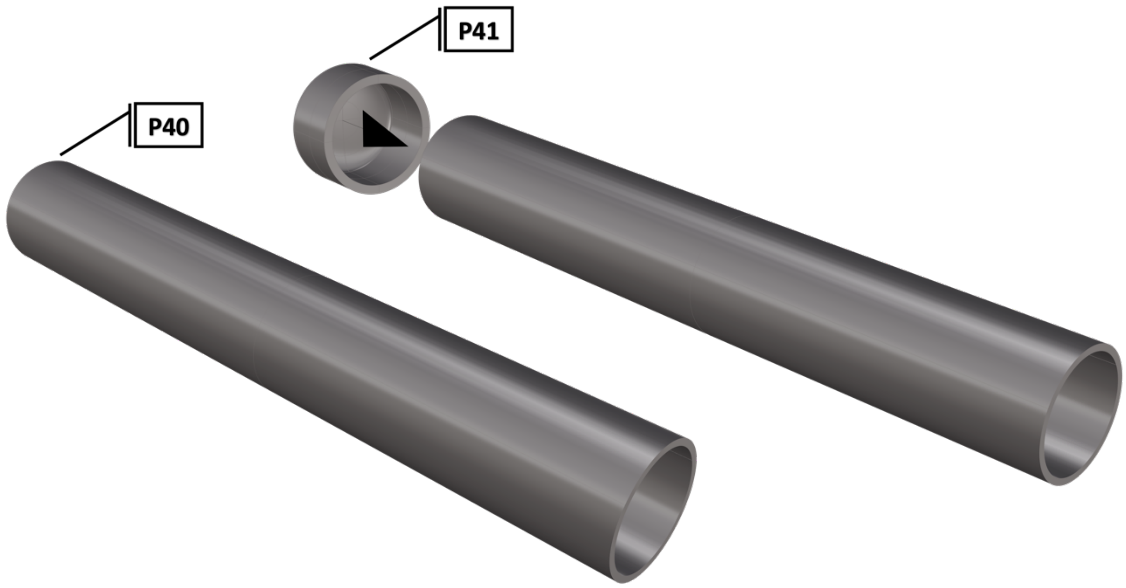
Appendix Figure 45: Diagram of wiring harness, adjacent to designated connectors.



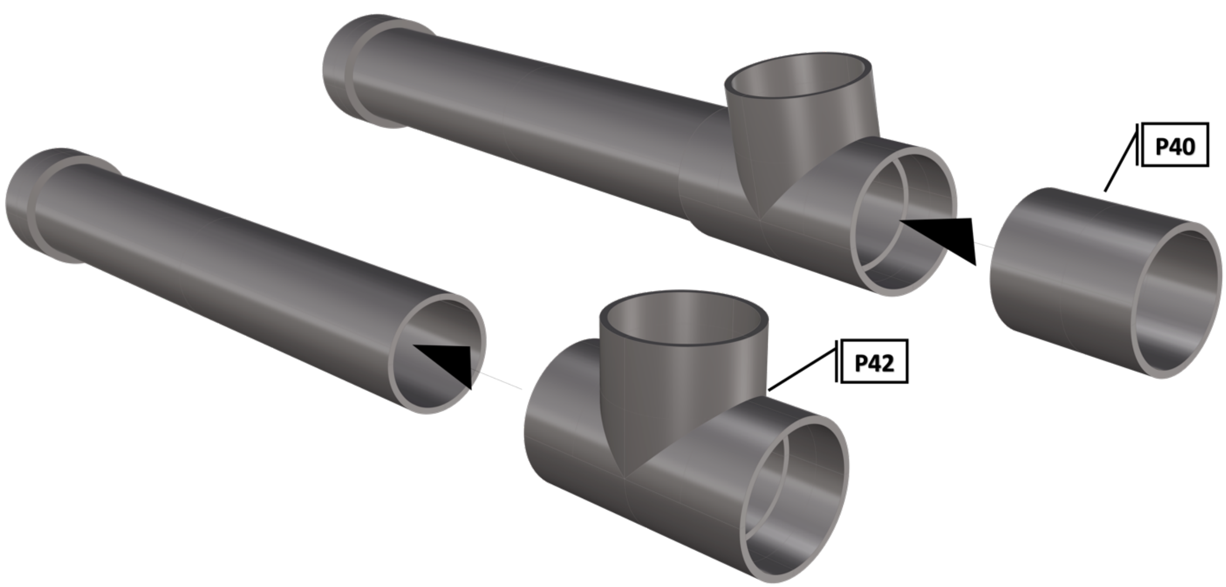
Appendix Figure 46: Wiring harness, plugs connect to female receptacles on battery packs and PCB.

5.6 Housing Construction

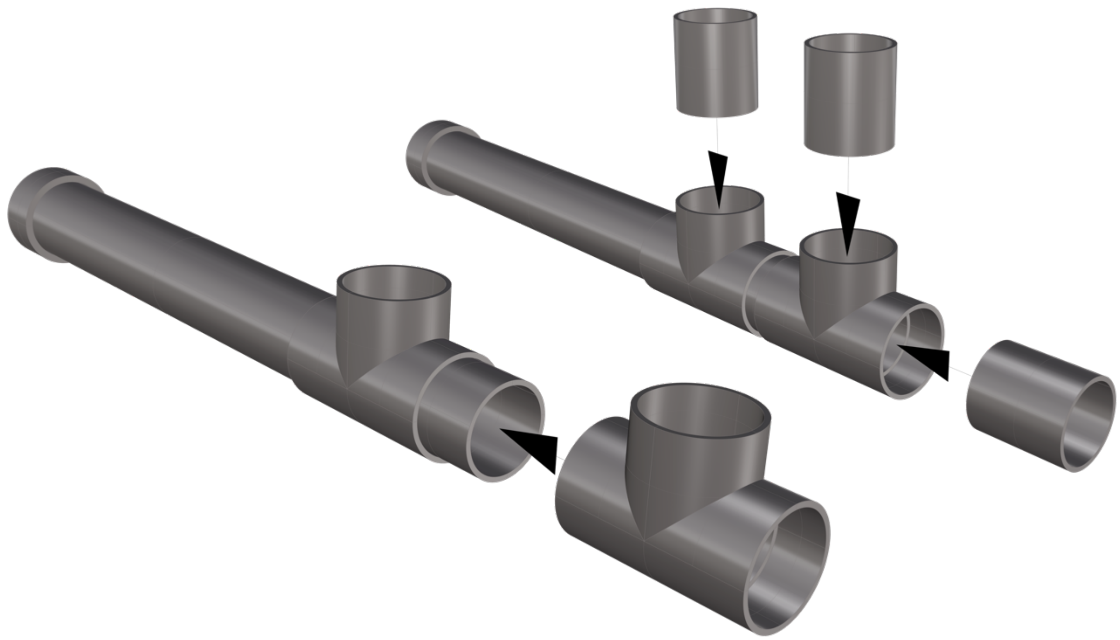
1. Cut schedule 80 PVC pipe (**P40**) into a 3ft length (using a table, belt or hand saw) and cap the end (**P41**), **Fig. 47** using schedule 80 PVC cement to secure components (**P48**).
2. Attach a T-joint (**P42**) to the PVC pipe with glue followed by a short 6 inch piece of pipe (**P40**), **Fig. 48.** Taper the insides of the 6 inch pipe segment using a sander or dremel to allow for easier loading and unloading of the camera system.
3. Attach a second T-joint to the 6 inch pipe segment and insert a 6 inch pipe into each of the 3 available female receptacles (**Appx. Fig. 49**).
4. Prepare 3 union joints (**P43**) for attachment by removing the union joint sleeve and replacing it with a plexiglass plate (**P44**) for the loading and camera ports and a glass plate (**P45)** for the strobe port (**Appx. Fig. 50, 51**).
5. The plexiglass plate used to cover the loading port requires the installation of a Blue Robotics high pressure switch (**P46**). Using the appropriate drill bit, make a larger hole ¼" through the plexiglass in the center of the plate, this corresponds to the non-threaded portion of the switch. Create a second smaller hole continuing through the plate which will allow a tapping set to thread the hole (**Appx. Fig. 52**).
6. Install the Blue Robotics switch by removing the nut and wrapping the threads with plumbers tape. Coat the O-ring with vacuum grease (**P49**). Using a wrench, screw the switch into the hole and replace the nut (**Appx. Fig. 53, 54**).
7. The completed housing should match the visualization shown in **Appx. Fig. 55**.
8. After camera and LED assembly install port mounts in designated ports (**Appx. Fig. 2)** by tightening center bolts until snug. Load cameras with desiccant packs (**P50**) to prevent moisture from corroding electronics.



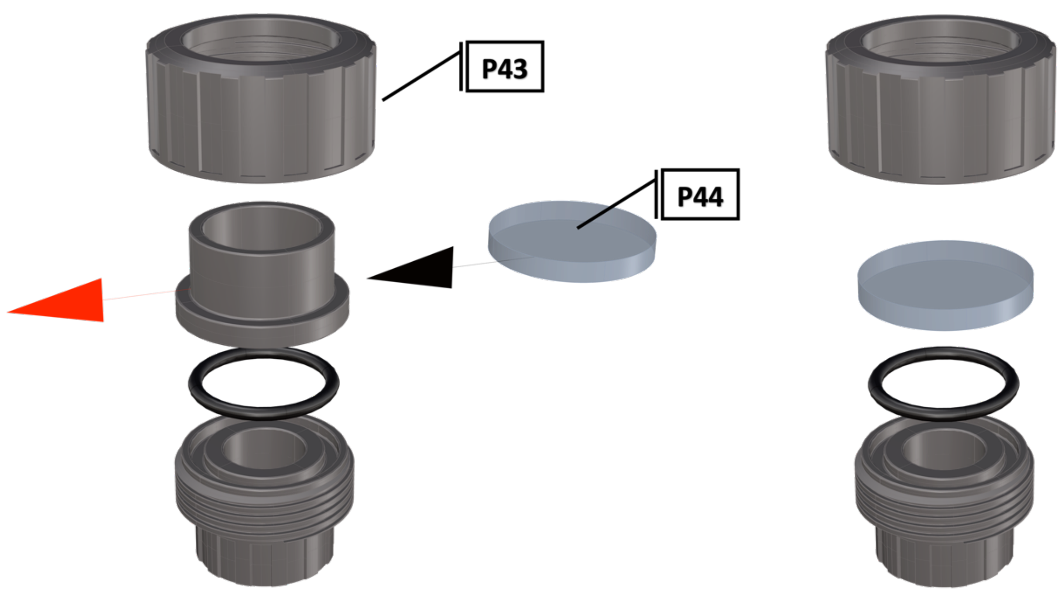
Appendix Figure 47: Cut to size schedule 80 PVC pipe with attached pipe cap.



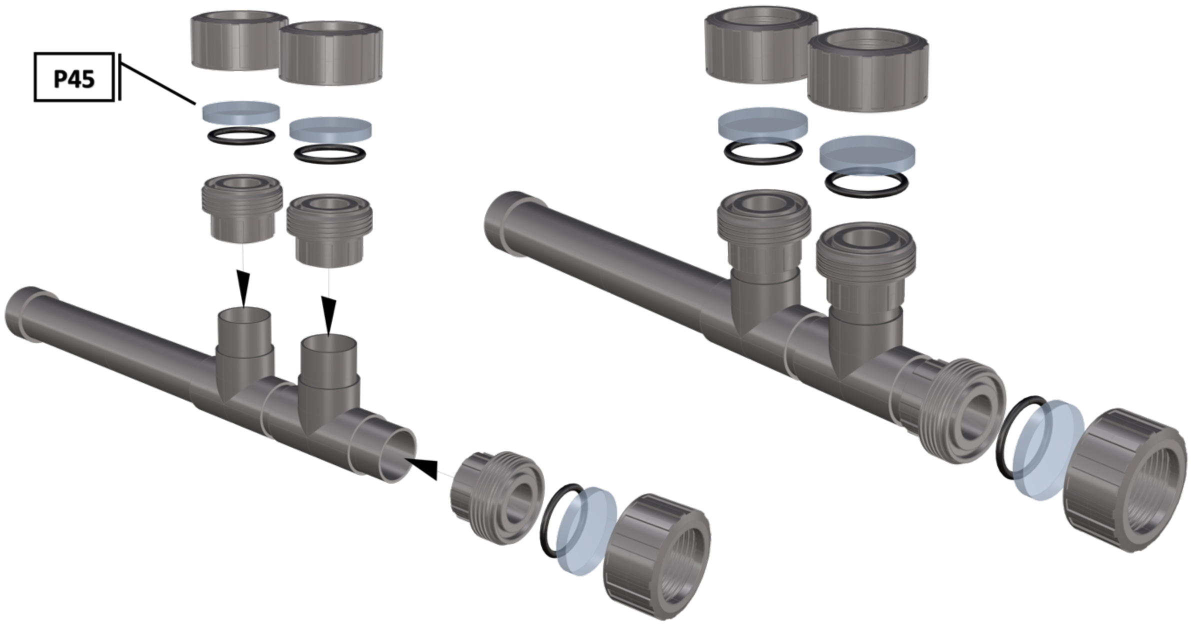
Appendix Figure 48: Installation of T-joint, followed by a 6 inch section of pipe (**P40**)



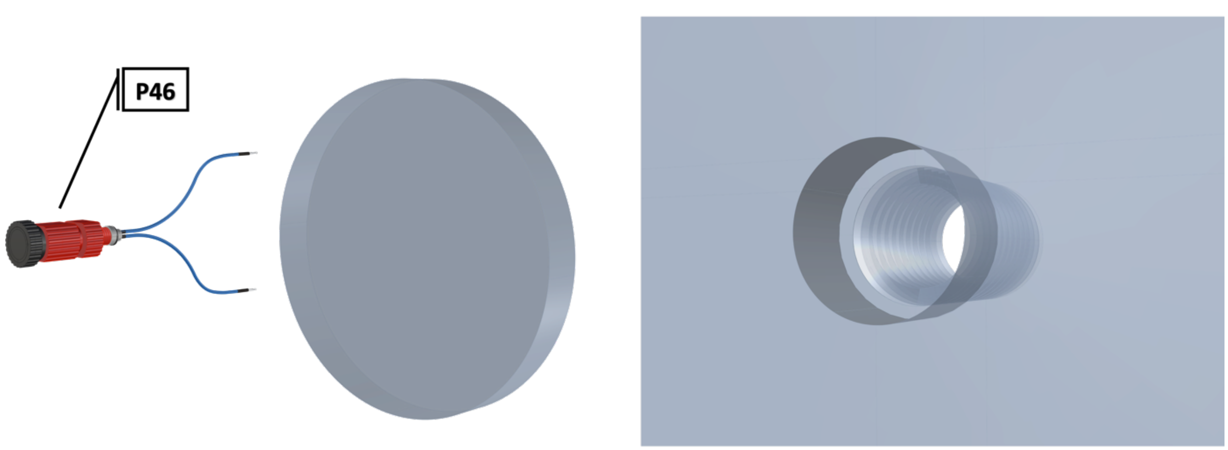
Appendix Figure 49: Attachment of second T-joint and three cut sections of 6 inch pipe.



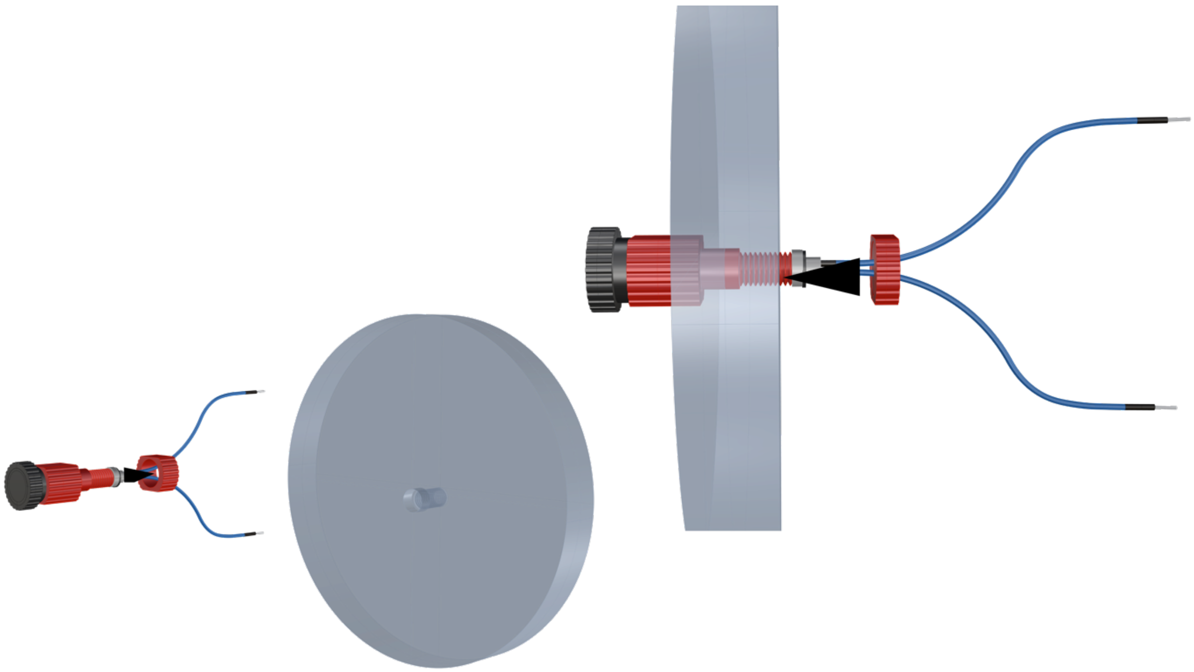
Appendix Figure 50: Removal of female Union sleeve to allow installation of Plexiglass or glass port cover.



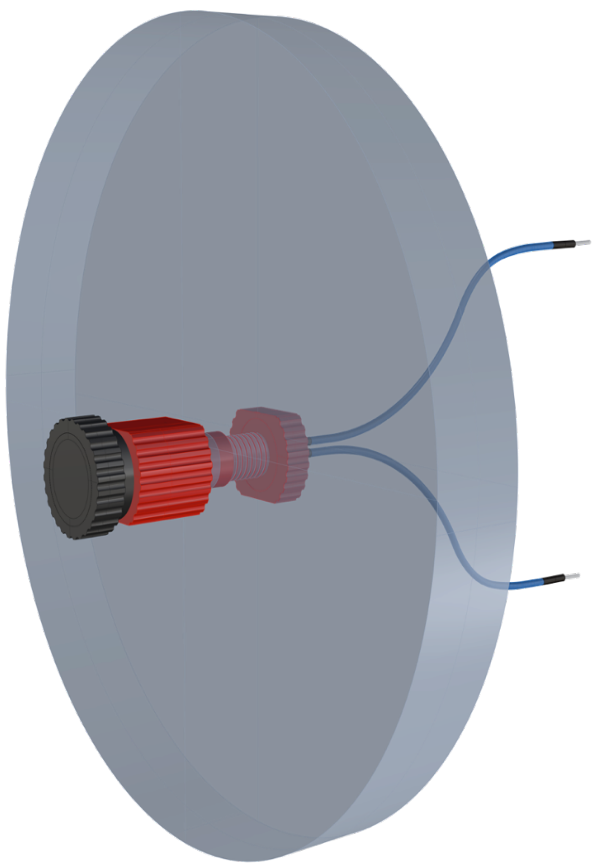
Appendix Figure 51: Installation of Union joints to 6 inch pipe sections, forming the ports and port covers.



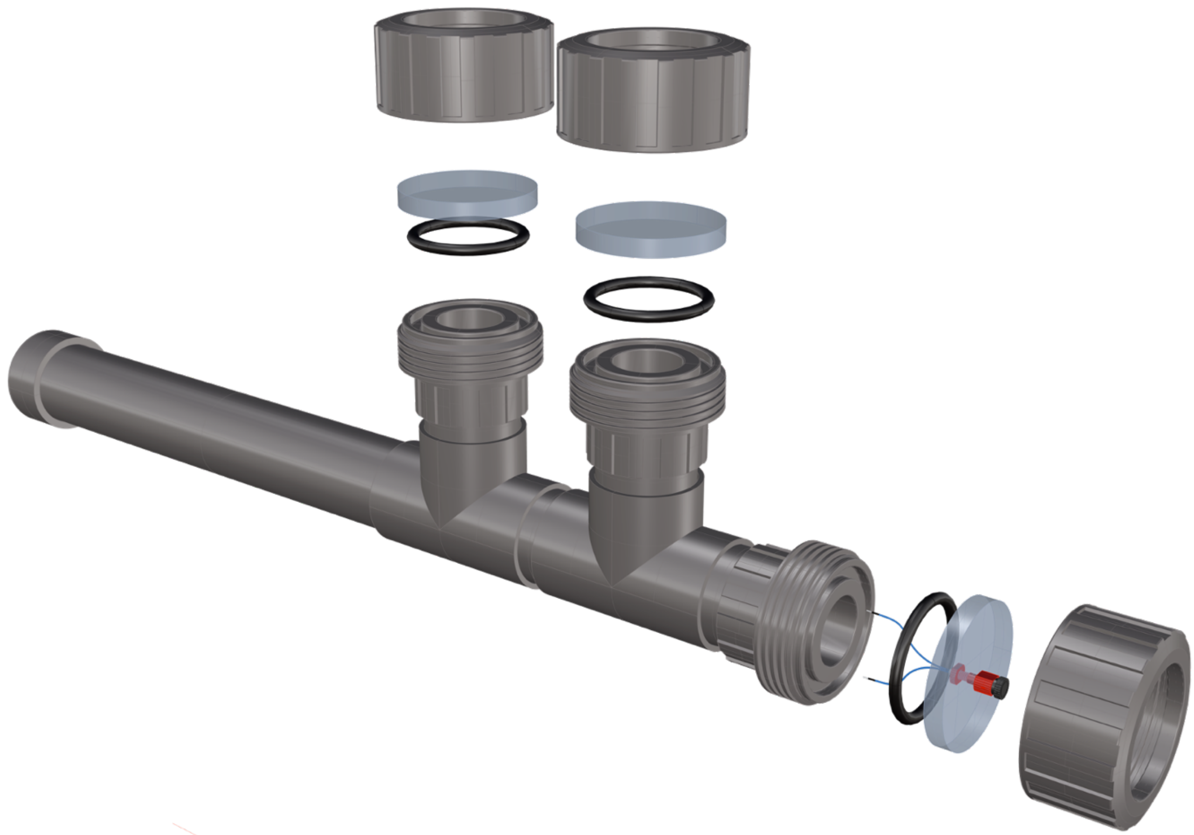
Appendix Figure 52: Drilling and tapping of ½” Plexiglass hole for installation of Blue Robotics switch.



Appendix Figure 53: Mounting of Blue robotics switch in prepared hole.



Appendix Figure 54: Completed switch installation in plexiglass port cover, allowing system to be powered on at depth.



Appendix Figure 55: Completed schedule 80 PVC housing with exploded ports.

6. Operation Instructions

6.1 Software

Software is written in the Python programming language (Version 3.5.3) using only open-source libraries. For ease of use, the main operating software is separated into two python scripts. The first, triggercam_main.py, contains the main operating code while the second, triggercam_functions.py, contains accessory functions required during runtime. The following table lists the additional required libraries:

Appendix Table 3: Software library

Library	**Description**	**Source**
adafruit-circuitpython-ina219	Library for the INA219 voltage and current monitoring integrated circuit by adafruit	https://github.com/adafruit
adafruit_blinka	Support for i2c communication with adafruit products using CircuitPython	https://github.com/adafruit
adafruit_ssd1306	Library for operating the adafruit PiOLED - 128x32 miniature screen	https://github.com/adafruit
Pillow (Python Imaging Library)	Required for operating the miniature screen	https://pypi.org/project/Pillow/
PyOpenCV (Version 3.4)	Required for all image analysis operations	https://pypi.org/project/pyopencv/

6.2 Configuration

The main operating parameters are specified in a JSON configuration file, which includes general program settings, image acquisition settings, and triggering thresholds. The table below lists all configurable parameters and default values.

Appendix Table 4: Operating settings

Section	Parameter	Default	Data type	Valid options	Description
general_settings	collection_type	trigger_using_red	string	still_intervalometer, trigger_using_red, trigger_using_ambient	Main collection mode - intervalometer is a simple set of timed images, trigger modes rely either on red strobe or ambient light for illuminating scene for evaluation target presence
	system_id	pi_triggercam_001	string	user specified	Name of the camera system to be used for metadata (if several systems are used)
	initial_wait	1	integer	unlimited	Rest period before system starts acquisition. Can be used if deployment takes a while.
	low_voltage_cutoff	11	floating point	unlimited	Voltage value for shutting the system down, typically ~11 V for 12 V battery systems
	shutdown_at_end	false	boolean	True/False	Flag for software power down pi at end of collection - false can be used for bench testing.
	shutdown_wifi_on_collection	true	boolean	True/False	Allows system to turn off wifi when collection starts - this can save battery life, wifi is available during initial wait to allow interaction for downloading or updates, etc.
intervalometer_settings	max_images	10	integer	unlimited	Allows for a fixed collection period
	image_interval	1	floating point	unlimited	Seconds between shots by intervalometer
	strobe_channel	red	string	red and white(UV in current implementation)	Strobe color, two possibilities - white and red
motion_detection_settings	motion_detect_interval	1	integer	unlimited	Seconds between evaluating for motion
	post_detection_rest	5	integer	unlimited	Minutes before starting motion sense again

	max_runtime	60	integer	unlimited	Can be used to limit trigger evaluation period
	foreground_threshold	8	integer	greyscale value (0-255)	Grayscale value for target detection threshold (MOG foreground mask)
	trigger_ROI	[20, 20, 280, 200]	integer	left bottom corner and width, height based on a 320X240 img	Only required if triggering is to be excluded from certain portions of the image - only targets inside this box will be evaluated, even if object is part way in
	frame_history	25	integer	20-100	Specifies how many frames to keep for background modeling.
	trigger_eval_method	object_size	string	object_size or pixel_total	Triggering mode: "object_size" method uses connected components to evaluate above threshold objects in image, and threshold is then used to decide if sufficient change has occurred to collect image. If "pixel_total" is used, all above threshold pixels are considered regardless of connected components.
	min_object_size	500	integer	unlimited	Size of object (connected components above threshold) to trigger (small objects ignored) - only relevant for "object_size" trigger mode
	min_pixel_count	10000	integer	unlimited	Setting for how many pixels above threshold constitute a trigger event, valid only with "pixel_total" trigger mode
camera_configuration	image_resolution	M	string	L = (1024,768) ~ 0.8 Mp, M = (2048,1520) ~ 3 Mp H = (4056,3040) ~ 12 Mp, default value is M	Image resolution for triggered images, specific to the Raspberry Pi HQ Camera
	auto_contrast	off	boolean	off/on- default setting is "off"	Flag to enable use of OpenCV clahe adaptive contrast on image to enhance performance
	exposure	0	integer	unlimited	Exposure duration in microseconds, if 0 then auto expose
	iso	800	integer	100, 200, 400, 800, 1600	Camera gain - higher values are more light sensitive but poorer quality (graininess)
	strobe	envelope	string	off, camera, envelope	Strobe mode - if not set to "off", strobe signal can originate from the camera itself ("camera" - only works with certain camera settings), typically a double flash (the first to meter scene, then second for image exposure), or "envelope" where strobe is independently triggered around exposure envelope
	pre_strobe_fire	0.1	floating point	0 - 0.5	Value for how far ahead of starting image capture to turn on the strobe, only relevant for "envelope" mode

	strobe_duration	0.5	floating point	0.5 - 2	Maximum in seconds of how long to leave strobe light on - LED strobes can overheat if left on form more than a few seconds
	image_depth	monochrome	string	color, monochrome	Image depth - color image is 24 bit (8 X 3 color channels), monochrome is single 8 bit channel
	image_type	JPEG	string	JPEG, PNG, BMP	Common file formats available for opencv.
	image_quality	95	integer	70 -100	Compression level for jpeg 70 - 100, default 95. If PNG format is used, than values are 0-9 with a default of 3

6.3 General operation

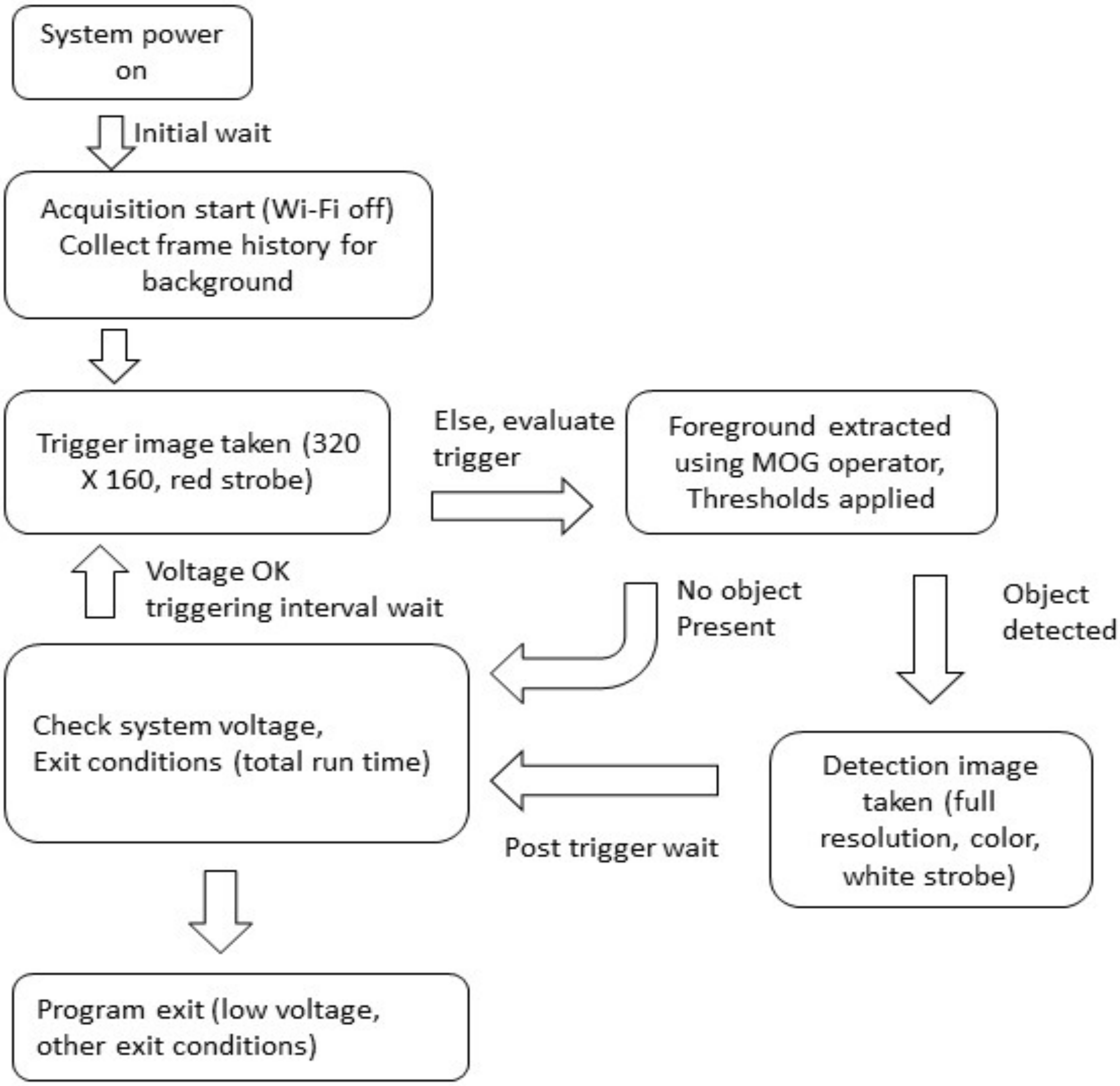
Each power up cycle is considered a “deployment”. The system is powered up by turning the Blue Robotics underwater switch knob to the “on” position. The system will then boot up the raspberry pi and display the IP address of the wifi interface for the raspberry pi, the system voltage and free disk space for 30 seconds. The system will count down for the pre-determined number of minutes before starting acquisition. Each new deployment is given a name based on the time using the format DMMDDYYYY-Thhmmss. A set of folders is created for storing images and log files related to each deployment.

6.4 Operation modes

In intervalometer mode, a single still image is taken at specified time intervals using preconfigured image settings and strobe channel up to a specified maximum number of images or until other exit conditions are detected, such as memory limitation for data writing or low voltage. In triggered mode, the camera takes a low resolution image (trigger evaluation image - TEI) using unobtrusive red light strobe or ambient light at specified intervals to evaluate the scene for change, such as the arrival of a target. The triggering process is illustrated graphically in Appendix Figure 56. When sufficient change is detected, a full resolution image is taken using the desired strobe channel for proper illumination. The TEI sequence is also stored to allow post-deployment evaluation of trigger performance and adjustment of trigger sensitivity.

6.5 Triggered operation details

The trigger operation relies on motion detection by using a background subtraction algorithm termed Mixture-of-Gausians (MOG; Bouwmans, et al, 2008). Within this system, a background model is constructed at the start of data collection from a series of TEI images (320×240 monochrome) of the scene background. TEI images can be illuminated using a lower detectability strobe such as red (600 nm) which is less detectable by many marine organisms, or collected at ambient lighting. After the TEI images are captured, a low pass filter is applied (Gaussian Blur) with kernel size 5x5 pixels, and the image is cropped to the boundaries specified in the region-of-interest (ROI) parameter. The low pass filtering eliminates noise and produces a more stable background, and the ROI allows for a focus area for motion detection to be specified, for example, away from the image edges. After the background model is established, each successive frame is evaluated for differences relative to the background (or image "foreground") based on an initial sensitivity threshold (in this systems configuration this is *foreground_threshold* parameter). The background model is continually updated as images are collected. Once the foreground is extracted as a binary mask image, it is subjected to a second level of scrutiny by using one of two approaches; 1) total pixel level – this is simply a count of non-zero pixels in the mask, or 2) by considering object size, where the foreground mask is evaluated using a connected components step, and looking form objects that meet a minimum pixel size. Before applying the connected components step, a dilation and erosion morphological operator is sequentially applied to the foreground mask to merge fragmented foreground objects that are likely a single target. If the total number of foreground pixels exceeds the min_pixel_count (approach 1), or any of the objects in the scene exceed the *min_object_size* parameter value (approach 2), conditions for a trigger event have been met. Capturing a triggered image consists of resetting camera resolution to the specified desired resolution (*image_resolution* parameter), and if desired, apply contrast enhancement using the Contrast Limited Adaptive Histogram Equalization (CLAHE) implementation in OpenCV. A secondary strobe channel can be specified for this operation, for example a white, full spectrum, strobe for capturing the true color properties of the target. The full resolution image is then written to disk.



Appendix Figure 56: System function flowchart for a complete camera deployment.

Supplemental Table 1: behavioral categorizations and terminology used for data analysis.

Deployment number	
was used to distinguish cameras when two cameras were being deployed simultaneously and shared similar Date and Time stamps.	
Date and Time	
each photo has a time and date stamp, allowing duration and daily cycles to be evaluated.	
Octopus ID	
if octopus were encountered that had been previously tagged they're VIE tagging patterns were cross-referenced with a photo library of octopus tags to determine individual specific data and track movements over time.	
Bottle ID	
each bottle was assigned an ID number in order to track resident octopus across multiple deployments and to determine if specific bottles were attracting a disproportionate number of resident octopus.	
Event types; 5 basic event types were used to broadly categorize behaviors	
non-resident non-interaction	any animal moving through the camera frame which fails to interact with an octopus regardless of presence.
resident non-interaction	any octopus behavior which fails to have a direct interaction with other species
nonresident/resident interaction	when a nonresident species has an interaction with a resident octopus.
nonresident/nonresident interaction	when two non-resident species interact with one another.
resident/resident interaction	when two resident octopus have a direct interaction, most commonly seen in adjacently denned octopus.
Species	
each species observed was labeled to allow for later identification and data analysis.	
Observed Behaviors	
frame_arrival	When a subject or part of a subject (ex:fin, claw, octopus arm) enters the area monitored within the scope of the cameras frame
frame_departure	When a subject or the last seen part of a subject (ex:fin, claw, octopus arm) leaves the area monitored within the scope of the cameras frame
fortify	When an octopus inhabiting a bottle is observed using discarded prey remains or debri to cover den openings or the resident can be observed moving prey remains or debri towards the den opening without fully implementing the barrier as an effective fortification
touch_bottle	When an animal touches the bottle exterior with an appendage
den_opening	An animal or appendage is observed within the den opening
evict_resident	When a non-resident octopus removes a resident octopus and inhabits its den location
touch_resident	When an organism makes direct contact with an octopus residing within the frame

exit_bottle	When an organism exits the bottle fully, with no appendages remaining inside the bottle or bottle opening
enter_bottle	When an organism enters the bottle fully, with no appendages remaining outside the bottle or bottle opening
periscope	When an organism keeps the majority of its body within the bottle and places its eyes or head outside the cell to observe the surrounding area. When observed within octopus this periscope behavior often involves the placement of the mantle outside the bottle opening while the arms remain inside the bottle opening.
predation	When any predatory event is observed within the frame of the camera
sat_outside	If an organism sits directly in front of the den opening for an extended period of time before departing the frame.
looking_in_bottle	If an organism sits adjacent to a bottle opening for an extended period with its head faced towards the bottle opening, and eyes angled directly towards bottle opening (observed in fish)
depositing_waste_outside_den	When a bottle resident demonstrates cleaning behavior and deposits den debri outside of the bottle opening
deploy_start	Time of deployment initialization, indicated by time stamp found on the first photo logged in system.
second_departure	Used for octopus departures when an octopus would repeatedly leave and enter the frame within a short period of time. Any frame departure within a 6 hour block of the first frame departure would be labeled as a second_department to allow isolation or exclusion of events during statistical analysis.
deploy_end	Used to indicate the termination of a deployment for later analysis
touch_uninhabited_bottle	When an octopus touches an uninhabited bottle
touch_inhabited_bottle	When an octopus touches an inhabited bottle
touch_nothing	When an octopus enters and exits the camera frame without touching any bottles or octopus
touch_resident	When an octopus comes in physical contact with a resident octopus
reach_inside_uninhabited_bottle	When an octopus reaches inside an uninhabited bottle
reach_inside_inhabited_bottle	When an octopus reaches inside an inhabited bottle
Additional parameters recorded for octopus interactions	
Is a resident octopus present within the frame?	(Y/N), used to determine if organisms adapted their visitation rate and/or duration when an octopus was present vs absent.
How long was the octopus within the frame?	Octopus visitations were evaluated for duration

No aquarium, no tank in a marine land, however spacious it may be, can begin to duplicate the conditions of the sea. And no dolphin who inhabits one of those aquariums or one of those marine lands can be considered normal.

-Jacques Yves Cousteau