Individually unique, fixed body patterns of *Octopus chierchiae* allow for photoidentification in long-term studies

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Abstract

The Lesser Pacific Striped Octopus, *Octopus chierchiae*, is a small iteroparous octopus known to inhabit intertidal regions of the Pacific coast of Central America. Many details about the species' life history in the wild remain unknown. For apparently rare and delicate animals such as *O. chierchiae*, non-extractive sampling methods are necessary to study individuals and populations over time. After photographically documenting the physical development of 25 octopuses from hatching, we have concluded that individual *O. chierchiae* have individually unique body patterns that remain constant throughout their lifetimes. Furthermore, using photographs taken of animals in captivity on different dates over many months, we show that untrained volunteers can accurately identify whether or not a pair of images depicts the same individual octopus. These results demonstrate that wild individuals could be recognized and observed for in situ longitudinal studies. In addition, our results imply potential for photoidentification and community science to be used as non-extractive, non-intrusive sampling methods for future studies of wild *O. chierchiae* and other octopuses.

Introduction

Constituting an estimated 99% of all marine species and spanning over 30 phyla, marine invertebrates are amongst the most diverse, ecologically-important, and understudied groups of organisms on the planet (Mather 2013). Cephalopods such as octopuses, squids, and cuttlefishes represent a peculiar class of marine invertebrates characterized by a bilateral body plan, a prominent head, and a set of muscular appendages surrounding a beaked mouth (Vendetti 2006); (Kaestner 1967). Their remarkable camouflage ability, complex behavior, and intelligence have

attracted vast scientific interest and pop-culture intrigue (Hackett and Harrington 2018). However, their soft body forms, elusive behavior, and ever-threatened habitats make it difficult to track individual cephalopods in the wild over time (Huffard et al, 2008).

Octopus chierchiae (Jatta, 1889) is no exception. Also known as the Lesser Pacific Striped Octopus or the pygmy zebra octopus, this species of dwarf octopus is found in shallow waters on the Pacific coast of the Americas, from Colombia to Baja California (Kerstitch, 1989). *Octopus chierchiae* and its relatives - the yet-undescribed Larger Pacific Striped Octopus and *Octopus zonatus* (Voss, 1968) - are commonly referred to as “harlequin” octopuses in reference to the black and white stripes and spots which cover the entire body [Fig 1]. However, unlike most other octopus species, which generally exhibit semelparity and only reproduce near the end of their lifespan (Mather, 2006), *O. chierchiae* is iteroparous (Rodaniche, 1984) with female individuals having been observed to produce up to eight clutches of eggs over the course of their lifespan in our laboratory. Another trait that sets *O. chierchiae* apart from most other octopus species is its lack of a planktonic stage in its development, suggesting a limited potential for dispersal. Hatchlings [Fig 2] have a mantle length of about 3.5mm (Rodaniche, 1984) and immediately begin their demersal lifestyle upon leaving the egg, making it possible to raise them in simple, conventional aquaria. Overall, *O. chierchiae’s* iteroparous reproductive strategy, observed lifespan of over two years, and lack of a planktonic developmental stage makes breeding and maintaining the species in captivity a practical and viable pursuit, contributing to its growing potential as a model organism (Grearson 2021). As its candidacy and status as the marine model organism develops, the need to locate, monitor, and study wild individuals of the species grows as well, especially because of the rapid change in abundance and distribution of intertidal species caused by anthropogenic climate change (Helmuth et. al., 2006). Specifically,

the ability to identify and monitor the octopuses on an individual basis would be crucial for both in situ studies (Arechavala-Lopez 2019) as well as controlled laboratory research involving multiple individual animals (Morse 2017).

Fig 1. One-year-old O. Chierchiae adult

Fig 2. One-day-old O. Chierchiae hatchling

Current studies on wild cephalopods most commonly involve tag-recapture studies (Semmens et al. 2007). However, as Semmens et al. discuss, external tags can harm octopuses' delicate tissues, cannot be applied to animals that are too small, and can fall off or be deliberately removed by the tagged animal. Chemical tagging, chemical analyses of cephalopod hard parts, geographic differences in parasite fauna, molecular genetics, and satellite data of detected signals are amongst some of the other rising technological tracking methods (Semmens et al. 2007). *O. chierchiae*'s delicate and diminutive bodies make longitudinal studies of wild *O. chierchiae* through the aforementioned techniques both difficult to complete and potentially dangerous to the animals.

Contrary to the techniques mentioned above, photography serves as a largely inexpensive, innocuous, non-intrusive, and widely accessible method to produce high-quality data. The harmless nature of this approach as well as the virtual ubiquitousness of smartphones that can record photos, videos, and geospatial data has enabled community science efforts to make increasingly significant and effective contributions to scientific research on the abundance, distribution, phenology, and ecology of multiple species (Ballance, 2018; Chandler et. al., 2017, Dickinson et al 2012). In situ research in particular has benefited tremendously from the growing

availability of photodata, as many inferences about wild animal populations can be drawn from both the content and the context of these photographic images, especially if members of the species of interest are individually and uniquely identifiable. The employment of such photography-analysis techniques has allowed researchers to estimate population dynamics (Karanth & Nichols, 1998), determine individual movement and home ranges (Oliveira-Santos et al., 2010) and quantify disease dynamics within populations (Geers et al., 1997). Besides its exceptional accessibility, the photography-based approach offers yet another distinctive advantage in that it may be applied retrospectively to existing photobanks (Johnston et al., 2017). All the aforementioned advantages along with the continual development of new computer-aided photo identification methods (Kelly 2001) and analytical techniques makes photography an ideal approach for monitoring and studying soft-bodied cephalopods like *O. chierchiae*.

As far as we know, *Wunderpus photogenicus* is the only octopus species in which individuals have been shown to be identifiable by photos of their individually unique body patterns, making it possible to use photoidentification methods to study this species (Huffard et al., 2008). Here, we test whether *O. chierchiae* also have identifiable, individually unique body patterns, and whether these remain constant throughout their lifetimes, allowing for photoidentification over long timespans by untrained volunteers.

Materials and Methods

Collection and Rearing

Two male and two female adult *Octopus chierchiae* were obtained from a fully-licensed commercial collector in Nicaragua during 2017 and 2018. We housed them individually in 1-

gallon(3.79 liter) glass aquaria lined with gravel, light sand and bivalve shell fragments [Fig 1, Fig 3c], provided PVC tubes, glass vials or hollow rocks as shelter, and fed them bait shrimp (*Crangon franciscorum* and *Palaemonetes sp.*) and yellow shore crabs (*Hemigrapsus oregonensis)* ad libitum daily. All aquaria in our laboratory were filled and maintained with artificial seawater (32-36 ppt) at 22-24 ℃.

Beginning in the summer of 2018, we allowed adult *O. chierchiae* (F0 generation) to mate with other wild caught individuals, assumed to be unrelated, by temporarily moving a male into a female's tank, waiting for up to one hour for copulation to occur, then moving the male back into his tank. Females usually laid eggs within two days of mating, which hatched after about 45 days. Upon hatching, individuals were transferred via a large pipette (turkey baster) into glass jars with screen lids (9 cm tall, 8 cm diameter) containing a variety of substrates including dark sand, light sand, bivalve shell fragments, snail shells, and gravel. To avoid issues of cannibalism, territorial disputes, and other aggressive interactions, the animals were reared in individual jars, all within the same 40 gallon tank(151.42 liter)[Fig 3a], initially on a diet of primarily marine amphipods and brine shrimp (*Artemia salina*). As they grew and matured, the animals were transitioned to a diet primarily consisting of bait shrimp (*Crangon franciscorum* and *Palaemonetes sp.*) and yellow shore crabs (*Hemigrapsus oregonensis)* and provided empty snail shells for shelter. Individuals were fed live foods ad libitum when available, and otherwise were offered a piece of shrimp every other day. We transferred mature animals of this generation (F1) individually to larger perforated plastic jars in a separate 20 gallon(75.71 liter) tank[Fig 3b]. In order to reduce the risk of genetic strain caused by inbreeding, we selected the least-related male-female pairs and moved them to 1-gallon(3.79 liter) tanks for mating[Fig 3c]. After copulation, we transferred the male back to his plastic jar and provided the female with hollow

rocks, PVC pipes, glass vials, and coral fragments in which to lay eggs. Following these methods we were able to successfully close the lifecycle and obtain a generation (F2) bred and raised in captivity. Captive-bred *O. chierchiae* that survived the first few weeks of life typically lived for at least a year, with our longest-lived captive-bred octopus being a male born in July 2018 who lived for 22 months and fathered at least 4 different clutches of offspring before his death in June 2020. We hatched a total of 156 individuals from 10 clutches over the course of this study (3 to 32 hatchlings per clutch) with an average body weight of 17.4 mg (min = 9.5 mg, max = 25.6 mg)[S1 Dataset].

Fig 3a. Nursery tank where animals were reared in individual glass jars with screen lids from hatching until sexual maturity

Fig 3b. General tank where mature animals were housed in individual perforated plastic jars

Fig 3c. Individual mating tank where matings occurred and eggs were laid

Photography

We photographed individual *O. chierchiae* with an iPhone 6s smartphone and a 4K Sony camcorder (model HDR-XR260V) from June 2018 to July 2020. In order to obtain a photographic record of each individual's development over the course of their lifetime, we photographed lab-reared *O. chierchiae* (n=25) weekly from a directly overhead angle [Fig 4], without removing them from their containers. All of the photographic images used in this study were taken via this described overhead method except for one pair of photographs which was taken from the side of the transparent glass jar. Body patterns of *O. chierchiae* become visible to

the naked eye when individuals are around two weeks old (~ 8 mm mantle length), but can be observed with magnification as early as the fifth day after they hatch (Fig 5).

Fig 4a. Timeline of *O. chierchiae* individual (hatched Nov 12, 2018) showing consistent body patternings over a 5 month timespan. The bottom row duplicates these photographs, with analogous patterns color coded.

Fig 4b. Timeline of *O. chierchiae* individual (hatched May 6, 2019) showing consistent body patternings over a 6 month timespan. The bottom row duplicates these photographs, with analogous patterns color coded.

Fig 4c. Timeline of *O. chierchiae* individual (hatched Nov 26, 2018) showing consistent body patternings over a 7 month timespan. The bottom row duplicates these photographs, with analogous patterns color coded.

Fig 4d. Timeline of Octopus individual (hatched May 6, 2019) showing consistent body patternings over a 4 month timespan. The bottom row duplicates these photographs, with analogous patterns color coded.

Fig 5. Five-day-old *O. chierchiae* hatchling already displaying a stripe pattern

<u>Survey Design and Evaluation</u>

We created two surveys following the methods of Huffard et. al. (2008) from this photobank, solicited participants via social media, and collected responses via google forms. Survey A[S2 File] consisted of a series of slides showing two photographs (taken from 0 days to 5 months apart) of *O. chierchiae* individuals side by side. Participants of Survey A were asked to mark whether the 2 photographs were of the same (Fig 6) or different (Fig 8) individuals. Survey

B[S3 File] consisted of the same series of slides as Survey A, but included pattern traces created in Adobe Illustrator below the photographs (Fig 7, Fig 9). We compiled survey responses into excel spreadsheets and graded them via a python script[S4 Code] using the pandas v1.3.3 package (McKinney 2010). Statistical analysis of our resulting data were calculated using the ttest_ind and ttest_1samp functions from the scipy.stats v1.7.1 library (Virtanen 2020) with a predetermined significance level of 0.05.

Fig 6. Question #17 of survey A. Matching pictures of the same *O. chierchiae* individual taken on Jul 26, 2019 (left) and Aug 2, 2019 (right)

Fig 7 Question #17 of survey B. Matching pictures of the same *O. chierchiae* individual taken on Jul 26, 2019 (left) and Aug 2, 2019 (right)

Fig 8 Question #1 of survey A. Non-matching pictures of 2 different *O. chierchiae* individuals, both taken on Aug 29, 2019

Fig 9 Question #1 of survey B. Non-matching pictures of 2 different *O. chierchiae* individuals, both taken on Aug 29, 2019

Results

Over the course of the study, we observed that *O. chierchiae* individuals' body patterns were consistent throughout their lifetimes, confirmed by photographic records [Fig 4]. All members of our research group were able to recognize individual animals based on their body patterns. Next we investigated if untrained observers would be able to do the same.

Both Survey A (1 sample t-test: t-statistic = 10.90, p-value = 4.17e-10) and Survey B (1 sample t-test: t-statistic=11.14, p-value=2.84e-10) yielded results significantly different from

50% – the expected value if participants were unable to distinguish individual *O. chierchiae*. Survey A (n = 22 responses [S5 Dataset]) had an average score of 75.54% correct answers, standard error of the mean = 2.34%. On average, participants missed 25.16% of total possible matches (false negative) and erroneously matched 22.11% of non-matching individuals (false positive), whereas Survey B (n = 22 responses [S6 Dataset]) had an average score of 74.27%, standard error of the mean = 2.18%. On average, participants missed 25.65% of total possible matches and erroneously matched 25.21% of non-matching individuals.

Discussion

Our results [Fig 10, Fig 11] show that *O. chierchiae* individuals are distinguishable to a majority of untrained observers, as 63.6% and 54.5% of all participants scored above 75% on surveys A and B respectively. There were five questions on each survey where over 50% of participants answered incorrectly and one question on each where it was an even split between correct and incorrect responses. Upon specific examination of the problematic questions, the increased difficulty could be explained by a combination of subpar photo quality, the animal being in dark morph [Fig 12] thus making its stripes harder to discern, body pattern being obscured in some manner, or the animal being viewed from different angles. Thus we conclude that *O. chierchiae* can be consistently identified throughout their lives via their individually unique body patterns by both trained and untrained observers, when a clear view of the body patterns is provided.

Fig 10. Scores of each participant of survey A (n=22 survey responses), illustrating total score (Blue), False Negative (Red) and False Positive (Orange) when assessing images.

Fig 11. Scores of each participant of survey B (n=22), illustrating total score (Blue), False Negative (Red) and False Positive (Orange) when assessing images.

Fig 12. A pair of images depicting the same *O. chierchiae* individual in dark (left) and light(right) morph

Interestingly, the average score was lower on the survey where body pattern traces were provided for assistance, perhaps showing variance in how individual participants recognise identifying features. Like the results for *Wunderpus photogenicu*s, false positives were more common than false negatives in both survey groups when photo identifying *O. chierchiae* (Huffard et. al., 2008). Because some participants responded to both surveys, we cannot combine the results of surveys B and C. Consequently, unlike Huffard et al. (2008), we considered the results of both surveys separately.

Having hatched, reared and documented the individuals used in the surveys in captivity over extensive time spans – often over the course of their entire life history – we observed no evidence that body patterns change over the lifetimes of individuals. Our surveys confirm that these permanent body patterns are a viable method of identifying the same individual at different points in time, even by volunteers with little to no experience with octopuses. To our knowledge, this is the first evidence that individually unique body patterns can be used by volunteers to consistently identify the same individual octopus throughout its development, from juvenile to adult.

This information could be used to design non-invasive studies monitoring populations of *O. chierchiae* over time in situ. Based on our results that participants could consistently identify whether or not two photos depicted the same octopus, we recommend that any future community

science effort to study *O. chierchiae* via photo identification should take the form of survey A, even if it means a large number of survey questions must be conducted to pair images. Smartphone apps or websites can be utilized to gather responses en masse and the process may even be automated with enough responses and high quality image data.

In our time working with this species, we have observed clutches of up to 32 hatchlings successfully leaving the nest, and an individual female who, in our care, laid 8 fertile clutches of eggs over the course of just over two years. Although they bear the rare distinction among octopuses of being iteroparous, *O. chierchiae* seems able to produce a maximum of around 240 offspring in its lifetime, as opposed to the many thousands other species might produce. Because *O.chierchiae* have so few young, which begin their demersal lifestyle from hatching and thus do not disperse as plankton, photo identification has potential to be a useful tool for in situ demographic studies even on very young individuals. While it is known that there can be variation in the founder chromatophores of individual octopus hatchlings of the same species (Ylitalo et. al., 2014), and that *Octopus chierchiae* can begin to display the characteristic stripe pattern as soon as five days after hatching (Fig 5), we did not begin photographing animals for this study until they were at least four weeks old. Thus, more work is needed to determine whether the individually unique body patterns of *O. chierchiae* are fixed from birth, and if not, at what point in early development the body patterns become permanent.

In addition, we hypothesize that the application of photo identification is not limited to *O. chierchiae* and *W. photogenicus*, or even the octopuses with conspicuously semi-permanent body patterns, like the blue-ringed octopuses (e.g. Figure 1 in Huffard et al 2008). Since it is known that octopuses have a fixed skin anatomy (Packard and Hochberg, 1977; Messenger, 2001), and that noticeable differences in skin anatomy between individuals can exist in octopuses with a

wide repertoire of body patterns (Huffard, 2006), we think it would be worthwhile to investigate whether individuals of other octopus species can be identified over their lifetimes by their semi-permanent or recurring body patterns or skin textures.

Lastly, we hypothesize that *O. chierchiae*'s individually unique, lifelong body patterning may play a role in intraspecific behavior in the wild. Octopus species are known to have the ability to detect conspecifics (Walderton 2011), high visual acuity, and the ability to remember (Hochner 2006). At least one species, *Octopus vulgaris*, is thought to have the ability of individual conspecific recognition (Tricario et. al., 2011). The harlequin octopuses, with their bold semi-permanent body patterns, also "rely less upon changes in chromatophores than upon perceptions of postures and positions," (Moynihan, 1985). In our view, these factors make it worthwhile to explore whether individual recognition based on visual cues plays a role in the lives of wild *O. chierchiae* and other octopus species.

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Supporting Information Captions

S1 Dataset. Octopus chierchiae Hatchling Weights

S2 File. Survey A

S3 File. Survey B

S4 Code. Survey Grader

S5 Dataset. Survey A Responses

S6 Dataset. Survey B Responses

Figure1

Figure2

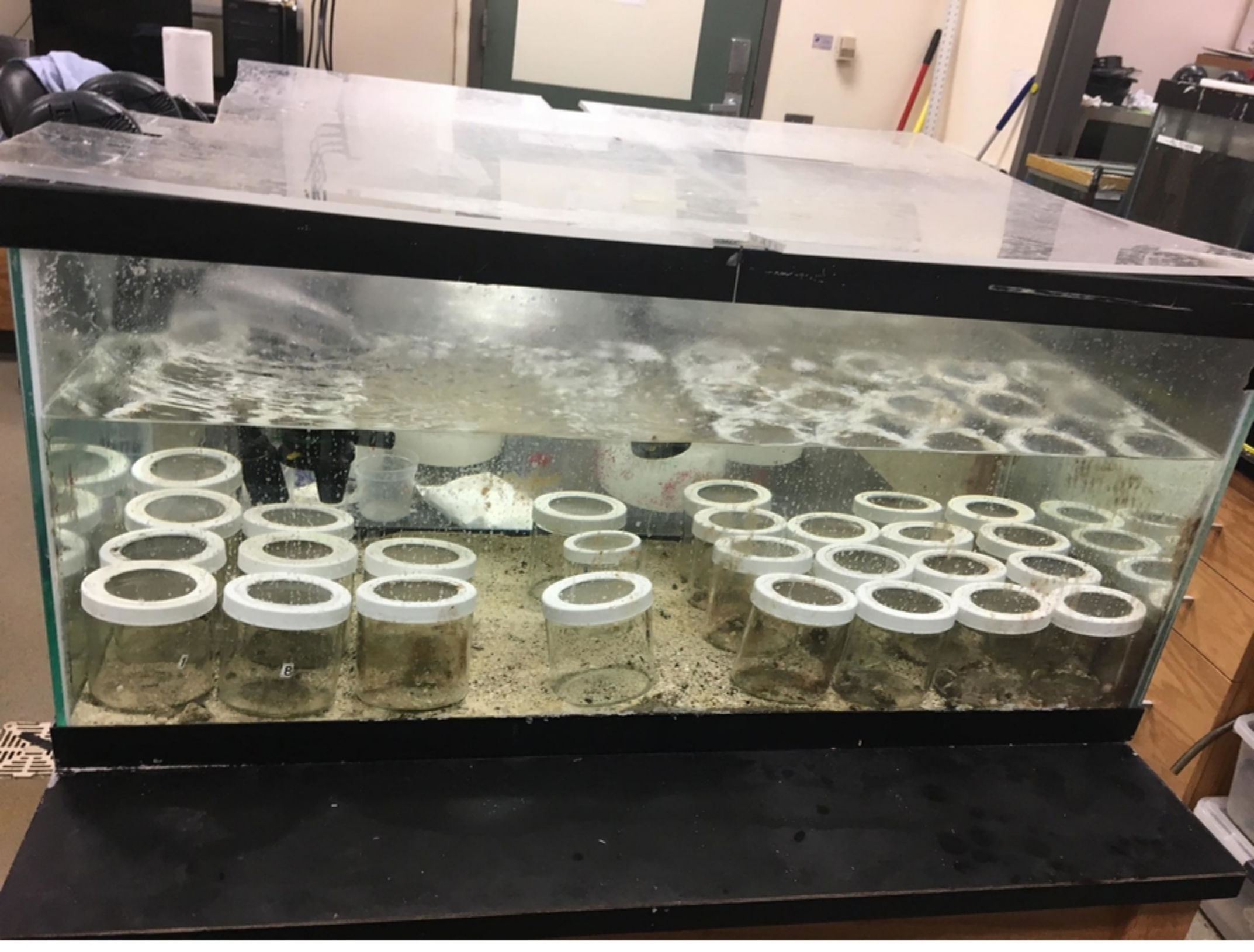

Figure3a

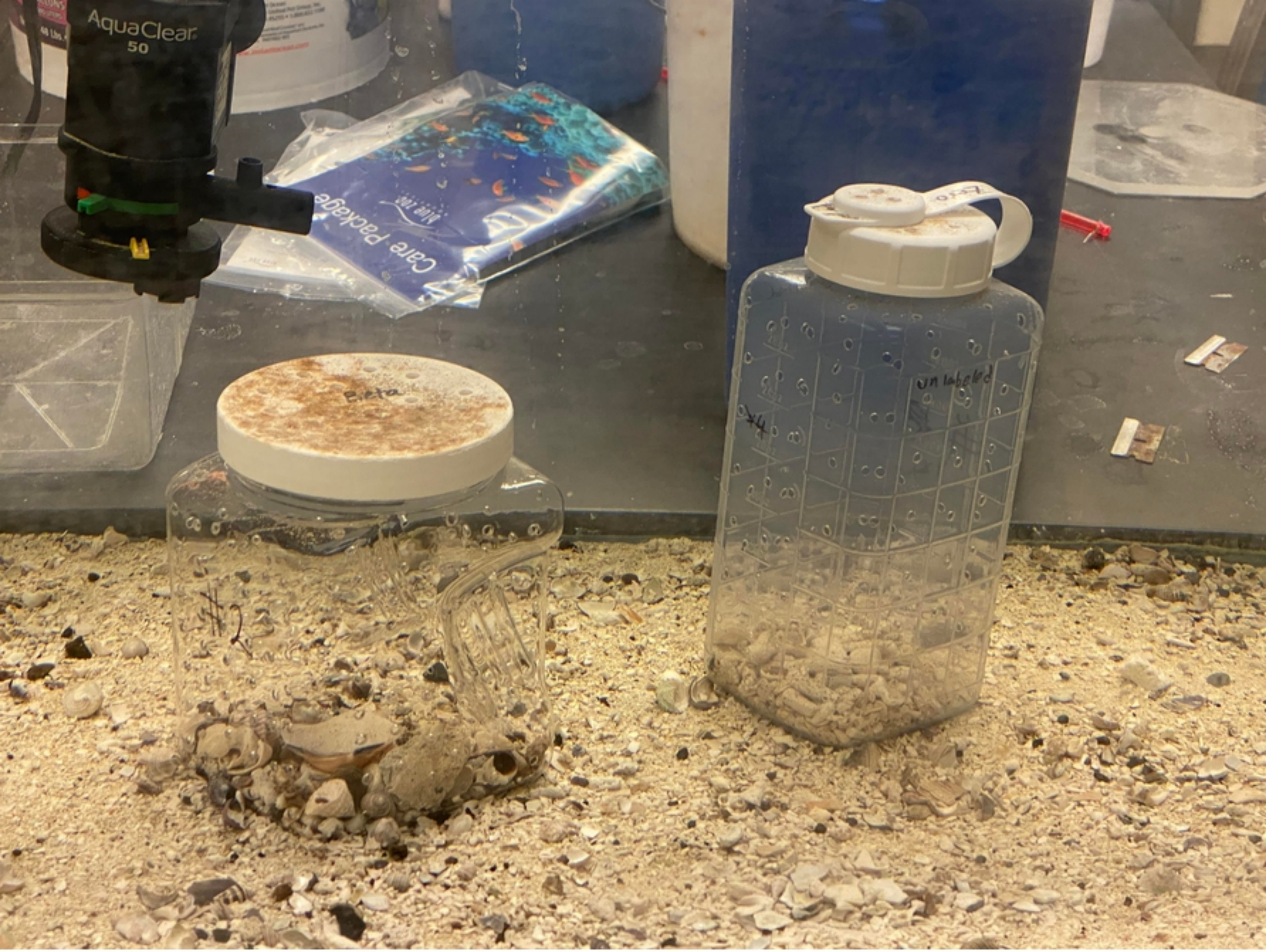

Figure3b

Figure3c

May 17, 2019
Jun 8, 2019
Aug 2, 2019
Oct 1, 2019
May 17, 2019
Jun 8, 2019
Aug 2, 2019
Oct 1, 2019

Figure4a

May 3, 2019
Jun 8, 2019
Jul 1, 2019
Aug 1, 2019
Sep 17, 2019
Nov 17, 2019
May 3, 2019
Jun 8, 2019
Jul 1, 2019
Aug 1, 2019
Sep 17, 2019
Nov 17, 2019

Figure4b

April 8
June 13
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October 1
November 10
April 8
June 13
July 1
October 1
November 10

Figure4c

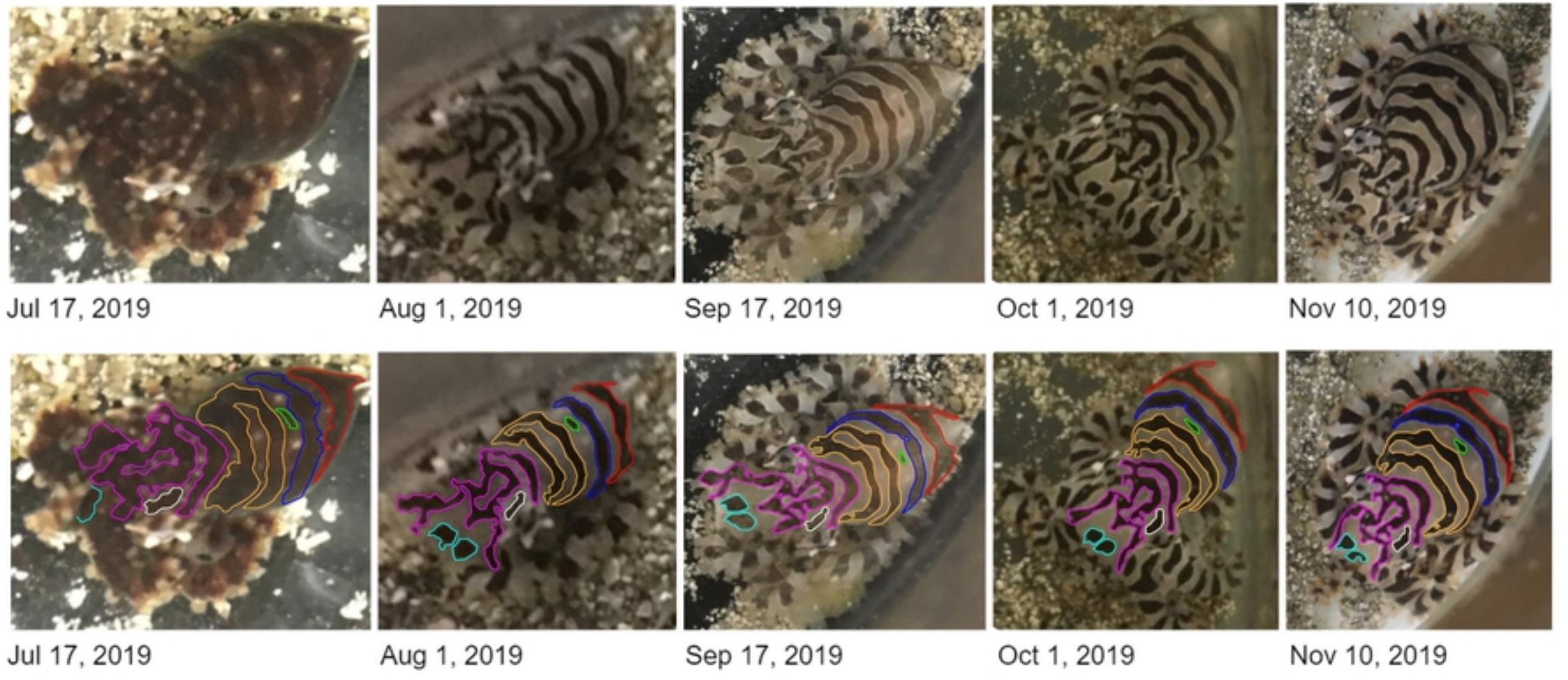

Jul 17, 2019
Aug 1, 2019
Sep 17, 2019
Oct 1, 2019
Nov 10, 2019
Jul 17, 2019
Aug 1, 2019
Sep 17, 2019
Oct 1, 2019
Nov 10, 2019

Figure4d

Figure5

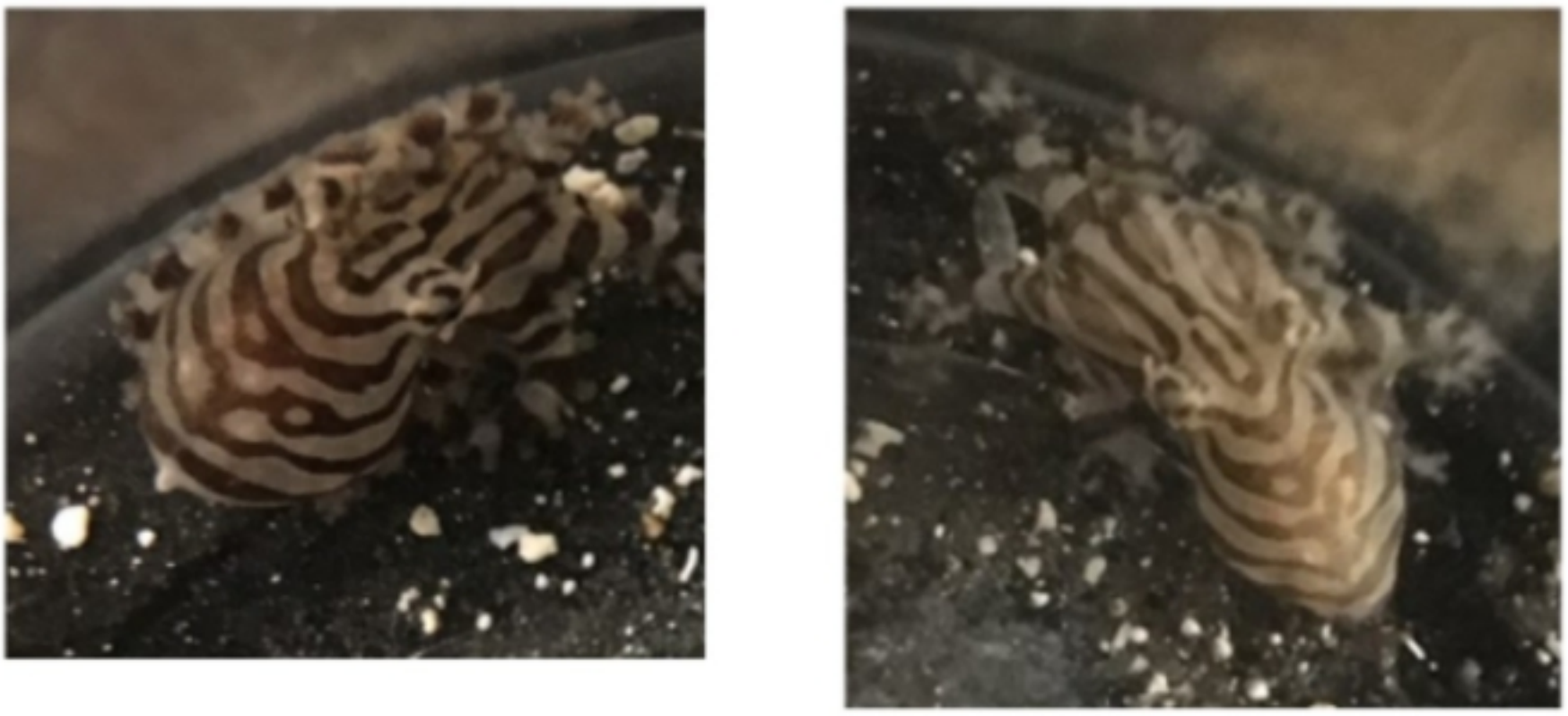
17

Question

- match
- no match

Figure6

17



- match
- no match

Figure7

1

Question

- match
- no match

Figure8

1

- match
- no match

Figure9

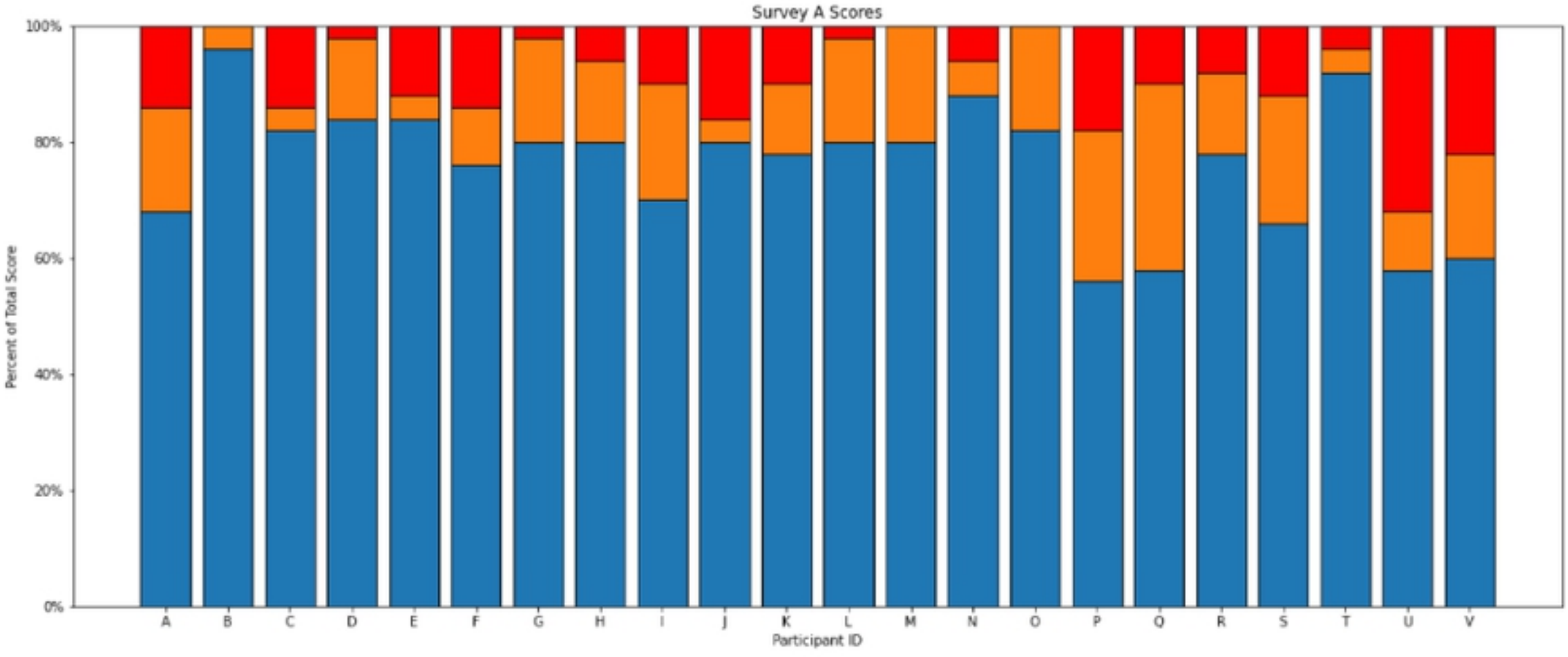

Survey A Scores
100%
80%
60%
40%
20%
0%
Percent of Total Score
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
T
U
V
Participant ID

Figure10

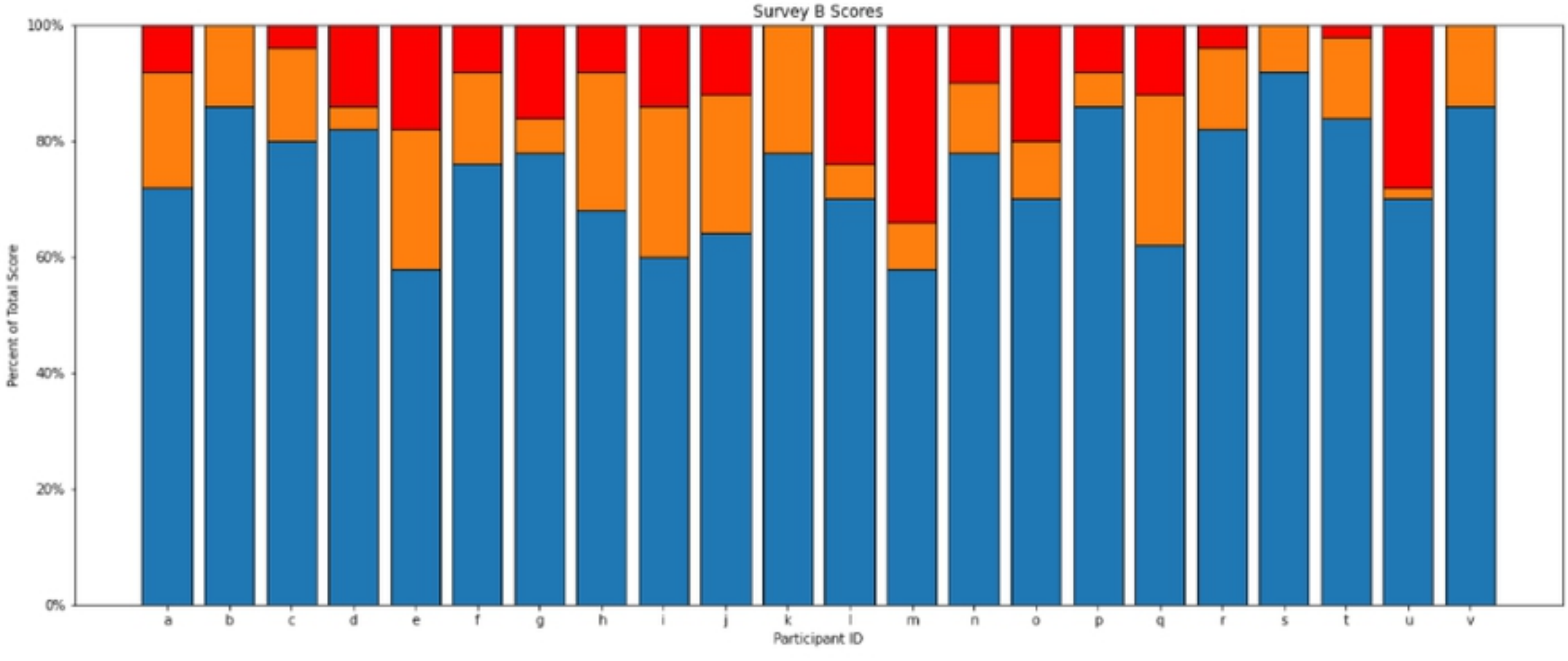
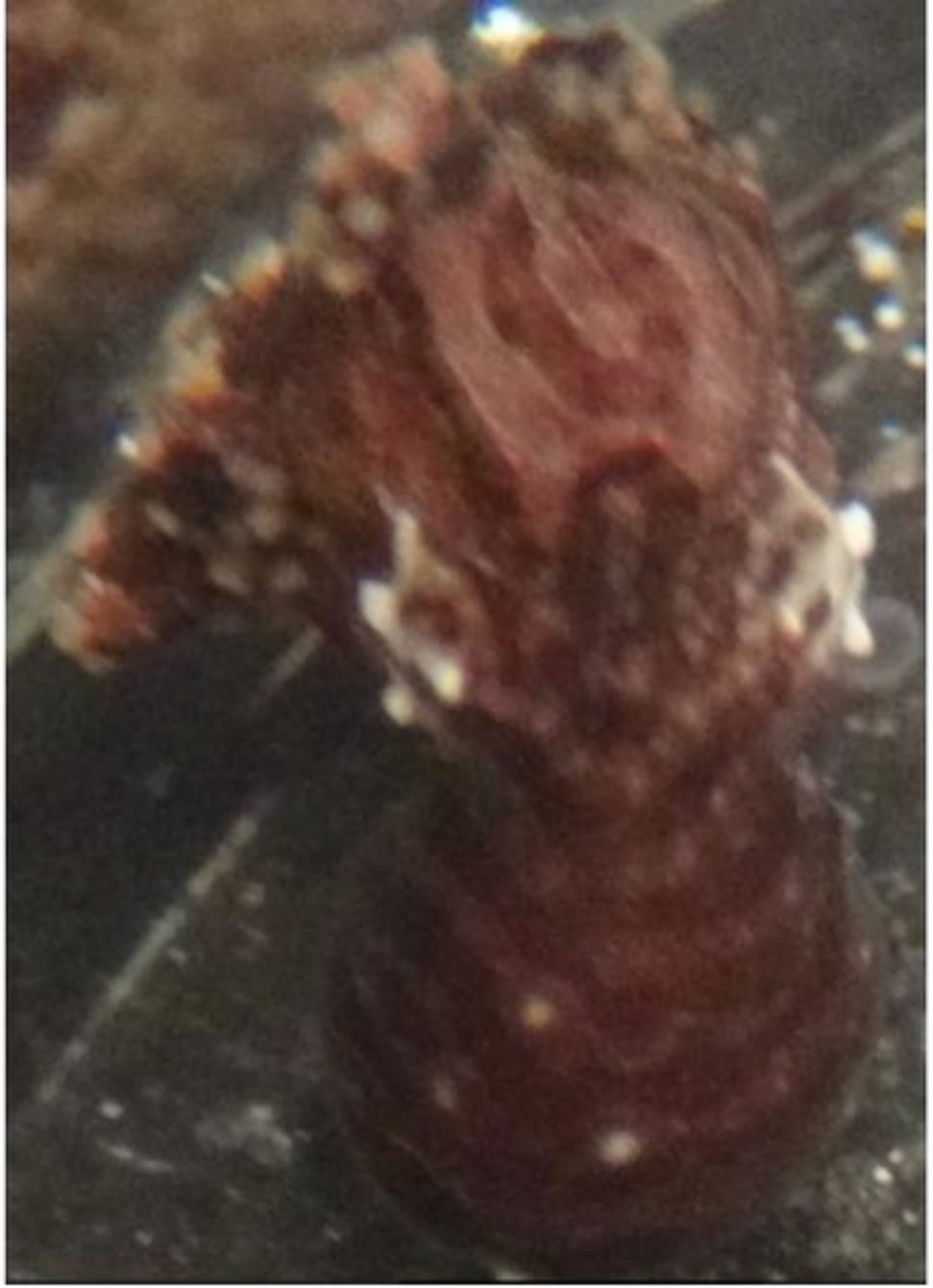

Survey B Scores
100%
80%
60%
40%
20%
0%
Percent of Total Score
a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
p
q
r
s
t
u
v
Participant ID

Figure11



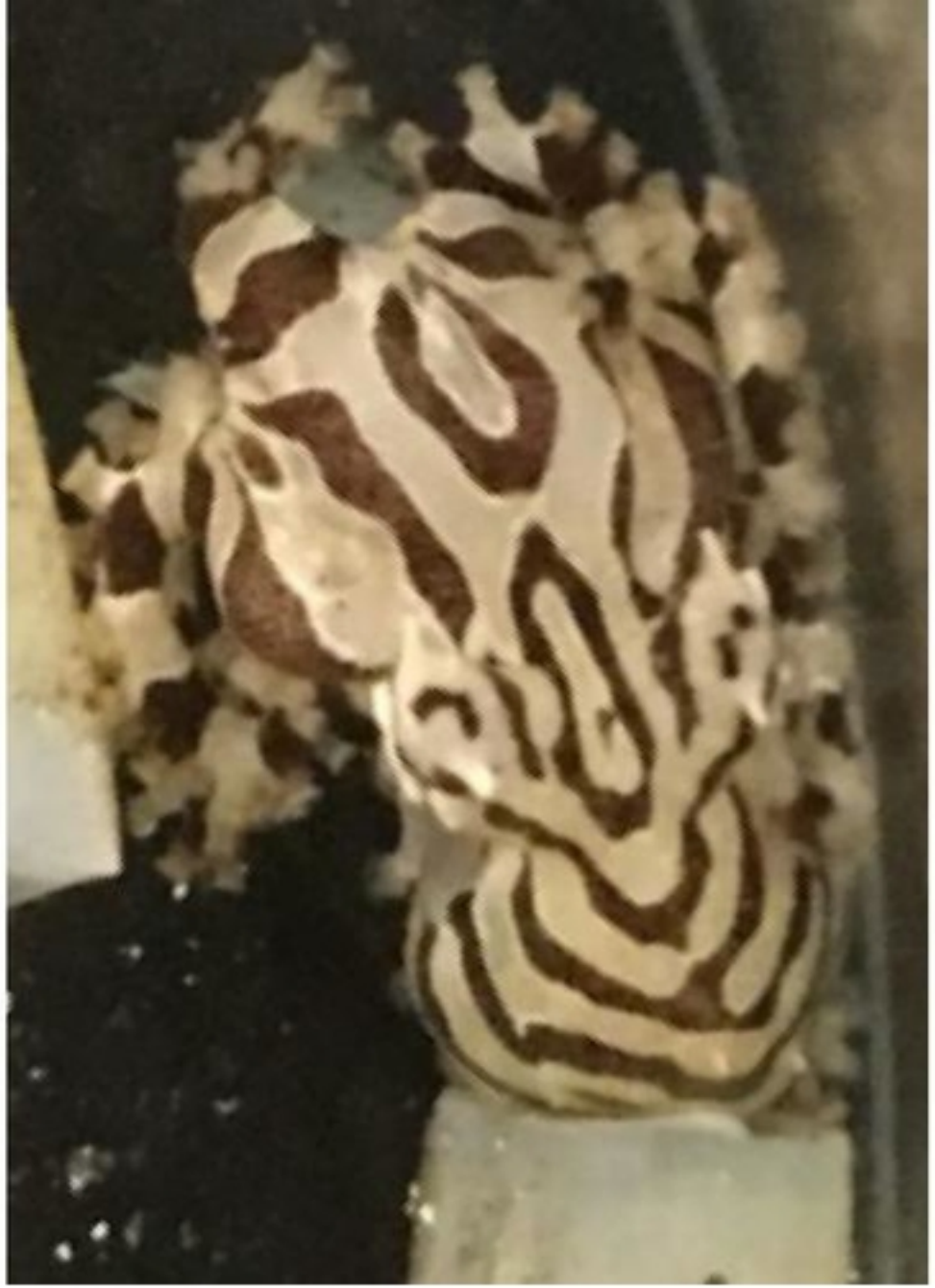


Figure12