JOURNAL OF
EMERGING INVESTIGATORS

Article

Chemoreception in *Aurelia aurita* studied by AI-enhanced image analysis

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SUMMARY

Jellyfish have not significantly changed in anatomy or physiology for over 500 million years. Their nervous system offers valuable insights into the function and evolution of more complex systems. Jellyfish were long believed to be passive feeders, capturing hapless prey with their tentacles. However, several groups have shown active responses to food and chemical cues as well as active movements within the water column, but these studies lacked a detailed quantification of jellyfish pulsation. Pulsation is a quantitative indicator of neural activity but is challenging to measure in moving jellyfish. To address this challenge in measuring pulsation, we developed Jellyfish Tracking and Analysis (JeTA) in Python, utilizing the YOLOv5m artificial intelligence (AI) to precisely identify, track, and quantify the pulsation of swimming jellyfish. We studied the chemoreception of the free swimming, bell shaped, adult stage (medusae) of *Aurelia aurita* towards a concentrated *Artemia* extract. We hypothesized that when *A. aurita* were exposed to a concentrated water-borne *Artemia* extract, they would demonstrate an active feeding response by increasing their pulsation behavior. We observed a strong behavioral response in the presence of the *Artemia* extract, with medusae increasing their pulsation rate. We also observed rotationally propagated contractions on the bells of some of the jellyfish after the stimulus was added. Together, these results present a more detailed analysis of *A. aurita*'s response to food and show that *A. aurita* have the capability to sense and actively respond to food within their immediate vicinity.

INTRODUCTION

Cnidarians are an ancient lineage of animals that likely shared a common ancestor with bilaterians (1). This diverse lineage includes corals and jellyfish that provide important ecosystem functions, such as coral reefs (2). Due to their position as sister-group to bilaterians, studying cnidarians offers intriguing insights into the evolution of complex structures, including the evolution of nervous systems (1,3). The jellyfish nervous system is characterized by neurons homologous to humans but lacks centralization. Instead, their neurons are organized in a nerve net, a diffuse network of interconnected neurons and ganglia on the basal side of the epithelium (3). Cnidarian neurons appear to be multifunctional as they have combined characteristics of sensory and motor neurons (4). Coordinated pulsing behavior in jellyfish underscores the efficiency and effectiveness of this neural arrangement. Understanding how non-centralized nervous systems coordinate animal behavior will deepen our understanding of nervous system evolution and is applicable to human health (5).

Within the Medusozoa, a lineage of cnidarians that is generally characterized by the presence of a swimming jellyfish (medusa) in its lifecycle such as a moon jellyfish or box jellyfish have been shown to exhibit complex behaviors including learning and sleep (6). As with all organisms, their success and distribution are reliant on their ability to acquire food. It has long been believed that jellyfish are passive feeders (7). Their tentacles act as a "drift-net", which capture hapless prey as they drift with ocean currents (8). However, studies have shown that the moon jellyfish, *Aurelia aurita,* can discriminate between prey based on taste and is attracted to water-borne cues associated with prey, e.g. waste excretions (9, 10). Similarly, Tamburri *et al*. showed that the jellyfish, *Mitrocoma cellularia*, will respond to food when placed in direct contact with the tentacles (surface-bound cue; homogenized *Artemia* in agarose) or water-borne cue, e.g. homogenized *Artemia* extract deposited away from the animal (11). Jellyfish have also demonstrated dynamic vertical movements within the water column, which is thought to reflect availability of prey (12). Pulsation rate reflects the jellyfish's neural activity and pulsation behavior directly impacts their feeding efficiency and survival. Pulsation equates to active feeding.

Jellyfish are effective predators when food is abundant and understanding how these creatures feed without the use of a centralized nervous system provides valuable insights for understanding the evolution of chemoreception and predation. While these studies collectively show that jellyfish can actively respond to food, their results lack a detailed quantification of jellyfish pulsation behavior, which is a direct indicator of neural activity (13). Jellyfish pulse by continuously relaxing and contracting their bell. The rhythmic pulsing behavior is controlled by pacemaker neurons in organs called rhopalia, which send signals directly to the motor nerve net. Recent advances in machine vision and artificial intelligence (AI) have provided novel methods of capturing and analyzing visual data. Previous studies have developed Python-based pulse detection image analysis to monitor jellyfish pulsation (6). In their study of the upside-down jellyfish *Cassiopea*, the authors utilized image detection to show that this jellyfish exhibits a sleep-like state at night. Additionally, AI models can be trained to identify and track the motion of marine animals (14).

However, studying the behavior of an organism suspended in seawater is challenging. Their movement

https://doi.org/10.59720/24-052

in two dimensions (forward and backward, left and right), known as 2-D translational motion, complicates the precise measurements of pulsing in non-sessile jellyfish (15). This is because existing pulse detection software has been primarily designed and tested for sessile jellyfish which remains stationary as it pulses (6) (**Figure 1**). To overcome this, we developed Jellyfish Tracking and Analysis (JeTA), an AI-enhanced image analysis system coded in Python. This system combines pulse detection with AI object identification and tracking. JeTA allowed us to precisely quantify the behavior of swimming, non-sessile jellyfish. We used JeTA to study the swimming behavior of *A. aurita* in response to a water-borne *Artemia* extract. We hypothesized that when *A. aurita* are exposed to a concentrated water-borne *Artemia* extract, they would demonstrate an active feeding response by increasing their pulsation behavior. Using JeTA, we were successful in quantifying a strong behavioral response in jellyfish exposed to *Artemia* extract compared to controls, with *A. aurita* increasing their pulse rate in the presence of the extract.

RESULTS

Individual *A. aurita* medusa were suspended in 200 ml of artificial saltwater in the presence or absence of *Artemia* extract and their pulse behavior was recorded using a high-resolution digital camera. Jellyfish pulsed with rhythmic contractions and relaxations of the bell, resulting in a predictable propulsion forward (**Appendix A)**. We occasionally observed rotationally propagated contractions on the jellyfish medusae bell after the introduction of the *Artemia* extract (**Figure 2**). Based on visual observation, the propagation created a temporary whirlpool-like vortex, which drew traces of stimulus into the bell. Based on visual observations from videos, we estimated the rotational speed of the observed contractions to be one full rotational contraction per second. This behavior lasted for about fifteen seconds at a time. In some preliminary trials, we observed defensive crumpling behavior of the medusae umbrella (**Figure 3**). Characteristics of the crumpling behavior is a bell contraction followed by cessation of pulsing behavior. Therefore, the bell remains in a contracted and tucked state

Figure 1: Adult *A. aurita*. *A. aurita* medusa swimming with tentacles extended.

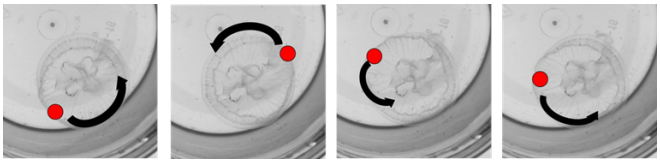

Figure 2: Observed rotational muscle contractions. Muscle contractions were observed on the subumbrella ring that propagated in a circular motion after the chemical stimulus was introduced. A representative sequence of one full rotation, shown above, occurred at an estimated speed of 1 RPS. A red dot and arrow show the progress and direction of the propagation.

(16). A previous description of this behavior speculates it to be a defense mechanism of the jellyfish when they feel threatened (17). Trials that exhibited this behavior were not included in the experiment. We removed two of such trials from the study.

Pulse rates were calculated using a novel AI enhanced image analysis software. Manual validation of the JeTA system showed it was able to accurately quantify pulse rate. We manually counted pulse rates for randomly selected 30 second recordings, and found the counts to differ by 6.48%, equivalent to a difference of a single pulse. This gave us confidence that the JeTA system is capable of accurately analyzing *Aurelia* pulse behavior. After the addition of the *Artemia* extract stimulus, we observed an increase in pulse rate, as compared to resting pulse rate (**Figure 4A**). The average inter-pulse interval (IPI), the time between each pulse, was significantly longer in the absence of stimulus, 1.67 seconds (SD = 0.50), compared to the presence of stimulus, 1.28 seconds (SD = 0.24; paired *t*-test, $p < 0.05$) (**Figure 4B**). The average pulse rate was significantly lower in the absence of stimulus, 25.6 pulses/minute (SD = 6.83), compared to the presence of stimulus, 30.9 pulses/minute (SD = 6.6; paired *t*-test, $p < 0.05$) (**Figure 4C**). We performed two negative control trials using artificial seawater (ASW) and found no change in pulse rate after the introduction of the ASW. The average pulse rate when the ASW was absent was 16.2 pulses/minute, and 16.5 pulses/minute the absolute average pulse rate for the control and *Artemia* extract trials likely reflect different animals being tested.

We also observed increased displacement of the bell edge from its relaxed position to the manubrium after the introduction of the *Artemia* extract (**Figure 4D**). We were able to determine this by calculating average pixel intensity during relaxed and contracted (**Figure 4E, F**). There was a significant increase ($p < 0.05$; paired *t*-test) in pixel intensity when the *Artemia* extract was present (mean = 2.24 pixel intensity, SD = 1.147), compared to when *Artemia* extract was absent (mean = 1.24 pixel intensity, SD = 0.548) indicating greater displacement of the bell margin (**Figure 4D)**. This increase in bell margin displacement may have been the result of the heightened pulsation activity of the medusa due to the *Artemia* extract inducing a feeding response (16).

DISCUSSION

We hypothesized that *A. aurita*, when subjected to a concentrated extract of *Artemia* suspended in water, would exhibit an intensified feeding response, evidenced by an increase in their pulsation activity. Our results support our

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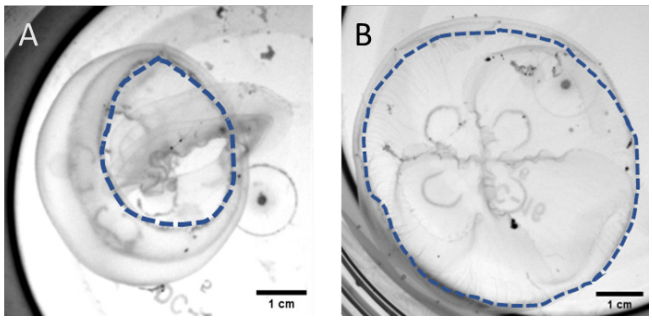

Figure 3: Crumpling behavior of a medusa. Representative images of a crumpled umbrella (A) and a relaxed umbrella (B). The umbrella edge is outlined in blue. Medusa that exhibited umbrella crumpling showed sporadic pulse activity.

hypothesis; we found that the addition of *Artemia* extract resulted in an increase in pulse frequency and pulse rate. In the presence of the *Artemia* extract the pulses were also found to be larger in terms of increased displacement of the bell edge. Previous studies have shown that Scyphozoans respond to amino acids, GSH, and glycylglycine. (18, 19). Since the main secretory product of *Artemia* is ammonium chloride (NH_4Cl), our results suggest that ammonia and its derivatives may be feeding activators of scyphozoans (10). Collectively, our results suggest that *A. aurita* sense and actively respond to water-borne chemical cues. Previous research has shown that jellyfish are capable of complex behaviors such as learning and sleep (6), we provide a detailed quantification of jellyfish behavior that was previously unrecognized.

Cnidarians are known to house a wide range of chemoreceptors, which can regulate the discharge of nematocysts (18). Chemoreceptors are found throughout the mesoglea and basal epithelial areas as well as along the lateral tracts of orals arms (20-22). Additionally, nematocysts themselves have been shown in hydrozoans to act as bimodal sensory cells that can send and receive signals in response to chemical and mechanical stimuli (22).

Chemoreceptors are activated by water-borne feeding activators. The most common feeding activators among cnidarians are the tripeptide reduced glutathione (GSH) and the amino acid proline (18). Scyphozoans are known to respond to amino acids, GSH, and glycylglycine, but the chemicals causing the response in *A. aurita* should be studied further.

This study demonstrates that jellyfish exhibit an active response to chemical stimuli, specifically by modulating their pulse rate and intensity. The pulsing mechanism in jellyfish is a crucial determinant of their swimming patterns. Therefore, changes in pulse behavior correlate to changes in their swimming behavior. Active locomotion, including swimming, has evolved independently in multiple lineages, which is not surprising in that active locomotion confers fitness advantages including the ability to actively seek food sources and environmental dangers. Our findings that *A. aurita* change their pulse behavior in response to food related chemical stimulus indicate that they may have the ability to actively seek prey. JeTA can track translational motion, but we were unable to measure directionality, as the small size of the experimental aquaria limited the movement capabilities of medusae. In future studies, we plan on using JeTA to measure chemotaxis on the same animals but in larger tanks to investigate *A. aurita*'s ability to actively navigate towards the *Artemia* nauplii stimulus.

The rotationally propagated contractions we observed provide further evidence of a feeding behavior induced by the *Artemia* extract (**Appendix A**).The propagated rotational wave travels around the margin of the bell at least two to three times without a resting phase. The circular propagation allowed the medusae to draw the stimulus into their manubrium due to the vortex effect it produced on the ambient water. Anecdotal observations of this behavior have been reported by aquarists but has not been formally characterized. We hypothesize this behavior to be a feeding mechanism of *A. aurita*, although we do not have concrete evidence to support this claim. Additionally, we speculate the reason this behavior was not present in all trials was due to physiological differences between individual jellyfish. In future studies, we plan to analyze this motion more precisely, made

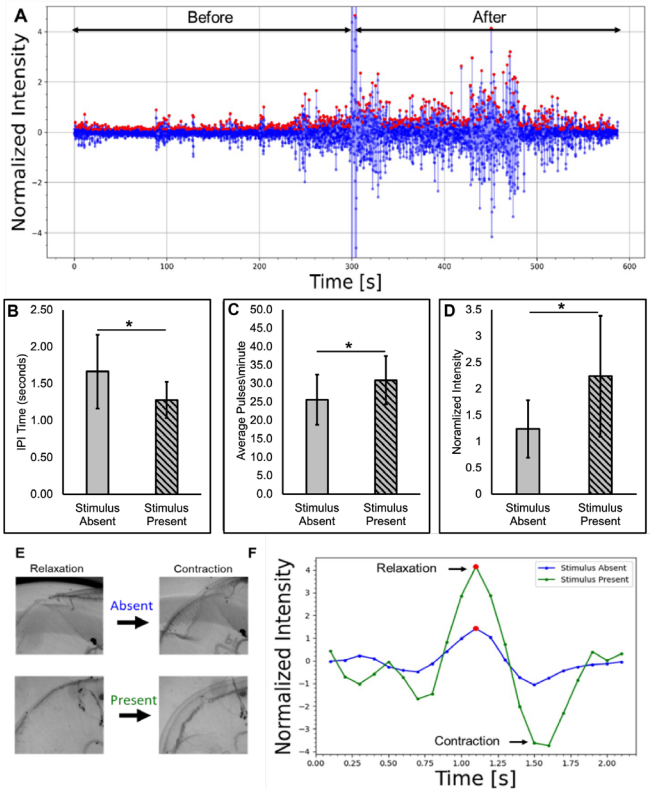

Figure 4: *A. aurita* pulse intensities and frequencies measured by AI (A) Measured pulse response of a representative trial generated from JeTA. Blue dots represent normalized pixel intensity values. Pulses are marked with red dots. (B) The average IPI among the five trials before and after the introduction of the chemical stimulus. (C) Average pulse rate among the five trials before and after the introduction of the chemical stimulus. (D) The average peak intensity of the largest pulses of the five trials before and after the introduction of the stimulus. Statistics for (B-D) were calculated using a paired *t*-test. Stars (*) are used to represent significance; * indicates $P < 0.05$. Error bars are standard deviation. (E) Representative ROIs comparing the maxima relaxation and contraction of the medusa bell with and without the chemical stimulus. (F) Normalized pixel intensity of the representative ROIs when the stimulus was absent (blue) and present (green), respectively. Blue and green dots represent normalized pixel intensity values and red dots indicate a pulse.

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possible by the use of JeTA, which is a novel tool for studying the pulsation behavior of pelagic jellyfish.

JeTA provides a robust tracking system to quantify pulsation, which overcomes inherent challenges associated with jellyfish behavior such as the drifting movements of these organisms. By integrating the YOLOv5m AI, JeTA achieved a high level of accuracy in tracking jellyfish movements, even in scenarios involving multiple organisms. Furthermore, the system's adaptability to rectify coordinate discrepancies among medusae ensures representation of individual movements. JeTA's image stabilization function also ensures that observed pulsation dynamics are not confounded by fluctuations in the spatial orientation of the animals. This study focused on tracking animals in two dimensions. In future experiments, we hope to extend JeTA tracking capabilities into three-dimensional space, which would allow for more precise studies of directionality.

We hypothesized that when *A. aurita* were exposed to a concentrated water-borne *Artemia* extract, they would demonstrate an active feeding response by increasing their pulsation behavior. We observed a strong behavioral response in the presence of the *Artemia* extract. We also observed rotationally propagated contractions on the bells of some of the jellyfish after the stimulus was added. Together, these results present a more detailed analysis of *A. aurita's* response to food and show that *A. aurita* have the capability to sense and actively respond to food within their immediate vicinity.

MATERIALS AND METHODS

Jellyfish Care and Maintenance

We analyzed the behavior of five *A. aurita* medusae, aged between three to six months, with undetermined sex, in response to an *Artemia* chemical stimulus. Medusae (4-5 cm diameter umbrella) were lab-grown and maintained in a 120 L acrylic Pseudo-Krisel tank with 33 ppt artificial salt water (ASW) at room temperature (68°F - 76°F). The medusae were fed live *Artemia* nauplii every two days, but they were starved 48 hours prior to the experiment (9). The chemical stimulus tested on medusae was made by grinding 10 mL of *Artemia* nauplii and filtering out the solids. We placed the medusae individually into a transparent 16 oz plastic aquarium, and we added 200 ml 33 ppt ASW to suspend the medusae upside down on the bottom of the container. After transferring the medusae to the containers, we observed signs of stress, characterized by erratic pulsation. To reduce their stress, we gave the medusae a one-hour recovery period. During the recovery period and the trial, the medusae remained upside down to expose their tentacles and sub-umbrellar area, facing the camera directly. This orientation was maintained as the water volume was sufficient to completely cover the jellyfish yet restricted enough to prevent them from flipping over. If the medusae were not oriented this way, JeTA could not detect pulses accurately.

Monitoring/Recording Jellyfish Behavior

After the one-hour recovery period, a high-resolution monochrome camera (Allied Vision Prosilica GT2750), positioned above the container, recorded *A. aurita* behavior for 5 minutes prior to the addition of the stimulus and 5 minutes after at 10 frames per second (FPS). JeTA functions with low-light and low-resolution cameras, but for enhanced precision, we used a high-resolution monochrome camera because of its high sensitivity to light intensity. The camera's sensitivity allowed us to analyze pulsation in more detail. We positioned a white LED light-board below the aquaria to increase the contrast of the medusae, making it easier for the tracking model to detect them. Two medusae were recorded at a time. Five minutes into the recording, 1 mL of *Artemia* extract was carefully added 4 cm away from the bell of each medusa (**Figure 5**). This procedure was repeated once for each of the five jellyfish. Using the same procedure, we used 33 ppt ASW in place of *Artemia* extract to conduct negative control experiments with two medusae.

Jellyfish Identification by YOLOv5m AI Model

We used the YOLOv5m AI model to locate and track medusa motion. YOLO (You Only Look Once) uses a convolution neural network (CNN) to detect objects. We chose the YOLOv5m model because it offers high precision with fast computation ability and training time. YOLO has three components that allow it to predict objects: a backbone, neck, and head. The cross stage partial backbone of the neural network (CSP-Darknet53) extracts features from the input (i.e., an image) and connects the spatial pyramid pooling (SPP) that enhances these features to improve their representation, with a path aggregation network (PAN), that processes the enhanced features to generate the desired output for the specific task (23). YOLO's backbone can be trained to detect specific classes of objects by integrating specialized weights and parameters. A weight tells the neural network what type of predictions it should make in an image. There are pre-installed weights that come with YOLO, such as weights to detect humans and cars. However, no such weight exists for detecting jellyfish. As such, we developed a custom weight for *A. aurita* medusae. To obtain the weight, the model was trained using frames from our recordings. From each trial we selected 6-10 random video frames; we used a total of 34 frames for training. For each frame, we identified, annotated, and labeled jellyfish manually using Roboflow software. The annotation procedure involved drawing a bounding box around the entire area of an open jellyfish. The labelling

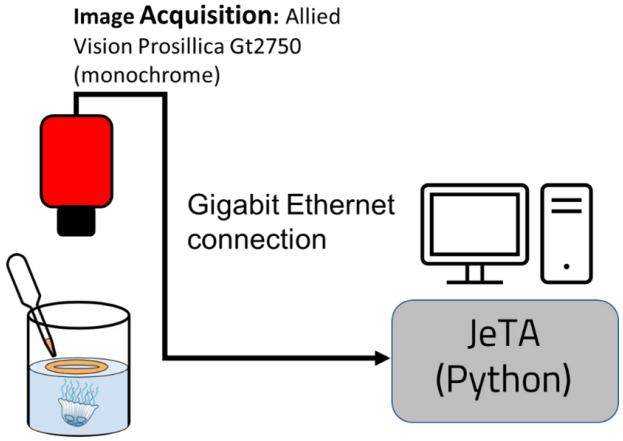

Figure 5: Experimental design setup. Jellyfish were placed in small plastic aquaria with a camera recording from above. Jellyfish activity was recorded for five minutes before and five minutes after the introduction of a chemical stimulus. Videos were recorded at 10 FPS and exported as a series of image frames.

https://doi.org/10.59720/24-052

process involved assigning the category of "jellyfish" to each box. The annotations and labels collectively provide the model weight with attributes about the jellyfish used to train Google Colab to identify jellyfish in image frames. We trained the model weight with a batch size of 16 for 600 epochs (**Figure 6**). The weight is specific to *A. aurita* and its orientation in our recordings, so deploying it on a different species of jellyfish would require training on a custom dataset of that animal.

Jellyfish Tracking by YOLOv5m AI Model

To enable tracking of jellyfish, we used the YOLOv5m AI one-stage detection algorithm (**Figure 7**). Recorded images were divided into S-by-S grids of cells, where each cell predicts the target bounding box centered on that cell and its corresponding class probability. Once enough cells with similar class probabilities overlap, a bounding box is drawn around them. By iterating through every frame, the algorithm successfully located and tracked *A. aurita* movement. We further validated the JeTA system (the combination AI and pulse tracker) by manually counting pulse rates and IPIs for three randomly selected 30 second clips. The code used for JeTA is available through Github along with reference videos used as input in the analysis **(Appendix A**).

During tracking, JeTA stored the coordinates of the bounding box as a list. While tracking single jellyfish is trivial, tracking multiple jellyfish can be challenging, as coordinates of one jellyfish can be appended to the list of a different jellyfish. To overcome this, we implemented tracking of multiple jellyfish by incorporating a pixel barrier into each frame. By separating each frame into two regions, JeTA successfully sorted the new coordinates of each individual jellyfish according to the region in which the medusae were detected.

One issue we faced during the development of JeTA was animals drifting out of the frame during filming. To solve this, we applied a basic image stabilization algorithm to the exported bounding box coordinates. The midpoint of each frame's bounding box was calculated by averaging the upper-right and lower-left coordinates. A 400-by-400 pixel square was then cropped around the midpoint. Using this process, we were successful in preventing fluctuations in the size of the region of interest and capturing the medusae in the same position for every frame.

Jellyfish Pulse Tracking

To quantify pulsation, we measured changes in pixel intensity within a defined region of interest (ROI), as changes in pixel intensity correspond with pulsation (**Figure 8**). The

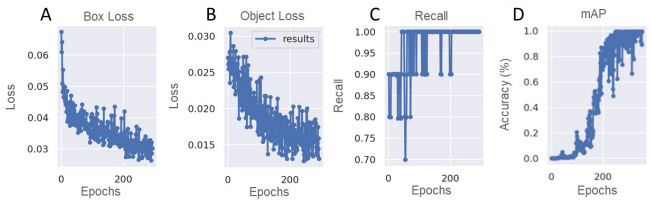

Figure 6: Training metrics of the model weight. Loss (A-B), the model's change in performance over time, recall (C), a measure of performance for a prediction system, and model average precision (mAP; D), a measure of how precise a model is at predicting classes. The reported mAP of 0.995 indicates a high level of precision in the model's predictions.

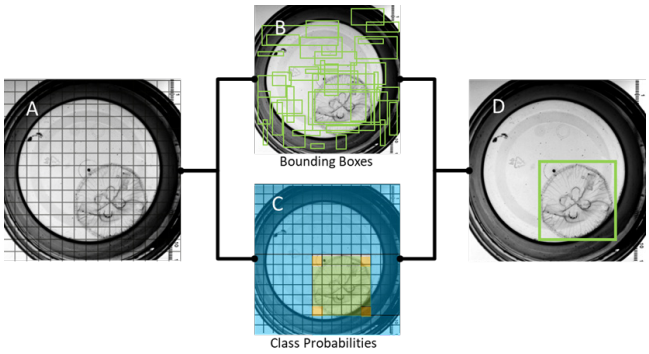

Figure 7: Visualized detection of *A. aurita* by JeTA. The process of detecting objects with JeTA is as follows: (A) frames are separated into a grid of cells; (B) the model generates bounding boxes based on its predictive algorithms, identifying potential locations where it anticipates the presence of a jellyfish. Each box has a different class probability; (C) bounding boxes with high class probabilities and spatial overlap are filtered and combined; (D) the model generates its final predication.

stabilized images were imported into Python as an array of pixel intensities using Python's Scikit-Image library. To measure the changes in pixel intensity, small ROIs were specifically selected on the subumbrella surface of the upside down medusae near the gonads. The software averaged the pixel intensity in the selected ROIs for each frame.

We analyzed the data using the model developed by Nath *et. al* (4). We applied a mean normalization to the data set to optimize pulse detection. The raw intensity measurements were smoothed using a Gaussian blur to reduce high frequency oscillations that result from medusae translational movement within the selected ROI. Normalization of the mean intensity values (T^n_{raw}) with the max mean intensity values (T_{max}) and the smoothed mean intensity values (T^n_{smooth}), where *n* is the frame index, are given by the formula below:

$$T^n = \frac{T^n_{raw} - T^n_{smooth}}{T_{max} - T^N_{smooth}}$$

To identify pulses, JeTA finds the time indices of local maxima and minima in the normalized data. For a pulse to be defined, a local maximum must be above a set threshold (to filter out local maxima due to noise) and must be far enough from the next local maxima (to prevent counting a single pulse twice). Each jellyfish can be individually configured using three adjustable parameters: the standard deviation of Gaussian smoothing, the threshold level, and the minimum distance between pulses. The normalized trace and pulse peaks were graphed using Python's Matplot library. For each jellyfish, we analyzed 6000 frames, resulting in an analysis of 30,000 total frames.JeTA works well on most systems, but its processing speed will vary based on the computing power of that system. JeTA will not compute well on systems that lack a graphics card or sufficient random-access memory (RAM). For our system, we installed an extra 16 GB of RAM to increase computation speed, which took around 30 minutes for each trial.

Data Analysis and Statistical Testing Methods

We utilized JeTA to quantitatively characterize the movement and pulsing of the jellyfish, for which we assessed multiple parameters. Pulse rate was measured as the number

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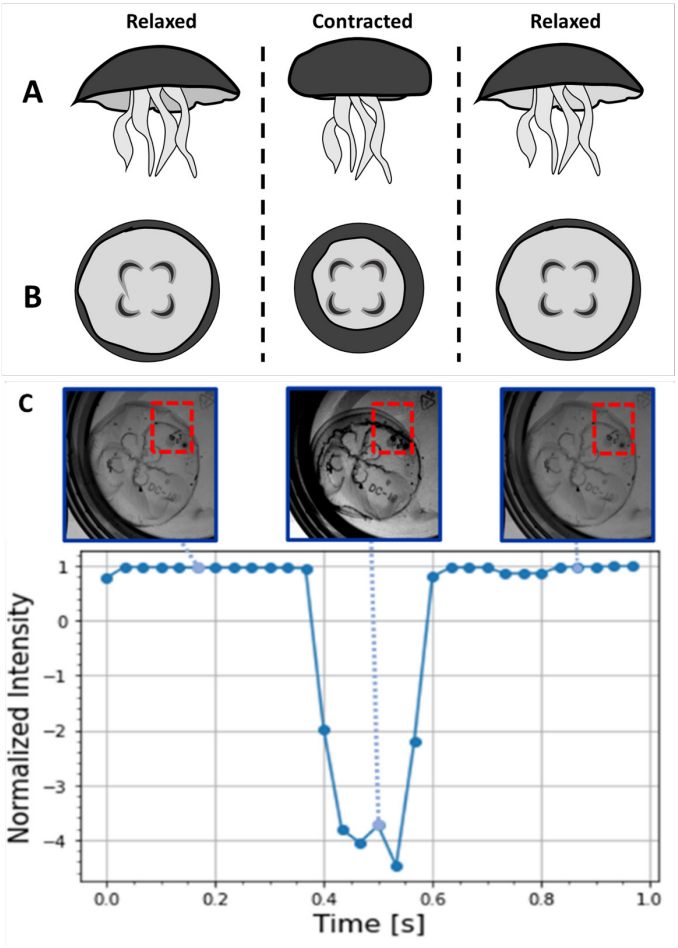

Figure 8: Overview of pulse detection. (A) side-view and (B) top-down view of pulsing. From left to right, diagrams show the relaxation and contraction of the bell. (C) Medusae pulsation corresponds to a change in total pixel intensity. The overlap of the umbrella over the subumbrella, illustrated for clarity in (A, B), increases the pixel intensity in those regions, which is measured within a ROI, marked in red. The graph shows the change in pixel intensity, marked with blue dots, within the ROI as the jellyfish pulses.

of full pulses, comprised of contraction and relaxation of the bell, took place per minute (pulses/minute). Inter-pulse interval (IPI) was calculated as the average time interval between successive pulses (seconds). We were also able to measure the rate and frequency of rotational contractions, which were muscle contractions on the subumbrella ring and a folded region that propagated in a circular motion around the mouth (**Figure 2**). These parameters were measured in the *A. aurita* medusae prior to, and immediately following, introduction of *Artemia* extract. Results were compared using two-tailed paired *t*-tests.

ACKNOWLEDGMENTS

We would like to thank Brady Weissbourd (Assistant Professor at MIT) for aiding our research over the summer. We thank the Flintridge Preparatory Jellyfish Lab for providing *A. aurita*. The Gradinaru Lab at Caltech is acknowledged for their pulse analysis scripts.

Received: February 6, 2024
Accepted: May 9, 2024
Published: June 23, 2025

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Appendix

A. JeTA Scripts and Reference Videos

Code used for JeTA and example videos of jellyfish behavior are available at https://github.com/wcab1/JeTA.git

B. YOLOv5 AI

Code used for the YOLOv5m AI is available at https://github.com/ultralytics/yolov5.git.