Behavior Analysis of *Oithona* in Hypoxic/Anoxic Conditions

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Abstract:

Oithona, one of the most abundant and ecological important copepods found in the oceans, is considered to be the base of many food chains. Hypoxia- defined as dissolved oxygen levels below 2.0 mL- has been rising and is projected to continue to rise in many marine environments. Hypoxia and anoxia are known to have diverse physiological and behavioral effects on many zooplankton, suggesting increasing hypoxia and anoxia events may also alter important characteristics of marine food webs. As hypoxia and anoxia events arise periodically, and are continuously rising, it would be valuable to understand what behavior changes occur in *Oithona* with such conditions to better understand what changes will be faced in global waters and its ecosystem. In this study, *Oithona* swimming behavior was quantified in shipboard video tracking observations, under normal oxygen conditions as well as hypoxic/anoxic conditions. *Oithona* swam significantly faster by about two orders magnitude, in hypoxic/anoxic water conditions compared to normal oxygen conditions. Modeling results based on these observations suggest this change in swimming behavior would enable *Oithona* populations to vertically migrate out of deep hypoxic/anoxic waters into oxygenated surface waters much faster than had they retained their swimming behavior in normal oxygen conditions. The observations and modeling suggest that *Oithona*'s behavioral response to hypoxia acts as an effective avoidance strategy.

Introduction

Over a period of decades, levels of dissolved oxygen have constantly been changing in the ocean’s waters (Diaz, 1995). Such changes in dissolved oxygen concentrations have led to hypoxic and anoxic conditions. Hypoxia occurs when concentrations of oxygen reach below 2.0 mL and anoxic conditions occurs when concentrations of oxygen reach below 0.0 mL (Diaz, 1995). These measurements are vital to current marine and oceanography studies because oxygen concentrations in the marine ecosystem heavily affects the ecological structure. Hypoxic and anoxic events are increasing around the world. Since the 1960’s, there has approximately been a six percent increase every year in reported hypoxic waters around the globe (Vanquer-Sunyer, 2008). This trend is expected to continue due to effects of eutrophication and a projected increase in temperatures. In turn, causing an increased production of organic matter, which leads to an increase in the oxygen demand of coastal ecosystems (Vanquer-Sunyer, 2008).

Hypoxia and anoxia events are also attributed to human-induced, deleterious effects on the marine environment (Diaz, 2001). Eutrophication is typically correlated to an anthropogenic input of excess nutrients and organic matter. Anthropogenic inputs can be from fertilizers, sewage, or any abnormal physical input of nutrients from humans (Richards, 2009). Organic matter usually comes from phytoplankton, zooplankton, or bacteria in the water column (Gray, 2002). Due to high abundance of zooplankton throughout oceanic waters, a high contribution of organic material in water columns is introduced. One common zooplankton species correlated to this event that should be studied is the copepod.

Hypoxia and anoxia events affects marine communities. Among the important species that are being affected are small copepods, which are important links in marine food webs and ecosystems. A specific and abundant genus of small copepods known are *Oithona*, which have been previously studied many times before and have been suggested to be an important and abundant copepod in the global ocean (Bigelow, 1926). Due to abundance and vast knowledge of *Oithona*, this makes the species a valuable and informative species to conduct a study on. This study will therefore focus on the study of *Oithona*'s behavior in hypoxic/anoxic waters.

In previous studies related to anoxic and hypoxic waters, there has been little observed information on how such water conditions may affect zooplankton, and in particular copepod behaviors. Up until now, major studies similar to this study were done by Sabia et al. 2014, Buskey et al. 2002, and the Svetlinchny et al. 2000. Sabia et al. 2014 looked into sex dependent changes in copepods in response with an absence of food. Observed changes in copepods are key since there are possibilities that in a low oxygen zone food will be limited for copepods. Buskey et al. 2002 determined how males versus female copepods change in escape behavior when placed in a water that is hydro dynamically disturbed. This observed difference in response from male and female could affect how copepods behave in this study as well. In Svetlinchny et al. 2000, copepods were observed to have metabolism rates about seven times slower, with a 3.4 to 9.5 fold in decreased locomotive power with the change in oxygen concentrations. This significant change in metabolism and locomotive power motivated this study on how *Oithona* behavior changes when exposed to oxygen depleted waters. Each previous study contributes as background information to this study, but there are still major knowledge gaps that makes this topic a valuable candidate for future research. Since copepods are found at the base of the food chain, understanding changes in copepod behavior will give us insight on future hypoxia related issues, and changes in food web dynamics.

Modeling is a tool for inferring the population-level consequences of individual-level behavioral observations to help explain and interpret patterns in our empirical results (Kriete, 2014). With the combined power of computation from mathematics and computers, and the understanding of biology systems, there is the power to analyze patterns and understand why patterns may occur. For this study, modeling not only provides a visual understanding of the migration and movement of *Oithona* populations between hypoxic/anoxic conditions, but it will also provide a general understanding or explanation of how much time occurs between the migration of the *Oithona* between the conditions.

The main objective of this study is to determine if there is a change in *Oithona* behavior in the presence of hypoxic/anoxic conditions. While there is little study or evidence on this subject, I hypothesize that there will be a change in behaviors such as *Oithona* swimming speed under hypoxic/anoxic conditions. With such significant change in locomotive and metabolic rates observed in the previous study by Svetlichny et al 2000, I hypothesize to see a similar and consequent corresponding behavioral difference in *Oithona* found in hypoxic or anoxic conditions. Furthermore, I hypothesize that the Oithona will move faster and more effectively to escape the hypoxic/anoxic conditions compared to normal oxygen conditions. If the results in this study show a variation in behavior of the *Oithona* in hypoxic or anoxic conditions, then it implies that hypoxic and anoxic waters make a significant impact on copepods and other zooplankton in terms of behavioral skills and survival rates.

Methods:

The study occurred in Puget Sound, WA on the evening of February 12th, 2016 aboard the R/V Barnes. Only one station, approximately 47° 42’12.77 N and 122° 24’20.81W, was used to gather samples due to constraints on time and unexpected experimental difficulties.

Set Up & Prepping:

For this study, the set up required preparation before the cruise date. There were several items that were altered in order to create an experimental set up. To be able to contain and record *Oithona* with a clear image, two 750 mL culture flasks were used. In order to prevent loss of oxygen or gas exchange, culture flasks caps were altered by drilling holes of 3/8 sizes in them to only allow tubing for the gases to be placed in them. An acrylic water jacket was created to maintain ambient seawater temperature in two culture flasks, while allowing them both to be imaged by cameras. A nitrogen gas tank along with tubing and an exchange rate controller was used to bubble dissolved oxygen out of the hypoxia treatment flask. In the oxygenated control culture flasks, a fish tank bubbler was used to provide a similar level of bubbling. A USB-camera was used to image zooplankton movements, adjusted prior to the cruise to be optimally focused on zooplankton placed in a culture flask given the distance between the camera and zooplankton. In Figure 1, shows an image of the set up used for the cruise and video analysis.

Gathering Samples on Research Vessel:

At the station, the water column oxygen was analyzed down to 50 m depth with a CTD oxygen probe to affirm that there were no hypoxic or anoxic conditions present down. Once this was confirmed, a hand phytoplankton net was cast to capture *Oithona*, along with other small and slow-moving plankton. Due to windy and rough water conditions (17 mph winds and gusts of 28 mph winds) during sampling, a hand held phytoplankton net was used rather than a zooplankton net. The phytoplankton net was cast down to approximately 20m. Once the phytoplankton tow was cast, zooplankton collected in the tow were placed into a 750 mL culture flask. The flask was then labeled with the station identification and placed into a sea water bath to acclimate the zooplankton for a one 1-hour minimum. This acclimation procedure followed methods found by Chen et al. 2012 to be appropriate for small zooplankton.

While the zooplankton acclimated, a camera for video analysis was positioned on the seawater bath so that each flask was framed. The video then recorded zooplankton in hypoxic/anoxic conditions and full or normal conditions of oxygen in the tank. In order to ensure light as a control variable, the zooplankton was kept in a room with only the room light as lighting.

The oxygen in the flasks was manipulated to stimulate hypoxic/anoxic scenarios and normal oxygen scenarios by bubbling nitrogen and oxygen. Following methods by Kristensen et al 1987, nitrogen was bubbled into the flask for approximately 12 mins to lower the oxygen levels in the flask. Similarly, in order to elevate the oxygen levels, a fish tank oxygen bubbler provided an input of bubbles into the flask for approximately 12 mins. After the bubbling stopped in both scenarios, three minutes was allowed for turbulence to dissipate to get an accurate video analysis. Then, the video camera was turned on and used to record approximately 10 mins of video for each hypoxic/anoxic conditions and normal levels of oxygen. I planned to use the oxygen probe and the CTD probe to measure initial and changing levels of oxygen present in the samples. The oxygen probe would help determine the sampling stations since it is attached to the ship. However, due to an unexpected difficulty, the oxygen probe failed to work aboard the ship. CTD casts occurred at the sampling station and determined the oxygen, salinity, and temperature of the water and were used as the main initial measurements of oxygen, salinity, and temperature for this study.

Video Analysis:

After video data was collected, video analysis was conducted by applying digital video filters using a customized version of open source video editing software avidemux (pers. Comm., Chris MacGregor, Wallingford Imaging Systems). In avideumx, a filter was first added to alter the video from color to grayscale. Afterwards, three more filters were added to make the video ignore pixels that were background noise and to only focus on groups of bright pixels approximately the size of Oithona. By switching back and forth between the filtered video product and the video, I was able to confirm which bright objects in the processed videos represented *Oithona.* Approximately 18 individual Oithona were visible on the filtered video. Once the video was processed using these filters, data about each bright pixel group were recording into a text format. This file contained for every frame, the location, size, and approximating ellipsoid of each bright pixel group to probable *Oithona*. Figure 2 shows the product of what the tracks of the *Oithona* look like after the video filters are applied and transferred into Python. By using python scripts to display and manually track individual across successive frame, I was able to determine *Oithona* swimming rates, direction of movement, and other metrics from the video data. Figure 3 helps show the results spatial plots of the *Oithona*, and how to identify an *Oithona*, and to track them for their swimming rates and direction of movements.

Modeling:

To assess the implications of observed movements for population distributions in the presences of hypoxic layers, I used a model implemented in MATLAB from a previous study by Danny Grunbaum, originally used to analyze harmful algal blooms. This model was modified to simulate an Oithona population's dispersion and diffusion, given the vertical migrations swimming movements extracted from the observations and video analysis. In this model, there are two layers of depth: a near-surface and a deeper layer. The model simulates population distributions assuming movement behaviors may differ in the two layers. In this study, the two layers were interpreted to represent a deep layer with hypoxic/anoxic conditions, and a surface (top) layer with normal oxygen conditions. Two coefficients were calculated from the video analysis to run the model; diffusion and up-swimming velocity. The formulas for these coefficients are as follows:

$$Diffusion\ (D) = \frac{dy^2}{2 * dt}$$

$$Up - swimming\ velocity\ (U) = \frac{dy}{dt}$$

These equations are needed to be calculated for both normal and hypoxic/anoxic conditions. The simulation was used to infer the differences in vertical distribution between populations initially in the hypoxic layer, with and without the behavior changes observed in response to hypoxic conditions.

Results:

Based on the video analysis and picking of 18 confirmed tracks of Oithona individuals each in normal level oxygen and hypoxic/anoxic conditions, significant differences were found between the swimming rates in the given *Oithona* in the presences versus absence of hypoxia/anoxia. The sample size was 18 *Oithona* for each different scenario due to the limited number of definite confirmations from the hypoxic/anoxic conditions and normal oxygen conditions. Under the hypoxic/anoxic conditions, swimming rates velocity (x, y) direction averaged to (3.51, 0.17) cm/s. Under normal oxygen conditions, swimming rates velocity averaged to (3.65, 0.003) cm/s. By using the Wilcoxon non-parametric rank order function in MATLAB to calculate significance of the data, the p-value was calculated to be .0413, which is considered to indicate a significant difference.

The model extrapolates the interaction and migration between the *Oithona* in the hypoxic/anoxic conditions and the normal oxygen conditions based on swimming velocities calculated earlier. The coefficients for normal oxygen conditions were calculated to be .003491 for diffusion and -.03022 for up-swimming velocity. For hypoxic/anoxic conditions the diffusion coefficient was calculated to be .17337 and the up-swimming velocity was .290235. Based on these coefficients the model shows that over time, the population of *Oithona* in the hypoxic/anoxic condition migrate towards the surface layer where the oxygen levels remain normal faster than the model with all normal oxygen conditions. Based on the model, which only ran to stimulate 72 hours, this trend can be seen when comparing figure 4, 5, and 6.

Discussion:

While the results of this study are based on a small sample size, the results of limited observations suggest behavioral responses by *Oithona* to hypoxia to be significant. A specific *Oithona* behavior to focus on was the vertical migration, or the y-component of the *Oithona* velocities. This component gives insight on how the copepods would move up and down in a water column. The results from this study from both the results and the model suggest that *Oithona* move quickly in an upwards direction in hypoxic/anoxic conditions, while *Oithona* in normal oxygen conditions move at a slower rate in a slight upwards direction. Given the observation that in most cases increase in depth also means an increase of hypoxic conditions till reaching anoxia, the results of this study suggest that *Oithona* reacts to hypoxia/anoxia by moving towards the surface, which is typically towards less hypoxic conditions. The migration in the upwards direction would reflect that *Oithona* prefer or need to be in more oxygenated waters.

Perhaps what is interesting to note that while in the Svetlinchny et al. 2000 study, it was found that small copepods tend to have less locomotive power in order to save energy in a lower oxygen environment. However, the results from this study was that *Oithona* will attempt to move much faster than in normal conditions to move towards surface waters. This is almost the opposite effect than what Svetlinchny et al. 2000 study found.

Given the small amount of data that was able to be collected, the next step in this study would be able to gather more data points. Collecting more *Oithona* and performing the study again would be key in being able to solidify the theory and results of what occurred from this study. During this study, several pieces of equipment malfunctioned, resulting in this study obtaining a smaller data set that is also quantifying Oithona movements with less accuracy. This included the loss of functionality of the pH probe on the day of the cruise and loss of functionality of a camera. Additionally, for future studies, adjusting the modeling time to be even longer to understand more of the interactions between the hypoxic/anoxic layer and normal oxygen layers would be a valuable insight.

It is also important to note that the model used in this study was a very simple model and made assumptions. However, some assumptions such as population, time, and layers may not accurately reflect true interaction and movements of *Oithona*. This model could be adjusted to become more complex and specific to fit the interactions that occurred more accurately, but more data and time would be needed to do so. Nonetheless, the implications of this simple model are still worth noting.

Given the results, swimming rates or swimming velocities show a difference given the two conditions of normal oxygen and low oxygen. A definitive finding that *Oithona* behave differently in hypoxic or anoxic conditions would be a key insight for scientists and researchers interested in understanding biological oceanography of human influenced marine environments. Because *Oithona* is abundant and ecologically important in waters around the globe, results of this study suggest that *Oithona's* behavioral responses to hypoxia would behave similarly by moving into shallower depths in lower oxygen situations.

Conclusions:

This study found a change of swimming rates between hypoxic/anoxic conditions and normal oxygen conditions, which were statistically considered significant with a p-value of .0413. This study found that *Oithona* tend to move quickly upwards towards the surface when in hypoxic/anoxic conditions, while *Oithona* tend to remain in their approximate depths when in normal oxygen conditions. These results support the original hypothesis that *Oithona* would behaved differently in the hypoxic/anoxic water conditions. The results were that *Oithona* do show a trend in vertical migration and swimming speeds when in the two different conditions. However, because of limitations in sample size and other experimental constraints, there is still a need to replicated this study to confirm results and hypothesis of this study.

Figures:

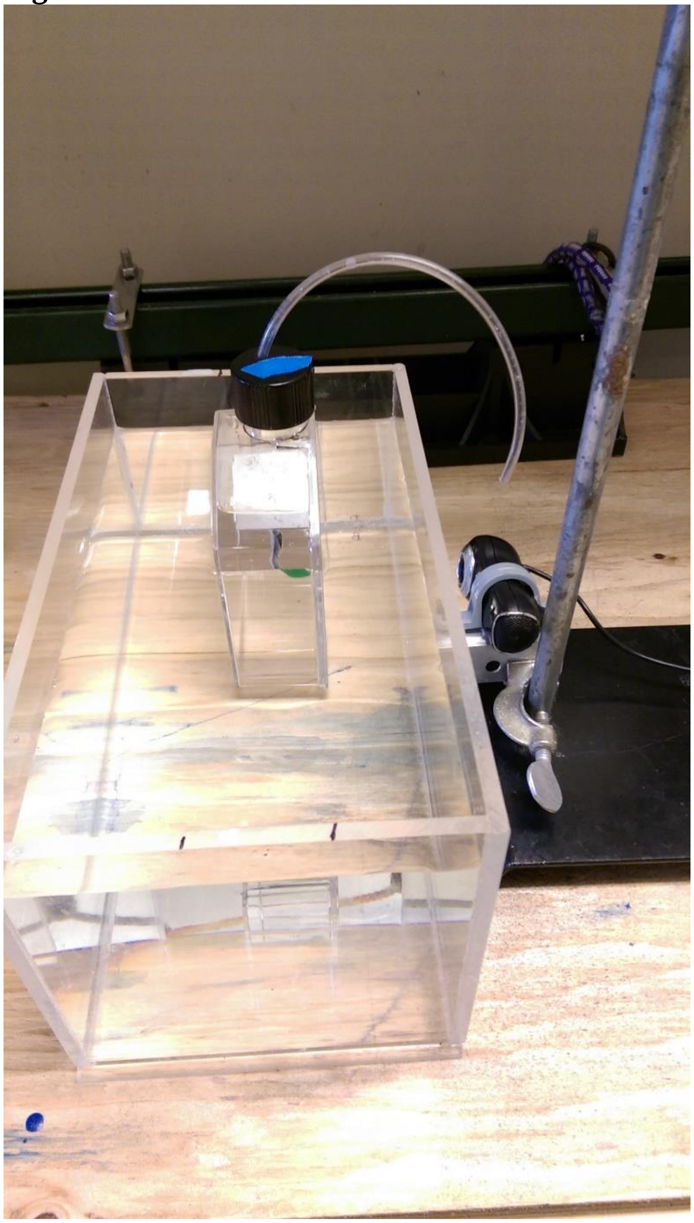

Figure 1. The set up for video analysis of Oithona. An USB-camera was clamped to a stand and centered to the culture flask. The altered culture flask lid already has the tubing for exchanging gases. The culture flask was in a seawater bath held in an acrylic box.

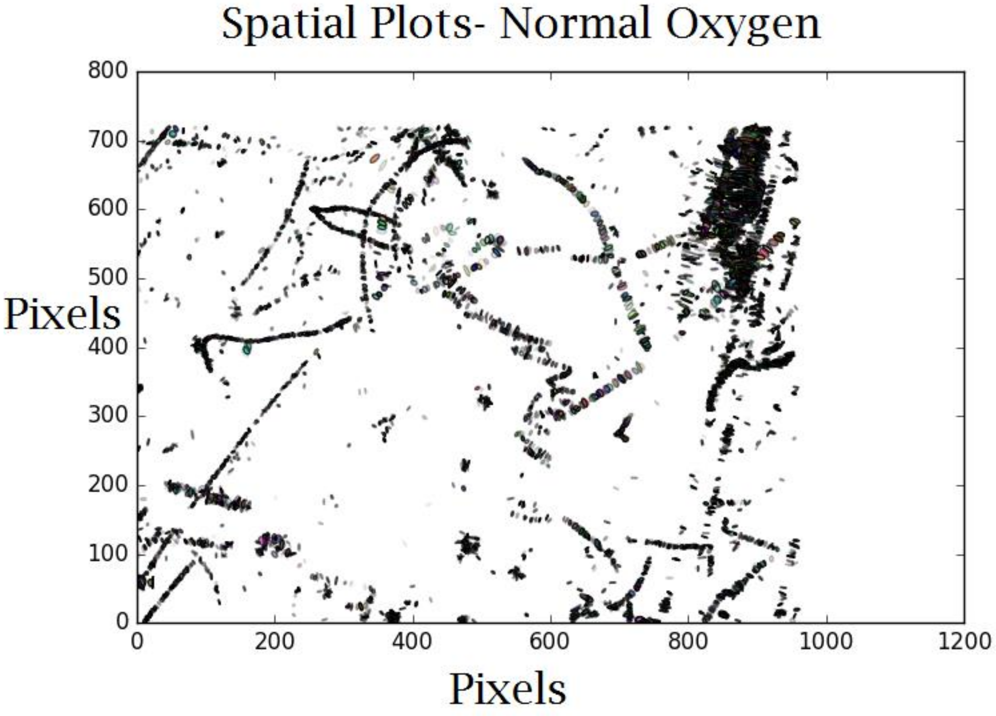

Figure 2. Spatial plot produced from given points of movement from the video after digital video filters were applied. Produced in Python.

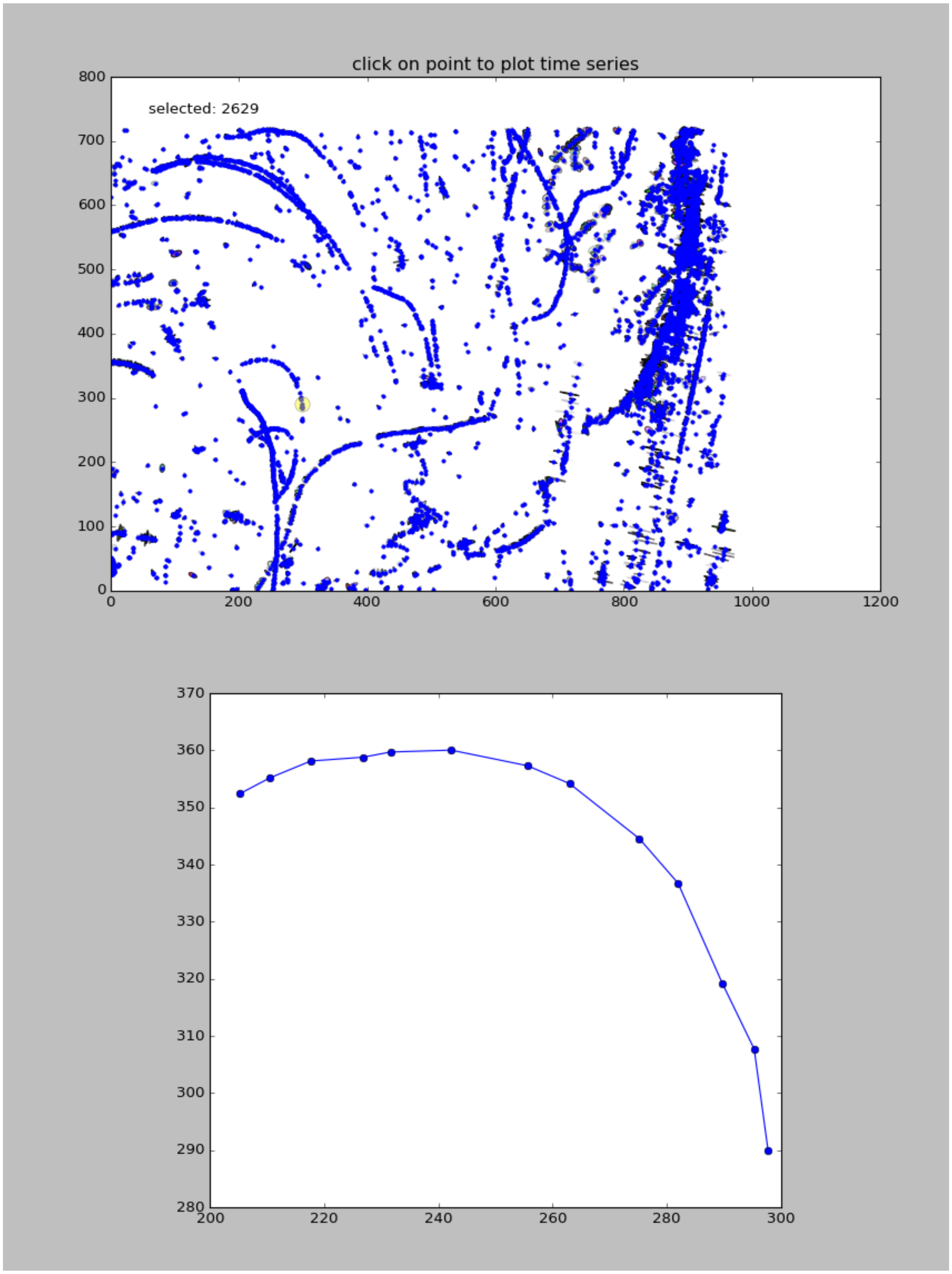

Figure 3. Given the various spatial plots (as seen in fig. 2), *Oithona* were found and tracked by clicking on their path. The top sub plot shows the full spatial plot with all points in the spatial plot. The bottom subplot shows just the tracked copepod points. Both axis in both subplots are pixels. Produced in Python.

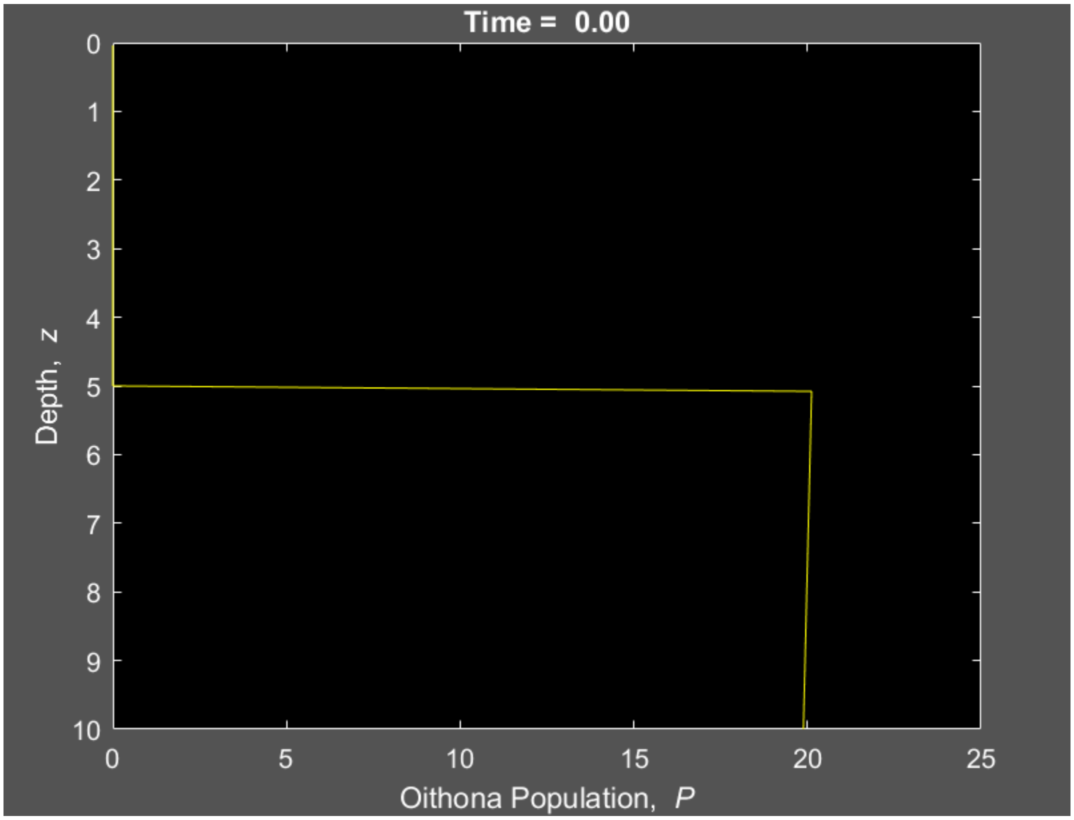

Figure 4. Results of model at time 0. The depth of 5 is the boundary between the surface and mixed layer. This depth has a full population of 100 Oithona.

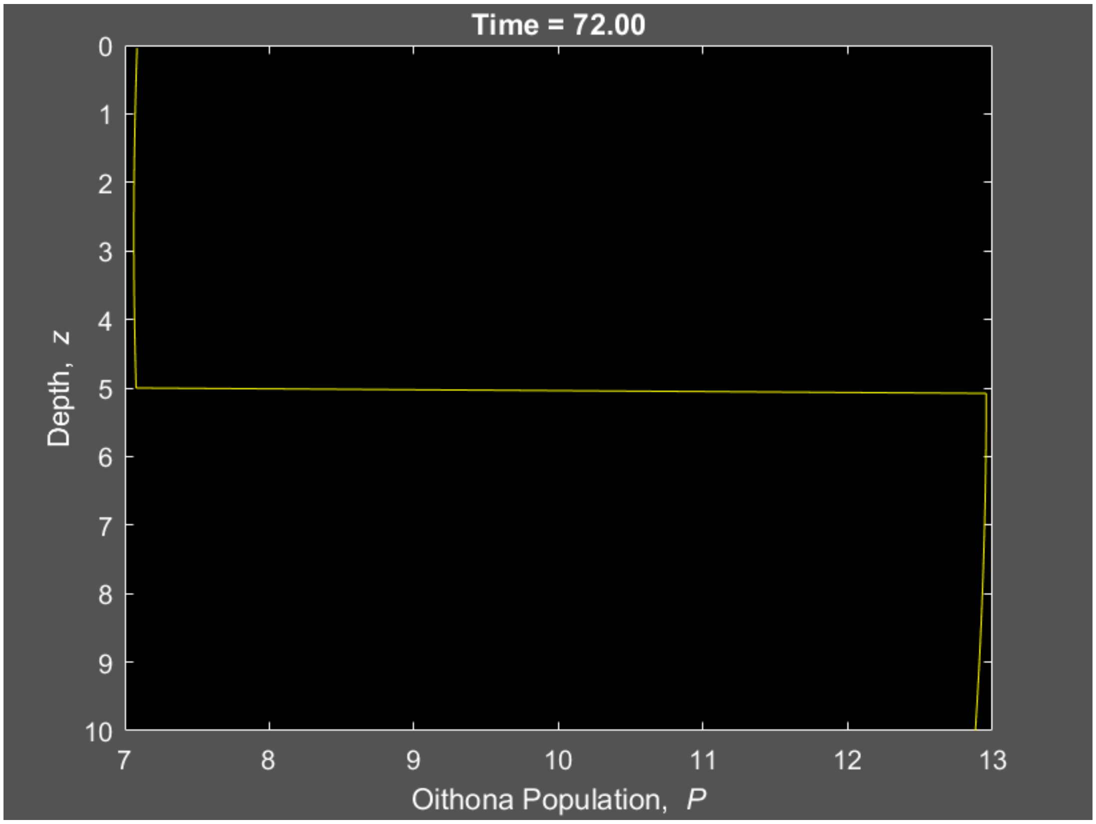

Figure 5. Results of model at time 72 hrs. The depths 0-5 are the surface layer where oxygen levels are 0 and initial population is 0. The depths 5-10 are the mixed layer depths where the oxygen levels are hypoxic/anoxic conditions. This depth has a full population of 100 Oithona. After 72 hours, the population from the hypoxic/anoxic layers have migrated up into the surface or normal oxygen layer.

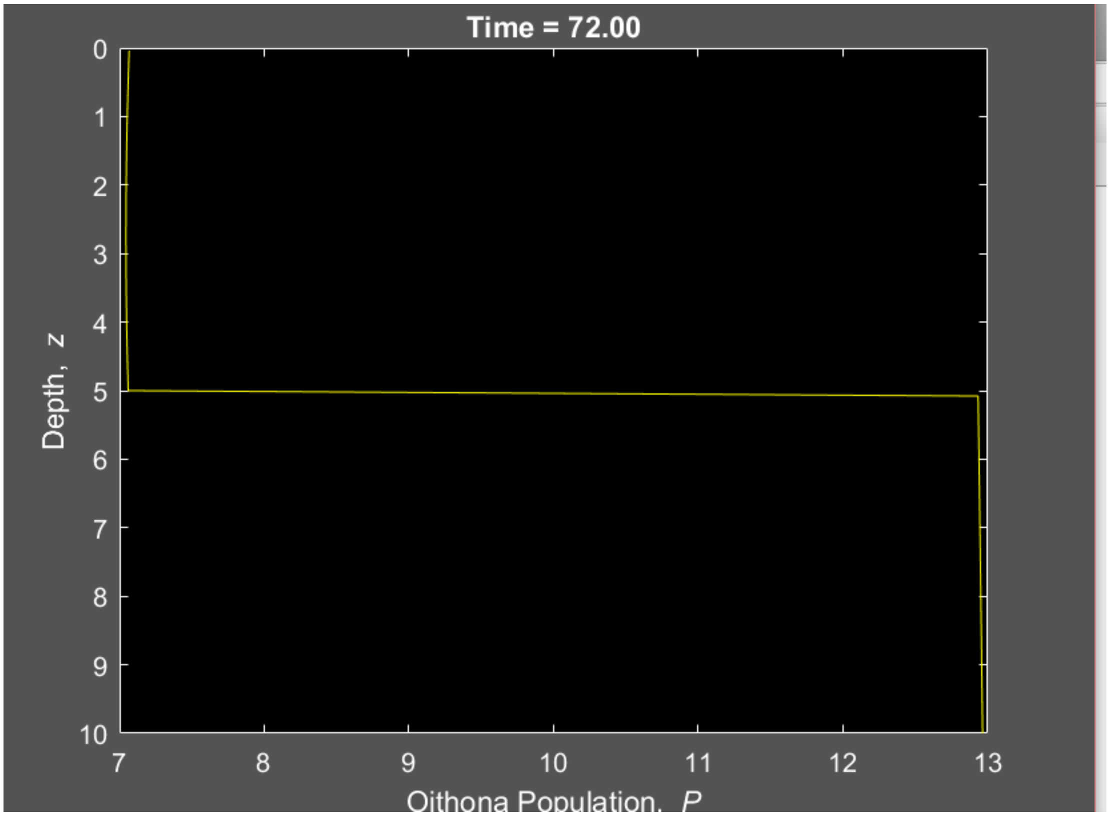

Figure 6. Results of model at time 72 hrs. The depths 0-5 are the surface layer where oxygen levels are 0 and initial population is 0. The depths 5-10 are the mixed layer depths where the oxygen levels are also normal conditions. This depth has a full population of 100 Oithona. After 72 hours, the some of the population has naturally moved up, but not as much as what is seen in fig. 5.

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