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Behavior of *Tergivelum baldwinae,* a benthic acorn worm, in relation to structure and food supply

Abstract:

Over the past 10 to 15 years, there has been a noticeable increase in particulate organic carbon flux reaching the seafloor at Station M (4000 m depth) in the Northeastern Pacific. This increase has been shown to correlate with changing surface conditions. Recently, the deep-sea enteropneust, *Tergivellum baldwinae*, has increased in population density allowing us to observe enough individuals to test hypotheses about their behavior. Enteropneusta are known for their fecal trails, usually found in a spiral, or less frequently a switchback pattern. Here we test whether there are correlations between *T. baldwinae* fecal trail characteristics, food availability, and individual footprint area (a proxy for body size). Time-lapse images taken at Station M were annotated using the open-source software program VARS. VARS allows for the digital annotation of each specimen, tracking of fecal trails, and area measurement of individual body size. We hypothesize that fecal trail size and the type of trail (i.e. spirals versus switchbacks) are positively correlated with an individual size, and food supply is correlated with the type of trail and number of spirals. We found no correlation between the length of path vs footprint area, or between food availability and the type of path. However our results suggest a correlation between the type of path and footprint area, with larger individuals more likely to create a spiral path. Given that the deep sea is the ultimate depository of a large fraction of surface-derived carbon, it's important we understand how the changing carbon cycle might impact deep-sea animals. As a deposit feeder, *T. badwinae* plays an important role within the carbon cycle by

consuming carbon that has settled from the water column to the benthos. Studying *T. baldwinae* allows for a better understanding of how climate change is potentially affecting deep-sea communities.

Introduction:

Once thought to be among pre-established enteropneusta, commonly known as the acorn worms, deep-sea enteropneusta are now within a family of their own that is defined by existing exclusively within the benthos (Priede et al., 2012). *Tergivellum baldwinae* is a benthic hemichordate belonging to the torquaratorid family and are most distinctly known for the patterns they create with their feces (Holland et al., 2012). Found commonly in the Eastern Pacific, *T. baldwinae* (Smith et al., 2005) has been rising in numbers at a time-series site on the California coast (Station M, 4000 m depth), potentially due to carbon influxes i.e. a larger food supply (Whelpley, 2016).

Based on research done at Station M, off the coast of central California, USA there have been changes in sinking particulate food supply influencing deep-sea communities (Smith et al., 2014.). Despite a time lag in which the food supply travels to the benthos, lasting from weeks to months, there have been larger fluxes in particulate organic carbon, POC, content that gives significant food input to the benthic communities (Smith et al., 2014.). Carbon influx has been linked to climate change as there is greater wind stress within coastal upwelling; this leads to an increase in mixing waters and causes greater primary production (Smith et al., 2014.). As a result there have been greater numbers of carbon fluxes at abyssal depths. Food supply is a key driver of community composition at abyssal depths, and due to greater carbon supply there is already a major shift in community composition that has been noticeable at Station M (Kuhnz et al., 2012).

This is particularly noticeable among holothurians, or sea cucumbers, which make up one of the densest megafauna communities in the benthos (Huffard et al., 2016).

It has been theorized that due to large influxes of carbon in the benthic communities, holothurian population has begun to rise considerably. There occurred a shift in population dynamics, where holothurians became the most dominant megafauna at Station M, as opposed to sponges which were most dominant previously (Kuhnz et al., 2012). The population growth seemed to be a result of opportunistic immigration and reproductive activity after carbon influx (Kuhnz et al., 2012; Huffard et al., 2016). With this increase in population and change in reproductive output it is worth questioning how other megafauna, including enteropneusts, might have responded with behavioral change as result of food supply.

Little is known about enteropneust behavior and how their fecal patterns might be linked to food supply, but it has been theorized more nutrient rich areas could result in specific patterns such as the spiral (Priede et al., 2012). It has also been hypothesized that taxon specificity might be more influential in enteropneust behavior than what was previously suspected. Research among different enteropneust species found that *Yoda purpurata* more commonly meandered irregularly as opposed to *T. cinnabarinum* which was more commonly seen beginning a spiral and then moving into a switchback pattern (Priede et al., 2012). This difference among individual species behavior could be a result of *Tergivelum* having more developed muscles, allowing for better steering than less-developed counterparts (Priede et al., 2012). There have also been links to fossil records in the Cretaceous age and younger, with spirals appearing in younger fossils and more switchbacks, or meandering patterns, in older fossils (Jones and Alt, 2013). With enteropneusta presence in the deep sea being suggested since the early Triassic period (Osborn et al., 2012), it is possible that benthic environment at the time may have influenced enteropneust behavior. This leads one to believe that it is likely a combination of both ecology and anatomy that influences enteropneusta behavior.

With this in mind we hypothesize a positive correlation between enteropneust spirals and greater food supply will be found. A positive correlation between smaller enteropneusta and switchbacks and/or meandering will be found, just as a positive correlation between enteropneust area and/or size and ability to make spirals will be found. This reasoning is based on previous path observations of *Tergivelum* creating more spirals, as opposed to *Yoda purpurata* which was found more commonly creating switchbacks. This was found in a study by Priede, et al. (2012) in which they hypothesized that *Tergivelum* created more spirals due to their muscular development being stronger, as opposed to *Y. purpurata.* Therefore, logically, it would make sense for a spiral to be created by a larger individual than a smaller one

Methods:

Tergivelum baldwinae images were found through observing images taken on the benthos with a tripod camera at Station M, off the coast of central California, U.S.A. This tripod camera captures hourly images of the benthos floor, allowing the researcher to observe what they can see. *Tergivelum baldwinae* behavior was observed using VARS for Images system through the Monterey Bay Aquarium Research Institute. This image annotation system allows the user to measure distance, area, and annotate images used for research. In order to maintain a greater amount of accuracy, the bottom half of each image was observed. This was due to the tripod camera's light not reaching as far as the top half, and the fact that each individual is quite small, so observing from further back is more difficult and the size, as well as the path may not be as accurate. This was also due in part to the fact that VARs for Images does not yet have a zoom feature, therefore to maintain greater accuracy only the bottom half of the image was used. Each image with an enteropneust was annotated at the point of first and last sighting, the spiral, switchback, or mixed path was tracked, and the footprint of the enteropneust was measured. The footprint was measured once for an image in the beginning of the enteropneust's spiral, one halfway through the path's creation, and one at the end of the path. In total, data from images taken between 2011 and 2016 were collected. After all images were annotated, and each enteropneust was identified, I noted the date each had arrived and left, as well as which path they had created: spiral, switchback, or mixed. If it was a mixed path, I noted type of path at the start and end. Throughout data analysis it was treated as the path it started with, as this was the point in time for which food was measured. Therefore, there was continuity between the two. For example, a path may have started as a spiral, but ended with a switchback, this path would then be counted as a spiral because for the majority of the path it was.

Relative change in the amount of food available to the benthic community was estimated separately by measuring the percent cover of detrital aggregates on the sea floor. These data allow for an understanding of the amount of carbon, or food supply, available to the enteropneust at the time. I compared percent cover at the time of arrival of the enteropneust to fecal trail characteristics.

Once annotatedall data I collected in VARS was extracted into a txt file using VARs Query for Images, and then converted to a Microsoft Excel spreadsheet. The measurements were sorted by individual, as this factor was also annotated in VARS. Length of path was added together for each individual, and the areas were averaged per individual, once again, this was all performed in Microsoft Excel. Continuing with the same program, a linear regression against the length of path and the average area of the individual was performed. Then, using the program R Studio, two logistic regressions were performed: the amount of food available and likelihood to create a spiral or switchback, and the average area of the individual against the likelihood to create a spiral or a switchback.

Results:

The type of path and the average area of the individual suggested a slight correlation, with a P value of 0.01724 and a sample size of 109 (Figure 1). The type of path did not correlate with the amount of food on the sea floor in terms of detrital aggregate coverage, as the logistic regression displayed a P value of 0.2855 with a sample size of 121 (Figure 2). Lastly the length of path did not correlate with the area of the individual. This is based on an R^2 value of 0.1748 with a sample size of 100. The linear regression did show a slight upward trend due to outliers (Figure 3).

Discussion:

As the R^2 value, 0.1748, for length of path vs. area of individual is closer to the value of 0, than it is to 1, the value is not strong, leading to the conclusion that area of the individual does not have a significant impact on the length of the path that is created. This could mean there is no significance on this factor at all.

As for type of path and amount of food available, the P value of 0.2855 is too large to be of significance in determining their behavior. This leads to the conclusion that the amount of food available at time of arrival of *T. baldwinae* does not determine the type of path the individual creates. This refutes the original hypotheses that a larger amount of nutrients, or more food, would more likely lead to a spiral. As there wasn't always data on the date of arrival for the amount of food available, the nearest previous date was used as it is possible food still may have been there upon arrival. There could also be a possibility that as the individual creates the path there may be more food available later on, than in the beginning of the path created, and as I was using the data on the date of arrival this was not accounted for. However, from the data collected in this study, it seems that the amount of food available does not have an influence on *T. baldwinae's* behavior.

The final test of average area of individual in relation to type of path resulted in a P value of 0.01724. This result is suggestive that the average area of individual does seem to predict in part the type of path the indicated individual will create. This lines up with the original hypothesis that body size can determine behavior. As mentioned previously, this could be due to the fact that larger individuals would have more developed muscles, and might have a different level of control over their movements, suggesting they are more likely to create a spiral path.

While two of the hypotheses were not supported by this study, there is still now a greater understanding of *Tergivelum balwinae* and its behavior. As not much was previously known about the predictability of a spiral vs switchback, we can be led closer to the idea that the size of the individual predicts what type of path it creates. It is also safe to assume that other factors may have an impact, perhaps there was an evolutionary reason to why these paths are created that we are not able to understand as there is no longer evidence for this. Or another type of nutrient could have a role in it that we don't have the means to measure right now. Either way, this study has now allowed for a greater understanding of *T. baldwinae* and its behavior.

Broader Impacts:

Through studying *T. baldwinae* there will exist a broader understanding of deep-sea creatures and how they may be affected by climate change. This topic relates to climate change as food input in the deep-sea is affected by warming waters. In coastal upwelling regions more carbon is being sent to the deep-sea and therefore there is a larger food supply. By noticing behavioral changes as a result of food supply, there is a greater understanding of how creatures in the most remote and unknown parts of the world are impacted by warming waters as a result of anthropogenic and natural influences.

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Figures:

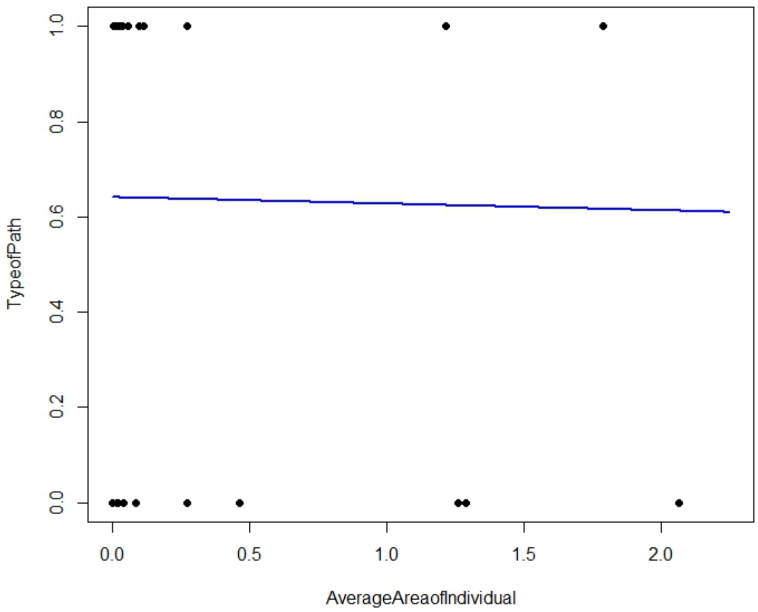

Figure 1: *Logistic regression of type of path and average area of individual. Sample size = 109, P-Value = 0.01724. In this figure, 0 would result in a spiral while 1 would result in a switchback.*

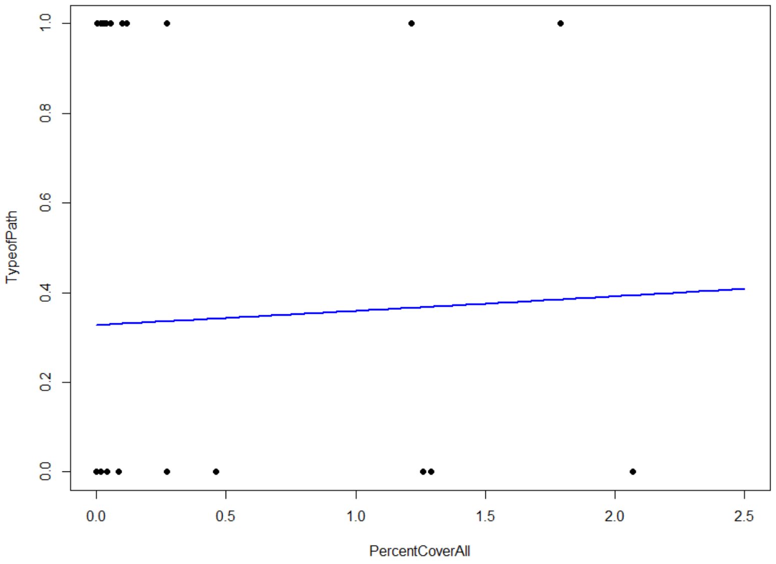

Figure 2: *Logistic regression of type of path and food available. Sample size = 121, P-Value = 0.2855. In this figure, 0 would result in a spiral while 1 would result in a switchback.*

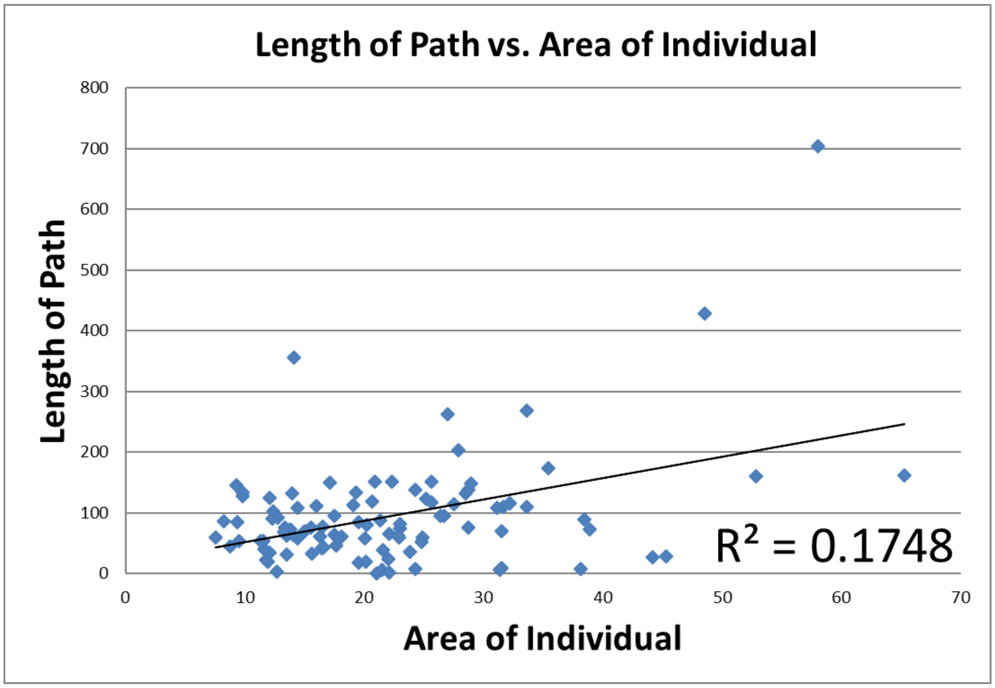

Figure 3: *Linear regression of length of path vs. area of individual*