



Population density of *Tergivelum baldwinae* correlated to particulate organic carbon flux at Station M

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ABSTRACT (Body text, Times New Roman, 12 pt, bold)

Here, we report on the findings of a nine-year study (2006-2007, 2009-2014) of the population density of *Tergivelum baldwinae*, a deep-sea enteropneust. A time-lapse camera was deployed at Station M, an abyssal long-term monitoring site in the NE Pacific (~4000 m depth), which photographed the same field of view at hourly intervals. A total of 37,067 images were reviewed and 44 individuals were observed. There were no *T. baldwinae* observed prior to late 2011 and the peak population density year was found to be 2014, with 30 individuals observed. This study shows an increase in observed feeding time (average = 63.5; min = 6; max = 357 hours) and reports on observations of multiple enteropneust feeding at the same time. Additionally, the findings from this current study show a significant correlation between food supply to the abyssal community and the population density of *Tergivelum baldwinae*. We found that the population density of *T. baldwinae* takes approximately four weeks to respond positively to a large input of POC to the seafloor.

INTRODUCTION

Abyssal depths cover 75% of the seafloor and are a key, but poorly understood, part of the global carbon cycle. Abyssal environments are considered relatively stable due to low temperatures and lack of nutrients. Particulate organic carbon (POC) from surface waters is the primary food available to organisms that inhabit this environment. With increasing average sea-surface temperatures that are primarily due to anthropogenic influence, it has been predicted that there will be a significant impact on the ocean surface community, that will in turn influence the deep-sea environment by altering the food input (Behrenfeld *et al.*, 2006; Ruhl & Smith, 2004; Smith *et al.*, 2008, 2009, 2013; Cias *et al.*, 2013; Kuhnz *et al.*, 2014; Howes *et al.*, 2015). Monitoring the pelagic-benthic relationship is thus essential to begin to understand the impacts of these influences.

Epibenthic megafauna are key deposit feeders in the deep-sea abyssal community. At the long-term abyssal monitoring site, Station M, Smith *et al.* (2014) described a large salp bloom in the spring of 2012, which resulted in a major POC flux. The sediment biological community responded immediately to the new influx of food source, which was shown by increased bacterial respiration rates. Additionally, there was a 7-fold increase in the population density of the dominant epibenthic megafauna, *Peniagone* sp. A.

Work by Kuhnz *et al.* (2014) at Station M, further described how the benthic community was influenced by this increased food supply. From 2006 to 2009 suspension feeders, such as sponges and tunicates, primarily dominated the environment. However, in 2011, the same time as the major POC flux event, they found a drop in suspension feeders and a shift to substrate deposit feeders, like holothurians.

The phylum Hemichordata is divided into two classes: Enteropneusta (acorn worms) and Pterobranchia. Hemichordata, together with their sister taxon Echinodermata, form the base of the deuterostome clade, which contains chordates (Turbeville *et al.*, 1994; Swalla and Smith, 2008). Due to their developmental similarities that they share with chordates [dorsal strand of nerve cells, stomochord, bilateral symmetry and gill slits (Lowe *et al.*, 2004)], acorn

worms have long been the focus for morphological and molecular studies in order to investigate the relationship between invertebrates and vertebrates as well as the evolutionary origins of chordates (Arendt *et al.*, 2008; Tagawa *et al.*, 2001; Anderson *et al.*, 2011). There are approximately 100 described coastal acorn worm species and prior to 2005 there were only three known deep-sea species. Since the description of the family Torquaratoridae in 2005, the grouping has become well established via molecular and morphological analysis, with at least eleven described species found worldwide and approximately fourteen undescribed species currently known (Osborn *et al.*, 2013).

Unlike the enteropneusts that inhabit the shallow water communities, torquaratorids are found at an approximate depth range of 1.6-8.8 km and live epibenthically on the deep-seafloor (Priede *et al.*, 2012). They have also been observed moving through the water column between feeding sites (Smith Jr. *et al.*, 2005, Osborn *et al.*, 2011, Priede *et al.*, 2012). Torquaratorids are found on a wide range of habitats ranging from the abyssal plains, where sediment is plentiful, to lava pillows where sediment cover is extremely thin. This family possesses the same three-part body plan as their coastal relatives (proboscis, collar and trunk), although it is primarily gelatinous, sometimes with a highly modified collar and anterior trunk. Unlike members of the three other acorn worm families, torquaratorids are relatively easy to identify based on gross morphology (confirmed with histology and genetics), and are thus identifiable in digital images and video.

In 2005, Smith *et al.* observed an undescribed enteropneust, (later described as *Tergivelum baldwinae* by Holland *et al.*, 2005), arriving at a feeding location at Station M where it fed in a spiral pattern for a period of approximately 36 hours. It then moved to the center where the animal appeared to rest prior to evacuating the remainder of its gut contents and moving into the water column. Prior to this observation, deep-sea enteropneust fecal trails had been identified in all of the ocean basins (Bourne and Heezen, 1965; Heezen and Hollister, 1971; Lemche *et al.*, 1976) and occasionally an enteropneust was present (Smith *et al.*, 2005). The observation that they are capable of swimming is important because it showed

that these animals, which we only recently began to notice in the deep-sea, have a greater range of mobility than previously thought. At this point in time it is unknown how and why they choose their feeding location. However, it is possible that the ability to move in the water column allows them to target areas of high food availability to maximize their feeding efforts.

Enteropneusts are a potentially important component of nutrient cycling in deep-sea benthic environments, however, very little is known regarding their distribution and abundance in this environment (Smith *et al.*, 2005; Anderson *et al.*, 2011). Building on our current knowledge of enteropneust ecology and deep-sea benthic environments, this study aims to fill in key data regarding the population density of *Tergivelem baldwinae* and its potential correlation to food availability over a nine-year period at the long-term monitoring site, Station M (34° 50'N, 123° 00'W; 4000 meters depth).

MATERIALS AND METHODS

STUDY SITE AND TIME-LAPSE IMAGE

Station M is located 220 km west of the central California coast (34° 50'N, 123° 00'W) at an average depth of approximately 4000 m below sea level (Figure 1A). This site has been monitored continuously since 1989 using a wide variety of tools developed by the Pelagic-Benthic Coupling Lab in order to study how particulate organic matter from the surface influences the deep-sea.

Images of the seafloor at Station M were taken using a time-lapse camera tripod (Figure 1B). The tripod camera, seen in Figure #, was developed as part of a long-term monitoring study at Station M and has been deployed since 1989 (Smith *et al.*, 2013). The camera photographs a 20 m² area of the seafloor at one-hour intervals.

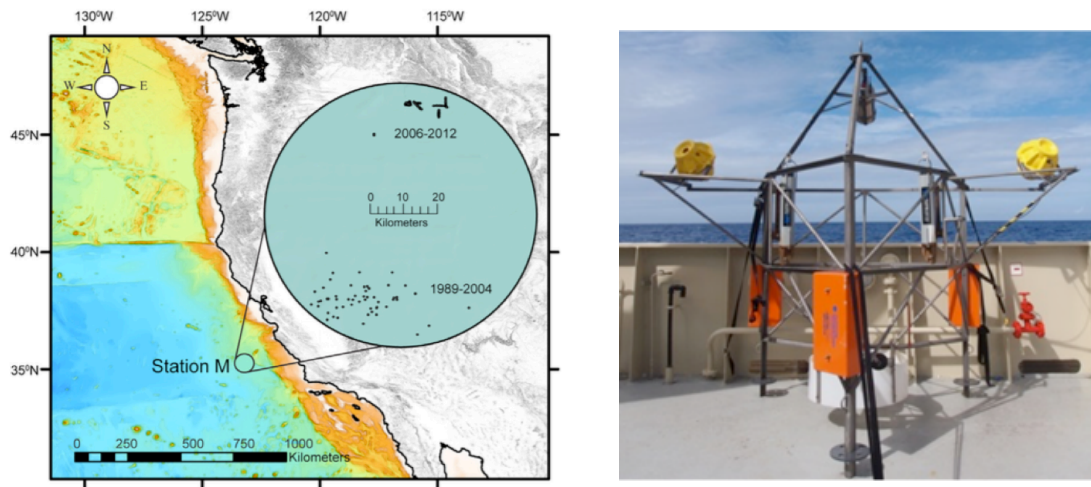


Figure 1A & 1B. (1A) Map showing location of Station M in the northeast Pacific (Kuhnz, *et al.*, 2014) and (1B) image of time-lapse camera prior to deployment (MBARI).

IMAGE ANALYSIS

Nine camera deployments from 2006-2007, 2009-2014 were analyzed for the presence of the enteropneusts *T. baldwinae* at Station M (Table 1). Image annotations were made using the Video Annotation and Reference System (VARS) image annotation software. Each animal was annotated in a similar manner by placing a marker on the center of the animal's proboscis. An example of *T. baldwinae* pre- and post- annotation can be found in Figure 2A and 2B. No images were analyzed from deployments 53, 54 and 61 because the time-lapse camera was not deployed, or the images have not been converted to digital format. The total feeding period was calculated from the timestamp on the images (1 image = 1 hour). During image review, annotations were made regarding information that would serve to enhance the natural history of *T. baldwinae*.

Table 1. Time-lapse camera image details

Deployment	Year	Images
	2006-	
52	2007	2500
55	2009	3585
56	2010	4217
57	2010	4721
58	2011	4273
59	2011	4321
60	2012	3718
62	2013	5203
63	2014	4529
Total Images		37067



Figure 2A and 2B. Example of *Tergivelem baldwinae* at Station M prior to annotation (A) and post annotation (B).

CORRELATION OF PARTICULATE ORGANIC CARBON FLUX

The POC was collected using two sediment traps suspended 600 m and 50 m in the water column above the time-lapse camera tripod. The sediment was collected over a 10-day sampling period. The average weekly POC flux was calculated from the end of 2005-2014. Spearman's Rho Correlation was used to measure the strength of association between the POC flux and the population density of *T.*

baldwinae. A time lag was applied in progressive weekly intervals to determine if there is a significant correlation between when the POC reaches the seafloor and the population density.

RESULTS

POPULATION DENSITY

A total of 37,067 images from the tripod camera were analyzed to determine the population density of *T. baldwinae* at Station M. Over the nine-year period a total of 44 individuals were observed. The population density is depicted in Figure 3. The population density and feeding rates of the observed individuals are displayed in Table 2. In the four deployments analyzed prior to November 2011 no *T. baldwinae* or fecal trails were observed in the 20 m² area. For all deployments there were no fecal traces observed without an animal present. Except for deployment 60 (n=0) in 2012, from 2011 on (deployments 58, 59, 62 and 63) *T. baldwinae* was present in all deployments. The highest concentration of *T. baldwinae* was during deployment 63 where a total of 30 individuals were observed.

There were nine instances where two individuals of *T. baldwinae* were feeding in the camera frame at the same period of time. There was one instance (Deployment 63: #26, #27 and #28) where three individuals were feeding in the camera frame at the same time. These instances occurred after the population density began to rise during the 2013 and 2014 tripod camera deployments.

FEEDING

The average time feeding for the 44 observed individuals was 63.5 hours. The least amount of time feeding (that the camera was able to capture) was 6 hours (Deployment 63, #11). The maximum time feeding was 357 hours (Deployment 62, #213). The total feeding time for the 44 individuals was 2,796 hours. From the total images analyzed, *T. baldwinae* was present and feeding 0.75% of the time.

Table 2. Population density and calculated feeding times of the 44 *Tergivelum baldwinae* at Staion M during the nine-year observed period. ^a through ^j each designate a single feeding event with multiple individuals.

Deployment	Count	Reference Number	Number of Total Hours Feeding	Deployment	Count	Reference Number	Number of Total Hours Feeding
52	0	-	-	63	30	1	21
55	0	-	-			2	21
56	0	-	-			3 ^c	49
57	0	-	-			4 ^c	23
						5	32
						6	46
						7	56
						8 ^d	17
						9 ^d	53
						10	31
						11	6
58	1		111			12 ^e	27
59	6	1	27			13 ^e	27
		2	50			14	35
		3	81			15	28
		4	70			16	83
		5	60			17 ^f	85
		6	156			18 ^f	23
60	0	-	-			19 ^g	68
62	7	211	119			20 ^g	38
		212	27			21	39
		213	357			22	39
		214 ^a	137			23 ^h	151
		215 ^a	35			24 ^h	8
		216 ^b	257			25	65
		217 ^b	24			26 ⁱ	26
						27 ⁱ	22
						28 ⁱ	113
						29 ^j	22
						30 ^j	31

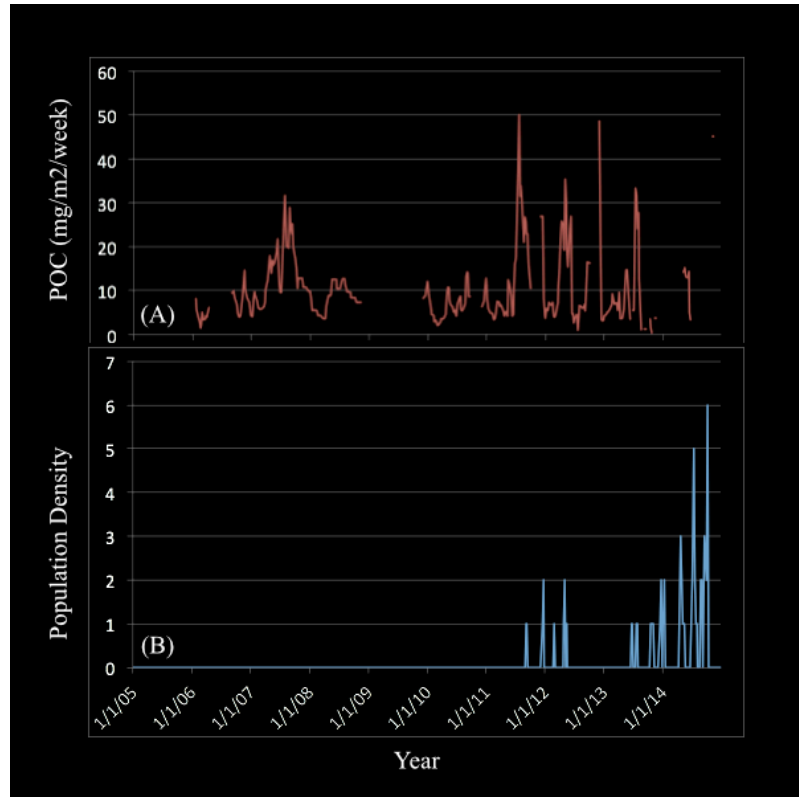


Figure 3. Time series measurements at Station M. The of weekly averages of Particulate Organic Carbon (A) measured 2005-2014 and the *Tergivelum baldwinae* population density (B) measured using the time-lapse camera during the nine deployments.

PARTICULATE ORGANIC CARBON FLUX

The POC flux was measured from the end of 2005-2014 (Figure #). For the following periods the sediment traps were not deployed, or there were gaps in the data due to mechanical error: March 2006 through the beginning of August 2006, October 2008 through the middle of November 2009, August 2010 through November 2010, September 2011 through the middle of November 2011, September 2011 through the beginning of November 2012, the end of October 2013 through April 2014. The gap from May 2014 through December 2014 is due to a major clogging event. Peaks in POC flux events ($> 30 \text{ mg/m}^2/\text{week}$) were measured June through July 2007, June 2011 through August 2011, April through July 2012, November 2012 and June 2013.

Correlations between food supply (POC flux) and *T. baldwinae* population density are listed in Table 3. The POC flux and population density had the most

significant correlation at lag week 4 (P-value = 0.0115; $\rho = 0.142$). Additionally, lag week 1 (P-value = 0.0413; $\rho = 0.015$), week 2 (P-value = 0.0247; $\rho = 0.126$) and week 5 (P-value = 0.0325; $\rho = 0.120$) were significant.

Table 3. Spearman rank correlations between POC flux (lagged 0 to 10 weeks) and *Tergivelum baldwinae* population density. Asteriks denotes significant P-value (> 0.5)

POC Average/Week	Spearman ρ	P-Value
Week 0	0.098	0.084
Lag 1*	0.015	0.041
Lag 2*	0.126	0.025
Lag 3	0.109	0.054
Lag 4*	0.142	0.012
Lag 5*	0.120	0.033
Lag 6	0.063	0.266
Lag 7	0.045	0.426
Lag 8	0.049	0.384
Lag 9	0.045	0.426
Lag 10	0.005	0.933

DISCUSSION

The 26-year monitoring of Station M provides the longest and most detailed look at a deep-sea abyssal community. This time series data is essential to help us answer questions regarding how the deep-sea community responds to periodic fluctuation of food input from surface waters. Previous work has shown that food supply is an important determinant on organismal abundance, diversity and the structure of the food web in the deep-sea ecosystem (Smith, 1987; Wigham *et al.*, 2003a; Robison *et al.*, 2005; Smith *et al.*, 2009; Lampitt *et al.*, 2010; Tecchio *et al.*, 2013; Kuhnz *et al.*, 2014). The findings from this current study show a significant correlation between food supply to the abyssal community and the population density of *Tergivelum baldwinae*. We found that the population of *T. baldwinae* takes approximately four weeks to respond positively to a large input of POC to the seafloor. Further work into the life-cycle and behavior of *T.*

baldwinae would need to be executed in order to understand what biological factors are at play that could potentially influence this four-week lagtime response. Extending the correlation analysis for a longer period of time would additionally enhance our understanding of this correlation.

Primary issues encountered with this study are due to the nature of deep-sea research. The number of individuals counted from the nine deployments could potentially be a higher value than the number of individuals actually present. When an animal left the camera's field of view, there was no way to ensure that same individual didn't return to the area to feed at a later point. While a total of 44 individuals were counted from the time-lapse images a maximum number of three were seen feeding in the camera frame at the same time.

Additional problems surround the gaps in the time-series particulate organic carbon data. There possibly were periods of high POC flux during these gaps and further statistical analysis and referencing surface data collected from satellite images could help to resolve the missing data.

In this study we observed an increase in the number of hours feeding for each individual when compared to the Smith *et al.* (2005) enteropneust feeding time observations. In the initial study, the observed enteropneust fed in a spiral pattern for a period of ~36 hours. By following up on the previous work from this study, we found that these animals can feed for a much longer period than previously thought, with the maximum feeding time found to be almost ten-fold the initial observation. Despite having a lower population density compared to other epibenthic megafauna found in abyssal environments, these organisms spend a long time feeding and mixing sediment in one area. Further work needs to focus on the calculation of their biomass and their rate of bioturbation and in order to determine their role in the carbon cycle process.

CONCLUSIONS/RECOMMENDATIONS

This study has demonstrated a significant correlation between an increase in population density for *T. baldwinae* due to the POC flux. While the population of this species is still low compared to other epibenthic megafauna, this study reinforces previous findings that a rare species can become more abundant over

time (Smith *et al.*, 2005). Additionally, we observed that some individuals are capable of feeding for a much longer time period than previously known so their importance in the functioning of the benthic ecosystem cannot be ignored. Long-term observations will help us to begin to understand and calculate their influence in this ecosystem and furthermore, their role in the global carbon cycle.

Building on this work we will be able to answer a multitude of questions regarding feeding behavior. We will calculate the frequency of a particular feeding pattern (spiral or switchback) and the frequency that their feeding pattern changes. We will determine if there is a correlation between their movement to/from a feeding location and the current pattern. We will calculate trace creation degradation rates and analyze feeding sites to determine if there is any pre- or post- conditioning to the site from other organisms. Additionally, we can use VARS to calculate the size, and therefore, the biomass of these animals. This biomass calculation can potentially be used in a global carbon cycle model to further aid in our understanding of the abyssal depth environment.

Collection and analysis of sediment pre-, during and post- processing could help to elucidate what attracts *T. baldwinae* to a particular feeding site and what nutrients they are extracting from their food.

Finally, the relationship between *T. baldwinae* and other animals found in deep-sea benthic abyssal communities needs to be further explored. During image analysis, interactions between holothurians and *T. baldwinae* were observed which caused some of the enteropneusts to relocate to a new feeding site. This indicates a possibility for food competition between different species and further image analysis needs to be executed to pursue this possibility.

An undescribed, possibly symbiotic polynoid, was observed on three individuals. We plan to collect and describe this animal in Fall 2015 or Spring 2016 so work from this study will help to fill-out ecological questions regarding this relationship.

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