Investigation of Holothurian Abundances at Station M

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*Summer 2011*

ABSTRACT

The PULSE project began at Station M in 1989. This 22 year long time series has provided data on how organic matter affected the epibenthic megafauna; especially echinoderms. In May 2011, a population bloom of the echinoderm taxa, Holothuroidea, was observed at Station M. The holothurians, an *Amperima-Peniagone Complex*, had an increase in abundance. This is very similar to the PAP site between 1996 and 2002, when the A. rosea increased in abundance by three orders of magnitude (Billett et al., 2010). The goal of the investigation was to determine if this perceived increase was statistically significant and further, to understand the significance of this apparent increase in holothurians in terms of the community and food supply. To provide a probable answer to the investigation, transect videos from dives D230-D232, dissection of ten individuals, and statistical analysis of the data collected, were used in the study. A total of 2,773 animals were counted in the annotations recorded. For two species, *Amperima-Peniagone Complex* and *P. sp. A*, there were more animals than ever recorded previously. This is also true for *S. globosa*, but for *P. sp. B* and *sp. C*, there are slightly fewer animals than the previous highest recorded number. This means that if there were actually a response in the population to some influx of food, only three out of the five observed species reacted strongly to the environmental stimulus. Results from statistical analysis indicated
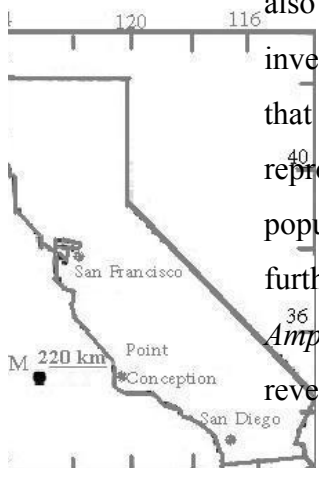
that *Amperima-Peniagone Complex* for D230 and D231 showed clumped distributions, also that *P. sp. A* showed clumped distributions for D230. Dissections performed to investigate the possibility that the aggregations were for reproductive purposes, showed that gametes were present in some of the individuals, indicating possible intention of reproducing again. This study has established that there was an increase in the populations of some holothurians. There was an increased food supply not long ago, but further investigation is required to see if the environment can support the new numbers of *Amperima-Peniagone Complex, P. sp. A,* and *S. globosa*; especially since the dissections revealed new gametes forming in over half the samples.

INTRODUCTION

In June of 1989, the PULSE project at Station M began. Station M is located 220 km off the coast of Point Conception, California, in the North East Pacific (Figure 1). Originally it was a time series project, monitoring and measuring particulate organic matter as it reaches the ocean floor (Smith and Druffel, 1998). The project soon expanded to study how the organic matter affected the epibenthic megafauna; especially echinoderms, since it was suspected that blooms in the population abundances correlated with the appearance of detrital aggregates on the sea floor. Unfortunately further study did not reveal a correlation (Lauerman and Kaufman, 1998). Although the relationship between populations and the organic matter was inconsistent, echinoderms were still found to be vital to the break down and redistribution of particulate organic matter (Kaufman and Smith, 1997).

One of the echinoderm taxa of interest are the Holothuroidea, or sea cucumbers. It has been recognized that the fluctuation of holothurian and other animal abundances are important to the abyssal benthic community (Ruhl, 2007). The holothurians have quite a history of abundance fluctuation. At Station M, *Elipidia minutissima* and *Peniagone vitrea* had steady or increasing populations starting in 1989, but then suddenly they decreased in 1999. On the other hand, species such as *Peniagone diaphana*, and

Scotoplanes globosa, had smaller populations and then suddenly spiked in 2001 (Ruhl and Smith, 2004). For some of the rises in abundance, it was suggested that the population was having an opportunistic response to ecological cues. Such was believed for *Amperima rosea*, not at Station M, but at a similar deep-sea location in the Atlantic Ocean, called the Porcupine Abyssal Plain (Figure 2; PAP), a few hundred kilometers off the coast of Ireland (Smith et al., 2009). No clear seasonal breeding was found when observed, so it was proposed that *A. rosea* withheld producing gametes until the proper conditions were available (Ruhl, 2007).

Between 1996 and 2002, *A. rosea* at the PAP site increased in abundance by over three orders of magnitude (Billett et al., 2010). Much of the rest of the community's populations also grew, but not to the degree that *A. rosea* did. It is thought that the changes were environmental factors, such as an increased amount of organic matter reaching the sea floor. The community took advantage of the more abundant food to reproduce (Billett et al., 2010).

Very similar to this event was a population bloom recently observed at Station M. The holothurians, a species likely to be *Amperima* or *Peniagone* (I refer to them here as *Amperima-Peniagone Complex*, until a verification can be obtained), increased in abundance. During the last transect that Ken Smith and his team videotaped in May 2011 with the Monterey Bay Aquarium Research Institute's (MBARI) *Doc Ricketts* remotely operated vehicle (ROV), they observed the largest *Amperima-Peniagone Complex* population thus far. When the videos were analyzed, it was discovered that it was not just one species but three. Despite being multiple species, the numbers of this complex were still significant enough to continue investigation into the suddenly increased abundances. The goal of the investigation was first to determine how many individuals were observed: which would provide a confirmation that there were more observed than in previous visits. Second was to understand why there were so many holothurians. Subjects of interest are: whether the animals were aggregated or randomly distributed, if the sudden increase in abundance was for reproduction, and whether this was an opportunistic response to a flux of organic matter such as in the Amperima Event (Wigham et al.,

2003). Finally it would be useful to determine the identification of the three species observed.

Transect videos from the dives D230-D232, dissection of ten individuals, and statistical analysis of the data collected, were used to provide a probable answer to each of the questions.
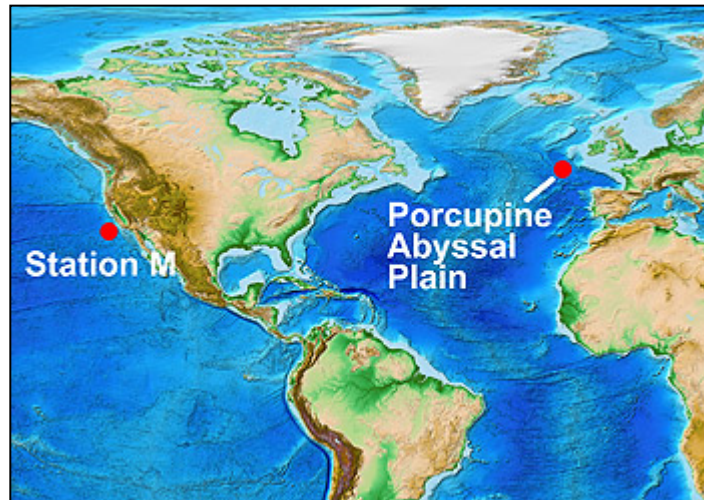

Figure 2: Location of the Porcupine Abyssal Plain in relation to Station M.
(Smith et al., 2009)

METHODS

VIDEO ANNOTATIONS

Video observations for abundances, measurements, and habitat observation were taken from transect tapes, recorded during Ken Smith's cruise at Station M on May 24-26, 2011. The tape numbers were Doc Ricketts dives 230- 7 hr. 231- 5 hr., and 232- 4hr. Transects were 4000 meters deep and covered approximately 4700 m^2. The videos were analyzed with MBARI's Video Annotation and Reference System (VARS).

When the videos were first reviewed, all of the holothurians were assumed to be one species. Some of the smaller individuals had morphological differences from the larger population of the holothurians of interest. I originally marked them with the comment "juv, or different species?" As soon as larger individuals with the same morphological

differences were observed, the frame grabs taken were compared to see if the different individuals were in fact different species (Figure 3). It was then I noticed there were different numbers of dorsal paripodia on the individuals; the body shapes and colors were also different. These were three distinct species, not just one. What the exact names are was unknown, so for each video, the holothurians were labeled as one of three different categories: The *Amperima-Peniagone Complex*, *Peniagone sp. A*, and *Peniagone sp. B* (Table 1). Later we recognized some of the *Peniagone sp. B* were actually a fourth species, and were therefore labeled as *Peniagone sp. C*.

Table 1: The morphological differences between the four species found on the videos are very clear and easy to see without needing a camera close up on the specimens.

| Title	Fused Dorsal Paripodia	Body Shape	Color	Feeding Tentacles
Amperima-Peniagone complex	Two short paripodia on the anterior end, where the body bends at a 90 degree angle	Turgid, tubular body, short "foot like" appendages elevating the body slightly	Orange/pink, partially transparent	Robust tentacles
Peniagone species A	Two longer and two short paripodia on the anterior end, where the body beds at a 45 degree angle	Flaccid, oval body, larger "foot like" appendages leaving body resting on the sea floor	Pink, transparent	Robust tentacles
Peniagone species B	Short, paripodia on the anterior end	Arched, tubular body, a few "foot like" appendages on posterior end	White/clear, transparent	Small, hard to see tentacles
Peniagone species C	Many slender paripodia around the outside circumference of the anterior end	Flaccid, oval body, barely visible "foot like" appendages	White, partially transparent, purple tinge in the center of the body	Several widely fanned out tentacles, dark-tipped
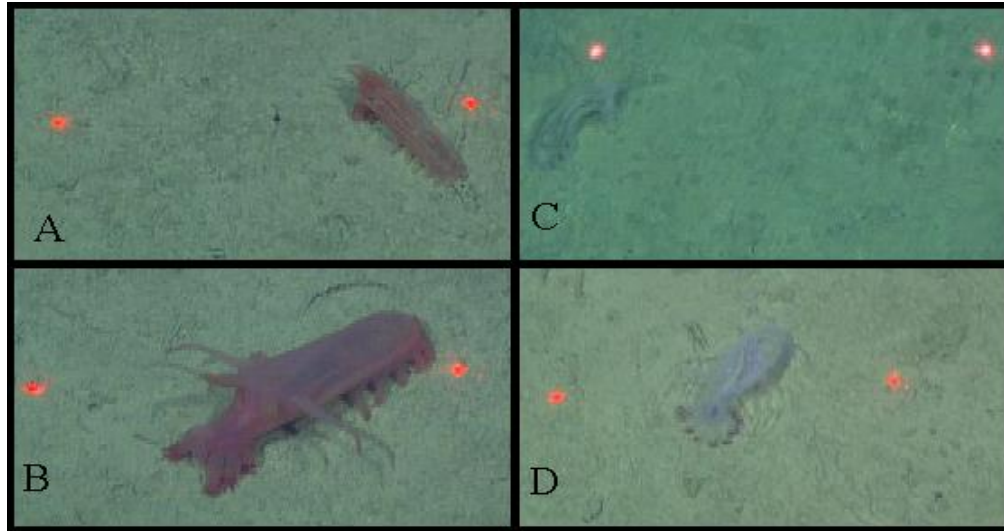
Figure 3: (A) *Amperima- Peniagone Complex*. (B) *Peniagone sp. A*. (C) *Peniagone sp. B*. (D) *Peniagone sp. C*. Red dots are 30 cm apart.

As each video was viewed in VARS, every individual of these four holothurian species was annotated in the system to record abundances. *Scotoplanes globosa* was also annotated (named and marked with a time code), for abundance purposes only. Frame grabs, were taken of animals I later measured.

Later in this study the videos were once again reviewed; this time looking for any habitat differences from one dive to another. All tapes were viewed in order, with random time code selections being watched and the habitat observed. Special notice was taken of habitat surrounding each group of holothurians, to see if there was something present near them that varied from the rest of the tapes.

In order to compare the abundances recorded during the last cruise in May 2011, to previously observed abundances, Henry Ruhl contributed his data on historical numbers of holothurians at Station M. His data, as mean number per square meter, was converted to the mean number per 10 m^2, and compared to the mean found for the most recent dives 230- 232. Due to previous dives having been recorded in standard resolution, it is likely that on older tapes, *Amperima- Peniagone Complex*, and *Peniagone sp. A* would have been indistinguishable from each other and therefore, in the past, counted as one species- *Peniagone vitrea*. The same would have been true for *Peniagone sp. B* and *C*, except these would most likely have been considered *Peniagone diaphana*. In order to compare

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my abundances to past abundances, I combined the means of each pair of holothurians and then compared the combined numbers to Ruhl's means for *P. vitrea* and the *P. diaphana*. *Scotoplanes* abundances were compared to the previous mean calculated for *Scotoplanes globosa.*

MEASUREMENTS

Frame grabs of each individual holothurian of interest were divided into the proper species group, and then measured using Photoshop. Because every frame grab has two red lasers that are always 30 cm apart, Photoshop can be calibrated for each separate picture.

Calibration is accomplished by using a software ruler tool, drawing a line from one laser to the other, and specifying, in the slots available, that the number of pixels selected was equal to 30 cm. The ruler tool was again used, starting at the feeding tentacles of the holothurian and drawing a parallel line along the body and ending, not on, but parallel to the posterior end. The computer then takes the amount of pixels selected and calculates the length of the holothurian, compared to the distance between the lasers.

DISSECTIONS

On the same cruise, ten holothurian specimens were collected. Two were preserved in 70% ethanol, one was preserved in 5% buffered formalin, and the other seven were preserved in 10% buffered formalin. Before the dissections were performed, I noted preliminary morphological differences.

The purpose of the dissections was to examine morphological differences, locate the gonads, look for presence of gametes, and to take samples for DNA and ossicle analysis. The first five dissections started with measuring the specimens the same way as the animals in transects. The distance from the fused dorsal to the first podia were also measured. Then the podia and the fused dorsal podia were counted and then the turgor and inflection was noted. Small tissue samples were taken from the dorsal side of the individual and an end of one of the podia for DNA and ossicle studies. The gonophore

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was located, scissors were used to dissect the body. Using the gonophore and the intestine as a reference, the gonads were located and it was noted whether or not gametes were visible. For several of the samples, the gonads were easily visible through the skin, so that was also recorded. The last five samples were determined to be the same as one of the previously dissected samples; so only the length and the presence of visible gonads were recorded.

MICROSCOPE WORK

After dissection samples A-E were observed more closely under a microscope. Close up pictures of the feeding tentacles, ovaries, and eggs were taken of samples A, B, C, and E. The eggs were measured to the nearest millimeter. Sample D had no visible feeding tentacles, ovaries, or eggs.

STATISTICS

In order to run the necessary statistical test to look for aggregations, transects were divided into 10 m^2 bins (sections), and the animals within the bins counted. The tests were run on the amount of animals within each bin, or section, and the results would reveal if aggregations were present. To look for distribution patterns, I calculated the distance between larger bins. The mean and variance for each species were calculated by dive.

In order to determine if aggregations were present, a Coefficient of Dispersion (CD), (the variance divided by the mean) was calculated for each of the five species on each dive. For this test a CD>>1 indicates a clumped distribution, a CD<<1 indicates a uniform distribution, and a CD of approximately 1 indicates a random distribution. To verify the results of the Coefficient of Dispersion, a G-test (maximum probability) was used to statistically test the number of individuals observed versus the number of expected individuals on the transects for each dive. For this test, a result with a number that is $p < 0.05$ is statistically significant. The Coefficient of Dispersion test was also repeated using 30 m^2 bins.

RESULTS

VIDEO ANNOTATIONS

A total of 2,773 animals were counted in the annotations recorded (Figure 4). The *Amperima- Peniagone Complex* is considerably more abundant than the other species, with a total number of 1,717 individuals. At the other end, *P. sp. C* is the least abundant with only three individuals. The mean number of animals per 10 m^2 was also calculated (Figure 5).
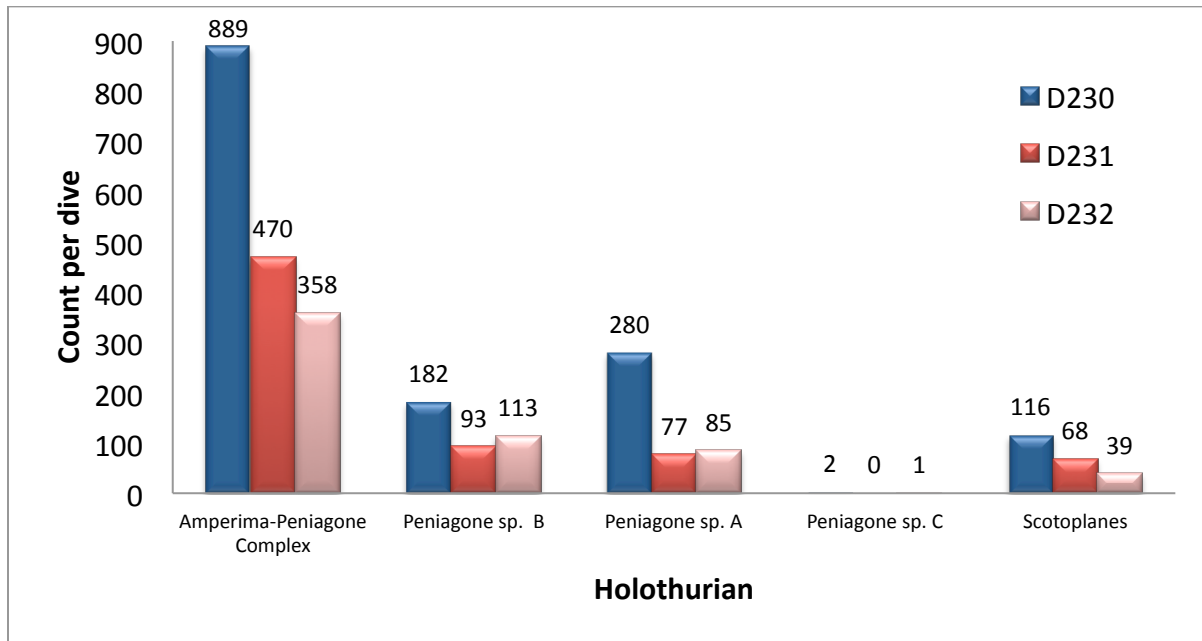

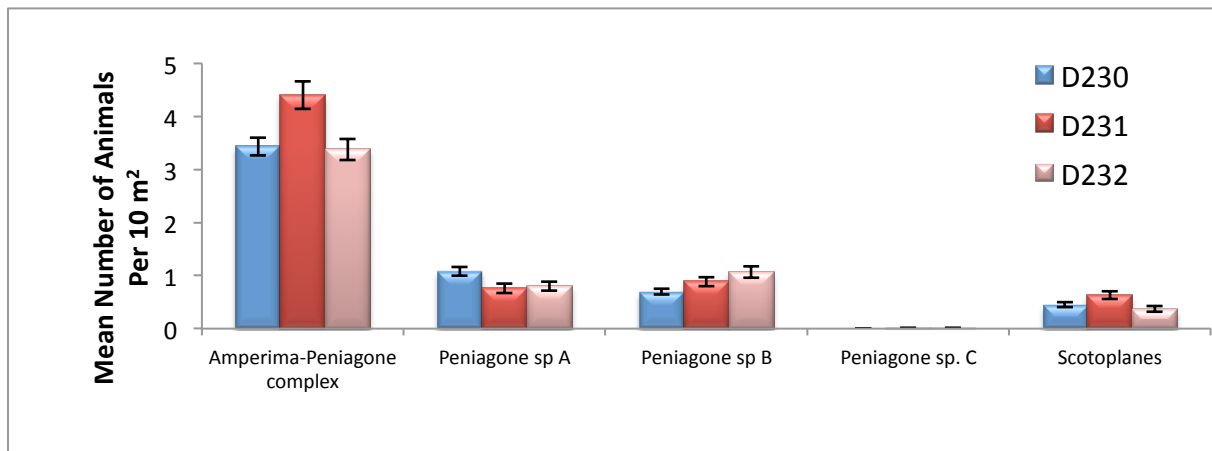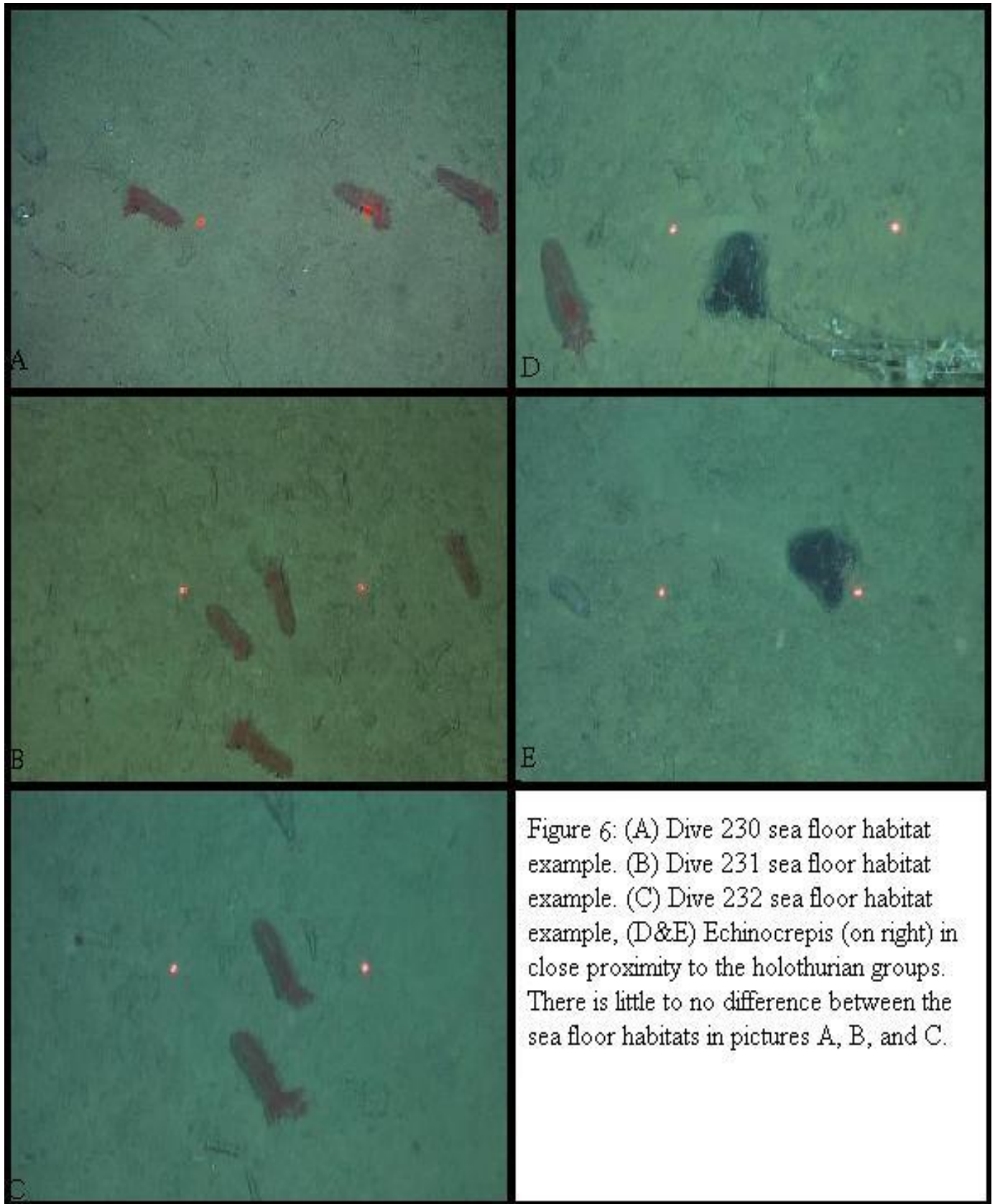Figure 4: Abundances of the five species on three dives.

Figure 5: The mean number of holothurians per 10 m^2, error bars = standard error.

Figure 6: (A) Dive 230 sea floor habitat example. (B) Dive 231 sea floor habitat example. (C) Dive 232 sea floor habitat example, (D&E) Echinocrepis (on right) in close proximity to the holothurian groups. There is little to no difference between the sea floor habitats in pictures A, B, and C.

Reviewing the habitat in the videos revealed little to no difference between the dives. The same general biological communities were present in all videos, and the habitat was not visually different around the larger groups of holothurians (Figure 4). The only slight difference was the presence of *Echinocrepis rostrata*. One to three echinoids were observed around some of the larger groups of holothurians. After reviewing abundances and habitats by individual dives, no considerable differences were found and all dives were treated together as one data set. All transects were relatively close together in a soft sediment, abyssal plain habitat.

With High definition video, we are able to tell the difference between some very similar species; while previously, they would have been indistinguishable with standard definition video. So I added together the means of *Amperima-Peniagone Complex* and *P. sp. A* in order to compare them to previously recorded *P. vitrea* at Station M. I also added together the means of *P. sp. B* and *C* to compare them to *P. diaphana*. Previous data for *P. vitrea*, the highest recorded mean per 10 m^2 is 1.161 animals, and for *P. diaphana* the mean was 1.568 animals (Ruhl, 2007). The mean calculated from the cruise in May 2011 for *Amperima-Peniagone Complex* and *P. sp. A* was 3.041 animals per 10 m^2 and the mean for *P. sp. B* and *sp. C* was 1.492 animals. The past mean for *S. globosa* was .367, and the recently recorded mean in May 2011, was 1.274 animals per 10 m^2.

MEASUREMENTS

The *Amperima-Peniagone Complex* had the most normal bell curve size distribution, with the middle sizes being the most abundant (Figure 7). The *P. sp. A* and *sp. B* both had very similar curves; there were far more small individuals than any other size. But there were still enough sizes smaller and larger, to make their distribution normal. *P. sp. C* only had three larger individuals so no conclusions can be drawn about size distribution.
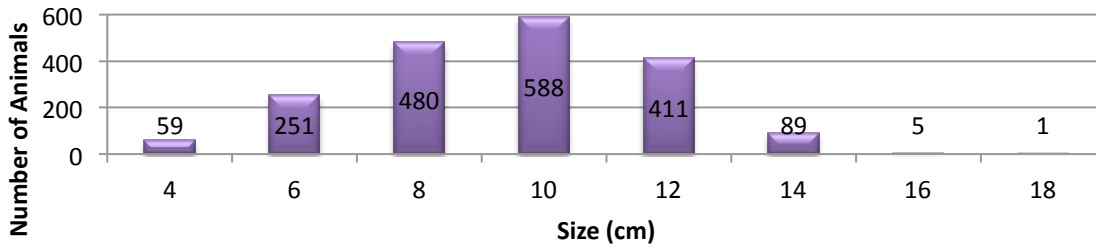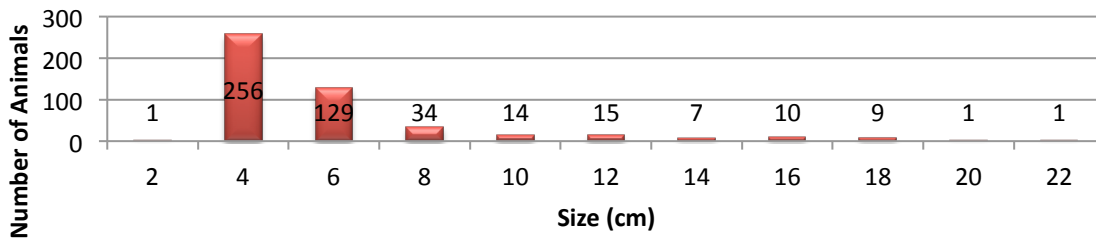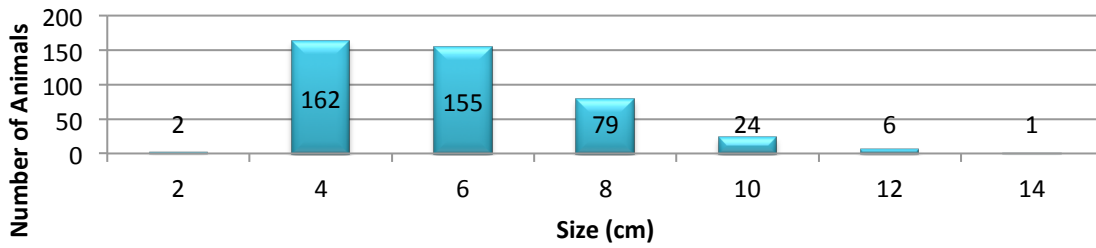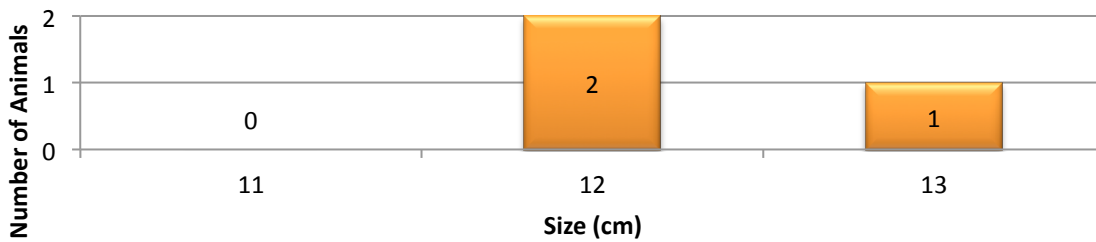
Figure 7: Distribution of size ranges for each of the four species.

DISSECTIONS

When examined prior to dissection for morphological differences, various colors, different numbers of fused dorsal paripodia, different sizes, presence of 90° body inflection, and visible gonads were observed (Table 2).

Table 2: The morphological differences between the specimens taken on dives 231 and 232. These were observed while still in the jar.

Solution and specimen	70% Ethanol- Specimen A	70% Ethanol- Specimen B	10% Formalin- Specimens E-J	10% Formalin- Specimen D	5% Formalin- Specimen C
Color	Brownish- pink	Brownish- orange	Orange- pink	White- pink	White- orange
Podia visible	Several	Several	Several	Very few	Several
Fused dorsal podia	4	2	2	0	4
Small/Large	Large	Small	Small	Small	Large
90 degree Inflection	n	y	y	n	y
Gonads Visible	n	n	y	n	y

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When the dissected specimens were measured, they proved to have a wide range of sizes, varying from 4 cm to 12.5 cm. When the specimens were examined further and dissected, they proved to be even more different than just size. It was confirmed that there were different numbers of dorsal paripodia, the gonophores were located in different spots on the anterior end of the animals, and even the color was different (Table 3). The first sample to be dissected (Sample A), had what appeared to be gametes (eggs) coming out of the gonophore (Figure 8). The visible orange spheres are most likely eggs, but since sperm are too small to see with the naked eye, specimens with no visible eggs will require microscopic examination in order to know if sperm are present. In the five dissected specimens, four contained eggs. In the five specimens not dissected, two contained eggs visible through the body wall and three did not.

Table 3: The differences and similarities found in specimens A-J of podia, colors, location of gonophore, presence of eggs, and length.

| Sample	size (cm)	dist to 1st podia(cm)	Inflection?	Turgid?	fused dorsal podia	fixative	preserved color	podia	gonophore	eggs
0232-A	9.5	2.5	n	n	2 l, 2 s	EtOH	dark, pinkish	5	right side of face	y
0232-B	9.0	3	y	y	2 s	EtOH	dark, pink-brown	5	between tentacles	y
0232-C	12.5	4	y	n	2 l, 2 s	Form	White- tips of feeding tentacles orange/pink	7	outside tentacles	y
0231-D	5.0	3	n	very	no	Form	clear, with light pink	Few?	right side of face	n
0231-E	7.0	2	y	n	2 tiny	Form	orange-pink	6	between tentacles	y
0231-F	6.5		y		2 tiny	Form	orange-pink	6	between tentacles	y
0231-G	7.5		y		2 tiny	Form	orange-pink	6	between tentacles	y
0231-H	5.5		y		2 tiny	Form	orange-pink	6	between tentacles	nv
0231-I	4.5		y		2 tiny	Form	orange-pink	6	between tentacles	nv
0231-J	4.0		y		2 tiny	Form	orange-pink	6	between tentacles	nv
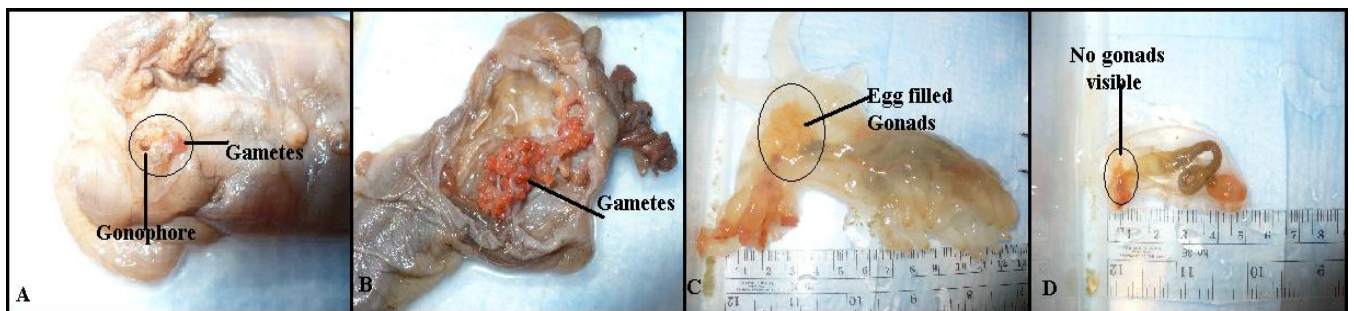
Figure 8: (A) Sample A, gonophore and possible gametes. (B) Sample A, dissected with gonads and gametes showing. (C) Sample C, Gonads visible through the skin. (D) Sample D, no gonads visible through the skin.

MICROSCOPE WORK

A total of eighteen microscope pictures were taken (Figure 9). Samples A, B, and C have piltate feeding tentacles, while Sample E has digitate feeding tentacles. Ovaries were branched sacks with eggs. The eggs from A were 1.1 x 1.1 mm, eggs from B and C were 0.5 x 0.5 mm, and eggs from E were 0.3 x 0.4 mm.
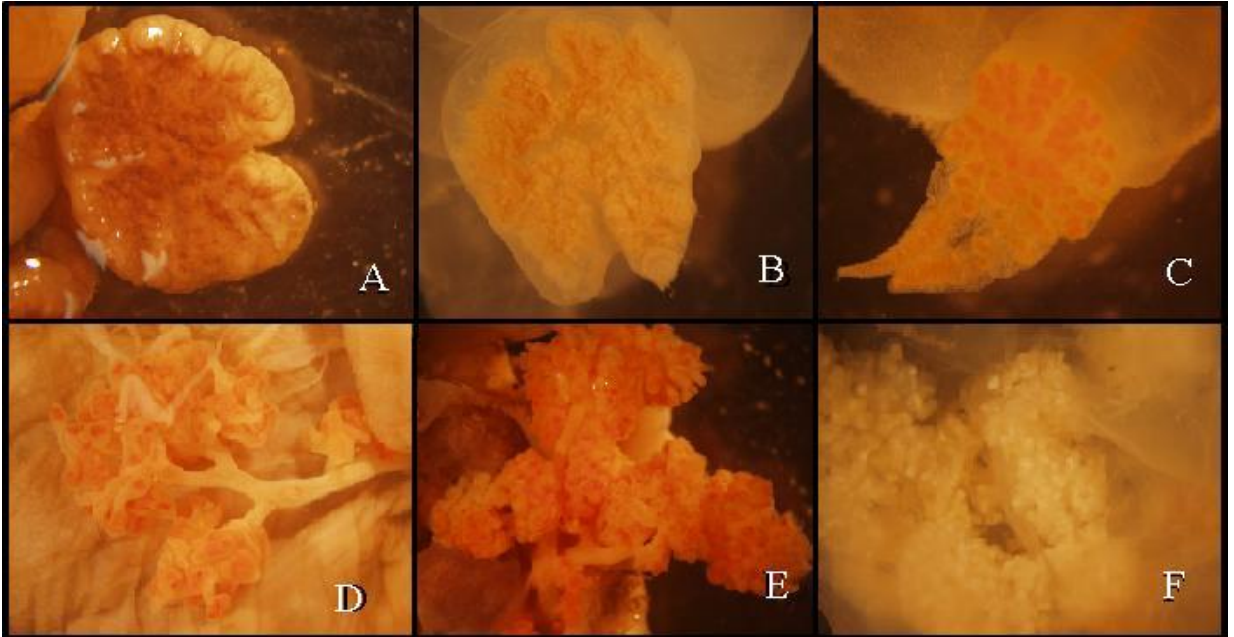

Figure 9: Example pictures from the microscope pictures. (A) Piltate feeding tentacle from Sample A. (B) Piltate feeding tentacle from Sample C. (C) Digitate feeding tentacles from Sample E. (D,E,F) Examples of the branched ovaries containing eggs.

STATISTICS

Transects were broken into four hundred and seventy 10 m^2 bins. The number of animals in each bin varied, but mostly there were smaller numbers in each bin (Figure 10). For *Amperima-Peniagone Complex* the most in one bin was seventeen and the least was zero. The largest for *P. sp. A* was eight animals in a bin and the smallest was again zero. *P. sp. B* had six in the largest bin and zero in the smallest. There were three bins together that had particularly large numbers of the *Amperima-Peniagone Complex*. With all three bins together, there were forty-four animals within 30 m^2. The distances between large bins varied widely; ranging from 0-750 m apart (Table 4). The average distances between the larger bins of *Amperima- Peniagone Complex* and *P. sp. A* were 134.09 m, and 170.77 m respectively.
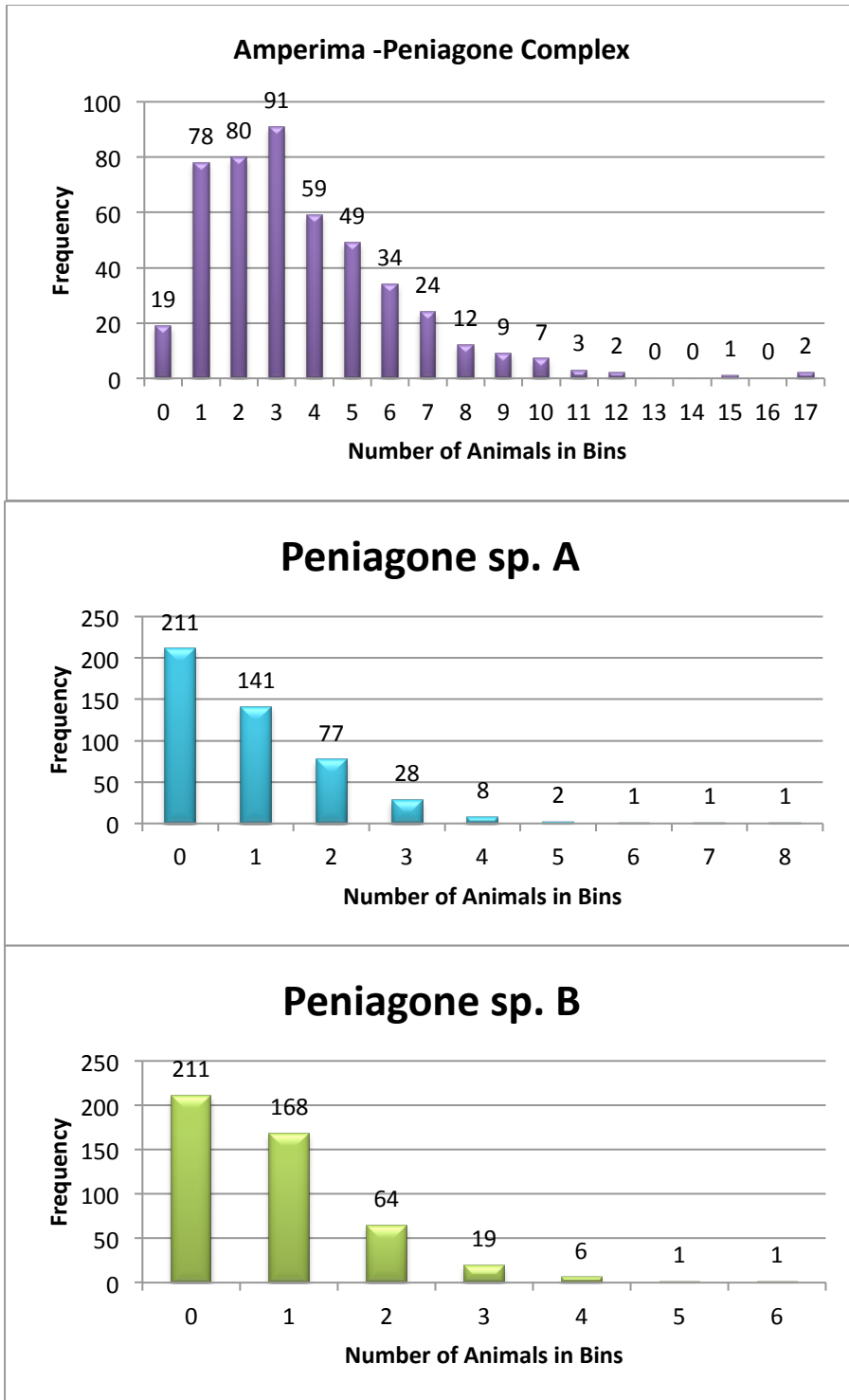
Figure 10: How frequently bins of certain sizes were seen. (A) *Amperima-Peniagone Complex.* (B) *Peniagone SP. A.* (C) *Peniagone sp. B.*

Table 4: Distances between larger bins of *Amperima-Peniagone Complex*, and *Peniagone sp. A*. The sizes of the large bins, and bin sizes around the large bins.

Bin #	Distance to Next Bin (m)	3 Bin Sizes Before	Bin Size	3 Bin Sizes After
Amperima-Peniagone Complex				
Dive 230				
A-230-07	0	5,8,5	9	9,3,1
A-230-08	240	8,5,9	9	3,1,2
B-230-16	200	1,5,3	10	2,5,1
C-230-11	310	1,3,3	11	1,2,7
D-230-04	230	3,2,3	11	2,2,2
D-230-28	750	1,3,5	9	4,1,5
G-230-22	30	3,3,6	10	6,2,4
G-230-26	530	6,2,4	10	4,1,5
I-230-02	90	6,3,3	9	8,5,1
I-230-12	0	4,5,1	17	17,10,5
I-230-13	0	5,1,17	17	10,5,7
I-230-14	0	1,17,17	10	5,7,8
Dive 231				
A-231-36	160	5,8,4	12	3,6,4
B-231-17	20	6,5,7	10	6,1,9
B-231-20	200	10,6,1	9	4,3,5
C-231-04	110	5,5,6	12	2,2,3
C-231-16	30	7,3,7	9	2,6,7
C-231-20	0	2,6,7	9	9,7,11
C-231-21	10	6,7,9	9	7,11,4
C-231-23	20	9,9,7	11	4,3,10
C-231-26	20	11,4,3	10	5,7,15
C-231-29	0	10,5,7	15	2,2,4
	Peniagone sp. A			
Bin #	Distance to Next Bin (m)	3 Bin Sizes Before	Bin Size	3 Bin Sizes After
Dive 230				
A-230-07	50	1,3,2	5	2,2,4
A-230-13	960	5,2,2	4	1,3,1
D-230-28	20	2,2,1	7	2,4,4
D-230-31	0	1,7,2	4	4,2,1
D-230-32	220	7,2,4	4	2,1,2
F-230-14	100	2,1,2	4	2,1,1
F-230-25	40	1,1,2	4	3,4,2
F-230-29	210	2,4,3	4	2,2,1
G-230-10	0	1,3,1	6	4,1,2
G-230-11	110	3,1,6	4	1,2,3
G-230-23	510	2,2,3	8	2,3,2
H-230-38	0	1,3,1	4	3,1,2
Dive 231				
B-231-18	0	1,1,1	5	2,1,1

Coefficient of Dispersion and g-test calculations for 10 m^2 bins indicated that *Amperima-Peniagone Complex* for dives D230 and D231 had clumped distributions, with a statistically significant difference between the observed vs. expected number of holothurians (Table 5). The CD performed with the 30 m^2 bins differed. This test claimed

that not just the first two dives, but all three were statistically significant for *Amperima-Peniagone Complex*. The 10 m^2 CD and g-test calculations for *P. sp. A* indicated that dive D230 had clumped distributions. The 30 m^2 CD test results were similar to the 10 m^2 results. There were insufficient observations for the g-test for *P. sp. C* on any of the dives, nor for *Scotoplanes* on D232. All other distributions were random.

Table 5: The results of the Coefficient of Dispersion and the g-test. *= Statistically significant.

Dive	Stats	Amperima Peniagone complex	Peniagone sp A	Peniagone sp B	Peniagone sp. C	Scotoplanes
230	Coefficient of dispersion	*2.080	*1.536	1.119	1.004	1.086
230	G-test	*0	*0.003	0.246	not enough obs.	0.0.156
231	Coefficient of dispersion	*1.736	1.204	1.003	1.017	0.944
231	G-test	*0	0.224	0.301	not enough obs.	0.224
232	Coefficient of dispersion	1.243	1.008	1.130	0.954	0.897
232	G-test	0.050	0.365	0.785	not enough obs.	not enough obs.

DISCUSSION

After reviewing abundances and transects by individual dives and no considerable differences were found, all dives were treated together as one data set. For the *Amperima-Peniagone Complex* and *P. sp. A*, which were compared to *P. vitrea*, there are clearly more animals than previously recorded. This is also true for the *S. globosa*. But for the *P. sp. B* and *sp. C*, which were compared to *P. diaphana*, there were fewer animals than previously recorded. This means that if there were actually a response in the populations of holothurians to some influx of food, only three out of the five observed species reacted strongly to the environmental stimulus.

When measured, most of *P. sp. A* and *sp. B* were between zero and six centimeters long, and are being considered as juveniles. This suggests that they reproduced sometime in the recent past. *P. sp. A* clearly had more localized juveniles than *P. sp. B*; which may be explained by the fact that the larvae of some species are planktonic, while other species' larvae are not, so the non-planktonic larvae would be more localized (Billett, 1991). After the aggregation statistics were completed, population size distributions were compared to the aggregation size distributions (Figure 11). All distributions were fairly similar and it was clear that the aggregations did not consist of only breeding adults. Meaning that if they did reproduce, it was quite some time ago and the holothurians just have not completely dispersed yet. For *P. sp. A* and *sp. B*, the reproduction was not as long ago as the *Amperima-Peniagone Complex's* reproduction, as made evident by the large amounts of juveniles.

Because dive D232 did not have aggregated holothurians, the tapes were reviewed for differences between the habitats in the aggregated and non-aggregated dives. No differences were found between the dives' habitats; and a check in Arch GIS showed all dives to be relatively close together, so the habitats would not be different. The only difference found between the aggregation's habitat and the rest of the population's habitat was that there were between one and three *Echinocrepis* bordering the aggregations. This could be explained by the fact that *Echinocrepis* consume holothurian fecal pellets (Figure 12).
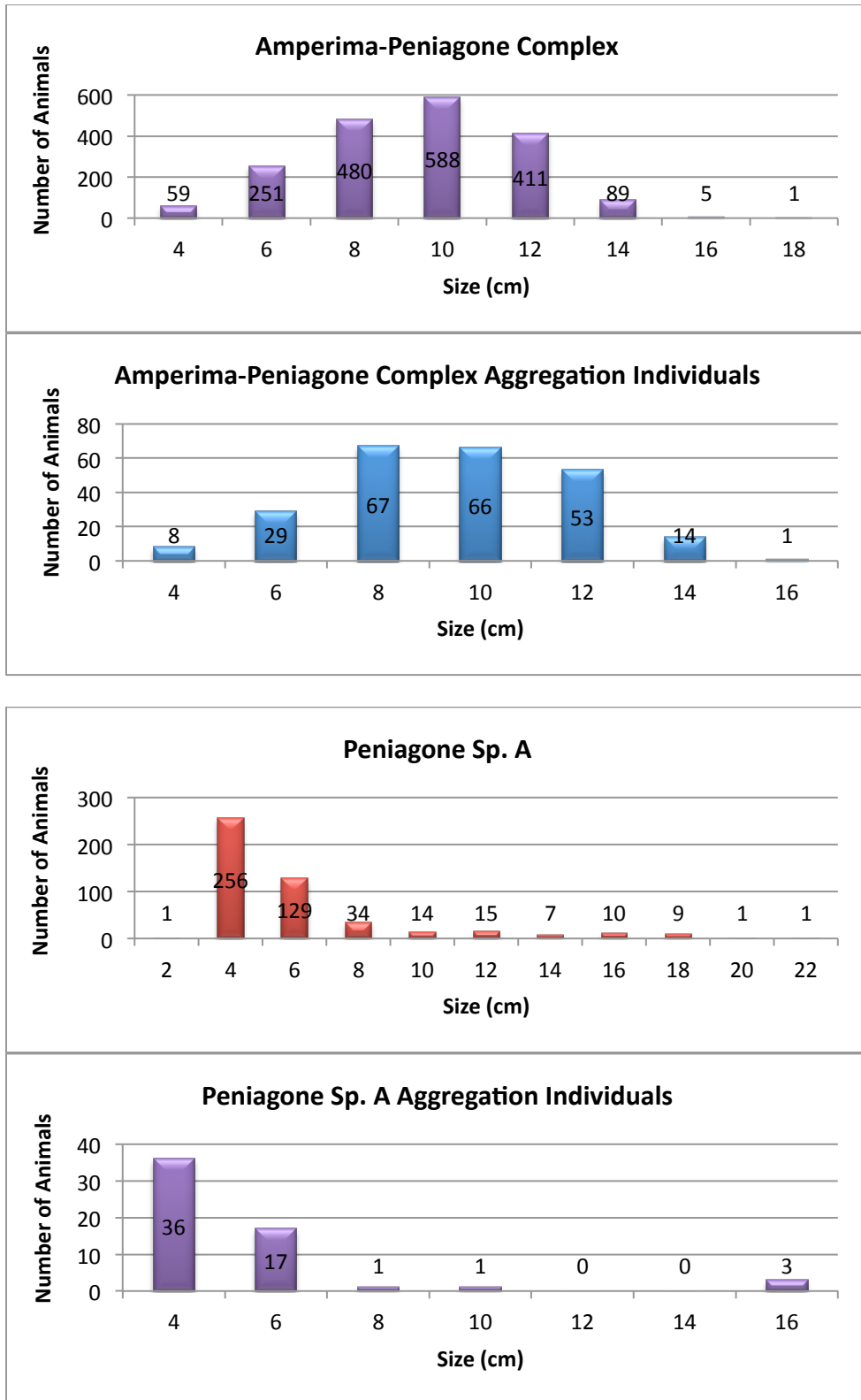
Figure 11: The population size distributions vs. the aggregation size distributions. The graphs are fairly similar and show no unusual patterns that differ from each other.
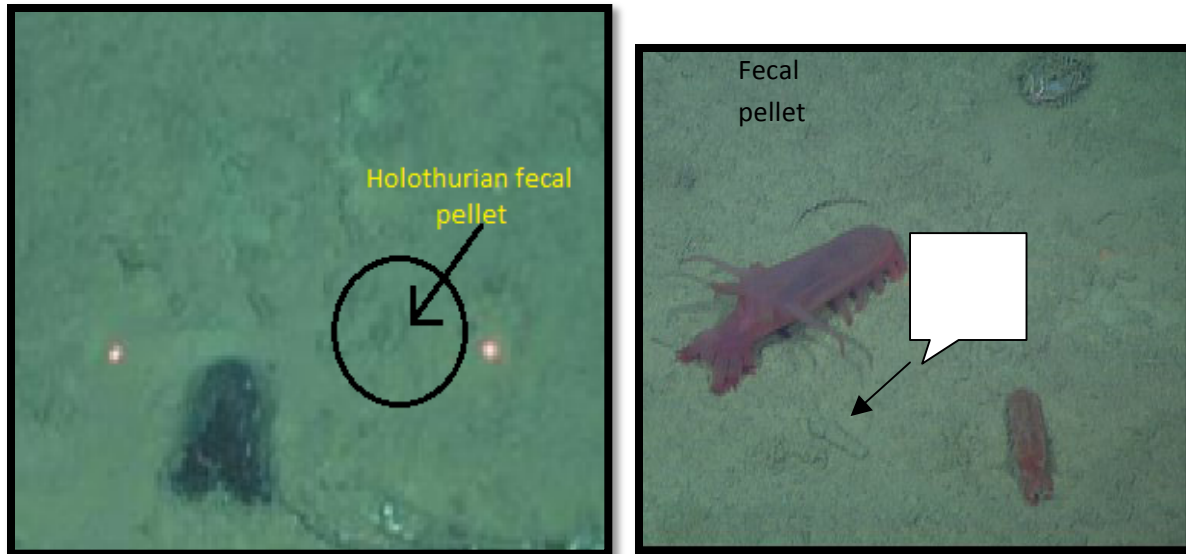
Figure 12: *Echinocrepis* (in left) and a holothurian fecal pellet (in both), which sea urchins are known to eat.

Dissection revealed that some of the specimens did have eggs developing in their gonads, which would lead me suggest that they are preparing for another round of reproduction. There was no food observed on the abyssal plain when transects were reviewed, so it is a possibility that the holothurians have already eaten it, turning that into the energy to produce gametes once again. Wigham et al. referred to this as episodic spawning- each episode is triggered by the environment providing the necessary nourishment to produce gametes (2003). If enough food is produced for more than one round of reproduction, then the holothurians could take advantage of that, which is why the dissected individuals contained developing eggs.

So far, the results from the efforts to identify which animal is what species are inconclusive. Taxonomist, Dr. Dave Pawson, will help determine the proper species names. While working with the microscope we believe we identified which sample was associated with each species designation, but no official names have been given to the four holothurians. We believe that Sample A and C are *P. sp. A*, Sample D is *P. sp. B*, and Samples E-J are *Amperima-Peniagone Complex*. Sample B may be yet another species, but that is still inconclusive.

CONCLUSIONS/RECOMMENDATIONS

This study has established that there was an increase in the populations of some holothurians. There was a increased food supply, not long ago, but we will see if the environment can support the new numbers of *Amperima-Peniagone Complex, P. sp. A,* and *S. globosa;* especially since the dissections revealed new gametes forming in some of the samples. Further studies that would help clarify some of the unknowns in this project would be first to finish with the microscope work by comparing the collected ossicles with previously documented ossicles. What would also help the study is to collect samples of the species we have been unable to dissect; such as the *P. sp. C*, and more of the *P. sp. B* since we were only able to observe one. Not only would further sampling be necessary, but future dives to check on the abundance status, and if the environment is continuing to sustain the increased numbers of holothurians. Continued monitoring of these abundances may reveal if this is a permanent change or just a response to a temporary influx of organic matter.

References:

Billett, D.S.M., 1991. Deep-Sea Holothurians. *Oceanography and Marine Biology Annual Review,* **29**: 259-317.

Lauerman, Lynn M.L., and Ronald S. Kaufman, 1998. Deep-Sea Epibenthic Echinoderms and a Temporally Varying Food Supply: Results from a One Year Time Series in the N.E. Pacific. *Deep-Sea Research II*, **45**: 817-842.

Ruhl, Henry A., 2007. Abundance and Size Distribution Dynamics of Abyssal Epibenthic Megafauna in the Northeast Pacific. *Ecology,* **88**: 1250-1262.

Smith, K.L., Jr. and E.R.M. Druffel, 1998. Long Time-Series Monitoring of an Abyssal Site in the NE Pacific: An Introduction. *Deep-Sea Research II*, **45**: 573-586.

Smith, K.L., Jr., H.A. Ruhl, B.J. Bett, D.S.M. Billett, R.S. Lampitt and R.S. Kaufmann, 2009. Climate, Carbon Cycling, and Deep-Ocean Ecosystems. *Proceedings of the National Academy of Sciences,* **106**: 19211-19218.

Wigham, B.D., P.A. Tyler, and D.S.M. Billet, 2003. Reproductive Biology of the Abyssal Holothurian *Amperima rosea*: An Opportunistic Response to Variable Flux of Surface Derived Organic Matter?. *Journal of the Marine Biological Association of the United Kingdom,* **83**: 175-188.