

***Anthopleura xanthogrammica* Behavior Studied Utilizing Time-Lapse Photography**

By

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A Thesis

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TIME-LAPSE PHOTOGRAPHY

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Animals living in a habitat affected by both tidal shifts and day night cycles display a wide variety of behaviors influenced by environmental factors and internal mechanisms. Sessile intertidal invertebrates exhibit extremely slow or subtle behaviors not noticeable during casual observation. This study aimed to observe, analyze, and describe the behaviors associated with the tidal and circadian rhythms of *Anthopleura xanthogrammica*, giant green anemones, in a tide pool at South Cove, Cape Arago State Park, Oregon. Time lapse video captured using a GoPro camera at a low- to mid-tidal range were used to test the hypothesis *A. xanthogrammica* opens on incoming tides. Percent open data were collected from videos and these data were used to evaluate the percentage of animals open in different light conditions. I also examined the data for individual sea anemones to determine if there were individual tendencies. The data suggest that there is a correlation between height of tide and anemone openness. As the tide rises fewer anemones are closed. Additionally the data suggest that anemones are more likely to be closed in direct sunlight. Furthermore, there is a slight, but insignificant difference in average time spent 100% open for each anemone. The most dramatic shifts in behavior occurred in the presence of direct sunlight.

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Table of Contents

Background _____	Error! Bookmark not defined.
Materials and Methods _____	5
Site Description _____	5
Field Data Collection _____	7
Analysis _____	8
Results _____	10
Discussion/Conclusion _____	Error! Bookmark not defined. 7
Appendix _____	Error! Bookmark not defined. 0
Works Cited _____	Error! Bookmark not defined. 2

Background

Numerous animal behaviors are dictated by environmentally or internally programmed clocks. Circadian rhythms, or behaviors associated with a 24-hour clock, and circannual rhythms, such as yearly hibernation, are well studied in numerous taxa from unicellular eukaryotes to humans (Tessmar-Raible et al. 2011). But the daily rhythms of marine animals can be based on a more complicated system than that of terrestrial organisms. Some intertidal animals have internal clocks that respond to the tidal cycle as well as the day-night cycle (Palmer 2001).

These rhythms are often entrained (initiated) by external (exogenous) cues and maintained (kept 'on time') by internal (endogenous) cues. For example, organisms behaving in direct response to changes in pressure or light changes exhibit a clock timed by exogenous cues. On the other hand, endogenous clocks depend on some kind of internal or instinctual mechanism. Some marine organisms behave according to a lunar cycle referred to as a "circatidal" clock (or circalunidian since the tides are caused primarily by the moon). On the west coast tides are semidiurnal with two high and two low tides per "day" (Tessmar-Raible et al. 2011). A tidal day refers to the 24.8-hour interval between moonrises.

Two existing schools of thought exist regarding the "mix" of clocks that dictate marine organismal behavior and these hypotheses are at odds with one another. The "circalunidian clock hypothesis" suggests that "two loosely coupled lunidian cycles run in antiphase to produce 12.4 hour intervals" (Wilcockson et al. 2008). This is based on observations of intertidal crab species that experience two activity spikes per day corresponding with the low tides. However, under constant conditions in the lab, the

activity peaks disappear after a few days, then reappear, suggesting that the rhythm is not the work of a single circa-tidal oscillator. The other argument postulates a “dedicated 12.4 hour circa-tidal clock” as well as a day/night circadian rhythm. This hypothesis explains the observations that many organisms display behaviors based on both circadian and circatidal schedules. For example, some intertidal invertebrates only spawn during a certain time of the tidal cycle (circatidal), but at night (circadian) (Tessmar-Raible, et al. 2011, Palmer 2001, Wilcockson et al. 2008).

Organisms in the intertidal must also grapple with abiotic changes as the tide shifts. A large body of literature shows that temperature changes throughout the tidal cycle can cause severe physiological damage to an organism (Helmuth et al. 2001, Hofmann et al. 1995). Intertidal organisms experience changes in temperature, salinity, pressure, turbulence, and food availability each time the tide goes out or comes in (Wilcockson et al. 2008). Therefore organisms have evolved these tidal and daily clocks to improve their chances of surviving rapid environmental changes. For example, many organisms change their physical location when the tide goes out to cope with temperature changes or to avoid desiccation. Other organisms may stay in the same place but employ a physiological protection such as a shell that closes tightly to survive during low tide.

Endogenous clocks are well studied in intertidal crab species. For example, it has been shown that a fiddler crab, *Uca pugnax*, maintained the same activity (foraging for food at low tide, immobile at high tide) even in the laboratory under constant light or constant dark (Palmer 2001, Thurman 2004). Furthermore, the crabs shifted this schedule forward 50 minutes every day to correspond with the daily shift in

the low and high tides. Field observations reveal that many organisms including, mollusks, arthropods, and fishes display some kind of intertidal migration. An intertidal migration can be as simple as moving from an area of a tide pool exposed to predators and sunlight to an area under cover of nearby rock or algae. An intertidal migration can also cover a relatively large distance. For example, the sea star *Pisaster ochraceous* travels to a higher zone of the intertidal to prey on *Mytilus californianus*, California blue mussels, during high tide, but then must traverse back down to lower tidal heights so as not to be exposed for all of low tide. The distance between mussel beds and water at low tide can sometimes be several meters. Migrating during the day may have several functions including feeding, avoidance of predators, reduction of desiccation, and reproduction. Feeding in some animals is only possible when the intertidal zone is submerged or when the tide has receded. Moreover, migration in some species avoiding predators only occurs when high or low tide is at night (Gibson 2003).

Recent research revealed that another anemone species, *Nematostella vectensis*, maintains a 24 hour behavioral cycle in which locomotion is predominantly nocturnal. Under constant light or constant dark conditions, the timing of locomotory behavior remained the same as in day/night conditions, suggesting the presence of some sort of endogenous mechanism (Hendricks et al 2012).

Anthopleura xanthogrammica, the study organism for the present research is a sessile invertebrate from phylum Cnidaria. *Anthopleura xanthogrammica* inhabits mid to lower tidal regions in cracks, crevices, in pools, and on rocks and is distinguishable by its size, bright green color, and ring of tentacles around its oral disc. *A. xanthogrammica* feeds on invertebrates, particularly mussels, dislodged by waves or by

the foraging activity of *P. ochraceous*. This allows me to postulate that anemones will be open more frequently when the tide is higher (Sebens 1986). The gastrodermal tissues of *A. xanthogrammica* contain at least one type of symbiotic algae that require light for photosynthesis. This leads to the prediction that anemones respond to both changes in tide (as they are more likely to catch prey at high tide), and to light because sunnier conditions provide an opportunity to expose symbionts to sunlight (Trench, 1971, Muscantine et al 1975).

Research on a congeneric anemone *Anthopleura elegantissima*, the aggregating anemone, revealed that presence or absence of symbiotic zooxanthellae affected their behavior. Those with the symbiont clustered near a light in an experimental setup whereas those without located themselves randomly in the sea table (Pearse-Buchsbaum, 1974). This experiment suggests that the symbionts have an effect on the behavior of the anemones perhaps due to the symbiont effects on anemone metabolism. Another experiment revealed the zooxanthellae slow weight loss of the anemones in low light conditions (Muscantine, et al. 1975)

In this study I tested the hypothesis that anemones will be more likely to open on rising tides. At higher water levels, the anemones are exposed to increased wave action and therefore more food should be available whereas at low tide invertebrates will not have a reliable food source as organisms are less likely to be knocked off nearby rocks into the pool by waves. Therefore, as the water level increases from low tide to higher tides I predicted that the percent openness of the anemones will increase.

Materials and Methods

Site description

Observations were made in a tide pool at South Cove in Cape Arago State Park (43.301627N, -124.399834W). South Cove is a large cove with a small beach at one end and a large rocky intertidal area. South Cove is oriented roughly to the south. With tall cliffs on both sides, large fallen sandstone rocks and obvious tidal zonation characterize Cape Arago intertidal areas. The study site was at the far west end of the intertidal area located in the middle to lower intertidal zone (Figure 1). This site was chosen for a number of reasons. The first reason was ease of access; it was possible to record video at various tide heights. Although this pool is located in a large surge channel (a deep narrow area between rocks where large waves enter at high tide) it is slightly elevated so waves did not crash directly in the pool at mid tide levels preventing video collection. On the other hand, the pool is low enough that the observation site is completely covered by a few feet of water at high tide. During the study this pool was shaded by adjacent rocks until around noon when it received direct light, until sundown.



Figure 1: Map of Cape Arago State park. Middle cove and South cove are visible on the map. The arrow indicates location of study site at high tide.



Figure 2: Image of study site at low tide. Far end of image shows where water flows into the pool. At high tide all of the area covered by algae is under at least two feet of water. Large rock on the left of pool shades pool until midday.

Furthermore, this pool was chosen because it is deep and clear even at low tide, which was ideal for underwater video photography. The pool was roughly two meters by three meters, and depth varied from one to 60 centimeters at low tide when there was no incoming water (Figure 2). During mid-tide, the depth could be as great as two meters. This pool is representative of pools in South Cove that remain covered even at low tide. Videos were recorded in the middle of the pool facing the source of incoming water. The camera was placed and oriented in the same position each time as there was a natural “shelf” in the pool which served as a good location for video recording. *A. xanthogrammica*, the giant green anemone was one of the more common larger invertebrates in the pool. Purple sea urchins, *Stronglyocentrotus purpuratus*, and a number of smaller invertebrates and fishes were also present and visible in the videos.

Field data collection

To test the hypothesis that anemones will be more open when the height of tide is higher, videos ranging in length from one to two hours were recorded using a GoPro camera in the tide pool at South Cove at Cape Arago State Park. Video was recorded at tide heights from -0.6 to 4.3 feet (0.18 to 1.3 meters) over the months April to June. Eleven videos recorded in the field ranged in length from 52 to 128 minutes. The majority of the videos were captured while the tide was coming in and while the pool was not in the direct sun (Table 2) (Table 3). Low tide observations were made at times when the study site was not receiving any incoming water whereas mid-tide observations were made when the site was subject to wave action and incoming water. Low tide observations commenced within one hour of low water whereas mid tide tests commenced at least two hours after low tide. A general species count was carried out to determine the makeup of the community in the pool using a ½ meter quadrat which made for easier counting. Notes on time, weather and tide height estimates were taken in the field, and analysis of the videos was carried out later.

Videos were obtained using a small waterproof GoPro camera capable of capturing HD video in two-hour intervals. The GoPro was mounted to a 2.25 kg weight and was attached to a rope and float. During low tide tests, the rope and float were not utilized. The camera was placed with as little disturbance as possible in the tide pool by hand and oriented towards the source of incoming water and a cluster of *Anthopleura xanthogrammica*. At mid-tide it was necessary to wade into the pool, behind the area of video capture, to position the camera correctly. In an extreme case the pool was filled waist high with water and it was necessary to fully submerge face and head to place the

camera. The camera rope was secured to rocks and cracks near the pool for stabilization. By securing the rope into a crack behind the camera, the setup was less likely to be dislodged by wave surge. At the end of data collection the camera was retrieved using the rope.

The camera, weight, and rope setup allowed for data collection without disturbing tide-pool organisms. In the field great care was taken to capture high quality images. Before and throughout video recording it was essential to check the orientation of the camera to make sure the camera was still in place, and that the lens was not obscured by bubbles on the camera or algae in the tide pool.

Analysis

The videos were edited and analyzed using Pinnacle Studio software. Videos were split into two to five sections by the GoPro camera but were edited together on the computer. Because most videos are an hour or longer videos were sped up five times for viewing. Eight individual anemones that were visible in most videos were chosen for study and assigned individual numbers (Figure 3). Data were collected in ten minutes increments in each video. Every ten minutes each of the visible anemones was assigned a “% openness” value ranging from 0 (closed) to 1 (completely open) by visual estimate. Percent openness numbers were assigned to one significant figure. For example 40% open was recorded as 0.4. “100% open” or a value of one was defined as full open and expanded. Likewise “0% open” or closed correspond to an anemone that was completely retracted (see appendix for additional figures). As the same eight anemones were scored in each of the videos, and anemones are circular, visual estimates were the most efficient way to measure % openness. If direct sunlight was on

the pool during any given data set this was recorded. No partial sunlight measurements were taken.



Figure 3: Representative sequence of images from the video data. Visible here are *A. xanthogrammica*, *S. purpuratus*, *Leptasterias hexactis*, and algae. This video was taken on an overcast day and therefore light is relatively low. The progression from anemones closed (frame one) to anemones open (frames two and three) is apparent as well as the movement of the sea star from left to right. These images were taken approximately twenty minutes apart and show changes in behavior over time.

Height of tide was calculated based on the tidal rate and time for each video.

The rate of the tide was calculated for each individual sample set using the following formula:

$$\text{Rate of tide} = \frac{\Delta \text{ between High and Low tide}}{\text{time elapsed between High and Low tide}} = \frac{ft}{min}$$

And the time at which the data were recorded was calculated:

$$\textit{time of day} = \textit{start time of video} + \textit{time elapsed}$$

(time elapsed was always in ten minute increments)

And thus the minutes past low or high tide were calculated and the approximate height of tide of the sample (data set or behavior) could be determined:

$$\textit{Height of tide} = \textit{Minutes past low tide} * \textit{Rate of tide}$$

The above formulas were customized to each video, if the tide was coming in or going out, for example, but the majority of data sets were collected after low tide during rising tides. Furthermore the time and approximate height at the first incoming wave was recorded. To check this method, the height of tide at which the first incoming wave enters the pool was calculated. A slight variation in these data was observed as the size of the waves dictates the height at which wave action will first enter the pool.

A T-test and an ANOVA were calculated using Microsoft Excel and double checked using SPSS statistics software. Graphs and tables were created in Microsoft Excel.

Results

In the pool there were several kinds of invertebrate organisms. Numerous purple urchins, *Strongylocentrotus purpuratus*, populated the rock into which they have burrowed. In addition to urchins, prominent green anemones, *Anthopleura xanthogrammica*, were visible and displayed states ranging from fully closed to fully open. It also included sea stars *Pisaster ochraceus* which were attached to rock near the surface of the pool (but out of the water) during low tide and *Leptasterias hexactis* (6-armed star). Mollusks *Tonicella lokii* (lined chiton), *Diodora aspera* (keyhole limpet),

and *Lottia sp.* (limpet), all coexisted within the cavities the urchins carved out of the rock. Annelids *Spirorbis sp.*, and *Dodecaceria sp.* were extremely small worms (only a few millimeters in length) which inhabited much of the open rock space in the pool. Various small crabs were visible ambulating on and around the *A. xanthogrammica* throughout many of the videos. Lastly, various algae such as encrusting coralline algae, as well as other more plant like algae were visible in the pool and can serve as shelter for smaller invertebrates. Species counts are summarized in Table 1.

Table 1: Species counts from field counts and video footage. Organisms identified in video do not include genus and species but only general name.

Species	Number
<i>S. purpuratus</i>	234
<i>A. xanthogrammica</i>	38
Sculpin	21
<i>T. lineata</i>	16
hermit crab	5
decorator crab	4
<i>L. pelta</i>	1
<i>Hemigrapsis sp.</i>	1
tube worm	1
<i>L. helianthoides</i>	1
<i>P. ochraceous</i>	1

The percent open of anemones was quantified and plotted against height of tide (Figure 3). Data points are clustered around one (open 100%) at all tide heights. From one to two feet anemones displayed a variety of openness. When water reached above 2.5 feet the majority of anemones were 100% open.

Table 2: Tabulated data detailing video observations. Start time recorded in field, video length determined during video analysis. Tide height at the start and finish were calculated from height of tide charts for Charleston, OR for given date. Rate of tide similarly calculated. Sun data collected from videos.

Date	Start Time	Video (min)	Start Height (ft)	End Height (ft)	Rate of tide (ft/min)	Tide
4/20/2013	14:35	84	1.5	2.3	0.01190	incoming
4/22/2013	15:05	52	2.4	1.7	0.01397	outgoing
4/27/2013	8:54	110	-0.6	1.9	0.02208	incoming
5/13/2013	8:44	115	-0.2	1.1	0.01634	outgoing/incoming
5/18/2013	12:50	118	1.1	2.6	0.01250	incoming
6/7/2013	10:00	96	2.9	4.5	0.01679	incoming
6/8/2013	9:35	63	1.8	2.9	0.01746	incoming
6/10/2013	9:43	127	0.8	3.0	0.01728	incoming
6/10/2013	20:25	54	3.3	4.3	0.01921	incoming
6/11/2013	10:59	116	1.4	3.2	0.01583	incoming
6/12/2013	11:30	128	1.5	3.6	0.01599	incoming

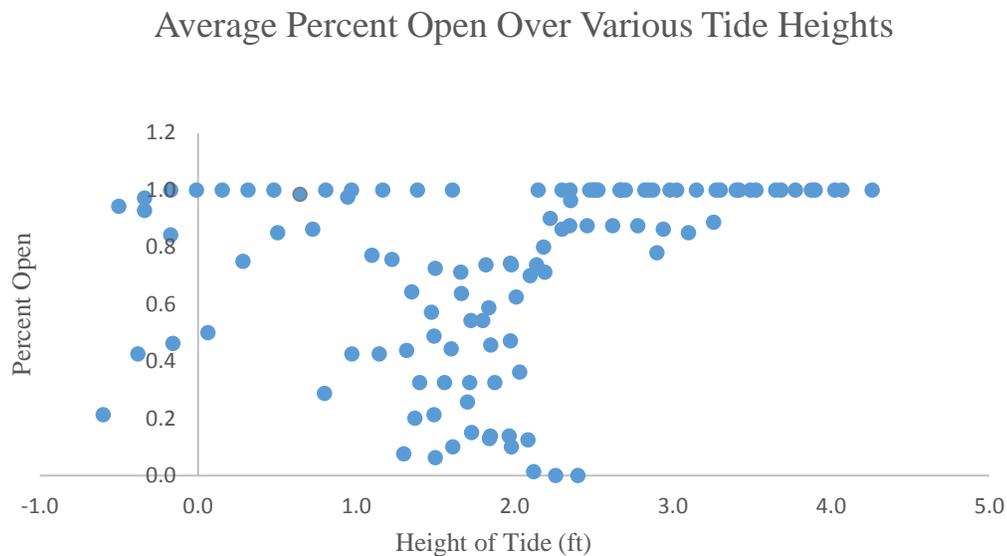


Figure 3: Graph depicting height of tide versus average percent open of anemones. Anemones are 100% open throughout most all of the heights of tide. 10-80% open is seen between water levels of 1ft to 2ft whereas above 2.5 feet the majority of anemones are completely open.

The videos showed few open anemones at lower tide heights and more at higher. Furthermore the observational data showed the presence of direct sun as having an effect on the state of the anemones. Thus the presence or absence of direct sunlight

in a video was tabulated (Figure 7-9 in Appendix, and Figure 4) (Table 3). The sequence of images (Figure 4) representing the pool in ten minute increments, show behavioral changed as the pool changes from shaded to direct sunlight. In image A all anemones are 80-100% open except the anemone (number 8) on the far right which is in direct sunlight. As the shade moves to the left, exposing more of the anemones to direct sunlight, the anemones close. Frame B shows one anemone half closed (on the side that was first exposed to the light). By frame E the entire pool is in direct sunlight and none of the anemones are fully open. Image F, taken 30 minutes later, shows the pool when the tide is coming in. All of the anemones have fully or partially reopened.

Table 3: Summarizes whether or not there was direct sunlight on the pool on a given video. Direct sunlight was not tabulated for individual anemone.

Date	Direct Sunlight
4/20/2013	yes
4/22/2013	yes
4/27/2013	no
5/13/2013	no
5/18/2013	no
6/7/2013	yes
6/8/2013	no
6/10/2013	no
6/10/2013	no
6/11/2013	no
6/12/2013	no



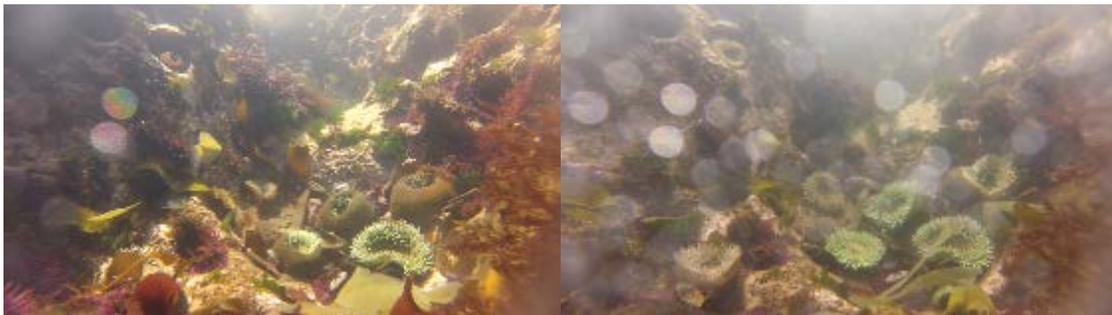
A

B



C

D



E

F

Figure 4: Video stills taken from video captured May 18th, 2013. Still A was taken from video captured at 12:50pm. The subsequent stills, B-E were taken every ten minutes. The final still, F was taken a full 30 minutes after image E when waves were hitting the pool. These stills show the pool changing from shaded to direct sunlight. Anemone behavior changes can be seen in these images as more of the pool is experiencing direct sunlight.

Average percent open for anemones in direct sunlight versus in shade or low light is plotted in Figure 5. On average, about 80% of anemones were open when there

was no direct sun and 50% were open when there was direct sunlight on the pool.

Standard error bars displayed on graph

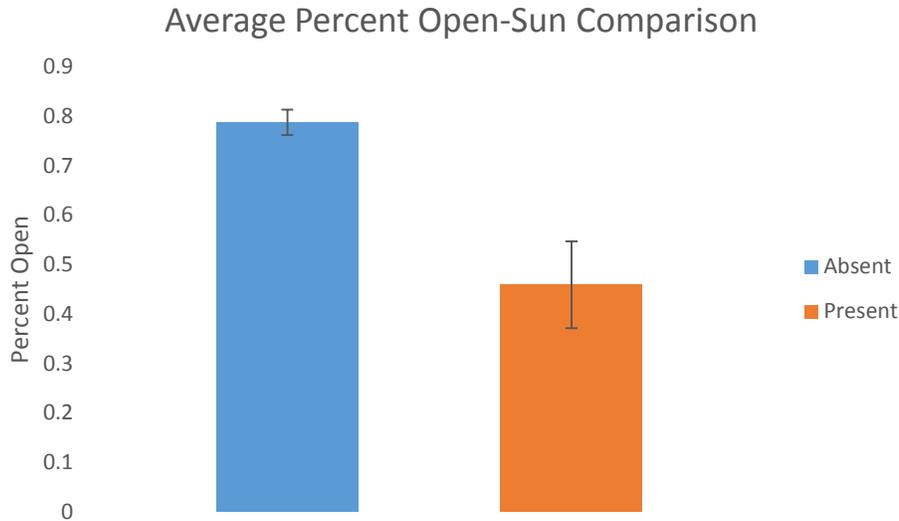


Figure 5: Graph comparing the percent open of all anemones when direct sunlight was present or absent. “Present” was clearly identifiable in the video data, whereas “absent” took on various forms. Examples in the appendix. Standard error bars are displayed on graph.

A T-test assuming unequal variances for the arcsine of average percent open data was calculated and the results are tabulated in Table 4. The p values indicate that the difference between presence and absence of sun is significant.

Table 4: T-test results for average percent open in direct sunlight and not in direct sunlight. The arcsine of the averages was taken in order to calculate the t-test.

	Absent	Present
Mean	1.193639	0.79462
Variance	0.122703	0.391362
Observations	85	25
Hypothesized Mean Difference	0	
df	29	
t Stat	3.051551	
P(T<=t) one-tail	0.002417	
t Critical one-tail	1.699127	
P(T<=t) two-tail	0.004833	
t Critical two-tail	2.04523	

Lastly, the average time spent 100% open for each individual anemone was calculated to measure the difference in behaviors of individuals. Results are graphed in Figure 6. Values range from an average of 40 minutes spent 100% open to 70 minutes.

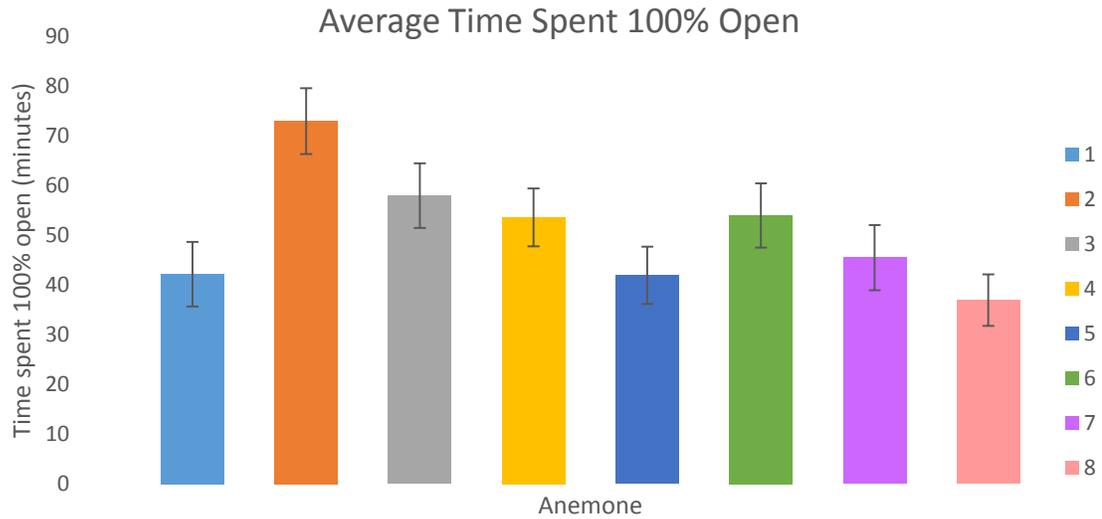


Figure 6: Average time spent 100% for all of the videos for each anemone. Error bars are displayed on graph. This graph shows a small but distinguishable difference in average time spent open by individual anemones.

A single factor ANOVA for average time spent 100% open for anemones was calculated and the results are tabulated in Table 5. The p indicates that these data are not significant.

Table 5: Results of the single factor ANOVA for the average time spent 100% open for individual anemones. The p value for this data suggests that these data are not significant.

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	8203.987	7	1171.998	0.782576	0.60375	2.123408
Within Groups	122804.5	82	1497.616			

Table 5: Results of the single factor ANOVA for the average time spent 100% open for individual anemones. The p value for this data suggests that these data are not significant.

Discussion/Conclusion

The trends in the percent openness data versus height of tide suggest that at lower heights anemones are in various states of openness ranging from fully closed to fully open and that after 2.5 feet nearly all anemones are fully open. That nearly all of the anemones were open beyond 2.5 feet when waves are hitting the pool was expected, as there should be more food availability when the tide is in. Perhaps some exogenous cues were at work, though, from tide heights 1.5 to 2.5 when many of the anemones were between 10% and 80% (presumably opening for higher tide). The trend that anemones were open throughout most all of the tide heights suggests that there were other factors dictating the open or closed state of anemones in addition to the height of tide. The stills from the video in which the shade was retreating from one side of the pool to the other suggests that sunlight had an effect on the pool. Likewise, the graph comparing percent open to the pool in direct sunlight versus not in direct sunlight suggests that there was some motivation to be closed when the pool is in direct sunlight. This is possibly because of the photosynthesizing symbiotic algae living in the gastrodermal tissue of *Anthopleura xanthogrammica*. As the symbionts live in numerous folds within their column, it is likely that they are exposed to light. The study site was most exposed to sunlight when the tide was low. As the tide increased waves and water movement prevented direct sunlight. Being fully open and feeding likely will provide greater metabolic material, therefore there is little reason for an anemone to be closed when the tide is coming in (unless it is already actively feeding).

The data which account for variations within individual *Anthopleura xanthogrammica* suggest that there is a slight difference in the time spent 100% open.

The anemone that spent the least time 100% open was the anemone highest in the pool (number one). But another that spent only 50% of its time 100% open was anemone five which is the most surrounded by other large anemones. Although there are variations visible in the data, the p-values suggest that these data are not significant.

The original intent of this project was to test the capabilities and limitations of using GoPro underwater time-lapse video capture for scientific study. This research was just one example of what GoPro modules could be used for in the field. The video quality is phenomenal considering the size and price of the module and with the appropriate accessories (some of which are still being developed) longer data sets could be obtained, and the camera could be cued remotely. In addition to quantifiable research, these videos have educational potential because of their ability to capture behaviors beyond what is seen at low tide. They not only provide an underwater view not normally seen, but the time-lapse effect shows that these invertebrates are truly alive, which the casual tide pool visitors might not get a sense of at low tide. Therefore it is my intention to further edit and attempt to distribute the video data collected in the field, perhaps to a visitor center or an educator so that further education will be inspired by South Cove.

Previous work asserts that *Anthopleura xanthogrammica* feeds on dislodged mussels as wave action increases, suggesting there is a circatidal rhythm associated with its feeding habits (Sebens 1986). Furthermore, the existence of a photosynthesizing symbiont is known, but little has been concluded regarding the effect of the symbiont on *A. xanthogrammica*. This research does not provide strong evidence to conclude anemone circadian and circatidal rhythms are dictated by the shifts in tide. Rather, the

data suggest a variety of exogenous cues that affect the state of the anemone. As there was not a laboratory portion of this study in which *A. xanthogrammica* was devoid of external stimulus, one cannot conclude whether or not endogenous cues were affecting the anemones. However, in addition to physical parameters, it is possible an endogenous mechanism could explain some of the variation between individual anemones in the fixed effects regression.

Future work is necessary at a larger variety of tide heights. Unfortunately this would require more setup to account for more wave action and deeper water. Additionally, information that would lead to a better understanding of behavior such as temperature, proximity to a potential food source etc. could shed more light on the circadian and circatidal cycle of *Anthopleura xanthogrammica*.

Appendix



Figure 7: 4/22 20 min, anemones displaying 0% openness. This video also characterized direct sunlight.



Figure 8: 6/10 (evening) 17min, anemones displaying 100% openness. This pool is also clearly not in direct sunlight.



Figure 9: 4/27 10 minutes, anemones displaying a variety of % open. Furthermore, although the pool is lit, this does not characterize direct sunlight.

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