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EFFECTS OF GLACIAL STRESSORS ON SPERM MATURATION IN COLONIES

OF THE RED TREE CORAL, PRIMNOA PACIFICA

by

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A Thesis Submitted in Partial Fulfillment of the Requirements for a Degree with Honors (Marine Science)

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ABSTRACT

The red tree coral, Primnoa pacifica, is a large, colony forming species of coldwater coral which is often an important habitat for many commercially important species of fish and crab. This keystone species is long lived and found at much shallower depths in the fjords of Glacier Bay National Park (GBNP) than elsewhere in the northern Pacific Ocean because of the phenomenon known as deep-water emergence. Due to their proximity to tidewater glaciers in GBNP, corals likely have to endure glacial stressors such as freshwater runoff and sedimentation that is not typical of populations in deeper water, which can affect physiological processes such as growth and reproduction. This study compared male colonies of Primnoa pacifica between three regions of GBNP to determine the correlation between glacial proximity and the colonies' ability to produce fully mature spermatocytes. This study found that there is no significant difference in the size of sperm nuclei in colonies from regions at different distances from the glaciers across GBNP. This could suggest that all male colonies across the West Arm of GBNP are synchronized in their reproductive cycles due to an environmental cue that is felt across the entire fjord. Further study to determine the nature of this potential environmental cue could be valuable in understanding how climate change and warming oceans could affect populations of cold-water coral.

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INTRODUCTION



Image 1: Map of southeastern Alaskan fjords including the study site, Glacier Bay. (Stone et al, 2018)

Glacier Bay National Park and Preserve (GBNP) is a system of deep, glacially derived fjords and forms the largest active glacier complex outside of Antarctica and Greenland (Hartill et al., 2020; Stone & Mondragon, 2018). It is located in southeastern Alaska and consists of three distinct portions, the east arm fjord, west arm fjord, and the central channel (Hartill et al., 2020) (Image 1). The west arm fjord shows signs of a rapid glacial retreat and is influenced by three tidewater glaciers, the Johns Hopkins, Lamplugh, and Margerie Glaciers (Stone & Mondragon, 2018). Several other grounded glaciers nearby also provide meltwater to the fjord. In the east arm, only one tidewater glacier is present, the McBride Glacier, and evidence of slower, more constant glacial retreat is present (Stone & Mondragon, 2018). A moraine at 55m depth guards the fjord while nearby grounded glaciers such as the Muir and Riggs glaciers provide substantial meltwater (Stone & Mondragon, 2018). Freshwater input from runoff or glaciers in these fjords can affect the stratification and flow dynamics of the water column as well as carry in suspended particles (Hartill et al., 2020). The glacial input works to create darker and colder conditions in the fjord system leading to deepwater emergence. This is a phenomenon where species such as the red tree coral, *Primnoa pacifica*, which normally live at great depths are found in shallower locations due to conditions within the fjords being similar to the deep sea (Hartill et al., 2020).

The red tree coral Primnoa pacifica (RTC) is a species of large tree coral found only in the North Pacific Ocean (Hartill et al., 2020). This gorgonian coral is a keystone species (Choy et al., 2020; Rossin et al., 2018; Stone et al., 2015) and is commonly found in large thickets at depths of 150-250m in the Gulf of Alaska (Stone et al., 2015; Stone & Mondragon, 2018). The full range of RTC extends from the Sea of Japan and the Sea of Okhotsk to the Aleutian Islands and into the Gulf of Alaska (Rossin et al., 2018). Across that range, these corals are found at depths ranging from 6-1029m but are most common around 500m (Hartill et al., 2020) in habitats dominated by sloping bedrock and moderate water currents (Rossin et al., 2018). RTCs are a long lived species with lifespans of over 100 years (Choy et al., 2020; Hartill et al., 2020) and there is a strong correlation between the age and the size of a colony. The growth rate of RTC declines logarithmically with age, averaging about 2.41 to 6.39 cm/yr, and old corals can reach a plateau in growth (Choy et al., 2020). RTC is a keystone species in the Gulf of Alaska as it provides a three-dimensional structure which is used by fish and crabs as essential habitat (Stone et al., 2015). Many of these species are commercially important (Hartill et al., 2020; Rossin et al., 2018) and support Alaskan fisheries.

Damage or dislodgment due to fishing activity is common with Alaskan RTC colonies. They are at risk of these disturbances from fishing due to their size and distribution, commonly caught as bycatch in Alaskan fisheries (Stone et al., 2015; Stone & Mondragon, 2018). Their large size and proximity to areas with commercially important fishing grounds makes them an easy target for disturbances. The colonies are easily damaged or dislodged by external forces, like those from long lines and bottom trawls, and their slow growth rate likely leaves little ability to recover quickly (Stone & Mondragon, 2018). Because of this, in June 2006 the North Pacific Fishery Management Council designated five areas known to support RTC thickets in the eastern Gulf of Alaska as Habitats of Particular Concern (Stone & Mondragon, 2018) in order to mitigate damage to corals. Use of bottom contact fishing gear is prohibited in these areas, but damage from fishing gear is still observed on RTC colonies throughout the Gulf of Alaska (Stone & Mondragon, 2018).

RTC are gonochoric broadcast spawners with asynchronous spawning cycles (Johnstone et al, 2021; Waller et al., 2014, 2019). Oocyte development in RTC follows a 16-month cycle while male gamete development takes only 3-4 months (Johnstone et al, 2021; Waller et al., 2014). Spawning is largely asynchronous with the majority of females spawning around January with scattered spawning year round and males spawning in three cycles: September-December, September-January, and March-June (Waller et al., 2014). Although fewer studies have documented cold water coral reproduction than that of shallow coral reefs, a small number of studies have shown how different factors can affect the reproduction of RTC. The effects of ocean acidification were detrimental to gametogenesis and reproduction as the corals showed a possible

inability to fully allocate resources to gametogenesis, and when pH was lower, sperm developed faster (Rossin et al., 2018). Colonies at different depths in fjord environments showed that oocytes were 2-3 times smaller at shallower locations, possibly due to plasticity in energy allocation to oogenesis in order to cope with the stressors of shallow waters (Waller et al., 2019). While this could be a reproductive dead end in the fjords (Waller et al., 2019), colonies in the Glacier Bay National Park and Preserve may be reproductively successful while other Alaskan populations aren't (Hartill et al., 2020).

In this study I will be investigating the sperm development of RTC colonies as a function of proximity to glaciers in Glacier Bay National Park and Preserve. Recent work has shown differences in sperm nuclear diameter in Southeast Alaska P. pacifica populations at different depths. Given that proximity to the tidewater glaciers likely influences the environment of P. pacifica colonies in GBNP (Stone and Mondragon, 2018), I investigated sperm development in P. pacifica colonies collected from sites at varying distance from the glacial front.

METHODS

Collection of Samples



Image 2: Map of study sites in Glacier Bay National Park& Preserve. Study sites are marked: far sites (>50km from tidewater glaciers), mid sites (20-40km from tidewater glaciers), and near sites (<10km from tidewater glaciers). Sills are indicated with arrows; tidewater glaciers are represented by blue boxes. (Hartill et al, 2020)

The samples used in this study were collected in 2016 during a research cruise through Glacier Bay National Park and Preserve. The ROV Kraken2 (University of Connecticut) was used to collect the samples used in this study. The ROV followed a dive plan of descending in the center of the fjord and ascending along the vertical fjord wall to take transects and collect samples. The samples used in this study were categorized into one of three zones depending on their proximity to the tidewater glacier in the west arm of the fjord: "near" within 10 km (Johns Hopkins), "mid" 20-40 km (Happy Knobb and Tidal Bulge), and "far" greater than 50 km away (Central Channel) (Image 2). Samples were collected from 250-300m, 340-390m, and 270-300m depth respectively.

Sample Name	Depth Collected (m)	Region	Area Collected	Date Collected	Colony Height (cm)	# Spermatocysts	# Spermatocytes Measured
GBNP-16-088	. 347	Mid	Tidal Bulge	23-Mar-16	177.201	40	3316
GBNP-16-098	378	Mid	Tidal Bulge	23-Mar-16	121.877	36	2518
GBNP-16-109	273	Close	Johns Hopkins	25-Mar-16	88.692	20	1379
GBNP-16-110	256	Close	Johns Hopkins	25-Mar-16	104.609	6	395
GBNP-16-111	297	Close	Johns Hopkins	25-Mar-16	127.652	5	209
GBNP-16-118	270	Close	Johns Hopkins	25-Mar-16	65.553	13	645
GBNP-16-123	278	Close	Johns Hopkins	25-Mar-16	82.322	33	1928
GBNP-16-140	286	Far	Central Channe	27-Mar-16	28.146	9	445
GBNP-16-141	295	Far	Central Channe	27-Mar-16	57.564	4	129
GBNP-16-143	286	Far	Central Channe	27-Mar-16	123.174	14	1144
GBNP-16-144	287	Far	Central Channe	27-Mar-16	103.946	44	3327
GBNP-16-145	276	Far	Central Channe	27-Mar-16	29.304	18	1032
GBNP-16-188	386	Mid	Happy Knobb	29-Mar-16	109.177	15	1038
GBNP-16-189	389	Mid	Happy Knobb	29-Mar-16	125.439	27	1860
GBNP-16-192	389	Mid	Happy Knobb	29-Mar-16	169.68	14	709

Table of Samples Used: Table of collection information for the 15 samples used in this study. All samples were collected from the West Arm and Central Channel of Glacier Bay National Park.

Histological Processing

Samples were already processed for this study using methods in Waller et al (2019). Briefly, samples were preserved in 4% neutral buffered formaldehyde and transferred to 70% ethanol for transport back to the Darling Marine Center (University of Maine) and once there were decalcified in Rapid Bone Decalcifier (Electron Microscopy Sciences), serially dehydrated in a graded ethanol series, cleared with toluene and infiltrated in paraffin wax. The wax blocks were sectioned at 5 microns using a Leica RM2235 rotary microtome while leaving 90 microns between slides. Each section was then stained using either Masson's trichrome or Hematoxylin Eosin stains and coverslipped. Due to COVID measures I was unable to do laboratory work myself but was supplied with images of slides for the purposes of this thesis.

Image Analysis

I analyzed images of sectioned reproductive corals provided to me by my thesis advisor. I examined a total of 15 samples over three locations within Glacier Bay: Johns Hopkins, Happy Knob/Tidal Bulge, and the Central Channel. From each sample 3 polyps were taken and the cross section with the most spermatocysts was selected to photograph. These samples were photographed under a microscope at a magnification of 60x.0 The images of each spermatocyst I received were analyzed using ImageJ on a laptop using a Wacom drawing tablet. I ran a script file named "GridRandomSelection.ijm " in ImageJ that selected three grid boxes randomly which allowed me to take unbiased measurements across each sample. I obtained feret diameters of individual sperm nuclei in order to assess the reproductive stage of corals from the three locations. Feret diameter is the computer analyzed diameter of a non-circular shape such as would be measured with calipers. ImageJ provides the minimum and maximum feret diameters which I averaged to obtain a mean feret diameter.

Statistical Analysis

All statistical data analysis was done in Microsoft Excel. First, I conducted size frequency analyses on individual polyps within each colony. I then determined that the data was non-normal using the XRealStats Excel add-in and performed Mann-Whitney tests to calculate P-values comparing differences in sperm nuclei diameter in each polyp. I then combined frequency counts of polyps that were not statistically significantly different and calculated new percent frequencies for each colony. I repeated this process

to compare sperm nuclei diameter within colonies in each region and subsequently to compare sperm nuclei diameter across the three regions.

RESULTS

Polyp Comparison

Mann-Whitney tests and size frequency graphs comparing polyps within each colony showed that there were no statistically significant differences between the distribution of spermatocyte nuclear diameters in polyps of a given colony. All p-values calculated from Mann-Whitney tests were above the threshold of 0.05 by (Table 1). Colonies 141 and 110 were different in that they had only one and two polyps respectively, so there were few comparisons to make for both colonies.

Colony	P1vP2	P1vP3	P2vP3
189	0.695254	0.383067	0.663336
123	0.695254	0.947969	0.679225
144	0.527625	0.571293	0.930663
118	1	0.777291	0.60131
140	0.647594	0.982646	0.586214
88	0.632006	0.844772	0.513449
141	x	x	x
110	0.711419	x	x
110 98	0.711419 0.913389	x 0.930663	x 0.827776
110 98 111	0.711419 0.913389 0.947969	x 0.930663 0.878969	x 0.827776 0.878969
110 98 111 145	0.711419 0.913389 0.947969 0.777291	x 0.930663 0.878969 0.527625	x 0.827776 0.878969 0.760654
110 98 111 145 143	0.711419 0.913389 0.947969 0.777291 0.861839	x 0.930663 0.878969 0.527625 0.647594	x 0.827776 0.878969 0.760654 0.485692
110 98 111 145 143 109	0.711419 0.913389 0.947969 0.777291 0.861839 0.632006	x 0.930663 0.878969 0.527625 0.647594 0.632006	x 0.827776 0.878969 0.760654 0.485692 0.947969
110 98 111 145 143 109 188	0.711419 0.913389 0.947969 0.777291 0.861839 0.632006 0.760654	x 0.930663 0.878969 0.527625 0.647594 0.632006 0.844772	x 0.827776 0.878969 0.760654 0.485692 0.947969 0.982646

Table 1:P-values from Mann-Whitney tests comparing the percent frequencies of spermatocyte nuclei size of *Primnoa pacifica*. Three polyps were compared within each colony and p-value results are shown in the table as P1vP2 means polyp one compared against polyp two, etc. Colony 141 had only one polyp so there are no p-values to report and colony 110 had only two polyps so there is only one reported p-value.



Figure 1: Size frequency graph of the three imaged polyps *Primnoa pacifica* colony 109 collected from GBNP. Each polyp is graphed as a separate series and the graph overall shows an example of the polyps within a given colony being in phase with one another.



Figure 2: Size frequency graph of the three imaged polyps *Primnoa pacifica* colony 145 collected from GBNP. Each polyp is graphed as a separate series and the graph overall shows an example of two polyps within a given colony being in phase with one another while the third is slightly shifted.

Size frequency graphs of polyps within each colony showed that most polyps were almost entirely in phase with each other (Figure 1). Some colonies, however, showed two polyps which were closely in phase while the third polyp was slightly shifted towards either a smaller or larger size (Figure 2). While these colonies appeared to have differing polyps on size frequency graphs, the differences were not statistically significant as p-values from Mann-Whitney tests comparing them were greater than 0.05.

Colony Comparison

P-values	C123	C118	C110	C109	C111
C123	x	0.777291	0.896155	0.896155	0.485692
C118	x	x	0.930663	0.744125	0.35961
C110	х	х	х	0.878969	0.40742
C109	x	x	х	х	0.60131
C111	Х	Х	Х	Х	Х

Table 2: P-values from Mann-Whitney tests comparing the percent frequencies of spermatocyte nuclei size in *Primnoa pacifica* colonies from the "close" region in GBNP, defined as within 10km of the nearby Johns Hopkins Glacier.



Figure 3: Size frequency graph of *Primnoa pacifica* spermatocyte nuclei from the five colonies that make up the "close" region of GBNP.

P-values from Mann-Whitney tests comparing colonies within the "close" region of GBNP showed that there were no statistically significant differences between the size of sperm nuclei between colonies (Table 2). All p-values calculated when comparing colonies within the "close" region were greater than 0.05 so they were not statistically significant. The size frequency plot of sperm nuclei diameter in colonies within the "close" region showed that the sizes were grouped consistently around 2.5-3um (Figure 3). Colony 111 showed a higher peak at this size as opposed to other colonies.

P-values	C192	C188	C098	C088	C189
C192	х	0.827776	0.695254	0.930663	0.794028
C188	х	x	0.485692	0.810859	0.965299
C098	x	x	x	0.663336	0.432654
C88	x	x	x	x	0.794028
C189	x	x	x	x	x

Table 3: P-values from Mann-Whitney tests comparing the percent frequencies of spermatocyte nuclei size

 in *Primnoa pacifica* colonies from the "mid" region in GBNP, defined as between 20 and 40km of the

 nearby Johns Hopkins Glacier.



Figure 4: Size frequency graph of *Primnoa pacifica* spermatocyte nuclei from the five colonies that make up the "mid" region of GBNP.

P-values from Mann-Whitney tests comparing colonies within the "mid" region of GBNP showed no statistically significant difference in sperm nuclei diameter between colonies. All p-values were higher than 0.05 by at least 0.38 as the lowest p-value calculated was 0.432654 (Table 3). A size frequency graph of sperm nuclei diameter from colonies in the "mid" range showed that the colonies were fairly well grouped together (Figure 4). Sperm nuclei diameter for all colonies were gathered generally around a sperm nuclei diameter of 2.5um but some colonies favored slightly towards 3um while others were shifted slightly towards 2um.

P-values	C143	C145	C140	C144	C141
C143	x	0.744125	0.827776	0.513449	0.744125
C145	х	x	0.930663	0.25599	0.982646
C140	x	x	x	0.294696	0.947969
C144	x	x	х	х	0.284678
C141	x	x	х	х	х

Table 4: P-values from Mann-Whitney tests comparing the percent frequencies of spermatocyte nuclei size in *Primnoa pacifica* colonies from the "far" region in GBNP, defined as greater than 50km away from the nearby Johns Hopkins Glacier.



Figure 5: Size frequency graph of *Primnoa pacifica* spermatocyte nuclei from the five colonies that make up the "far" region of GBNP.

For colonies within the "far" region of GBNP, p-values from Mann-Whitney tests showed that there was no statistically significant difference in sperm nuclei diameter between any of the colonies. All p-values were above the threshold of 0.05 as the smallest value calculated for a colony was 0.25599 (Table 4). The size frequency graph of sperm nuclei diameter for colonies in the "far" range of GBNP showed close grouping for all colonies except for colony 141. Colonies were centered around a sperm nuclei diameter of 2.5-3um (Figure 5) but colony 141 was shifted right towards larger sperm nuclei diameters. Colony 141 showed two peaks in percent frequency, one at 3um and another between 3.5 and 4um. Although colony 141 looks to have a different distribution on a size frequency graph, p-values from Mann-Whitney tests showed that there was no significant difference in sperm nuclei diameter.

Region Comparison

P-values	Close	Mid	Far
Close	x	0.930663	0.513449
Mid	x	x	0.571293
Far	х	x	х

Table 5: P-values from Mann-Whitney tests comparing the percent frequencies of spermatocyte nuclei size

 in *Primnoa pacifica* colonies from three regions in GBNP. Regions are defined by their distance to the

 nearby Johns Hopkins Glacier: "close" within 10 km, "mid" 20-40 km, and "far" greater than 50 km away.



Figure 6: Size frequency graph of *Primnoa pacifica* spermatocyte nuclei from three regions in GBNP. Regions are defined by their distance to the nearby tidewater glacier: "near" within 10 km, "mid" 20-40 km, and "far" greater than 50 km away.



Figure 7: Comparison of the average feret diameter of sperm nuclei across colonies from three regions within GBNP. Regions are defined by their distance to the nearby tidewater glacier: "close" within 10 km, "mid" 20-40 km, and "far" greater than 50 km away.

P-values from Mann-Whitney tests comparing the size of sperm nuclei between the three regions of GBNP showed that there were no statistically significant differences between regions. The p-values of tests comparing the "close" region to the "mid" region, the "close" region to the "far" region, and the "mid" region to the "far" region were 0.930663, 0.513449, and 0.571293 respectively which all are above the threshold of 0.05 (Table 5). A size frequency graph comparing sperm nuclei diameter across the three regions also showed that there was close grouping of the regions. The three peaks of percent frequency are all around 2.5-3um, but the peak of the "mid" region is slightly shifted towards 2.5um more than the other two regions (Figure 6). When comparing average feret diameter of the sperm nuclei of colonies across the three regions, the size grouping can be clearly seen (Figure 7). While the majority of the sizes appear to be within 2 and 4um, the "far" region has more small sperm nuclei as its line reaches farther down than the other two regions, giving it a wider spread of spermatocyte sizes than other regions.

DISCUSSION

In this study I compared male *Primnoa pacifica* colonies between three regions of GBNP to determine the correlation between proximity to glaciers and the colonies ability to produce fully mature spermatocytes. Although colonies closer to the tidewater glacier in my study likely have to cope with glacial stressors such as meltwater runoff and sedimentation, there were no statistically significant differences in sperm nuclei diameter between any of the regions.

While there were no significant differences in sperm nuclei diameter between polyps within each colony, there was small amounts of variation in size from one polyp to the next. Most commonly, one of the three polyps would have slightly smaller, more mature sperm sizes. It makes sense that most polyps in a colony would be synchronized in sperm development as Primnoa pacifica are gonochoric broadcast spawners (Johnstone et al, 2021; Waller et al 2019) and would likely release sperm during a discrete spawning period (Waller et al., 2014). Colony 141 and colony 110 were outliers in that they had less than three reproductive polyps which could be imaged and analyzed. Colony 141 had only one while colony 110 had two. This could be due to other polyps already releasing their sperm or other polyps simply not having the energy to invest in successful reproduction. Interestingly, colony 141 is from the "far" region of GBNP while colony 110 is from the "close" region, so these colonies with non-reproductive polyps were rare and most colonies in all three regions had reproductive polyps. Although the size frequency graphs of some colonies suggested that individual polyps had larger or smaller sperm sizes than the rest of the colony, p-values from Mann-Whitney tests showed that the differences were not statistically significant.

There was no apparent difference in the diameter of sperm nuclei for colonies in the close region, as the p-values from Mann-Whitney tests comparing them were all greater than 0.05. Since all colonies from this region had sperm nuclei diameters around 2.5-3um in size, it appears that the colonies could be synchronized in their spermatogenesis processes in order to spawn. While most colonies had a peak percent frequency of 20% for sperm nuclei around 2.5-3um, colony 111 had a higher peak percent frequency at this size. That colony's sperm were more closely grouped around the 2.5-3um size mark as most other colonies had smaller amounts of variation in their size.

All of the colonies in the "mid" region of GBNP had sperm nuclei diameters which were grouped generally around the size of 2.5um. Although some colonies had sperm nuclei diameters shifted slightly larger towards 3um or slightly smaller towards 2um, no colonies had a distribution of sperm nuclei diameters that were significantly different. This implies that the studied colonies in this region are possibly synchronized in their spermatogenesis in order to spawn at the same time and whatever environmental signal that drives that reaches every colony in the region.

Colonies within the "far" region of GBNP were found to have sperm nuclei that were grouped around 2.5-3um in size. These colonies followed similar trends of size grouping in relation to each other as the colonies in other regions did. There were no significant differences in sperm nuclei diameter between any of the colonies which implies that all the studied colonies could be in the same stage of spermatogenesis. Although all the colonies were similar in their sperm nuclei diameter, colony 141 was visually different and its diameters shifted towards larger nuclei sizes when compared in a size frequency graph. Interestingly, colony 141 showed two peaks in percent frequency

when plotted. One at 3um and another between 3.5 and 4um, both of which are larger sizes than the peaks of other colonies in the region. A likely cause of this is that colony 141 had only one polyp with reproductive activity, so it may have been a colony with stunted reproductive growth.

At the regional scale, colonies showed no statistically significant difference in sperm nuclei diameter between any of the regions. Each region showed percent frequencies that peaked around sperm nuclei diameters of 2.5-3um when plotted as a size frequency graph suggesting that the three regions may be fairly synchronized in their spermatogenesis cycles. This could suggest that although the colonies found in these three regions are living in different environments at different distances from Johns Hopkins Glacier, they are following the same reproductive schedule. There may be an environmental cue that happens in all three regions that triggers or contributes to this reproductive pattern in all of these colonies. Differences in reproduction between regions may be attributable to local factors. For example, when the range of sizes is examined (Figure 7) the "far" region has a larger range of spermatocyte sizes, indicating a wider range of developmental stages present in the sperm from this region. It may be that living in a less stressed environment, farther from the glaciers with adequate resources enables these colonies to reproduce more continuously in this region. The "mid" region also showed slightly smaller, more mature sperm development when plotted on a size frequency graph than either the "close" or "far" region. This difference is not statistically significant, but it may have interesting implications in the differences between regions. The samples collected from the "mid" region were collected from slightly deeper depths than the other two regions. These deeper depths could be better living conditions for the

corals and could explain the slightly smaller, more mature sperm found in that region. The deepest depths that samples were collected from in either the "close" or "far" region was about 300m, but the shallowest depth a sample from the "mid" region was collected from was 340m. The average feret diameters of sperm nuclei across the three regions also showed that the regions closely matched each other. The "far" region had a greater range of sizes, especially towards the smaller sizes. This is interesting because it suggests that the colonies in the "far" region are not as synchronized as other regions. Although the "far" region has smaller, more mature sperm nuclei present, the majority of sperm nuclei in colonies there were in the 2.5-3um range which was similar to what was observed in other regions. It was noted during observations that none of the spermatocytes appeared to have undergone a head shape change to a more pointed morphology. This indicates that none of the colonies examined were producing fully mature sperm at the time of collection.

CONCLUSIONS

While tidewater glaciers in GBNP cause the input of freshwater runoff and sedimentation as well as other glacial stressors, there is no significant difference between sperm maturity in *Primnoa pacifica* colonies across different regions of the fjord at varying distances from the glaciers. Since spermatogenesis is fairly uniform across the study area, it is likely that there is an environmental cue that triggers the response at the same time in each of the colonies. An interesting exception to that is the wider range of spermatocyte sizes found in the "far" region which suggests that corals in the region are able to reproduce more continuously. Further study to determine what this environmental cue is and how it affects the entire fjord could be useful to understand especially as climate change may affect this cue.

WORKS CITED

- Choy, E., Watanabe, K., Williams, B., Stone, R., Etnoyer, P., Druffel, E., Lorenson, T., & Knaak, M. (2020). Understanding growth and age of red tree corals (Primnoa pacifica) in the North Pacific Ocean. PLoS ONE, 15(12 December), 1–18. https://doi.org/10.1371/journal.pone.0241692
- Goldsmith, D. B., Kellogg, C. A., Morrison, C. L., Gray, M. A., Stone, R. P., Waller, R. G., Brooke, S. D., & Ross, S. W. (2018). Comparison of microbiomes of cold-water corals Primnoa pacifica and Primnoa resedueformis, with possible link between microbiome composition and host genotype. Scientific Reports, 8(1), 1–15. <u>https://doi.org/10.1038/s41598-018-30901-z</u>
- Johnstone, J. W., Waller, R. G., & Stone, R. P. (2021). Shallow emerged coral may warn of deep sea coral response to thermal stress. *Scientific Reports*, 1–10. https://doi.org/10.1038/s41598-021-01948-2
- Hartill, E. C., Waller, R. G., & Aust, P. J. (2020). Deep coral habitats of Glacier Bay National Park and Preserve, Alaska. PLoS ONE, 15(8 August). <u>https://doi.org/10.1371/journal.pone.0236945</u>
- Hartill, Elise C., "Deep Benthic Coral Habitats of Glacier Bay National Park and Preserve, Alaska" (2019). Electronic Theses and Dissertations. 2996. <u>https://digitalcommons.library.umaine.edu/etd/2996</u>
- Rossin, A. M., Waller, R. G., & Stone, R. P. (2018). The effects of in-vitro pH decrease on the gametogenesis of the red tree coral, Primnoa pacifica. BioRxiv, 1–17. <u>https://doi.org/10.1101/407403</u>
- Stone, R. P., Masuda, M. M., & Karinen, J. F. (2015). Assessing the ecological importance of red tree coral thickets in the eastern Gulf of Alaska. ICES Journal of Marine Science, 72(3), 900–915.
- Stone, R. P., & Mondragon, J. (2018). Deep-sea emergence of red tree corals (Primnoa pacifica) in Southeast Alaska glacial fjords. NOAA Professional Papers NMFS 20, 33.

- Waller, R. G., Stone, R. P., Johnstone, J., & Mondragon, J. (2014). Sexual reproduction and seasonality of the Alaskan red tree coral, Primnoa pacifica. PLoS ONE, 9(4). https://doi.org/10.1371/journal.pone.0090893
- Waller, R. G., Stone, R. P., Rice, L. N., Johnstone, J., Rossin, A. M., Hartill, E., Feehan, K., & Morrison, C. L. (2019). Phenotypic Plasticity or a Reproductive Dead End? Primnoa pacifica (Cnidaria: Alcyonacea) in the Southeastern Alaska Region. Frontiers in Marine Science, 6(November), 1–14. https://doi.org/10.3389/fmars.2019.00709

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