Does the nutritional state of jellyfish vary with season along the Pacific Northwest Coast?

by

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DOES THE NUTRITIONAL STATE OF JELLYFISH VARY WITH SEASON ALONG THE

PACIFIC NORTHWEST COAST?

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Kelly Sutherland

Cnidarian jellyfish are ubiquitous predators of pelagic communities, but little is known about their phenology and how food availability affects their nutritional status. Starved medusae tend to decrease somatic growth to allocate resources towards gonad development, thus a ratio of gonad to bell size might help determine the nutritional state of hydromedusae. We hypothesized that when food is scarce, *C. gregaria* and *E. indicans* will have larger gonads relative to their body size. I conducted starvation experiments to directly test how bell diameter and gonad area vary with food availability. The same two species of hydromedusae were also collected in a period of low primary productivity (winter) and high primary productivity (summer) along the North California Current System. ImageJ was used to analyze photos of the formalin-preserved specimens to obtain morphological measurements and create a gonadal index (gonad area/bell area). As the preservation method caused a loss in biomass of the collected medusae, we made a correction factor to convert the measurements of the preserved organisms to live ones. The medusae showcased slightly higher gonadal index in the medusae during winter than summer indicating an increased effort towards reproduction when resources are depleted. Understanding the links between oceanographic conditions and population dynamics of gelatinous predators will allow us to better predict their effects on zooplankton community dynamics.

Introduction

Hydrozoans are a class within the phylum Cnidaria. These medusae are known for their small size and transparency (Purcell et al, 2013; Madin & Harbison, 2001), and as carnivorous predators that prey on a variety of gelatinous animals, crustaceans and larval forms of fish (Madin & Harbison, 2001). Despite their wide distribution, very little is known about their phenology and how food availability affects their nutritional status and development. What is well known about hydrozoans is their role as predators of zooplankton communities in various marine ecosystems like the well-studied Northern California Current (Larson, 1986).

In the Pacific Northwest, wind-driven upwelling during the summer months along the coast enhances primary production and causes the neritic and ocean zooplankton communities to vary significantly (Smith, 1974; Peterson and Miller, 1976). This causes seasonal blooms of phytoplankton during the months of April and May that continue into the summer months of June and July (Peterson and Miller, 1976). While in the winter, a process called downwelling occurs due to storms pushing oceanic water onto the neritic zone causing oceanic plankton to come along with it (Morgan et al., 2003). These seasonal differences in plankton between seasons can indirectly affect the nutritional state of organisms within the intermediate levels of the pelagic food web that prey on the plankton such as hydrozoans.

When food becomes limiting, cnidarian jellyfish will increase reproductive efforts while reducing somatic growth to increase survivorship of offspring, whereas when food is abundant the jellyfish will increase somatic growth to increase future offspring output (Lucas, 2001; Olive, 1985). This pattern has been observed in various species of jellyfish where growth is slow during the winter and early spring but increases exponentially starting in mid-spring (Lucas & Williams, 1994). The slow growth during the winter causes the medusae to reach sexual maturity at a

smaller size during the winter months compared to the summer months. It has even been found in *Aurelia aurita* that the number of sexually mature individuals decrease with increased food abundance despite the overall larger size of the organism (Lucas, 1996). There has also been an observed shrinkage period between the summer and autumn season due to the release of gametes (Lucas, 2001). Scyphomedusan jellyfish have also been found to be capable of shrinking and reabsorbing gonadal tissue when food is scare to allow survival during periods of starvation. Once food availability increases, the medusae return to their original size with without a decrease in their fitness (Lucas, 2001). For instance, starving *A. aurita* can go through a process of degrowth that regresses the entire animal until it resembles minute adult. Even the gonads of fully sexually mature jellyfish can regress into an immature state in as little as 5 days (Hamner and Jenssen, 1974).

While the adaptations of strategic food usage and growth have been well studied in Scyphomedusae, few studies have been conducted around this topic for hydromedusae. Growth of hydromedusae has been shown to be correlated with temperature (Matsakis, 1993; Lucas et al., 1995) with the maturing and spawning cycle being regulated by light (Lucas et al., 1995). The medusae grow quickest during their immature phase months with growth decreasing as they enter the mature phase (Chiaverano, 2004). Most medusae are mature by early summer, however, some continue to grow throughout the autumn and winter and mature the following summer season (Lucas et al., 1995). Food supply has also been found to be important in regulating fecundity with the gonads of medusae being largest when food is abundant (Larson, 1986). However, more still needs to be discovered about the relationships between the somatic growth and gonad growth in hydromedusae as this knowledge remains thin (Chiaverano, 2004).

Therefore, in this study, we compared the gonad to bell ratio of two abundant hydrozoans, *Clytia gregaria* and *Eutonina indicans,* between the seasons of winter and summer along the Newport Hydrographic line (NH) and the Trinidad Head line (TR) within the Northern California Current, USA to infer if these jellyfish have the ability to strategically allocate their consumption. Morphological analysis of these jellies provides insight into the effect of surrounding nutritional availability on nutritional status. Though modern molecular tools allow us to accurately determine nutritional state and metabolic rates of organisms at any given time (Chícharo and Chícharo, 2008), these tools are not widely available. In contrast, the collection of zooplankton and preserving samples in formalin is a widely used and accessible method to generate ecological data (Jankowski and Anokhin, 2019). Therefore, we used this simpler method in our analysis of the hydromedusan jellyfish. But as formalin has been known to cause changes in the size and volume of organisms over time (Ahlstrom and Trailkill, 1963), we corrected for these changes by measuring the effects of formalin on samples over time.

Additionally, we conducted starvation experiments where we fed groups of medusae varying levels of plankton to observe changes in their morphology across different food availabilities. We hypothesized that hydromedusae present during the winter will have larger gonads in relation to their bell size due to prioritization of gonadal growth during seasons of low food availability. For summer, we hypothesized smaller gonads in relation to bell size due to focus on stomatic growth during times of high food availability. Therefore, for our starvation experiments we predict larger gonads in comparison to bell size in the medusae that are starved compared to those that are well-fed.

By comparing the two species of *C. gregaria* and *E. indicans* we can further discover (1) if the effects of starvation in morphology are universal across all medusae or if they vary

between species and (2) if the seasonal variation in plankton assemblages drive changes in morphology of these medusae. In doing so, we can begin to infer the potential impact that upwelling has on the nutrition state of hydrozoans and the impact that these medusae will have on the zooplankton communities. Furthermore, using morphometry in preserved samples to approximate the nutritional state of medusae could yield valuable comparative datasets when more expensive tools are not available and provide a population-level estimate of the physiological state of jellyfishes.

Methods

1.Starvation Experiments

To observe real time changes of food availability on gonadal development, individual medusa were collected in Coos Bay, Oregon on August 28, 2020 using dip netting. The medusae were kept in 1000ml jars with sea water and kept in a sea table at a temperature of 12° C. They were observed for a period of 1 week, which was chosen as adequate time to observe change as hydromedusae are known to have a life span of no more than three months (Roosen-Runge, 1970; Lucas et al., 1995). The medusae were separated into 3 treatment groups with 7 individuals in each group: fed twice a day, fed once a day, and unfed. The food was a solution of plankton in sea water with 10 ml given at each feeding. The prey was obtained from the same location as the medusae using a 100 μm plankton tows. Prey were kept in 2-liter plastic beakers with seawater with a constant rotation of 2 rpm (Corrales-Ugalde and Sutherland, 2020). New plankton was captured on day four of the experiment to provide new and live prey to the medusae. Before each feeding, debris was removed from the bottom of the jar to prevent nitrogen build-up. Jars were cleaned and given fresh sea water on day four.

To capture the photos of the preserved medusae, a Sony handycam HDR'CX560 was attached to the trinocular port of a stereo microscope (Fig. 1). Each individual preserved medusa was laid out, mouth-side facing up, on a petri dish arranged in a manner that allowed for bell diameter and gonads to be easily measured. Immediately following the photo of the medusae, a photograph of a clear ruler was taken at the same zoom for future morphological measurements. The gonad and bell area measurements were obtained using image analysis in ImageJ (version 2.0.0-rc-69/1.52pc; Fig. 2). The areas for each gonad for each specimen were added together to obtain the total gonad area. The bell area was calculated using the formula $A=3.14*r^2$. The gonadal index was calculated by taking the total gonad area divided by the bell area. On day 0, initial photos were taken of each individual, and then once again at the end of the week on day 7. Two individuals from each group were photographed on days 1, 3, and 5. The average and standard deviation of the bell diameter, gonad area, and gonadal index of each group in day 0 and day 7 was found using Microsoft Excel (Version 16.42) with the functions "Average" and "STDEV."
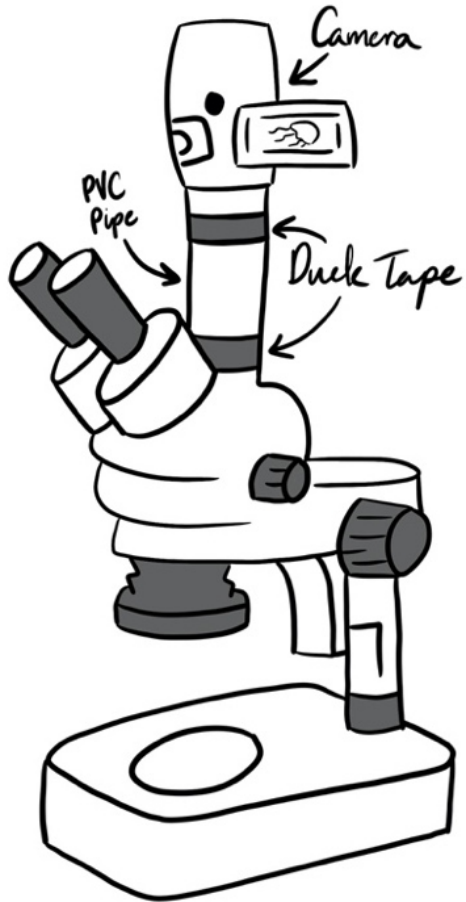
Fig. 1: Diagram of camera set up with Sony handycam HDR'CX560 attached to stereo microscope via a PVC pipe and duct tape.
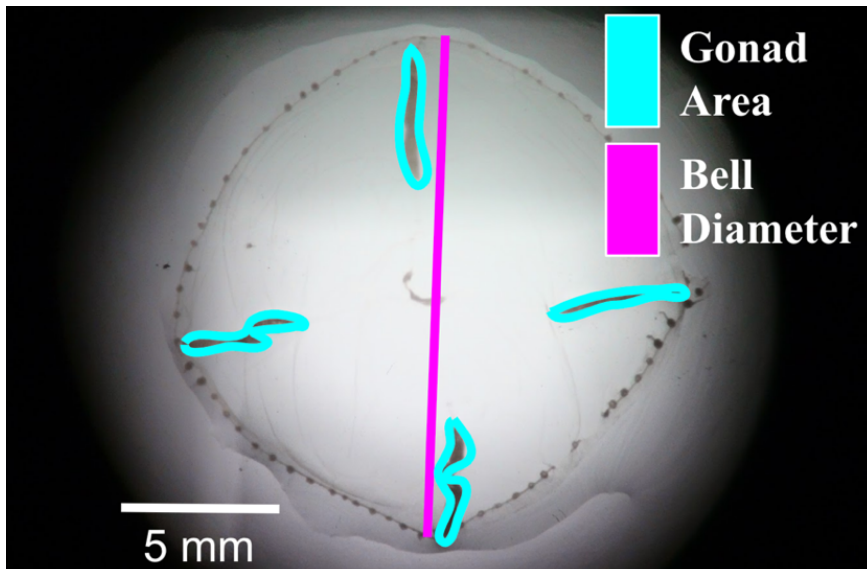

Figure 3: Diagram showing how the gonad area (blue) and bell diameter (pink) were obtained in ImageJ.

2. Correction Factors for Biomass Loss of Preserved Organisms

To account for the biomass losses due to formalin (Ahlstrom and Trailkill, 1963; LaFontaine and Leggett, 1989), we observed the changes in the bell and gonad weights, bell diameter, and gonad area of preserved *C. gregaria* and *E. indicans* over time. The medusae were captured by dip netting from the docks in Coos Bay, Oregon near the Oregon Institute of Marine Biology. The specimens were separated into 2 groups of dissected and un-dissected. For the dissected organisms, gonads were surgically removed from the bell under a microscope using a scalpel and dissecting scissors. The dissected gonads and bells were each kept in their own separate container to prevent cross-contamination. The un-dissected organisms were preserved whole with each individual also being kept in their own container.

Photos of the organisms were taken prior to being weighted. To obtain bell diameter and gonad area, the same methods described in the starvation experiments were utilized (Fig. 1 and Fig. 2) with initial photos being labeled as day 1. To capture weight measurements, organisms were placed on a petri dish and dabbed with a Kim wipe until there was no longer a visible ring of water around the organism. Afterwards, they were weighed on an analytical balance to get an initial measurement, which was labeled as day 1. Immediately after initial measurements all specimens were placed in jars with the preservation liquid of a solution of formalin in seawater (~4% v/v).

Both the undissected and dissected *C. gregaria* and *E. indicans* were weighed and photographed periodically over a timeframe of either 81 days or 52 days to find the correction factors for the amount of loss in weight, bell diameter, and gonad area. Keeping track of the time of preservation is important to make adequate estimates of the original size and volume of the organism before preservation as the rate of change varies depending on the preservation time

(Mills et al., 1982; LaFontaine and Leggett, 1989). We then fitted logarithmic functions with the y-variable as the percent of weigh or area loss with days of preservation as the x-variable (lm(weight or area/diameter) ~ log(day)) using R Studio Version 1.3.1093 (RStudio Team, 2020) with packages tidyverse (Wickham et al., 2019), reshape2 (Wickham, 2007), Rmisc (Hope, 2013), fitdistrplus (Delignette-Muller and Dutang, 2015), ggpmisc (Aphalo, 2020). and gglot2 (Wickham, 2016).

3. Morphological Measurements

Selected individuals of *Clytia gregaria* and *Eutonina indicans* were collected during winter of 2019 (March 2-14, 2019) and summers of 2018 and 2019 (July 3-12, 2018 and July 14-27, 2019) from five stations along both the Newport Hydrographic line (NH) and Trinidad Head Line (TR) within the North Califonria Current (Fig. 3). Due to the narrower and more pronounced shelf slope of the TR transect, stations were put much closer to one another. The samples were collected at fixed locations during the day using a coupled multiple opening-closing net and environmental sensing system with different openings and mesh sizes (MOCNESS; MOC 1= 1 m^2 aperture, 333 μm mesh, MOC 2= 4 m^2 aperture, 1-mm mesh; Guigand et al., 2005). After the nets were recovered, a subsample was placed in a cold petri dish to select hydromedusae. The selected *C. gregaria* and *E. indicans* were rapidly fixed in a solution of formalin in seawater (~4% v/v).

The same methods for photographing the medusae in the starvation experiments was used to obtain the morphological measurements of these samples (Fig. 1 and Fig. 2). To compare the size of the gonads in relation to the size of the bell, we utilized a gonadal index (gonad area/bell area). This gonadal index provides a better estimate of whether medusae during the winter prioritize the growth of their gonads when food is scare compared looking at the raw

measurements of the bell diameter and gonad area. Medusae that were observed to have damaged or incomplete gonads (<4 gonads), as well as those with damaged bells that compromised the bell diameter, were excluded from the final gonadal index data set. All the data for the bell diameters and gonad area, including those that were considered "damaged," was used to calculate mean bell diameters and mean gonad area separately.

Time since preservation was estimated to the closest month (1 month = 30 days) by comparing the approximate time of collection from the Newport Hydrographic line (NH) and the Trinidad Headline (TR) to the time of photo capture. The calculated days since fixation for each individual was used to estimate the biomass loss of each individual collected from the 2018 and 2019 samples. This calculated date was then inputted into the formulas created from the biomass loss experiments to find the percentage of decrease since fixation to back-calculate estimated live measurements. A student t-test was performed in Microsoft Excel (Version 16.42) to test for the significance in the gonadal index between winter and summer.
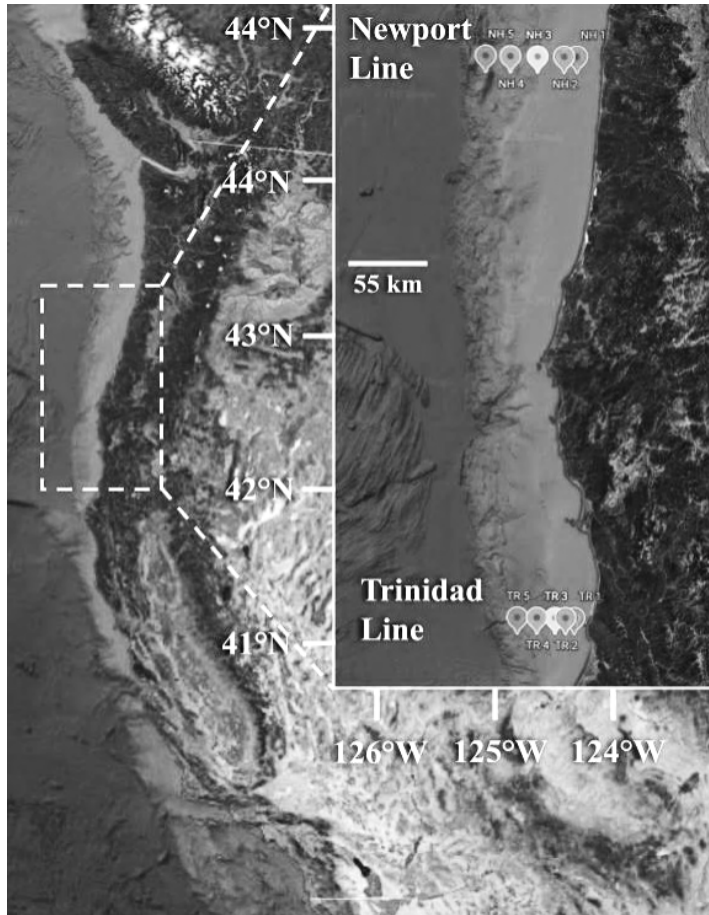
Fig. 3: Sampling stations along the Newport Line and Trinidad Line within the Northern California Current where hydromedusae were collected in the summers of 2018 and 2019 and winter of 2019.

Results

1.Starvation Experiments

Bell diameter and gonad area of *C. gregaria* decreased across all medusae in every treatment group between day 0 and day 7 (Fig. 4). The level of decrease of the bell diameter was similar amongst all groups with an average decrease of approximately 2 mm between day 0 and day 7 for all feeding groups. The gonad area decrease was most significant in the unfed group with an almost a four-fold decrease between day 0 and day 7. The gonad area of the medusae fed once a day had a two-fold decrease while those fed twice a day had an almost three-fold decrease (Table 1). This intense decrease of the gonads in the unfed individuals can also be seen in the

decrease of the gonadal index where the unfed individuals decreased by an average of around

80% compared to the 55% decrease of those fed once a day and the 45% decrease of those fed
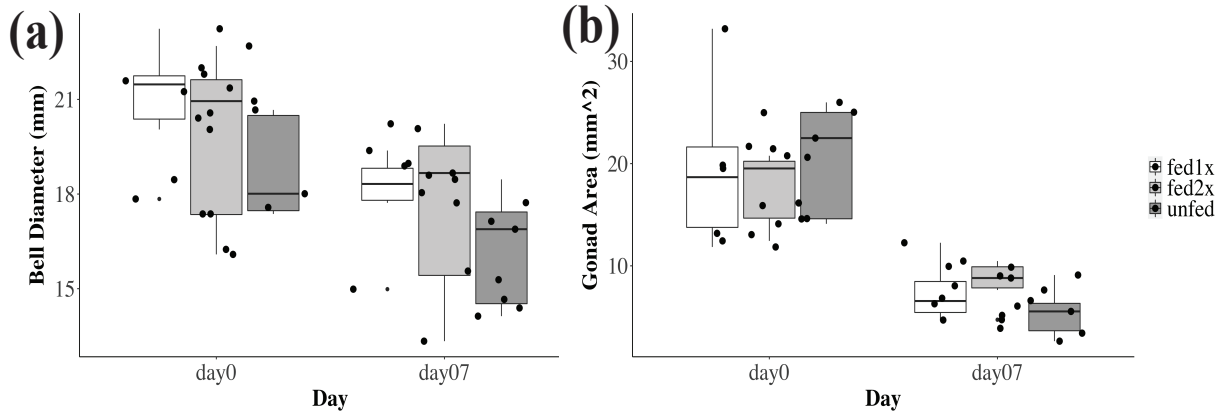
twice a day (Fig. 5).

Fig. 4. Comparison of the average **(a)** bell diameter and **(b)** gonad area between day 0 and day 7 of *C. gregaria* who were either fed 10 ml of plankton solution once a day, fed 10 ml of plankton solution twice a day, and those that were unfed.

Table 1. Average and standard deviation of the bell diameter and gonad area from all feeding groups on day 0 and day 7.

| Type	Day	Feeding Group	Average	Standard Deviation
Bell Diameter	0	Fed 10 ml twice a day	20.98 mm	± 1.84 mm
Bell Diameter	0	Fed 10 ml once a day	19.67 mm	± 2.73 mm
Bell Diameter	0	Unfed	18.85 mm	± 1.60 mm
Bell Diameter	7	Fed 10 ml twice a day	17.94 mm	± 1.56 mm
Bell Diameter	7	Fed 10 ml once a day	17.45 mm	± 2.69 mm
Bell Diameter	7	Unfed	16.20 mm	± 1.77 mm
Gonad Area	0	Fed 10 ml twice a day	19.52 mm^2	± 7.86 mm^2
Gonad Area	0	Fed 10 ml once a day	17.50 mm^2	± 3.56 mm^2
Gonad Area	0	Unfed	20.26 mm^2	± 5.55 mm^2
Gonad Area	7	Fed 10 ml twice a day	7.37 mm^2	± 2.83 mm^2
Gonad Area	7	Fed 10 ml once a day	8.50 mm^2	± 1.86 mm^2
Gonad Area	7	Unfed	5.32 mm^2	± 2.22 mm^2
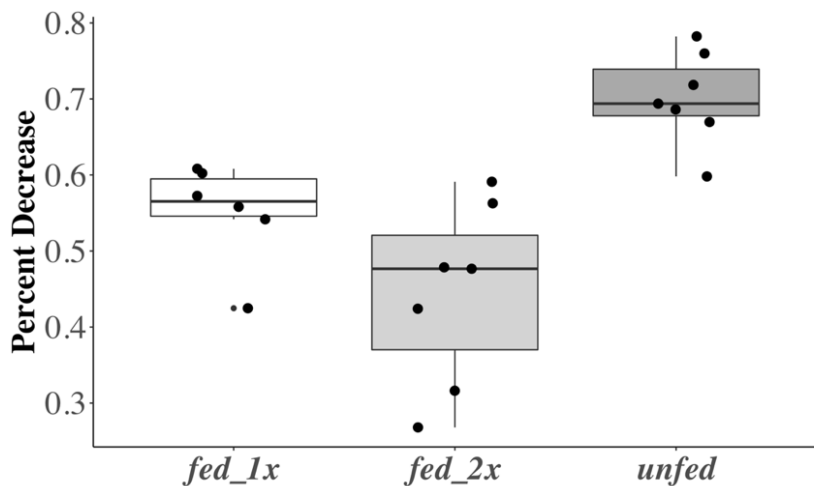
Fig. 5. Percent decrease of the gonadal index (gonad area/bell diameter) between day 0 and day 7 of medusae that were fed 10 ml of plankton solution once a day (average = 0.5513±0.0670, N = 7), medusae fed 10 ml of plankton solution twice a day (average = 0.4453±0.1195, N= 6) and those unfed (average = 0.7012±0.0609, N = 7).

2. Correction Factors for Biomass Loss of Preserved Organisms

While all formulas were found to be significant ($p < 0.05$), the levels of error varied between the species and between bell diameter, gonad area, and weight (Table 2, Figure 6). Overall, the bell weight and bell diameter formulas were more significant than the formulas for the gonad weight and gonad area. *E. indicans* showcased higher levels of variation in bell diameter, gonad area, and gonad weight in comparisons to the *C. gregaria. C. gregaria* has about twice the amount of decrease in both bell diameter and gonad area than *E. indicans.* Gonad area and gonad weight both garnered low R^2 despite the significance of their p-values. The gonad weight had the highest amount of deviation and was the only one of the groups to be observed for only 52 days compared to the 81 days of the other groups. Both species started showing stabilization in the bell diameter around days 60-80. Stabilization of the gonad area came quickly for *E. indicans* at around days 10-20, but gonad area of *C. gregaria* didn't stabilize until around days 60-80. The wet weights of both the bell and the gonad didn't show signs of stabilizing within the 82 days of observation but the amount of decrease did lessen over time.
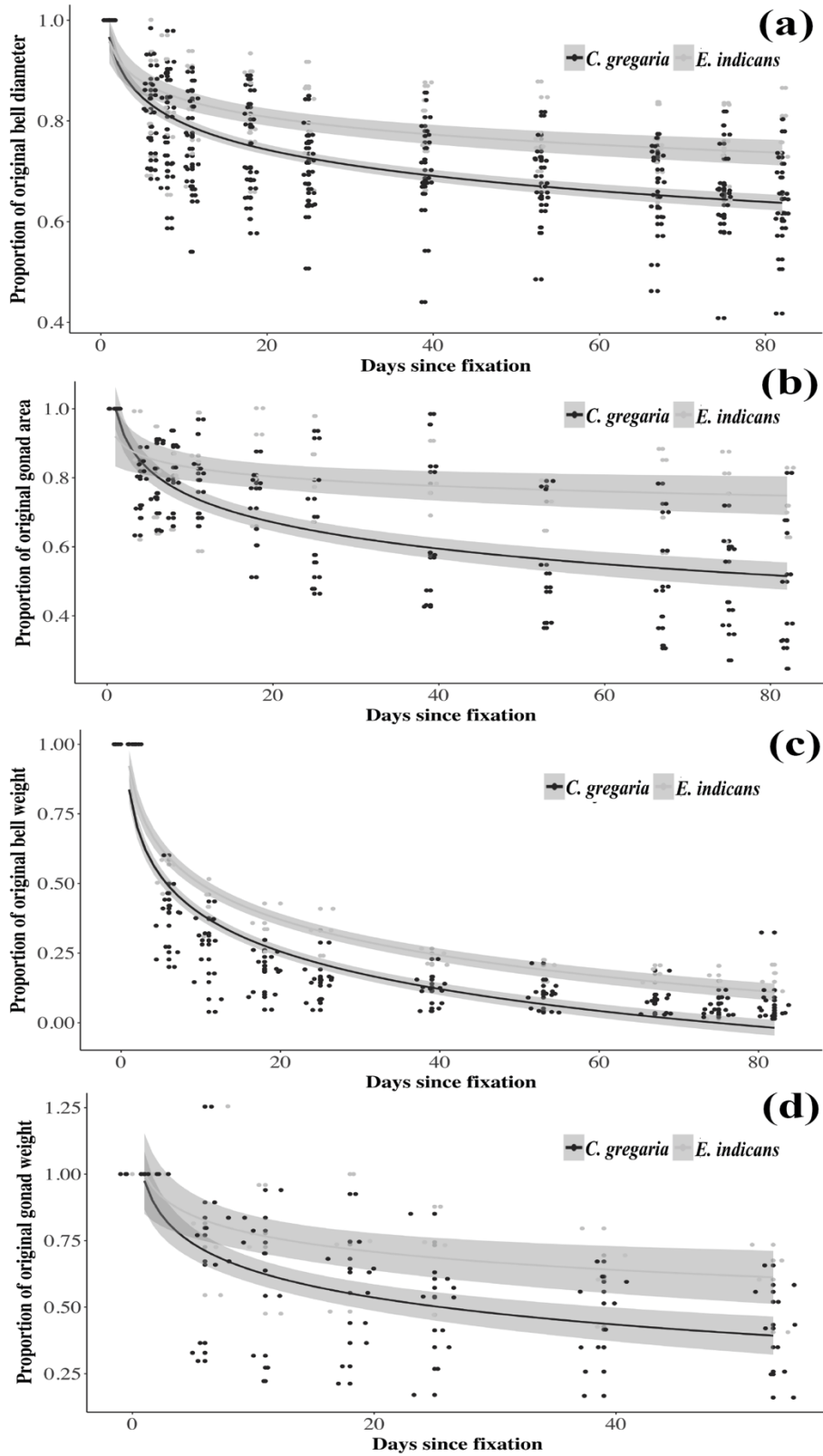
Fig. 6. Change in the bell size (whole medusae) and gonad size over the course of 81 or 52 days of preservation for *C. gregaria* and *E. indicans* **(a)** Change in bell diameter **(b)** Change in the gonad area **(c)** Change in the bell weight **(d)** Change in the gonad weight

Table 2. Logarithmic regression fits of bell diameter, gonad area, bell wight, and gonad weight over time.

Species	Y	X	Formula	F	p-value	R²	N
C. gregaria	Proportion of Original Bell Diameter	Days since fixation	-0.110*log(day)+ 1.002	125	<2.2e-16	0.51	23
E. indicans	Proportion of Original Bell Diameter	Days since fixation	-0.050*log(day)+ 0.958	56.84	4.486e-11	0.39	8
C. gregaria	Proportion of Original Gonad Area	Days since fixation	-0.075*log(day)+ 0.967	327.2	<2.2e-16	0.56	10
E. indicans	Proportion of Original Gonad Area	Days since fixation	-0.039*log(day)+ 0.920	8.122	0.0065	0.13	4
C. gregaria	Proportion of Original Bell Weight	Days since fixation	-0.194*log(day)+ 0.837	652.2	<2.2e-16	0.83	13
E. indicans	Proportion of Original Bell Weight	Days since fixation	-0.184*log(day)+ 0.922	520.3	<2.2e-16	0.93	4
C. gregaria	Proportion of Original Gonad Weight	Days since fixation	-0.147*log(day)+ 0.975	59.53	7.198e-11	0.46	10
E. indicans	Proportion of Original Gonad Weight	Days since fixation	-0.098*log(day)+ 1.002	14.43	0.0008	0.33	4

3.Morphological Measurements

We analyzed a total of 157 medusae with 82 being *C. gregaria* and 75 being *E. indicans*.

When the bell diameter and gonad area were corrected using the formulas from Table 2, the

gonadal index decreased considerably with the uncorrected data being between 35 and 80-fold

larger than the corrected data. Both *C. gregaria* and *E. indicans* had a higher gonadal index

(gonad area/bell area) in winter compared to summer, however, the difference was not

significant in both the corrected and uncorrected data (Fig 7, Table 3). But there was a significant

difference between gonad area and the bell diameter between summer and winter (Fig. 8, Table
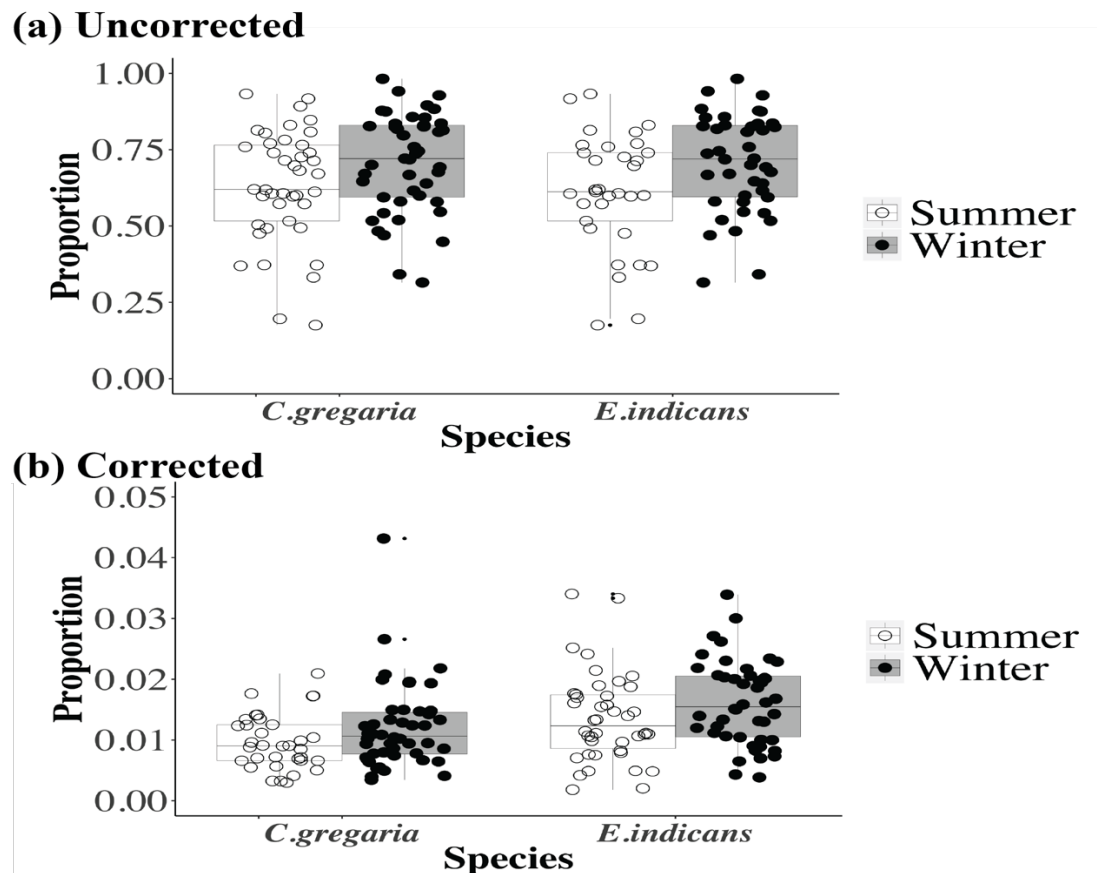
4).

Fig. 7. (a) Comparison of the gonadal index (gonad area/bell area) during a time of high food availability (summer) and low food availability (winter) of preserved *C.* and *E. indicans* preserved and collected from the North California Current in summer of 2018 and 2019, and winter of 2019 **(a)** before corrections and **(b)** after bell diameter and gonad area were corrected using correction factors from Table 2.

Table 3. Average gonadal index (gonad area/bell area) of both the uncorrected and corrected values from the *C. gregaria* and *E. indicans* from summer and winter. P-values are the p-wise comparisons of the indices between winter and summer.

| Uncorrected/ Correct	Species	Season	Average Index	Std. Deviation	p-value between winter and summer	N
Uncorrected	*C. gregaria*	Summer	0.5374	± 0.1489	0.0639	34
Uncorrected	*C. gregaria*	Winter	0.5770	± 0.2510		48
Uncorrected	*E. indicans*	Summer	0.6051	± 0.1683	0.4263	33
Uncorrected	*E. indicans*	Winter	0.6205	± 0.2192		42
Corrected	*C. gregaria*	Summer	0.0067	± 0.0045	0.0830	34
Corrected	*C. gregaria*	Winter	0.0136	± 0.0073		48
Corrected	*E. indicans*	Summer	0.0120	± 0.0069	0.1035	33
Corrected	*E. indicans*	Winter	0.0161	± 0.0069		42
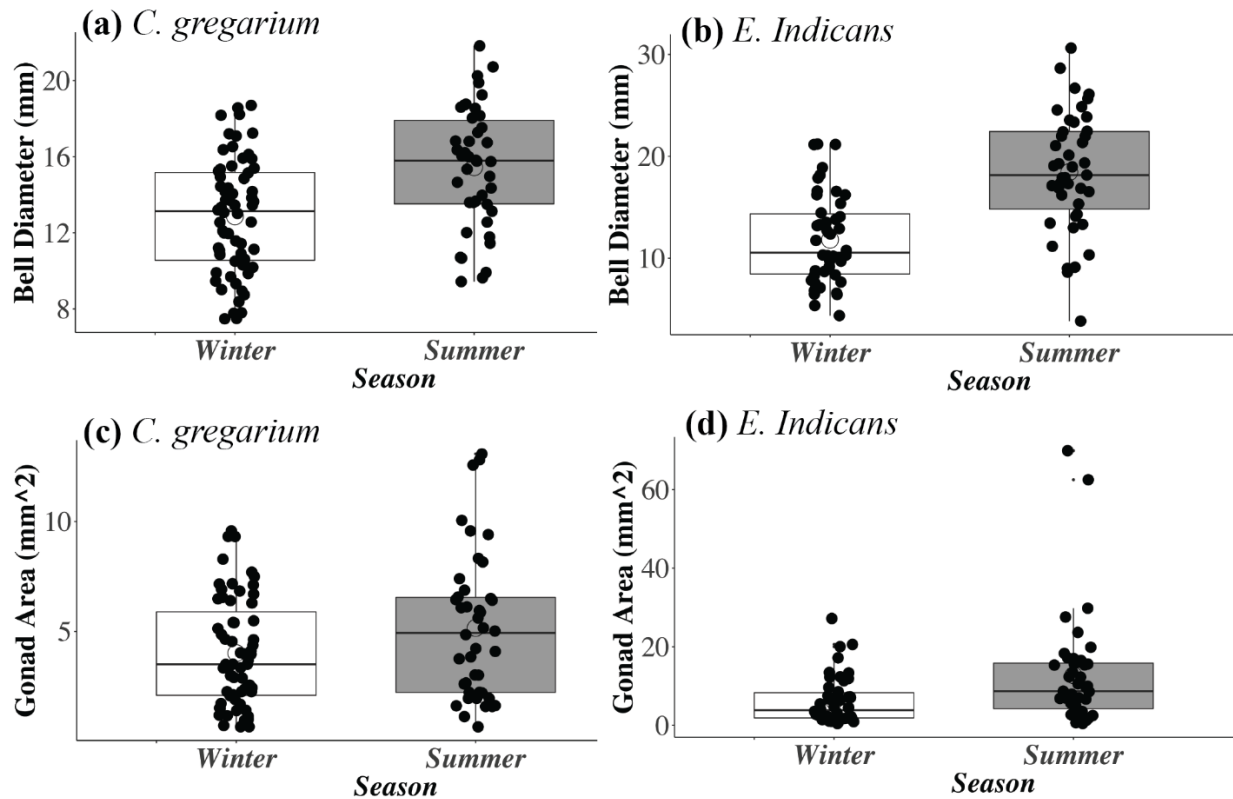
Fig. 8. Comparison of the uncorrected bell diameter of **(a)** *C. gregaria* and **(b)** *E. indicans* and gonad area of **(c)** *C. gregaria* and **(d)** *E. indicans* during a time of high food availability (summer,) and low food availability (winter) preserved and collected from the North California Current in summer of 2018 and 2019, and winter of 2019.

Table 4. Average and standard deviations of the raw morphological measurements of the bell diameter and gonad area of C. gregaria and E. indicans from both winter and summer. P-values are the p-wise comparisons of the bell diameter or gonad area between winter and summer.

Species	Season	Bell Diameter or Gonad Area	Average	Std. Deviation	p-value	N
C. gregaria	Summer	Bell Diameter	18.9274 mm	± 5.6458 mm	5.1142×10^{-5}	42
C. gregaria	Winter	Bell Diameter	11.6988 mm	± 4.3846 mm		63
C. gregaria	Summer	Gonad Area	5.1608 mm^2	± 3.2995 mm^2	0.0427	42
C. gregaria	Winter	Gonad Area	4.0136 mm^2	± 2.4256 mm^2		63
E. indicans	Summer	Bell Diameter	18.4813 mm	± 5.8077 mm	8.6804×10^{-5}	43
E. indicans	Winter	Bell Diameter	11.8179 mm	± 4.4111 mm		45
E. indicans	Summer	Gonad Area	12.7336 mm^2	± 13.39946 mm^2	0.0005	43
E. indicans	Winter	Gonad Area	6.2029 mm^2	± 6.1143 mm^2		45

Discussion

1.Starvation Experiments

The starvation experiments showcased that the growth/development of the bell and gonads of *C. gregaria* vary with food availability. Unlike predicted, the gonad and bell diameter of all observed medusae decreased, however, those that were unfed decreased most drastically. This could be due to the lack of food that was provided to the different groups that even those fed 10 ml twice a day was still insufficient compared to the amount they would ingest in the natural environment. Past experiments with hydromedusae in laboratory environments have found that the amounts of food provided are often insufficient to stimulate feeding (Roosen-Runge, 1970). Therefore, all the groups could have been starved leading to a decrease in size, which does match up with past studies which observed a decrease in bell diameter in jellyfish when starved (Ishii and Båmstedt, 1998).

Another issue in the feeding was that the plankton was not caught daily, which could have led to feeding inactive or dead plankton. As *C. gregaria* do not actively pursue their prey (Mills, 1981), providing live prey is essential in ensuring that they encounter and capture their

prey as they prefer prey that is actively moving (Corrales-Ugalde and Sutherland, 2020). This is especially important when food concentrations are low, as they were in our experiments, as the ingestion rate of *C. gregaria* depends primarily on their encounter rate with their prey (Matsakis and Nival, 1989). Water circulation also plays a role (Matsakis, 1993; Roosen-Runge, 1970; Lucas et al., 1995) as continuous water flow has been found to be essential in maintaining the medusae (Lechable et al., 2020), as well as increasing the likelihood they encounter their prey due to their passive feeding nature (Adamík, 2006; Lucas et al., 1995: Mills, 1981). Another factor influencing growth could have also been temperature as there is a positive correlation between the growth rates of Clytia *spp.* and temperature up to 25 degrees Celsius (Matsakis, 1993; Lucas et al., 1995).

Nevertheless, we did still find that there were variations in the change in bell diameter and gonad area between the different feeding groups. This was most prevalent in the gonads where the gonad area of those fed once a day decrease less than those fed twice a day, while those that were unfed decreased the most (Table 1). Scarcity of food has shown to decrease the growth rate of Scyphomedusae into the negatives (Ishii and Båmstedt, 1998), and this experiment showcases that hydromedusae can do the same. We might have seen the medusae going through a process of degrowth, which has been shown to occur even in sexually mature adults during starvation (Hamner and Jenssen, 1974). The line between starved and complete starvation is unknown, which makes it harder to infer when we would see the prioritization towards gonadal growth due to starvation compared to a complete negative growth.

2.Correction Factors for Biomass Loss of Preserved Organisms

The hope with the preservation experiments was to create a formula to estimate live measurements from our preserved ones. In addition, we discovered how differently the gonads

and the bell react to preservation. The change in bell weight and gonads weight formulas were not utilized, as weights were not obtained from the samples taken from the Newport Hydrographic line and Trinidad Head Line. However, the observation of the changes in weight over time gave us an insight into the declines of the samples that were not observed in the change in bell diameter and gonad area observations. The weights showed how the bell changes more dramatically than the gonads in formalin, which is likely due to the chemical composition of the tissue in the bell having a much higher water content than the gonads (Larson, 1986).

Despite that wet weights were used which could cause overestimation of weight, estimates using wet weight are still fairly precise (Ahlstrom and Thrailkill, 1963). Declines of all samples were most prevalent during the first few days which correlates with past studies where the most rapid rate of shrinkage occurred during the first 24 hours and then slowed down (Ahlstrom, 1963; LaFontaine and Leggett, 1989). The rate of reduction in both the bell diameter and wet weight of *C. gregaria* and *E. indicans* in formalin matched that of previously recorded changes of other jellyfish species in formalin with similar trends in stabilization (Fig. 1; LaFontaine and Leggett, 1989).

The lack of stabilization and the high variation in the gonad data might indicate the amount of loss from the gonads in the formalin. Due to the fragility of the gonads, especially those of the *C. gregaria,* the gonads slowly broke apart both from the formalin and from being moved by the forceps. As gonads broke apart over time, it would lead to an increase in area due to the newly made edge, as well as a higher decrease in weight due to tissue loss. This physical break-up of the samples could have also stemmed from blotting using Kim wipes (Ahlstrom and Thrailkill, 1963). While preservation in formalin does have its caveats, it is one of the most

feasible ways to measure the size and weight of marine plankton as it is often not possible to do with fresh specimens (LaFontaine and Leggett, 1989).

3. Morphological Measurements

The gonadal index varied between season with medusae collected in winter having a slightly higher gonadal index compared to medusae collected in summer. This is comparable to the higher gonad to bell ratio of *A. aurita* in low food availability than in high food availability (Fig. 4; Lucas 1996). Despite that the difference in the gonadal index was not significant, there was a significant difference between the raw data of the gonad area and bell diameter between summer and winter (student t-test, $p<0.05$). The difference between seasons was most prevalent in the gonads, which has been shown in past studies where growth rate of the gonads was always significantly higher than the bell (Chiaverano et al., 2004). This means that while we rejected our hypothesis that the medusae would have a larger gonadal index during the winter compared to summer, we found that there was still a force driving differences in the bell diameter and gonad area between the two seasons.

As the data on the plankton abundance along the North California Current in the years of 2018 and 2019 is rather thin, it is difficult to truly know the difference in plankton abundance between the summer and winter of 2018 and 2019. What has been reported is the difference in the copepod assemblages along the Newport Hydrographic Line between summer and winter. The more fatty acid rich "northern copepods" are dominant during the summer, while the "southern copepod," that has lower nutritional quality and fat content, is more prevalent during the winter (Harvey et al., 2019). In general, the copepods present during the summer have a higher per capita bioenergetic content than those in the winter (Hoof and Peterson, 2006). As hydromedusae are known to be prevalent predators of copepods, especially *C. gregaria* (Daan,

1989)*, this difference in the nutritional value of the copepods present between the two seasons might indicate that the medusae are similarly well fed during both seasons, but obtain less nutritional value during winter.

Additionally, it has been found that *C. gregaria* tend to ingest in excess of their maintenance needs during the spring season (Larson, 1987), which also might explain the larger sizes present during the summer. There has also been evidence that hydrozoans can increase their growth with a small increase in carbon investment (Larson, 1986), therefore any increase in the nutritional content of their food could cause a drastic change in growth. This might explain why we did not find a significant difference between the gonadal index between the two seasons, while the gonad area and bell diameter were significantly higher during summer than winter for both *C. gregaria* and *E. indicans*.

Whether this difference is driven solely by seasonal production isn't clear. Another factor observed to affect the size of medusae in any given season is the population density (Lucas and Williams, 1994). In years of high medusae abundance, the medusae tend to be smaller in size compared to years of low abundance. This leads to a density dependent mechanism of regulating adult medusae size due to increased competition for food resources (Schneider and Behrends, 1994). Therefore, small size at maturity with a high population abundance is usually indicative of food scarcity (LaFontaine and Leggett, 1989). While there is evidence of this in Scyphomedusae, specifically *Aurelia aurita*, it is also likely that there is a population density dependent mechanism regulating the size of hydromedusae.

Conclusion

This study shows that there is some variation between the growth and reproduction of hydromedusae during a season of high production and a season of low production. Additionally,

differences in food availability and/or the nutritional value of food affect the nutritional state of hydromedusae. With the correction factors created based on formalin-preserved samples, we can begin to infer more about hydromedusae morphology while utilizing preserved samples.

Recent increases in eutrophication may lead to increases of jellyfish populations in some areas (Mills, 2001). Various species of jellyfish, including hydromedusae, have been associated with fish killing events in marine-farmed fish due to their predation on plankton populations including fish larvae and pelagic eggs (Purcell et al., 2013; Purcell and Arai, 2001). Hydromedusae also compete with juvenile fish as they have the shared prey of euphausiid eggs and other larvae (Larson, 1987). It is vital that we garner more knowledge of the seasonal dynamics of hydromedusae to aid in our understanding of how these population increases will affect plankton populations and commercial fisheries.

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