The Life of A Polyp: Reproduction in a deep-sea coral

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Abstract

Deep-sea ecosystems represent some of the most complex and least explored environments on Earth, characterized by their unique and diverse faunal communities and extreme conditions. This study investigates the reproductive biology of the deep-sea octocoral *Isidella tentaculum*, focusing on the seasonal dynamics of egg release and maturation through a comprehensive 15-month time-lapse imaging analysis conducted at Sur Ridge, off the coast of Monterey, California. Our findings reveal continuous egg production year-round, with notable peaks in late summer and fall, particularly from July to mid-October. We identified four distinct stages of egg maturation, that through annotation and analysis revealed overlapping cohorts of eggs present throughout the year. Remarkably, only 41.67 percent of the 25 tracked polyps released eggs, averaging a release less than one egg per polyp annually. While the study could not disclose the drivers of seasonal egg production, correlations with temperature and net primary productivity were explored. This research enhances our understanding of deep-sea coral reproductive biology and highlights the need for further studies in histology and morphology to fill critical knowledge gaps, ultimately informing conservation strategies for these vital marine organisms.

Keywords · Coral · polyp · eggs · Maturation stages

1 Introduction

Deep-sea ecosystems are among the least understood and most challenging environments to study. Coral and sponge communities at great depths differ significantly from shallow-water corals due to their distinct ecological niches, inaccessibility and extreme conditions. Despite these challenges, understanding deep-sea systems has become increasingly crucial in recent decades. From being considered a vast, empty void, the deep sea is now known to consist of complex systems of faunal communities such as cold-water coral reefs, sponge beds, and hydrothermal vents hosting a biodiversity rivaling the world's richest tropical rainforests (deep-sea-conservation.org).

Additionally, deep-sea environments play a vital role in key ecological and oceanographic processes, including thermohaline circulation, carbon sequestration, and nutrient regeneration (NASA, 2009; oceanprotect.org). While considerable progress has been made in studying shallow-water corals, most aspects of the life history of deep-sea corals remain unknown. The life history details of most deep-sea corals remain unknown and thus there is a need for continued research in the field. One particularly underexplored area is the reproductive patterns of deep-sea corals. Though challenging, understanding coral reproductive behaviors is essential for understanding their role in benthic communities and their resilience to increasing environmental change and human impacts. Increased interest in deep coral reef restoration techniques similarly requires further research in coral reproduction (noaa.org).

Shallow water corals have been widely studied, but little research exists on the maturation and life histories of deep-sea water corals. Generally, corals are long lived with species in the family Isididae. being estimated to be older than 829 years, based on radio-carbon dating (Wang et al. 2004). Corals remain reproductive throughout most of their lives and in orders such as Alcyoniidae reproductive age is reached between 7 and 37 years after settlement (Waller et al. 2023). When maturity is reached, there are two central modes of sexual reproduction for these sessile cnidarians: broadcast spawning and brooding. Broadcast spawning corals release gametes into the water column during mass spawning events when eggs are fertilized and develop into planula larvae. In brooding corals, fertilization occurs internally within the gastrovascular cavity of female polyps (noaa.gov). Most species known spawn only once a year, triggered by environmental cues such as temperature and productivity (Waller et al. 2021)

Deep-sea corals, particularly those in the class Octocorallia, and including our study organism Isidella tentaculum, exhibit diverse reproductive strategies. Most species are gonochoristic, with mature colonies displaying only male or female reproductive structures. (Kahng et al. 2020). The reproductive modes in octocorals are nearly evenly divided between brooding and spawners, but notably deep-sea Octocorallia tend to have a higher proportion of brooders. (Kahng et al. 2020). Furthermore, reproductive output and seasonal patterns also vary widely, with deep-water Octocorals exhibiting both punctuated and continuous reproductive periods throughout the year (Kahng et al. 2020).

While it is known that shallow water coral colonies can release over short periods during synchronous yearly spawning events, sparse information exists on the reproductive output of deep-sea coral colonies. Similarly, knowledge of gametogenesis and reproductive morphology and output is very limited for most species. Studies on alcyonaceans [lower case, I think for alcyonaceans, but upper case for Alcyonacea] have revealed continuously developing eggs at various stages of maturation throughout the year within female colonies (Eckelbarger 1998, Mohammed et al. 2016). In Octocorals, previous research has found development of eggs is initiated within polyps in gonads located along the ventral and lateral mesenteries which release clusters of primordial germ cells that adhere to the mesentery walls (Simpson 2009). As maturation progresses, these gametogenic cells are released into the coelenteron, where they become single differentiable individual eggs that are ready for fertilization (Eckelbarger 1998). It remains unknown whether the eggs are fertilized after separation or at a later point before release, and it is understood that the eggs ascend through the oral disc and are ultimately released from the polyps' tentacles (Simpson et al. 2009). While studies are limited, in general octocoral development begins with an increase in the size of the gonads located on the ventral and lateral sides of the mesentery (Eckelbarger et al., 1998; Benayahu et al., 1989). Gonads extend into the polyp cavity and release undifferentiated clusters of primordial germ cells (stage 1) from the basal region of the polyps (Benayahu et al., 1989). At this stage, the germ cells are indistinguishable and form clusters. As development progresses, these clusters separate (stage 2) into differentiable eggs. In stage 3, eggs move toward the upper coelenteron where the eggs are retained in the upper coelenteron until they are fully mature (Benayahu et al., 1989). Significant growth and differentiation occur during this period, preparing the eggs for the final stage. Once maturation is complete, the eggs move out of the mesentery and are released onto the tentacles from the oral disc of the polyp (stage 4). In stage 4 mature eggs remain on the tentacles until the polyp and colony experience optimal environmental conditions for release.

Despite the complexity of these reproductive processes, it is important to note that not all polyps within the same colony participate in reproduction. Some polyps remain non-reproductive throughout their entire lifespan. This phenomenon can be attributed to the dimorphic nature of octocorals, which possess two types of polyps: siphonozooids and autozooids. In Alcyonaceans, the reproductive structures are specifically located in these siphonozooids while autozooids is in charge of allocating energy within the colony between polyps (Simpson et al. 2009). While the reproductive characteristics of Alcyonaceans are well-documented, there remains a significant gap in our understanding of the temporal patterns in gamete development and seasonal cycles of reproduction in these deep-sea corals.

Previously, it has been difficult to collect continuous temporal data on their reproductive behavior and thus so far only less than 7% of any deep coral taxa have had any aspect of reproduction reported. Advancements in technology, such as time lapse camera systems, are improving the feasibility of such studies (Girard et al. 2022). These advances allow scientists to conduct detailed observations of organismal behavior over a longer period making it possible to understand the life history of organisms at a higher level.

The goal of this study is to characterize the reproductive seasonality and maturation stages in the octocoral Isidella tentaculum by tracking reproductive behaviors in both the Isidella colony and individual polyps through time lapse images collected over a 15-month duration. This research will contribute to a more comprehensive understanding of deep-sea coral reproductive behavior, ultimately aiding in our understanding of their biology and critical ecological functions.

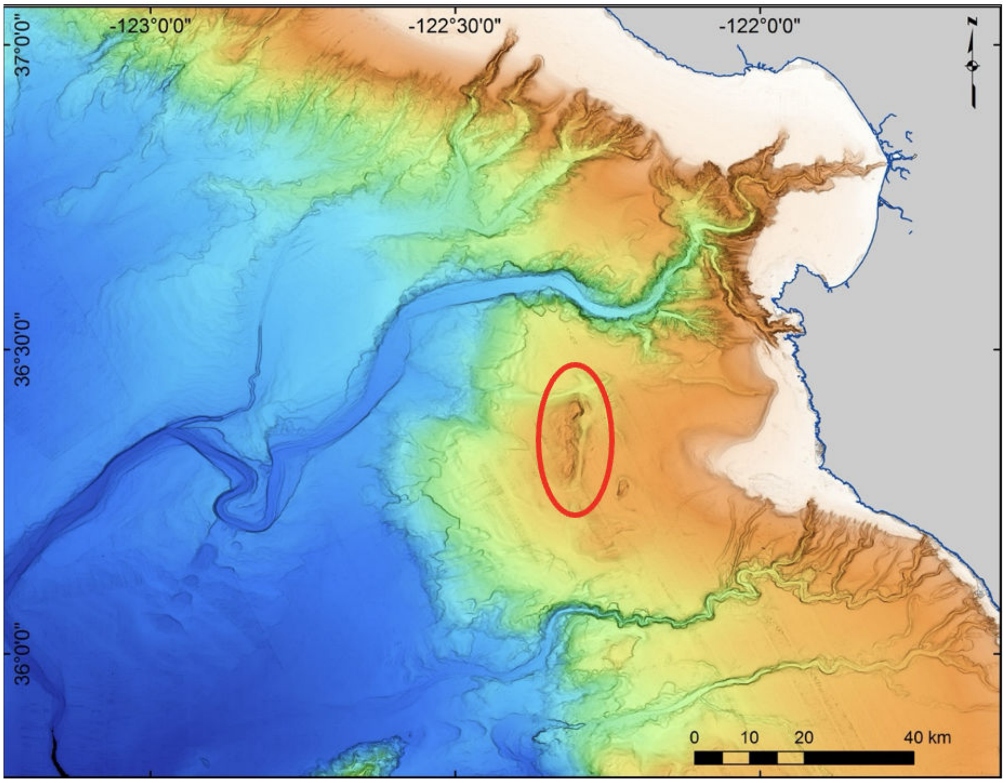
2 Methods

2.1 Materials and study area

Time lapse imagery was collected at Sur Ridge which lies off the coast of Monterey, California (figure 1a,b). Sur Ridge offers abundant deep-sea communities of sponges and corals at depths of 800-1500 m. In November 2022, between November 15th 2022 and January 30th 2024, a time lapse imagery system was deployed on the sea floor of Sur Ridge at a depth of 842 m to investigate deep sea coral behavior. Images were collected with the Macro Coral Camera System (MCcam) which consists of a digital Canon EOS 5D Mark IV, lens (focal distance 1m) and control electronics, all contained within a pressure housing with a view port, coupled to dual strobe lights. The system was mounted on a steel frame and for the second deployment a thermistor was mounted to the frame. (figure 1c).

2.2 Image collection

The camera was deployed facing the bamboo coral Isidella tentaculum, capturing images at 15-minute intervals. The short interval allows for nearly continuous remote observations of the colony's reproductive behavior and potential seasonality. This interval was chosen to balance the need for power conservation with the necessity of capturing critical behaviors that might be missed with longer intervals. Over a course of 15 months the camera captured ca. 40.000 6720 x 4480 72 dpi 24-bit images at f/10 1/20 sec ISO 400. The field of view of each image measures 29x43 cm, corresponding to 1/5 of the entire colony size. The camera was initially deployed at Sur Ridge for seven months until June 5th, 2023, at which time it was retrieved for battery replacement. It was then redeployed on June 27th, 2023, for an additional six months, remaining in place until January 30th 2024. All pictures were cropped to maintain a consistent frame as the redeployment resulted in a slightly different fields of view.



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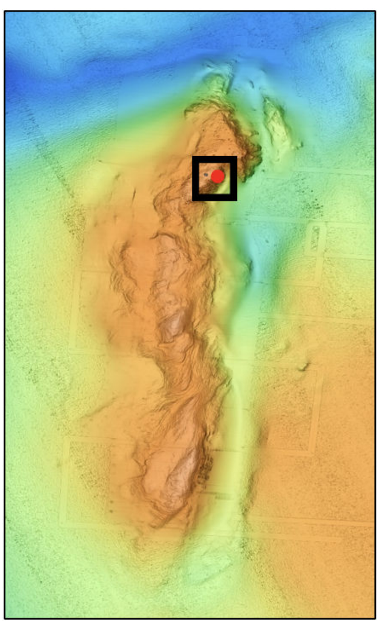

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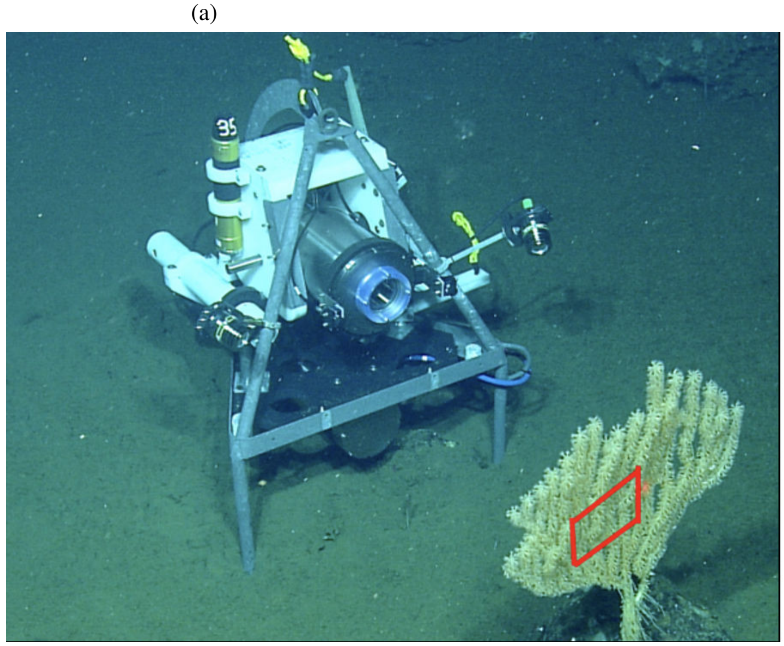


(c)

Figure 1: Study area and camera system. **a)** Map of Monterey Bay Canyon, with study site, Sur Ridge, outlined, **b)** Map of sur ridge with camera system location shown with the red dot. **c)** camera system facing the Isidella colony.

2.3 Annotation

2.3.1 Colony reproduction

To quantify the total colony reproductive output and investigate for seasonality, a total of 320 of the 4000 images, corresponding to 1 every week for the 15 months period, were selected. Within each week, the image corresponding to Tuesdays at 2:30 pm was analyzed. Using Python packages os, numpy, and cv2, a code was created to randomly select 5 Regions of Interest (ROI) with a 5x5 cm size. Reproductive output was then manually determined by counting the number of polyps and the total number of eggs within each ROI (figure 2). A polyp was included if any part of its structure fell within the bounds of a given ROI. If a polyp only had its stalk within the bounds, but not the oral disc, it was excluded from counts. If most polyps within the ROI were closed or in a position that concealed the oral disc and tentacles, an image as close to the original Tuesday 2:30 pm as possible was chosen to determine the number of eggs visible on that polyp.

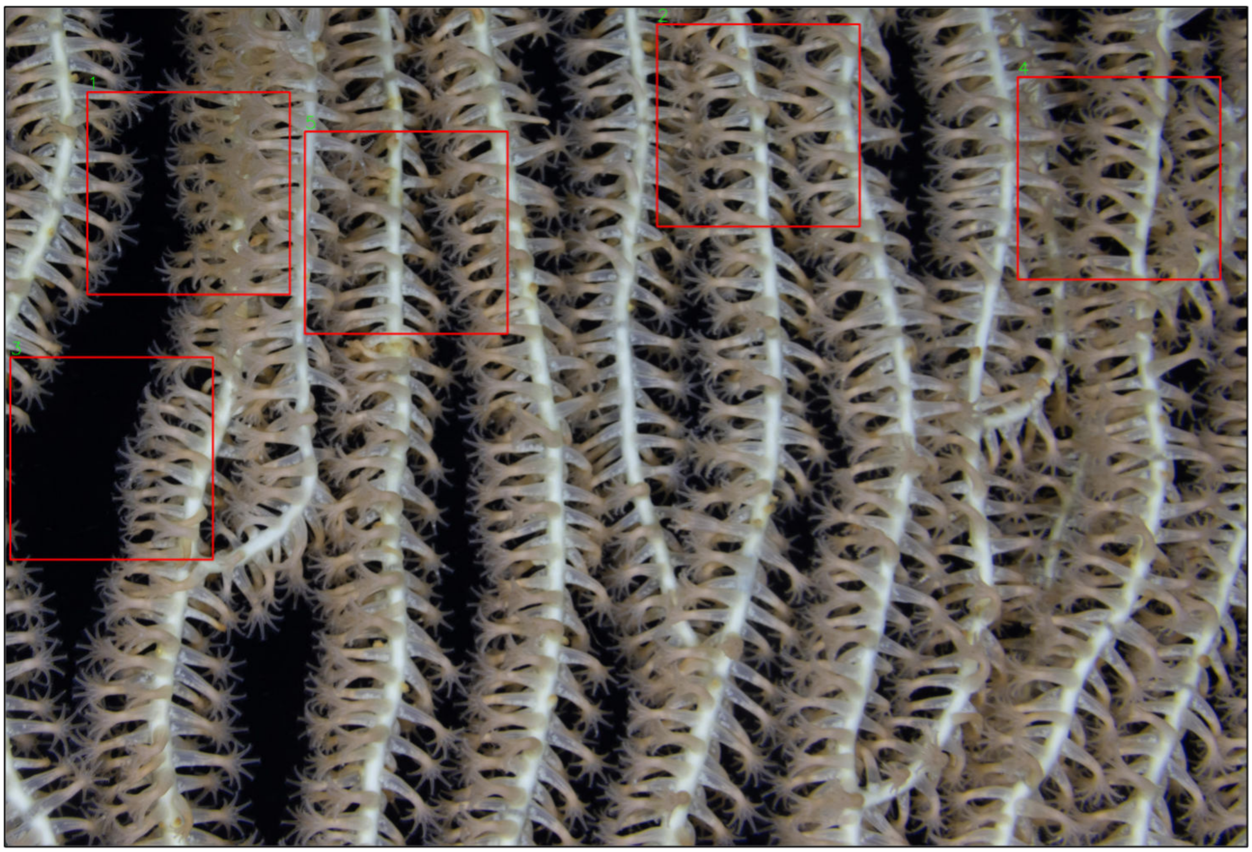


Figure 2: Example from 9/21/24 of the 5 ROIs that were annotated to account for colony reproductivity during that week.

2.3.2 Egg maturation stages

To describe the development and maturation of eggs in detail throughout a longer temporal scale, 25 polyps were identified and monitored through a one-year period (see figure 4 & 5). Every 7th day for 48 of the 52 weeks, each polyp was annotated for the number of eggs found in each stage of maturation and tracked for the duration an individual egg remained on a polyp tentacle. Four weekly sampling dates were not quantified due to polyps being closed or obscured from view for extended periods.

To help ensure all developing eggs within a sampling date were accurately staged, all 90 images recorded within that day were examined to determine the best image to score development across all polyps. In addition, two additional images with clear views of all 25 polyps were examined to help ensure eggs development scoring was accurate. To estimate the total number of eggs released during the entire year, we calculated the average number of eggs released from the tracked polyps, multiplied that value by the average number of polyps within the five ROIs across all dates (in the coral reproductivity analysis) and scaled that value by determining the ratio of the ROI size relative to the total colony size.

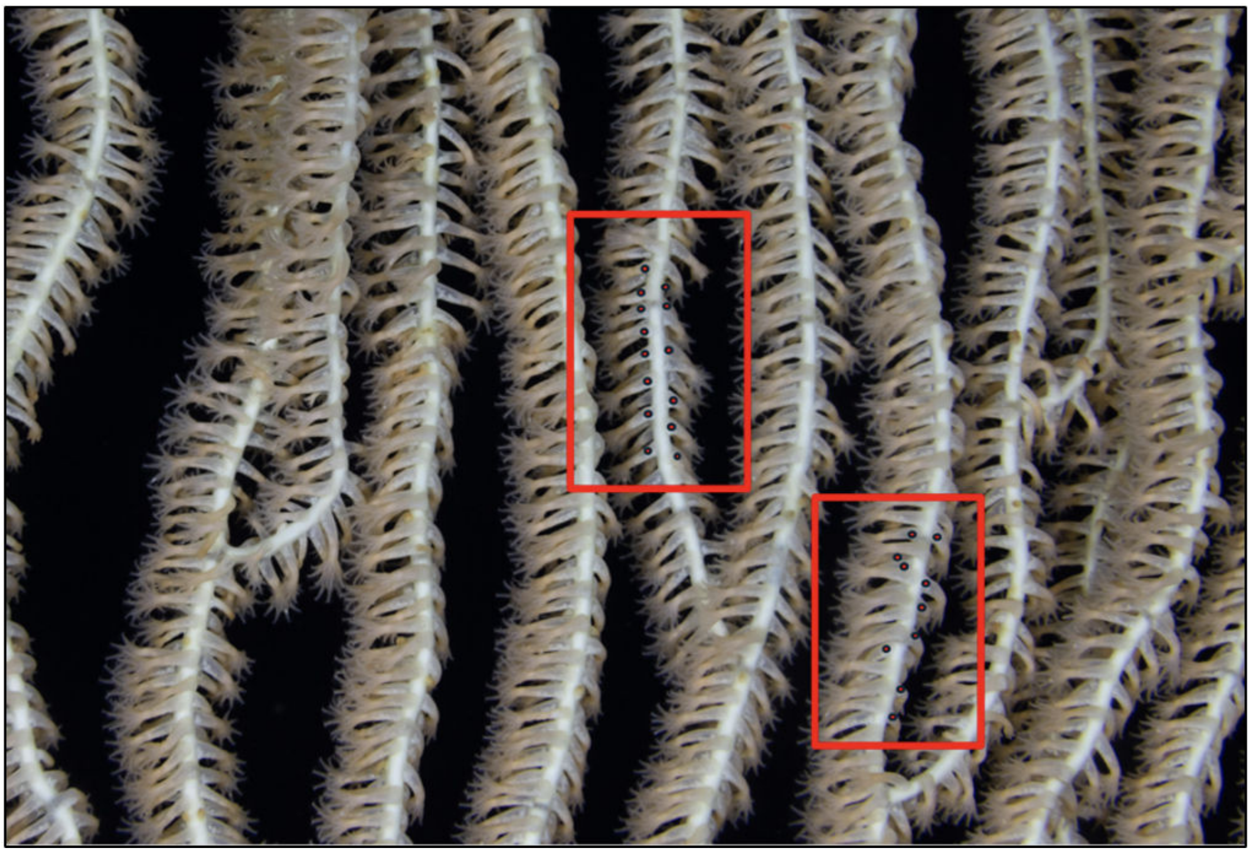
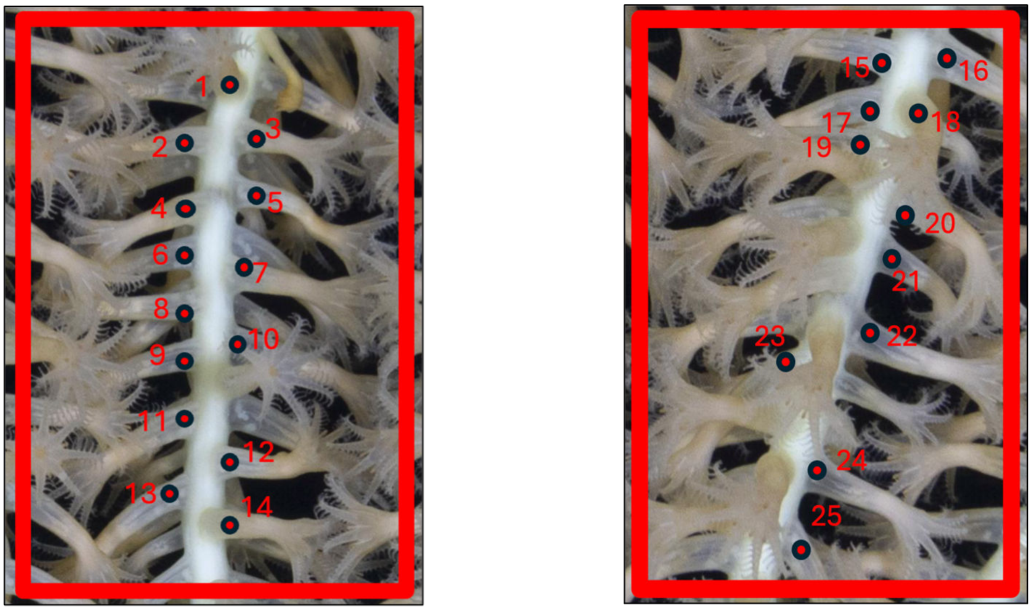


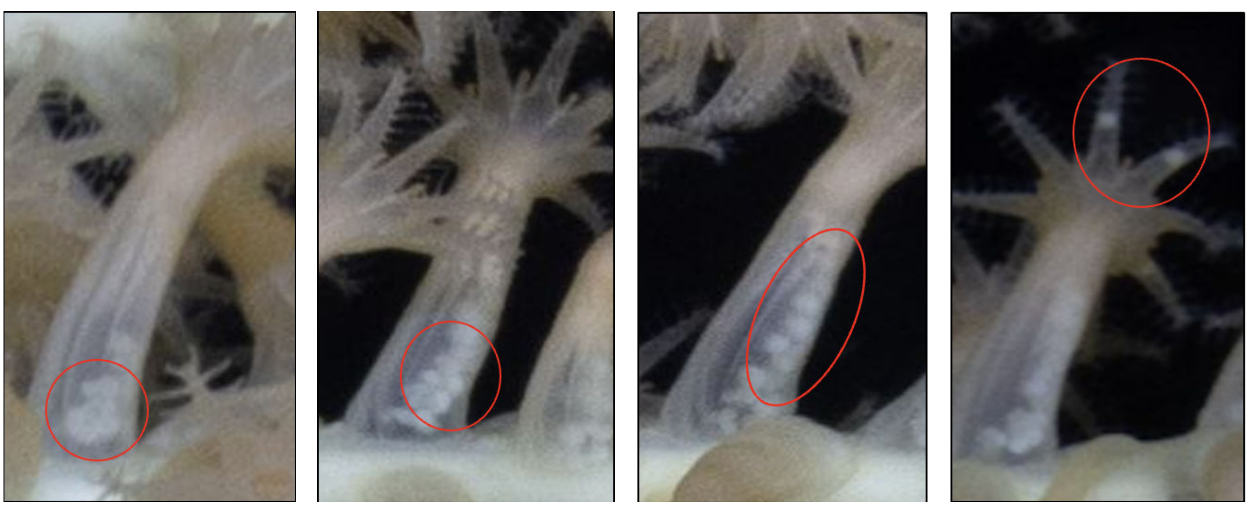
Figure 3: Tracked individuals seen in the entire FOV.

To quantify and track the development and maturation of eggs, four distinct stages of egg maturation were manually identified and characterized (figure 3). These stages were based on previous studies focusing on octocoral sexual maturation and gamete development described in the introduction. For each polyp the maximum stage at which the polyp had eggs in was annotated



(a) (b)

Figure 4: Tracked individuals numbered from 1-25.



(a) (b) (c) (d)

Figure 5: Stages of polyp maturation. a) Stage 1, undifferentiable clusters of primordial eggs at the base of the polyp. b) Eggs separating in stem. c) Eggs moving up the mesentery in the stalk toward oral disc. d) Mature Eggs in the tentacles ready for release.

2.4 Additional data

We explored correlations between seasonal patterns in reproductive status and in-situ temperature and net photic zone primary production, two factors that may be important in determining patterns of deep-sea coral reproduction (Waller et al. 2009) Net primary productivity (NPP) data was obtained from the Oregon State Ocean Productivity homepage using the VGPM algorithm and MODIS satellite data (REF). To estimate NPP in the region of our study site, we averaged monthly estimates of NPP within a 100x100 km area centered on Sur Ridge (36.33°N, -122.33°W) from January 2022 to July 20th, 2023. NPP files were download in HDF format, converted to text files and opened in Excel. NPP data is provided across a +90° to -90° latitude and -180° to +180° longitude grid with a 1/6-degree resolution cells. The cell number corresponding to our study area was identified by calculating the center of each grid cell. For a grid cell at row i and column j, the latitude and longitude of the center were calculated as:

$$Row = \frac{90 - latitude}{\frac{1}{6}}$$

$$Column = \frac{180 + longitude}{\frac{1}{6}}$$

NPP for a 6x6 matrix of cells, representing 100km x 100km, surrounding the grid cell most closely centered above Sur Ridge was extracted and summarized for this study.
Available temperature data was collected from a thermistor attached to the camera system. The CTD performed temperature measurements every 5 minutes starting June 23rd,2023, and ending January 31st, 2024. As this period only covered the second half of the study period, additional temperature data was added from a temperature logger incorporated in an Aquadopp current profiler located near the camera system at Sur Ridge. Available added data was collected between October 18th, 2022, and May 4th, 2023, leaving a month in between the two-temperature dataset for which no data was recorded.
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3 Results

3.1 Temporal dynamics of polyp egg release

Egg production was evident, but variable throughout the year. Egg release was highest in late summer and fall, from July through mid-October, with the peak in early October. A second, smaller, intra annual peak occurred in January or 2023 and 2024. The lowest periods of reproductive output were from October through December, as well as between February and May. The lowest proportion of ROI's with no eggs was seen in the late summer and early fall.

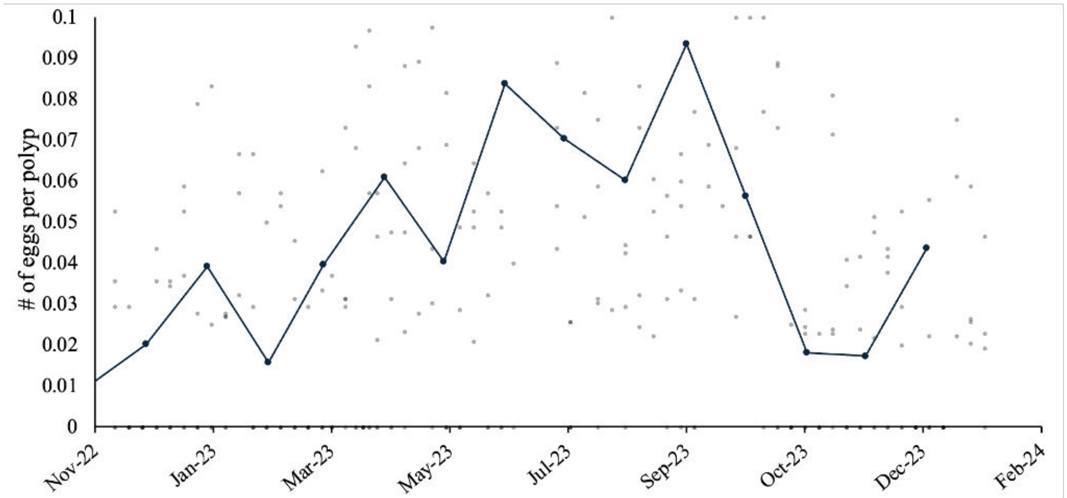


Figure 6: Time series of number of eggs per polyp released by the colony between November 2022 and February 2023. Line represents monthly averages.

3.2 Seasonal cycles in maturation stages of tracked polyps

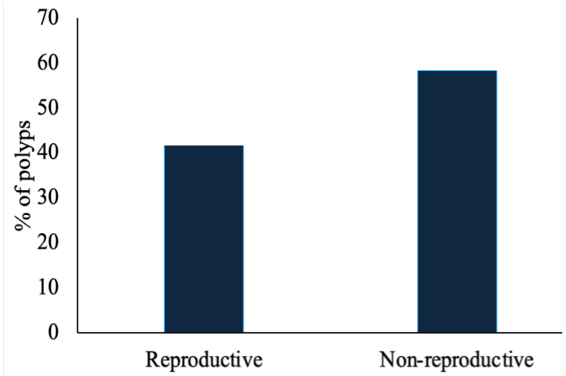
Tracked individual polyps showed overlapping seasonal cycles of production, development and egg release. Of the 25 tracked polyps, one died mid-year and from these remaining 24 live polyps, including the unreproductive polyp, only 14 (41.67%) released a total of 18 eggs over the one-year annotated period (figure 7). From this pattern it can be estimated that each polyp releases less than one egg each year, 0.75 eggs/year. The entire coral colony consists of approximately 2,811 polyps, considering the size of the field of view relative to the colony's overall size. Of these polyps, 1,171 (40% of the total amount) are expected to release a total of 2,108 eggs in one year (see table 1).

Total	Tracked individuals	Upscaling Calculation	Colony
# of polyps	24	$(37.45) \cdot (14.4) \cdot (5.21)$	2811.41
# of eggs released	18	$\frac{18}{24} \cdot 2811.41$	2108.56
# of reproductive polyps	10	$0.583 \cdot 2811.41$	1639.99
# of non-reproductive polyps	14	$0.4167 \cdot 2811.41$	1171.42

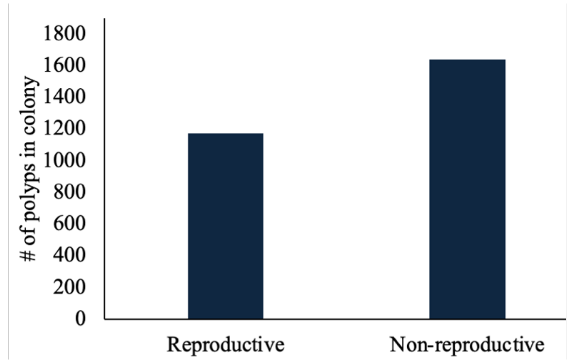
Table 1: Table showing tracked individuals, upscaling calculations, and colony results.

The duration of time that eggs remained on the tentacles before release showed considerable variation. While most polyps released their eggs after approximately one week, many eggs remained on a tentacle between two and eight weeks before release. Additionally, 2 polyps retained an egg on their tentacles for an extended period, three to four months respectively (Figure 8).

Tracked individual (figure 9) polyps revealed a continuous maturation process, with all egg maturation stages present simultaneously throughout most of the year, punctuated by seasonal periods of egg release. Notably, eggs in stages 2 and 3 of development remained consistently present throughout most of the study period and had higher and more variable percentages of presence in polyp. In contrast, the prevalence of stage 1 eggs showed a marked decrease around May. Stage 0 was included in our classification to account for polyps that had either released their eggs or remained reproductively inactive throughout the year. Seasonal patterns in the occurrence of fully matured (stage 4) eggs were observed, characterized by a minor peak in early January and a more pronounced peak in August-September, indicating



(a)



(b)

Figure 7: Proportion of reproductive polyps. (a) percent of polyps that are reproductive or non-reproductive. (b) Estimated number for reproductive and non-reproductive polyps in the entire colony.

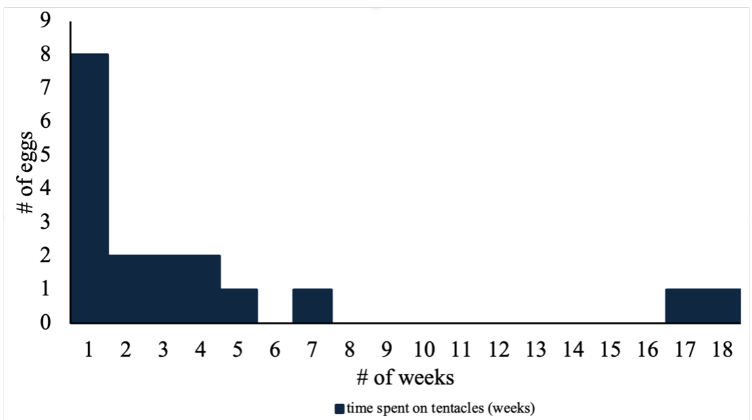


Figure 8: Number of weeks eggs remained on tentacles before release

specific periods of reproductive activity. Following the August-September peak, a rapid decrease in stage 4 eggs was observed in September-October.

Egg development and release from tracked polyps was seasonal with a peak in fall, as detected using ROIs. A small peak, with 2 eggs released, was seen in January, then in May egg release started to increase with a peak in late September to early October. Egg release declined quickly after October after which no additional eggs were released. Notably, a key difference in development among polyps was observed in May 2023. For polyps with eggs that reached stage 4, eggs began to rapidly develop and moved onto tentacles (fig 11b). In contrast, eggs within polyps where development only proceeded as far as stage 3 began to decline and were often resorbed. (figure 11a) .

3.3 Relation between food availability and temperature on the reproductive seasonality.

Overall, net primary productivity (NPP) at Sur Ridge, averaged over an 8-day period, exhibited a clear seasonal pattern. The highest productivity was observed in June 2022, with three smaller peaks occurring during the fall (Figure 12). A year-to-year comparison of average May NPP revealed that 2023 was particularly low in, ranking among the four lowest averages since 2003. Combined temperature data showed an overall trend from October 2022 to February 2024. Temperature ranged between 4.0 and 4.5 °C, with an increase from January to late August, followed by a significant drop in the fall. Despite the fluctuations there was a loose correlation between temperature changes and the number of eggs per polyp. This correlation is particularly evident where temperature trends appear to influence or coincide with changes in egg counts. Specifically, there was a lag of about 30 days between the low temperature observed in late August and the subsequent drop in colony reproductive output in late September (Figure 14). Generally, egg production seems to rise with small rises in temperature and drop when temperature drops.

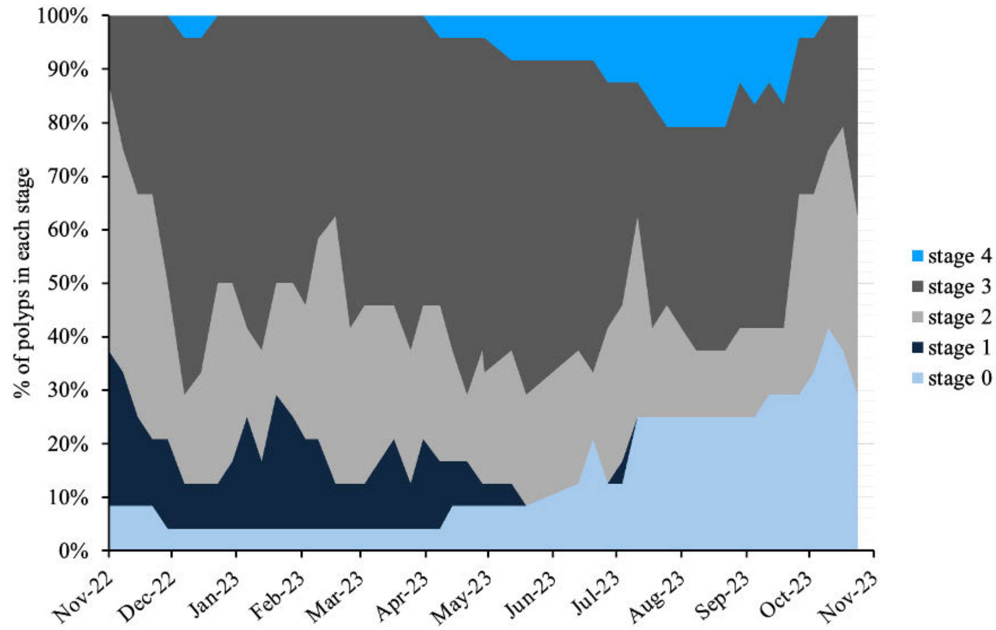


Figure 9: Temporal distribution of polyps across different reproductive stages between November 2022 and November 2023. Percentage of polyps in each stage over time, with stages 0 through 4 represented by different colors. Stage 0 (light blue), stage 1 (blue), stage 2 (gray) and stage 3 (dark gray), stage 4 (dark blue).

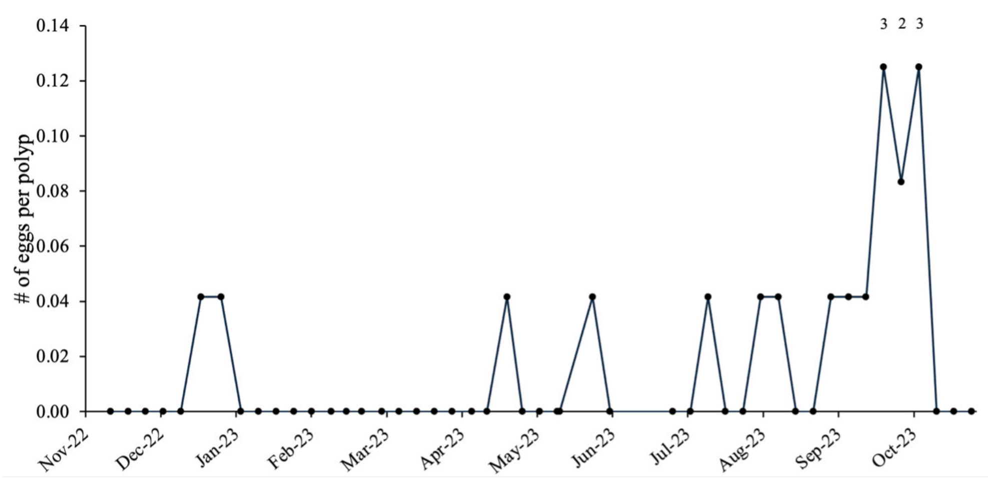


Figure 10: Time series of number of eggs per polyp in stage 4 (released) by the tracked individuals between November 2022 and February 2023. Numbers indicate when more than 1 eggs were released

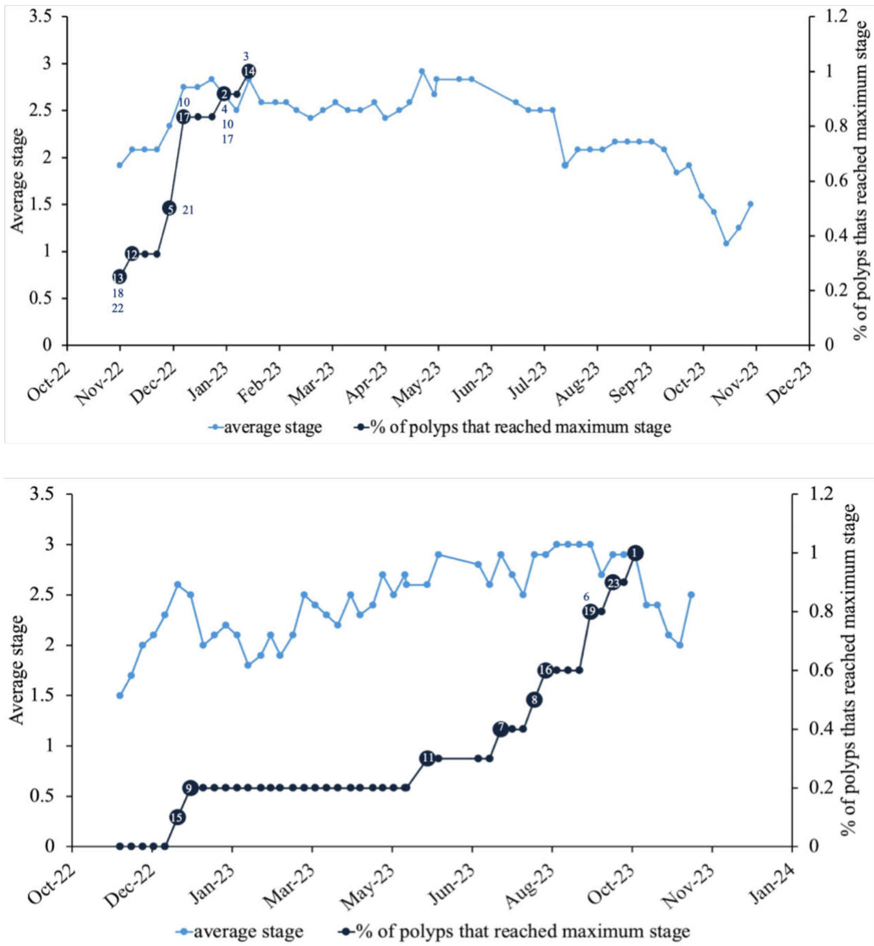


Figure 11: Temporal trends in the reproductive development of coral polyps that reached a maximum stage of 3 (top) or 4 (bottom) from October 2022 to December 2023. For both graphs, the blue line represents the average reproductive stage of the polyps while the dark blue line indicates the percentage of polyps that reached the maximum reproductive stage. Numbers indicate which polyp number reached the maximum stage.

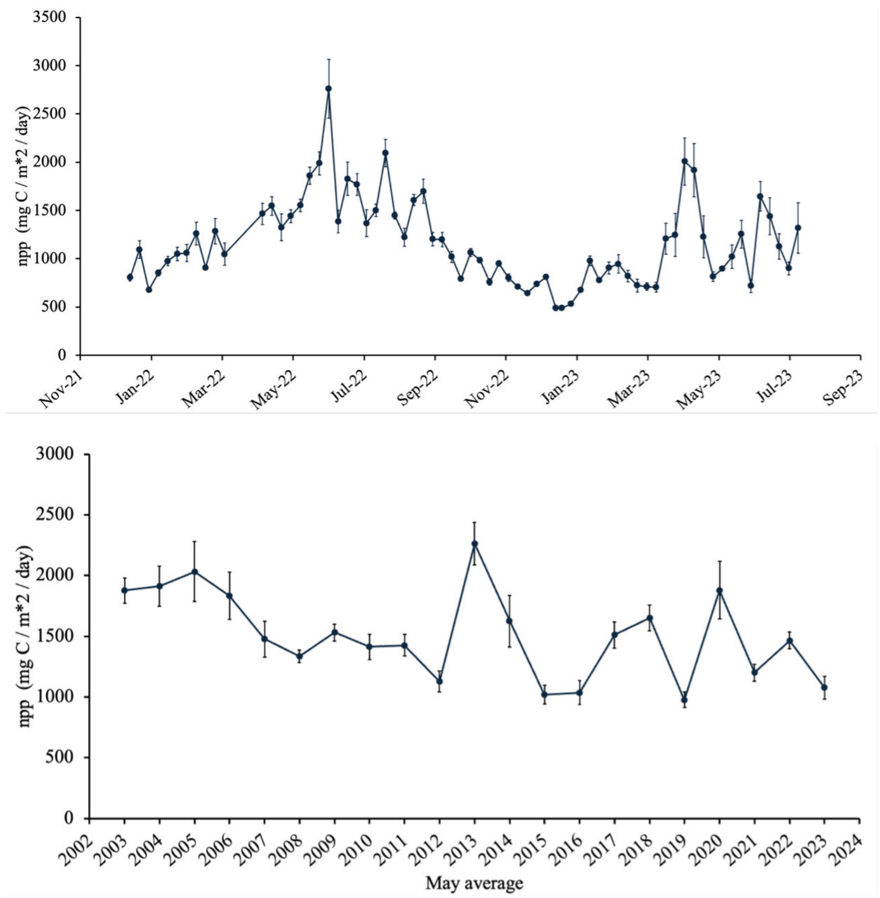


Figure 12: Time series of net surface primary production within a 100 x100 km area centered above Sur Ridge between (a) Dec 2021 and Feb 2023 (b) May average between 2003-2023. Time points represent 8-day averages (a) and monthly averages (b) (± s.e.). Data were downloaded from the Ocean Productivity website (http://sites.science.oregonstate.edu/ocean.productivity/).

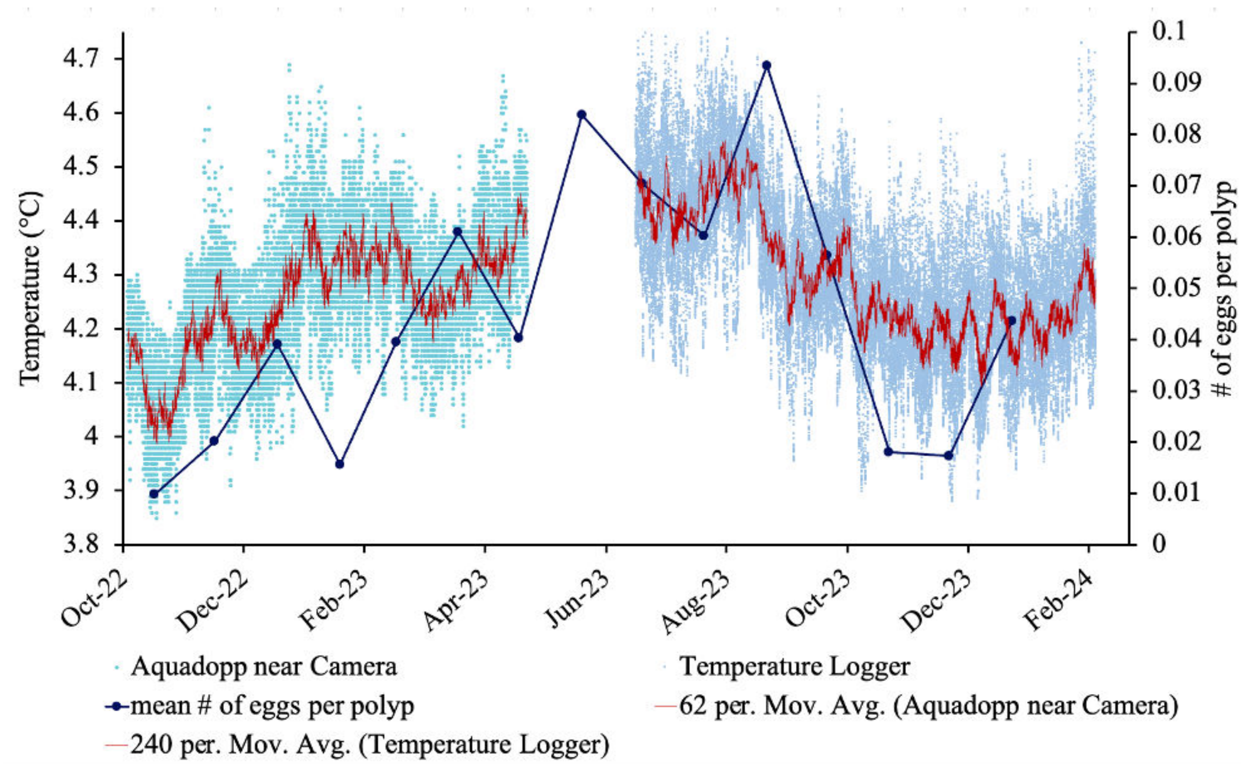


Figure 13: Time series of combined temperature from two different data logger locations close to the coral at Sur Ridge as well as temporal total colony reproductive output represented by a running average, blue line, with a 25 period. Red lines indicate running averages of temperature with a period of 240 data point.

4 Discussion

This study provides one of the first comprehensive description of the reproductive pattern of the deep-sea coral *Isidella*, based on an extensive analysis of the total number of eggs released by the colony and the maturation stages of polyps within in individual polyps over a one-year period. Utilizing advanced time lapse technology, we detailed the reproductive behavior of *Isidella*, describing the continuous development and seasonal cycles of egg release. Our results indicate that *Isidella* exhibits continuous individual polyp maturation with seasonal colony cycles of egg production peaking from late summer to fall. While the specific reproductive strategy and sexual system (i.e., brooding or broadcast spawning) of *Isidella* could not be definitively determined, we propose the most likely reproductive systems based on our findings in this discussion.

4.1 Seasonality in coral reproductive activity

Isidella inhabits environments characterized by perpetual darkness and diminished fluctuations in food availability and temperature. Despite these mildly seasonal conditions, our study revealed a distinct seasonal reproductive pattern in *Isidella*, marked by a gradual increase in reproductive activity between May and October with smaller intra-annual peaks and a significant peak in early October. Though the reproductive modes of most alcyonaceans are not known, the few studies available indicated considerable variation in reproductive strategies (Waller et al. 2023). In contrast, we found that this colony of *Isidella* released a surprisingly low number of eggs compared to other octocorals.

For polyps that release eggs in fall, the delayed egg release and failure of eggs to reach stage 4 could be caused by two reasons; either eggs are awaiting fertilization or, if fertilized, some eggs are too energetically poor to move up and out of the coelenteron. If egg release depends on fertilization, it suggests a key factor may be seasonal sperm availability. We do not know how sperm development and release occur in *Isidella*, or if sperm limitation affects egg development and release in the observed female *Isidella* colony. In some alcyonaceans, eggs of different maturation stages are known to be present in female colonies and oogenesis is estimated to take 23 months, while spermatogenesis and sperm release followed a 10–12-month release pattern (mohammed 2016). If this pattern is present in *Isidella*, the fall peak in egg release likely represents a single cohort of developing eggs and maturation may have overlapping cycles of release among polyps. Moreover, if cycles of sperm productivity ultimately provide a continuous supply of sperm, even if there are seasonal peaks, it may be advantageous for some proportion of female polyps within a colony to always have some well-developed eggs awaiting potential fertilization.

The low egg fecundity (i.e., numbers of eggs released per polyp), at 0.75 eggs per polyp, suggests that *Isidella* is a brooder, with fertilization likely occurring internally or externally on the tentacles. By avoiding the release of gametes into the water column and ensuring fertilization before release, *Isidella* may achieve higher reproductive success, offering larvae a greater chance of survival in the harsh deep-sea environment.

4.2 Effect of temperature and net primary productivity on seasonal reproductive pattern

Temporal variation in egg release could be an adaptive response to local environmental conditions, potentially linked to seasonal variation in sperm production / release Though mild compared to surface water, temperature and productivity vary over annual, seasonal, and shorter time scales, and may be important cues for egg development and release. A small, but rapid drop in temperature during the fall of 0.3 degrees Celsius (figure 14) does cooccur with the sudden drop in egg release which could indicate there is an environmental cue at play to move onto the tentacles to be released. The seasonal pattern in NPP (figure 12) aligns with typical cycles for NPP fluxes, as similar monthly variations have been documented in other studies (Girard et al., 2022)

The small increase in NPP? Egg release? Temp? For both January 2022 and 2023 may indicate a link to the intra-annual peak in egg release However, the highest peak in productivity was seen in May through June while peak egg release was during the fall (figure 12). While this study does not see a direct correlation between productivity and egg release previous literature has highlighted that surface productivity has a lag of 24 days (Girard et al. 2022) before reaching the deep sea, which could account for lags between NPP and egg release, even if they are linked. Comparing yearly differences between 2003 and 2023 revealed 2023 to a notably low year for food production, which might correlate with the relatively low total colony reproductive output observed (Figure 13). Although no data are available to assess the influence of interannual variation in NPP on *Isidella* reproduction, the coincidence the surprisingly low number of eggs released during the 2023 study period with the low regional NPP in that year (Fig 13) suggests that food availability may limit fecundity for *Isidella*.

4.3 Maturation stages in tracked polyps

The surprisingly low fecundity observed in tracked individual polyps is inconsistent with the general pattern of reproduction expected for broadcast spawners and suggests that *Isidella* may brood embryos prior to release. Assuming the colony is a brooding female, release of a small number of planula larva is plausible given the likely greater maternal care investment compared to broadcast spawning. The relatively short period that many eggs remained on tentacles suggests brooding with internal, versus external, fertilization. Though the extend period some eggs remain on tentacles make it unclear if they are released in response to external fertilization, an environmental que, or a combination of both factors. The environmental cues driving egg release, while not inferred by this study, could be linked to current speed, direction, temperature, and food availability (Waller et al., 2023).

Analogous to when tracking the number eggs released by the colony, peak reproductive output was seen in the tracked individuals during the fall (figure 9+10), with and a small peak in January (figure 10). Tracked individuals showed high constant presence of eggs present in either stage 2 or 3 supporting the hypothesis that egg production is continuous and either an environmental cue or fertilization to move into the last stage of maturation (release). The sudden increase of stage 0, resulting from polyps that decrease in stage suggests that some polyps await for later intra-annual spawning periods or are from different cohorts. This raises questions about whether egg retention is due to lack of fertilization or insufficient energy to move a fertilized stage 3 egg to stage 4 for release.

However, not all polyps have eggs that reach stage 4. While almost all polyps have eggs that develop through stage 3, only 40% have eggs which move to the tentacles are released. For those polyps who reach stage 3 but do not progress to stage 4 it could be due one of two reasons. One, sperm limitation results in low fertilization rates; therefore stage 3 eggs were aborted and resorbed. Stage 3 eggs being resorbed and the the rapid development of stage 4 polyps notable cooccurrence in May therefore might just represent that they are different cohorts that are at different points in maturation. Or two, we are seeing the results of a low energy year for which some more mature and developed eggs are capable (i.e., energy is sufficient) to complete development, but for most, energy is insufficient and remaining eggs are resorbed. Alternatively, we are observing two different overlapping cohorts of eggs and stage 3 eggs have simply not ready to take the step to stage 4 yet and will do so in a following intra-annual peak. Again, these questions become a matter of whether maternal energy investment or fertilization or both are key limitations for reproductive success (development to stage 4, fertilization, and egg/larval release). A combination of oscillations in temperature and net primary productivity could also be important in the success of polyp reproduction (Waller et al. 2023). As eggs reach stage 3, we suggest that they await moving out the oral disc onto the tentacles until fertilization occurs within the mesenteries like previous studies have demonstrated (Coma et al. 1995; Dahan and Benayahu 1997). This would explain why we see some polyps being successful in release and some not.

This study demonstrates deep-sea coral reproduction is complex with multiple patterns of egg development and spawning occurring across a single colony. The pattern and magnitude of reproduction are not completely clear but are likely linked to environmental conditions including food availability and temperature (Waller et al. 2023). Reoccurring patterns in reproductive behavior were detected when comparing results from the seasonal tracked colony and tracked individuals. This includes the presence of high amount polyps in stages < 4 during the spring and winter which coincided with results from tracking the colony where there was most ROI with 0 eggs during the spring and winter. Vice versa, less ROI had 0 number of eggs in the fall which were like results of tracked individuals in that more polyps reached stage 4.

5 Conclusion

The results suggest that the bamboo coral *Isidella tentaculum* exhibits dioecious gonochorism. Specifically, the coral colony under investigation is likely a brooding female colony that exhibits continuous but seasonal release of eggs with a pronounced peak in the fall. Maturation occurs continuously, likely with overlapping cohorts of eggs. Though this study could not shed light on the exact driver shaping the intra-annual differences in egg production and release, sperm supply and in terms of development, the transition from stage 3 to 4 is seemingly critical. The results demonstrating eggs that reach stage 3 but are never released and those who rapidly develop from earlier stages through stage 4 both hint at a plausible role for both fertilization and energetics in governing the patterns of spawning, though their relative importance may vary intra-annually. This makes histology imperative for future research within deep-sea coral biology, as this would able researchers to investigate the reproductive morphology of these complex invertebrate animals. This would able scientists to account for the number of eggs more accurately and tell whether they are fertilized. Incorporating histology together with image analysis would provide an improved and broader perspective addressing those questions image analysis cannot shed light upon alone. More quantitative image analysis is likewise needed to properly assess individual polyp maturation stages. Tracking more than 25 polyps could deliver a more accurate presentation of larger quantities of polyps and their maturation stages. With these techniques future research can help determine key features of anthozoan reproduction biology, such as whether the eggs fertilized before release, and could confirm whether *Isidella* is dioecious. More quantitative and morphological analysis would also aid in revealing whether the remarkably low number of eggs released, found in this study, is an accurate depiction of the reproductive productivity of the species *Isidella*. Recent technological advancements, such as time-lapse camera systems, offer unprecedented insights into the behaviors and dynamics of the organisms inhabiting the deep-sea. Understanding how corals live and survive in these extreme environments is essential for comprehending the impacts of human-induced effects on deep-sea ecosystems. Continued research in this area, alongside efforts to understand the role of environmental factors to coral reproduction, is vital for assessing and mitigating the consequences of environmental changes on these important sessile marine species.

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