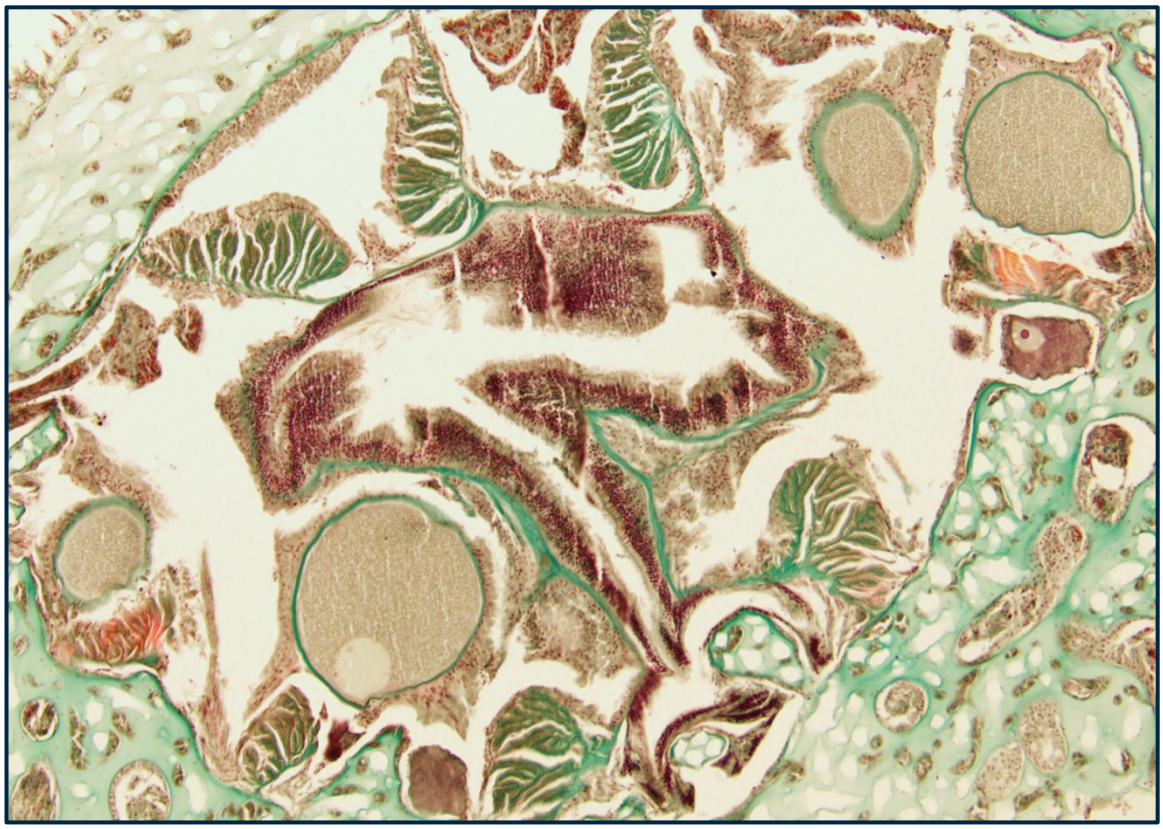
UNIVERSITY OF GOTHENBURG

DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES

Comparative reproductive assessment of the native and replanted pink sea fan *Eunicella verrucosa*



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Abstract

Sea fans are known to play a large role in ecosystem engineering and in keeping marine environments biologically versatile. The pink sea fan *Eunicella verrucosa* is found in the northeast Atlantic and the western Mediterranean Sea. It is a common octocoral, which many other species rely on at one point in their lifecycle, either for shelter or food. However, mechanical damage, such as bottom trawling with large fishing nets, can cause damage to these corals, with bycatch fragments brought to the surface in nets often being discarded, despite their viability. A common conservation effort to support the recovery of impaired coral colonies is the replantation of fragments, as it offers an inexpensive and accessible way to support decreasing coverage. This method could, however, limit genetic diversity within the population, making their sexual reproductive ability crucial for surviving changing environmental conditions. This study examined replanted and naturally growing *Eunicella verrucosa* individuals for reproductive differences by examining oocyte size and polyp fecundity. Histological and microscopical analysis of both groups was conducted on samples from the eastern Atlantic. It was found that the oocyte size distributions differed a lot between the groups. Additionally, the fecundity analysis showed differing numbers of previtellogenic and vitellogenic oocytes in replanted, compared to natural corals, as well as a change in total fecundity. These results show that the reproduction and spawning pattern is altered in the replanted coral fragments. It is, however, not possible to say if these changes stem from the replantation process or from the change in environment the corals experienced. Future research observing colonies at several time points might be able to uncover the reproductive changes and adaptations the corals go through during transplantation.

Introduction

Benthic ecosystems are made up of complex interactions between a myriad of organisms. Building the foundation of these systems are so-called ecosystem engineers, which, through their growth and three-dimensional shape, provide habitat for other species. One such foundational group is the corals. Their branched three-dimensional structure offers protection and habitat, which an estimated 25% of marine organisms rely on at some point throughout their life (Plaisance *et al.*, 2011). With this number being an estimate based on tropical coral reefs, the global percentage is likely even higher, making these ecosystems extremely valuable and irreplaceable.

Anthropogenic stress on corals

In the last decades, anthropogenic pressure on corals globally has become increasingly observed at the same time as more knowledge is gained about their essential roles in many ecosystems. Issues such as ocean acidification, global warming, and the impact of fisheries, as well as their compounding effects, have led to corals and many other marine species suffering declining populations. Due to the reliance of many species on the habitats that corals form, their presence in most benthic ecosystems is not replaceable.

One major concern for coral health is ocean acidification, which weakens the calcium carbonate skeletons of many hard corals. Additionally, it has been found that recovery and competition amongst corals are also strongly impaired in acidic environments (Doropoulos *et al.*, 2012; Webster *et al.*, 2013; Evensen *et al.*, 2021). A study by Agostini *et al*. (2013) has shown that increasing temperatures combined with acidification are proving to have even worse effects on coral health, demonstrating the complex nature of combined climate stressors, in a way that they are already manifesting in our oceans (Agostini *et al.*, 2013). It is important to note that research in this area is vastly centered around hard corals in warm waters, leaving a major gap in knowledge around soft corals, especially in temperate and cold waters. Bellin and Rossi (2024), however, conducted a study on sea fans, specifically *Eunicella spp,* to predict future changes in communities in response to several environmental stressors, such as climate change. The study combined species distribution models and machine learning to predict future outcomes, as they are likely to arise. The outcome they found likely to happen was a major decrease of area in which *Eunicella spp* grows, affecting some species with a decrease of up to 49% (Bellin and Rossi, 2024).

Many environmental stressors on marine ecosystems are passively caused by humans, however the direct impact we have cannot be neglected. Human activity in marine environments is proving to impact coral health and their populations globally. Tourism and recreational activities such as diving are centered around the aesthetic of coral reefs. Additionally, fisheries rely on corals as habitat for many commercially and recreationally fished species. With the decrease of these ecosystems, coastal fisheries in some areas have already started to struggle with decreased catch and loss of jobs as a result of this (Speers *et al.*, 2016; Rossi *et al.*, 2017). One major threat to corals has been fishing lines and nets breaking off parts of coral colonies in large numbers and over big areas, especially in a type of fishing called bottom trawling. This refers to the dragging of large fishing nets across the ocean floor. One study has shown that in gorgonian forests around the continental shelf, individuals living on the shelf break are larger in size than those on the shelf at shallower depths (Grinyó *et al.*, 2016). This is due to the drag of nets damaging the corals and limiting their size at shallower depths. Additionally, the gorgonian diversity has also been shown to be higher at the shelf break, possibly due to more sheltered conditions at deeper depths, allowing settlement and growth of coral larvae (Grinyó *et al.*, 2016).

The corals broken by fishing nets are usually pulled up as bycatch and subsequently discarded, as they provide no direct value to the fisheries. However, 80% of bycatch gorgonians are brought up alive, and 61% of them have no necrosis or epibionts, showing that they do have a high potential for becoming a self-sustaining, healthy organism again, if they were to be replanted (Montseny *et al.*, 2025).

Coral reproductive strategies

There are vastly different reproductive strategies among corals, with many mechanisms still unknown. They can reproduce both sexually and asexually, with each species having its own methods. Most coral species can reproduce asexually in more than one way. One common strategy is called budding and refers to a polyp growing directly out of a previously existing polyp (Lin *et al.*, 2022). A similar strategy is called polyp bailout, where one polyp separates itself from the parent individual to form a new colony (Paul W. Sammarco, 1982). Lastly and most known are fissions and fragmentation. The first of them refers to an individual splitting into two, and the latter meaning a broken-off piece becoming its own colony (R. Highsmith, 1982). This strategy is often used in conservation efforts to fight decreasing coral coverage.

Corals, however, do not only utilize asexual reproduction, as this would limit the genetic variation of a population. Some species have distinct single sex colonies, while others are hermaphrodites (Anne Simpson, 2009). Hermaphroditic species have both sexes in a polyp and can thus produce both sperm and oocytes. It is important to note that while they do possess both sexes, they are not necessarily present at the same time. Regardless of sex, corals have similar ways of fertilization. Some species utilize a method called broadcast spawning, which is a synchronised release of oocytes and sperm into the water. Others reproduce via brooding, which implies internal fertilization of the oocyte and subsequent release of larvae (Anne Simpson, 2009).

In the context of conservation, sexual reproduction is especially important, as successful population recovery depends not only on coral coverage but also on the population's ability to withstand future changes in the environment.

Eunicella verrucosa the pink sea fan

In the case of *Eunicella verrucosa*, the ability to survive environmental changes is becoming more relevant by the day, as it is one of the most frequently found Mediterranean and East Atlantic bycatch corals (Dias *et al.*, 2020). It is an octocoral in the family of G*orgoniae*, which is found at depths of 2 to 200 meters (Chimienti, 2020; Canessa *et al.*, 2022). The sea fan of light yellow to deep pink colour variation often grows in large aggregations, referred to as coral forests (Chimienti, 2020). This coral contributes to marine biodiversity in several ways, primarily through their importance as habitat formers (Gibson, Atkinson and Gordon, 2006; Rossi *et al.*, 2017). In addition to this, *E. verrucosa* plays an important role in the food web, as they link pelagic systems to benthic systems due to their suspension feeding. Gorgonians mostly feed on plankton and organic matter, which they passively sequester out of the water column. Through the binding of this organic matter, they support the benthic food system, clean the water, and build a foundation for other species to survive and thrive off of (Gili and Coma, 1998). Similarly to *E. verrucosa* is *Leptogorgia sarmentosa,* which comes from the same family and oftentimes shares the same habitats. This species, too, is a commonly damaged and bycaught coral (Dias *et al.*, 2020). Known for their bright orange to reddish colour, these corals occupy the same depth range and environments as *E. verrucosa*.

Conservation efforts for *Eunicella* species

Due to *Eunicella* species frequently being caught as bycatch, their native populations have consequently been declining at an alarming rate. To combat the deterioration of coral forests, a new mode of restoration called the 'the badminton method' has been developed. It utilizes the bycatch fragments, by gluing them to cobbles and dropping them off boats, to have them land in locations where the natural populations are declining or no longer present. The stone ensures the coral pieces land the right way on the seafloor and are not further damaged (Montseny *et al.*, 2020). Even though it appears effective, the long-term success of this method of restoration is not yet fully realised, and the question remains whether growth and reproduction of the corals are affected. Edery *et.al*. (2025) examined the reproduction of transplanted *Eunicella sigularis* fragments, finding the size of the fragment to be highly relevant to the survival of the coral. This study also found the reproduction of female fragments to be impaired for up to three years after transplantation, regardless of size (Edery *et al.*, 2025).

What we know about *E. verrucosa* reproduction

It is known that *Eunicella verrucosa* releases gametes via broadcast spawning. A study by Egger *et.al*. (2025) showed that this is done through split-spawning, leading to three spawning events, which are two weeks apart each. In this study, the average oocyte size was measured to be around 300-400 μm in diameter (Egger *et al.*, 2025). It is important for conservation efforts to acknowledge the relevance of sexual reproduction in a coral community, in addition to asexual growth. Fragment replantation alone may help decreased coverage but limits genetic diversity and may ultimately decrease the community's ability to adapt to environmental changes, which are inevitably going to occur. Only through sexual reproduction can the coral communities evolve to adapt to changing environmental pressures.

Bellin and Rossi (2024) conducted a study to predict future changes in *Eunicella spp* communities in response to several environmental stressors, such as climate change. The study combined species distribution models and machine learning to predict future outcomes, as they are likely to arise. The most likely outcome modelled was a major decrease of area in which *Eunicella* grows, affecting some species with a decrease of up to 49% (Bellin and Rossi, 2024). Bellin and Rossi (2024) conducted a study to predict future changes in *Eunicella spp* communities in response to several environmental stressors, such as climate change, moving research towards soft corals. The study combined species distribution models and machine learning to predict future outcomes, as they are likely to arise. The outcome they found likely to happen was a major decrease of area in which *Eunicella spp* grows, affecting some species with a decrease of up to 49% (Bellin and Rossi, 2024).

The impact of fishing has led to many coral species being damaged and suffering a decrease in coverage. Among the most common species in the North Atlantic are *Leptogorgia sarmentosa* and *Eunicella verrucosa*. While these damages continue to occur, conservation efforts are being made. Coral fragment replantation is a widely used method to increase coral coverage and support benthic ecosystems. While this appears to be an easy and effective method, the effect on coral reproduction remains largely unexplored.

Aim

This study aimed to find out if there are reproductive differences in replanted *Eunicella verrucosa* and *Leptogorgia sarmentosa* individuals, respectively, on the southern coast of Portugal, when compared to native colonies. Oocyte measurements as well as fecundities were used as an indicator of reproductive capability and spawning patterns.
The results of this study could provide valuable insights into the effectiveness of current conservation efforts and how they may impact the sexual reproduction of individuals. This knowledge can be used to adapt future methods of conservation of gorgonian species and support the recovery of an ecologically and economically invaluable ecosystem.

Material and methods

Collection

The samples were collected on May 22nd, 2025, by CCMAR - Centro de Ciências do Mar do Algarve, Universidade do Algarve - Campus de Gambelas. Divers collected branches of replanted and natural corals at the Algarve Coast in Portugal (Figure *1*). The cut branches were transported back to shore in bags with clean seawater (Table *1*). The replanted samples were bycatch corals caught at 60-100 m depth, which were subsequently replanted at 20-32 m depth.

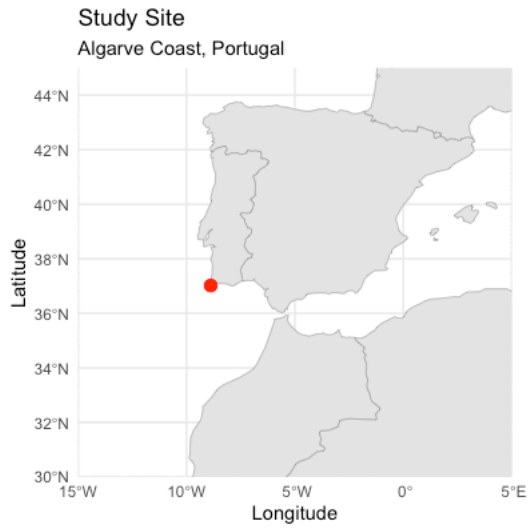

Figure 1: Map of study site, with the red dot marking the precise latitude and longitude of sampling location.

Eunicella verrucosa						
Group	Date	Site	Female	Male	Unknown	Total
Replanted	22.05.2024	Torove	7	2	3	13
Shallow	22.05.2025	Pontao	9	3	3	15
Deep	22.05.2026	Torove	5	2	8	15
Leptogorgia sarmentosa						
Group	Date	Site	Female	Male	Unknown	Total
Replanted	22.05.2024	Torove	0	0	8	8
Shallow	22.05.2025	Pontao	0	0	9	9
Deep	22.05.2026	Torove	0	0	14	14

Table 1: Sample overview for Leptogorgia sarmentosa and Eunicella verrucosa, listing group, collection date, site, sex, and total number of colonies.

Histology

Preservation

After the collection, the samples were initially preserved in neutral buffered 4% formalin. After 24 hours, the formalin was changed to 70% ethanol, in which the samples remained for several months until further preparation.

Preparation

The initial development of the methods for *Leptogorgia sarmentosa* followed a basic histological protocol. This protocol was known to work for the species *Primnoa pacifica*, which is similar in size and shape (Waller *et al.*, 2014). The first step was dissecting the samples into pieces that fit the cages selected for wax molding and removing the calcium carbonate sclerites. After cutting suitable pieces, the decalcification was done in DC2 (hydrochloric acid-based decalcifier) before being dehydrated in increasing concentrations of ethanol. Following this, the samples were cleared with toluene and incubated in three different paraffin wax jars in the oven set at 55 degrees Celsius for several days in total. When the preparation was done, each sample was cast in wax and left to cool overnight (Table *2*).

Some adaptations were made to make the incubation times more time-efficient for *Leptogorgia sarmentosa.* The successful protocol was then tested and adapted for *Eunicella verrucosa*. Due to the significantly larger polyp size of this species, some incubation times were increased to ensure proper penetration of the chemicals (Table *2*).

	A) Primnoa pacifica protocol		*B) Leptogorgia sarmentosa*		*C) Eunicella verrucosa*	
	Chemical	**Duration**	**Chemical**	**Duration**	**Chemical**	**Duration**
Decalcification	DC2	60 min	DC2	50 min	DC2	50 min
Dehydration	30% Ethanol	2 x 10 min	30% Ethanol	2 x 10 min	30% Ethanol	2 x 10 min
	40% Ethanol	2 x 10 min	40% Ethanol	2 x 10 min	40% Ethanol	2 x 10 min
	50% Ethanol	2 x 10 min	50% Ethanol	2 x 10 min	50% Ethanol	2 x 10 min
	60% Ethanol	2 x 10 min	60% Ethanol	2 x 10 min	60% Ethanol	2 x 10 min
	70% Ethanol	2 x 10 min	70% Ethanol	2 x 10 min	70% Ethanol	2 x 10 min
	80% Ethanol	2 x 10 min	80% Ethanol	2 x 10 min	80% Ethanol	2 x 10 min
	90% Ethanol	2 x 10 min	90% Ethanol	2 x 10 min	90% Ethanol	2 x 10 min
	95% Ethanol	2 x 10 min	95% Ethanol	2 x 10 min	95% Ethanol	2 x 10 min
	100% Ethanol	overnight; 10min	100% Ethanol	overnight; 10min	100% Ethanol	overnight; 10min
Clearing	Toluene	3 x 10 min	Toluene	3 x 10 min	Toluene	3 x 15 min
Embedding	Paraffin wax 1	overnight	Paraffin wax 1	7 h	Paraffin wax 1	8 h
	Paraffin wax 2	8 h	Paraffin wax 2	overnight	Paraffin wax 2	overnight
	Paraffin wax 3	overnight	Paraffin wax 3	7 h	Paraffin wax 3	8 h

Table 2: Overview of protocol development for Leptogorgia sarmentosa and Eunicella verrucosa. Column A) shows the initial protocol as it was known from Primula pacifica, now used for L. sarmentosa. Column B) shows the final protocol with adapted incubation time.

Sectioning

Slicing was done the day after casting, to ensure the wax had solidified. The initial samples of *Eunicella verrucosa* and *Leptogorgia sarmentosa* were cut at 7 microns on a Leica rotary microtome and later reduced to 6 microns to identify female individuals. After this was done for *Eunicella verrucosa*, nucleus measurements were taken and used to calculate how many slices to discard during serial sectioning. The purpose of this is to avoid double-counting and/or measuring oocytes by leaving space approximately the size of the nucleus between each section retained on a slide. Sections were then kept on slides for analysis, while 20-24 microns were discarded in between slides, utilizing all the coral tissue inside a wax block.

Staining

Several stains were used for this project, with Gomoris Trichrome being the initial test stain. As oocytes were hard to see, Haemotoxylin and Eosin was the second stain. This was the one that was utilized for all samples analysed. For final images and better visualization Masons trichrome was used (Table *3*). The final step to all stains was coverslip gluing with DPX mounting media and letting the samples dry overnight.

Gomoris Trichrome		**Haemotoxylin & Eosin**		**Masons trichrome**	
Chemical	**Duration**	**Chemical**	**Duration**	**Chemical**	**Duration**
Neoclear	5 min	Neoclear	5 min	Neoclear	5 min
Neoclear	2 min	Neoclear	2 min	Neoclear	2 min
100% ethanol	2 min	100% ethanol	2 min	100% ethanol	2 min
70% ethanol	2 min	70% ethanol	2 min	70% ethanol	2 min
Gomoris Trichrome	5 min	Haemotoxylin	5 min	Haemotoxylin	25 min
95% ethanol	5 min	Water	5 min	Water	10 min
100% ethanol	3 min	Eosin	2 min	Ponceau S	5 min
100% ethanol	3 min	Water	dip	Water	dip
Neoclear	5 min	100% ethanol	5 min	Phosphomolybdic acid	5 min
Neoclear	5 min	100% ethanol	2 min	Light green	5 min
		Neoclear	5 min	Water	dip
		Neoclear	5 min	100% ethanol	5 min
				100% ethanol	2 min
				Neoclear	5 min
				Neoclear	5 min

Table 3: Protocols for all three stains used. When water is mentioned, this refers to running water.

Analysis

After confirming female individuals of *Eunicella verrucosa*, the oocytes were photographed under the microscope and measured.

Analysis of the samples was conducted with a ToupTek camera attached to an Olympus BX60 microscope. Pictures were taken of all oocytes with a visible nucleus, until 100 or more were photographed. In samples with many oocytes, random slides were selected and photographed until a number of around 100 was reached. The images were given a scalebar in the native application WaveImage and imported into Fiji (Schindelin, J.v, 2012). After setting the scale, the freehand selection tool was used to circle the oocyte and calculate the ferret diameter.

For the fecundity analysis, 5 random polyps with oocytes from each sample were selected. A slide that contained the most central section of each polyp was photographed and measured. One measurement on the X-axis was taken across the widest point of the polyp, while the Y-axis measurement was taken from the mouth to the base of each polyp. The oocyte counts were done at the microscope, by going through each slide and selected polyps and noting the numbers of previtellogenic and vitellogenic oocytes in each. Only oocytes with a visible nucleus were counted, preventing double-counting. The measurements and numbers were compiled in Microsoft Excel and further analysed using R-studio (Posit team, 2024). The packages used for statistical analyses were `tidyverse´ (Wickham H, 2019), `ggplot´ (H. Wickham, 2016).

Statistics

To get an initial idea of relationships between fecundity metrics and group effects, Welch two-sample t-tests were used. The relationships between polyp size and fecundity between groups were analysed this way. In this paper, `groups´ refers to the three sampling groups: replanted shallow (replanted), shallow natural (shallow), and deep natural (deep). The relationships between polyp size and fecundity between groups were analysed this way.

To get a better and more accurate idea of the relation between fecundity and the three groups, linear mixed-effects models (LMMs) were used, with ‘individual’ as a random effect, due to repeated measurements in colonies. In these tests, total fecundity, previtellogenic fecundity, and vitellogenic fecundity were analysed as response variables, while the groups were fixed effects. The goal of this was to find out if individual variance leaves any group effects.

Additional mixed-effect models were used to test whether polyp size would account for the fecundity measurement differences. In these tests, total fecundity, previtellogenic fecundity, and vitellogenic fecundity were analysed as response variables, while X-size and Y-size of the polyps were set as fixed effects. The goal of this was to find out if polyp size and individual variance leave any group effect.

The statistical relevance of the individual fixed effects was evaluated using Type III ANOVA tables with Satterthwaite’s method.

For any statistically significant results in these analyses, a post-hoc pairwise comparison test was done using estimated marginal means with Tukey-adjusted p-values to identify between which groups a statistical difference occurs.

Results

Microscopy: *Leptogorgia sarmentosa*

The initial microscopy analysis was conducted on *Leptogorgia sarmentosa* samples. The aim of this was to identify female individuals for further reproductive analysis. However, after testing over thirty samples, none of them had any oocytes. Because of this, this species was not further used in this study.

Microscopy: *Eunicella verrucosa*

In the second species, *Eunicella verrucosa*, oocytes were found, and female colonies were confirmed (Figure *2*). Staining methods were adapted to best visualize the oocytes and their stages (Figure *3*). After determining which of the samples were female, the lowest number of female individuals in one of the groups was five. Due to this, all further analyses were done with five females of each of the three groups.

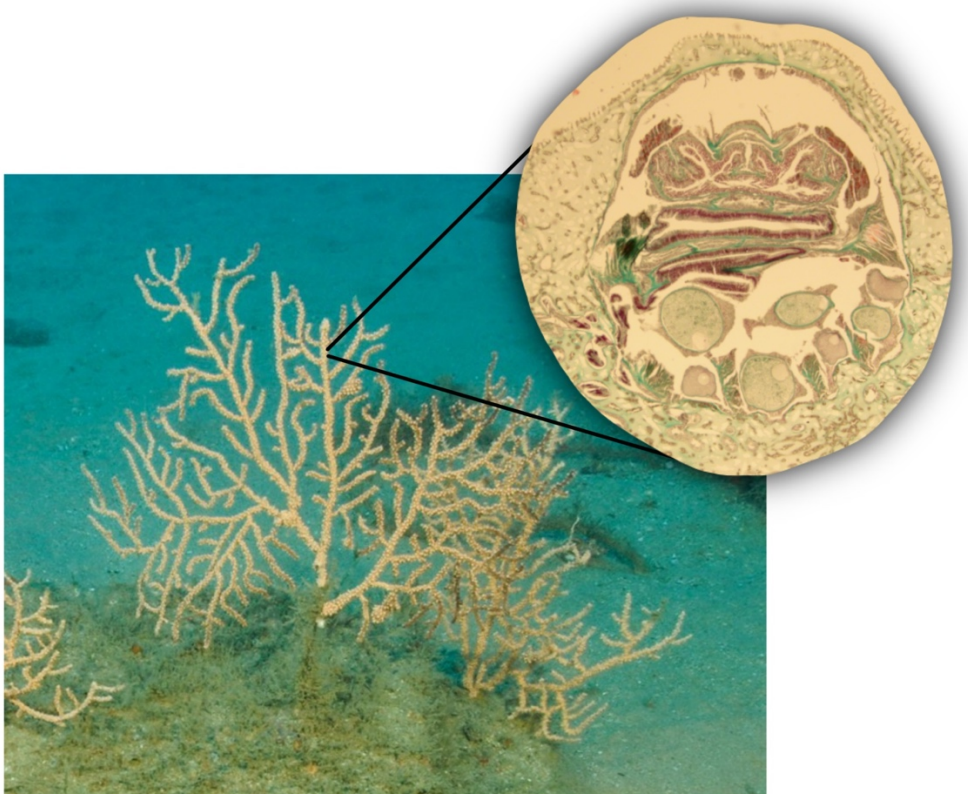

Figure 2: Picture of transplanted Eunicella verrucosa colonies, with a section of a polyp. (Transplant photo by Diogo Paulo)

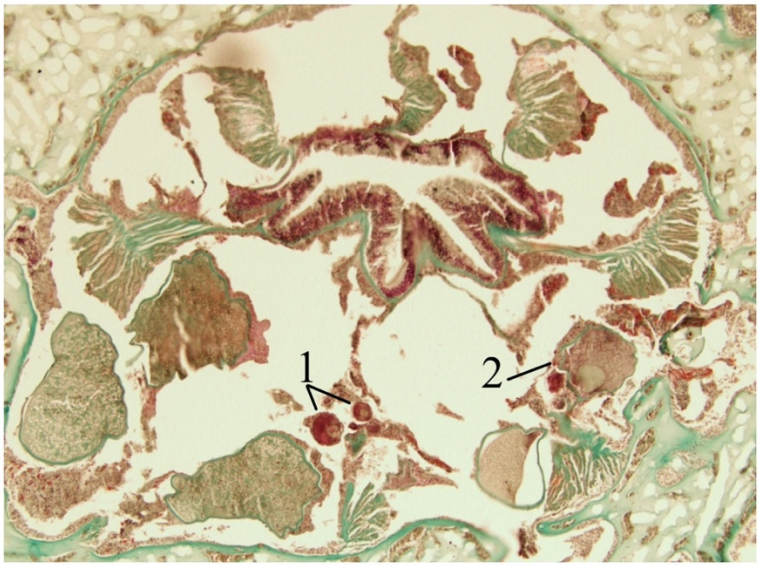

Figure 3: Image of a polyp with both previtellogenic (1) and vitellogenic (2) oocytes.

Fecundity Welch two-sample t-tests

The initial T-tests showed that there are differences between replanted and shallow-natural (shallow), as well as natural-deep (deep). They differ in different factors, however. The total fecundity is significantly different between replanted and shallow ($t = 2.0958$, $df = 41.024$, p-value = 0.04232) as well as replanted and deep ($t = -3.5804$, $df = 38.908$, p-value = 0.00094). When looking closer at the individual fecundities of previtellogenic and vitellogenic oocytes, differences became clearer. The amount of previtellogenic oocytes differed significantly between replanted and deep ($t = -5.5964$, $df = 32.571$, p-value = 3.307e-06), while between shallow and replanted, the vitellogenic oocytes differed ($t = 3.9245$, $df = 40.498$, p-value = 0.0003291). These results gave a broad overview of tendencies in the data, but did not yet confirm or deny any variances, due to a lot of remaining factors, namely polyp size and individual variance.

Linear mixed-effects models (LMM)- Fecundity

The LMMs used with individual variance as a random effect showed that total fecundity does have statistically relevant differences between the groups ($F = 4.15$, $p = 0.0427$). When analysing the individual fecundities of previtellogenic and vitellogenic oocytes, the group effect remained, with both previtellogenic ($F = 4.35$, $p = 0.0379$) and vitellogenic ($F = 5.71$, $p = 0.0181$) group differences being statistically relevant.

Post-hoc pairwise comparison

After finding group effects in the LMMs, the post- hoc test showed that, depending on oocyte stage, the group effect differs. The total fecundity of shallow and deep was found to be statistically different ($p=0.0343$), while none of the other groups were found to be significantly different when compared directly.

When comparing previtellogenic fecundities, the only significant difference occurred between replanted and deep ($p=0.0465$). While the other groups again did not differ significantly from each other, there was a trend between shallow and deep, with a p-value of 0.0842.

In the vitellogenic oocyte fecundity, shallow and deep differed significantly ($p=0.0239$). This test also showed that there is a significant difference in vitellogenic fecundity between replanted and shallow ($p=0.0440$).

Linear mixed-effects models (LMM)- Polyp size

The LMMs used with X-size and Y-size as fixed effects showed that the X measurement can show a trend in fecundity, but does not exclude group effects. This model showed that the main effect of X-size is very significant ($F = 10.25$, $p = 0.0021$), while the main effect of Group did not show any significant relevance. When testing the interaction between X-size and Group, however, a significant difference was found ($F = 3.26$, $p = 0.0448$).

When the same tests were done for Y-size, the main effects of the size and Group were not relevant. The interaction between Y-size and group did show a statistically relevant difference, though ($F = 3.21$, $p = 0.0470$).

Due to the significance of X-size, the next step was to determine if it varies between groups. The results of this showed that there is no relevant difference ($F = 0.042$, $p = 0.959$). In addition to this, it visualized the close similarity in mean X-size between the three groups, with replanted=1145µm, shallow=1124µm, and deep=1122µm.

When doing the same test for Y-size, a difference between groups was found ($F = 4.31$, $p = 0.0389$). When using a pairwise comparison to find the significant difference, it was found that

the biggest difference is between replanted and deep (p = 0.0314). The means of the Y-size by group further illustrates this, with replanted=817µm, shallow=753µm, and deep=674µm.

Figure 4 visualizes the differing effects polyp size has on total fecundity based on group. For both X and Y-size, replanted and shallow tend to have a positive relation, where a larger size leads to higher fecundity (Figure *4*). For deep, this is the opposite, where larger polyps lead to a decrease in total fecundity. It is important to note that the only statistically relevant p-values lie in the deep samples, where X-size shows p=0.0256, and Y-size has p=0.032. The remaining graphs have higher p-values, although the Y-size of shallow shows a slight significance in relation to fecundity.

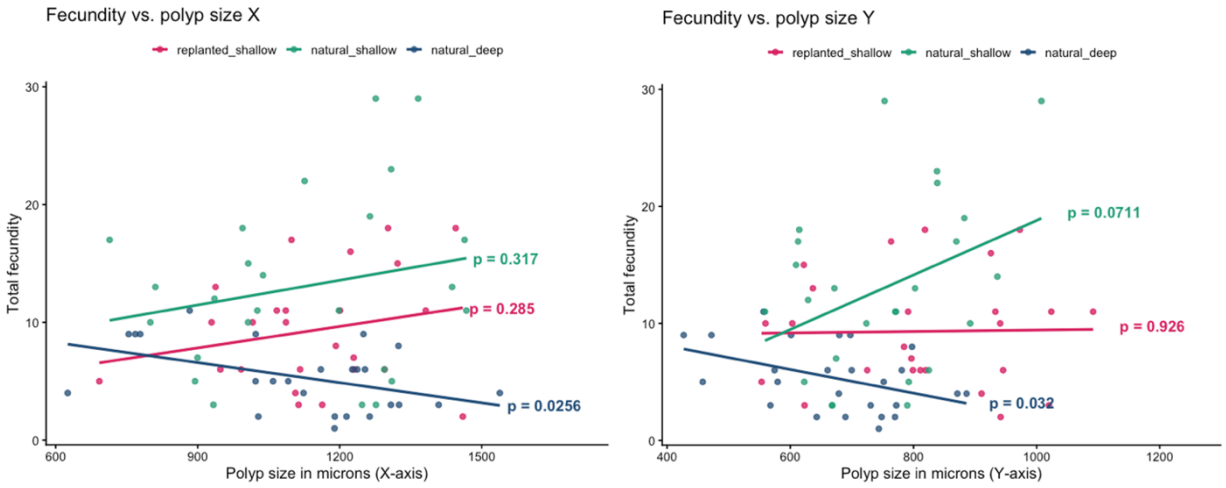

Figure 4: Total fecundity in relation to X-size (left) and Y-size (right). N=5 in each group, with replanted = pink, shallow = green, and deep=blue. P-values for the relations are depicted on the right end of each line.

Group and morphology effects on fecundities

When testing LMMs with both individual variance and polyp size factored in, it was shown that the group still has a significant effect on fecundity
Table 4). Effects did, however, vary between groups and in relation to the X- and Y-size, respectively.

		Replanted vs shallow		Replanted vs deep		Shallow vs deep		
		p-value	F-value	p-value	F-value	p-value	F-value	LEGEND
Total	X-size	0.0007059	13.3616	0.21750	1.5628	0.003867	9.3160	Significant
	Group	0.2847260	1.3186	0.08636	3.9672	0.059925	4.8776	
	Y-size	0.05712	3.8196	0.85845	0.0322	0.10422	2.7590	Marginally significant
	Group	0.19904	1.9531	0.05295	4.9308	0.04936	5.2941	
Previt	X-size	0.01921	5.9091	0.459124	0.5575	0.01094	7.0465	Group significant
	Group	0.84005	0.0435	0.004581	15.8806	0.08159	4.0051	
	Y-size	0.07074	3.4287	0.679418	0.173	0.01254	6.8081	
	Group	0.95792	0.0030	0.004097	13.722	0.11311	3.1378	
Vit	X-size	0.002643	10.1871	0.3933	0.7422	0.05293	3.9498	
	Group	0.064156	4.6844	0.7044	0.1572	0.05214	5.4092	
	Y-size	0.1742	1.9067	0.4169	0.6707	0.6063	0.2695	
	Group	0.0330	6.565	0.4722	0.5631	0.0306	6.7263	

Table 4: The table visualizes a summary of mixed effect models comparing different fecundities between two groups

at a time, while accounting for polyp size and individual variance. Models were run separately for previtellogenic, vitellogenic, and total fecundity, as well as between each possible group combination. Shallow vs deep serves as a control in this case. Within each group comparison, X and Y-size were tested separately as fixed effects, while individuals were a random effect. Green values indicate statistically significant effects ($p < 0.05$), blue values indicate marginal significance ($0.05 < p < 0.10$), and red values highlight significant group effects specifically ($p < 0.05$).

Shallow vs deep

When comparing shallow and deep to get a baseline idea of their reproductive differences in the natural habitat, X-size was shown to be the reason for the difference in total (p=0.003867) and previtellogenic fecundity (p=0.01094). Still, with the X-size accounted for, a marginally significant group effect remains in both total (0.059925) and previtellogenic (0.08159) fecundity.

The same result was shown for the Y-size in the previtellogenic fecundity (p=0.01254), although no significant group effect remained. When analysing the Y-size in relation to total and vitellogenic fecundity, however, the group effect was the only significant effect in both total (p=0.04936) and vitellogenic (p=0.0306) fecundity.

Replanted vs shallow

In the comparison of replanted and shallow, the X-size was the significant effect in total (p=0.0007059), previtellogenic(p=0.01921), and vitellogenic fecundity (p=0.002643). Group effects were insignificant in all cases, except for vitellogenic fecundity (p=0.064156), where a marginal significance was shown.

The Y-size had marginal effects on the difference in Previtellogenic(p=0.07074) and total (p=0.05712) fecundity, with no group effects. When analysing the vitellogenic fecundity difference between replanted and shallow, the effect of the Y-size was insignificant, while the group effect was significant (p=0.0330).

Replanted vs deep

The results of the comparison between replanted and deep were very different from the comparison to shallow. The X-size in his case showed no relevant effect on the difference in fecundity of any oocyte group. Group effects were marginally significant for the total fecundity in comparison to both X- (p=0.08636) and Y-size (p=0.05295). The strongest effect was found when analysing fecundity differences in previtellogenic oocytes, where group effects of both X (p=0.004581) and Y-size (p=0.004097) were strongly significant. No differences were found in the vitellogenic group in this comparison, indicating that the fecundity was similar between these groups.

Oocyte size distributions

When sorting the oocyte sizes in each group by size bins, all three groups have a distinct and unique pattern (Figure *5*). When comparing the two natural groups' size distributions, big differences become clear (Figure *6*). The shallow distribution has two peaks of almost equal height, with a slight dip in between, while the deep group has a very high peak of previtellogenic oocytes and then falls steadily. When looking at the replanted groups' distribution, similarities to both shallow and deep are clear. A very high peak at the start is followed by a dip and a second peak.

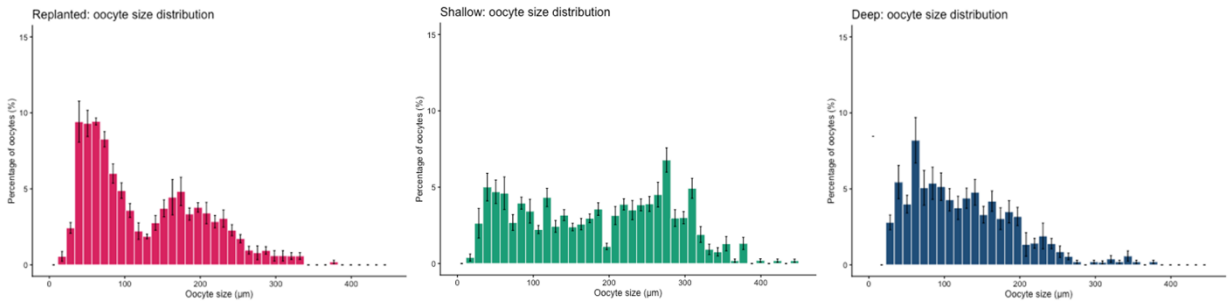

Figure 5: Oocyte percentage size distributions of each group, respectively, with replanted=pink, shallow = green, and deep=blue. N=5 for each distribution. Percentages of oocytes per bin size are plotted against oocyte size in microns. Error bars depict +-1 SE of the mean percentage of oocytes per bin across individual colonies.

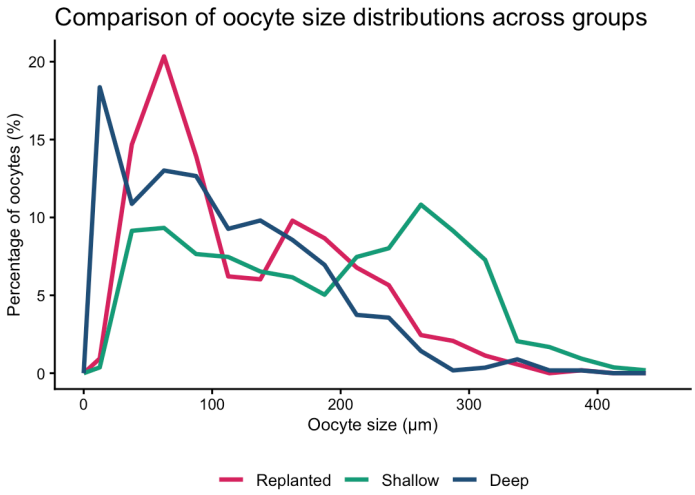

Figure 6: Overlaying distributions of oocyte number over size in each group. N=5 in each group.

Table 5 visualizes the peaks in each group, where each group was split in half into oocytes over and under 200µm (Table *5*). This size was chosen due to several groups having a dip in oocyte count per bin at about 200µm, and this size corresponds to the change from previtellogenic to vitellogenic oocytes. The means of all three groups in the under 200µm category are very similar, with the highest variability between them being only 7µm. The medians, however, differ more, with the replanted group having a lower median than the others. This indicates that some larger oocytes are outliers, which increased the mean. The number of oocytes differs strongly as well, with replanted and deep having numbers over 400, while shallow has 277 oocytes.
In the over 200µm category, the means of both replanted and shallow are close in size and are bigger in comparison to deep. The medians between all three groups are, however, very similar. The number of oocytes here is highest in shallow, about half as many in replanted, and again about half the amount in deep. This is coherent with the distribution visible in Figure 6, where shallow has the highest second peak, followed by replanted.

Oocytes under 200 µm			
Group	**Mean**	**Median**	**Number of oocytes**
Replanted	97 µm	81 µm	428
Shallow	102 µm	96 µm	277
Deep	104 µm	99 µm	401
Oocytes over 200 µm			
Group	**Mean**	**Median**	**Number of oocytes**
Replanted	346 µm	236 µm	103
Shallow	300 µm	271 µm	259
Deep	247 µm	234 µm	59

Table 5: Mean and median values of each group, for oocytes under and over 200µm, respectively, with oocyte number per group. N=5 in each Group.

Discussion

Leptogorgia sarmentosa was non-reproductive

When looking at this species, no females or males were found, even after processing and analysing around thirty samples. This can have several reasons, one of them being that they possibly just spawned. It is, however, unlikely to have anything to do with the stress of replantation, as neither natural nor replanted samples had any gametes.

Eunicella verrucosa

While several statistical analyses were done for *E. verrucosa,* the most accurate results stem from the linear mixed model (LMM), which took polyp size into account (Table 4). The tests prior to this showed that polyp size does play a significant role in fecundity, so it cannot be neglected as a factor when comparing between groups. Additionally, the T-tests offered some great initial insight into tendencies of the dataset, but cannot be taken at face value. This is due to the T-test assuming that the datasets are compiled of independent values, while it is in fact replicates of different individuals. Because of these reasons, the most reliable statistical test is the LMM, including polyp size and individual variation.

X-axis polyp measurements indicate total fecundity

The first result of this test was a strong relation between X-size and total fecundity (Table 4). This finding was shown fairly universally, independent of the group. Biologically, this could make sense, as polyps that expand laterally (X-axis) rather than further out of the coral (Y-axis) could have an increased feeding surface. A larger polyp also provides more reproductive tissue and thus a higher number of oocytes. While research has found that some coral species do have a correlation of polyp size and fecundity, it seems that energy allocation and colony size are generally more important factors affecting fecundity (Nozawa and Lin, 2014; Foster and Gilmour, 2020).

In the case of previtellogenic and vitellogenic oocytes, the X-size started to become less relevant, depending on which groups were compared. This shows that, individual variance and polyp size considered, there are still significant differences between some of the groups. One such difference was found between the fecundity of previtellogenic oocytes between replanted and

deep. The replanted samples showed a significantly higher fecundity in comparison to the deep samples. When compared to shallow, there was no significant difference. This indicates that the replanted corals are more like shallow in regard to the number of previtellogenic oocytes. Due to the samples initially coming from a deeper depth and being replanted shallow, this could also be an indicator of the corals adapting to their new environment. They have become statistically no different from the native shallow individuals, while differing significantly from the native deep samples.

The comparison of vitellogenic fecundities between the groups gave quite the opposite result, however. This analysis showed that the replanted individuals differ marginally from the shallow ones, while there is no significant difference between replanted and deep. Although the significance of the difference is not as strong as in the previtellogenic comparison, it shows a tendency. The vitellogenic fecundity is more like the deep native samples, even after the corals have been in the shallow environment for 4.5 years. This opens a discussion about how certain traits adapt to changing environments.

Implications of differences between groups

The findings of the fecundity analysis could mean many different things, and as the samples are all from only one time point, it becomes difficult to say which is the reason for the results. Multiple time points of sampling are needed to identify, for example, spawning patterns. When considering the oocyte size distribution of the three groups, a pattern becomes visible (Figure *5*). The replanted corals' oocyte size distribution appears almost like an intermediate between shallow and deep. The first high peak is similar between all three groups, but replanted has the highest peak, shared with deep. The graph of the deep samples then continues to steadily drop, with the replanted samples showing a very similar tendency. What becomes clear, however, is that the replanted distribution is starting to show a second peak, which is becoming similar to shallow, but is still much lower. These tendencies in the oocyte size distribution could indicate an adaptation of the replanted samples to their new environment. Even though the overall outline of the distribution is very similar to the deep samples, a second peak, like the shallow ones, becomes visible. One possible explanation for this could be different spawning patterns between deep and shallow environments. This is very common in corals generally, due to different light and nutrient availability (Martinez *et al.*, 2020). A paper by Eckelbarger & Watling (1995) illustrates that while genetically inherent traits may stay the same across individuals of a species, their environment can have a strong influence on them. The environment a species lives in can change traits like fecundity, spawning patterns, and oocyte size, showing that these factors are not purely defined by genetics (Eckelbarger and Watling, 1995).

Similar to the size of the polyp playing a possible role in fecundity, it is likely that environmental factors like nutrient availability are more significant (Nozawa and Lin, 2014). The replanted corals' first peak is similar to deep, but significantly higher, which could be due to higher nutrient availability at shallower depths. These factors could give the replanted samples the energy needed to produce more previtellogenic oocytes than they did initially when at a deeper depth. The second peak, which is similar to shallow, might show a start of adaptation towards shallow native spawning patterns. The deep samples have no second peak, which indicates that they may have released their vitellogenic oocytes shortly before the collection of samples, as it is known that this species has multiple spawning events (Egger *et al.*, 2025). The shallow samples, however, have a higher vitellogenic fecundity, which shows that they might be getting close to releasing their oocytes. The replanted samples are between the two native groups in the vitellogenic fecundity. This could mean that they have either released a part of their mature oocytes already or are still preparing to release. As *E. verrucosa* is known to have three spawning events with about two

weeks in between each, it is very possible that these samples were collected during these events (Egger *et al.*, 2025).

Previous research suggests that lower food environments, in this case deep, lead to a colony having fewer but larger oocytes. In contrast, high nutrient environments, like shallow, tend to have more and smaller oocytes (Eckelbarger and Watling, 1995). While the results of this study show very few large oocytes in the samples from the deep environment, the total fecundity trend is in line with the research. The deep samples have an average total fecundity of 5.85 oocytes per polyp, while replanted has 9.32 and shallow has 12.80 (Appendix 6). The lack of large oocytes is likely due to a recent spawning event. A project with a larger scope of data and sampling times would need to take place to monitor spawning patterns and changes in fecundity.

Reproductive differences were found

Returning to the research question, if there are reproductive differences between the natural and replanted samples, the answer is yes. However, it is not possible to say to which extent changes in reproduction are due to the process of replanting or the change of environment. These results would likely look completely different if the replanted samples from deep were replanted in deep water, all the same with shallow. It is very likely that the change of depth is responsible for a large part of the differences which were found between the replanted samples and the two native groups. When trying to evaluate the efficacy of the method of conservation, which was another goal of this study, it is still possible to form a based view. The replanted samples show a reasonable fecundity in relation to both natural groups, which indicates reproductive ability. While the oocyte size distribution was biased towards smaller oocytes, it cannot be proven that this is an effect of replanting, since the deep native samples behave very similarly. In summary, the replanted corals show reproductive patterns which would fit an intermediate of deep and shallow. This means there is likely no significant impairment of reproductive ability due to replantation.

What is important to note however, is that other studies have shown the size of the fragment to be highly correlated to the survival after replantation (Edery *et al.*, 2025). Many of the smaller fragments do not survive replantation in the studies that have been done on this method. While it is still an effective way to help with decreasing coverage, fragment survival does limit the efficacy of this method. The transplantation project, which was done before these samples being collected, used multiple sites for replantation. At one of these sites no replanted corals ended up surviving, showing again sensitivity to not only size, but also location.

While this method of replanting is effective in cost and can be used on a large scale, results may vary. This study indicates no strong impairment in reproduction but also considered only the surviving fragments. The largest loss of colonies happens before this point due to size of fragment and location. This is very important when considering this method in future conservation efforts. Further studies could help uncover more specific details about what it takes for replanted corals to survive. Aspects such as salinity, mechanical disruption and predation may be relevant. In addition to this, research into the specific spawning patterns between deep and shallow *Eunicella verrucosa* colonies could help with the interpretation of the results of this study, as well as shed light onto a species which we lack scientific knowledge about.

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Appendix 1: Popular science summary

Marine environments offer invaluable resources and sceneries humans depend on. They are the base of our survival, from being the planet's largest carbon sink to providing us with food and work. At the very base of these environments are so-called ecosystem engineers. These are organisms that, through their structure, build habitats for other species. Corals are known to be one of the most important ecosystem engineers, as their wide variety of species ensures their presence in every ocean around the globe. Through their ability to provide habitats, corals are invaluable not only to marine ecosystems but also to human economies. Fisheries, tourism, or recreational activities boost coastal economies, which rely on the ecological functionality of reefs and coral forests.

Corals have been subject to a lot of discussion in recent years, with anthropogenic stresses like ocean acidification, global warming, and mechanical damage having led to a large decrease in coral coverage and overall health globally. Yet, a large knowledge gap remains surrounding cold and temperate waters, as well as sea fans. Even though they occur globally, from tropical reefs to cold, deep waters, little is known about how sea fans react to and recover from anthropogenic pressures.

A large threat to these species has been fishing activities such as bottom trawling, which is where fishing boats drag large nets over the sea floor. Unfortunately, fish are not the only thing being caught, with corals often falling victim to large-scale breakage and thus exponentially decreasing coverage. A frequently observed bycatch coral in the North Atlantic is *Eunicella verrucosa*, the pink sea fan, and although research has shown that a large percentage of the coral fragments are viable and healthy, most of them are discarded by the fisheries. A common conservation method tackles this problem by using the bycatch fragments for replantation.

This helps with the decreasing coverage of coral forests by increasing the number of individuals at a large scale. A known problem with this method, however, is the fact that the replanted fragments are genetically identical clones of their mother colony. Because of this, the genetic variety in a coral population decreases, leading to a limited ability to withstand environmental changes long-term. Due to this, it is very important that the replanted corals can sexually reproduce to diversify the gene pool.

This study aimed to uncover any reproductive differences between natural and replanted *Eunicella verrucosa* individuals. Using histological methods and microscopy, the oocyte sizes and numbers (fecundity) per polyp were analysed. Through this, it was found that the average oocyte size of replanted individuals is much smaller than that of the colonies from the natural populations. This may be strongly related to the results of the oocyte counts, which showed that the number of young oocytes was close to the same, but mature oocyte counts were almost halved in replanted corals, effectively moving the average size toward the smaller end. The lack of mature oocytes may indicate that the corals are not yet ready to spawn and are functioning on a different spawning pattern than the shallow or deep natural samples.

These results show that even though replantation helps improve the density of corals, it may take years before they fully adapt to their new environment. This highlights the importance of preservation and conservation measures for natural populations.

Appendix 2: AI usage

AI was utilized for generating and correcting code for R Studio. Usage of this was supportive of the statistical analyses. In a few cases, AI was used to aid the search for research papers for specific or related topics.

Appendix 3: Acknowledgements

First and foremost, I would like to thank my supervisor, Rhian Waller, for the utmost trust and support throughout this project. Thank you for trusting in my abilities and allowing me freedom within this work.

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Appendix 4: Sample Overview *Eunicella verrucosa*

Sample number	Date	Site	Site type	Specues	Type	Depth (m)	Sex
001	2024-05-22	Torvore	Deep	*Eunicella verrucosa*	Transplant	30-32	unknown
002	2024-05-22	Torvore	Deep	*Eunicella verrucosa*	Transplant	30-32	Female
003	2024-05-22	Torvore	Deep	*Eunicella verrucosa*	Transplant	30-32	Female
004	2024-05-22	Torvore	Deep	*Eunicella verrucosa*	Transplant	30-32	Female
005	2024-05-22	Torvore	Deep	*Eunicella verrucosa*	Transplant	30-32	unknown
006	2024-05-22	Torvore	Deep	*Eunicella verrucosa*	Transplant	30-32	unknown
007	2024-05-22	Torvore	Deep	*Eunicella verrucosa*	Transplant	30-32	Male
008	2024-05-22	Torvore	Deep	*Eunicella verrucosa*	Transplant	30-32	Male
009	2024-05-22	Torvore	Deep	*Eunicella verrucosa*	Transplant	30-32	Female
010	2024-05-22	Torvore	Deep	*Eunicella verrucosa*	Transplant	30-32	Female
011	2024-05-22	Torvore	Deep	*Eunicella verrucosa*	Transplant	30-32	Female
012	2024-05-22	Torvore	Deep	*Eunicella verrucosa*	Transplant	30-32	Female
106	2024-05-22	Pontao	Shallow	*Eunicella verrucosa*	Natural	15-20	Female
107	2024-05-22	Pontao	Shallow	*Eunicella verrucosa*	Natural	15-20	Female
108	2024-05-22	Pontao	Shallow	*Eunicella verrucosa*	Natural	15-20	unknown
109	2024-05-22	Pontao	Shallow	*Eunicella verrucosa*	Natural	15-20	Female
110	2024-05-22	Pontao	Shallow	*Eunicella verrucosa*	Natural	15-20	Male
111	2024-05-22	Pontao	Shallow	*Eunicella verrucosa*	Natural	15-20	unknown
112	2024-05-22	Pontao	Shallow	*Eunicella verrucosa*	Natural	15-20	Female
113	2024-05-22	Pontao	Shallow	*Eunicella verrucosa*	Natural	15-20	unknown
114	2024-05-22	Pontao	Shallow	*Eunicella verrucosa*	Natural	15-20	Female
115	2024-05-22	Pontao	Shallow	*Eunicella verrucosa*	Natural	15-20	Female
116	2024-05-22	Pontao	Shallow	*Eunicella verrucosa*	Natural	15-20	Male
117	2024-05-22	Pontao	Shallow	*Eunicella verrucosa*	Natural	15-20	Female
118	2024-05-22	Pontao	Shallow	*Eunicella verrucosa*	Natural	15-20	Female
119	2024-05-22	Pontao	Shallow	*Eunicella verrucosa*	Natural	15-20	Male
120	2024-05-22	Pontao	Shallow	*Eunicella verrucosa*	Natural	15-20	Female
031	2024-05-22	Torvore	Deep	*Eunicella verrucosa*	Natural	30-32	unknown
032	2024-05-22	Torvore	Deep	*Eunicella verrucosa*	Natural	30-32	Female
033	2024-05-22	Torvore	Deep	*Eunicella verrucosa*	Natural	30-32	unknown
034	2024-05-22	Torvore	Deep	*Eunicella verrucosa*	Natural	30-32	unknown
035	2024-05-22	Torvore	Deep	*Eunicella verrucosa*	Natural	30-32	Female
036	2024-05-22	Torvore	Deep	*Eunicella verrucosa*	Natural	30-32	unknown
037	2024-05-22	Torvore	Deep	*Eunicella verrucosa*	Natural	30-32	Male
038	2024-05-22	Torvore	Deep	*Eunicella verrucosa*	Natural	30-32	unknown
039	2024-05-22	Torvore	Deep	*Eunicella verrucosa*	Natural	30-32	unknown
040	2024-05-22	Torvore	Deep	*Eunicella verrucosa*	Natural	30-32	Female
041	2024-05-22	Torvore	Deep	*Eunicella verrucosa*	Natural	30-32	unknown
042	2024-05-22	Torvore	Deep	*Eunicella verrucosa*	Natural	30-32	Male
043	2024-05-22	Torvore	Deep	*Eunicella verrucosa*	Natural	30-32	Female
044	2024-05-22	Torvore	Deep	*Eunicella verrucosa*	Natural	30-32	unknown
045	2024-05-22	Torvore	Deep	*Eunicella verrucosa*	Natural	30-32	Female

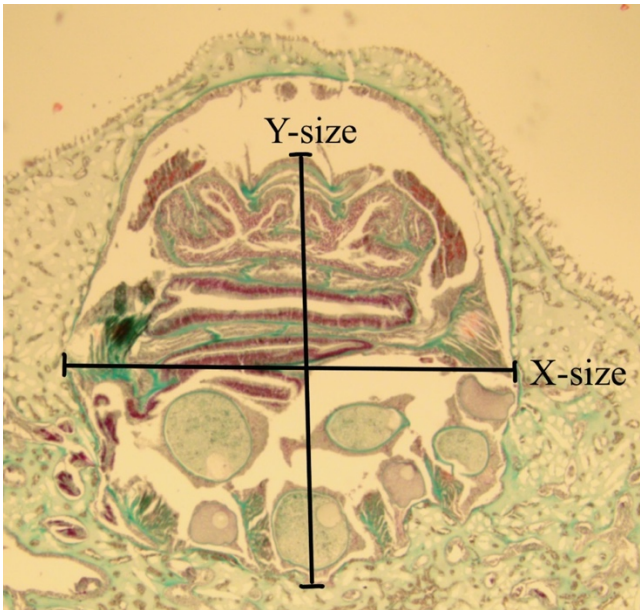
Appendix 5: Sample Overview *Leptogorgia sarmentosa*

Sample number	Date	Site	Site type	Specues	Type	Depth (m)	Sex
016	2024-05-22	Torvore	Deep	*Leptogorgia sarmentosa*	Transplant	30-32	—
017	2024-05-22	Torvore	Deep	*Leptogorgia sarmentosa*	Transplant	30-32	—
019	2024-05-22	Torvore	Deep	*Leptogorgia sarmentosa*	Transplant	30-32	—
020	2024-05-22	Torvore	Deep	*Leptogorgia sarmentosa*	Transplant	30-32	—
021	2024-05-22	Torvore	Deep	*Leptogorgia sarmentosa*	Transplant	30-32	—
022	2024-05-22	Torvore	Deep	*Leptogorgia sarmentosa*	Transplant	30-32	—
023	2024-05-22	Torvore	Deep	*Leptogorgia sarmentosa*	Transplant	30-32	—
024	2024-05-22	Torvore	Deep	*Leptogorgia sarmentosa*	Transplant	30-32	—
025	2024-05-22	Torvore	Deep	*Leptogorgia sarmentosa*	Transplant	30-32	—
046	2024-05-22	Torvore	Deep	*Leptogorgia sarmentosa*	Natural	30-32	—
047	2024-05-22	Torvore	Deep	*Leptogorgia sarmentosa*	Natural	30-32	—
048	2024-05-22	Torvore	Deep	*Leptogorgia sarmentosa*	Natural	30-32	—
049	2024-05-22	Torvore	Deep	*Leptogorgia sarmentosa*	Natural	30-32	—
050	2024-05-22	Torvore	Deep	*Leptogorgia sarmentosa*	Natural	30-32	—
051	2024-05-22	Torvore	Deep	*Leptogorgia sarmentosa*	Natural	30-32	—
052	2024-05-22	Torvore	Deep	*Leptogorgia sarmentosa*	Natural	30-32	—
053	2024-05-22	Torvore	Deep	*Leptogorgia sarmentosa*	Natural	30-32	—
054	2024-05-22	Torvore	Deep	*Leptogorgia sarmentosa*	Natural	30-32	—
055	2024-05-22	Torvore	Deep	*Leptogorgia sarmentosa*	Natural	30-32	—
056	2024-05-22	Torvore	Deep	*Leptogorgia sarmentosa*	Natural	30-32	—
057	2024-05-22	Torvore	Deep	*Leptogorgia sarmentosa*	Natural	30-32	—
058	2024-05-22	Torvore	Deep	*Leptogorgia sarmentosa*	Natural	30-32	—
059	2024-05-22	Torvore	Deep	*Leptogorgia sarmentosa*	Natural	30-32	—
060	2024-05-22	Torvore	Deep	*Leptogorgia sarmentosa*	Natural	30-32	—
091	2024-05-22	Pontao	Shallow	*Leptogorgia sarmentosa*	Natural	15-20	—
092	2024-05-22	Pontao	Shallow	*Leptogorgia sarmentosa*	Natural	15-20	—
093	2024-05-22	Pontao	Shallow	*Leptogorgia sarmentosa*	Natural	15-20	—
094	2024-05-22	Pontao	Shallow	*Leptogorgia sarmentosa*	Natural	15-20	—
095	2024-05-22	Pontao	Shallow	*Leptogorgia sarmentosa*	Natural	15-20	—
096	2024-05-22	Pontao	Shallow	*Leptogorgia sarmentosa*	Natural	15-20	—
097	2024-05-22	Pontao	Shallow	*Leptogorgia sarmentosa*	Natural	15-20	—
098	2024-05-22	Pontao	Shallow	*Leptogorgia sarmentosa*	Natural	15-20	—
099	2024-05-22	Pontao	Shallow	*Leptogorgia sarmentosa*	Natural	15-20	—
100	2024-05-22	Pontao	Shallow	*Leptogorgia sarmentosa*	Natural	15-20	—

Appendix 6: Fecundity raw data with averages and SD

	replanted				deep native				shallow native		
Female	Previt	Vit	Total	Female	Previt	Vit	Total	Female	Previt	Vit	Total
3	5,00	6,00	11,00	32	4,00	7,00	11,00	106	2,00	8,00	10,00
3	10,00	7,00	17,00	32	2,00	2,00	4,00	106	1,00	4,00	5,00
3	6,00	7,00	13,00	32	2,00	7,00	9,00	106	4,00	7,00	11,00
3	4,00	2,00	6,00	32	3,00	6,00	9,00	106	4,00	9,00	13,00
3	6,00	5,00	11,00	32	0,00	9,00	9,00	106	6,00	5,00	11,00
Average	6,20	5,40	11,60	Average	2,20	6,20	8,40	Average	3,40	6,60	10,00
SD	2,28	2,07	3,97	SD	1,48	2,59	2,61	SD	1,95	2,07	3,00
4	3,00	2,00	5,00	35	1,00	5,00	6,00	107	0,00	5,00	5,00
4	9,00	9,00	18,00	35	1,00	7,00	8,00	107	0,00	3,00	3,00
4	2,00	8,00	10,00	35	2,00	7,00	9,00	107	1,00	2,00	3,00
4	2,00	1,00	3,00	35	2,00	3,00	5,00	107	0,00	6,00	6,00
4	1,00	5,00	6,00	35	2,00	4,00	6,00	107	1,00	2,00	3,00
Average	3,40	5,00	8,40	Average	1,60	5,20	6,80	Average	0,40	3,60	4,00
SD	3,21	3,54	5,94	SD	0,55	1,79	1,64	SD	0,55	1,82	1,41
9	3,00	1,00	4,00	40	5,00	4,00	9,00	115	5,00	12,00	17,00
9	1,00	2,00	3,00	40	1,00	5,00	6,00	115	2,00	9,00	11,00
9	2,00	0,00	2,00	40	1,00	4,00	5,00	115	3,00	9,00	12,00
9	4,00	2,00	6,00	40	2,00	4,00	6,00	115	11,00	11,00	22,00
9	4,00	4,00	8,00	40	1,00	1,00	2,00	115	13,00	16,00	29,00
Average	2,80	1,80	4,60	Average	2,00	3,60	5,60	Average	6,80	11,40	18,20
SD	1,30	1,48	2,41	SD	1,73	1,52	2,51	SD	4,92	2,88	7,46
10	11,00	7,00	18,00	43	1,00	3,00	4,00	117	2,00	8,00	10,00
10	5,00	6,00	11,00	43	1,00	1,00	2,00	117	3,00	12,00	15,00
10	2,00	4,00	6,00	43	0,00	1,00	1,00	117	3,00	11,00	14,00
10	5,00	1,00	6,00	43	0,00	3,00	3,00	117	4,00	9,00	13,00
10	6,00	1,00	7,00	43	1,00	2,00	3,00	117	7,00	11,00	18,00
Average	5,80	3,80	9,60	Average	0,60	2,00	2,60	Average	3,80	10,20	14,00
SD	3,27	2,77	5,13	SD	0,55	1,00	1,14	SD	1,92	1,64	2,92
12	3,00	7,00	10,00	45	2,00	2,00	4,00	118	8,00	15,00	23,00
12	5,00	10,00	15,00	45	1,00	2,00	3,00	118	9,00	10,00	19,00
12	7,00	3,00	10,00	45	0,00	2,00	2,00	118	10,00	19,00	29,00
12	10,00	6,00	16,00	45	0,00	2,00	2,00	118	6,00	1,00	7,00
12	7,00	4,00	11,00	45	1,00	4,00	5,00	118	7,00	10,00	17,00
Average	6,40	6,00	12,40	Average	0,80	2,40	3,20	Average	8,00	11,00	19,00
SD	2,60768096	2,73861279	2,88097206	SD	0,83666003	0,89442719	1,30384048	SD	1,58113883	6,74536878	8,1240384
Average	previt	vit	total		previt	vit	total		previt	vit	total
	4,92	4,40	9,32		1,60	4,25	5,85		4,65	8,15	12,80

Appendix 7: Visualization of X and Y-size measurements



Appendix 8: GitHub

The R-script as well as the R-project and excel raw data can be found at:
https://github.com/sawe0123/E.-verrucosa-reproductive-study