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BIOLOGICAL CHARACTERISTICS AND EXPERIMENTAL ASEXUAL REPRODUCTION OF THE SOFT CORAL SPECIES *Sarcophyton serenei* TIXIER–DURIVAULT, 1958

ABSTRACT OF THE DOCTORAL THESIS IN BIOLOGY

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INTRODUCTION

Soft corals are key components of tropical coral reefs, contributing to biodiversity and providing habitats for numerous marine species. Their high adaptability enables them to act as pioneer species in reef formation, particularly in turbid waters. The genus *Sarcophyton* comprises approximately 80 species, including *Sarcophyton serenei*, which occurs in Nha Trang Bay and other marine areas. In addition to their ecological importance, soft corals are a valuable source of bioactive compounds with significant application potential. However, overharvesting for the ornamental aquarium trade has caused declines in natural populations, threatening biodiversity and coral reef ecosystems.

Therefore, soft coral cultivation has gained increasing attention as a sustainable approach to reduce pressure on natural populations while supplying the ornamental aquarium trade, pharmaceutical industry, and coral reef restoration programs. Although research on soft coral reproduction has mainly focused on the Red Sea and the Great Barrier Reef, information from the Western Pacific remains limited. In Vietnam, studies on coral reproduction are scarce and have largely focused on hard corals. Low-cost propagation and cultivation methods, particularly asexual techniques optimized through factors such as substrate type, feeding regime, lighting, and culture systems, offer promising solutions for commercial production and conservation. However, the reproductive biology and asexual propagation of *Sarcophyton serenei* have not yet been investigated.

Currently, the cost of producing a coral colony through asexual propagation is only about 20% of that associated with sexual reproduction. This suggests that research and application of artificial sexual reproduction require substantially greater investment. Therefore, this thesis focuses on clarifying the biological basis and improving asexual propagation methods as a more practical and efficient approach under current conditions.

Research Objectives

- To investigate the habitat characteristics and reproductive biology of *S. serenei* as a scientific basis for artificial propagation.
- To develop an effective asexual propagation protocol for *S. serenei*.

Research Content

Content 1: Biological characteristics of the soft coral species *S. serenei*

Content 1.1. *Morphological characteristics and taxonomy*

Content 1.2. *Habitat characteristics*

Content 1.3. *Reproductive biology characteristics*

Content 2: Study on the asexual propagation of the soft coral species *S. serenei* under artificial conditions

Content 2.1. *Effects of Lugol's solution concentration and fragment treatment duration*

Content 2.2. *Effects of substrate type and fragment size*

Content 2.3. *Effects of feed type and feeding regime*

Content 2.4. *Effects of light intensity*

Scientific and Practical Significance

This study on the reproductive biology and asexual propagation of the soft coral *Sarcophyton serenei* is the first of its kind in Vietnam, providing a scientific basis for conservation, population restoration, and the exploitation of bioactive compounds.

New Contributions of the Thesis

This thesis is the first study to investigate the biological characteristics and asexual propagation of the soft coral *S. serenei*. The study also clarified the reproductive biological characteristics of *S. serenei*, including sex ratio, developmental stages of oocytes and spermatocytes, reproductive capacity, and spawning season. In addition, the study identified the optimal technical conditions for asexual propagation of *S. serenei*, including treatment of fragments with 0.5 $mL \cdot L^{-1}$ Lugol's solution for 15 min or 1.0 $mL \cdot L^{-1}$ for 10 min; fragment sizes ≥ 1.5 cm; the use of ceramic tile or cement substrates; feeding with rotifers every two days; and light intensity ranging from 90 to 120 μmol m^{-2} s^{-1}.

Chapter 1. OVERVIEW OF THE STUDY

1.1. Taxonomic Characteristics of the Genus *Sarcophyton* and the Species *S. serenei*

1.1.1. Genus *Sarcophyton*

Sarcophyton is a genus of soft corals belonging to the phylum Anthozoa, class Octocorallia, order Malacalcyonacea, and family Sarcophytidae. Currently, the genus *Sarcophyton* comprises 50 valid species, 3 species with uncertain taxonomic status, and 27 species with revised classifications.

Taxonomic Characteristics of Species Belonging to the Genus *Sarcophyton*

Species of the genus *Sarcophyton* have a characteristic mushroom-like morphology, with a capitulum bearing polyps that may be flat, lobed, cup-shaped, or funnel-shaped. Sclerites on the capitulum and stalk are typically club-shaped, occasionally with a central wart, and their morphology and size are important taxonomic characters. Colonies contain two types of polyps: retractile autozooids, responsible for feeding and reproduction, and siphonozooids distributed between them. The spacing and density of these polyps are key diagnostic features for species identification.

1.1.2. Classification System of the Soft Coral *S. serenei*

Figure 1.1. Soft coral *S. serenei* used in the study

The taxonomic classification of the soft coral *S. serenei* is as follows:

Kingdom: Animalia
  Phylum: Cnidaria
    Class: Anthozoa
      Subclass: Octocorallia
        Order: Malacalcyonacea
          Family: Sarcophytidae

Genus: *Sarcophyton*

Species: *S. serenei* Tixier–Durivault, 1958

Figure 1.1. Soft coral *S. serenei* used in the study

1.2. Distribution of the Genus *Sarcophyton* and the Species *S. serenei*

Species of the genus *Sarcophyton* are widely distributed from intertidal zones to depths of 6,400 m and from tropical to polar regions. Their highest diversity occurs in the Indo-Pacific, particularly within the Coral Triangle (Indonesia, Malaysia, the Philippines, Papua New Guinea, Timor-Leste, and the Solomon Islands), where strong water currents provide favorable feeding conditions. *Sarcophyton serenei* has been recorded in the Gulf of Suez (Egypt) and several Vietnamese marine areas, including Nha Trang Bay, Ly Son Island, and the Cu Lao Cham Marine Protected Area.

1.3. Overview of International Research

1.3.1. Ecological Characteristics of Soft Corals and the Genus *Sarcophyton*

The distribution and growth of soft corals are strongly influenced by environmental factors, including light intensity, water currents, sedimentation, depth, distance from shore, temperature, salinity, and nutrient availability. Species differ in their tolerance and adaptability: zooxanthellate soft corals typically inhabit clear, shallow waters, whereas azooxanthellate species are more common in deep or low-light environments. Stable water currents enhance nutrient supply, waste removal, and photosynthesis, while excessive sedimentation can cause tissue smothering, necrosis, and mortality. The genus *Sarcophyton* is widely distributed throughout the tropical Pacific, Indian Ocean, and Red Sea, where it commonly inhabits nearshore habitats with moderate turbidity and soft substrates and exhibits high environmental adaptability.

1.3.2. Growth Characteristics of Soft Corals

Studies on coral growth, particularly in soft corals of the genus *Sarcophyton*, remain limited. Growth rate is a key indicator of environmental impacts and coral reef dynamics, but research on soft corals is complicated by their flexible morphology and hydrodynamic plasticity. Consequently, most studies have focused on hard corals, leaving information on soft coral growth scarce. Existing studies report growth rates of approximately 0.5 cm $year^{-1}$ or 3.95 g $month^{-1}$ during summer, with growth strongly influenced by temperature, turbidity, sedimentation, and water movement. Coral growth has been assessed using direct measurements, photographic analysis, X-ray imaging, Alizarin staining, and digital image modeling, of which photographic analysis with ImageJ is considered a non-destructive and accurate method.

1.3.3. Reproductive Biological Characteristics of Soft Corals

1.3.3.1. Reproductive Modes

Reproductive strategies play an important role in determining the abundance and distribution of octocorals (*Octocorallia*). This group reproduces through both asexual and sexual mechanisms. Asexual reproduction is common among soft corals and occurs through processes such as fragmentation, fission, and budding. Sexual reproduction is essential for maintaining genetic diversity and population stability, with species exhibiting either gonochoric or hermaphroditic reproductive patterns.

1.3.3.2. Size at First Reproduction and Reproductive Season

Octocorals show considerable variation in age, colony size, sexual maturity, and reproductive traits. Colony size strongly influences reproduction, as gonads generally develop only after colonies reach a minimum size. Oocyte size, gametogenic duration, and reproductive cycles vary among species according to reproductive mode, larval characteristics, and environmental conditions. Many species exhibit gametogenic cycles of 9–20 months and reproduce synchronously with lunar cycles, while geographic and habitat differences further influence reproductive timing and oocyte development. However, the reproductive biology of *S. serenei* remains poorly understood.

1.3.4. Relationship Between Symbiotic Algae, Chlorophyll-a, and Soft Corals

Soft corals of the genera *Sarcophyton*, *Sinularia*, *Xenia*, and *Lobophytum* maintain intracellular symbioses with *Symbiodinium*, obtaining much of their energy through photosynthesis. Photosynthetic pigments, including chlorophyll-*a*, chlorophyll-c_2, and carotenoids, support light harvesting and protect the symbiosis from ultraviolet radiation. Light intensity directly influences chlorophyll content and symbiont density, with stress potentially causing color changes or bleaching. *Symbiodinium* comprises seven major clades (A–G), of which clades C and D are most commonly associated with soft corals and enhance their environmental adaptability. However, the symbiotic algae and chlorophyll characteristics of *S. serenei* remain poorly understood.

1.3.5. Research on Asexual Propagation of Soft Corals

Asexual propagation is a commonly used method in soft coral aquaculture, allowing the maintenance and expansion of coral colonies with high survival rates while reducing dependence on natural harvesting. This technique involves separating fragments from parent colonies and attaching them to suitable substrates for the development of new colonies.

During fragmentation, corals secrete mucus that may increase susceptibility to infection; therefore, wounds are commonly treated with antiseptic agents such as Lugol’s iodine solution. Lugol’s solution possesses strong antimicrobial properties and is widely used in marine aquaculture and aquarium systems for wound treatment and disease prevention.

Fragment size is an important factor affecting coral survival and recovery. Studies on soft corals such as *Sarcophyton glaucum* have shown that smaller fragments containing a greater number of polyps and obtained from peripheral regions of the colony exhibit higher survival rates. Substrate type also plays a crucial role in fragment attachment and subsequent growth. Commonly used substrates include aragocrete, live rock, ceramic materials, resin, and cement.

Nutrition is essential for the growth and survival of soft corals, particularly species associated with symbiotic zooxanthellae. Under artificial culture conditions, corals are commonly fed planktonic organisms or alternative live feeds such as *Brachionus plicatilis* and *Artemia salina*. Some studies have reported that supplemental feeding improves survival, recovery, and acclimation, whereas others have found no significant differences between fed and unfed treatments.

Light intensity directly influences the photosynthetic performance of zooxanthellae and the growth of soft corals. Previous studies have indicated that the optimal light intensity for *Sarcophyton* species ranges from approximately 80 to 120 $\mu mol\ m^{-2}\ s^{-1}$. Current studies on asexual propagation of soft corals have mainly focused on *S. glaucum* and *S. flexibilis*, whereas technical parameters for *S. serenei* remain largely unknown. Optimizing factors such as fragment size, substrate type, light intensity, feeding regime, and Lugol's solution treatment may improve the propagation efficiency of this species.

1.4. Overview of Coral Research in Vietnam

Previous coral research in Vietnam has mainly focused on species diversity, biological characteristics, chemical composition, bioactive properties of soft corals, coral diseases, and associated bacteria. More recently, reproductive biology has gained attention as a basis for artificial propagation. Studies in Nha

Trang Bay showed that *Acropora* species reproduce from March to May, with spawning closely synchronized to lunar cycles. Experimental studies on soft coral propagation further demonstrated that substrate type influences fragment attachment and survival, with dead coral substrates performing best, whereas fragment size mainly affects survival and morphology rather than growth; fragments of approximately 1.5 cm are considered optimal for fragmentation. However, research on *Sarcophyton serenei* in Vietnam has so far been limited to taxonomic classification and distribution.

Chapter 2. RESEARCH SUBJECTS AND METHODS

2.1. Research Subjects, Time, and Location

2.1.1. Research Subject

The research subject was the soft coral species *Sarcophyton serenei* Tixier–Durivault, 1958.

2.1.2. Research Period

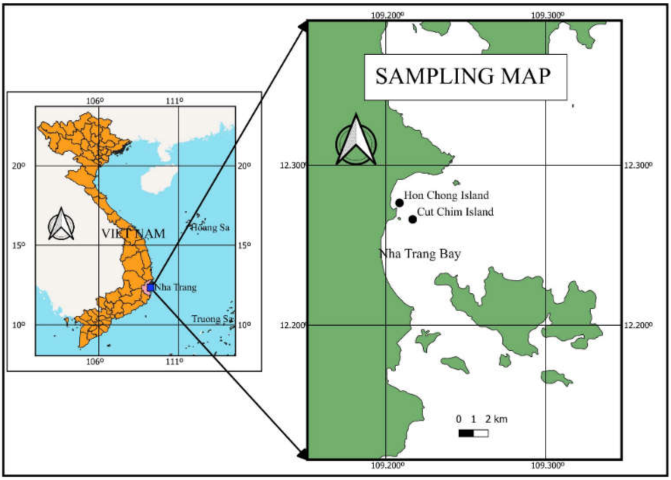
The study was conducted from November 2022 to October 2025.

2.1.3. Research Location

- Sampling locations: Coral samples were collected at depths of 6–8 m from sites extending between Hon Cut Chim (12°15′55.9″N, 109°13′29.3″E) and the Hon Chong area (12°17′11.0″N, 109°13′15.0″E) in Nha Trang Bay.

- Research facilities: Experimental farm and laboratory facilities of the Institute of Oceanography.

Figure 2.1. Sampling locations in the study area



2.2. Research Methods

Content 1: Biological Characteristics of *S. serenei*

Content 1.1. Morphological Characteristics and Taxonomic Identification of *S. serenei*

External morphological characteristics, including colony color, colony shape, polyp structure, stalk morphology, capitulum morphology, and sclerite morphology, were analyzed based on the taxonomic descriptions and identification keys provided by Tixier–Durivault (1958) [1], Verseveldt (1982) [2], and Dautova and Savinkin (2013) [3].

Content 1.2. Habitat Characteristics of *S. serenei*

Environmental parameters, including turbidity, temperature, salinity, light intensity, and dissolved oxygen (DO), were measured monthly for 12 consecutive months at depths of 6–8 m between 08:00 and 09:00 a.m.

Content 1.3. Reproductive Biological Characteristics

Samples of the soft coral *S. serenei* were collected monthly during the full moon period from August 2023 to July 2024. The reproductive biological characteristics analyzed included sex ratio, oocyte and spermatocyst size, developmental stages of oogenesis and spermatogenesis, reproductive capacity, and spawning season.

Content 2: Study on the Asexual Propagation of *S. serenei* Under Artificial Conditions

The evaluated parameters included attachment time, survival rate, zooxanthellae density, and chlorophyll-a content.

Content 2.1. Experiments on the Effects of Lugol's Solution Concentration and Treatment Duration

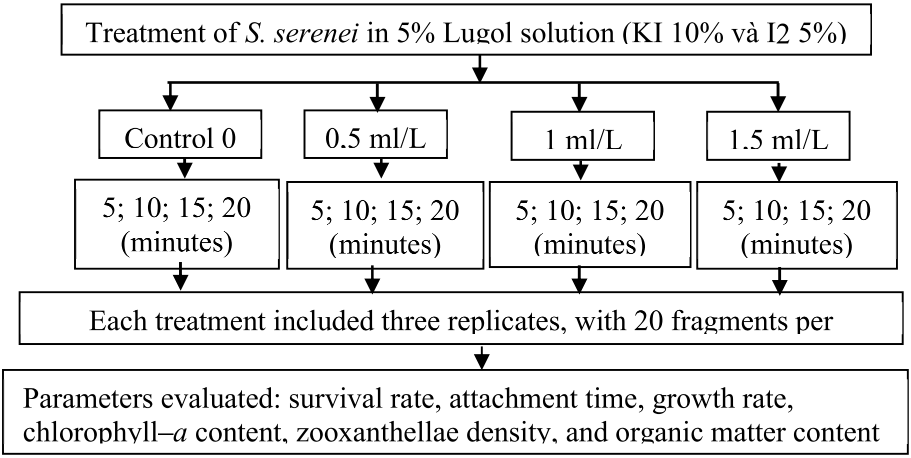


Figure 2.2. Schematic diagram of the experimental design evaluating the effects of Lugol's solution concentration and treatment duration on the growth and physiological characteristics of *S. serenei*.

Experimental treatments evaluating the effects of Lugol's solution concentration and treatment duration were designed as illustrated in Figure 2.2.

Content 2.2. Experiments on the Effects of Substrate Type and Fragment Size

The experimental design evaluating the effects of substrate type and fragment size was conducted as illustrated in Figure 2.3.

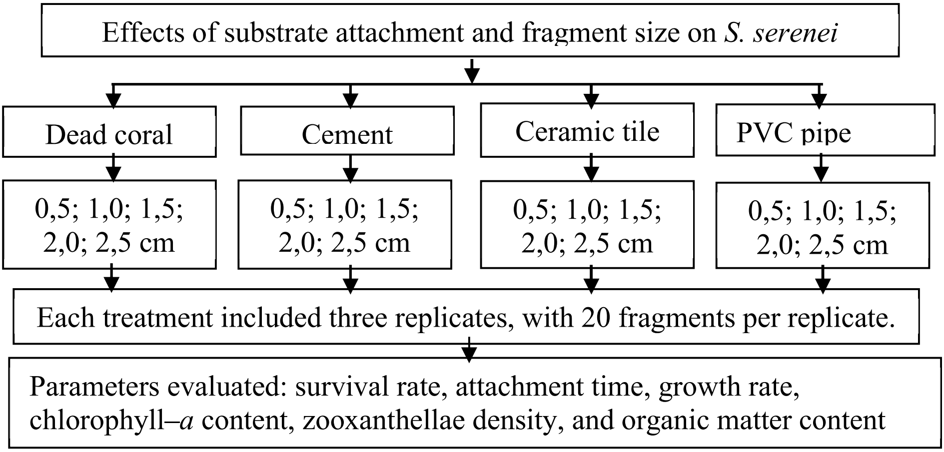


Figure 2.3. Schematic diagram of the experimental design evaluating the effects of substrate type and fragment size on the growth and physiological characteristics of *S. serenei*.

Content 2.3. Experiments on the Effects of Feed Type and Feeding Regime

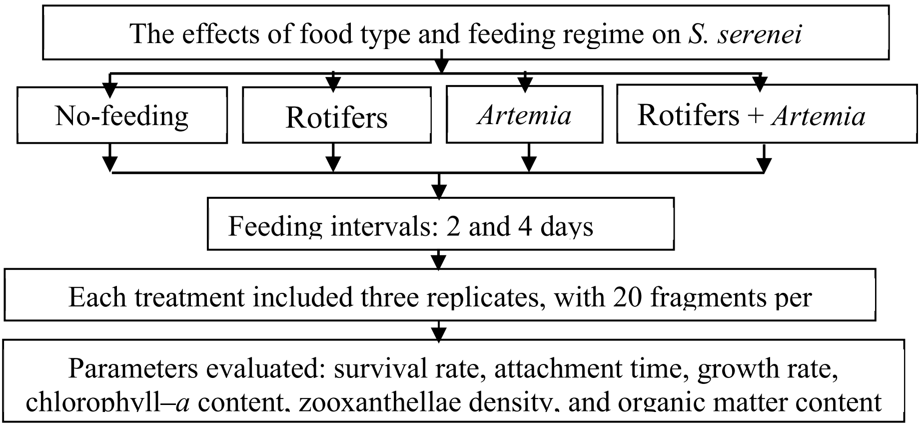


Figure 2.4. Schematic diagram of the experimental design evaluating the effects of feed type and feeding regime on the growth and physiological characteristics of *S. serenei*.

The experimental design evaluating the effects of feed type and feeding regime is illustrated in Figure 2.4.

Content 2.4. Research on the Effects of Light Intensity on the Growth and Physiological Characteristics of *S. serenei*

The experimental design evaluating the effects of light intensity on the growth and physiological characteristics of the soft coral species *S. serenei* is illustrated in Figure 2.5.

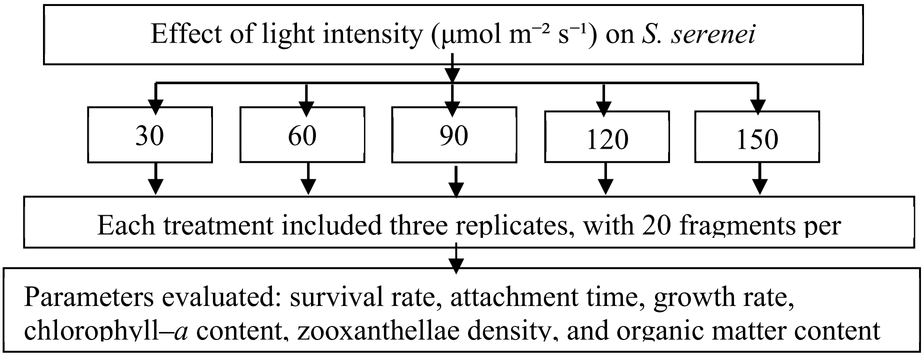


Figure 2.5. Schematic diagram of the experimental design evaluating the effects of light intensity on the growth and physiological characteristics of *S. serenei*.

Data Collection Methods

Attachment time**:** Determined according to the third-order scale proposed by Cunha (2006) [4].

Survival rate (%)**:** Recorded at 3, 6, 9, and 30 or 60 days after fragmentation, depending on the experiment.

Coral mass**:** Measured using the wet-weight method with an electronic balance (Sartorius) accurate to 0.001 g.

Oral disc diameter**:** Measured using *ImageJ* software based on photographs taken from above the water surface with a calibrated scale.

Zooxanthellae density**:** Determined according to the method described by Pupier et al. (2018) [5].

Chlorophyll-a content**:** Determined according to the method described by Dong et al. (2023) [6].

Organic matter content of coral fragments**:** Determined according to the method described by Costa et al. (2016) [7].

Data Analysis

Sex ratios were analyzed using the Chi-square test of independence.

One-way analysis of variance (ANOVA) was applied for Content 2.4, whereas two-way ANOVA was performed for Contents 2.1, 2.2, and 2.3 using SPSS version 18.0 at a significance level of $p = 0.05$. Data are presented as mean ± standard deviation (Mean ± SD).

Chapter 3. RESULTS AND DISCUSSION

3.1. Biological characteristics of the soft coral species *S. serenei*

3.1.1. Morphological characteristics and classification of *S. serenei*

3.1.1.1. External morphology of the soft coral species S. serenei

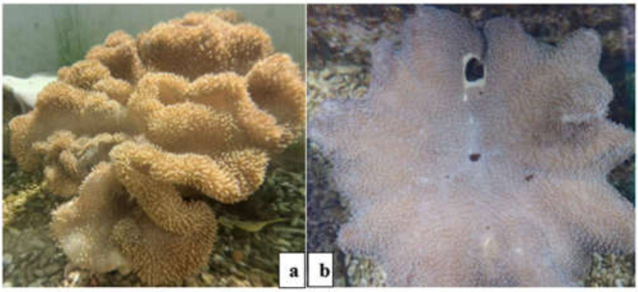


Figure 3.1. Soft coral *S. serenei*

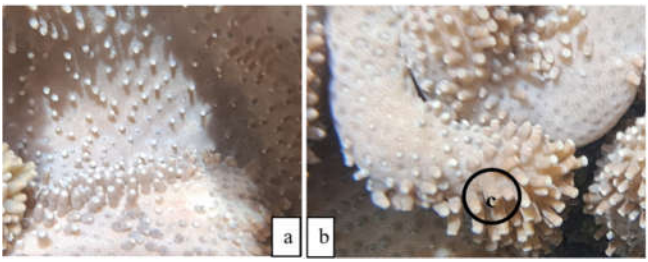


Figure 3.2. Distribution of autozooids and siphonozooids on the capitulum

(a) Central region of the capitulum; (b) marginal region of the capitulum; and (c) anthocodia

3.1.1.2. Sclerite characteristics of the soft coral species S. serenei

The sclerite morphology of 35 specimens of *S. serenei* is illustrated in Figure 3.3.

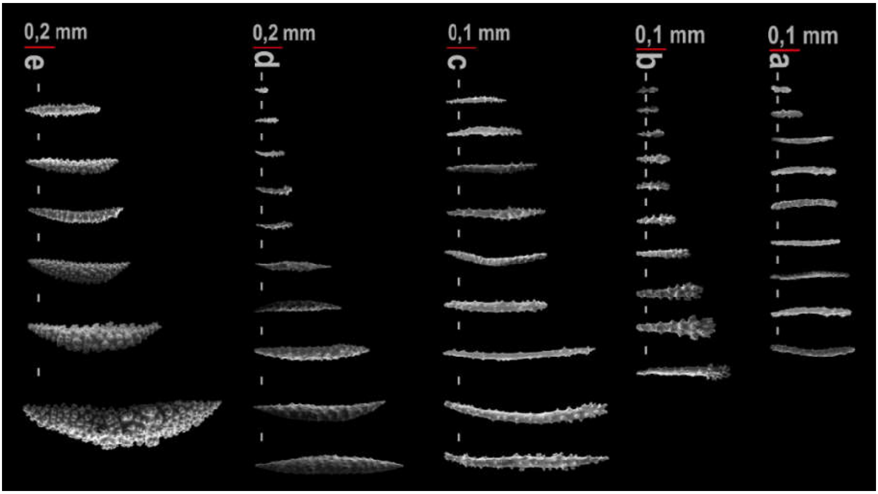


Figure 3.3. Sclerites of the soft coral species *S. serenei*.

(a) Tentacles and anthocodiae; (b) surface of the capitulum; (c) interior of the capitulum; (d) surface of the stalk; and (e) interior of the stalk.

The soft coral *S. serenei* is bright yellowish-brown to grayish-brown in color. Its capitulum bears numerous folds along the margin and is smooth in the central region (Figure 3.1). The cap surface is slightly rough to the touch and does not secrete mucus. Colonies are approximately 5 cm in height, with a stalk diameter of 10–12 cm, and generally lack a distinct overall shape. When touched, the autozooids do not retract completely. The tips of the anthocodiae are pale white and do not fully expand (Figure 3.2c).

3.1.2. Habitat characteristics of *S. serenei* in Nha Trang Bay

3.1.2.1. Environmental characteristics of the habitat of S. serenei

The environmental parameters, including light intensity, salinity, temperature, dissolved oxygen (DO), chemical oxygen demand (COD), turbidity, NH_4^+, and NO_3^- concentrations, measured at Hon Chong and Hon Cut Chim are presented in Table 3.1. All measured environmental parameters were within the range suitable for coral growth and development.

Table 3.1. Environmental parameters at the two survey sites

Parameters	Hon Chong	Hon Cut Chim
Temperature (°C)	28,38 ± 1,54	28,04 ± 1,53
Salinity (‰)	32,35 ± 1,12	32,53 ± 0,94
Light (µmol $m^{-2}s^{-1}$)	42,36 ± 23,15	51,40 ± 21,07
pH	8,02 – 8,18	8,04 – 8,13
DO (mg/L)	6,65 ± 0,34	6,68 ± 0,34
COD (mg/L)	1,34 ± 0,42	1,32 ± 0,38
Turbidity (NTU)	2,21 ± 0,86	1,76 ± 0,54
NH_4^+(mg/L)	0,017 ± 0,006	0,017 ± 0,006
NO_{3-}(mg/L)	0,039 ± 0,011	0,037 ± 0,008

Bottom substrate characteristics:

The coral distribution area investigated in this study encompassed Hon Cut Chim and the surrounding waters extending to Hon Chong in Nha Trang Bay, at depths ranging from 5 to 8 m. These two sites are located in the northern part of Nha Trang Bay and are approximately 2.35 km apart; therefore, they share similar topographic and geomorphological characteristics. Field surveys and sediment sampling revealed that the seabed in the coral distribution area consisted primarily of sand, coral rubble, and mud, with sand and coral fragments accounting for the largest proportion. The soft coral *S. serenei* was observed growing on substrates composed mainly of dead coral rock.

3.1.3. Reproductive Biological Characteristics of *S. serenei*

3.1.3.1. Sex Ratio of the Soft Coral Species S. serenei

Analysis of 380 colonies of *S. serenei* indicated that this species is gonochoric, with 217 male colonies and 163 female colonies recorded. No hermaphroditic colonies or colonies of undetermined sex were observed. The male-to-female ratio was 1.33:1.

3.1.3.2. Stages of Oocyte and Spermary Development in S. serenei

The development of oocytes and spermaries in *S. serenei* can be divided into four stages.

3.1.3.3. Reproductive Capacity of S. serenei

The reproductive capacity of *S. serenei* ranged from 14 to 26 oocytes per polyp.

3.1.3.4. Maturity Rate and Reproductive Season of S. serenei

Oocyte size increased progressively over time and reached its maximum in late March (20 March), when 100% of the oocytes had a diameter greater than 450 µm, indicating maturity. Thereafter, oocyte size decreased markedly by the end of March (30 March) and throughout April. This pattern suggests a synchronous reproductive strategy, with spawning occurring once annually and concentrated in March.

3.2. Study of Asexual Reproduction of the Soft Coral Species *S. serenei*

3.2.1. Effects of Lugol's Solution Concentration and Immersion Time

3.2.1.2. Survival Rate

The results showed that the survival rates of coral fragments treated with 0.5 mL L^{-1} Lugol's solution for 15 min (T:0.5–15) and 1.0 mL L^{-1} for 10 min (T:1.0–10) were significantly higher than those of the control treatment without Lugol's solution after 9 days of experimentation.

3.2.1.3. Attachment Time

The shortest attachment time was recorded in treatment T:0.5–15 (8.73 ± 0.43 days), whereas the longest attachment time was observed in treatment T:0–10 (14.47 ± 0.41 days) ($p \leq 0.001$). No significant difference in attachment time was detected between treatments T:0.5–15 and T:1.0–10 ($p \geq 0.965$).

3.2.1.4. Coral Fragment Growth

The results indicated that Lugol's solution concentration, immersion time, and their interaction had no significant effects on the specific growth rates based on weight (SGRW) ($p \geq 0.348$) or length (SGRL) ($p \geq 0.390$). The highest SGRW value was recorded in treatment T:1.5–5 (0.45 ± 0.06% day^{-1}), whereas the lowest value was observed in treatment T:0–15 (0.35 ± 0.04% day^{-1}) ($p \geq 0.055$). Similarly, the highest SGRL value was recorded in treatment T:0.5–15 (1.29 ± 0.09% day^{-1}), while the lowest was observed in treatment T:1.5–15 (1.05 ± 0.09% day^{-1}), with no significant difference among treatments ($p \geq 0.883$).

3.2.1.5. Zooxanthellae Density

Zooxanthellae density decreased following coral fragmentation and reached its lowest level on day 6, after which it gradually recovered by day 9 and continued to increase until the end of the experiment (day 30). However, neither Lugol's solution concentration, immersion time, nor their interaction significantly affected zooxanthellae density at any sampling time ($p \geq 0.215$).

3.2.1.6. Chlorophyll–a Content

Chlorophyll–*a* content declined markedly following fragmentation, particularly during the period from fragmentation to day 6, and gradually recovered after day 9. Nevertheless, Lugol's solution concentration, immersion time, and their interaction did not significantly influence chlorophyll–a content throughout the experimental period ($p \geq 0.153$).

3.2.1.7. Organic Matter Content

Organic matter content was significantly influenced by both Lugol's solution concentration and immersion time ($p < 0.001$). The highest values were recorded in treatments T:1–10 (42.96 ± 1.04% OW) and T:1–15 (42.18 ± 0.89% OW). In contrast, the lowest organic matter contents were observed in treatments T:0–5, T:0–10, T:0–15, T:0–20, and T:1.5–20, ranging from 34% to 35% OW ($p \leq 0.001$).

3.2.2. Influence of Substrate Type and Fragment Size

3.2.2.1. Survival Rate

The survival rate did not differ significantly among treatments with fragment sizes of 1.5 cm or larger attached to different substrate types ($p > 0.550$). However, treatments with fragment sizes greater than 1.5 cm exhibited significantly higher survival rates than those with smaller fragments ($p < 0.05$). No mortality was recorded after day 9 of the experiment.

3.2.2.2. Attachment Time

Fragments larger than 1.5 cm attached significantly faster than smaller fragments ($p < 0.05$). However, no significant differences in attachment time were observed among treatments with fragment sizes of 1.5, 2.0, and 2.5 cm attached to different substrate types ($p > 0.05$).

3.2.2.3. Growth of Coral Fragments

The specific growth rates based on weight and length were lowest in treatments with fragment sizes of 0.5 cm, followed by those with 1.0 cm fragments, and highest in treatments with fragment sizes of 2.0 and 2.5 cm ($p < 0.05$). Nevertheless, no statistically significant differences were detected among treatments with fragment sizes of 1.5, 2.0, and 2.5 cm attached to different substrate types ($p > 0.05$).

3.2.2.4. Zooxanthellae Density

At the beginning of the experiment (day 0), zooxanthellae density ranged from 4.87×10^6 to 5.09×10^6 cells g^{-1}. The density decreased markedly by day 3 and gradually recovered after day 6. By day 9, zooxanthellae density had returned to initial levels and increased further by day 60 (the end of the experiment), reaching values ranging from 4.81×10^6 to 5.05×10^6 cells g^{-1}. No significant differences were observed between the beginning and the end of the experiment ($p > 0.05$).

3.2.2.5. Chlorophyll–a Content

At the beginning of the experiment (day 0), chlorophyll–a content ranged from 4.61×10^2 to 4.81×10^2 µg g^{-1}. The chlorophyll–a content decreased sharply by day 3, began to recover on day 6, and increased substantially after day 9. By day 60, chlorophyll–a content ranged from 4.63×10^2 to 4.81×10^2 µg g^{-1}. No significant differences were observed between the beginning and the end of the experiment ($p > 0.05$).

3.2.2.6. Organic Matter Content

The results showed that the organic matter content of *S. serenei* was significantly affected by fragment size, substrate type, and the interaction between these factors ($p < 0.05$). The highest organic matter content was recorded in treatment T:2.5–S, in which 2.5-cm fragments were attached to coral substrate, whereas the lowest values were observed in treatments with 0.5-cm fragments ($p < 0.05$). However, no significant differences were detected among treatments with fragment sizes of 1.5 cm or larger attached to different substrate types ($p > 0.05$).

3.2.3. Effects of Food Type and Feeding Frequency

3.2.3.1. Survival Rate

The experimental results showed that neither food type nor feeding frequency significantly affected the survival rate of *S. serenei* fragments ($p \geq 0.082$).

3.2.3.2. Specific Growth Rate Based on Weight

The results indicated that treatment LT–2 achieved the highest specific growth rate based on weight (SGRW) ($0.46 \pm 0.02\%$ day^{-1}), which was significantly higher than those of the control treatment and the Artemia-fed treatments ($p \leq 0.035$). However, no significant difference was observed between treatments LT–2 and LT–4 ($p \geq 0.124$).

3.2.3.3. Zooxanthellae Density

The results showed that zooxanthellae density did not differ significantly among treatments throughout the experimental period ($p \geq 0.059$). During the initial stage, zooxanthellae density decreased markedly compared with the initial value, reflecting the temporary physiological response of coral fragments following the fragmentation process. From day 6 onward, zooxanthellae density gradually increased, indicating recovery of the coral fragments. By the end of the experiment, zooxanthellae density in all treatments had returned to levels comparable to, or slightly lower than, the initial values ($p \geq 0.059$).

3.2.3.4. Chlorophyll a Content

Chlorophyll a content did not differ significantly among treatments throughout the experimental period ($p \geq 0.111$), as shown in Table 3.22. These results indicate that food type and feeding frequency had no significant effect on chlorophyll a content in *S. serenei*. In all treatments, chlorophyll a content decreased markedly from day 0 to day 3, followed by a gradual increase from day 6 to day 60. By the end of the experiment, chlorophyll a content had nearly recovered to its initial level in all treatments.

3.2.3.5. Organic Matter Content

The organic matter content of *S. serenei* was significantly affected by both food type and feeding frequency ($p \leq 0.031$). The highest organic matter content was recorded in treatment LT–2 (46.68 ± 1.53% OW), whereas the lowest value was observed in the control treatment (DC) (42.11 ± 1.78% OW) ($p \leq 0.003$). A significant difference was detected between treatment LT–2 and both the control and Ar–4 treatments ($p \leq 0.003$). However, no significant differences were observed among the remaining treatments ($p \geq 0.057$).

3.2.4. Effects of Light Intensity

3.2.4.1. Survival Rate

After 60 days of experimentation, the highest survival rate was recorded in the treatment exposed to a light intensity of 90 µmol m^{-2} s^{-1} (83 ± 2%), whereas the lowest survival rate was observed in the treatment exposed to 30 µmol m^{-2} s^{-1} (73 ± 2%) ($p \leq 0.007$). No statistically significant differences were detected among the treatments with light intensities of 30, 60, and 150 µmol m^{-2} s^{-1}, nor among the treatments with 90, 120, and 150 µmol m^{-2} s^{-1} ($p \geq 0.124$). These results indicate that survival of *S. serenei* fragments was enhanced under light intensities ranging from 90 to 120 µmol m^{-2} s^{-1}.

3.2.4.2. Attachment Time

Light intensity significantly affected the attachment time of *S. serenei* fragments ($p < 0.05$). The longest attachment time was observed in the treatment exposed to 30 µmol m^{-2} s^{-1}, whereas the shortest attachment times were recorded in the treatments exposed to 90 and 120 µmol m^{-2} s^{-1} ($p < 0.05$). No significant difference was found between the treatments exposed to 60 and 150 µmol m^{-2} s^{-1} ($p > 0.05$); however, both treatments differed significantly from the remaining treatments ($p < 0.05$).

3.2.4.3. Growth

The specific growth rates based on weight (SGR_W) and length (SGR_L) differed significantly among light intensity treatments ($p \leq 0.009$). Both SGR_W and SGR_L were significantly higher in the 90 and 120 µmol m^{-2} s^{-1} treatments than in the 30, 60, and 150 µmol m^{-2} s^{-1} treatments ($p \leq 0.009$). Furthermore, no significant difference in SGR_W was observed among the 30, 60, and 150 µmol m^{-2} s^{-1} treatments ($p \geq 0.302$). In contrast, SGR_L reached its highest values under 90 and 120 µmol m^{-2} s^{-1} and its lowest value under 30 µmol m^{-2} s^{-1} ($p \leq 0.001$), whereas no significant differences were detected among the remaining treatments ($p \geq 0.055$). These findings suggest that light intensities of 90–120 µmol m^{-2} s^{-1} are optimal for the growth of *S. serenei*.

3.2.4.4. Zooxanthellae Density

Zooxanthellae density recovered more rapidly in the 90–120 µmol m^{-2} s^{-1} treatments than in the other treatments, reaching levels comparable to the initial density by day 10. At this time, zooxanthellae density was highest under 90–120 µmol m^{-2} s^{-1} and lowest under 30 µmol m^{-2} s^{-1} ($p < 0.05$), although no significant differences were detected compared with some of the intermediate treatments ($p > 0.05$). By day 12, zooxanthellae density in the 90–120 µmol m^{-2} s^{-1} treatments was significantly higher than that observed under low (30 µmol m^{-2} s^{-1}) and high (150 µmol m^{-2} s^{-1}) light intensities, whereas no significant differences were found among the remaining treatments ($p > 0.05$). After 60 days, zooxanthellae density had recovered in all treatments, and no significant differences were observed among light intensity levels ($p > 0.05$).

3.2.4.5. Chlorophyll a Content

Significant differences in chlorophyll a content among treatments were first observed on day 6 and persisted until day 12 ($p < 0.05$). The highest chlorophyll a content was recorded in the treatments exposed to 90 and 120 µmol m^{-2} s^{-1}, whereas the lowest value was observed under 30 µmol m^{-2} s^{-1} ($p < 0.05$). By day 10, chlorophyll a content had begun to recover in the 90–120 µmol m^{-2} s^{-1} treatments. After 60 days of experimentation, chlorophyll a content had returned to levels comparable to the initial values in all treatments, with no statistically significant differences among them ($p > 0.05$).

3.2.4.6. Organic Matter Content

Organic matter content was highest in the treatments exposed to 90–120 µmol m^{-2} s^{-1} and lowest in the treatment exposed to 30 µmol m^{-2} s^{-1} ($p < 0.05$). No statistically significant differences were detected among the remaining treatments ($p > 0.05$).

CONCLUSIONS AND RECOMMENDATIONS

1. Conclusions

Biological characteristics of the soft coral species *S. serenei*

Based on its morphological and taxonomic characteristics, *S. serenei* has a mushroom-shaped capitulum with distinct marginal folds, a rough surface, no mucus secretion, and partially retractable polyps. Its sclerites vary in shape and size, with numerous club-shaped forms in the tentacles and capitulum and large wart-like forms in the stalk. These morphological and sclerite characteristics are key diagnostic features for species identification and classification.

S. serenei is mainly distributed in areas with dead coral reef substrates and calcareous sandy–muddy sediments in the coastal waters of Nha Trang Bay. Environmental conditions at the study sites included water temperature ranging from 25–30 °C, salinity from 30–34 ‰, light intensity from 14.77 to 89.64 µmol m^{-2} s^{-1}, pH from 8.02–8.10, dissolved oxygen (DO) from 5.93–7.44 mg L^{-1}, chemical oxygen demand (COD) from 0.88–1.79 mg L^{-1}, turbidity from 0.80–3.5

NTU, NH_4^+ from 0.009–0.030 mg L^{-1}, and NO_3^- from 0.022–0.062 mg L^{-1}, all of which were within suitable ranges for coral growth.

The soft coral *S. serenei* is dioecious, with a male-to-female ratio of 1.3:1. Reproductive output ranged from 14 to 26 oocytes per polyp. Mature oocytes had an average diameter of 685 µm, while spermaries had an average size of 235.29 µm. Oocytes and spermaries developed through four distinct stages. This species exhibits a synchronous annual reproductive cycle, with spawning occurring once per year, concentrated in March.

Research on the Asexual Reproduction of *S. serenei*

Research on the effects of Lugol's solution concentration and immersion time on coral fragments showed that treatments with 0.5 mL L^{-1} for 15 min or 1.0 mL L^{-1} for 10 min were most suitable, as they increased survival rates and shortened attachment time, while having no significant effect on growth rate or symbiotic algal density.

Studies on the effects of fragment size and substrate type indicated that fragments of 1.5, 2.0, and 2.5 cm exhibited the highest survival and growth rates. Ceramic tiles and cement were identified as suitable artificial substrates that can replace dead coral rock or PVC in reef restoration models.

The results on feeding regime effects showed that feeding with rotifers (*Brachionus plicatilis*) every two days enhanced growth rate and organic matter content compared with feeding with Artemia or no feeding.

Studies on the effects of light intensity indicated that the optimal range was 90–120 µmol m^{-2} s^{-1}, which improved survival, growth, and attachment time, increased organic matter content, and shortened the recovery time of chlorophyll a content and symbiotic algal density following fragmentation and handling stress.

2. Recommendations

Research conducted during 2023–2024 indicates that *S. serenei* reproduces in March. However, because coral reproductive timing may shift in response to environmental variation and climate change, long-term monitoring is needed to accurately determine the species' reproductive season. Such information will facilitate the optimization of gamete collection schedules and the design of experiments on sexual reproduction.

Based on these findings, further studies are required to develop reliable protocols for the asexual propagation of *S. serenei* and to conduct larger-scale evaluations of their technical and economic feasibility.

Pilot restoration programs using asexually propagated *S. serenei* colonies should be implemented on coral reefs in Nha Trang Bay and in other coastal areas with similar environmental conditions. These programs would assess the species' adaptability, survival and recovery potential in natural habitats, as well as the ecological risks associated with restoration efforts.

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