



Review

Restoration of Marine Sponges—What Can We Learn from over a Century of Experimental Cultivation?

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Abstract: Marine sponges are the driver of many critical biological processes throughout various ecosystems. But anthropogenic and environmental pressures are rapidly compromising the diversity and abundance of Porifera worldwide. In our study, we reviewed the main experiences made on their cultivation to provide a roadmap of the best methodologies that could be applied to restore coastal sponge populations. We synthesized the results of experimental trials between 1950 and today to facilitate information on promising methods and materials. We detected a strong geographical imbalance between different ecoregions, as well as a shift of scientific effort from the investigation of "bath sponge" mariculture towards the rearing of bioactive compounds from sponges. Although sponge cultivation is arguably highly species-dependent, we further found that skeletal consistency in combination with taxonomy may be used to decide on appropriate techniques for future restoration initiatives.

Keywords: Porifera; aquaculture; transplantation



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1. Introduction

The evolutionary history of metazoans started with Porifera, the most ancient animal phylum [1]. The first adopted biological solutions maintained even in the more evolved taxa. Sponges are generally considered very simple, an aggregation of eukaryotic cells with low specialization, a primordial organization, and with a peculiar morphological plasticity [2]. Thanks to these features, they can be found both in marine and freshwater ecosystems, from polar to tropical regions, and from shallow to deep habitats. Being effective sessile suspension feeders, sponges play a key functional role in marine ecosystems. Moreover, they can also reach big sizes, with massive and erect growth forms, providing structural complexity to benthic habitats. Their highly variable morphologies are due to the secretion of mineral and/or organic skeletons, offering shelters and food to an impressive number of species, during different phases of their life cycles [3,4]. Their plasticity is mirrored also in their reproductive strategies that can be both sexual and asexual. Thanks to the latter, sponges can be handled to create fragments and amplify their biomass both in the field and in aquaria.

The exploitation of sponge populations has been occurring for over centuries. In ancient Rome, these species were highly valued and used as bath utensils (so called "bath sponges") [5]. Although historical data on global trade is scattered and often incomplete, it is likely that the trade had its peak in the beginning of the 20th century. The uncontrolled harvest, together with heavy outbreaks of diseases, ended up in a critical decline of sponge populations resulting in drastic effects that were seen throughout the 20th century, even causing regional extinctions of certain species [6].

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The high demand through the 20th and 21st century coupled with the disappearance of natural stocks have led to an increase of interest in the cultivation of commercially valuable species. For over 100 years researchers have been investigating potential cultivation techniques to develop successful and economically viable farming structures, especially in the case of "bath sponges".

In recent history, the number of studies in this field has increased exponentially, testing different synthetic materials and methodologies such as farming sponges that are threaded on either horizontal or vertical ropes, e.g., [7–12], and farming sponges that are attached to or inside mesh panels, e.g., [7,13–16]. Other approaches have included the use of steel cages that are close to the seafloor [12] or attaching sponges to natural (e.g., coral boulders, rocks) or artificial surfaces (e.g., concrete discs and boulders) [17,18].

This increase of interest in sponge mariculture, especially over the last two decades, has been driven also by the discovery of bioactive metabolites in many marine sponges [13,19–21], which are of high interest to the pharmaceutical industry due to their often associated antiviral, anti-inflammatory, and anticancer properties [22]. As a result, a shift could be observed from the investigation of sponge mariculture techniques for the production of "bath sponges" towards the development of protocols and methodologies in order to sufficiently and sustainably rear bioactive metabolites for pharmaceutical purposes. Simultaneously, the number of natural products and clinical trials for pharmaceuticals that are derived from marine invertebrates has been constantly rising over the past two decades [23]. For this reason, many authors have also attempted cultivation under strictly controlled laboratory environments rather than *in situ* cultivation, e.g., [18,19,24–27].

Besides the above, sponges have further been identified as potential bioremediator of organic waste, such as that which is produced by commercial aquaculture farms due to their extraordinary filtering capacities [28,29]. There has been an increasing interest over the past decade in this field with multiple studies indicating the positive effects of sponge-integrated aquaculture systems [21,30–33].

Although a small number of early reviews of different cultivation techniques have been published [20,22,34–36], there seems to be no comprehensive roadmap synthesizing previous results and giving recommendations on the best techniques for the transplantation of different groups of sponges with similar physiological characteristics. To fill this gap, the aim of this manuscript is to facilitate information and state-of-the-art knowledge on marine sponge cultivation in order to provide guidance and recommendation on potential best combinations of materials and methodologies with regards to sponge taxonomy and skeletal consistency. This will benefit future cultivation prospects and, most importantly, provide valuable information for future marine benthic habitat restoration efforts. Here we:

- 1. compile information on the methodologies that have been applied previously for the transplantation and cultivation of marine sponges,
- 2. synthesize the results of the compiled literature to identify potential best techniques in consideration of taxonomic differences and skeletal features,
- 3. provide guidelines and recommendations on *in situ* transplantation and cultivation methods for sponges with different skeletal consistency.

2. Materials and Methods

A literature review was carried out in order to compile the state-of-the-art of transplantation techniques for marine sponges and identify the potential best techniques. The search was carried out using the databases and terminology that are summarized in Table 1. Publications between 1950 and 2021 were assessed to extract information about experimental approaches that are related to the transplantation of marine sponges (Porifera). However, if any other relevant literature was identified, it was further added for assessment when the publications addressed the cultivation of marine sponges. Applying the same principle, the compilation was completed by additionally assessing the complete reference lists of the previously published reviews that were related to the topic [20,22,34–36] and by hand-searching based on expert opinion in order to obtain the most complete list of literature.

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Finally, all the transplantation-related information (e.g., methodologies, materials, species, and results) were extracted and compiled in a table to allow for comparison and analysis. For the analysis, we focused on *in situ* cultivation techniques.

Table 1. Search procedure and inclusion-exclusion criteria.

Criteria	Specification	
Years	1950–2022	
	"marine sponge transplant(-ation)"	
	"marine sponge cultivation"	
	"marine sponge explant(s)"	
Search terms	"restoration marine sponge(s)	
	"sponge aquaculture"	
	"sponge farm(-ing)"	
	Web of Science	
Databases	Google Scholar	
	ResearchGate	
	Studies published between the period 1950–2021	
	Studies including information about transplantation of marine sponges	
T 1	Studies experimenting transplantation methods	
Inclusion criteria	Peer-reviewed studies	
	Published in English, French, or German language	
	Available in selected databases	
	Studies outside marine sponge transplantation-cultivation domain	
Exclusion criteria	No peer-reviewed studies	
	Duplicated among databases	

The collected information allowed us to first analyze the geographical distributions and differences in economic interests of the studies and, secondly, the potential relationships between survival rates and method, materials, taxonomy, and skeletal consistencies. In the second part of the analysis, studies that were carried out in polluted environments were excluded due to the particular environmental conditions and potential effects on survival rates.

3. Results

3.1. Geographical Distribution and Commercial Interests

A total of 48 studies were compiled during the review part, of which 40 were directly addressing the $in\ situ$ cultivation of marine sponges that were used for the first part of the analysis (see Appendix A, Table A1). A strong difference in the number of publications between different marine and coastal ecoregions (based on [37]) was detected (see Figure 1). Most studies were conducted in the Temperate Northern Atlantic (n=15), which also includes the Mediterranean Sea, and the Central Indo-Pacific (n=9). For Temperate Australasia (n=7), the western Indo-Pacific (n=4), and the Tropical Atlantic (n=4) lower numbers were recorded. The lowest number of publications was obtained for the Tropical Eastern Pacific with n=1. For all other regions (Arctic, Eastern Indo-Pacific, Southern Ocean, Temperate Northern Pacific, Temperate South America, Temperate Southern Africa) no publications were found.

Regarding sponge cultivation for commercial interest, 21 studies (53%) focussed on the production of bioactive metabolites, 8 (20%) on methodologies to farm "bath sponges", and 4 (10%) on integrated mariculture approaches as an approach to produce bioactive compounds while reducing organic waste pollution. Only three (7%) studies were found to be related to the restoration of marine benthic communities, as well as only one (3%) addressing bioremediation individually without the aim of producing sponge biomass or bioactive compounds Another three publications were not directly addressing any of the

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above and therefore titled "n/a". These were mostly focussed on the general ecology of marine sponges.

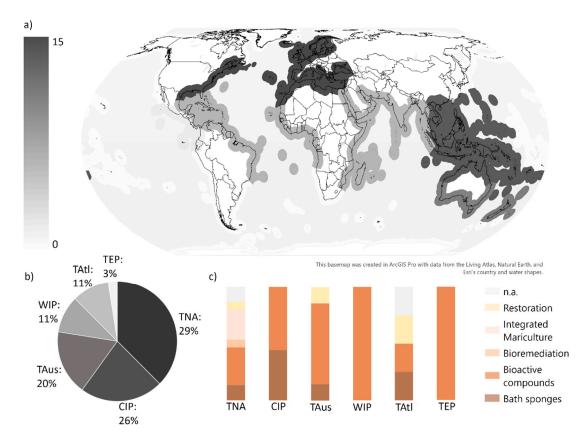


Figure 1. (a) Geographic distribution of publications that were related to the *in situ* cultivation of marine sponges (gradient shows number of publications for different ecoregions (based on [36])). (b) Distribution of all publications in percent for different ecoregions. (c) Percentage of publications that were related to different commercial interests (TNA = Tropical Northern Atlantic, CIP = Central Indo-Pacific, TAus = Temperate Australasia, WIP = Western Indo-Pacific, TAtl = Tropical Atlantic, TEP = Tropical Eastern Pacific).

Across all six regions, bioactive metabolites were the dominating commercial interest, except for the Tropical Atlantic where there was an equal interest in four different categories (Figure 1). For the Central Indo-Pacific, the interest in bath sponges was nearly as high as for bioactive compounds (44% to 56%, respectively). Further, a change in main commercial interest was observed over time. In the beginning of the 1990s, sponge farming trials were mostly related to the rearing of bath sponges, whereas the interest seemed to shift more towards the production of secondary metabolites during the early 2000s, which is now the dominating interest (Figure 2).

3.2. Transplantation Techniques

For further analysis, all the transplantation techniques were categorized as shown in Table 2.

The highest effort was found for the method "Mounted on artificial substrate" as 30% of all the trials were using this technique. "Mesh systems" and "Rope systems" accounted for 26% and 28%, respectively. "Mounted on natural substrate" and "Cage systems" were applied arguably less often with only 7% each. The combined "Mesh-rope systems" approach was only used in one study making up 2% of all trials.

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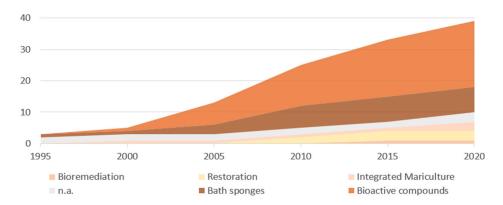


Figure 2. The number of publications on sponge *in situ* cultivation (included in this review) that were related to different selected commercial interests over time.

Table 2. Description of categorized transplantation techniques.

Technique	Description
Cage systems	Sponges cultivated inside a cage structure with a solid (not mesh) bottom part. Cage may be covered or uncovered on the top.
Mesh systems	Sponges cultivated in enclosed mesh arrays (completely surrounded by mesh). Can be "squeezed" between panels or held loosely in more spacious arrays (e.g., oyster/mussel panels or lanterns).
Mesh-rope systems	Sponges initially cultivated in mesh arrays (see Mesh systems) before transferred to a rope array (see Rope systems)
Mounted on artificial substrate	Sponges cultivated attached/on top of artificial substrates (e.g., cement blocks, plastic trays). Category also assigned when farmed on top of mesh arrays (e.g., mesh grid) instead of enclosed by it.
Mounted on natural substrate	Sponges are farmed attached/on top of artificial substrates (e.g., live rock, coral boulder, oyster shells)
Rope systems	Sponges cultivated using ropes. Can either be single lines or large arrays spanning across artificial frameworks. No differentiation between vertical or horizontal orientation. Sponges may be threaded directly on ropes, attached to ropes (e.g., using zip ties or thin ropes) or hung on ropes using small pieces of rope (or other material such as zip ties) forming a loop

All the sponge species that were included in the reviewed studies belonged to the class Demospongiae, while species of the subclass Heteroscleromorpha were assessed most frequently accounting for 49% of all the trials. All the other investigated species were either members of Keratosa or Verongimorpha making up 34% and 18%. Sponges of 12 different orders were represented, with Dyctioceratida alone accounting for 33% of all the trials. Haplosclerida and Poecilosclerida represented 16% and 13%, respectively, whereas all other orders were relatively equally distributed showing lower percentages. In total, 52 species of 29 families were tested across the 40 reviewed articles. The representation of each family across all the trials was comparatively equally distributed with the exception of members of Spongiidae, which were assessed in 20% of all the trials.

An overview of the survival rates in relation to different methodologies, materials, skeletal consistencies, as well as different methodologies and subclasses is shown in Figure 3. For the analysis, only "Mesh systems", "Mounted on artificial substrate", and "Rope systems" were included as for the others the sample size was arguably low (n < 7). The best results were seemingly obtained for sponges that were transplanted using the "Mounted on artificial substrate" with a mean survival rate of 77%, while for "Mesh systems" 72%, and for "Rope systems" 69% was obtained. However, differences between the methodologies proved to be non-significant (p-value = 0.5599). The highest variation in the survival rates was observed for the rope systems.

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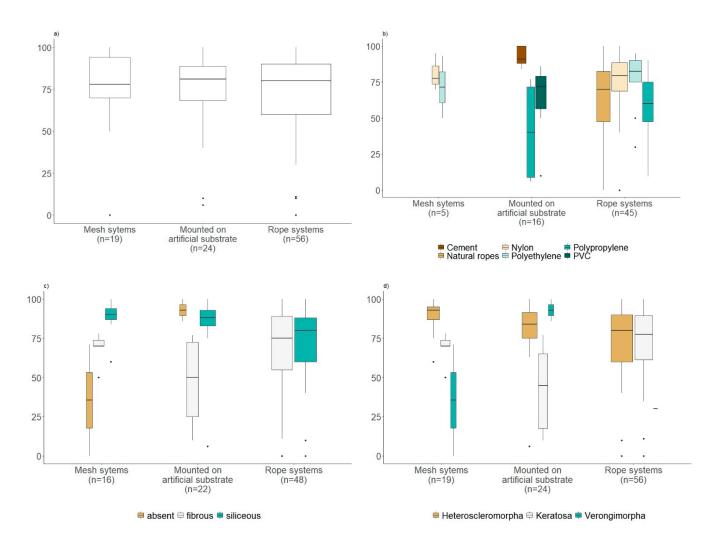


Figure 3. (a) Survival rates in percentage in relation to each technique. (b) Survival rates in percentage in relation to different techniques and materials (the materials with the highest sample effort were selected where n > 5. Not further defined plastics have been excluded). (c) Survival rates in percentage in relation to different methodologies and skeletal consistencies. (d) Survival rates in percentage in relation to different techniques and subclasses. (Note: The width of the box plots corresponds to sample size for each group. Only techniques with n > 5 were included in the analysis. Records occurring only once for a certain method in (a–c) were also excluded, hence the varying sample sizes).

The "Mesh systems" seemed to perform best on average using materials that were made of nylon, followed by polyethylene with mean survival rates of 81% and 72%. For "Mounted on artificial substrate", the survival rates were highest on average when cement was used (93%). The obtained values for PVC were 63% and the observed variation for this group was relatively high. Polypropylene scored the lowest value of 41%. Using "Rope systems", polyethylene and nylon showed the highest mean survival rates with 77% and 70%, respectively. The natural ropes reached an average survival rate of 62%, although the variation for this group was extremely high with some individual trials resulting in survival rates of 0% as well as of 100%. Polypropylene seemed to perform less well with a mean survival rate of 58%.

Regarding the results for the different skeletal characteristics, there was a high variation among the groups for "Mesh systems". Sponges with siliceous skeletons performed best with a mean survival rate of 87%, while 69% of the sponges with fibrous skeletons survived on average. Only two replicates for sponges with absent skeletons were recorded, one with a survival rate of 71% and a second one with 0%. Opposed to the "Mesh systems", sponges with no skeleton performed arguably well using the "Mounted on artificial

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substrate"-technique with survival rates averaging at 93%. The obtained value for siliceous sponges was 82% and the lowest one was obtained for fibrous sponges with 49%. Only siliceous and fibrous sponges were tested using "Rope systems" with very similar results. The mean survival rate for the first was 66% and 69% for the latter.

Last, the relationship between the methodology and subclass was investigated. For "Mesh systems", the subclass of Heteroscleromorpha reared the best results with a mean survival rate of 89%. Keratosa reached a mean survival rate of 69%. Only two trials for Verongimorpha could be included in the analysis with survival rates of 71% and 0%. However, the latter performed arguably better when "Mounted on artificial substrate" with a mean survival rate of 93%, whereas Heteroscleromorpha and Keratosa reached 80% and 43%, resepctively. Especially for the latter, strong variations were observed. For "Rope systems", Heteroscleromorpha showed a slightly smaller variation than Keratosa, although the mean survival rate for both was 71%.

4. Discussion

On 1 March 2019, the United Nations (UN) General Assembly (New York) declared 2021–2030 the "UN Decade on Ecosystem Restoration", a declaration that coincides with the UN Decade of Ocean Science for Sustainable Development. If harmonized with a holistic strategy, signatory nations could reverse deterioration in ocean health, mitigate the climate crisis, protect biodiversity on the planet, provide clean water, and enhance food security. It is a unique opportunity, and we will not have a second chance.

While on land there is a quite good experience regarding restoration actions, in aquatic habitats we are still in a testing phase, with a lot of uncertainties regarding both the best methodologies and the right spatial scales. Nevertheless, some examples of large-scale conservation efforts can be recognized in the literature, such as the project called Marine Ecosystem Restoration in Changing European Seas (MERCES) or the LIFE project ECOREST. These projects aimed to restore marine habitats of the continental shelf and slope by transplanting and recovering engineer species such as seagrasses, sponges, corals, and bivalves. We urgently need robust roadmaps and guidelines showing the best technical approaches to transplant at least the main foundation species [38] to be able to speed up the recovery of the lost habitat integrity and the relative ecosystem services. Papers focusing on gaps analysis [39] demonstrate the lack of knowledge on the natural history of the majority of species is compromising the effectiveness of these actions but it is important to amplify these experiences to increase knowledge on species phenology.

The results that are provided by this wide review have the main scope to offer a comparative analysis on the most effective transplant and cultivation techniques to support future restoration actions having sponges among the key habitat forming species.

Although many authors discussed the importance of species that are dependent cultivation designs, valuable lessons can be learned from past experiments. Sponge growth forms and general consistencies are highly variable even among individuals of the same species and potentially only of limiting value when having to choose adequate transplantation techniques. During the review, it was also found that the description of sponge features is often sparse and not concise, which made it challenging to establish condensed categories to use for recommendations. However, information on skeletal consistencies was well accessible and straightforward to categorize, which potentially increases the benefit of this indicator for future restoration and transplantation efforts. Together with taxonomy, both may be used as potential first indicators when deciding on suitable techniques.

Across the reviewed experiments, Heteroscleromorpha seemed to perform comparatively well (see Figure 3). It may be attributed to their siliceous skeletons, which seem to be less influenced by the different transplantation techniques. Based on our results, mounting sponge transplants on cemented substrates for transplantation may deliver the highest success when trying to restore sponges of this subclass and skeletal consistency. Additionally, cement or concrete blocks offer the benefit of reducing the amount of plastic and other harmful materials that are introduced to the ecosys-

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tem when restoring these species. Successful examples of this technique are presented in [17,18]. The exact same approach is further already being implemented by the team of the Behringer Lab (https://behringerlab.com/sponge-community-restoration-2/, accessed on 15 December 2021) in southeast Florida aiming to restore sponge communities on a larger scale. Although excluded in our analysis due to the low number of experiments, natural substrates may further be worth considering when transplanting sponges for restoration purposes as disturbances to the natural environment will be reduced to a minimum. Previous trials actually showed promising results in terms of survival, although underlining at the same time that the knowledge of the sponge's ecology is crucial for the success [17,18]. Depending on the life strategy, it may be recommended to place sponge explants slightly elevated compared to the seafloor to avoid smothering [17].

During the analysis it became evident that members of the subclass Keratosa may pose the greatest challenges to effective restoration efforts. Some cultivation experiments have been successful in the past, resulting even in the commercial farming of "bath sponges". For example, commercial farming systems have been implemented in Zanzibar and Micronesia (maricultures.org, https://www.marinecultures.org/en/projects/spongefarming/ spongefarming/, accessed on 15 December 2021; MERIP, https://www.meripmicronesia. org/sponge-farming/, accessed on 15 December 2021). However, cultivating sponges on rope arrays for commercial purposes offers only limited direct benefits to ecosystem restoration. This form of cultivation may provide small-scale benefits by enhancing natural recruitment of cultivating species due to larval spill-over [40,41], but is unlikely to promote ecosystem restoration on a larger scale. It is worth mentioning that for this subclass, considerably less experiments were conducted "Mounted on artificial substrate". This is due to the fact that most trials were carried out in order to produce marketable "bath sponges", where the shape of the sponge is one of the most important factors to consider. Therefore, future research is needed to provide a better understanding of how to best transplant sponges of this subclass possessing fibrous skeletons in terms for restoration aspirations.

The results of this review may also be used as a guide for future cultivation prospects, which may not directly impact restoration efforts but benefits sponge conservation indirectly. The increase of frequency and severity of extreme climate events, marine heat waves, and the subsequent porifera disease outbreaks [5,8] drew attention to the critical situation of sponge communities worldwide. Mass mortalities are arguably most well documented for the Mediterranean Sea, with strong declines for multiple species [42–44]. Similar declines were reported by [45] for the Florida Bay Region following heavy cyanobacterial blooms with mortalities ranging between 23% to 80%, as well as by [4] as the result of a long-term study of tropical sponge assemblages in Panama's coastal waters (Caribbean Sea) where 50% of species and 40% of biomass were lost. In other areas, scientists detected more dynamic changes in the abundance of sponge species with rapid declines followed by increases again returning to original composition, e.g., [46]. Conclusively, the exploitation of natural sponge populations can at best only sustain small-scale operations in certain regions. Integrated multi-trophic aquaculture on the other hand, may hold the potential to enhance marine coastal habitat recovery and it could play an important future role as restoration and conservation tool [47]. The positive effects include bioremediation of organic pollution as well as the potential larval dispersal supplying local wild populations. The latter process is still under debate regarding oyster reefs and still to be assessed for other taxa including sponges.

Despite the above, the sheer number of different species, materials, and methodologies very visually highlight the complexity of this topic and the results of experimental sponge cultivation are to be interpreted with great care. Low survival rates may, in some cases, be products of unsuitable environmental conditions rather than an inappropriate technique. For example, Verdenal and Vacelet in 1990 [48] recorded very high variations in the survival rates of *S. officinalis*, despite using the same technique at different locations. Similar observations were also made by Oronti and colleagues [49] and Ruiz and colleagues [50]. Schiefenhövel and Kunzmann in 2012 [17] also reported very low survival rates for *Neopet*-

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rosia sp. when attaching it to natural substrates (opposed to 100% when using cement blocks). Per contra, this was most likely the result of unfavorable environmental conditions. Based on these findings, it remains evident that no optimal "one-fits-all"-method for sponge transplantation and cultivation exists. To minimize failure, it is, therefore, most important to understand as best as possible the ecology and natural history of the targeted species as well as its habitat when aiming to transplant and cultivate sponges.

To our knowledge, the very few economically operating (bath) sponge farms are exclusively using rope systems. However, in the long term, these seem to be very susceptible to heavy disease and predator outbreaks as well as they are associated with relatively high maintenance costs. To overcome these problems, experience from terrestrial approaches could be implemented such as rotational farming sites. This is practiced in Micronesia, where local farms offer the most reliable yields so far. The maintenance costs are arguably kept lower when operating in low-income countries, where these farms may still offer an alternative source of income for local communities while keeping the operational costs at an economically viable level.

Additionally, more research is needed to provide guidance and establish thresholds for minimum survival rates for successful restoration. For commercial purposes a minimum survival rate of at least 90% is generally recommended [49]. However, without facing certain economic pressures slightly lower survival rates may be sufficient to initiate successful restoration actions. Precise information will, therefore, be useful when moving on from initial small-scale experiments to final large-scale operations. In most publications, growth was used as a measure for success. For restoration on the other hand, assessing potential natural reproduction of transplants may be a more useful indicator. Growth may be neglected here (as long as individuals do not decrease and die off), as slow-growing individuals may very well be able to reproduce as reported, for example by the work of Baldacconi et al. [51].

5. Conclusions

The growing awareness that marine sponges play a key ecological role in many marine ecosystems is increasing the attention of scientists on their inclusion during restoration projects. The present review shows that all the transplant techniques can be organized into six main categories and that sponge skeletal stiffness represents an important feature to select the best methodology. Until now, these approaches have been developed mainly on species of commercial interest for cultivation purposes. Now we need to speed up new experiments and tests including sponge species with both functional and/or structural roles, to restore benthic habitat integrity, and to recover their ecosystem services.

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Data Availability Statement: The row data we referred to in this review are available on reasonable request from the corresponding author. No new data were created or analyzed in this review.

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Conflicts of Interest: The authors declare no conflict of interest.

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Appendix A

Table A1. List of reviewed articles.

References	Technique	Species	Environment
Wilkinson & Vacelet (1979) [52]	Mesh systems; Mounted on artificial substrate	Aplysina aerophoba Aplysina cavernicola Chondrilla nucula Chondrosia reniformis Petrosia ficiformis	In-situ
Barthel & Theede (1986) [53]	In-vitro	Halichondria (Halichondria) panicea	Ex-situ
Verdenal & Vacelet (1990) [48]	Rope system	Spongia agaricina Spongia nitens Spongia officinales	In-situ
Kaandorp & De Kluijver (1992) [54]	Mounted on artificial substrate	Haliclona (Haliclona) oculata	In-situ
Duckworth et al. (1997) [7]	Mesh systems; Mounted on artificial substrate	Psammocinia hawere Raspailia topsenti Raspailia (Clathriodendron) arbuscula	In-situ
Müller et al. (1999) [55]	In-vitro	Geodia cydonium	Ex-situ
Pronzato et al. (1999) [30]	Rope systems	Agelas oroides Axinella damicornis Cacospongia mollior Chondrosia reniformis Hippospongia communis Ircinia variabilis Petrosia ficiformis Spongia agaricina Spongia officinales	In-situ
Nickel & Brümmer (2002) [24]	In-vitro	Chondrosia reniformis	Ex-situ
Belarabi et al. (2003) [25]	In-vitro	Crambe crambe	Ex-situ
De Caralt et al. (2003) [19]	In-vitro	Corticium candelabrum	Ex-situ
Duckworth & Battershill (2003) [56]	Mesh systems; Rope systems	Latrunculia sp. nov. Polymastia crocea	In-situ
Duckworth et al. (2003) [57]	In-vitro	Axinella corrugate	Ex-situ
Thoms et al. (2003) [58]	Mounted on natural substrate	Aplysina cavernicola	In-situ
Van Treeck et al. (2003) [13]	Mesh systems	Axinella damicornis Axinella verrucose Chondrosia reniformis Ircinia variabilis	In-situ
Corriero et al. (2004) [9]	Rope systems	Spongia officinalis	In-situ
Duckworth et al. (2004) [59]	Mesh systems	Latrunculia (Biannulata) wellingtonensis Polymastia crocea	In-situ
Kelly et al. (2004) [14]	Mesh systems	Spongia (Heterofibria) manipulates	In-situ
Hadas et al. (2005) [60]	Mounted on artificial substrate; Rope systems	Negombata magnifica	In-situ
Hausmann et al. (2006) [26]	In-vitro	Aplysina aerophoba	Ex-situ
De Caralt et al. (2007) [61]	In-vitro	Crambe crambe Dysidea avara Hippospongia communis Ircinia oros	Ex-situ
De Voogd (2007) [10]	Mounted on artificial substrate; Rope systems	Callyspongia (Euplacella) biru	In-situ

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Table A1. Cont.

References	Technique	Species	Environment
De Voogd (2007) [62]	Rope systems	Aaptos suberitoides Amphimedon paraviridis Callyspongia (Euplacella) biru Hyrtios reticulatus Ircinia ramose	In-situ
Duckworth & Wolff (2007) [15]	Mesh systems; Mesh-rope systems; Rope systems	Coscinoderma sp.	In-situ
Duckworth et al. (2007) [63]	Mesh systems; Mounted on artificial substrate; Rope systems	Coscinoderma sp. Rhopaloeides odorabile	In-situ
Johnston & Clark (2007) [64]	Mounted on artificial substrate	Tedania (Tedania) anhelans	In-situ
Louden et al. (2007) [11]	In-vitro; Rope systems	Coscinoderma sp. Rhopaloeides odorabile	Ex-situ; In-situ
Lipton & Sunith (2009) [65]	In-vitro; Cage systems	Callyspongia (Chladochalina) diffusa Callyspongia (Chladochalina) subarmigera Clathria (Clathria) gorgonoides	Ex-situ; In-situ
Baldacconi et al. (2010) [51]	Mounted on artificial substrate	Spongia officinalis	In-situ
Carballo et al. (2010) [66]	In-vitro; Mounted on artificial substrate	Mycale (Carmia) Cecilia	Ex-situ; In-situ
De Caralt et al. (2010) [67]	Mesh systems; Mounted on artificial substrate, Rope systems	Dysidea avara	In-situ
Osinga et al. (2010) [12]	Cage systems; Rope systems	Chondrosia reniformis Dysidea avara	In-situ
Bergman et al. (2011) [68]	Mounted on artificial substrate	Diacarnus erythraenus	In-situ
Page et al. (2011) [69]	Mesh systems	Mycale (Clamia) hentscheli	In-situ
Webster et al. (2011) [27]	In-vitro; Mesh systems	Rhopaloeides odorabile	Ex-situ; In-situ
Oronti et al. (2012) [49]	Rope systems	Hyatella pertusa Spongia (Spongia) tubulifera	In-situ
Schiefenhövel & Kunzmann (2012) [17]	Mounted on artificial substrate; Mounted on natural substrate; Rope systems	Neopetrosia sp. Stylissa massa	Ex-situ; In-situ
Biggs (2013) [70]	Mounted on natural substrate	Aplysina cauliformis Aplysina sp.	In-situ
Ruiz et al. (2013) [50]	Mesh systems	Discoderma dissolute	In-situ
Di Bari et al. (2014) [71]	In-vitro	Tethya citrina	Ex-situ
Ledda et al. (2014) [31]	Rope system	Agelas oroides Ircinia variabilis	In-situ
Kiruba-Sankar et al. (2016) [18]	Mounted on artificial substrate; Mounted on natural substrate; Rope system	Liosina paradoxa Stylissa massa	Ex-situ; In-situ
Meyer et al. (2016) [16]	Cage systems; Mesh systems	Ecionemia alata	Ex-situ; In-situ
Avila & Briceno-Vera (2018) [72]	Mounted on artificial substrate	Halichondria (Halichondria) melanadocia	In-situ

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Table	Δ1	Cont
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References	Technique	Species	Environment
Padiglia et al. (2018) [73]	Mounted on artificial substrate; Mounted on natural substrate	Crambe crambe	In-situ
Gökalp et al. (2019) [21]	Mounted on artificial substrate; Mesh system	Chondrosia reniformis	In-situ
Santiago et al. (2019) [74]	Mounted on artificial substrate; Mesh systems	Xestospongia sp.	In-situ
Giangrande et al. (2020) [32]	Mesh systems	Sarcotragus spinosulus	In-situ
Gökalp et al. (2021) [33]	Cage systems	Chondrosia reniformis	In-situ

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