Underwater Sampling, Monitoring and Assessment Methods of Marine Biodiversity

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Marine bidiversity represents a layered complexity of living organisms, ranging from diversity within species to entire ecosystems. It is integral to all dimensions of sustainable development—economic, social, and environmental—supporting the Earth's well-being and providing essential services for humanity's health, happiness, and prosperity. Regrettably, human-induced pressures such as overfishing, climate change, invasive species, and pollution have led to a steep decline in marine biodiversity worldwide. Therefore, it is crucial to monitor and evaluate biodiversity across different marine habitats. This evaluation can assist in identifying species, habitats, and communities that are significant due to their rarity, those that serve as biological indicators, or those crucial to local human societies. Such assessments help pinpoint species and areas in need of conservation or restoration efforts.

Monitoring of ecosystem

Monitoring is the systematic acquisition of data and information pertaining to a designated 'ecosystem under consideration' within a scientific framework. This process also involves the comprehensive examination of resource utilization by both direct and indirect stakeholders associated with the ecosystem. To ensure the robustness of the monitoring endeavor, it is imperative to conduct periodic assessments, ideally spanning an extended temporal scope.

Initiating the monitoring protocol entails the execution of a baseline survey, a foundational undertaking designed to gauge parameters that may undergo temporal fluctuations. The fundamental data procured during the baseline survey encompass

1. Cartographic representation of the ecosystem's geographical extent, delineating spatial details.
2. Comprehensive inventory of local and migratory organisms, obtained through primary and secondary data collection, inclusive of their ecological status.
3. Identification and characterization of the various direct and indirect stakeholders, elucidating their interactions with the ecosystem and targeted resources.
4. Examination of existing regulatory frameworks governing the ecosystem, encompassing the extent of surveillance and adherence.
5. Analysis of decision-making processes and governance structures within local communities, providing insights into factors influencing ecosystem conservation.
6. The overarching objective of monitoring is to furnish data that substantiates the formulation of effective management strategies for the designated ecosystem. By employing a scientifically rigorous approach, monitoring contributes essential insights that underpin informed decision-making and facilitate the sustainable conservation of the ecosystem in question.

In this chapter, we aim to understand the monitoring of coastal and marine ecosystem. Hence, the various underwater sampling methods with are commonly follow is detailed along with the sample collection tools, along with cleaning and preservation methods.

Underwater Sampling Methodologies: A Scientific Exploration

The exploration and understanding of underwater ecosystems demand sophisticated methodologies that can unravel the complexities of marine environments. From the broad-scale insights offered by the Manta Tow and Timed Swim techniques to the nuanced observations facilitated by Transects, Quadrats, and Underwater Videography, this scientific exploration navigates the methodologies essential for comprehending the intricate dynamics of life beneath the waves. Every technique brings its own distinct value to the scientific arsenal, facilitating the exploration of underwater ecosystems and the enhancement of

our understanding of the intricate equilibrium supporting marine life.

1. **Manta Tow Technique:** The Manta Tow method is a preeminent approach employed for acquiring a comprehensive, broad-scale depiction of an aquatic site. This technique involves towing a diver behind a watercraft, circumnavigating the designated site. The objective is to obtain a general overview of the ecological characteristics prevalent in the underwater environment.
2. **Timed Swim Technique:** The Timed Swim technique entails a diver swimming for a predetermined duration or distance, offering a holistic assessment of the overall site. This method provides valuable insights into the spatial distribution and ecological features of the surveyed area.
3. **Transect Methodology:** Transects serve as an intermediary approach, furnishing information of moderate scale. These involve the placement of lines on the reef floor, beneath which corals and other objects are systematically tallied. Various survey methods include:

a. **Line Transects:** Encompassing line intercept transects (for horizontal plane) and chain transects (for three-dimensional surveys, with the chain conforming to the contour).
b. **Point Intercept Transects:** Involving measurements at specific intervals beneath or beside the transect tape.
c. **Belt Transects:** Measuring entities within a belt adjacent to the transect, facilitated by structures like PVC poles or T-bars.

To demarcate transects, materials such as waterproof fiberglass tape on a spool with a winding handle, marked rope with coloured indicators or knots denoting distance, or plastic chains with links of known length are commonly utilized.

4. **Quadrat Sampling:** Quadrats, defined as square or rectangular sampling units, are instrumental in enumerating or measuring organisms within a specified area. The appropriate size of a quadrat depends on the organism's size and spatial abundance. Commonly, quadrats of 0.5-1m² or larger are employed for assessing species diversity, while smaller 25 cm by 25 cm quadrats are suitable for fine-scale investigations of sessile benthic juvenile recruits and other diminutive organisms. Permanent quadrats serve the purpose of longitudinally observing specific coral colonies.

5. **Underwater Videography:** Utilizing video footage to capture the general reef area offers qualitative insights. For quantitative studies, videos are particularly advantageous for large-scale ecological monitoring using techniques such as belt transects. The width of the belt in video transects is determined by the distance the camera is held from the coral reef benthos. Replicating the exact path, speed, and distance from the substrate for repeated video sampling poses challenges but is essential for maintaining consistency in data collection.

Sampling and Data collection

Data collection emerges as an indispensable component in scientific endeavours aimed at unravelling the mysteries of underwater life. They give quantitative as well as qualitative insights of the ecosystem. By systematically collecting data from various locations within the ecosystem, scientists can extrapolate population densities, assess species richness, and discern patterns of spatial distribution. This quantitative information is fundamental for formulating accurate ecological models, understanding population dynamics, and gauging the health of marine communities. Qualitative data derived from sample analysis contribute to the identification of species, assessment of biodiversity, and discernment of ecological niches within the marine habitat.

Sample collection plays a crucial role in data collection. Scientific research, aimed at unravelling the mysteries of marine life, relies heavily on accurate and representative data obtained through sample collection. Sample collection is, therefore, a foundational step in the scientific process that empowers researchers to contribute meaningfully to marine conservation and ecosystem preservation. Based on the type of research activity envisioned, commonly used sampling devices and methods employed for collecting organisms are given in Table 1.

Table 1. General Sampling Techniques for collecting/ monitoring of Marine Organisms

Sampling Device/ methods	Targeted Organisms
Nets: Sieve, gillnets, trammel nets, trawl, dipnet	Marine organisms especially from minute microscopic planktons to larger nektons.
Traps	Fishes, crabs, octopus, lobsters
Visual observation	Larger megafauna visible on the water surface (large pelagic fish shoals, Jellyfish blooms etc.)
Visual observation by underwater video, Scuba assisted dives	All marine organisms especially sessile epifauna, invertebrates up to large Nektons
Aerial survey	Larger megafauna visible on the water surface
Acoustic (echo-sound)	Fish/ marine mammals' species
Dredges, sledges	Benthic organisms and Epifauna (both sessile and motile)
Remotely Operated Vehicle (ROV)	Coastal area not accessible by Scuba dives, Deep water species (generally >200m depth)
Light Traps	Organisms attracted by light (Photosensitive organisms in plankton, mobile benthos, nektons)
Hand held shovels and rake	Intertidal organisms
Tagging and marking	Nektons (mammals/whales/ fishes), Sessile benthic fauna (large corals)

While employing these sampling techniques, we tend to collect samples for analysis in laboratory. Good quality preservation methods are essential for maintaining the quality of biological samples, thereby enabling researchers to conduct thorough analyses and make informed conclusions. The direct preservation of various organisms in fixatives or preservatives is not universally suitable due to the diverse physiological responses exhibited across taxonomic groups. The employment of a relaxant serves the purpose of inducing a state of anaesthesia in specimens, rendering them unresponsive and incapable of contraction upon immersion in fixative solutions. Commonly used relaxant with their dosage is given in Table 2.

Table 2. Relaxant with dosage and target organism

Sl No:	Relaxant	Targeted Organism	Dosage
1.	Magnesium Chloride (MgCl2)	Most marine organisms	Prepared with freshwater at a concentration of 7.5% by weight, matching the osmotic pressure of seawater. A blend of isotonic MgCl2 solution and seawater in a 50:50 ratio is recommended as a suitable general mixture. It is advisable to add the solution gradually when dealing with delicate organisms.
2.	Menthol	cnidarians and ascidians	Sprinkling crushed menthol crystals or adding

			drops of concentrated menthol solution, which is prepared in ethanol.
3.	Chloretone/ chlorobutanol	echinoderms, including large holothurians	Few drops of saturated solution of Chloretone in ethanol
4.	Clove oil (eugenol)	crustaceans	25% solution of clove oil in ethanol
5.	Cooling/freezing	Crustaceans, like crabs, and shelled molluscs.	Fast freezing is advised, as slow freezing can negatively impact anatomy and histology. It is not advisable for soft-bodied organisms.
6.	Propylene phenoxitol	Bivalves	1 to 2 drops

Type of preservatives used depends on the type of analysis intended. In case of genetic analysis, Ethanol, a prominent choice for preservation. A small quantity of the fresh specimen is preserved in higher ethanol concentrations, ranging from 95-100%.

For general preservation, Formalin, typically employed at concentrations ranging from 5-10%, constitutes a solution derived from the industrial "formalin" mixture, containing 38% formaldehyde dissolved in water. To ensure iso-osmotic conditions for marine animal specimens, it is recommended to blend formalin with seawater. Specifically, for taxa characterized by calcareous components (a prudent practice for all taxa), formalin necessitates buffering with laundry borax (sodium borate), or alternatively, by the addition of calcium carbonate powder/sand. For enhanced histological and anatomical fixation, utilizing buffer recipes or specialized fixatives such as Bouin's is advisable. Common fixatives and preferred concentration for various taxa are detailed in Table 3.

Table 3. Common fixatives and preferred concentration for various taxa

Sl no	Group	Relaxant	Cleaning agent (if any)	Fixative
1.	Porifera	Nil	Nil	70-95% Ethanol
2.	Hard corals	Nil	Bleached with a solution of sodium hypochlorite	Dried for preservation. For DNA based studies colony to be preserved in 95% Ethanol
3.	Soft Coral	Nil	Nil	Preserved in alcohol or buffered formalin solution. Storage: Alcohol For DNA based studies colony to be preserved in 95% Ethanol
4.	Sea fans	Nil	Nil	Preserved in alcohol or buffered formalin solutionthenquickly dried.

				For DNA based studies colony to be preserved in 95% Ethanol
5.	Black corals	Nil	Nil	Preserved in alcohol or buffered formalin solution. Expanded polyps are necessary for taxonomy. For DNA based studies colony to be preserved in 95% Ethanol
6.	Anemones	menthol	Nil	Ideally, specimens are preserved in formalin while fully expanded and then stored in alcohol.
7.	Flatworms	Nil	Nil	Let the animal crawl and expand on a moistened paper, then gently transfer both the paper and the animal onto frozen formalin for preservation; thereafter, preserve in ethanol. A small piece of live tissue extracted from the worm using a razor serves as the subsample for DNA and can be preserved in 95% ethanol.
8.	Nemertean worms	MgCl2	Nil	Fix the worm straight in formalin, preserve in ethanol. Tissues in 95% ethanol for DNA work
9.	Crustaceans	clove oil, freezing	Nil	Fixation and preservation in alcohol is ideal. Tissues in 95% ethanol for DNA work
10.	Molluscs	MgCl2 and propylene phenoxitol	Shells can be cleaned in Aluminium thioglycolate or solution of sodium hypochlorite	Fixation can be in alcohol or formalin
11.	Bryozoans	Nil	Nil	Preservation is by Air drying.
12.	Ophiuroids, asteroids, echinoids	MgCl2		Preserved in either ethanol or formalin. Ethanol fixation is preferable when specimens are intended to be kept moist,

				whereas formalin offers a better fixative for specimens that will be dried.
13	Crinoids	Nil		Fixed and preserve in 70-95% EtOH
14.	Holothuroids	MgCl2 or chloretone		Fixed and preserve in 70-95% EtOH
15.	Urochordates	menthol		Preserved and kept in formalin for storage. Tissues stored in 95% ethanol for DNA analysis
16.	Fishes	Nil	With running water	Preserved and kept in formalin for storage. Tissues stored in 95% ethanol for DNA analysis
17.	Planktons	Nil	-	Fixed and stored in 5% formalin

Biodiversity Assessment

Quantifying biodiversity at the species or population level aims to establish an index reflecting the number of species present and their relative abundance within a specific ecosystem. These assessments are crucial for identifying key conservation areas and establishing priorities. Areas are ranked based on various criteria such as rarity, diversity, habitat condition, fragmentation, resilience, threats, and ecosystem processes, offering a comprehensive view of the ecosystem. Optimal levels of biodiversity enhance ecosystem resilience against stressors like climate change and invasive species. Monitoring biodiversity over time is vital for detecting changes and informing sustainable development strategies.

Numerous biodiversity indices have been developed to mathematically evaluate biodiversity. There are various methods for measuring biodiversity, with richness and evenness being the primary considerations.

Species richness

Species richness refers to the number of different species present in a particular area or habitat. It is a measure of biodiversity that quantifies the diversity of species within a given community or ecosystem. Species richness considers only the number of species and does not take into account the relative abundance of each species. Higher species richness indicates a greater variety of organisms coexisting within an ecosystem, while lower species richness suggests a more limited diversity of species. It is one of the fundamental metrics used to assess and compare biodiversity across different environments.

Species evenness

Species evenness is a measure of how evenly or uniformly the individuals of different species are distributed within a community or ecosystem. It considers not only the number of species (species richness) but also the relative abundance of each species. In a community where species evenness is high, all species are represented in relatively equal proportions, meaning that no single species dominates the community. Conversely, in a community with low species evenness, one or a few species may be significantly more abundant than others, resulting in an uneven distribution of individuals across species.

Species evenness complements species richness in providing a more comprehensive understanding of biodiversity within an ecosystem. It helps to assess the balance and stability of a community by indicating the degree to which resources are shared among different species. Communities with high species evenness are often considered more stable and resilient to environmental changes compared to those with low evenness.

Diversity indices

A diversity index is a mathematical measure used to quantify the diversity of species within a community or ecosystem. These indices provide a way to summarize various aspects of biodiversity, including species richness (the number of different species present) and species evenness (the distribution of individuals among those species). They encompass aspects such as rarity, commonness, and the composition of the community.

***1. Simpson's Index*(D)**

The Simpson Diversity Index, also known as the Simpson Index is a measure of biodiversity that quantifies the diversity within a community by considering both species richness and species evenness. It provides a single numerical value that reflects the probability that two randomly selected individuals from the community belong to different species.

Simpson's Index is calculated as follows:

$$D = 1 - \sum_{i=1}^{S} \left(\frac{ni}{N}\right) \text{^}2$$

Where S is the total number of species in the community, ni is the number of individuals of the i-th species and N is the total number of individuals in the community.

Simpson's Diversity Index ranges from 0 to 1, where:D=0 indicates low diversity (or high dominance by one species).D=1 indicates high diversity (or perfect evenness, where all species are equally abundant).

Simpson's Dominance Index is the inverse of the Simpson's Index (1/D).

2. *Shannon-Weiner Index* (H)

Shannon-Weiner Diversity Index, originally proposed by Claude Shannon in 1948, is a measure of biodiversity that quantifies the diversity within a community. It takes into account both species richness (the number of different species present) and species evenness (the distribution of individuals among those species.

Shannon -Weiner Index (H) is calculated as follows

$$H = \sum_{i=1}^{S} pi * \ln pi$$

Where, S is the total number of species in the community, pi is the proportion of individuals belonging to the i-th species relative to the total number of individuals in the community and ln denotes the natural logarithm.

The Shannon-Weiner Index provides a single numerical value that reflects the uncertainty associated with predicting the species identity of a randomly selected individual from the community. Higher values of the index indicate greater species diversity within the community.

3. *Margalef's Diversity Index* (I_{Mg})

This index was developed by the Spanish ecologist Ramon Margalefin 1958. Margalef's Diversity Index, also known as the Margalef Index or IMg, is a measure of biodiversity that quantifies the richness of species within a community relative to the total number of individuals present.

$$\text{IMg} = \frac{\text{S} - 1}{\ln N}$$

Where S = total number of species N = total number of individuals in the sample In = natural logarithm.

Margalef's Diversity Index measures the species richness of a community by taking into account the logarithmically transformed number of individuals. Higher values of the index indicate greater species richness within the community. It provides insight into the complexity and diversity of ecological communities.

4. *Menhinick's Diversity Index* (DMn)

Menhinick's Diversity Index is a measure of biodiversity that focuses on the species richness of a community in relation to the total number of individuals sampled. It is particularly useful for assessing biodiversity in ecological studies, especially when comparing the diversity of different communities or sites.

$$DMn = S/\sqrt{N}$$

Where S = the total number of species in a community and N = total number of individuals sampled.

Menhinick's Diversity Index provides a measure of species richness that accounts for the size of the sample. It gives more weight to communities with a higher number of species relative to the size of the sample, thus providing a more accurate representation of biodiversity. Higher

values of the index indicate greater species richness within the community.

5. Evenness Index (J)

The Evenness Index (J), also known as Pielou's Evenness Index, is a measure of how evenly individuals are distributed among different species within a community. It complements measures of species richness by considering the relative abundance of each species. The evenness of a community can be represented by Pielou's evenness index (Pielou 1966):

$$J = H / \ln S$$

where H = Shannon – Wiener diversity index S = total number of species in the sample In = natural logarithm

The Evenness Index (J) ranges from 0 to 1, where:

J=0 indicates low evenness, meaning that one or a few species dominate the community.

J=1 indicates perfect evenness, where all species are equally abundant.

The Evenness Index provides a measure of how equitably individuals are distributed among different species, allowing for comparisons of community structure and diversity across different habitats or ecosystems. J and D can be used as measures of species dominance (the opposite of diversity) in a community. Low J indicates that 1 or few species dominate the community.

6. Community Similarity Index

The Community Similarity Index, also known as the Bray-Curtis Similarity Index, is a measure used to quantify the similarity between two or more communities based on their species composition and relative abundances. It is widely used in ecology and biodiversity studies to compare the similarity of species assemblages across different sites or habitats.

The Bray-Curtis Similarity Index is calculated using the following formula:

$$BC = \frac{2C}{S1 + S2}$$

Where:

BC is the Bray-Curtis Similarity Index.

C is the total abundance of species shared between the two communities.

S1 and S2 are the total abundances of species in the first and second communities, respectively.

The index ranges from 0 to 1, where:

BC=0 indicates no similarity between the communities.

BC=1 indicates complete similarity, meaning that the species composition and relative abundances are identical between the communities.

The Bray-Curtis Similarity Index provides a quantitative measure of community similarity that accounts for differences in species abundance, making it particularly useful for comparing communities with varying levels of species richness and evenness.

Biodiversity at different scales

Alpha Diversity: Alpha diversity refers to the diversity of species within a particular habitat or ecosystem. It focuses on the variety of species present within a defined area or sample, without considering the differences between habitats or ecosystems. In other words, alpha diversity measures the richness (number of different species) and evenness (relative abundance of each species) within a single community or location. It provides insight into the local biodiversity and is often used to assess the health and ecological complexity of specific habitats.

Beta Diversity: Beta diversity is a measure of biodiversity that quantifies the differences in species composition between different habitats or ecosystems within a larger geographical area. It reflects the turnover of species from one habitat to another and provides insight into the heterogeneity or similarity of species composition among different sites. In other words, beta diversity compares the diversity of species between different locations or habitats, highlighting the degree of variation in species composition across spatial gradients. It helps ecologists understand patterns of biodiversity distribution and assess the degree of habitat heterogeneity within a region.

Gamma Diversity: Gamma diversity refers to the overall diversity of species across a large geographical area or region, encompassing multiple habitats or ecosystems. It represents the total number of species present within the entire geographic extent, regardless of the individual habitats or ecosystems within that area. In

essence, gamma diversity reflects the combined alpha and beta diversities across all habitats or ecosystems within a region. It provides a comprehensive understanding of biodiversity at a broader scale, considering the variety of species present across different habitats and landscapes within a given geographic range.

Gamma Diversity= Alpha diversity + Beta diversity

Conclusion

In conclusion, the intricate marine biodiversity, ranging from intra-species diversity to entire ecosystems, plays a pivotal role in sustaining the planet's health and supporting economic, social, and environmental pillars of sustainable development. Despite its significance, a precipitous decline in global marine biodiversity is evident due to various anthropogenic threats. Recognizing the urgency, this chapter emphasizes the importance of monitoring and assessing various ecosystems. Through systematic baseline surveys and sophisticated underwater sampling methodologies, scientists can unravel the complexities of marine life. Ultimately, the integration of robust monitoring, advanced sampling methodologies, and comprehensive biodiversity assessments is fundamental to the sustainable conservation of marine ecosystems.

Suggested Reading

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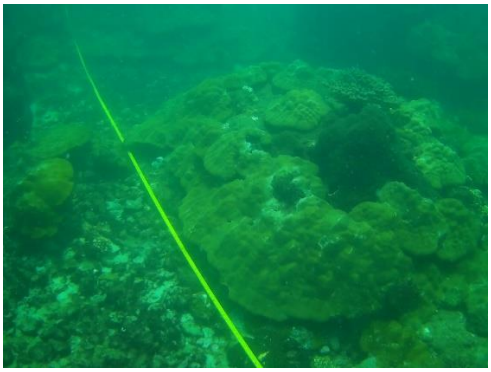

Fig. 1. Line transects for studying corals

Fig. 2. Common quadrats (50cm x 50cm)