The Regional Organization for the Conservation of the Environment of the Red Sea and Gulf of Aden

(PERSGA)

Standard Survey Methods:
Seagrasses and Seaweeds
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1.0 INTRODUCTION

1.1. A brief history of research on seaweeds and seagrasses of the region

The Red Sea has been a region of natural history exploration by European scientists for more than 200 years. Previous to the completion of the Suez Canal in 1869, travellers generally started their journeys of exploration from one of two points. From the east coast of Egypt (usually Suez), they could travel by vessels to the Arabian coast and then on to the Ethiopian coast, or they entered the Red Sea from the south, through the Strait of Bab el Mandeb, coming by ship via the Cape of Good Hope. After the completion of the Suez Canal many expeditions passed through the Red Sea on their way to other parts of the Indian Ocean. During that time numerous marine algae were collected, resulting in the description of many species with the Red Sea as type locality. A historical review of phycological research in the Red Sea is given by Papenfuss (1968). The first record of marine algae from the Red Sea dates back to 1756, and since then there have been a number of important contributors to the knowledge of the marine algae of the Red Sea. These include Forsskål (18th century); Turner, Delile, Lamouroux, Decaisne, Agardh, Montagne, De Notaris, Zanardini, Piccone, Hauck, and Bornet (19th century); Reinbold, Lyle, Christensen, Bærgesen, Nasr, Newton, Rayss, and Dor (20th century). A milestone in macro-algal research in the Red Sea was the catalogue and bibliography of the Red Sea benthic algae, compiled by Papenfuss (1968). Recent studies include Walker (1987) and Ateweberman (1997). Price et al. (1988) studied the ecology of seagrasses in the Red Sea. Global taxonomic and biogeographical studies on seagrasses have been carried out by Den Hartog (1970) and Phillips & Meñez (1988).


1.2. An overview of the significance of seaweed and seagrass communities in the region

Seaweed communities. Seaweeds can grow as individuals, but they more frequently live together in communities with other seaweed and animal species. Seaweed communities affect and are affected by the environment and are some of the most productive marine plant communities in the world. Together the intertidal and subtidal zones give rise to a narrow coastal area that accounts for less than one percent of the Earth’s surface. However the productivity of this region can equal or exceed that of most terrestrial communities (Dawes 1998).

Several areas with hard substrate in the Red Sea and the Gulf of Aden are not dominated by corals but by macroalgal assemblages. Shallow coral reef areas of the northern and central Red Sea are often dominated by filamentous greens, small browns and tuft forming red algae. In upwelling regions (e.g. south coast of Yemen) large brown algae may dominate. Perennial brown algae (such as Sargassum, Cystoseira and Hormophysa) are dominant over extensive parts of shallow hard substrata.
in the southern Red Sea. In most of these areas algal communities show a strong seasonality; many seaweed species appear to be annual. Seasonality is correlated with water temperature that, for the Red Sea, is coldest in winter but for the Arabian Sea, is coldest during the summer upwelling (Sheppard et al. 1992; Banaimoon 1998).

**Seagrass communities.** Seagrass communities (also called seagrass beds or meadows) often characterise sandy and muddy biotopes. Seagrasses are monocotyledonous angiosperms adapted to marine life both through their physiology and morphology. The most obvious characters of seagrass species are the extensive rhizome and rooting systems, and the very flexible, generally strap-like leaves. Tropical seagrass beds on mud, sand or coral rubble can consist of a single species, but often contain members of different genera. According to some phytosociologists, seagrass beds are the most simply structured communities of rooted plants, as they are mostly composed of only one or a few rooting species. This often results in a rather monotonous appearance of seagrass meadows. However, the structure of these apparently uniform seagrass beds disguises a great diversity of floral and faunal components. Seagrass ecosystems provide habitats for a wide variety of marine organisms, both plant and animal. These include meiofauna and flora, benthic flora and fauna, epiphytic organisms, plankton and fish, not to mention microbial and parasitic organisms. The relatively high rate of primary production of seagrasses drives detritus-based food chains, which help to support many of these organisms. Birds, fishes and turtles also directly consume seagrasses. Four main sub-habitats can be recognised in seagrass beds. These are:

- The leaf epiphyton, comprising the microflora with associated small animals including nematodes, polychaetes and crustaceans, together with sessile fauna, such as hydroids, anemones, snails, echinoderms and small fish;
- Stem and rhizome biota, which include larger epiphytic algae, various polychaetes, amphipods and bivalves;
- Species swimming among the leaves including fish and crustaceans;
- Sediment fauna, although this may differ little from that of the surrounding benthos.

The distribution and complexity of seagrass habitats in the Red Sea and the Gulf of Aden is probably controlled by habitat availability and extremes of temperature and salinity. Seagrass beds develop to their fullest extent in the south of the Red Sea. This area is characterised by a wide and shallow shelf, a high prevalence of unconsolidated sediments, and low temperature and salinity fluctuations (Sheppard et al. 1992). Limited areas of dense seagrass beds have been recorded in the Gulf of Aden (Hirth et al. in Sheppard et al. 1992).

**1.3. Species recorded in the area**

**Seaweeds.** The catalogue of the Red Sea benthic algae by Papenfuss (1968) contains more than 500 seaweed taxa. The proportion of species endemic to the Red Sea is about nine percent. On the other hand, 64 percent of the species are pan tropical. Walker (1987) separated the known species into four geographical regions, the gulfs (i.e. Gulf of Aqaba and Gulf of Suez), northern, central and southern regions, and showed that about 8-40% of species known from the Red Sea occur in only one region. Many of the southern species are typical of warm waters from the tropics, while the northern species include members typical of slightly cooler areas. The boundary between the two species assemblages is drawn approximately through the middle of the Red Sea. The seaweeds of the Gulf of Aden remain understudied, especially from the north coast of Somalia. From the south coast of Yemen, 163 seaweed taxa have been recorded (Ormond & Banaimoon 1994).
Seagrasses. Ten species of seagrasses have been recorded from the Red Sea. These belong to seven genera, the total number known for the tropical Indo-West Pacific region. On the eastern Red Sea coast, seagrass assemblages have been identified from cluster analysis using species cover data (PRICE et al. 1988). At a broad level, this revealed three groupings separated by latitude, suggesting biogeographic trends. A more detailed study carried out in the southern Red Sea indicated six distinctive assemblages. Three of these were dominated by a single seagrass species (*Thalassia hemprichii*, *Halophila ovalis* and *Halodule uninervis*) (SHEPPARD et al. 1992).

2.0 METHODOLOGY

2.1. Qualitative assessment of the macroalgal and seagrass flora of an area

A qualitative assessment of the marine flora involves collecting specimens from a specific area, resulting in a list of species. Depending on the study, the coastal area can vary from small (e.g. a coastal band of 10 m, a rock outcrop, etc.) to large (e.g. one to several kilometres of coastline, or a small offshore island). The resulting species list is important for calculating biodiversity indices for the area. When comparing species numbers or biodiversity indices for different coastal areas, these areas should be of comparable size. A major disadvantage of qualitative collection data is that species abundance is not taken into account. This can be corrected, partially, by making the sampling method semi-quantitative. This implies that each species is ranked based on its abundance, evaluated by visual observations. An example of such a ranking is the Tansley scale (Table 1). The growth form (sociability) of seaweeds can also be taken into account. Here the Braun-Blanquet's sociability scale can be used for each species (Table 2).

<table>
<thead>
<tr>
<th>Tansley scale</th>
</tr>
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<tbody>
<tr>
<td>d</td>
</tr>
<tr>
<td>c</td>
</tr>
<tr>
<td>a</td>
</tr>
<tr>
<td>f</td>
</tr>
<tr>
<td>o</td>
</tr>
<tr>
<td>r</td>
</tr>
<tr>
<td>s</td>
</tr>
</tbody>
</table>

Table 1. The Tansley scale, an indication of species abundance in a quadrat (quantitative sampling) or larger area (semi-quantitative sampling); after SCHAMINÉE et al. (1995) and SCHILS (2000).

<table>
<thead>
<tr>
<th>Braun-Blanquet's sociability scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
</tbody>
</table>

Table 2. The Braun-Blanquet's sociability scale for the indication of a species' life form; after SCHAMINÉE et al. (1995) and SCHILS (2000).

2.1.1. Field collecting (intertidal and subtidal) and preservation of marine plants

Extensive and well-prepared collections are the basis of all studies of marine organisms. The importance of good collections for taxonomic studies is evident, but it is equally important that representative collections - often referred to as ‘voucher specimens’ - be kept of each species recorded during an ecological survey. Without such specimens, there is little possibility of checking and confirming identification on the basis of names used in publications. Such specimens should be numbered, labelled and be deposited in a recognised herbarium (WOMERSLEY 1984).
Collecting. Intertidal habitats can be surveyed by wading during (extreme) low tide or by snorkelling at high tide. Subtidal collecting can be done by snorkelling or SCUBA diving. For the non-diver, subtidal seagrass and algal beds can be sampled in calm waters (at least down to several metres) using a dredge. Whether making subtidal or intertidal collections, similar water-resistant equipment will be required. If wading, collecting shoes, or boots should be available. Many algae and some seagrasses can be removed by hand, but a scraper or a stout knife may be necessary. A spade is useful in seagrass beds. Some thick encrusting algae can be removed with a knife, but many (especially the crustose coralline algae) must be collected along with the substrate. This can only be done with heavy instruments such as a hammer and chisel. Specimens can be kept in a variety of field containers such as buckets, (zip-) bags, perforated plastic bags or mesh bags. Small plastic vials can be useful for minute specimens. The collected material should be kept in water to avoid decay by temperature rise or desiccation. Each container should be given a serial number on a water-resistant label, and recorded on a clipboard with wet-dry paper or on a SCUBA board. Ecological data (intertidal zone, substrate type (rock, epiphytic, sand, silt, etc.) and inclination (horizontal, vertical, overhanging) should be noted for each collecting site. Additional information on collecting seaweeds and seagrasses is given by Dawes (1998), Tsuda & Abbott (In Littler & Littler 1985), and Womersley (1984).

Preservation. Seagrass and algal specimens can either be preserved in formalin (wet preservation), or prepared on herbarium sheets (dry preservation). Each specimen to be preserved is given a serial number that corresponds with a number in a notebook. The notebook contains the data recorded with each specimen; this information is placed on the label at a later stage (see below).

Formalin is about 40% by volume formaldehyde, and is diluted 1/10 with seawater, giving a solution of 10% formalin or 4% formaldehyde (the concentration is not critical and half the above will usually give good preservation). Formalin is a strong irritant and carcinogenic so it should be handled with care, avoiding inhalation or direct contact with the skin.

Herbarium sheets can be prepared directly in the field using fresh plant material or in the laboratory using material preserved in formalin. The preparation of fresh material should be done as quickly as possible after leaving the field (preferably the same day) because seaweeds die off very quickly. The material is first sorted in plastic trays and complete specimens (including the holdfast and reproductive structures, if available) are selected for preparation. These plants are then mounted by "floating out". The specimen is immersed in a tray with seawater and arranged with forceps on an immersed sheet of stiff herbarium paper on which the specimen number has been written in pencil. The herbarium sheet with specimen is removed horizontally from the seawater, drained of the surplus water and deposited on newspaper, covered by a piece of cloth and a newspaper again. A stack of herbarium sheets and newspapers is built up and placed in a plant press under moderate pressure. Adding undulated cardboard between stacks of newspapers and specimens enhances drying. The newspapers must be changed at frequent intervals (twice a day) until the specimens are dry.

Labelling. Unless specimens are properly and accurately labelled, they are of little value. Data recorded with each specimen should include:

- The locality (latitude and longitude are useful, especially for remote sites);
- Ecological notes, including the zone (intertidal) or depth (subtidal), slope, exposed at low tide or submerged in an
Mounting dried specimens. The dried specimens are mounted on herbarium sheets of a standard format and the labels are added. Specimens that do not stick on the paper should be stuck with adhesive paper (not with adhesive tape or glue).

2.1. Identification of seaweeds and seagrasses

No marine flora guides exist for the study area, hampering identification of seaweeds. Some field guides from adjacent areas which may be helpful to identify the seaweeds of the Red Sea and the Gulf of Aden are: JAASUND (1976: Tanzania) and COPPEJANS et al. (1997: East Africa). Since 64% of the seaweed taxa in the Red Sea are pan tropical, floras or field guides from other tropical or subtropical regions can be used, certainly for identification to genus level. These include guides prepared by ABBOTT (1999: Hawaii, red algae); COPPEJANS (1983: Mediterranean); CRIBB (1983: Australia, red algae); DE CLERCK & COPPEJANS (1996: Jubail, Saudi Arabia); LAWSON & JOHN (1987: West Africa); LITTLER & LITTLER (2000: Caribbean); SILVA et al. (1996: Indian Ocean); TAYLOR (1960: tropical eastern coast of America); TRONO (1997: Philippines).

Seagrass identification can be carried out with DEN HARTOG (1970) or PHILLIPS & MEÑEZ (1988).

2.2. Remote sensing combined with groundtruth observations (phytosurvey)

Survey techniques include creation of landscape and vegetation maps through remote sensing (aerial photography or scanning systems) and groundtruth observations. Surveys are particularly useful for the study of large areas (e.g. kilometres of coastline). The procedures are simple and yield repeatable results in studies of seaweed and seagrass communities. Groundtruth observations can be carried out by qualitative or quantitative assessment of the marine flora of the area. Quantitative assessment is carried out using sample plots that are selected along the coast based on visual observations. The combination of remote sensing and groundtruth observations offers information for the creation of vegetation maps. A concrete example is given in DAHDOUH-GUEBAS et al. (1999).

2.2.1. Remote sensing

Remote sensing uses sensors to identify or measure parameters of an object according to variations in the electromagnetic radiation (EMR) reflected or emitted by the object. EMR can be natural, either reflected radiation from the sun or emitted heat from the earth. It can also be man-made such as a radar system. The wavelength of electromagnetic radiation spans many orders of magnitude and is conveniently divided into several arbitrary regions (e.g. ultra-violet, visible, near infrared, infrared, etc.). The amount and type of radiation reflected or emitted depends upon incident energy (e.g. thermal radiation) and the nature of the earth's surface. Remote sensing can be carried out by aerial photography or scanning systems (airborne spectral scanners or satellite sensors). GUILLAUMONT et al. (1997) discuss spectral properties of seaweeds in their natural habitat and provide a critical review of sensors and data processing for remote sensing of seaweed communities. Methods for distribution and mapping of seagrass communities using remote sensing and groundtruth observations are dealt with by KIRKMAN in PHILLIPS & McROY (1990).

Aerial photography. Aerial photography can be carried out from fixed-wing aircraft (light or medium altitude aircraft), or helicopters. Photography is carried out using
several types of photographic emulsions simultaneously. Films are chosen according to their respective performances: colour and infrared for intertidal, colour for submerged areas, colour and false colour film for floating algae. Photographs have little spectral capacities (infrared and visible field). However, they provide high spatial resolution, allowing texture analysis and good geometric quality.

**Airborne spectral scanners.** Image spectrometers have a good to excellent radiometric and spectral resolution but are much more expensive than photographic systems. They are also more expensive and complex to use over large regions than satellite data.

**Satellite sensors.** Satellite imagery provides reliable synoptic information reaching the user cheaply at regular intervals. It is a consistent and repeatable method. Historical data are available since the 1970's. Radiometric calibration can be produced in good conditions. However, satellite sensors have limited performance in seaweed studies because of their low spatial and spectral resolution, frequency and sensitivity. Moreover, bands are not optimal for underwater studies.

### 2.2.2. Data acquisition

Qualitative images obtained from the methods discussed above need to be transformed to quantitative information. This requires measurement of the areas covered by the various identified populations. Different techniques have been developed. Classical methods, such as manual measurement of the areas covered by the various identified populations, are time consuming. Other methods include planimeter methods, grid count methods and scannerisation. **GUILLAUMONT et al.** (1997) have reviewed data processing techniques. The most significant advances in the use of remote sensing data are in the field of Geographical Information Systems (GIS).

### 2.2.3. Groundtruth observations and creation of vegetation maps

Once aerial photographs have been examined, some form of groundtruth survey must be carried out. Groundtruth observations can be conducted through qualitative or quantitative assessment (or a combination of the two) of the marine flora of the area. Qualitative assessment implies general collection over a large area: several metres to kilometres of coastline. Quantitative assessment implies selecting sample plots (1-10 m²) along the coast (DAWES 1998). The choice of location of the sample plots is determined by the data from the remote sensing. In these sample plots each dominant species is ranked for abundance, cover, and growth form (see 2.3.1. investigation of spatial community variation - quadrat sampling). The combination of data acquired from remote sensing and groundtruth observations can then be used to draw up vegetation maps.

### 2.3. Quantitative sampling methods

#### 2.3.1. Investigation of spatial community variation

**Transect sampling.** Transects are used in plant zonation studies of intertidal communities (seaweeds) or where line quadrats are used (across seagrass beds). Stakes are aligned from the highest to lowest zone and a metric tape stretched between them. Samples for identification can be taken along the transect in each zone, or at every unit of measurement (every centimetre to every few metres, depending on the slope and detail required). Percent species is determined by dividing the number of individuals within a zone, by the total number present along the entire transect. Percent species cover is calculated by dividing the length (in centimetres or metres) of the transect (or zone) species cover by the total length of the transect (or zone).

**Quadrat sampling.** Measurements of unit-area can be done using quadrats ranging in size from 25 cm² to 1 m² squares; larger or
smaller areas can be used according to the community structure and the accuracy required. Determination of the quadrat size is crucial. The frame size is a reflection of the size of the patches in the population. For instance, if seagrass shoots or seaweeds are clumped in 1 dm² patches, frames considerably larger (e.g. 1m²) should be used to ensure the inclusion of several patches. Quadrat frames can be easily and inexpensively constructed from plastic pipe (PVC works well). Quadrats may be subdivided if detailed sampling is required. Quadrat samplers are useful to determine changes in species composition in areas with major shifts in abiotic factors (e.g. a temperature gradient along a stretch of coastline). They also can be used in zonation studies to develop a more accurate determination of percentage cover, frequency and abundance.

To avoid bias in sampling, random or haphazard methods can be used for quadrat placement. Figure 1 shows a fictional example of a sample strategy to determine changes in species composition along a stretch of coastline. Quadrats can also be placed at regular intervals along each transect.

Species abundance in each quadrat can be determined in a number of ways: 1) counting individuals of each species, 2) estimating cover of each species, or 3) determining biomass (standing crop). Other vegetation parameters that can be recorded for the species in a quadrat are sociability and phenology. For sociability, the Braun-Blanquet’s sociability scale can be used (Table 2). The phenology of a species can be indicated as: g = germling, v = vegetative, f = fertile (if possible with indication of the life stage), dis = old thallus parts remain, dth = thallus almost vanished.

Counting the number of individuals of each species can be problematic; in many algal species individuals cannot be distinguished as they grow in a diffuse manner forming algal tufts. Counting the number of individuals in seaweed communities should only be considered with large distinct species, e.g. large browns. Instead of absolute numbers, a scale, e.g. the Tansley scale (already mentioned in 2.1) can be used (Table 1).

In seagrass communities, species abundance is often determined by estimating the number of seagrass shoots in a quadrat.

Shoot density refers only to the above ground, leafy portions of the plant. The density of roots is correlated to the density of

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Figure 1. Fictional example of a sample strategy to determine changes in species composition along a stretch of coastline. Every 10 km, 5 quadrats (1 m² each) haphazardly placed in the infra-littoral fringe are examined. See text for explanation.
shoots, but due to difficulty in measurement, is seldom quantified. Both destructive and non-destructive means of estimating shoot density can be used. A destructive technique commonly used involves clipping a quadrat of shoots at the sediment surface and measuring leaf surface area in the laboratory. The advantage of using destructive sampling is that samples can be processed in the laboratory and leaf area (see 2.3.2. - leaf surface area) and biomass determination (see below) can be conducted on the same sample. Non-destructive estimates of shoot density allow for minimal perturbation of the meadow, which is useful for repeated sampling (see below: investigation of temporal community variation, permanent quadrat method). Counting shoots within a quadrat can be accomplished at low tide in intertidal meadows and with SCUBA equipment in subtidal meadows (DENNISON in PHILLIPS & MCROY 1990).

Percentage cover can be estimated using broad categories (e.g. a Braun-Blanquet’s scale, Table 3). Seaweed communities are often characterised by different layers of algal growth forms: e.g. crustose species, an algal turf layer overgrown by a layer of larger foliose or filamentous algae, overgrown by large fucoid algae or kelp. In such a case the percentage cover of species is somewhat more complicated to estimate. Moreover the total cover (i.e. the sum of all species cover in a quadrat) can exceed 100%. Cover estimates can also be applied in seagrass beds but the estimation of shoot density is more widely used (see above).

Species abundance can also be determined by biomass or standing crop measurements. There are a number of ways of expressing biomass or standing crop: wet weight, dry weight, weight of organic carbon or inorganic nitrogen.

The most widely used unit is dry weight in g/m². Dry weight of seaweed and seagrass species can be determined by oven-drying the specimens at 70°C for 72 hours. To allow comparisons, this unit should be given whenever possible, specifying whether it applies to pure stands or to a larger area including bare substrate patches. In the latter case, percentage-cover of the seaweed or seagrass bed in the area considered should be noted. If only wet weight can be determined routinely, at least one series of wet weight/dry weight (wwt/dwt) correlations per dominant species should be made, since this ratio may vary considerably between different seaweed and seagrass species according to the texture of the plant tissue.

**Table 3. Braun-Blanquet’s combined estimation of species’ abundance and cover; after SCHAMINÉE et al. (1995) and SCHILS (2000).**

<table>
<thead>
<tr>
<th>No of individuals</th>
<th>Cover</th>
</tr>
</thead>
<tbody>
<tr>
<td>r very few</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>+ few</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>1 numerous</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>2 very numerous</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>or arbitrarily</td>
<td>5 - 25%</td>
</tr>
<tr>
<td>3 arbitrarily</td>
<td>25 - 50%</td>
</tr>
<tr>
<td>4 arbitrarily</td>
<td>50 - 75%</td>
</tr>
<tr>
<td>5 arbitrarily</td>
<td>75 - 100%</td>
</tr>
</tbody>
</table>

All data recorded in each quadrat should be written down in a standardized format. Table 4 shows an example of such a data entry form.
2.3.2. Specific techniques for the investigation of seagrass communities

**Root/shoot ratios (R/S)** (FONSECA, THAYER & KENWORTHY in PHILLIPS & McROY 1990). The R/S ratio has been used operationally to include both root and rhizome components. R/S ratios are relatively simple to measure and enhance estimates of total production by seagrass species. R/S ratios should be derived from plant material separated into shoot and root (plus rhizome) components at the meristem where cell differentiation occurs. R/S ratios are usually presented on a weight/weight basis, although area and volume ratios can also be determined. Weight data should be presented.
on an ash-free dry-weight basis, since inorganic contamination may account for up to 50% of the dry weight. The leaves and roots should be placed in 5% phosphoric acid for 10-15 minutes to remove carbonates and encrusting epiphytic organisms, and then rinsed in tap water. Plant components should then be dried in an oven at 80°C. Subsamples of the dried plant material should be ashed at 550°C for 4-6 hours to determine ash-free dry weight (AFDW). Field methods for R/S collections may vary depending on the required precision and accuracy. Clipping shoots out of a quadrat at the sediment surface will suffice for above ground estimates. Running a sharp blade around the inside edge of the quadrat and harvesting the portions of the plant in the sediment provides the below ground part for the ratio. This method may well leave some deep-root material behind (e.g. Thalassia roots can extend 4 m into the sediment). Usually, however, the majority of root and rhizome is found in the upper 20-40 cm of the sediment.

Wide variations in the R/S ratio occur among habitats as well as throughout the year. These are related to seagrass-development processes and to prevailing environmental conditions. Seasonal changes in weight also occur. Hence, there is an age-dependent mechanism contributing to the rate, proportions and mass of observed R/S ratios, causing these values to change with time.

Static measures (one point in time) of seagrass R/S ratios can be used to assess the degree of development of a seagrass system. Because some seagrass components take a long time to decompose, it is necessary to quantify living versus dead material. An older seagrass meadow generally has a lower ratio of living to dead seagrass components (especially roots and rhizomes). For foliar portions, a visual examination of the shoot will suffice to distinguish living green blades from dead ones. For roots and rhizomes, a visual plus a physical examination is needed. Most roots decompose relatively quickly compared with rhizomes and will no longer appear white or succulent after senescence. Rhizomes may appear to be intact, but flexing the rhizome to the point of breaking should produce a brisk snap if it is still alive.

**Leaf surface area** (BULTHUIS in PHILLIPS & McROY 1990). The leaf area of seagrasses in a quadrat must be known in order to calculate the leaf-area index (one-sided leaf area per unit ground area), and to calculate photosynthesis (moles of carbon or oxygen per unit leaf area). Leaf area can be measured directly using an area meter, planimeter or digitiser, calculated from length, width and diameter measurements. The easiest and most accurate method of measuring leaf area, irrespective of whether leaves are linear or irregularly shaped, is by using an area meter (e.g. LICOR LI-3100). This instrument is an automatically integrating planimeter developed for measuring leaves. If an area meter is not available the alternative method chosen depends on leaf shape and the number of leaves to be measured. Most seagrasses have flattened, linear leaves so that leaf area can be estimated by measuring length and width. The width is measured at three or more locations along the leaf. The area is calculated by multiplying length by mean width.

**Leaf production** (DENNISON in PHILLIPS & McROY 1990). The principal methods of measuring leaf production are by change in standing stock over time and by leaf-marking techniques. The first method is problematic with seagrasses because of their perennial growth and the loss of leaf material during growth due to rapid leaf turnover.

Leaf-marking techniques can be used to distinguish leaf tissue formed before and after marking due to the growth form of seagrasses. Seagrass leaves have a basal meristem, which is near the sediment surface for most species. Above this region of dividing cells is a region of elongating cells, which are usually protected by leaf sheaths.
Above the dividing and elongating cells, leaf tissue moves away from the basal meristem as long as leaf growth occurs, even though no cell division or elongation occurs in this region. Hence, the distance between the basal meristem and leaf marks made above the region of cell elongation can be used as a measure of leaf growth. Several techniques of leaf marking have been employed. One method involves stapling individual leaves. This marking technique is fast and easy but requires relatively large leaves. Several modifications of the staple technique have been developed for seagrasses with small leaves. A small wire inserted into the leaf, hole punches, or a water-insoluble pen can be used to mark seagrass leaves (Figure 2). Marking with a pen has the advantage of minimal damage to the leaf. The time interval between marking and collection is constrained by the growth rate of the plant, and the leaf turnover time sets the upper limit.

Several leaf production values can be calculated from leaf-marking techniques. Leaf material can be separated into leaf tissue produced before (above mark) and after marking (below mark and unmarked leaves), dried for 24 hours at 80°C and weighed. The ratio of leaf material produced before marking to that produced after marking divided by the time interval yields a relative production rate (e.g. g g⁻¹ day⁻¹). For each shoot the leaf material produced after marking divided by the time interval yields leaf production per shoot (e.g. mg shoot⁻¹ day⁻¹).

Figure 2. Hole-punch method of leaf marking in which a syringe needle hole is created several centimetres above leaf bundle (a) and the resultant scars used to distinguished leaf tissue which arose from the leaf before (stippled) and after (unstippled) marking (b); after DENNISON in PHILLIPS & MCROY (1990).
2.3.3. Investigation of temporal community variation

In order to investigate the temporal variation of seaweed and seagrass communities, permanent quadrats (PQ) can be used. The methods used in this survey technique are explained by Polderman in Price et al. (1980). In principle, the procedures for a general survey (e.g. quadrat sampling as explained above) and for the monitoring of one particular station (permanent quadrat sampling) are the same, the difference being that the latter procedure is repeated at regular time intervals. The choice of time interval depends on the objective of the investigation. If, for example, the study is to determine seasonal changes in species composition, then the permanent quadrats should be examined at least once a month. Quadrats measuring from 25 cm² to 1 m² are placed in a homogeneous vegetation patch. The different measurements for species abundance used in the quadrat sampling (2.3.1) can also be used here.

2.3.4. Primary productivity

In any study of seaweed or seagrass ecology or physiology, a measurement of fundamental interest is the primary production of the population or community. Carbon fixed in photosynthesis, and organic matter accumulated with plant growth, constitute the very basis for the seaweed or seagrass community, its physical structure, its food supply and its mineral cycle. Numerous techniques are available for measuring primary production. The oldest of these methods involves monitoring increase in plant biomass over a growing season. Direct measurements of changes in dissolved oxygen (O₂) and dissolved inorganic carbon (DIC) in water surrounding seaweeds or seagrasses have been used successfully for various macrophyte systems. The radioactive C14 technique has had widespread use for measuring seaweed and seagrass production. For a description of the different techniques to measure primary production we refer the reader to Kemp, Murray & McRoy in: Phillips & McRoy (1990); Arnold & Littler in Littler & Littler (1985); and Kennish (1989).

2.4. Measurement of environmental variables

Water temperature. It has been shown that individual seaweed species distributions over a biogeographic scale are overwhelmingly limited by seawater temperature regimes. In the ideal situation, temperature should be recorded continuously daily or several times a day in order to calculate minimum, maximum and average temperatures per day, month and year. If daily temperature recording is not possible, it should be recorded at regular time intervals (e.g. once a month) over several years. Temperature varies with water depth, currents and waves, the amount that seaweeds or seagrasses retard water motion, as well as with local insolation. It is recommended that the temperature be measured near the substrate at different water depths. Temperature should be recorded using the Celsius scale. A glass thermometer, protected in a steel case, can be used for these measurements. For many studies, combination sensors recording temperature as well as salinity or conductivity simplify in situ measurements.

Light. The measurement of the sun's energy for photosynthesis is complex under any circumstances. The complexity is compounded when the light is filtered through water. Different measurement techniques (such as Secchi disk measurements, hours of daylight, total solar radiation and total irradiance under water) can be used in combination. The simple long-standing Secchi disc method measures the depth at which light, reflected by the Secchi disc, disappears from the view of a human observer as the disc is lowered into the water (Figure 3). Measurement of the hours of daylight are useful when making comparisons over many years of overall light conditions that may have contributed to the presence, growth, or disappearance of a seaweed or
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Figure. 3. Use of a Secchi disc to measure water transparency. The Secchi disc is lowered in the water until it disappears from the view of a human observer. This depth is a measure for water transparency, which is in its turn a measure for light penetration to the bottom.

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Shore height above the low water mark (mean or extreme low tide) is measured using a level meter (or theodolite) and a surveyor's rod (Figure 4). Height is measured relative to the low water mark on a specific day and time. These relative measurements have to be transformed to absolute measurements by using tide tables and curves. For example in Figure 4 the height of plot 1 relative to the extreme low water mark is \( b - a^1 + c \).

Depth below low water mark can be measured using a depth sounder on board a vessel, or a depth meter while scuba diving.

Sand inundation can be determined by estimating the percentage of sand cover in a quadrat or by removing all sand in a quadrat and measuring the wet or dry weight.

Substrate. Seaweeds grow on different types of substrate including rock, fossil coral, or artificial substrates such as plastic buoys or wooden constructions. Type as well as texture of the substrate should be determined. Seagrasses grow on sand or mud flats. Here the substrate type is determined by measuring particle size. This is done by taking a core of the sediment, which is dried, and then sieved using a set of standard screens (0.063 mm to 2 mm pore size). Particle-size distribution is obtained by dividing the dry weights of each size class by the total dry weight of the sample.

Slope of the substrate should be measured in degrees: 0° (horizontal) - 90° (vertical) - 180° (overhanging substrate). Instead of numerical values, broad categories can be used (e.g. horizontal, sub-vertical, vertical, overhang).

Water movements, including waves, tides and currents can be important factors determining the structure of a seaweed or seagrass community. Different types of measurement include cumulative water motion, maximum force and continuous measurement of water velocity. An overview of techniques is presented by Denby in Littler & Littler (1985).

Salinity can be measured using a refractometer. Refractometers are small and portable and give reliable readings. Measurements of salinity do not generally suffer from the daily variations experienced by water temperature except for smaller intertidal pools (evaporation versus rain).
Rather, seasonal variation and changes associated with storm events are among the primary sources of variation to consider in designing a sampling protocol for a given site.

**Nutrients.** Four primary elements necessary for plant growth are oxygen, carbon, nitrogen and phosphorus. Nitrogen and phosphorus can be limiting nutrients to marine plants. Sample and analytic methods for determining nutrient concentration are elaborate and will not be discussed here. The reader is referred to Wheeler in Littler & Littler (1985) for a review of analytical techniques.

### 3.0 DATA ANALYSIS

Different sample methods require different analysis techniques. This section focuses on the analysis of data collected to assess structural patterns in seaweed and seagrass vegetation, and spatial or temporal community variation.

#### 3.1. Sample data input: spreadsheets and databases

In order to analyse collected data, it must be entered in a data matrix. This can be done in a spreadsheet or a database programme. In the case of quadrat sampling, sample plots are placed in columns and species in rows. The species abundance values can be presence/absence values, cover estimates, biomass data, etc. If environmental variables are incorporated in the analysis, they should be placed in a second matrix: sample plots in columns, environmental variables in rows. Table 5 shows an example of a species and environmental data matrix.
Table 5. Example of a matrix with species data (a), and environmental data (b), in an Excel spreadsheet. See text for explanation. In the second matrix abbreviations are used for the environmental variables: temperature variables (average temperature of warmest month, average year temperature, average temperature of coldest month) (°C), sponge cover (%), sand cover (%), slope (°), grazers (number of grazing animals), exposure rate.
3.2. Preliminary data analysis:
exploratory data analysis by means of
descriptive statistics

Descriptive statistics can be defined as the
enumeration, organisation and graphic
presentation of data (data reduction). Preliminary data exploration can be done with
a simple spreadsheet programme or with
statistical programs (e.g. STATISTICA®).
Simple graphs can often clarify the large and
disorderly amount of data. Questions that can
be answered with descriptive statistics for
example are: what are the dominant species;
what is the distribution of the dominant
species; what is the minimum, maximum,
average and standard deviation of the
environmental variables; which locations are
species rich or species poor, etc.

3.3. Multivariate statistics

Studies in environmental biology usually
involve more than one variable (e.g. large
number of species, plots and environmental
variables). Analytic techniques that deal with
such kind of data are called multivariate
analysis techniques or multivariate statistics.
Techniques that are effective in revealing
patterns in data (e.g. structural and
distribution patterns) are ordination and
classification. An overview of multivariate
analysis techniques in vegetation studies is
given by KENT & COKER (1992).

3.3.1. Transformation of data

The number of species is usually not
evenly distributed over the plots. This is
problematic for the implementation of
statistical techniques. Transformation of data
is a technique to correct this. Likewise,
environmental variables can be transformed.
The question of optimal transformation of
species abundances in ordination has not yet
been fully addressed. Techniques such as
Detrended Correspondence Analysis (DCA)
and Canonical Correspondence Analysis
(CCA) appear to work well for raw data
values (e.g. percentage cover, biomass,
presence/absence data etc.) as well as for
logarithmic transformations, square root
transformation, or transformation into a
traditional cover-abundance scale. Apart from
the problem of normal distribution of data,
logarithmic and square root transformation
are often used to dampen the effects of
dominant species.

3.3.2. Ordination

Ordination is a widely used family of
methods, which attempt to reveal the
relationships between ecological communities.
These multivariate techniques arrange sites
along axes on the basis of species
composition. The mathematical theory behind
the different ordination techniques is quite
complicated. A comprehensive overview of
ordination techniques used in community
analysis can be found in KENT & COKER

Indirect ordination methods arrange sites
along axes only on the basis of species
composition. Widely used methods in
community studies are Principal Component
Analysis (PCA), Correspondence Analysis
(CA) and DCA. In direct ordination methods
the arrangement of the sites is constrained by
environmental factors, which are incorporated
in the analysis along with the species
composition. Widely used methods are CCA
and Redundancy Analysis (RDA). The choice
of environmental variables greatly influences
the outcome of CCA and other constrained
ordinations. For an exploratory analysis, one
should certainly include variables that are
related to the most important determinants of
species composition. However, it is also often
desirable to include other variables that are
easy and inexpensive to measure - one may
be surprised and find out that previously
unsuspected factors are quite important in
determining species composition (PALMER
1993).

The final result of an ordination is a two-
dimensional diagram with samples, species
and environmental variables plotted (Figure 5).
In simple terms, samples that are grouped
together are characterised by similar species composition and environmental conditions. Samples that are plotted far away from each other have very different species composition. Correlations can be examined between environmental variables, species and plots.

### 3.3.3. Numerical classification

Agglomerative methods (cluster analysis) proceed from individual samples or quadrats and progressively combine them in terms of their similarity in species composition until all the quadrats are in one group. The combinations are made by similarity coefficients that measure how alike any two quadrats are in terms of species composition or by dissimilarity coefficients that assess how unalike any two quadrats are. Different cluster methods are used in community studies: single-, complete-, and average-linkage clustering. A much used similarity index in plant community studies is the squared Euclidean distance. An overview is given by Kent & Coker (1992).

Divisive methods start with the total population of individuals and progressively divide them into smaller and smaller groups. Two-way indicator species analysis (TWINSpan) is now the most widely used technique for divisive classification in plant community studies. The method is based on progressive refinement of a single axis ordination from reciprocal averaging or correspondence analysis. The output of a TWINSpan is a computer generated two-way table, which can be transformed into a classification (Figure 6). For each group of sample plots indicator species (i.e. species typical for a group of plots) are defined.

An overview of classification techniques used in community analysis can be found in Kent & Coker (1992).

When groups of plots can be clearly distinguished (from ordination and classification analysis), they indicate distinct community types, each being characterised by its typical species composition and environmental variables.
3.4. Calculation of species richness

Biodiversity indices are an overall measure of diversity that usually combine aspects of species richness and evenness. Species richness is the number of species in a given area. Evenness, or equitability, is the uniformity of abundance in an assemblage of species. Equitability is greatest when species are equally abundant. Two commonly used indices used to express biodiversity are the Simpson index and the Shannon-Weaver (Weiner) index.

Simpson's index assumes that the proportion of individuals in an area adequately weighs their importance to diversity. The equation for this index is:

\[ D = 1 - \sum (p_i^2) \]

where \( D \) is the diversity and \( p_i \) is the proportion of the \( i^{th} \) species in the total sample. Values for \( D \) can range from one to the total number of species (\( S \)). An index of one indicates that all of the individuals in the area belong to a single species. When \( D = S \) then every individual belongs to a different species and species are equally abundant.

The Shannon-Weaver index is very similar to the Simpson index, except for the underlying distribution. The Simpson index assumes that the probability of observing an individual is proportional to its frequency in the habitat whereas the Shannon-Weaver index assumes that the habitat contains an infinite number of individuals. The equation for this index is:

\[ H = -\sum(p_i \ln(p_i)) \]

\( H \) is high when equitability and species number are high.

The terms alpha, beta and gamma diversity are used to refer to biodiversity on different spatial levels. Alpha diversity, or local diversity, is the diversity within a site or quadrat. Beta diversity is determined to measure the rate of species turnover between adjacent sites or areas. Beta diversity can be defined as a measure of how different (or similar) a range of samples are in terms of variety of species found in them (Magurran 1988). A widely used method for measuring beta diversity using presence and absence data is the Wilson and Shmida measure, \( \beta T \):

\[ \beta T = \frac{g(H) + l(H)}{2\alpha} \]
where \( g(H) \) is the number of new species encountered and \( l(H) \) the number of species which are lost along a transect; \( \alpha \) is the average sample richness (i.e. average species number per area). A high \( \beta_T \) number indicates a high species turnover between adjacent sites. Gamma diversity or regional diversity is the diversity of a landscape, or all sites combined.

Different types of curves are used to visualise species diversity. A species-individual curve is a plot of the cumulative number of species encountered, versus the cumulative number of individuals captured. A species-area curve is a plot of the (cumulative) number of species encountered, as a function of area. Species-area curves can be used to compare different regions.

### 3.5. Computer software

This section gives a short overview of existing software available for the above-mentioned analytic techniques.

**Construction of data matrices** can either be done in a spreadsheet or database program. MS Excel can be used as a spreadsheet program, MS Access as a database. MS Excel can also be used for exploratory data analysis (i.e. descriptive statistics and graphic presentation) and transformation of data.

**Descriptive statistics and graphic presentation** can be carried out with a large variety of statistical software packages. Two widely used programs are STATISTICA and SPSS.

**Ordinations** can be carried out with the FORTRAN program CANOCO (TER BRAAK 1988). This program offers many possibilities but it is not easy to use. More recently, Windows versions of this program, which are much more user-friendly, have become available. A complete overview of ordination software can be found at [http://www.okstate.edu/artsci/botany/ordinate/software.htm](http://www.okstate.edu/artsci/botany/ordinate/software.htm).

Two software packages will be discussed below.

CANOCO for Windows 4.5, developed by the Centre for Biometry, Wageningen (CBW), offers the same possibilities as the FORTRAN program but is much easier to use. A disadvantage of CANOCO is that it cannot directly display ordination diagrams. Therefore another program: CANODRAW 3.1 is used. The software can be ordered from [http://www.microcomputerpower.com/cfw/](http://www.microcomputerpower.com/cfw/).

PC-ord, a programme developed by Bruce McCune, offers a wide variety of multivariate statistical analysis methods for ecological communities, including cluster analysis, ordination, and species diversity. PC-ord is an easy-to-use programme that directly displays ordination diagrams.

**Cluster analysis** can be carried out with statistical programs such as STATISTICA and SPSS. When using STATISTICA, cluster diagrams are directly displayed. A demo version of the program can be downloaded from [http://www.statsoftinc.com/](http://www.statsoftinc.com/). A demo version of SPSS can also be downloaded, from [http://www.spss.com/](http://www.spss.com/).

**Divisive classification** can be carried out with the FORTRAN program TWINSPAN (HILL 1979). No Windows-version of this program is yet available.

TWINSPAN, written by Mark Hill, is a program for classifying species and samples, producing an ordered two-way table of their occurrence. The two-way table generated by the program has to be transformed to a classification by hand. The software package can be ordered from [http://www.ceh.ac.uk/](http://www.ceh.ac.uk/).
4.0 REFERENCES


SCHILS T., HUISMAN J.M. & COPPEJANS E. 2003b. Chamaebotrys erectus sp. nov. (Rhodymeniales, Rhodophyta) from the


**Ter Braak, C.** 1988. *CANOCO - a FORTRAN program for canonical community ordination by (partial) (detrended) (canonical) correspondence analysis, principal component analysis and redundancy analysis (version 2.1).* Wageningen. 95 pp.


**5.0 OTHER RECOMMENDED LITERATURE**


