Eelgrass biomass and production

Objectives
- To introduce methods for assessing basic parameters fundamental to many seagrass studies such as shoot size and stand structure expressed as biomass and shoot density
- To introduce methods for sampling of epiphytes and macroalgae associated with seagrass ecosystems
- To introduce techniques for measurements of seagrass growth and production
- To discuss the measured seagrass parameters in relation to growth condition at the two study sites
- To provide the participants with an understanding of seagrass growth dynamics at the level of the shoot, the whole plant and the population

Methods

Seagrass biomass and shoot density
Shoot density is determined from the biomass samples. Biomass samples of above- and belowground plant parts are measured by harvesting the total biomass within sampling frames. Samples should be taken randomly in areas where the vegetation is well established and representative of the area. At each location, 5 subsamples are collected using a circular frame with an inner area of 0.0625 m$^2$ (1/16 m$^2$). The frame is placed on the vegetation ensuring that only plant parts rooted inside the frame are placed inside the frame. To collect the plant parts the rhizomes must be cut with a knife around the inside of the frame. The contents of the frame are then removed to a sediment depth of more than 15 cm and put into a mesh bag and washed free of sediment under water.

In the laboratory, the samples are rinsed of remaining sediment, debris and dead tissues, and epiphytes are gently removed from the leaves. The number of shoots, seedlings and flowering shoots are counted. The biomass is separated into shoots (leaves), rhizomes and roots and the dimensions of a selection of shoots is measured (see below). All biomass fractions are rinsed in freshwater, placed in numbered paper bags, and dried to constant weight at 60 °C for 24 hours. The dried samples are weighted (including shoot biomass used for LAI measurements) and related to the area of the sampling frame.
Data analysis:
Following data are presented as mean and standard error from replicated samples:
1) Shoot density (shoots m$^{-2}$)
2) Relative number of reproductive shoots
3) Leaf, rhizome and root biomass (g dry wt m$^{-2}$)
4) Above-/belowground biomass ratio

Shoot dimensions and leaf area index (LAI)
Shoot length (maximum leaf length) and the number of leaves per shoot are measured on all shoots within the quadrates or on subsamples (10-20 shoots), depending on the shoot density.

The total leaf area per shoot is measured in order to estimate leaf surface area available to epiphytes and to calculate the leaf-area index (LAI; cm$^2$ cm$^{-2}$ ground area), a descriptor of the degree of leaf packing with the canopy. Collect randomly a total of 20 shoots and measure the leaf length from base of sheath to leaf tip and the leaf width at ¼, ½ and ¾ of the leaf length. The shoots are rinsed in freshwater, placed separately in paper bags, dried and weighted. The shoot weights must be added to the respectively biomass quadrate samples above.

Data analysis:
1) Average shoot length (cm shoot$^{-1}$) and number of leaves shoot$^{-1}$
2) Leaf surface area (cm$^2$ shoot$^{-1}$) is calculated from the product of leaf length and leaf width for all leaves in each shoot.
3) LAI (cm$^2$ leaves cm$^{-2}$ ground area) is calculated as the product of the ratio between leaf surface area and shoot weight (cm$^2$ g$^{-1}$ dry wt) and the total leaf-biomass per ground area.

Epiphytes and macroalgal biomass in seagrass meadows
Algae and epiphyte biomass are estimated as a mean of assessing their relative importance in the trophic structure of the eelgrass habitats examined.

Macroalgae, loose lying within the quadrates sampled for eelgrass biomass measurements, are removed and identified using a field guide. Samples are separated and sorted by genus/species, rinsed in freshwater, dried and weighted.

To quantify the epiphyte biomass 10 eelgrass shoots are randomly sampled within the eelgrass meadow by placing a plastic bag over each shoot and cutting off the leaf cluster at the base of the shoot. The plastic bags are sealed and stored in a cooling box until processing. In the laboratory loosely attached epiphytes are removed with forceps and the leaves are gently washed in a closed container to remove loosely bound inorganic particles and detritus. Detached material, if present in significant amount, is collected onto pre-weighed filters and processed as describe below.
Leaves with adhering epiphytes are then placed under a dissecting microscope and the main algae groups are identified. The epiphytes are then carefully scraped off without injuring the leaf cells using a razor blade or scalpel while keeping the leaves in a Petri dish with filtered seawater, and transferred to pre-weighted glass filters (Whatman GF/C). If there are many calcareous algae the CaCO$_3$ should be removed from the samples by placing them in a dilute solution of 5% hydrochloric acid.

The filters are dried at 60 °C for 24 hours (or until the weight has stabilized) and re-weighed. Alternatively, the epiphyte biomass can also be determined as ash free dry weight or klorofyl a (see Kendrick and Lavery 2001). The epiphyte biomass is given by the difference between the weight of the filter and sample after drying and the dry weight of the filter. Eelgrass leaf length and width, leaf biomass is determined. The leaf area of each shoot is measured as described above and the ephiphyte biomass is expressed as mg dry wt cm$^{-2}$ if eelgrass leaf.

**Data analysis**

1) Macroalgae biomass (g dry wt m$^{-2}$)
2) Epiphyte biomass (g dry wt cm$^{-2}$ eelgrass leaf)

**Seagrass shoot growth and biomass production**

*Shoot growth estimated from leaf marking technique*

Leaf, rhizome and root growth is measured using the in-situ leaf-marking technique. This method is based on marking all leaves at a fixed reference point and relocating the marks at a later time (Fig. 1). As the younger leaves of the shoot elongate, the mark moves upwards and new leaf growth during the marking period is any growth that occurs between the reference point and the mark on the leaf plus the length of new leaves appearing.

The method used here is a modification of the method applied by Sand-Jensen (1975). Shoots in three different plots within each site should be marked, yielding a minimum of 15-20 marked shoots per site. The shoots are tagged and all leaves present in a shoot are marked by punching a hole through the upper part of the enclosing leaf sheath of the oldest leaves using a syringe needle. The punched hole (scar) in the leaf sheath is used as the reference point.

After approximately 14 days (1 new leaf per leaf should be produced as a minimum), the marked shoots are harvested and brought back to the laboratory. The marking holes are relocated in each leaf and the length of new leaf material produced is measured. The appearance of new unmarked leaves is recorded and their length, measured from the leaf meristem at the base of the shoot to the leaf tip, is also included and total leaf elongation is expressed as mm shoot$^{-1}$ day$^{-1}$. 
Leaf elongation is converted to leaf biomass growth (g dry wt shoot\(^{-1} \text{ d}^{-1}\)) by applying the weight to length ratio of the youngest mature leaf (leaf \# 3). The length of this leaf is measured from meristem to leaf tip, dried and weighted. Leaf growth is calculated from the product of the weight to length ratio and the length of new leaf material produced.

The leaf plastochrone interval (PI, days), which is the time between emergence of successive leaves on a shoot, is calculated as the number of days since marking divided by the total number of new leaves produced. Rhizome and root growth are then estimated as the weight of a rhizome internode (the rhizome piece in between 2 consecutive leaf scars) and a root bundle, respectively divided by the rhizome plastochrone interval (equivalent to the leaf plastochrone interval). Rhizome weight is determined by drying and weighing the youngest fully expanded rhizome internode (usually 3rd or 4th internode) and root weight is determined by weighing all roots attached to a single node on the same rhizome internode.

**Data analysis**

1) Leaf elongation rate (mm shoot\(^{-1} \text{ day}^{-1}\)) equals length of new leaf tissue summed for all leaves per shoot.

Figure 1. Leaf marking method to determine seagrass leaf elongation and the leaf plastochrone interval. From Short and Duarte 2001.
2) Leaf biomass growth calculated as total length of new leaf tissue multiplied by the dry weight:length ratio of leaf number 3
3) Leaf plastocrone interval (PI) is calculated as the marking period (days) divided by number of new leaves formed per shoot
4) Rhizome and root growth is calculated as the weight of a rhizome internode/root bundle divided by the leaf PI

Leaf production using reconstructive technique
The strong seasonality of eelgrass growth results in a clear annual cycle in rhizome internode lengths, with short rhizome internodes being produced in winter and long ones in summer (Fig. 2). The number of internodes between two consecutive minima or maxima comprises a full annual cycle. Since rhizome internodes are formed at the same rate as leaves a full annual cycle can be used to derive an estimate of the number of leaves produced annually and the average annual plastochrone interval.

Figure 2. Seasonal variation in length of rhizome internodes of eelgrass. Collected by C.G.J Petersen in May 1912

The method requires examination of intact rhizome pieces produced over at least one year and is, therefore most suitable for seagrass species with long-lived rhizomes. In the case of eelgrass the
rhizome longevity is moderate and intact rhizomes dating more than one year back in time may be difficult to retrieve.

Intact rhizomes extending into unvegetated sediments in the seagrass meadow are excavated from the apical shoot to the oldest portion possible. A minimum of 5 replicate rhizomes should be collected. In the laboratory rinse the rhizome free of sediments and remove remnants of old leaves. Measure to nearest mm the length of the rhizome segments from the apex to the oldest part retrieved. Plot the sequence of intermodal lengths and determine the number of rhizome segments between two consecutive minima or maxima. Determine the number of rhizome segments in an annual cycle and calculate the average plastochone interval (days leaf$^{-1}$ shoot$^{-1}$) as 365 days divided by the number of rhizome segments produced annually for each replicate.

Data analysis
1) The number of leaves formed per year estimated as the number of rhizome internodes produced during an annual cycle
2) Average annual leaf plastochrone interval (days leaf$^{-1}$)
3) Rhizome elongation rate (cm year$^{-1}$) estimated as the length of the rhizome piece representing one annual cycle of growth

References
