SEAGRASS-WATCH
THAILAND

Guidelines for Community Groups & Volunteers

CRC Reef Research Centre

SEAGRASS ECOLOGY GROUP

Queensland Government

DPI QUEENSLAND DEPARTMENT OF PRIMARY INDUSTRIES FISHERIES

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OVERVIEW

SEAGRASS-WATCH is a new program being developed by the Seagrass Ecology Group (Queensland Department of Primary Industries, Northern Fisheries Centre, Cairns). The program is being developed with the assistance of community groups and volunteers.

The ultimate aim of the **SEAGRASS-WATCH** program is to collect information on changes in seagrass meadow characteristics (eg., area, position & depth of habitat, seagrass species and composition, estimates of biomass, presence of dugong feeding trails, notes on other fauna and possible impacts). The specific methodologies for the **SEAGRASS-WATCH** program will be developed with co-operation of community groups, volunteers and government departments.

SEAGRASS-WATCH programs will establish a reliable early warning system on the status of our seagrass resources, and a broad measure of changes in these resources.

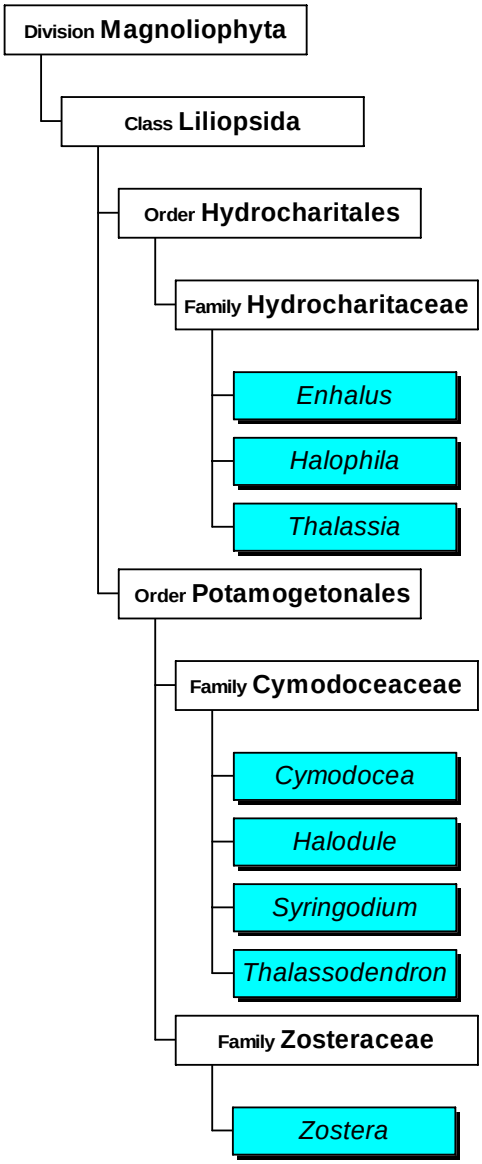
The aim of the training exercise is to give community groups & volunteers an understanding of the principles behind the techniques which are being proposed for the **SEAGRASS- WATCH** program. The success of the participants in the training program will dictate the methods that are adopted. We envisage that the methods that are finally used in the program will be modifications from what participants will experience during the training exercises.

The following information is provided as

- a training guide and
- a basis from which a monitoring manual can be developed.

GENERAL INTRODUCTION

Seagrasses are angiosperms (flowering plants) more related to terrestrial lilies and gingers than to true grasses. They grow in sediment on the sea floor with erect, elongate leaves and a buried root-like structure (rhizomes). There are only 58 described species of seagrasses worldwide, within 12 genera, 4 families and 2 orders. There are several genera of seagrasses in Queensland, *Cymodocea, Enhalus, Halodule, Halophila, Syringodium, Thalassia, Zostera and Thalassodendron*. The small number of species however, does not reflect the importance of seagrass ecosystems which provide a sheltered, nutrient-rich habitat for a diverse flora and fauna.



Taxonmic classification of Queensland's seagrasses.

Seagrasses are unique amongst flowering plants in that all but one genus, *Enhalus*, can live entirely immersed in seawater. *Enhalus* plants must come to the surface to reproduce, all others can flower and be pollinated under water. Adaptation to a marine environment imposes major constraints on morphology and structure. The restriction to seawater has also influenced geographic distribution and speciation.

Seagrass meadows occur in most shallow, sheltered soft-bottomed marine coastlines and estuaries of the world. These meadows may be monospecific or may consist of multispecies communities of up to 12 species.

Seagrass meadows physically help to reduce wave and current energy, help to filter suspended sediments from the water and stabilise bottom sediments. The habitat complexity within seagrass meadows enhances the diversity and abundance of animals. Seagrasses on reef flats and near estuaries are also nutrient sinks, buffering or filtering nutrient and chemical inputs to the marine environment. The high primary production rates of seagrasses are closely linked to the high production rates of associated fisheries. These plants support numerous herbivore- and detritivore-based food chains, and are considered as very productive pastures of the sea. The associated economic values of seagrass meadows are also very large, although not always easy to quantify.

Seagrass/algae beds are rated the 3rd most valuable ecosystem globally (on a per hectare basis), only preceded by estuaries and swamps/flood-plains. The average global value of seagrasses for their nutrient cycling services and the raw product they provide has been estimated at ^{1994}US$ 19,004 ha^{-1} yr^{-1} (Costanza *et al.* 1997). This value would be significantly greater if the habitat/refugia and food production services of seagrasses were included. In seagrasses meadows of western Cairns Harbour for example, the estimated landed value of the three major

commercial penaeid prawns (*Penaeus esculentus, P. semisulcatus* and *Metapenaeus endeavouri*) was ^{1992}AUS$3,687 ha^{-1} yr^{-1} (Watson, R.A., Coles, R.G., and Lee Long, W.J. (1993). Simulation estimates of annual yield and landed value for commercial penaeid prawns from a tropical seagrass habitat, northern Queensland, Australia. *Australian Journal of Marine and Freshwater Research*. **44(1)**, 211-220.)

Tropical seagrass meadows vary seasonally and between years. The potential for widespread seagrass loss has been well documented. The causes of loss can be natural such as cyclones and floods, or due to human influences such as dredging, agricultural runoff, industrial runoff or oil spills.

Destruction or loss of seagrasses has been reported from most parts of the world, often from natural causes, eg "wasting disease", or high energy storms. More commonly destruction has resulted from human activities, eg. as a consequence of eutrophication or land reclamation and changes in land use. Anthropogenic impacts on seagrass meadows are continuing to destroy or degrade coastal ecosystems and decrease their yield of natural resources.

It is important to document seagrass species diversity and distribution and to identify areas requiring conservation measures to prevent significant areas and species being lost.

In order to determine the importance of seagrass ecosystems and to detect changes that occur through perturbations (man-made and natural), it is necessary to first map the distribution and density of existing seagrass meadows. These maps must be monitored to determine natural variability in the extent of seagrasses (e.g. seasonal dieback) before estimates of loss or gain due to perturbation can be made. Coastal management agencies need to know what levels of change are likely to be ecologically or economically important, and sampling designs for baseline and monitoring surveys need to be sufficient to measure changes that are statistically significant.

Spatial and temporal changes in seagrass abundance and species composition must be measured and interpreted with respect to prevailing environmental conditions. These may need to be measured seasonally, monthly, or weekly, depending on the nature of their variability, and the aims of the study. Physical parameters important to seagrass growth and survival include light (turbidity, depth), sediment type and chemistry, and nutrient levels.

Detailed studies of changes in community structure of tropical seagrass communities are essential to understand the role of these communities and the effects of disturbance on their composition, structure and rate of recovery. Seagrass meadows should be mapped as a first step towards understanding these communities.

FIELD TRAINING EXERCISE

We would first like you to sign the attendance sheet with your name and address.

A short talk will be given prior to the field exercise

In this exercise you will learn how to estimate seagrass biomass using the method of **Mellors (1991).** This method visually estimates above-ground dry weight biomass. The method calibrates these standing crop estimates against a set of pre-selected quadrats which are harvested at the end of the exercise. The visual technique is more precise than some traditional harvesting methods due to the larger number of replicates that can be taken.

YOU WILL NEED THE FOLLOWING EQUIPMENT.

- ☐ Quadrat (50 centimetre x 50 centimetre).
- ☐ Clipboard with pre-printed data sheets on A4 size underwater paper. The sheets are attached to the and kept as a permanent record.
- ☐ Pencils.
- ☐ Waterproof labels. Pre-printed labels ensure that all essential data are recorded for each sample.
- ☐ Plastic bags - for seagrass samples
- ☐ 50-100 metre fibreglass measuring tapes.
- ☐ Dive knife.
- ☐ Diver's mesh bags.

GENERAL PROCEDURE

As a group, we will first set up 5 reference quadrats.

1. Select **5 reference quadrats,** we will help!. The quadrats should represent the range of seagrass biomass (most to least) likely to be encountered during sampling. Remember to estimate **dry weight biomass** and not percent cover. You must consider the area of bare ground between plants, plant height and the moisture content of each species.

2. Rank the 5 reference quadrats on a linear scale, 1 (least) to 5 (most).

3. Select the reference quadrats for Rank 1 and Rank 5 first, followed by Rank 3, and finally Rank 4 and Rank 2.

Rank	Estimate
0	nil
1	least
2	half-way between Ranks 1 and 3
3	half-way between Ranks 1 and 5
4	half-way between Ranks 3 and 5
5	most

4. The reference quadrats must be agreed to by all observers (*photograph for future reference*).

5. Leave the reference quadrats in place until the entire exercise is completed.

We will now set up the transect and survey the seagrass meadow.

1. Select the position for a transect after a visual survey of the area, we will help!. The transect should be representative of the entire seagrass area.

2. Record the position of the transect on a map if you have one. The origin (inshore end) of the transect is the most useful reference.

☞ *The length of the transect will depend on the size of the seagrass meadow, and should extend to outer limits of the bed (where the seagrass disappears).*

3. The transects should be separated from each other by a reasonable distance (50 to 100 metres).

4. Starting at the transect origin, haphazardly toss 3 quadrats within an area of approximately 5 metre radius.

5. For each quadrat, first estimate (rank) the above-ground biomass as per the 5 reference quadrats (*you may want to check to refresh your memory*). Then determine the seagrass species present and their respective percent covers. Record all data legibly onto the data sheets provided.

6. Record the sediment code and write any comments if any (eg. *lots of algae, 5% live coral*).

7. Proceed along the transect recording the ranks of 3 quadrats at each site. Sites should be taken at regular intervals (usually 20 metres) along the transect, so that gradients in community structure are described.

☞ *In a large uniform (homogeneous) seagrass meadow which extends out from the shore for more than 100 metres, the sample interval may be every 15 to 20 metres. In mixed (heterogeneous) meadows, intervals may be less than 5 metres.*

8. Collect a voucher specimen of each seagrass species you encounter (only 1 or 2 shoots which have the leaves, rhizomes and roots intact). Label each specimen clearly and bag.

9. When you have completed the transect, check with Rob, Warren or Len.

We will now calibrate your seagrass ranks.

1. You will be provided with photographs of seagrass quadrats (labelled **A** to **I)**. Rank the above-ground biomass of the seagrass in each photograph, and record on the **calibration data sheet**.

2. As a group, we will select 10 quadrats at the completion of the surveys, which represent the ranks (1 to 5) encountered along the transect. These 10 **calibration quadrats** cover the range of biomasses at the location.

3. Rank the above-ground biomass of the seagrass within each of the labelled calibration quadrats, the same as you did when surveying along the transect.

4. Photograph each of the calibration quadrats for future reference (ensure that each quadrats label is clearly visible).

5. Dig up all the seagrass from the 10 representative quadrats, for calibration of the rank estimates. First cut around the inner edge of each quadrat using a dive knife and then carefully loosen the vegetation inside the quadrat. Collect all the vegetation inside the quadrat (including roots and rhizomes).

6. Place the seagrass from each quadrat inside a separate plastic bag with a waterproof label clearly identifying the sample number.

7. Take the seagrass samples back to the laboratory for analysis.

Remember, the laboratory exercise is where you will learn how your data will be used to aid management. See you there!

Filling in a data sheet - an example:

FIELD TRAINING EXERCISE

Trainee name: *J Citizen* **Date:** *1 April 1998*

Transect number: *1*

Seagrass species codes: CR= *Cymodocea rotundata*, **CS**= *Cymodocea serrulata*, **EA**= *Enhalus acoroides*, **HB**= *Halophila beccarii*, **HD**= *Halophila decipiens,* **HM**= *Halophila minor*, **HO**= *Halophila ovalis*, **HP**= *Halodule pinifolia*, **HU**= *Halodule uninervis*, **SI**= *Syringodium isoetifolium*, **TH**= *Thalassia hemprichii.*

Sediment codes: M=Mud, **S=**Sand, **CS=**Coarse Sand**, G=**Gravel

Distance (Site)	Quad	Biomass rank	Seagrass species / % cover for each species	Sediment code	Comment
0	1	*1.3*	*HO/90 HU/10*	*M*	*lots of algae*
	2	*2.5*	*HU/40 HO/60*	*M*	
	3	*1.9*	*HO/90 HU/10*	*M*	
20m	1	*2.1*	*HU/30 HO/70*	*M/S*	
	2	*2.3*	*HU/40 HO/60*	*M/S*	
			HO/90 HU/10		

LABORATORY EXERCISE

The laboratory exercise follows directly on from the field exercise.

In the lab you will learn how to measure seagrass biomass, how to identify seagrass species using a taxonomic key, how to make a seagrass press specimen and how the data you collected is analysed and interpreted.

TO DETERMINE ABOVE-GROUND SEAGRASS BIOMASS:

1. Process each calibration quadrat sample one at a time.

2. Rinse the seagrass sample from each quadrat in water, and work on sample in sorting tray. Always keep the label with the sample.

3. Clean any attached debris (sediment) off the samples.

4. Separate sample into above-ground (leaves, stems & sheaths) and below-ground (roots & rhizomes) portions. Check with Rob, Warren or Len if unsure.

5. Blot above-ground portion of sample dry with paper towel, place in labelled paper-bag/dish and weigh (wet weight in grams to 2 decimal places). Record the wet weight on data sheet.

6. Blot below-ground portion of sample dry and weigh (wet weight in grams to 2 decimal places). Record wet weight on data sheet.

7. To measure the dry weight of above & below-ground portions, place the labelled paper bag or dish in an oven at 40 to 50 'C to constant weight (dry weight in grams).

SEAGRASS TAXONOMY AND PRESSING A SEAGRASS SPECIMEN FOR THE HERBARIUM COLLECTION:

1. Wash voucher seagrass specimen and carefully remove any debris or epiphytes.

2. Identify specimen to species level if possible with taxonomic keys provided. Most of the morphological characters used can be seen with the naked eye. A hand lens is useful for some minute (microscopic) features.

3. Layout specimen on a clean sheet of white paper, spreading leaves and roots to make each part of the specimen distinct.

4. Place specimen label on lower right hand corner of paper (label must include location, lat/long, depth, %cover, substrate, other species present, collector and comments).

5. Place another clean sheet of paper over the specimen, then place between several sheets of newspaper.

6. Place this assemblage of specimen/paper between two sheets of cardboard and then place into the **Seagrass Herbarium Press**, winding down the screws until tight (*do not over-tighten*).

7. Allow to dry in a dry/warm/dark place for a minimum of two weeks.

8. For best results, it is advisable to replace the newspaper after 2-3 days.

ANALYSING THE DATA.

These are demonstrations by Rob, Warren or Len showing

1. **how to convert field biomass estimates (ranks) into dry-weight:**

- Calibration curves will be establish for each observer by regressing the above-ground dry weights against the corresponding rank for the 10 calibration quadrats. We will do this in groups.

- Regressions for each observer will be used to transform field biomass estimates (ranks) into dry-weights.

2. **how to use Geographic Information Systems for mapping and monitoring seagrasses.**

PLEASE DO NOT HESITATE TO ASK ROB, WARREN OR LEN FOR ASSISTANCE OR INFORMATION.

WE VALUE YOUR FEEDBACK/COMMENTS.

A GUIDE FOR MAPPING SEAGRASSES

The most important information that is required for management of seagrass resources is their *distribution,* ie. a map. It would be inappropriate to set up a monitoring program if the most basic information is unavailable - that is, whether seagrass is present or absent.

The **SEAGRASS-WATCH** program is essentially people going to an area, establishing the edges/boundaries of any seagrass meadows and recording information on species present, % cover, sediment type, and depth (*if subtidal*).

YOU WILL NEED THE FOLLOWING EQUIPMENT.

- ☐ Quadrat (50 centimetre x 50 centimetre).
- ☐ Clipboard with pre-printed data sheets.
- ☐ Pencils.
- ☐ Waterproof labels. Pre-printed labels ensure that all essential data are recorded for each sample.
- ☐ Plastic bags - for seagrass samples

PRELIMINARY ASSESSMENT OF AN AREA

- Aerial photographs will help identify the location and extent of seagrass meadows, or
- A preliminary (*general*) visual survey of the area is required to map out, establish and adequately represent differences and the real extent of the seagrass meadows.

TO MAP THE MEADOW EDGES:

Select random points/sites on the edge of the meadow and either

1. mark/draw on a map or
2. record with a GPS (*Global Positioning System*) if available, or a hand-held compass to determine the bearing, with reference to at least 2 permanent landmarks or markers established as reference points.

TO RECORD INFORMATION ABOUT THE MEADOW.

1. First, locate a sampling site. Sites can either be:
   a) within transects across the meadow. (*Transects do not have to be accurately measured using a tape. You can estimate distances between sites depending on the size of the meadow. eg. in a small meadow you can have sites 20m or 50m apart, but in a large meadow sites may be 100m or 500m apart*), or
   b) haphazardly scattered over the entire meadow

2. Record the position of each site using a map, GPS or compass
3. At each site, haphazardly toss 3 quadrats within an area of approximately 5 metre radius.

4. Record site information. Information that is collected from each site can be either *basic* or *detailed*.

BASIC SITE INFORMATION

(*this is the minimum information required for mapping*):
For each quadrat,
1. first determine the seagrass species present and
2. their respective percent covers. (Percent cover may be in broad categories such as <10%, 10-50%, >50%. Record all data legibly onto a data sheet.)
3. Record the sediment type and write any comments if any (eg. lots of algae).
4. Record the water depth if the site is subtidal.

or

DETAILED SITE INFORMATION

(this is when accurate data is needed for monitoring) :
For each quadrat,
1. first determine the seagrass species present
2. visually estimate the above-ground biomass
3. estimate the respective composition that each species contributes to the above-ground biomass (record all data legibly onto a data sheet.)
4. Record the sediment type and write any comments if any (eg. 80% algae).
5. Record the water depth if the site is subtidal.

Remember: *If you have chosen to conduct visual estimates of biomass for each quadrat, at completion of the survey you will need to calibrate your ranks (see training exercise for detailed methodology).*

5. If time in the field is limited, we suggest taking a video of the quadrats. We also recommend that every now and again, you take a photograph of a quadrat that you have examined (*make sure this is noted on the data sheet so the photo can be matched with the quadrat details*). Because photographing every quadrat would be expensive, we recommend that you photograph a quadrat from every 10^{th} site (ie. 10% of the sites will have a quadrat that has been photographed). It is best to photograph a quadrat from two angles:
   1) from directly above and
   2) from 45-60 degrees (navel height?)

6. Collect a voucher specimen of each seagrass species you encounter (only 1 or 2 shoots which have the leaves, rhizomes and roots intact). Label each specimen clearly and put in bag.

7. Select the next survey site. The number of sites you survey will be entirely up to you. If you need to accurately monitor an area, then we recommend intensive surveys (*lots of sites*).

APPLICATION OF THE VISUAL ESTIMATES TECHNIQUE

A DETAILED WORKED EXAMPLE:

A group of 3 observers was asked to map the distribution and abundance of seagrass meadows within a bay. The survey was conducted over a 1 week period. At the beginning of the survey, the 3 observers gathered together to decide on the "*standard ranks*" for the study. As one of the observers had been to the area before, they went to a meadow which had both the greatest and lowest above-ground biomasses that they expected to see within the bay. They placed a quadrat over an area they all agreed was the highest biomass (referred to as "**standard rank 5"**) then another quadrat over an area they all considered was comparatively low biomass (referred to as "**standard rank 1"**). Then using this approach they found an area they all agreed was mid-way between the 5 and 1 (referred to as "**standard rank 3"**), and similarly set up standard ranks **2** and **4**. The standard ranks they set up were what they believed to be a "linear" relationship between the ranks and the above-ground seagrass biomass. They also took photos of the standard rank quadrats so they could refer back during the week of surveying if required.

The observers then proceeded to survey the bay. Each observer recorded their own visual estimate ranks independently of the other observers estimates, and ranks were each estimated to one decimal place. The observers surveyed 1100 sites with 3 biomass estimates at each site (a site was agreed to be an area of 5 m radius). **At the end of the survey** the observers gathered at another meadow which had the highest and lowest biomasses, **similar to those found during the survey**. At this location the observers threw down 10 quadrats, spread over the range of biomasses observed. Each observer then **independently** ranked the above-ground biomass in each quadrat, in the same way as they did during the survey. After each observer had ranked each quadrat (being careful not to discuss and compare ranks with other observers), each quadrat was harvested and taken back to the laboratory for sorting.

In the laboratory, the above-ground biomass was separated from the below-ground biomass for each harvested calibration sample (the entire sample was separated, no subsampling). The above-ground component was then dried and weighed to 2 decimal places.

The observer's ranks of the calibration quadrats were then regressed against the actual above-ground biomass for the calibration quadrats (g dry wgt m^{-2}) (see Table 1).

Table 1. Biomass and respective observer ranks for each calibration quadrat.

Calibration Quadrat	Above ground Biomass (g dry wgt 0.25m^{-2})	Observer1	Observer2	Observer3
1	1.55	1.3	1.1	0.5
2	1.95	0.2	0.2	0.1
3	8.75	4.5	4.6	4.8
4	10.93	3.9	3.6	4.3
5	7.18	4.3	4.2	4.4
6	4.93	2.4	2.20	2.1
7	6.53	2.5	3.8	2.4
8	3.95	2.1	2.4	1.4
9	0.7	0.8	0.6	0.2
10	1.01	0.5	0.8	0.4
r^2		0.89	0.94	0.92

A regression is a mathematical equation that allows us to predict values of one dependent variable (in this case the actual above-ground biomass) from known values of one or more independent variables (ie. the observers ranks).

From a plot of each observers ranks against actual above-ground biomass (Figure 1), it appears that quadrat # 4 was an outlier (it was well outside the 95% confidence limits). This means that all the observers had ranked quadrat # 4 too low - possibly because many of the shoots may have been covered with sediment, making estimation difficult, *etc*). After quadrat # 4 was removed, a regression for each observer was calculated (Table 2).

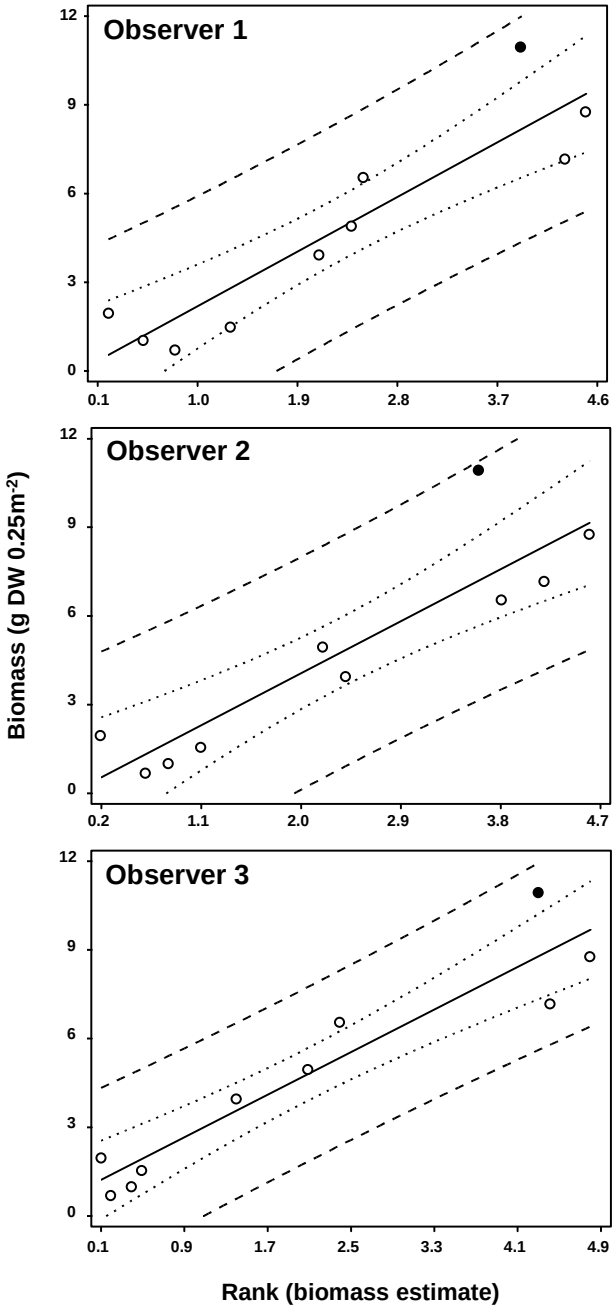


Figure 1. Linear regressions to explain the relationship between observer rank and above ground seagrass biomass. (filled circles signify outlier).

Table 2. Regression of observers ranks

Observer	Regression
Observer1	*Biomass* = 1.7908 x *Rank* + 0.3601
Observer2	*Biomass* = 1.7227 x *Rank* + 0.2520

Observer3	*Biomass* = 1.5888 x *Rank* + 1.1836

Using the regression for each observer, the field ranks estimated by each observer were converted to above-ground biomass (g dry wgt m^{-2}). All calculations of seagrass abundance within the bay were then done using the g dry wgt m^{-2} values.

Further comments:

- Mellors (1991) *does not recommend using integers, or categories.* An observer can estimate to 1 decimal place without difficulty (I suppose if you rank on a scale from 0.1 to 5.0 you in fact have 50 categories??)
- There is no need for observers to agree in the field after the standard ranks have been established. You do not want a single regression for all observers pooled. This is because *observers will always differ* - there is no point observers practicing to get the same rank. What is important is that **each observer has their own regression**, and that **each observer rank the same way each time**. In fact it is best that observers do not compare ranks at all when surveying an area, as this causes *bias*.
- The only values you are concerned with in the end is the above-ground biomass (g dry wgt m^{-2}). The ranks only mean something to the particular observer who estimated them. **Only the converted biomass estimates should be used for analysis**.
- Re-calibration should be done for each sampling/survey event (*what an observer ranks this week may differ from what they rank next month*) and at different locations.
- There are instances when 2 sets of standard ranks have to be used within the same survey (1 set for low abundance meadows (eg. *Halophila*), 2^{nd} set for high abundance meadows (eg. *Zostera*)) as this allows greater accuracy for biomass estimates.

KEY FOR STERILE MATERIAL OF QUEENSLAND SEAGRASSES

1. Leaves petiolate or compound, or strap-shaped without a ligule (i.e. a tongue-like structure at the junction of leaf blade and sheath) (Hydrocharitaceae) 2
   Leaves linear to strap-shaped and ligulate, neither petiolate nor compound 4
2. Leaves strap-shaped, neither compound nor petiolate 3
   Leaves compound or petiolate *Halophila*
   A. Plants with erect lateral shoots bearing a number of leaves B
      Plants without erect, lateral shoots, but one pair of petiolate leaves at each rhizome node C
   B. 10-20 pairs of distichous leaflets on an erect lateral shoot, blade with dense serrated margin *H. spinulosa*
      3 leaves per erect lateral shoot node; blade with sparse serrated margin *H. tricostata*
   C. Leaf blade longer than petiole; blade margin finely serrated, blade surface usually hairy *H. decipiens*
      Leaf blade normally shorter than petiole; blade margin entire, blade surface naked D
   D. Leaf blade oval to oblong, less than 5mm wide, cross veins up to ten pairs *H. minor*
      Leaf blade oval to elliptical, more than 5mm wide, cross veins more than 10 pairs *H. ovalis*
3. Rhizome more than 1cm in diameter, without scales, but covered with long black bristles (fibre strands); roots cord-like *Enhalus acoroides*
   Rhizome less than 0.5mm in diameter, covered with scales, but no fibrous bristles; root normal *Thalassia hemprichii*
4. Leaf blade more or less terete *Syringodium isoetifolium*
   Leaf blade linear, flat, not terete 5
5. Plants with elongated erect stem bearing terminal clustered leaves; rhizome stiff, woody; root stiff *Thalassodendron ciliatum*
   Plants with a short or no erect stem, bearing linear leaves; rhizome herbaceous; root fleshy 6
6. Rhizome bearing short erect stems; leaf sheath finally falling and leaving a clean scar, blade apex usually serrated or dentated; roots arising not in groups 7
   Rhizome without erect stems; leaf sheath persistant, remaining as fibrous strands covering rhizomes; blade apex truncate, neither serrated nor dentated; roots arising in 2 distinct groups of 4-8 at each node *Zostera capricornii*
7. Leaf blade with 3 veins *Halodule* 8
   Leaf blade with more than 7 veins *Cymodocea* 9
8. Leaf apex tridentate, with median tooth blunt and well developed lateral teeth *H. uninervis*
   Leaf apex more or less rounded, lateral teeth weak *H. pinifolia*
9. Leaf scars closed; blade apex rounded with no or weakly serated *C. rotundata*
   Leaf scars open; blade apex blunt with strongly to moderately serrated *C. serrulata*

(Prepared by J Kuo, UWA, Apr. 94)

Seagrass Ecology Group, Northern Fisheries Centre, Cairns

Queensland Department of Primary Industries
PO Box 5396
Cairns Q 4870
ph 07 40 529 888, fx 07 40 351 401

The Seagrass Ecology Group, based at the Northern Fisheries Centre, Cairns, is an internationally recognised and industry-funded team deeply committed to the QDPI's vision of a Fishing Industry Sector based on sustainable use of resources. They undertake pure and applied research and provide management advice directly related to the priority fisheries areas of maintaining marine fish habitats, improving fisheries productivity, coastal and regional environment planning for sustainable resource use, and the development of recreational fisheries.

The Group's nine staff focus on seagrass management and research and in May 1997, the Seagrass Ecology Group were presented one of the eight DPI Client Services Awards. Projects include mapping of seagrass and juvenile prawn nursery grounds for the fishing industry (managed by the Queensland Fisheries Management Authority), dugong management and for Marine Park zoning plans (GBRMPA), and monitoring change in fisheries productivity and marine plants. Seagrass habitat maps have:

- enabled the prawn trawling industry to avoid trawling on these sensitive habitats and protect juvenile prawn nursery grounds and recruitment to the fishery;
- provided fisheries and marine park managers with new knowledge on the status of our seagrass resources and likely trends in these habitats; and
- highlighted the necessity for sustainable land-use practices in catchments to ensure maintenance of these valuable coastal fisheries habitats;
- been invaluable to the understanding of resource sharing between dugong and humans in areas such as Shoalwater and Hervey Bay.

Group members regularly speak at national and international conferences and committees on biodiversity, restoration, and monitoring of coastal habitat. In 1995 the group was asked to develop the Australian Standard for monitoring change in seagrasses and has developed an innovative and internationally accepted method of visual assessment of seagrass habitats. These methods provide a national protocol for seagrass habitat mapping and monitoring that will lead to nation-wide awareness of the resource status and management priorities for sustainable seagrass habitats.

As an example of the Groups acceptance as an international authority on coastal marine science in the Asia - Pacific region, Group members were invited as the regional experts to Hawaii in 1994 to present two papers to an international committee on biodiversity. These papers were on the taxonomy and systematics of Pacific seagrasses and on the effects of development and conservation of the coastal zone. Maintaining biodiversity is the basis for protecting the complex marine ecosystems that support our fisheries. While there is much information for temperate systems only a few agencies worldwide research tropical systems. The Group is recognised as one of those lead agencies that can represent fisheries issues and the complex issues of habitat productivity. By contributing to the international understanding of tropical Pacific systems and their sustainability the Group are assisting DPI's vision of primary industries confidently competing in a world market.

The Seagrass Ecology Group, as a participant in the CRC for Reef Research brings Government and Industry together in a forum which helps meet DPI's mission of ensuring marine primary industries are managed in a sustainable way. The program involves research on determining the status of seagrass resources within the Great Barrier Reef and monitoring seagrass productivity and response to

terrestrial influence as well as research on recovery after loss of seagrass. Key issues in this research are the long-term viability and competitiveness of the Great Barrier Reef region tourist industry and fishing industries in the world market.

With CRC Reef Research support, the Group has developed and evaluated new sampling and research methodologies to conduct a Great Barrier Reef wide survey of deep water seagrass, to overcome the enormous logistic problems of surveying vast areas of water deeper than 30 metres. The project will help determine much of the zoning for fishing in deep-water inter-reef areas of the Great Barrier Reef lagoon. In doing so it will ensure the long-term sustainability of the coastal ecosystem, the marine habitat, and the commercial and recreational fisheries that depend on the viability of the inter-reef ecosystems.

Since its inception in 1985 the Seagrass Ecology Group has maintained a reputation as the leading advisers on seagrass management in north-eastern Queensland. The Group is about 80% externally funded. Almost all research is to a contract timetable and the Group has delivered a quality product on time.

Since 1989 the group has received funding from:- the Ports Corporation of Queensland; The CRC for Reef Research; the Australian Fisheries Management Authority; the Fishing Industry Research and Development Corporation; the Trinity Inlet Management Plan Technical Committee; Connell Wagner Engineering; Department of Economic Trade and Development; The Program on Environment East-West Centre; The Department of Primary Industries and Energy; the Department of Environment and the Great Barrier Reef Marine Park Authority. Continued funding from external agencies has been achieved by keeping a high level of client and funding body support by timely publication of reports; by excellent quality control; and by many public appearances to maintain commercial acceptance and goodwill.

The Seagrass Ecology Group always fosters a spirit of team research, and gets the best out of staff by including them in the whole process - from project planning; to analysis; to write up. Group publications always include those staff that contributed to the science. The Group has a strong commitment to provide information to schools and public awareness programs. The Group provides information for Integrated Catchment Programs and is currently advising and training community and government agencies to establish a statewide network of seagrass habitat monitoring programs.

Researchers in the Seagrass Ecology Group at Northern Fisheries Centre are:
Dr Robert Coles (Snr Principal Scientist, Group Leader)
Mr Warren Lee Long (Biologist, Project Leader)
Mr Len McKenzie (Snr Research Scientist, CRC Program)
Ms Jane Mellors (PhD Student)
Mr Anthony Roelofs (Biologist)
Ms Chantal Roder (Biologist)
Mr Michael Rasheed (PhD Student)
Mr Paul Daniel (Temp. Biologist)
Ms Wendy Baker (Scientific Assistant)

REPRINTS OF RELEVANT ARTICLES

The following reprints are provided for further reading

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(http://www.erin.gov.au/marine/natmis/standards/biology/leelon.htm)

A Standard for Seagrass Resource Mapping and Monitoring in Australia

Robert G. Coles, Warren J. Lee Long and Len J. McKenzie
[A]Queensland Department of Primary Industries, Northern Fisheries Centre, PO Box 5396, Cairns Qld 4870
Tel. 070 529888 Fax. 070 351401
CRC Reef Research, James Cook University, Townsville, Qld 4811
[A]*Address for correspondence*

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Abstract

Seagrass habitat loss and recognition of the value of seagrass habitats to fisheries in the 1970's and 1980's were the cause for early growth in seagrass research. Developments in seagrass research and data collection standards quickened in pace from the mid-1980's. Turbid, low visibility waters of much of Australia's tropical north coast require different data collection and data protocols to those of clear-water temperate regions. Further differences in approaches between temperate and tropical Australia are also necessary because of differences in seagrass species and habitat types. Measures of seagrass depth range, plant productivity, tissue condition/ nutrient content, biomass, shoot density, etc., can be chosen or adapted to suit the habitat types of any particular region. Regardless of locality, a minimum set of data required for seagrass collection would include:- a sample of seagrass plant lodged with a herbarium for future reference; a latitude and longitude; collector; depth; sediment type; samples of reproductive material and other species present. If collected in addition, seagrass biomass is recorded as g dw m^{-2}. Biomass may be recorded separately for above- and below-ground parts of the plant, although the components measured depend on the species and its growth habit. It may be necessary to record separately leaves and stems for some large species. Other useful measures of abundance include shoot density and leaf-area index or a simple estimate of percentage cover of the bottom. Change in seagrasses can be measured as a change in shoot density; a change in biomass, above- or below-ground; increase or decrease in productivity; species composition; depth range or location of a meadow; change in area or shape of meadows and in associated flora and fauna. Sampling designs for monitoring can include:- stratified; random; systematic or adaptive approaches; and transects, randomised or fixed location of sampling sites according to local conditions and needs. A sampling design for monitoring is tailored to the question being asked, the precision required and the parameters of the habitat being studied. Baseline surveys may need intensive data collection so that initial estimates of spatial variability are available for developing an effective monitoring program. Collection of data on physical attributes such as temperature, salinity, light and nutrients are useful in interpreting changes. Satellite and aerial photo-imagery and use of rectified digital images on GIS basemaps makes for quicker, more precise, drafting and mapping, and more useful data presentation, analysis, interpretation and storage. Differential GPS is a quick method for position fixing during mapping and reduces point errors to <3m in most cases. It is essential that estimates of error and reliability accompany each seagrass map, measure of seagrass aerial extent, and other seagrass parameter estimates. Metadata should be attached to GIS archives to describe data source, data reliability, conditions of use, limits on interpretation and use-by date, and usually includes the correct form of citation to be used for acknowledging the data source.

Introduction

Seagrasses play a vital role in supporting coastal marine communities and in maintaining diverse flora and fauna. They support coastal fisheries productivity and play a role in maintaining coastal water quality and clarity. Fisheries and coastal zone planners in Australia today take into account these values in planning for conservation management of seagrass resources.

Seagrass research in Australia has only recently included a range of studies from cellular to organism, population, community and regional resource level. There has been little formal development and testing for national data collection standards. A standard for seagrass data collection was developed for the ASEAN-Australia Marine Science Project: Living Coastal Resources workshops (English *et al.*, 1994) and a UNESCO guide to seagrass research methods (Phillips and McRoy, 1990) describes techniques for a wide range of research needs from applied to theoretical applications.

We draw on this information for the present paper which addresses the protocols for seagrass resource mapping and monitoring and comments on the collection of essential voucher or reference specimens for taxonomy. Earlier standards for seagrass mapping (eg., Walker 1989) are now part of a growing selection of alternative approaches as improvements in navigation and remote sensing technology and sampling design lead to more efficient and precise methods for mapping. In particular, accessibility to differential global positioning system (GPS) technology has given easy access to more precise position fixes. New methods of assessing seagrass abundance (eg., estimates of biomass techniques, *cf.* Mellors, 1991) enable more sites to be sampled within less time and with considerably less destructiveness. Modifications of grab designs (eg., Long *et al.* 1994) may improve opportunities for sampling in localities where diving is unsafe because of sharks or crocodiles or ineffective because of poor visibility. New equipment for improving divers' visual range in turbid conditions will have impacts on sampling in tropical coastal waters.

The present paper summarises and discusses methods for seagrass data collection and resource mapping and monitoring in Australian waters. The issues, methods and techniques detailed are also relevant to macroalgae.

Sampling Strategy

Published descriptions of methods for mapping and monitoring coastal seagrasses are very recent, eg., Kirkman (1996), Coles and Lee Long (1995) and Lee Long *et al.* (1996). Recognising the differences between tropical and temperate seagrass biology, there will be differences in sampling design and methodology. Our suggested national standard sampling strategy for seagrass resource mapping and monitoring is based on the following background principles.

Background principles for sampling strategies

Baseline mapping programs are best designed with monitoring in mind, and include intensive sampling to allow for the possibility of high levels of temporal and spatial variability. Measures of spatial variability calculated within baseline mapping will influence the design of monitoring programs and the statistical rigour of any tests for detecting change. Baseline data sets must therefore include sufficient density of seagrass data points to enable a reasonable measure of the natural spatial, and temporal variability within the habitat. Monitoring (routine measuring to determine status or condition) requires a different set of information to mapping, and the temporal and spatial scales most suitable for monitoring depend on the questions asked.

Techniques used for sampling aquatic vegetation are variations of those used for terrestrial communities. The difference is that for seagrasses and algae a sampling strategy takes into account the problems of working on the sea-bed. These include limited time for sampling (based on dive tables, or exposure at low tide), limited visibility, difficulty in relocation of sites, high costs of vessel charter and variable sea states. Typically, seagrass habitats in Australia can be in remote locations and may include the added thrill of dangerous marine animals.

Seagrasses can change in several ways. There can be a change in:- shoot density; biomass; meadow area; meadow shape; species

composition; plant productivity and depth distribution. There can be changes in the location of a meadow or a change in the associated fauna and flora, or a combination of some or all of these at small or large spatial and/or temporal scales. These changes may occur naturally and possibly on a regular seasonal basis. There is little information on the range of natural seasonal and year-to-year variability in seagrasses, and this information is a prerequisite to distinguishing human impacts. The seagrass parameters chosen for study depend on the questions to be answered. Seagrass parameters which represent indexes of impact can be monitored at local scales on permanent sites or throughout the meadow. These parameters can include seagrass tissue nutrients/elements (eg., Chlorophyll a, CHO's, C:N:P), plant productivity (eg., growth rates) or seagrass depth range. If it is necessary to know the changes in size of seagrass resources, distribution (maps) and abundance measures (eg., biomass, shoot density) are necessary for the whole meadow. The required precision and intensity of sampling effort will be less for regional scale studies.

Designing sampling programs

We suggest a hierarchy of information is required. To scope the extent of the existing resource, remotely captured (eg. satellite or aerial photography) images combined with ground truthing and specimen collection would be a priority. Locations and areas which support seagrass resources of special importance which are under threat or areas for which more information is required could be identified from this data. At these select sites, detailed sampling would include species composition and estimates of means and variances for parameters such as above-ground biomass or percent cover. The choice of sampling designs (eg. systematic, stratified, multistaged or adaptive), and location of sites (eg. transects, haphazard, random or fixed approaches), will depend on the peculiarities of each study situation. Attention should be drawn to the problems of pseudo-replication, spatial autocorrelation, assigning suitable controls and the difficulties in meeting all the requirements for parametric tests.

Seagrass biomass (above-ground), total area, percent ground cover, and species composition have been the most commonly chosen parameters for monitoring. Measuring seagrass growth parameters (eg. plant growth rates, plant tissue C:N:P, carbohydrate composition) provides greater insight into the causes of change in seagrass abundance. Physical environmental parameters which most often influence seagrass growth are:- light (Photosynthetically Active Radiation), turbidity, depth, temperature, salinity and sediment nutrients. Information on these parameters help in assessing the causes and scale of seagrass loss and the mechanisms for seagrass recovery. Turbidity, light (PAR), salinity and temperature are often included in monitoring, but require more frequent measurements according to the time periods over which they vary and affect seagrass growth and survival (Dennison *et al*. 1993).

The type of information to be collected on coastal habitat types such as seagrass meadows is dependent on the use expected for the data; the questions likely to be asked of the data; and the accuracy and precision of the answers required. Monitoring is easiest to apply to a specific environment concern such as the change likely to seagrasses from a port or harbour development. To measure regional changes it is our view that mapping using qualitative information on spatial distribution and repeated twice a year or at a suitable pre-determined time interval may provide a broad but sufficient indication of change. If changes in the area of seagrass measured this way continued in one direction for three or more sampling intervals, resources could be diverted to investigate the cause of change and, if possible and necessary, to remove the causal agent and at that point in time establish a more detailed monitoring program.

A useful basis for sampling is that adopted recently by the ASEAN-Australia Marine Science Project: Living Coastal Resources (English *et al*., 1994). This details the physical and biological parameters to be monitored, and provides examples of field sampling design, sampling methodology, sample processing, data recording, processing and analysis, with notes on safe procedures. Sampling methodologies detailed in the UNESCO monograph 'Seagrass Research Methods' (Eds. Phillips and McRoy 1990) are also recommended.

Equipment and Field Techniques

Remotely captured (satellite and vertical air-photo) images for seagrass distribution and abundance can be digitised and rectified to geo-coordinates for use on a Geographic Information System (GIS). Acoustic survey techniques are showing promise for mapping and monitoring densely vegetated meadows, but require much more improvement to detect low vegetation cover.

We have regularly used methodologies developed by Mellors (1991) to measure and record change in seagrass biomass and species composition (McKenzie *et al*., 1995). Other methods are described by Long *et al.* (1994) and Saito and Atobe (1970). The method adopted by any particular study will depend on the biological, logistic, cost-benefit, environmental and safety priorities of the study.

A technique developed for intertidal algae (Saito and Atobe, 1970) uses ranked estimates of vegetation cover in quadrats, including detailed assessments of species composition, for each sampling site. Rank estimates of above-ground biomass can also be used, as in Mellors (1991), and this technique is recommended for collecting seagrass biomass estimates from numerous sites, without harvesting large numbers of samples. 5 to 10 reference quadrats can be harvested at the end of a sampling event, to calibrate each persons' visual estimates against actual seagrass biomass measures. Incorporating estimates of species composition in quadrats (Saito and Atobe, 1970), makes the Mellors (1991) method even more useful. Care is required during every estimation of vegetation biomass and composition, but the errors inherent in visual estimates are acceptable if a sufficiently large number of sites are observed.

Where poor visibility prohibits visual estimates, grabs are an alternative for sampling seagrasses. Long *et al.* (1994) tested the use/efficacy of a modified "orange-peel" grab in different sediment and vegetation types, and report acceptable results. We have recently however developed an apparatus for making visual estimates in low visibility waters in northeastern Queensland and expect to publish this method in the near future.

Equipment needed for sample collection

Satellite and aerial-photo images are commercially available, or special aerial photo runs can be arranged. Minimum requirements for ground surveys, include maps/charts (and aerial photos), GPS units (with differential capability if possible), depth measuring instruments, compass, quadrats and data sheets. We regularly use quadrats 50 cm x 50 cm as they are the largest size comfortable for diving operations, although smaller quadrats may be necessary in some circumstances, depending on the seagrass species. The researcher must also be aware of cumulative errors when multiplying measures from small quadrats to per metre square units. Vessels and diving gear are needed for subtidal work. Equipment for harvesting seagrass for biomass measures include:- 5 - 10 quadrats; collecting bags; knives (for cutting rhizomes around edges of quadrats); labels and plastic bags.

Calibration of equipment and samples

Within the Mellors (1991) method, 5 to 10 quadrats - equal in size to the sample quadrats, and across the full range of biomasses observed during the survey - are ranked by each observer, harvested and biomass measured. Estimates of seagrass biomass are calibrated by calculating a regression equation for each observer. The regressions are for observer rank against actual dry weight biomass. Calibrations may need to be repeated for different seagrass species if plant physiology varies. As the Mellors (1991) visual estimates of seagrass biomass are calibrated to actual biomass measures within each survey, data can be cross calibrated with other surveys of seagrass biomass.

Depth measuring instruments are regularly calibrated and depth measures are standardised to depths relative to mean sea level (MSL), using the tidal plane information for each survey locality. The depth of the echo-sounder transducer below the water surface needs to be accounted for.

Spatial resolution

The scale decided upon for mapping or monitoring may determine the overall approach to sampling intensity and influences what is possible with a limited set of financial and human resources. If mapping for resource inventories is on a large scale (eg. the Great

Barrier Reef World Heritage Area) then the intensity of sampling will be low and may detect only broadscale changes. Satellite imagery and aerial photography are useful for mapping where dense seagrasses can be seen on large scales (Kirkman, 1996; Hyland, Courtney and Butler 1989; Long *et al*., 1994), but cannot always be used to successfully map or monitor seagrass biomass (Walker, 1989) or identify seagrasses of low density, or in water too deep or too turbid for remote sensing (Hyland, Courtney and Butler 1989). This may include vast areas of important seagrass in northern Australia.

If examination of seagrass meadows is required at a finer scale (eg., a port or harbour), the sampling intensity can be higher with greater precision than large-scale or remote areas and smaller levels of change may be detectable. If good quality remote sensing information or aerial photographs are available a stratified sampling design may be possible, requiring less field samples for the same resolution.

Temporal resolution

Seagrass abundance and distribution can change quite dramatically depending on time of year (a six-fold increase in biomass was recorded by McKenzie (1994) between seasons). This information is necessary in designing monitoring programs to measure inter-annual variability of seagrass meadows. A pilot study is recommended if time permits. Seagrass leaf turnover rates can be as quick as 15 days in tropical conditions but much slower (up to hundreds of days) in temperate regions (Hillman *et al.* 1989). Sampling during only one season may miss seasonal seagrass species, and sampling in Winter is likely to record the smallest sustainable distribution for the year. Sampling during the period late Spring to early Summer, at least in the tropics, gives an idea of the highest abundances and greatest distributions.

It is important to ensure seagrass abundance is measured during a period of little seasonal change, and/or monitored at the same time each year and/or measured frequently. Sampling intensity can be concentrated and unevenly spread if the expected change is related to a point source or seagrass species respond differently to the same environmental change. It may be possible to monitor on a different spatial scale to that in the original baseline if sufficient information is available on the likely response of the system. In some cases it is difficult to find a statistical difference in biomass and abundance between adjacent months. Sampling twice or three times a year may be necessary.

Sample storage & labelling

Historically, seagrass voucher specimens have been stored dry pressed on herbarium paper. Specimens can be kept damp in cold storage for short term or fixed in a preservative for longer terms. Freezing larger specimens may result in a deteriorated, "mushie" end-product and is not recommended for taxonomic specimens. Standard procedure is to fix and store in 5-10% seawater formaldehyde. Specimens collected for reproductive section can be stored in 5-10% gluteraldehyde, or in alcohol : acetic acid (3:1) for chromosome analysis. Specific requirements are best discussed with the taxonomist as methods may vary with species type and size or with the investigative procedure. Minimum requirements for labelling include species name, preservative, collector, date, location, latitude and longitude, depth, sediment type and co-occurring species.

Sample and data storage in the field

Seagrass biomass samples for calibrating divers' estimates are stored refrigerated in plastic bags but should be processed within days. We use manually completed hard-copy field data sheets so that special notes and sketches can be incorporated. Total reliance on electronic data may not be possible in a small vessel. Electronically collected GPS data can be downloaded and backed up frequently in the field.

Measuring problems and data quality

It is important to be aware of possible sources of errors that can occur in the field as they directly influence the quality of the data. It is important to document these errors and ensure that this documentation travels with the data. Commonly encountered problems in the field when using the Mellors (1991) visual estimates technique require the following precautions to be taken.

1. Two sets of standard ranks may be necessary when the biomass between meadows varies greatly due to the species composition of a meadow (eg., a high biomass *Zostera*

meadow verses a low biomass *Halophila* meadow). In such a circumstance it is often better to assign standard ranks to individual observers who are instructed to only examine meadows of equivalent biomass (eg., one observer ranks the *Zostera* meadows, while another observer ranks the *Halophila* meadows). This allows finer resolution of biomass estimation and finer levels of detectable change.

2. A photographic record of the standard set of ranks is useful for observers to review when mapping is over several days. This eliminates the chances of 'drift' in estimation.
3. It is necessary to calibrate after every mapping exercise, to eliminate the effects of any "drift" in estimations.
4. When position fixing with a GPS it is important for the observer to be as close as possible to the GPS aerial to minimise position fix error. This can be difficult in small boats under conditions of strong wind and current.
5. Conduct the calibration exercise in the same type of environment as the sampling was conducted so that visual estimates for calibrations reflect the conditions experienced during sampling.

Some Practical Guidelines for Field Work

Guidelines for seagrass sampling are site dependant and local knowledge may be required. Safety should be foremost when sampling the marine environment, paying particular attention to tidal regimes, turbidity, sea-state, dangerous marine animals and other human activities and impacts. Local knowledge of the above factors should always be sought. We strongly recommend that diving policies be developed by each organisation and national safety standards be met.

Documenting physical conditions during sampling

Climatic conditions, sea state, water visibility may effect the quality of data collected and should be recorded. Notes on any peculiarities of a site are also very useful in later validation of data and for general interpretation of patterns observed during field studies.

Data Processing and Reporting

Database management

Relational databases are useful for storage and management of data. A protocol for verification of data and a reliability index is required. The data should be accompanied by any caveats on data reliability, eg., changes in data quality during sampling because of physical changes such as sea state. This is important when data is loaded into a GIS system which is used by managers. GIS data also requires a use-by date. Taxonomic data should be associated with a collector and source of reference material so species revision can be included, or species identification checked at a later date. Original (master) copies of final GIS maps should be stored in two places: the source laboratory and a regional or central archive. Always attach metadata and 'readme' files to GIS files the above-mentioned information on data source, data reliability, conditions of use, limits on interpretation and use-by date. Metadata also includes the correct form of citation to be used for acknowledging the data source.

Assessing change

The size of change in the seagrass habitat that can be detected will depend on the resources available. Measuring a change induced by human activity against a background of natural variability can be difficult as little information is available on natural variability in the tropics and variability may be site and species specific. When assessing the downstream effect of coastal development the amount of change that is economically important may be different to what would be considered ecologically important. Even in countries with advanced research resources, detecting induced year-to-year changes of up to 25% in the tropics is in most cases unrealistic. A 50% year-to-year change in seagrass biomass normally would be detectable against natural change and would be important enough to prompt habitat management concern.

The level of significance (based on the Type I error) and level of assurance (based on the Type II error) in measuring and detecting changes are also important in calculating the most appropriate monitoring design. While it is preferable for the probabilities of both Type I and II errors to be as small as possible, a reduction in the probability of a Type I error

inevitably results in an increase in the probability of a Type II error. In monitoring environmental factors such as seagrass abundance, accepting a high probability of Type II error is likely to be more costly in environmental terms than the risk of a Type I error (Peterman, 1990; Fairweather, 1991), ie., it is better to say there is a difference when one does not exist (being over-cautious) than to say there is no difference when in fact a difference does exist. The probability of a Type I error is best risked in an attempt to reduce the probability of a Type II error.

Summary and Conclusions

The use of standards/ guidelines for seagrass data collection and management in Australia is ad hoc and accords to regional and local conditions and available resources. Standards can be adopted across regions of similar species groups, climatic or ecological patterns. Differences between tropical and temperate seagrass systems may require minor regional variations to the implementation of a national standard.

The recommended minimum procedure for ground surveys is use of the Mellors (1991) visual estimates of above-ground vegetation biomass, with estimates of species composition included. This has advantages of sampling numerous sites without having to harvest and process large numbers of samples. It is also the preferred method in sensitive or protected seagrass/ algae meadows. Quantitative (harvested) samples may be more appropriate for smaller experimental studies. The most commonly utilised measures for species which form high canopies still appear to be estimates of percent ground cover or shoot density. Remote sensing is less effective for mapping and monitoring for low vegetation cover, deep water or high. Cost, safety, remoteness, spatial and temporal scale and the questions being asked influence sampling design. Estimates of error and a use-by date are essential, and should where possible be attached to all archived databases and GIS maps.

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Wasting Disease in seagrass - review of current literature.

Paul A Daniel
Seagrass Ecology Group,
Queensland Department of Primary Industries
Northern Fisheries Centre
PO Box 5396
Cairns Q 4870

Massive die-back or wasting of seagrasses has been recorded around the coasts of the world since the 1930's. A marine slime mold, *Labyrinthula sp.* was identified as the suspected pathogen in this wasting disease. *Labyrinthula* usually plays a non-aggressive role in the senescence of seagrass leaves. Small brown spots which develop in lesions and spread, becoming much darker, throughout the leaf are characteristic symptoms of this disease. It is believed that one or a combination of external influences, both natural and anthropogenic, stress the health of seagrass communities triggering these events. Transmission is most likely via direct contact of infected leaves with healthy ones. The die-back of seagrasses has been recorded right around Australia.

The occurrence of a wasting disease or dieback, in seagrass meadows has been recorded worldwide (Muehlstein et al, 1988; Wnuczynski, 1996). It was initially and most dramatically observed in the eelgrass, *Zostera marina*, in the early 1930's along the coasts of North America and Europe. By 1933, in virtually one year, the disease had decimated 90% of the eelgrass in the North Atlantic (Anon, 1997).

Similar events where noted in eelgrass populations along the US Pacific coast in the late 1930's, New Zealand in the early 1960's and has re-occurred since 1984 in specific localities along both the east and west coasts of the USA and Europe (Muehlstein *et al.*, 1988). This disease has been particularly studied in the turtle grass, *Thalassia testudinum* in Florida Bay, southern Florida, USA., where rapid and widespread recurring mortality has been found since 1987 (Durako and Kuss, 1994).

In Australia, losses of seagrass have been extensive since the 1960's, but documented as principally human-induced (Walker and McComb,1992). The presence of *Labyrinthula* however was found throughout Lake Macquarie and the Tuggeron(?) lakes in NSW in the mid 1970's without any signs of a wasting event (West, pers. comm).

More recently in Great Sandy Strait, south-east Queensland, a decline of seagrass began in the early to mid 1980's, with a loss of *Zostera capricorni* in the upper region of Tin Can Bay. This loss had spread extensively out into large seagrass meadows of the straits by 1988. Five of the predominant species of seagrass present in Great Sandy Strait; *Zostera capricorni*, *Halodule uninervis*, *Halophila spinulosa*, *H. ovalis* and *Cymodocea serrulata*, have undergone periodic decline over the past three years and the symptoms associated with wasting disease noted (McLeod pers comm., in Wnuczynski, 1996). Wnuczynski(1997), isolated *Labyrinthula* from seagrass meadows of the Great Sandy Strait and Moreton Bay in 1995.

Reports of seagrass die back have also come from Torres Strait (Pitcher and Bishop, 1994). An unusually large run-off of freshwater from the Papuan mainland is suspected to be the cause of this event (Long and Skewes, 1996). Although the presence of *Labyrinthula* was not recorded in this location, it has been recorded in tropical mangrove ecosystems (Ulken, 1986; Ulken *et al.*, 1990; Bremer, 1993).

Species of *Labyrinthula*, commonly referred to as marine slime molds, are widely

distributed in coastal areas around the world (Vergeer and den Hartog, 1994). They have been isolated from a variety of marine habitats and substrates including organic detritus, diatoms, macro-algae and marine vascular plants. Infection experiments have shown isolates to be genus specific. *Labyrinthula zosterae* for example, has only been isolated from species of *Zostera* (Short *et al.,* 1993). The *Labyrinthula sp*. isolated from *H. ovalis* is the most aberrant and may represent another genus. Currently eight to nine species of *Labyrinthula* have been recognised (Porter in Vergeer and den Hartog, 1994), with one freshwater species reported (Zopf in Muehlstein *et al.,* 1991).

Labyrinthula spp. are characterized by spindle or fusiform shaped cells surrounded by an ectoplasmic network which serves a role in cell adhesion, motility, communication and nutrition (Porter, in Muehlstein *et al.,* 1991). The development of wasting disease symptoms have been recorded and most recognized in *Z. marina*. Lesions develop which cause some air lacunae to fill with water. Small brown spots and stripes develop in these lesions which then spread along the leaf and become darker. In very diseased plants, these characteristics are evident in even the youngest leaves but are usually restricted to the oldest leaves in most populations. Similar lesions have been found on almost every seagrass investigated (Vergeer and den Hartog, 1994).

Cytological studies of the pathogen have shown it to be most frequently associated with marginal areas of the disease symptoms. In early stages of infection, *Labyrinthula* cells were located in the mesophyll cells taken from marginal areas of necrosis of small necrotic spots. *Labyrinthula* cells were rarely observed in epidermal cells. The mesophyll cells may be nutritionally more advantageous or easier to penetrate. They appeared to move rapidly through the tissue, directly penetrating the cell walls of the host. The ectoplasmic network that surrounds *Labyrinthula* cells appears to have an important role in the enzymatic degradation of the host plant cell walls and then presumably a role in the destruction of cells contents. In leaf pieces from marginal areas of larger necrotic patches, *Labyrinthula* cells had invaded the vascular tissue. Later phases of infection are characterised by leaf tissue that is completely brown, with pathogen cells more common in the epidermal cells and occasionally in the lacunae (Muehlstein, 1992).

Direct contact of diseased leaves with healthy leaves is thought to be the most probable mechanism of disease transmission. In laboratory conditions, direct contact was necessary for disease symptoms to appear. In nature, water currents could facilitate a diseased leaf coming in contact with healthy tissue. The pathogen was never isolated from the roots or rhizomes (Muehlstein, 1992).

Durako and Kuss (1994), recorded the pathogenic effect of *Labyrinthula* on *T. testudinum*. They noted that when *Labyrinthula* infected lesions where present, there was a reduction in photosynthetic capacity. The maximum photosynthetic rate decreased to below zero when lesions covered 25 % or more of the leaf tissue. At the same time the oxygen demand of the leaves increased, with respiration rates being up to three times higher in infected leaves than in non infected leaves. Severely infected tissues exhibited net respiration, even in high light levels. This may then reduce the availability of oxygen for transport to below ground tissues, possibly making *Thalassia* more susceptible to hypoxia, a proximal cause of death.

The presence and activity of a slime mould, *Labyrinthula zosterae*, was initially generally thought to be the pathogen and the sole agent responsible for this massive wasting of seagrass communities world wide (Muehlstein *et al.,* 1991; Short et al 1993). Although wasting disease has been recognised as a natural event (den Hartog, 1987), further studies have shown *Labyrinthula* spp., to be associated with seagrasses, without necessarily large scale epidemics comparable to the 1930's (Muehlstein *et al.,* 1988), or no damage at all (Vergeer and den Hartog, 1994). The

occurrence of the disease does not always result in the death of the plant (Short *et al.*, 1993).

The omnipresence of Labyrinthulacae in seagrasses has suggested it has a functional role. Labyrinthulacae was found in all 11 seagrass species investigated, belonging to nine genera (See Appendix One). In all species, *Labyrinthula* was isolated only from wasting disease like lesions in the oldest leaves. The only exception to this was with *H. ovalis*, where it was found on healthy green leaves. Thus it is thought that *Labyrinthula* normally plays a part in the senescence of the leaves. This supports the view that other factor(s) are also required to catalyze an outbreak of the wasting disease. This could be through increasing the susceptibility of the seagrass or stimulating the growth of the slime mold (Vergeer and den Hartog, 1994).

That *Labyrinthula* is normally a non-aggressive secondary decomposer of seagrasses is well accepted within the scientific literature (Young, in den Hartog 1987, Wnuczynski 1996, Landsberg *et al.*, 1996). Exactly what triggers an outbreak of a wasting or die back event though still remains unclear (den Hartog 1987, Nienhuis 1994, Vergeer *et al.*, 1995). A local explanations appear to be necessary, rather than a global cause (den Hartog, 1987).

Natural phenomena such as floods, droughts or hurricanes produces stress in specific localities. The decline of *Zostera* in the US, for example coincided with a period of very low precipitation, while conversely another more localised decline in seagrass correlated with extremely high rainfall. The decline of *Zostera* in Denmark in the 1930's, related to high summer water temperatures which supported the drought correlations, where drought is accompanied by high water temperatures, salinity and light intensity (Martin, in den Hartog, 1987).

However due to the surprisingly virulent and aggressive nature of *Labyrinthula* in a wasting event, many researchers see anthropogenic influences as the primary catalyst (den Hartog 1987, Wnuczynski 1996). Reduction in water quality through eutrophication, chemical input, thermal and sewerage effluent and such events as oil spills, increased turbidity from dredging and salinity changes are some man induced factors that cause a reduction in seagrass meadows (Wnuczynski, 1997). The initial die back of seagrass communities in the Great Sandy Straits region, coincided with township development and population increases along the adjacent coast. The development of sewage treatment plants, rubbish dumps and industrial estates are thought to more than likely have had a negative influence on coastal aquatic ecosystems (Wnuczynski, 1996).

Any environmental circumstances prevailing at the time of the wasting events that altered light intensity and water temperature may as in the case of *Zostera* make the seagrass more susceptible to *Labyrinthula*. Phenolic compounds in eelgrass for example act in the chemical defence of the plant against invading organisms. Plants grown under high light intensity show higher levels of these phenolic compounds, than those in low light. Whereas an increase in water temperature leads to a decrease in these compounds. An infection with *Labyrinthula* itself also greatly effects the phenolic compounds (Vergeer *et al.*, 1995).

The one element in the wasting disease enigma that is uniformly agreed upon is the link between salinity and disease severity. In a Wasting Index developed by (Burdick et al, 1993), the disease was found to rapidly spread above a certain salinity threshold. Declines below this salinity, due to rainfall or run off allowed recovery. Tests at various salinities have demonstrated that below 10 ‰ the disease symptoms rarely appear, and not at all below 5% (Muehlstein *et al.*, 1988).

In the example of Durako and Kuss (1994), density-dependant studies on *Thalassia*, drought conditions in addition to diversion of upland run off, had resulted in the lagoon becoming hypersaline. This was compounded by a reduction in the frequency of hurricanes in the region, reducing low

salinity pulses through the system and allowed an increased accumulation of sediments. These changes allowed *Thalassia* to develop to high densities. When the outbreak of wasting disease occurred it was absent in the lower salinity basins in the northeast of the bay even though these populations where chronically stressed. This study suggests that a combination of factors trigger a wasting event.

Little information was available on recovery seagrass. In the North Atlantic, it was noted that recovery of eelgrass from the 1930's epidemic was slow taking several decades. Even then it did not reappear in all of its previous locations. In 1988, the symptoms of wasting disease was again noted in many widespread eelgrass populations. There have been several local declines but non-comparable to the earlier epidemic (Muehlstein *et al.,* 1988). It has been suggested that this may be a developmental cycle of 50-55 years (Glemarc, in den Hartog, 1987). As already discussed a sufficient decline in salinity would facilitate inactivating the pathogen and allow recovery (Burdick *et al.*, 1993) but no time frame has been investigated to date.

Muehlstein *et al.* (1991) found *Labyrinthula* easy to isolate using modified techniques of Watson and Ordal (1957) and Koch's postulates (Brock, 1961) to test its pathogenicity. Species identification is facilitated primarily by substrate or host specificity, growth patterns and cell morphology.

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Appendix One

List of 11 species of seagrass and their locations from which *Labyrinthula* was isolated during investigations by Vergeer and den Hartog (1994).

Zostera marina	Exmouth, England
Zostera mucronata	**Swan River, Perth, Western Australia**
Heterozostera tasmanica	**Whitfords area, Mullaloo pl. Western Australia**
Posidonia oceanica	Gallipoli, Italy
Halodule uninervis	Mombasa, Kenya
Cymodocea nodosa	Taranto, Italy
Syringodum isoetifolium	Mombasa, Kenya
Thalassodendron ciliatum	Mombasa, Kenya
Ruppia cirrhosa	The Fleet, England
Thalassia testudinum	Curacao, Netherlands Antilles
Halophila ovalis	**Whitfords area, Mullaloo pl. Western Australia**

FEEDBACK

We value your suggestions or any comments you may have to improve our **SEAGRASS-WATCH** program.

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Send your comments to

Seagrass Ecology Group
Northern Fisheries Centre
PO Box 5396
Cairns Q 4870
Australia

or contact us on
☎ 07 40 529 888
fx 07 40 351 401
mckenzl@dpi.qld.gov.au
colesr@dpi.qld.gov.au
leelonw@dpi.qld.gov.au