Net Primary Production in Giant Kelp (NPP) Protocol

Outline

7.2 Calculate loss
7.3 Calculate NPP (change + loss)

1.0 Overview

One of the central goals of the SBC LTER is to understand how spatial and temporal patterns of production by the giant kelp *Macrocystis pyrifera* are driven by terrestrial and oceanic inputs. In 2002 we initiated field studies designed to measure the standing stock and demographic rates of *Macrocystis*. At present monitoring activities include**:** (1) tracking the sizes and numbers of fronds on marked individuals over time, (2) developing frond length/weight regressions for different seasons and sites, and (3) estimating changes in population density over time. We use these data to estimate net primary production as the growth of surviving fronds + the biomass of new fronds in a population. This approach will be useful in determining the relative contributions of the birth of new fronds vs. the death of old fronds (i.e.,population turnover) to NPP.

Net primary production of giant kelp is estimated monthly at three sites by the SBC LTER. Data collection consists of two main components. In the field, we measure the number and size of new fronds, track the longevity of each cohort, and estimate the size of all plants on fixed transects each month. Lab measurements are used to develop monthly frond length/weight regressions, kelp wet-weight to dry-weight conversions and to determine what percent of dry weight is carbon, nitrogen and hydrogen. These different datasets are integrated using a unique processing protocol into a single net primary production estimate for each month and site.

2.0 Field Site Descriptions

Sites Arroyo Quemado (AQUE-34^0 28.12' N 120^0 07.28' W) and Mohawk (MOHK-34^0 23.66' N 119^0 43.80' W) consist of an array of five parallel, north to south 40 meter long transects. These are centered and evenly spaced every 8 meters on a main east-to-west running transect (SBC LTER Kelp Forest Community Dynamics transect 1 at both sites) (Fig. 1a). One meter to the west of each transect line is sampled. Each transect is broken up into two 20 m long subsections (a north and south subsection). The total sampling area at these two sites is 200 m^2.

Site Arroyo Burro (ABUR-34^0 24.00' N 119^0 44.65' W) consists of three parallel east-to-west running transects of varying lengths (transect 1-60 m, transect 2-25 m, transect 3-40 m). A 2 m-wide area is sampled on both the north (N) and south (S) sides of each transect, resulting in a total sampling area of 500 m^2 (Fig. 1b). The patchy distribution of plants at Arroyo Burro necessitated this sampling design and large area of survey in order to obtain a comparable number of plants to the other sites.

Figure 1.

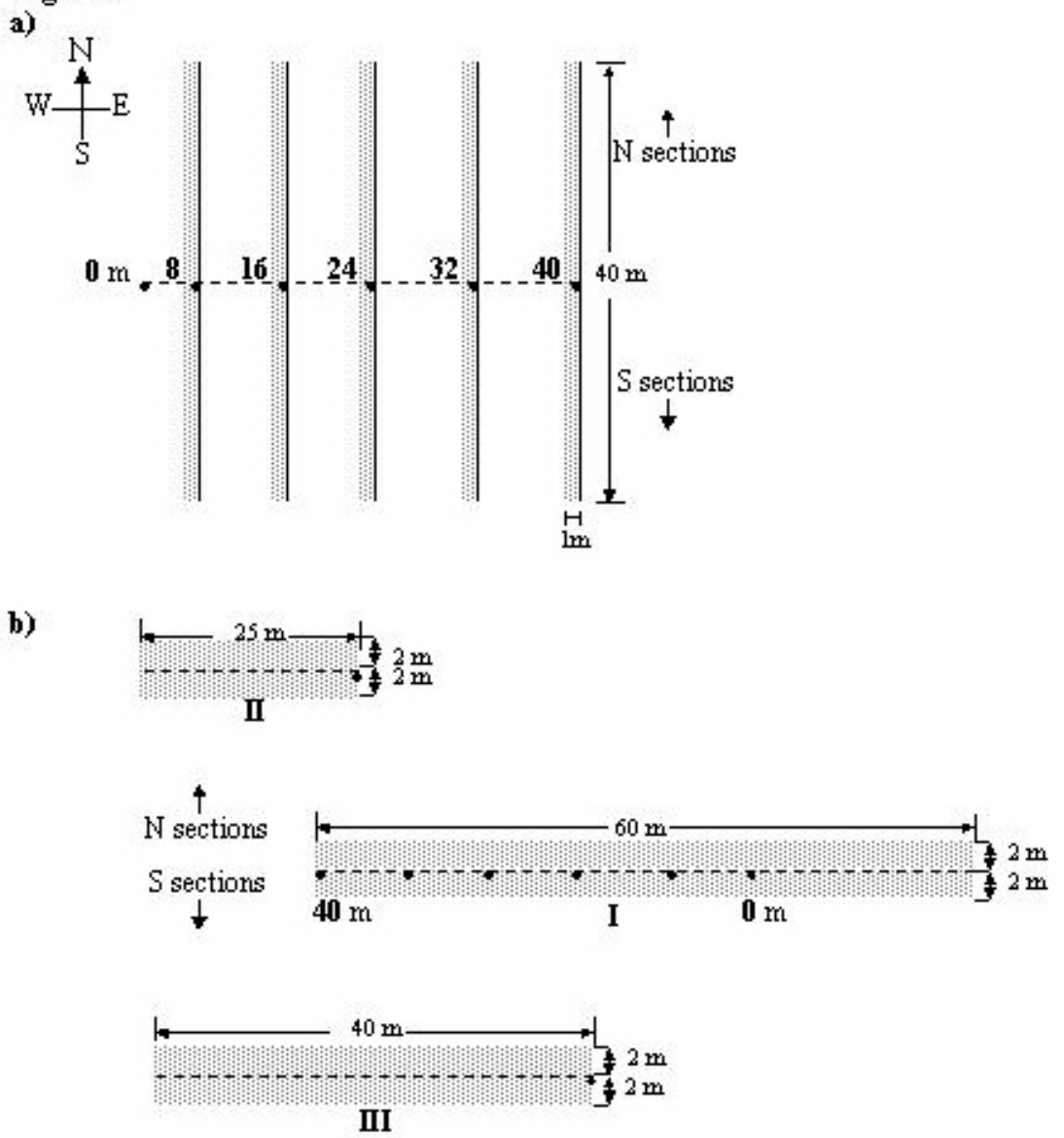


Figure 1. Transect layout and sampling areas of kelp NPP sites at (a) Arroyo Quemado, Mohawk and (b) Arroyo Burro. Bold numbers denote distances (meters) of permanent bolts along SBCLTER transect 1 at each site. Shaded areas are kelp NPP sampling areas.

3.0 Field Methods

3.1 Measuring the sizes and numbers of fronds on tagged individuals over time

Frond "birth" and loss rates are estimated using 10-14 uniquely tagged individual plants at each site. Each month, the frond "birth" rate is estimated by a diver marking near the base (using a unique color-coded tag denoting the month of appearance) and measuring (to the nearest 0.5 m) any new fronds greater than 1 m in length appearing on the tagged

plants. Frond loss rate is estimated by searching the plant and recording the number of remaining tags for each color code (each month cohort) of previously tagged fronds.

3.1.1 Tissue Sample Collection: Field

Tissue samples of the tagged plants are collected for CHN analysis. A single blade from each tagged plant is collected approximately 1 meter from growing tip of a surface canopy-reaching frond (N=10-15 blades per site per month). The samples are brought back to the lab for processing (See section 4.2 below).

3.2 Developing frond length/weight regressions for different seasons and sites

Frond length to wet weight relationships are calculated by dissecting 1-2 plants totaling at least 50 fronds from each site each month. Plants are collected by a diver selecting an appropriately sized plant and then cutting the holdfast near the substrate. The plant holdfast is then brought to the surface, placed in a large plastic garbage can on the boat and then the fronds are gathered and gently pulled into the can. The plants are transported in this manner to a large and flat concrete surface for dissection.

3.3 Estimating changes in population density over time

All kelp plants encountered in the defined sampling areas (Section 2.0) with at least 1 frond greater than 1 m in length are sampled. The following measurements are recorded for each sampled plant: depth (D), measured at the top of the holdfast using a submersible pressure gauge, number of fronds 1 m above the holdfast (NB), number of fronds reaching the surface (NS) and the surface length of the longest frond (max) (Figure 2). Divers use one of two methods to count the number of fronds at the surface and measure the length of the longest frond. When sampling smaller plants (usually those less than 50 fronds), the diver remains stationary on the substrate at the base of the plant. (S)he pulls the plant down a distance equal in length to the water depth of the holdfast and counts the number of fronds at that linear distance. Similarly, the diver measures the surface length of the longest frond by continuing to pull the plant down (in 1 m intervals) until the end of the longest frond is reached. Alternatively, the diver measures large plants (typically greater than 50 fronds) by gathering all the fronds of the plant as he/she swims to the surface to count the number of fronds at the surface. Plants with fronds that do not reach the surface but are greater than 1 m in length “subsurface” fronds (Fig. 2a), (typically young plants with less than 10 total fronds) are also sampled by counting the number of fronds at 1 m and then simply estimating the average length of the fronds. These measurements are used to estimate an average frond length for the particular site and month (see sections 5.0-7.3).

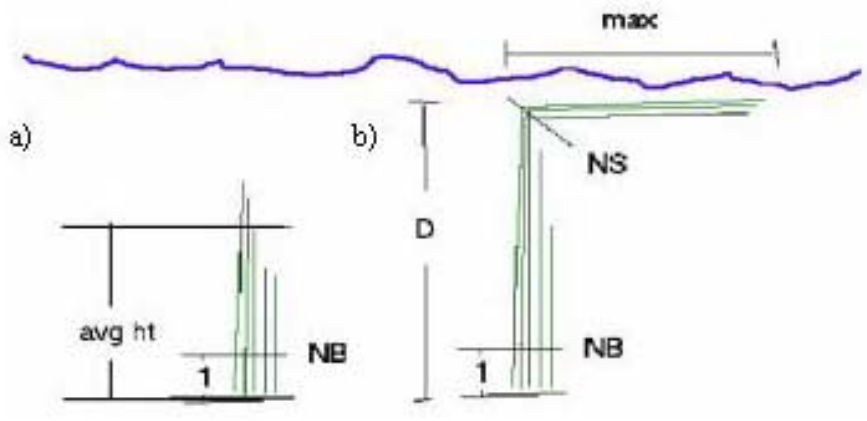


Figure 2. *Macrocystis pyrifera* section abbreviations used for a) subsurface and b) canopy-reaching plants in SBC LTER Kelp NPP data processing.

4.0 Laboratory Methods

4.1 Developing frond length/weight regressions for different seasons and sites

Macrocystis plants collected from each site are brought into the laboratory for length/weight dissection. The large flat concrete area used for dissection is rinsed to remove foreign material and debris. A plant is emptied from the garbage can and stretched out as if it were in the water column to facilitate separation of the individual fronds. The holdfast is removed by cutting each frond directly above the holdfast; individual fronds are then separated from the plant. Each frond is individually measured (to the nearest 0.1 m) and weighed (to the nearest 0.001 kg) in two sections. The "water column" section is defined as the section of the frond beginning at the holdfast and ending where the frond reaches the surface and is operationally determined by the water depth where the plant was collected in the field. The "canopy" section is defined as the surface-floating section of the frond and is operationally determined as the remaining portion of the frond after the "water column" section has been removed.

4.1.1 Frond Condition Assessment

For each frond, a qualitative assessment condition is recorded. "Growing" fronds are still actively dividing at the apical meristem. "Terminal" fronds have ceased growing and lack an apical meristem. "Cut" fronds are obviously recently cut by a man-made source such as a boat propeller or kelp harvester. "Senescent" fronds are defined as near the end of the frond life cycle and would be lost from the plant by the next month. Senescent fronds are degraded in quality, typically lighter in color and often have grown into a spiral-like form. Senescing fronds in the spiral growth form are measured un-stretched, so that any decrease in length as a result of the curling is not accounted for in the total length.

4.1.2 Plant Processing Remains

After all fronds have been measured, a "scrap" weight is taken. "Scrap" is a composite measurement of all remaining blades that were lost from fronds during the processing of the plant and is used as a relative measure of plant degradation during the laboratory processing. All plant material is discarded after processing.

4.1.3 Supplementary Data: Frond Stipe Diameter and Blade/Stipe Proportions

In August 2002, September 2002 and March 2003 several supplemental physical measurements were taken from dissected plants. For both the water column and canopy sections, a representative section of the frond was selected and calipers were used to measure the stipe diameter to the nearest millimeter. The number of nodes, that is the number of branching sections from the main stipe that produce blades, was also recorded for both the water column and canopy sections. Finally, after initial weighing, all blade material was removed from the stipe leaving the pneumatocyst attached to the stipe, and the weight of the stipe only was recorded.

4.1.4 Tissue Sample Collection: Lab

Tissue samples of the dissected plant are also collected for CHN analysis. Nine representative tissue samples are collected from each site; mature blade and stipe samples are collected from a subsurface frond and the water column and canopy sections of a canopy-reaching growing frond. Apical meristem samples are collected from both a subsurface frond and the canopy section. A sample of live haptera is also collected.

4.2 CHN Procedure: General Notes

The purpose of the kelp CHN sampling is to track the elemental composition of *Macrocystis pyrifera* tissue at the SBC LTER monthly giant kelp production sites. For this analysis a single *Macrocystis* blade is collected from each uniquely tagged plant in the field (3.1.1) and nine tissue samples are collected from the length/weight plant dissections (4.1.4).

4.2.1 Tissue Sample Processing

Prior to obtaining samples, the surface of all kelp tissue is cleaned by scraping it of epiphytes (e.g. bryozoans) and other foreign debris. Blade samples are cored by placing them on a wooden surface and firmly pressing a 2.9 cm diameter sharpened stainless steel pipe down near the pneumatocyst end of the blade. Stipe, growing tip and haptera samples are cut to appropriate sizes using scissors. Each sample is then submerged in a 10% HCl solution to remove any latent calcium carbonate and then immediately rinsed in a DI water bath to remove any HCl residue. The samples are then "patted" dry on a paper towel to remove excess water. The field collected blade cores are then added to a composite sample for the date and site of collection. The composite and nine tissue samples obtained from the plant dissection for each site are then placed in individual pre-weighed plastic weigh boats and labeled with the tissue type or number of core samples included, date, site and sample consecutive number information.

4.2.2 Tissue Sample Wet Weights

Wet weights of all samples (including the site composite sample) are then obtained using a Mettler AE 200 Analytical balance. Weights are recorded to the nearest 0.0001 gram.

4.2.3 Tissue Sample Drying and Dry Weights

All samples are then dried in a 60^0 C drying oven for a period of 2-5 days until the kelp samples are completely dry. Dry samples are then re-weighed and recorded.

4.2.4 Tissue Sample Grinding

Each sample is individually placed in a mortar and pestle and ground into a fine, uniform powder. The powder is then transferred to a 1.5 mL plastic centrifuge tube labeled with a consecutive number used to track samples at the University of California Santa Barbara Marine Science Institute (MSI) Analytical Lab. The mortar and pestle are wiped out between samples with a dry Kimwipe tissue.

4.2.5 Tissue Sample Labeling

Each individual plant sample is then assigned a consecutive number for sample tracking at the MSI Analytical Lab. Consecutive numbers are logically assigned using the date and site of sample collection. The consecutive sample number is then attached to the top of the 1.5 mL vial and is tracked throughout the processing steps.

4.2.6 Tissue Sample Storage

After the samples are dried, ground and assigned consecutive sample numbers, they are stored in sealed jars with moisture-retarding silica crystals for short-term storage before analysis. The samples may also be stored in the 60^0 C drying oven short periods.

4.2.7 Tissue Sample Submission

Samples are submitted to the MSI Analytical lab in monthly batches. A submission cover sheet accompanies the samples describing the number and consecutive number assignments to the Analytical lab.

4.2.8 Tissue Sample Analysis

Samples are analyzed using the Automated Organic Elemental Analyzer (Dumas combustion method) to determine the elemental composition (weight percent) of organic material in solid samples. See the MSI Analytical Lab website (http://www.msi.ucsb.edu/analab/analab texts/ analab.htm) for sample processing details.

4.2.9 Data Reporting

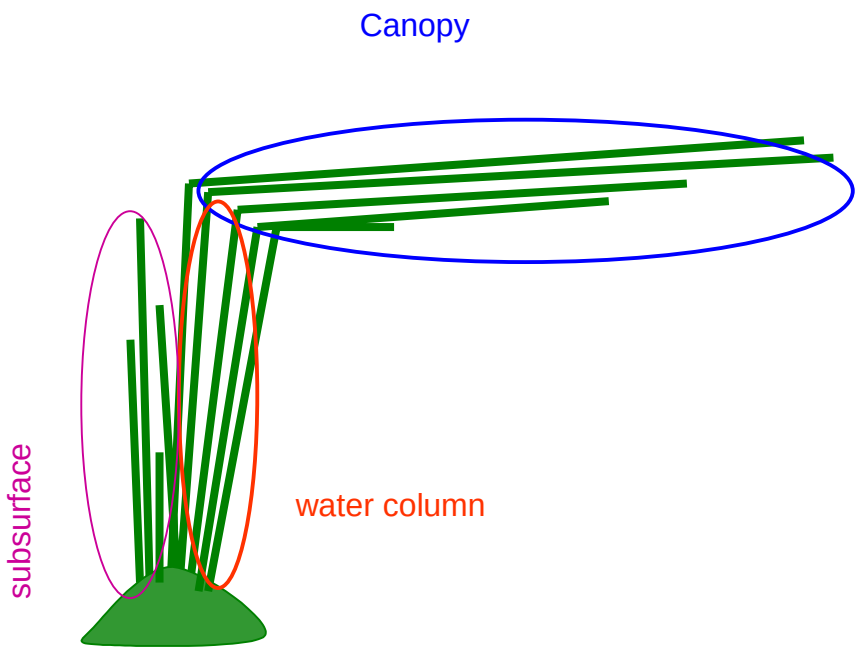
The MSI Analytical Lab emails the data in ascii or excel format to SBC LTER personel. These data are then incorporated into the appropriate datasets.

5.0 Kelp NPP Data Processing

The field and laboratory methods described in sections 2.0-4.2.9 produce the raw data for the Kelp NPP dataset. Sections 5.0-7.3 describe how these raw data are processed to produce estimates of Kelp Net Primary Production.

5.1 Estimate total length of kelp on each transect

Our biomass estimation relies on converting the lengths of fronds measured in the field into weights. This process is only appropriate if fronds are broadly similar across their whole length. Since the morphology of *Macrocystis pyrifera* fronds differs according to age, length and position in the water column, we have separated the plant into three subsections: subsurface, water column and canopy.



Young fronds which have not yet reached the surface are treated as the **subsurface** section of the plant. Older fronds which do reach the surface are divided into two parts. The **canopy** section is defined as the part of the fronds which lies along the surface of the water. The part of these fronds which is below the surface is defined as the **water column** section. (Figure 3)

Figure 3. Schematic diagram of the three kelp frond subsections used in SBC LTER Kelp NPP estimates.

Field plant measurements (Section 3.3) are used to calculate the total length of each section:

For a plant where no fronds reach the surface (Fig. 2a):

Total length all subsurface fronds = (NB)(estimated average length)

For a plant with fronds which reach the surface (Fig 2b):
Total length all subsurface fronds = (NB – NS)(1+½ [D – 1])
Total length all water column fronds = (NS)(D)
Total length all canopy fronds = (NS)(½ max)

In some cases, one or more of the plant measurements are missing because of logistical difficulties in the field. For these instances, the amount of kelp in each section is estimated based on regressions (created from data where all plant measurements were available) of the length of each section as predicted by the number of fronds at 1 m.

Finally, the three sections for all plants on a transect are added together, giving the total length in meters of subsurface, water column and canopy fronds on each transect.

5.2 Calculate conversion factors

5.2.1 Meters to kilograms wet weight

Three conversion factors are calculated for converting meters of kelp into kilograms (kg) wet weight, one for each section (subsurface, water column and canopy). The conversion factors are calculated by performing linear regression on pooled data from all plants collected and dissected in the lab (4.1). "Senescent" status fronds are excluded from the pooled data.

5.2.2 Kilograms wet weight to kilograms dry weight

A conversion factor relating wet to dry weight is calculated by linear regression, using wet weight to predict dry weight (4.2.2-4.2.3). Pooled data from all tissue samples is used to derive a single conversion factor which is applied to all three sections of the plant.

5.2.3 Percent carbon and nitrogen

The *average* %C and %N in the stipe relative to the blade is determined from the tissue samples as *ratio* of the average %C and %N in the stipe relative to that in the blade.

The weight of the plant that is stipe is then calculated from the plant dissection data by summing the weight of all non-senescent fronds with blades removed and then dividing by the weight of the same fronds with the blades attached (4.1.3).

Average %C and %N from the composite samples (3.1.1) are then adjusted for the presence of stipe, for example:
percent N is %N from the composite * stipe correction where the stipe correction for nitrogen is (1 – fraction of frond weight which is stipe) + (fraction nitrogen in the stipe relative to blade)* (fraction of frond weight which is stipe)

5.3 Calculate biomass per transect

5.3.1 Wet weight (kg)

Total kg wet $_{subsurface}$ = meters kelp $_{subsurface}$ (5.1) * kg wet/m $_{subsurface}$ (5.2.1)

Total kg wet $_{water\ column}$ = meters kelp $_{water\ column}$ (5.1) * kg wet/m $_{water\ column}$ (5.2.1)

Total kg wet $_{canopy}$ = meters kelp $_{canopy}$ (5.1) * kg wet/m $_{canopy}$ (5.2.1)

5.3.2 Dry weight (kg)

Total kg dry $_{subsurface}$ = Total kg wet $_{subsurface}$ (5.3.1) * kg dry/kg wet (5.2.2)

Total kg dry $_{water\ column}$ = Total kg wet $_{water\ column}$ (5.3.1) * kg dry/kg wet (5.2.2)

Total kg dry $_{canopy}$ = Total kg wet $_{canopy}$ (5.3.1) * kg dry/kg wet (5.2.2)

Sum kg dry = Total kg dry $_{subsurface}$ + Total kg dry $_{water\ column}$ + Total kg dry $_{canopy}$

5.3.3 Kg carbon

kg carbon (B_C) = Sum kg dry (5.3.2) * (%C in composite samples, adjusted for the stipe) (5.2.3)

5.3.4 Kg nitrogen

kg nitrogen (B_N)= Sum kg dry (5.3.2) * (%N in composite samples, adjusted for the stipe) (5.2.3)

6.0 Calculate Loss Terms

6.1 Calculate loss of fronds from tagged plants

The number of fronds lost over the previous month is calculated separately for each site (3.1). For each surviving plant, the total number of surviving (tagged) fronds is divided by the total number of fronds for that plant in the previous month to give the fraction of fronds surviving. The fraction of fronds lost (*f*) is simply one minus this number. The fraction of fronds lost for all surviving plants at a site are then averaged to yield a frond loss rate for the site in each month.

6.2 Calculate loss of plants based on transect data

The fraction of plants lost (*p*) is calculated using the changes in population density for each transect in each month (3.3). The proportional change in plant number is calculated as the number of plants this month minus the number the previous month, all divided by the number the previous month. If the change is positive, then the loss of plants is assumed to be zero. If it is negative, the loss of plants is (-1)(fractional change in plant numbers).

7.0 Calculate NPP

7.1 Calculate change in standing stock

The change in biomass for each transect is the total carbon (B_C) (5.3.3) or nitrogen (B_N) (5.3.4) content in a given month minus the carbon content in the previous month.

$$\text{Change in standing stock} = [B_{t+1} - B_t]$$

7.2 Calculate loss

The amount of biomass lost over the previous month can be calculated by combining estimates of the loss of plants with the loss of fronds from remaining plants for each transect. Note that the fraction of plants lost is specific to the transect in that month while the fraction of fronds lost is specific to the site but the same for all transects within the site in a month.

loss = loss of plants (*p*) (6.2) + loss of fronds from remaining plants (*f*) (6.1)

so the amount of biomass lost is:

previous biomass (B_t)* fraction of plants lost (*p*)+ remaining biomass (B_r)* fraction fronds lost by surviving turnover plants (*f*)

where remaining biomass (B_r) = previous biomass(B_t) * (1 - fraction of plants lost)

$$\text{Lost biomass} = [B_t p + B_t(1-p)f]$$

7.3 Calculate NPP (change + loss)

The primary production on each transect is then calculated by adding the change in carbon or nitrogen for the past month to the estimated loss of carbon or nitrogen during that month.

Net primary production (NPP) of giant kelp is estimated for each transect as the change in biomass (7.1) + the lost biomass (7.2).

$$NPP = [B_{t+1} - B_t] + [B_t p + B_t(1-p)f]$$

This gives an amount of carbon or nitrogen produced in kilograms, which is then divided by the area of the transect and the number of days elapsed between sampling periods to give the kg c produced per m^2 per day.