# Plankton Toolbox User's Guide

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Illustration on cover page: photo by Ann-Turi Skjevik

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# Plankton toolbox Users Guide

## Table of contents

## Introduction

Plankton form the base of the food web in most aquatic ecosystems. There is a need to estimate the biomass, abundance and the biodiversity of plankton organisms. Eutrophication, climate change, invasive species and harmful algal blooms are some of the reasons to monitor plankton. Microscope based methods are currently the standard in several monitoring programs including HELCOM-COMBINE, for the Baltic Sea, and OSPAR-JAMP, for North Eastern Atlantic Ocean covering the area between the Azores and the Arctic Ocean. Phyto- and zooplankton samples are collected using e.g. water sampling devices, hoses or nets. Data have been collected for decades and large data sets are available e.g. at international and national data centres. To work with the data in a consistent way may be difficult without the right tools.

The Plankton Toolbox is a free tool for aquatic scientists, and others, working with phyto- and zooplankton data. It is available for MacOS and Windows. Plankton Toolbox makes it relatively easy for non-programmers to work with large data sets on the diversity, abundance, biovolume and carbon content of plankton efficiently. The software is useful for working with datasets emanating from quantitative and qualitative analyses of phytoplankton and zooplankton. Phytoplankton, including harmful algae, are enumerated and identified in numerous ways; see e.g. Karlson et al. (2010). One of the most popular quantitative methods is water sampling, preservation of the sample and subsequent microscope analysis using the sedimentation chamber method (Utermöhl, 1958; Edler and Elbrächter 2010). These methods produce data on the abundance of plankton. The cell volume of the taxa is also often included to facilitate the calculation of biomass. Plankton toolbox offers a work flow for calculating biovolume of organisms based on Olenina et al. (2006) and also carbon content based on the algorithms by Menden-Deuer and Lessard (2000).

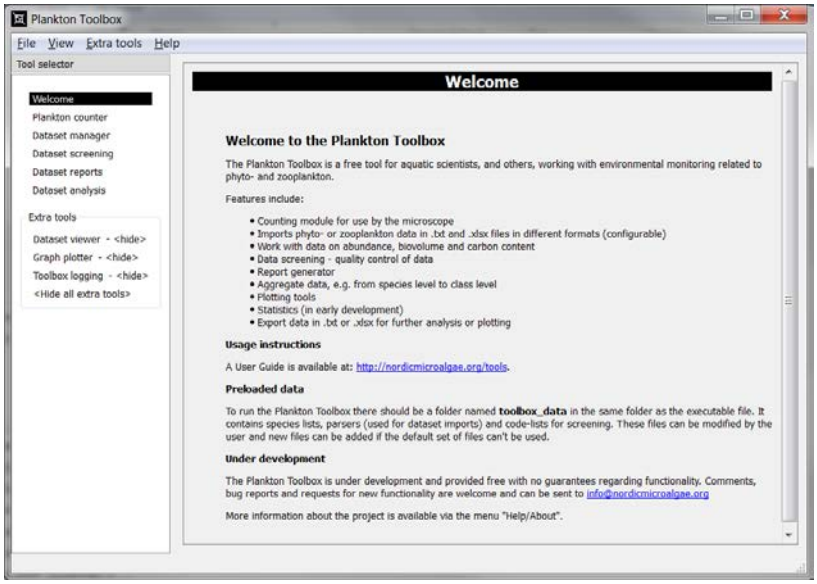
## Lifewatch

The development of the Plankton Toolbox is part of the Swedish Lifewatch project funded by the Swedish Science Council Vetenskapsrådet. Lifewach is also a European Union programme on sharing biodiversity data across Europe.

## Plankton Toolbox is free software

Plankton Toolbox is free software and comes with ABSOLUTELY NO WARRANTY.

## Uses for Plankton Toolbox




The software Plankton Toolbox has many features, here follows the main ones:

1. The **Plankton counter** provides
    a. A tool for the microscopist analysing (counting) plankton samples in a consistent way.
    b. A graphical user interface designed for counting samples using a computer by the microscope. If you prefer pen and paper by the microscope you may find the Plankton counter module useful anyhow.
    c. A way to record metadata such as sampling data, station name etc.
    d. A way to store templates with some metadata pre entered, e.g. when samples from different dates from a certain station are analysed
    e. A way to work with lists of organisms
        i. The HELCOM-PEG list
        ii. The Nordic Marine Phytoplankton group list
        iii. A zooplankton list (ZEN – in development)
        iv. A list of your own choice
        v. User defined subsets of lists mentioned above
    f. Information on traits such as trophic type, cell volume, harmfulness etc.
    g. Easy calculations of cell abundance and biomass based on concentrated volume, counted area, sedimentation chamber size etc.
    h. A data format for storing results together with metadata and methods used
    i. A way to save the results as a report
2. With the **Data set manager** you can :
    a. Select results from the Plankton counter module
    b. Import data sets in various formats, e.g. data sets downloaded from data centres.
        i. Text files
        ii. Microsoft Excel (.xlsx) files
    c. Combine different data sets
3. With **Data set screening** you can:
    a. Carry out some quality control of the data, e.g.
        i. Screen your data
        ii. Make plots of the raw data
4. With **Data set reports** you can:

   a. Select what data you want to include in the report
      i. Export data in various ways, e.g. in special formats
5. With **Data set analysis** you can
   a. Clean up your data, e.g.
      i. to remove some data in you data set
         1. to select a certain species
         2. to select a time period
         3. to select a station
         4. to select a depth interval
   b. Aggregate/complement your data
      i. If a sample has been counted at a high level of detail, e.g. at the size group level, you may want to aggregate to a higher taxonomic level, e.g. species, order or class level. Also non-taxonomic plankton groupings may be used.
      ii. Add zeroes – when combining data from several sampling occasions it is often useful to add zeroes for taxa that have not been observed
   c. Plot your results
   d. Calculate statistics

## Mac, Windows and Linux

Plankton Toolbox is available for Mac, Windows. A Linux version will be made available upon request. The software has been tested extensively on Windows 7 and to a smaller degree on MacOS 10.1. The user's guide provides examples, i.e. screen shots, from Windows 7.

## Getting the latest version of the software and the user's guide

The software and the user's guide may be downloaded from http://nordicmicroalgae.org/tools

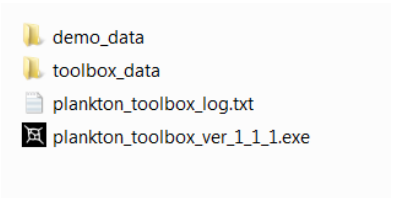
## Installing the software

Download the zip file. Unpack it and place it in a convenient place on your computer. It should also be possible to run the software on a server or on a virtual machine.

**Windows**

Place the whole folder with the software and supplied subfolders and files e.g. on the C: drive or the D: drive if you have multiple partitions on your hard drive.

Start the software by double clicking on the black and white icon.




In some computing environments you need to be a local administrator of your computer to be allowed to install or run software. Check with your IT-department if this is needed.

**Mac**

The instructions below are preliminary but should work for most users.

After unzipping the folder downloaded from http://nordicmicroalgae.org/toolse you need to do the following:

1. Place the whole folder with the software and supplied subfolders and files in the logged in user's folder, e.g. in Peter, if you are logged in as Peter on your Mac. The folder icon looks like a small house.
2. Move the application *plankton_toolbox_1_1_1_2015-12-03.app* to the main Applications folder, i.e. the folder with the name "Program" in Swedish.
3. Start the software by double clicking on the file "plankton_toolbox_mac-terminal_2015-12-03". This file should be in the logged in user's folder, e.g. in Peter, if you are logged in as Peter on your Mac.
4. This will start the window for terminal on the Mac. A few seconds later Plankton Toolbox starts. The terminal window will run in the background. The information shown in the terminal window is not of importance for the user of Plankton Toolbox.

Screen shots from Mac will be added in a later version of the user's guide.

## The Plankton toolbox folder

In the same folder as the software two other folders reside:

toolbox_data
demo_data

**toolbox_data**

This folder must not be moved or deleted. Essential species lists etc. reside in the folder. Read more about this in the section on taxonomic lists near the end of the user's guide.

**demo_data**

Example datasets with plankton data downloaded from the Swedish Oceanographic Data Centre (http://sharkweb.smhi.se) is found in this folder.

## Getting help

The user's guide is at present the only help system for Plankton Toolbox. The user community is encouraged to use the forum at http://nordicmicroalgae.org/forum to post questions and answers and to suggest improvements for the software. Also use the e-mail address info@nordicmicroalgae.org for questions.

# Basic concepts

## Data sets

Plankton Toolbox treats data as datasets. A data set may contain results from one or several samples. Datasets may be combined, e.g. when working in the *Data manager* and *Dataset analysis*.

## Taxonomic hierarchy

One of the features of Plankton Toolbox is the ability to aggregate data to different taxonomic levels, e.g. to class level. This requires a taxonomic tree, i.e. a hierarchy. There are two different taxonomic hierarchies supplied with the package. You may also create your own hierarchy. The hierarchies are user selectable, i.e. the user can use a tree of his or her own choice.

1. The taxonomic hierarchy used in Nordic Microalgae, http://nordicmicroalgae.org. This is based on AlgaeBase, http://algaebase.org (Guiry and Guiry 2015).
2. The taxonomic hierarchy used in the HELCOM-PEG list.

## Cell volumes and trophic types

Another feature of PTBX is the ability to work with biovolumes of phytoplankton. A list of cell volumes for phytoplankton taxa from the Baltic Sea region based on Olenina et al. (2006) is supplied with PTBX. This list is updated yearly by the HELCOM Phytoplankton Expert Group and is available for download at www.ices.dk. The list also includes information on the trophic type of the organisms, e.g. autotrophic, mixotrophic or heterotrophic. The term not specified (NS) is used for cells that have an unknown trophic type. In addition to the standardised lists support for lists handling synonyms and user defined lists is part of PTBX. Calculation of carbon content is part of PTBX. The equations used for phytoplankton were developed by Menden-Deuer and Lessard (2000).

# The user interface

The user face consists of one or a few window panes. There is a min window pane and window panes called Extra Tools. You may show and hide window panes as you please. If you have a large computer monitor you may choose to have all open. It is also possible to tear off window panes and place them on the same or on another computer monitor. The Extra Tools may easily be moved around by clicking and dragging them. They may float in front of the toolbox, or placed where one wishes, far right or below the work space.

### The Extra Tools

*Toolbox logging*

In this text file activities are logged and errors reported. Keep the window open when importing new data sets to note problems with species names etc.

*Dataset viewer*

You can see the original data, the filtered data or choose to not see any data for increased speed.

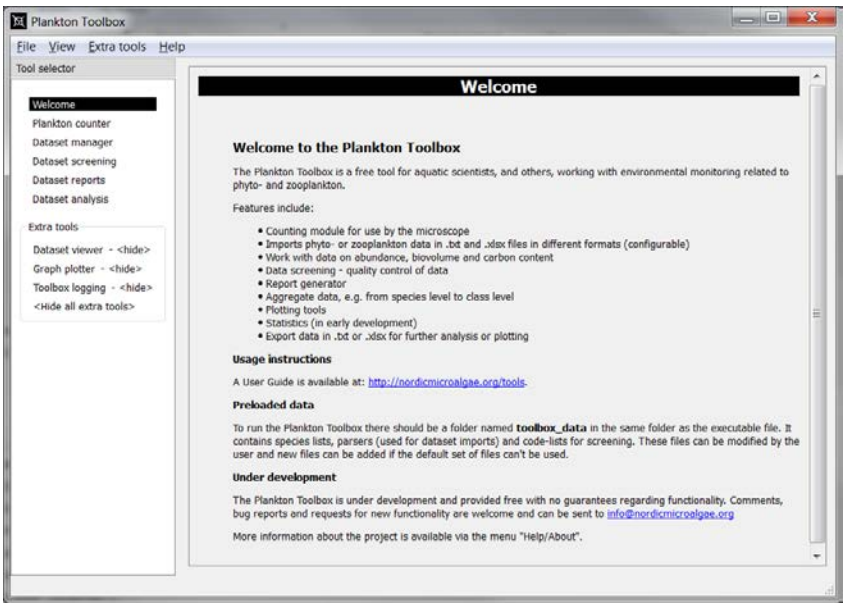
Data may be exported through the clipboard and pasted into other software such as a text editor or Microsoft Excel.

Data may be exported using the save function as text files or xlsx files.

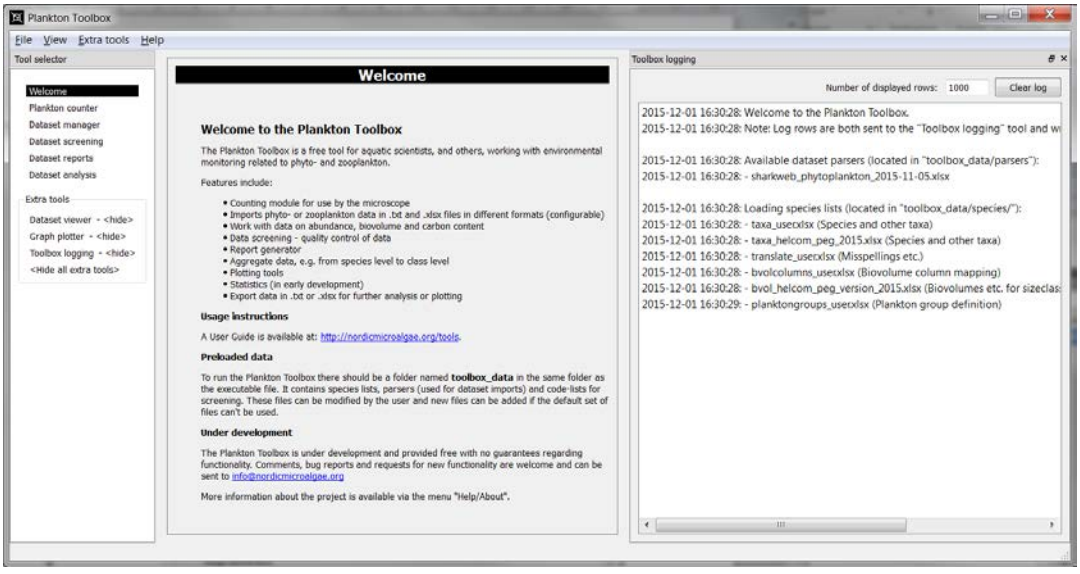
*Graph plotter*

In this window pane new plots are shown. Plots may be exported in various formats, e.g. jpg and png.

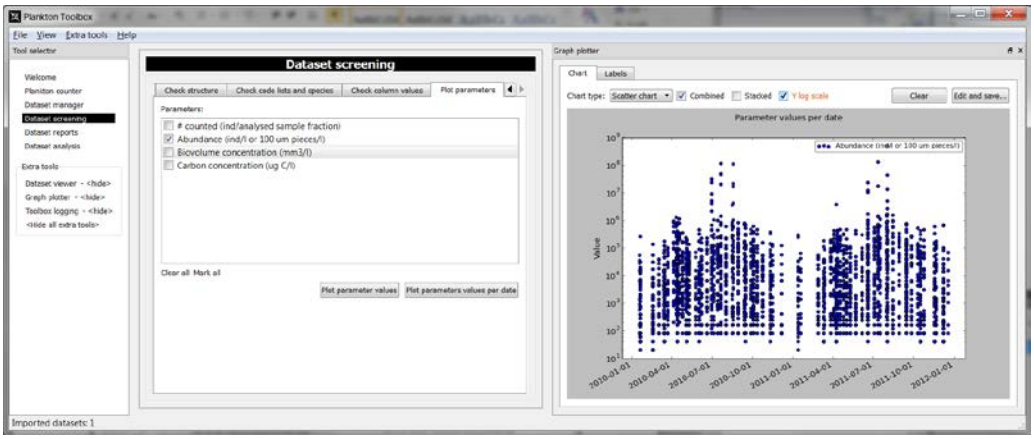
Here follows some examples of how you may configure the user interface.



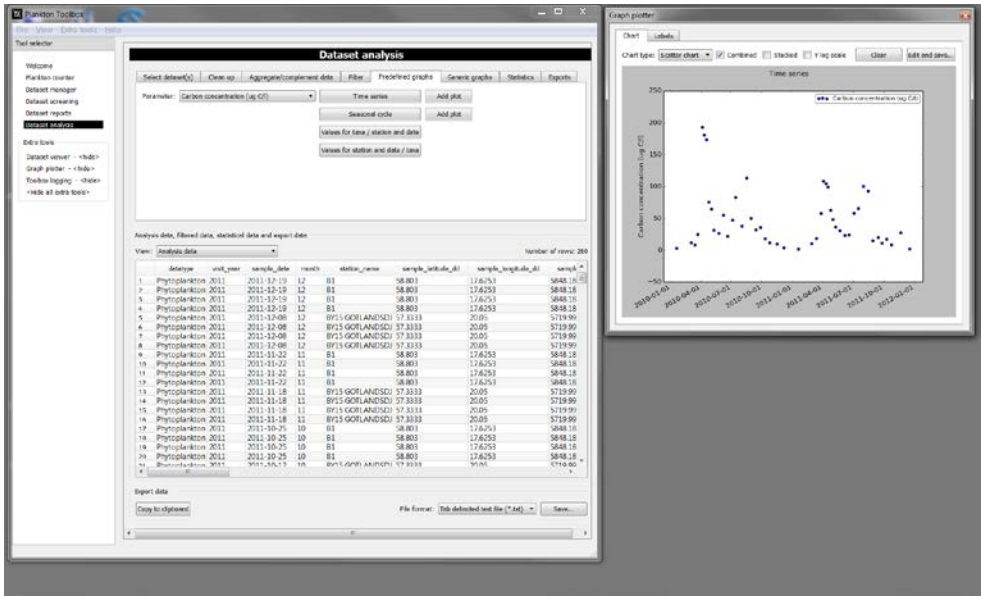

Main window only



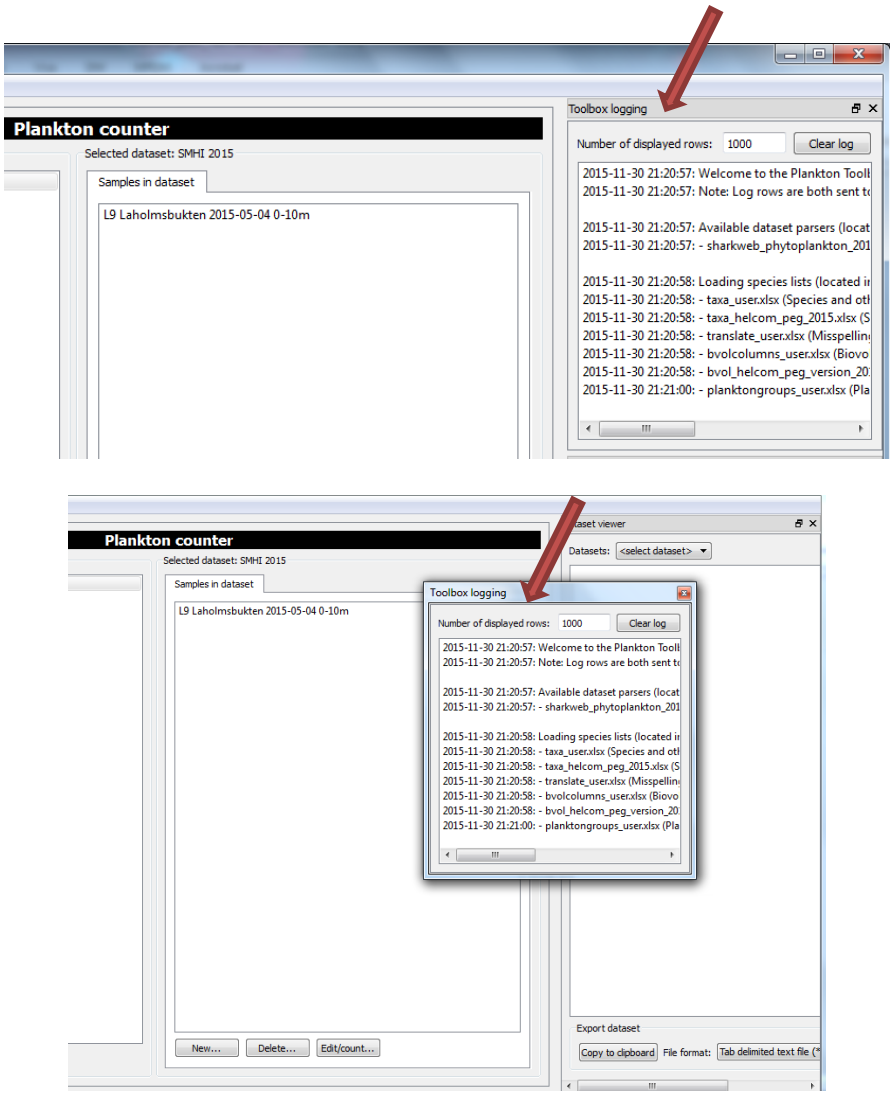

Main window pane with window pane *Toolbox logging* open



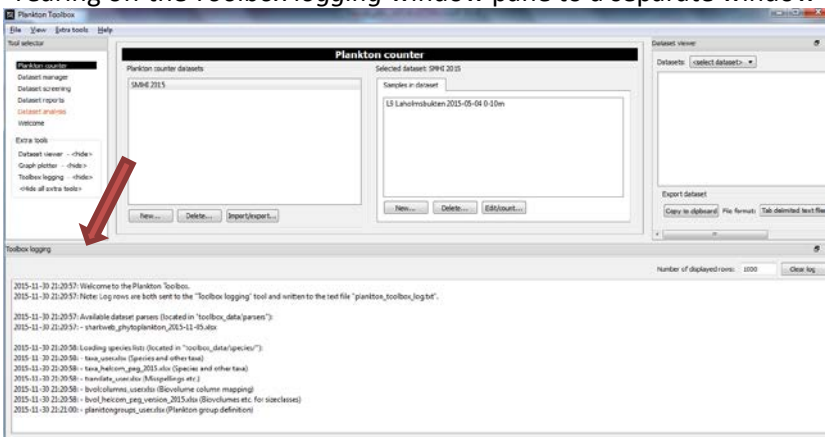

Main window with window pane *Graph plotter* open




Main window pane with *Graph plotter* in a separate window.

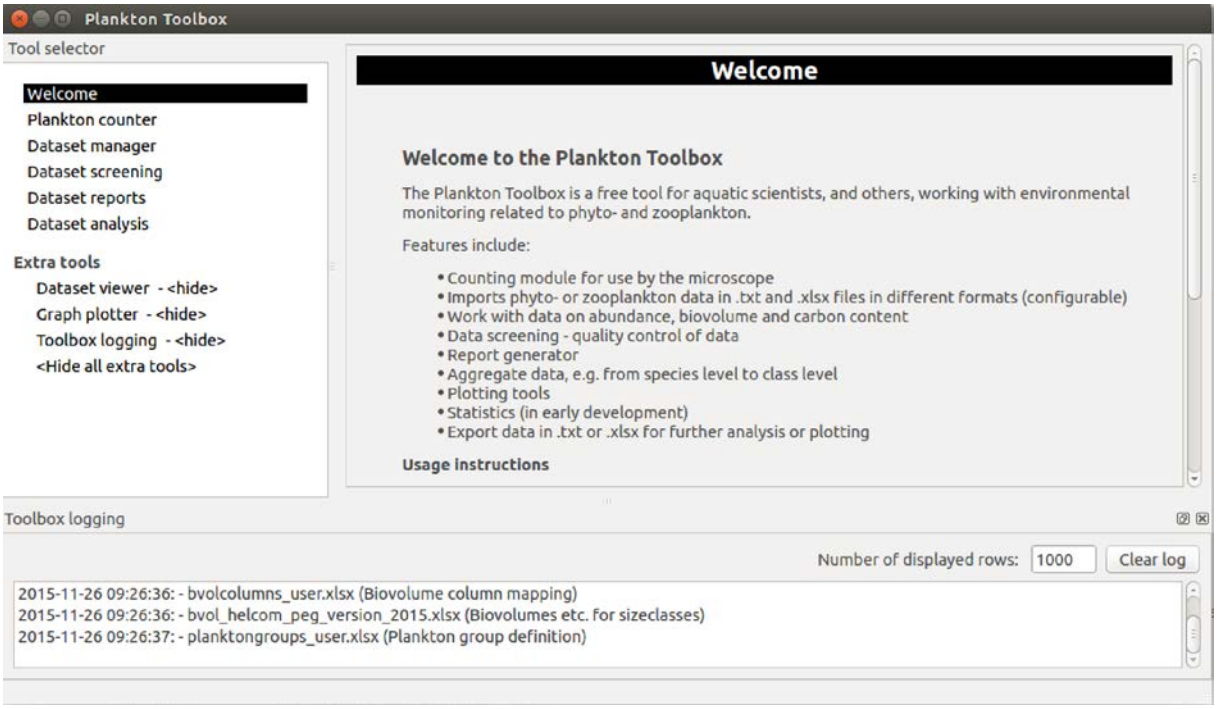



Tearing off the *Toolbox logging* window pane to a separate window




Docking the *Toolbox logging* window pane below the main window pane.

# Welcome




The ptbx welcome page. Text and links will guide you to, hopefully, useful information.

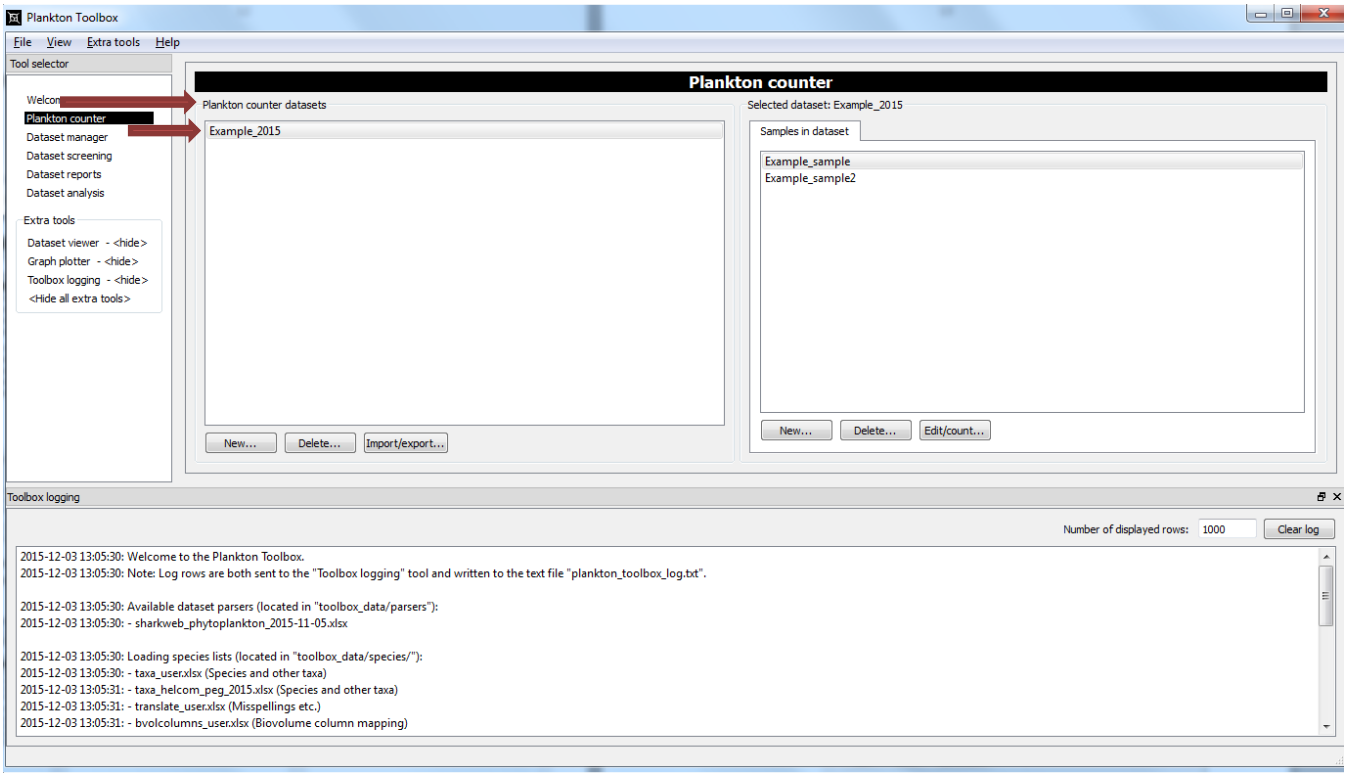
## Getting started with Plankton counter

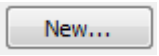
To get started counting a plankton sample you need the following information:

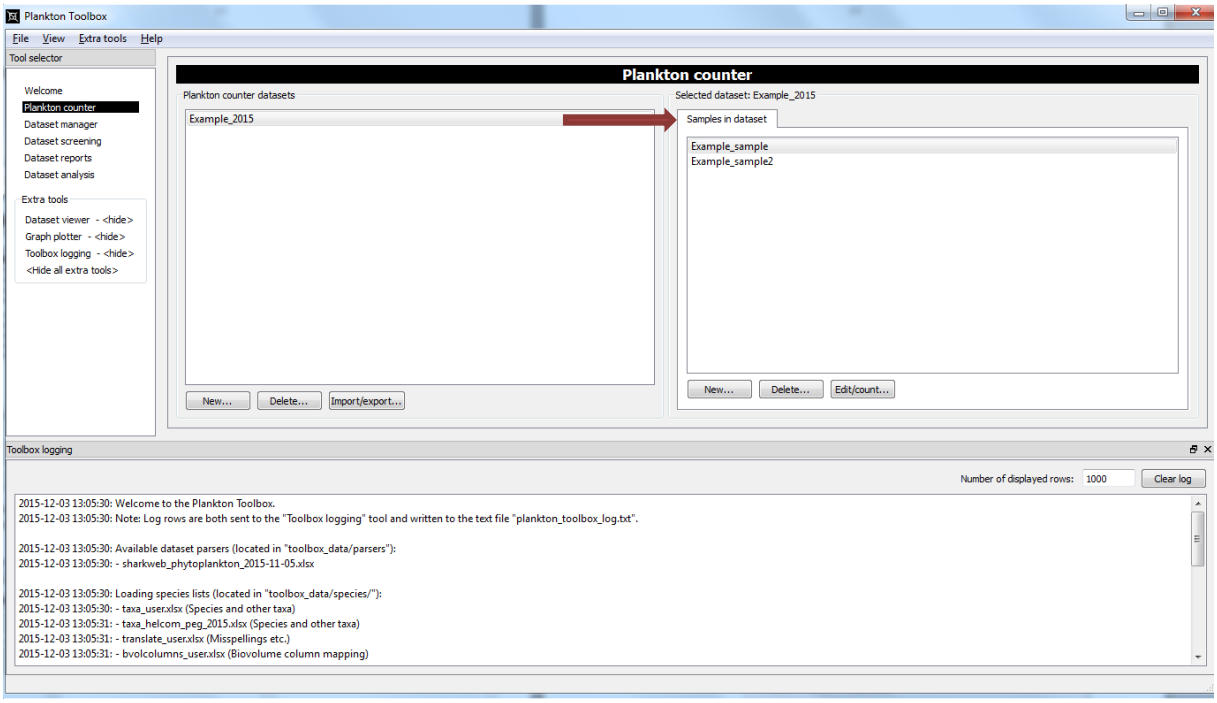
1. Meta data about the sample, where it was collected, when, how it was preserved etc.
    a. You may want to save the data as a *a template sample*
2. A list of taxa that you expect to find in the sample, A useful list for the Baltic Sea area is provided (see managing species lists for details)
3. Information on your counting device e.g.
    a. Volume of sedimentation chamber or filtered volume, e.g. 20 mL
    b. The diameter of sedimentation chamber or filtered area, e.g. 26 mm
4. Information on your microscope. You may need to use a stage micrometer ( a small ruler) to check the diameter of the field of view in your microscope at a certain magnification. The information may look like this:
        i. 5x objective xx mm
        ii. 10x objective yy mm
        iii. 20x objective zz mm
        iv. 40x objective åå mm
        v. 100x objective ää mm
5. Information on sample volume and the volume of preservative added is needed to calculate the dilution of the sample that is a result of adding preservative.
6. The next thing to do is to set up a *method* that suits your work. You may want to set up methods for different magnifications and for different counting styles. Some examples:
    a. 5x whole chamber
    b. 10x whole chamber
    c. 20x transect counting (=counting diameters)
    d. 40x transect counting (=counting diameters)
    e. 100x field of view counting (useful for counting autotorophic picoplankton in the fluorescence microscope)
7. Start counting!

## Plankton counter

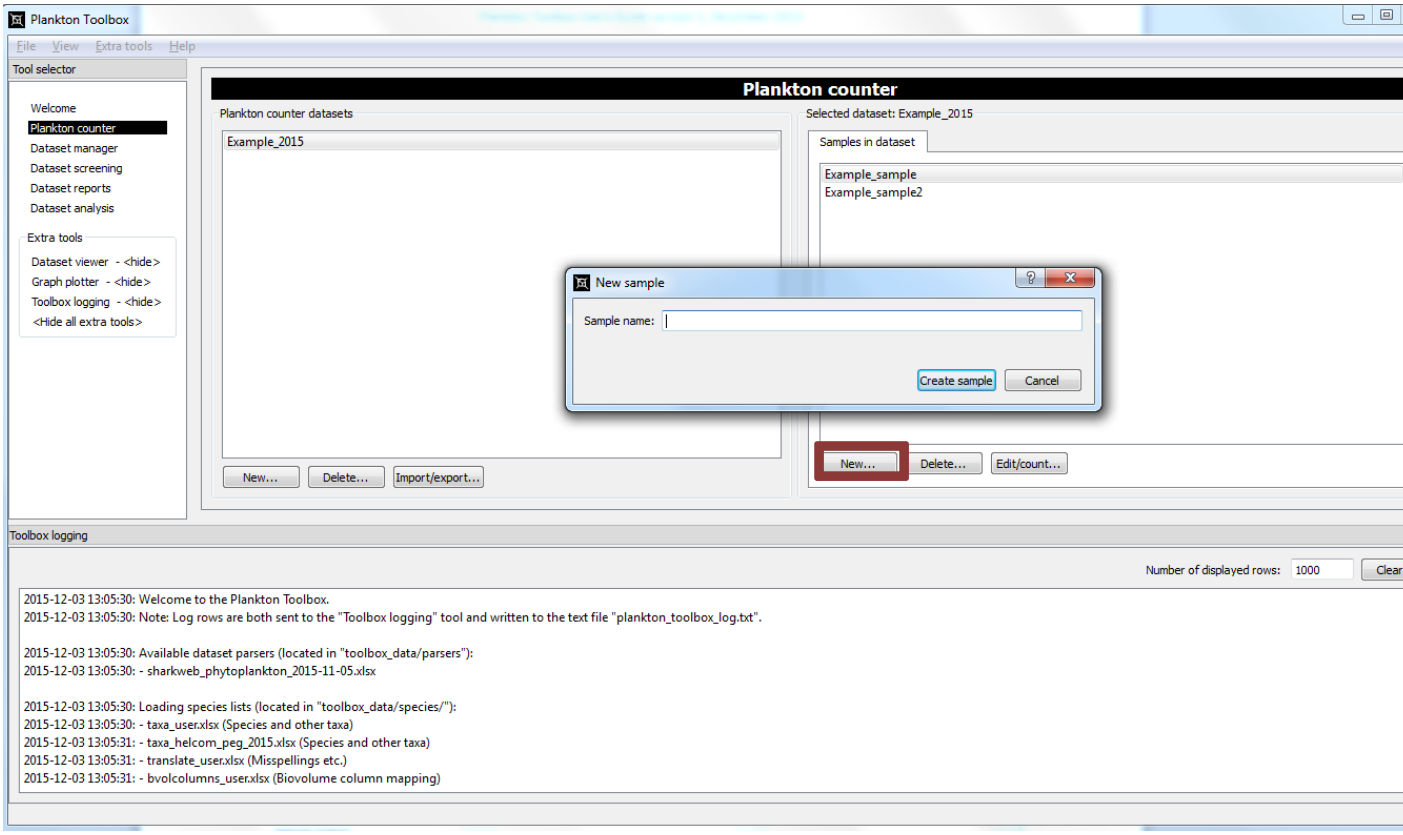
The plankton counter is a tool for counting zooplankton or phytoplankton samples. The module enables you to record your results while analysing.




Choose one of the existing Plankton counter datasets or create a new one by clicking  and naming the dataset. In the dataset several samples may be added (samples are added in the right window), so if you have a program for a whole year, you may add all dates and depths analysed.

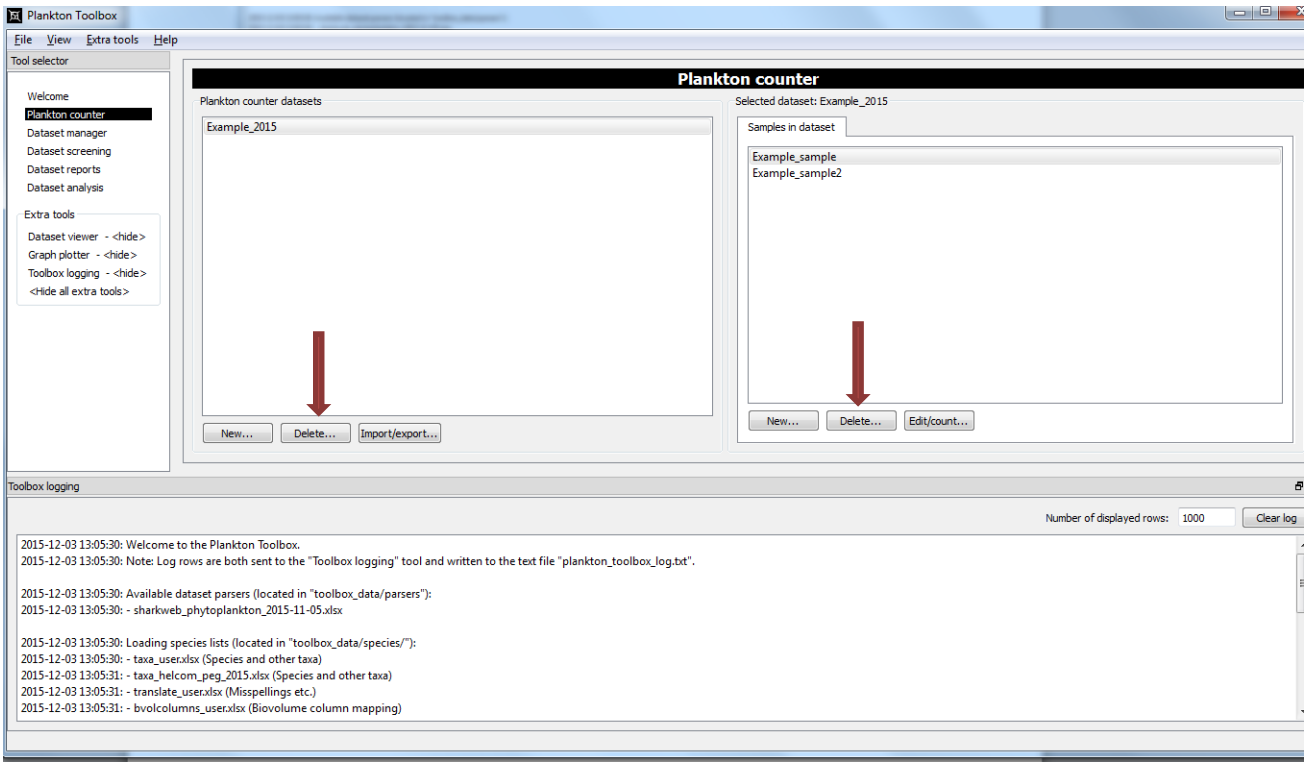


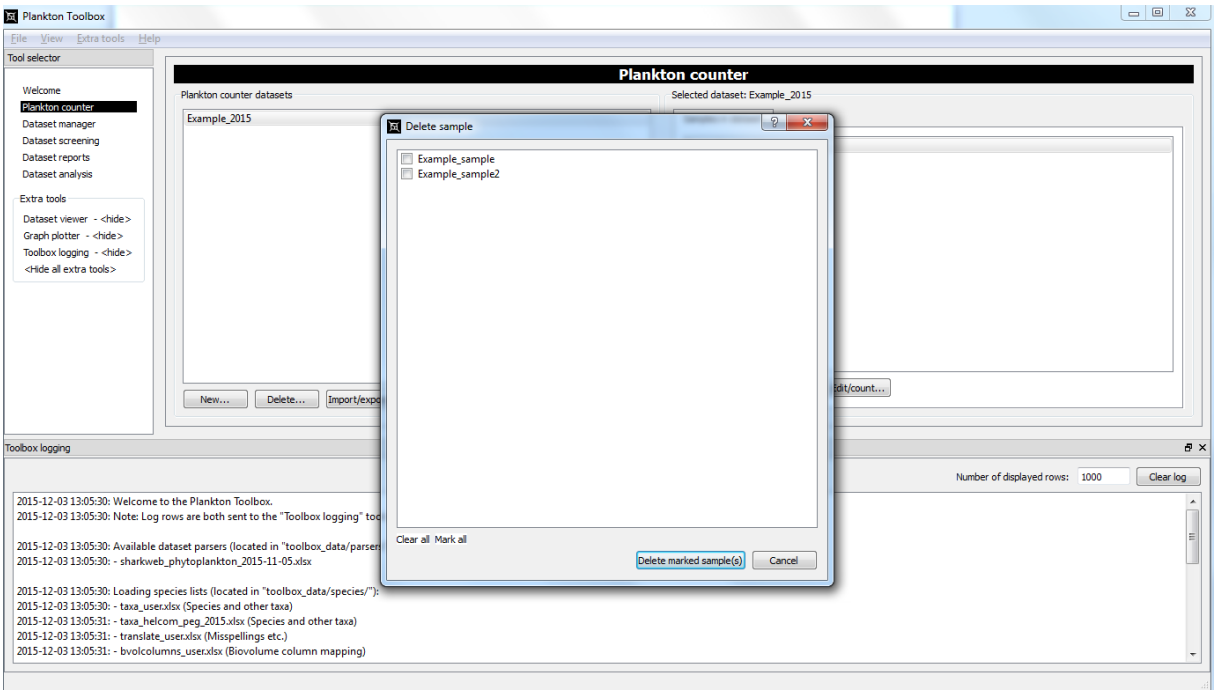
Choose one of the existing “Samples in dataset” or…



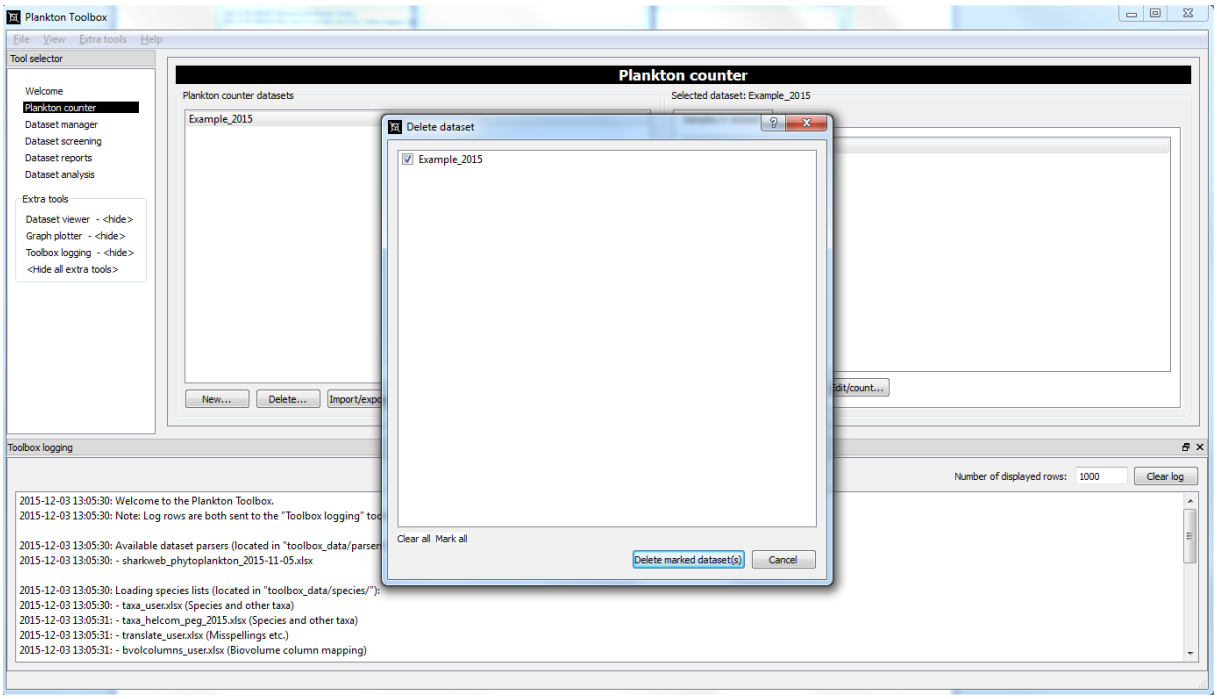

…create a new sample.

The delete buttons make it easy to delete an unwanted sample or dataset:

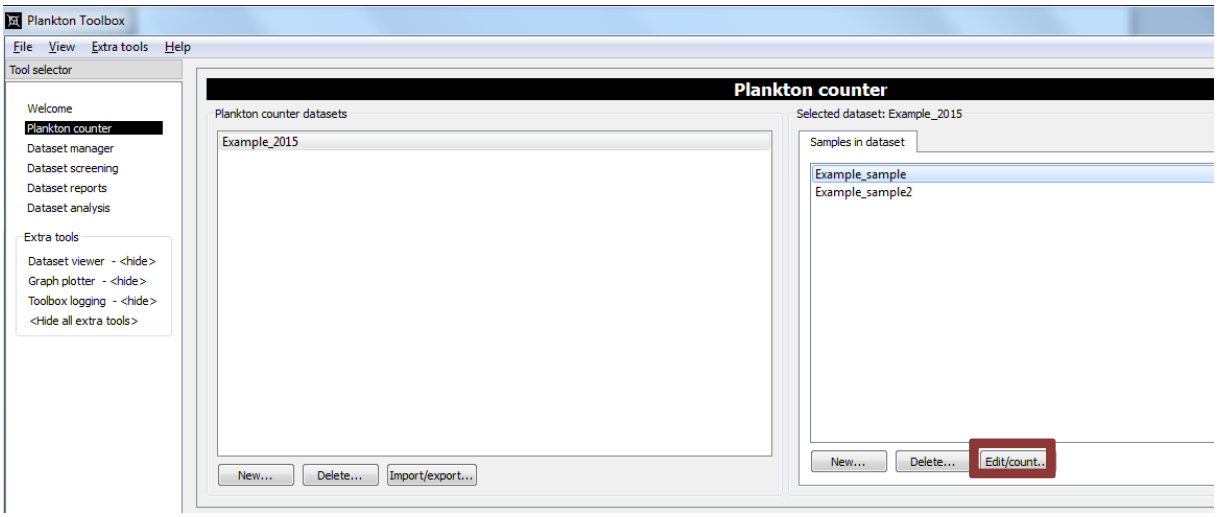





Delete sample.

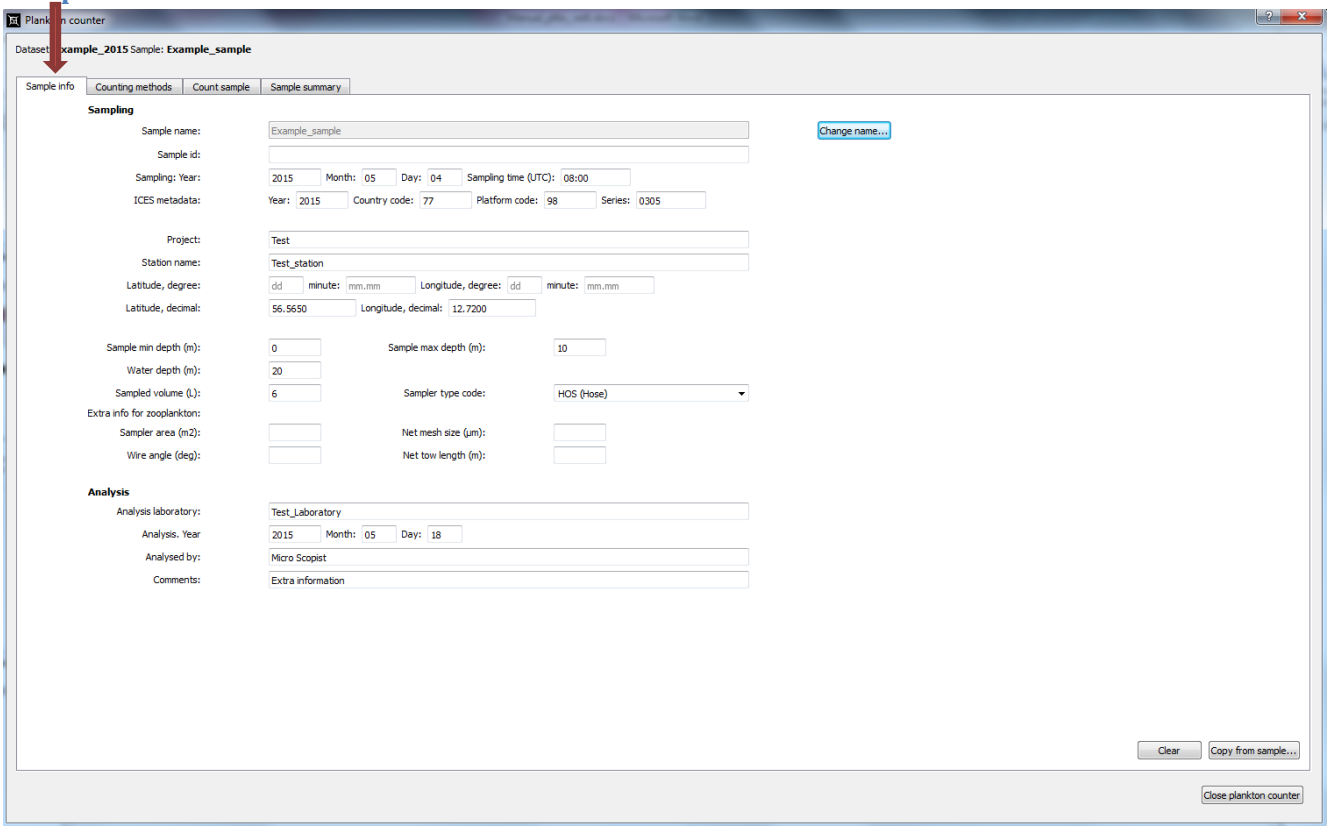



Delete dataset.




To enter an existing or new count, click the Edit/count button or double click the selected dataset.

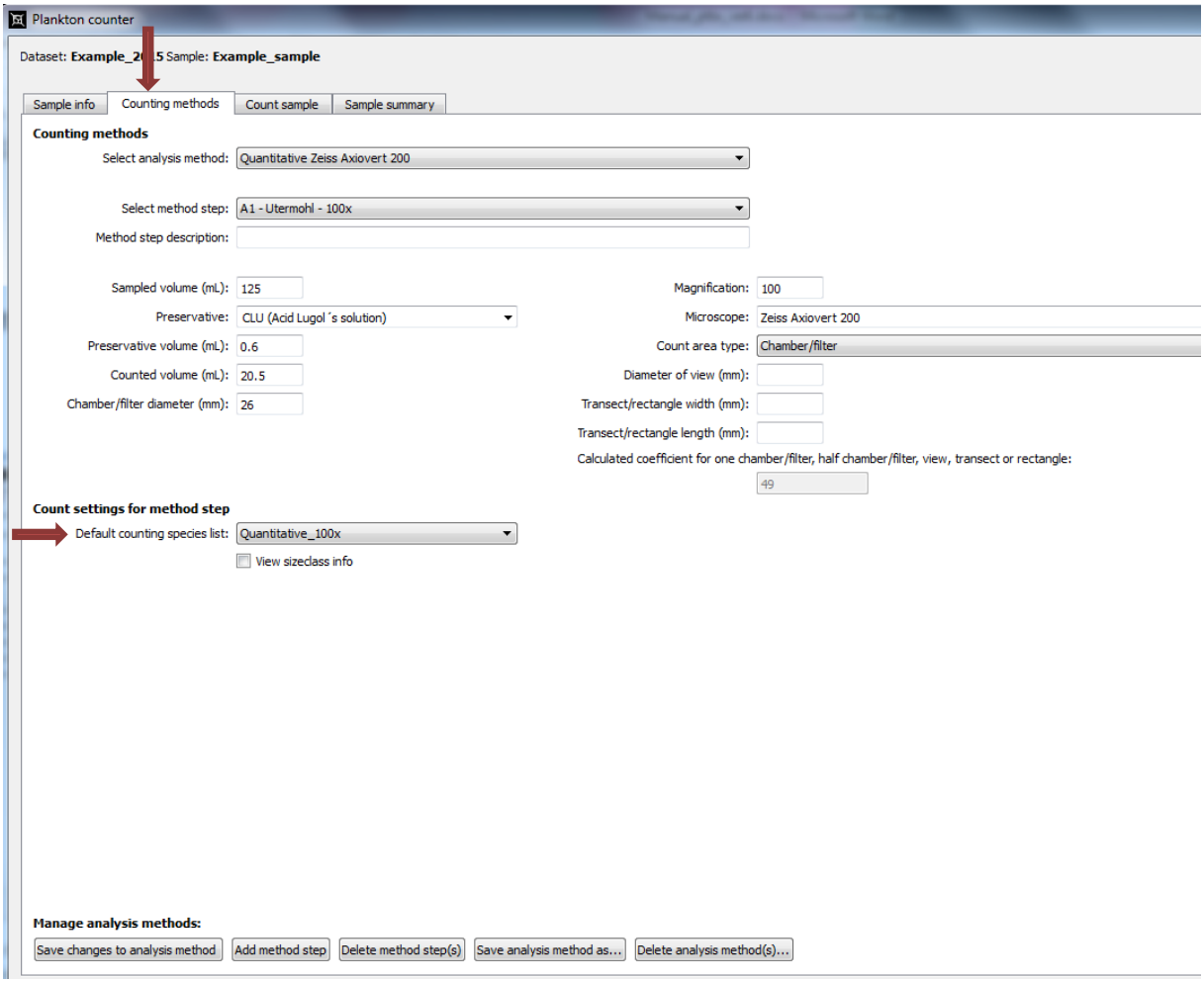
## Sample info



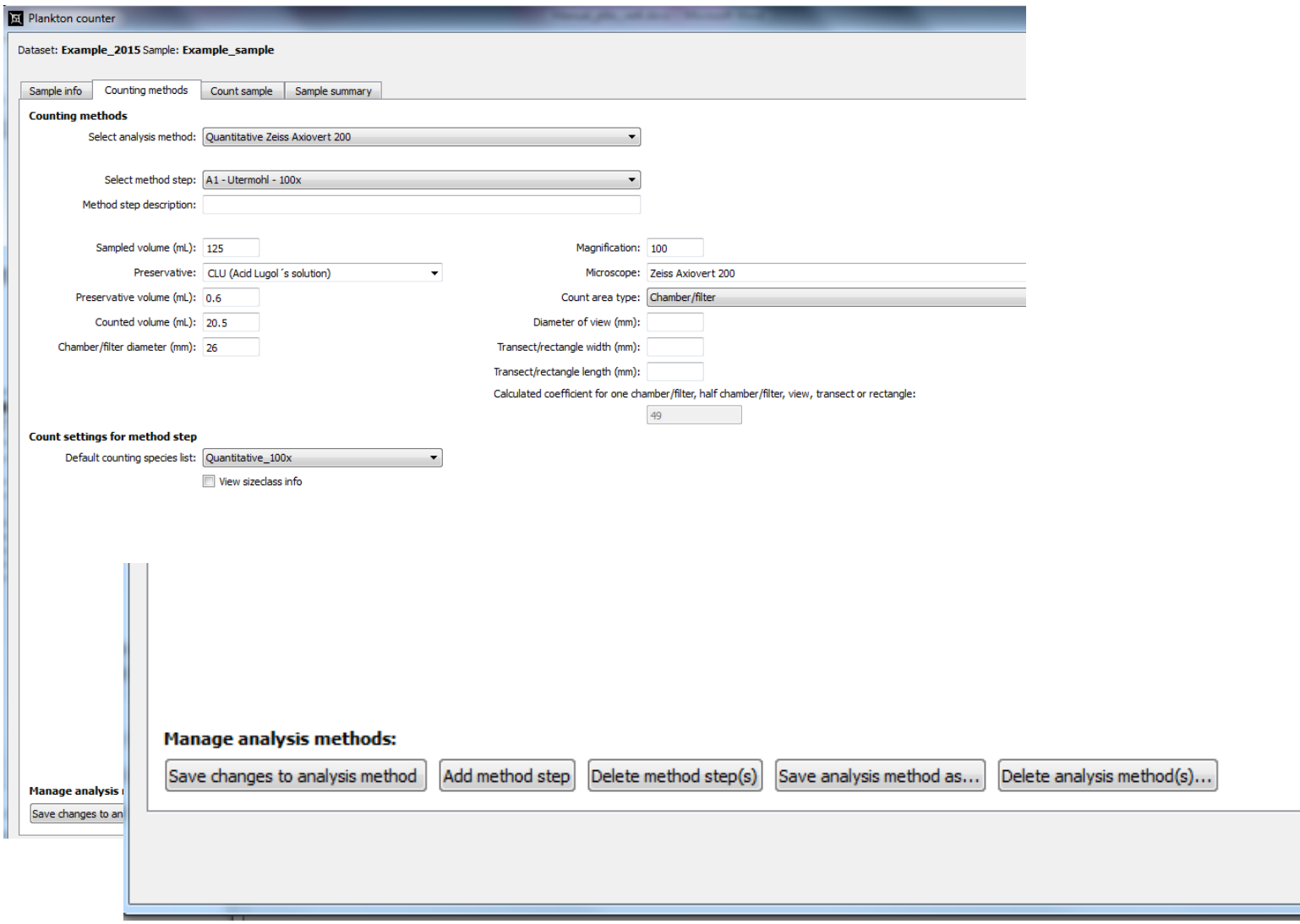

Fill in all relevant info about this sample. If you have a template already, just click 

and find the template from an earlier count/sample. The information is automatically saved and you can proceed to Counting methods.

## Counting methods

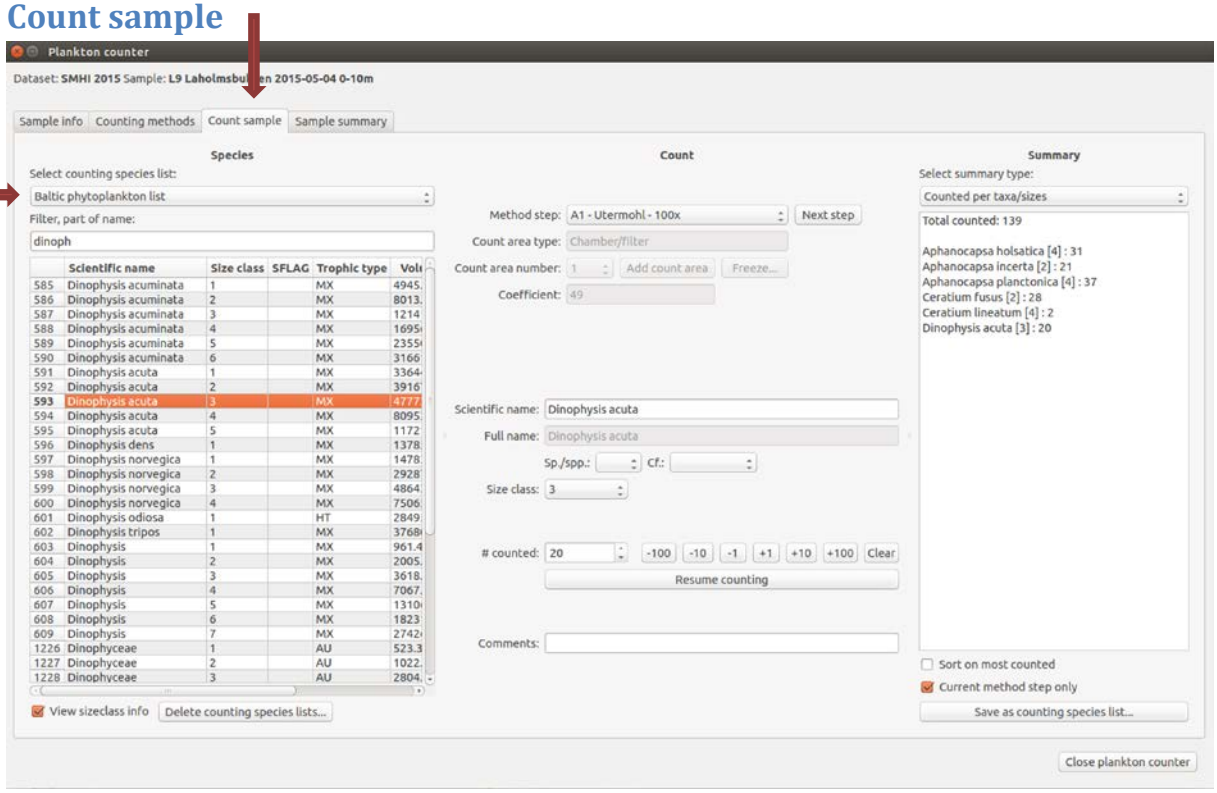



Fill in/choose the relevant info. Choose species list for the count (Default counting species list), mark the “view sizeclass info” if this is an analysis including biovolumes. All cells may be filled in with information of your own choice, even the drop down menu.




If you want to custom make analysis method and method steps, these are the functions to use. Add Add method step or Delete method step(s), then click Save analysis method as... and choose a name, we suggest to add the microscope used.

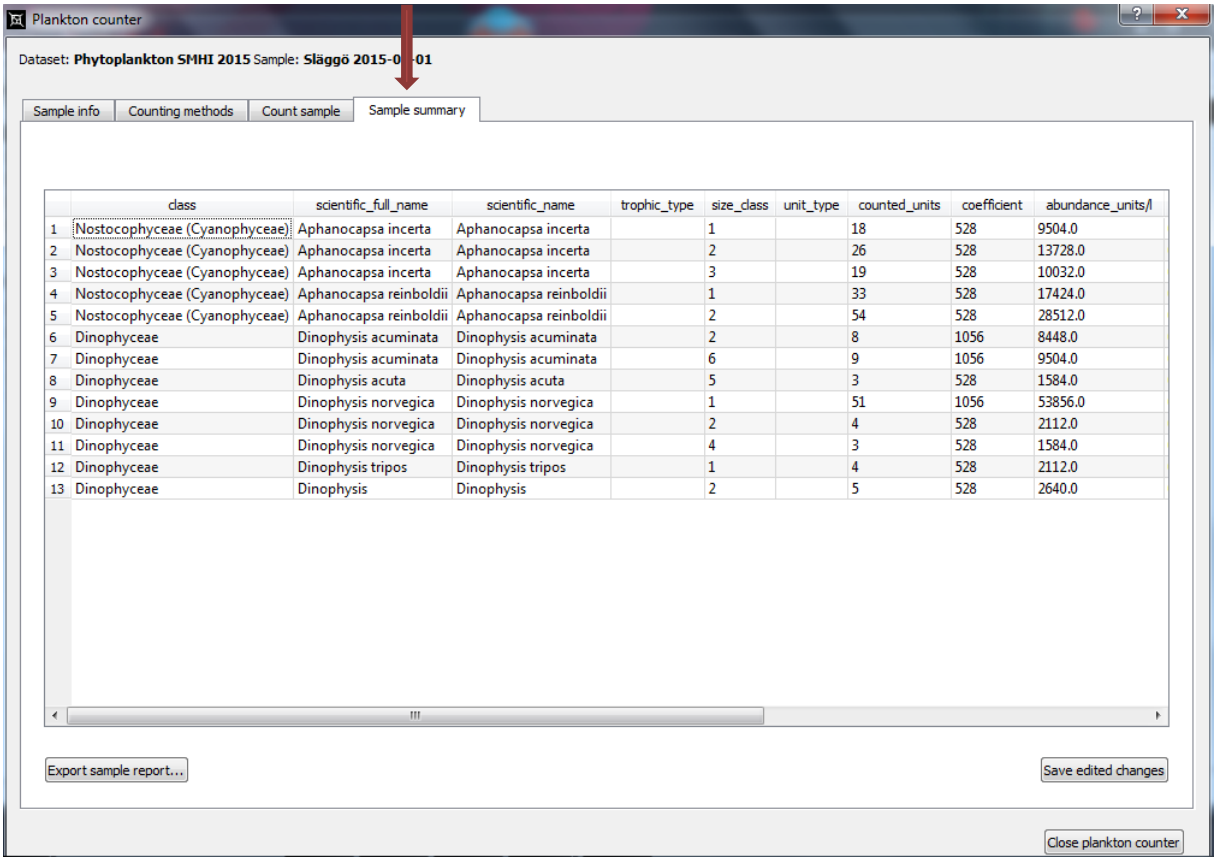
## Count sample




Now you can start your count. Select species list. Find the species you want to count in the species list (left column). Count by clicking the space bar or by marking the buttons on the screen. The species you count end up in the right column, where you can mark species already in the existing count. You can also save the right hand list as a template for later counts. If you want to create a counting species list before you start, you can create a .txt-file and save in the following folder:

Plankton Toolbox -> toolbox_data\plankton_counter\config\counting_species_lists

## Sample summary

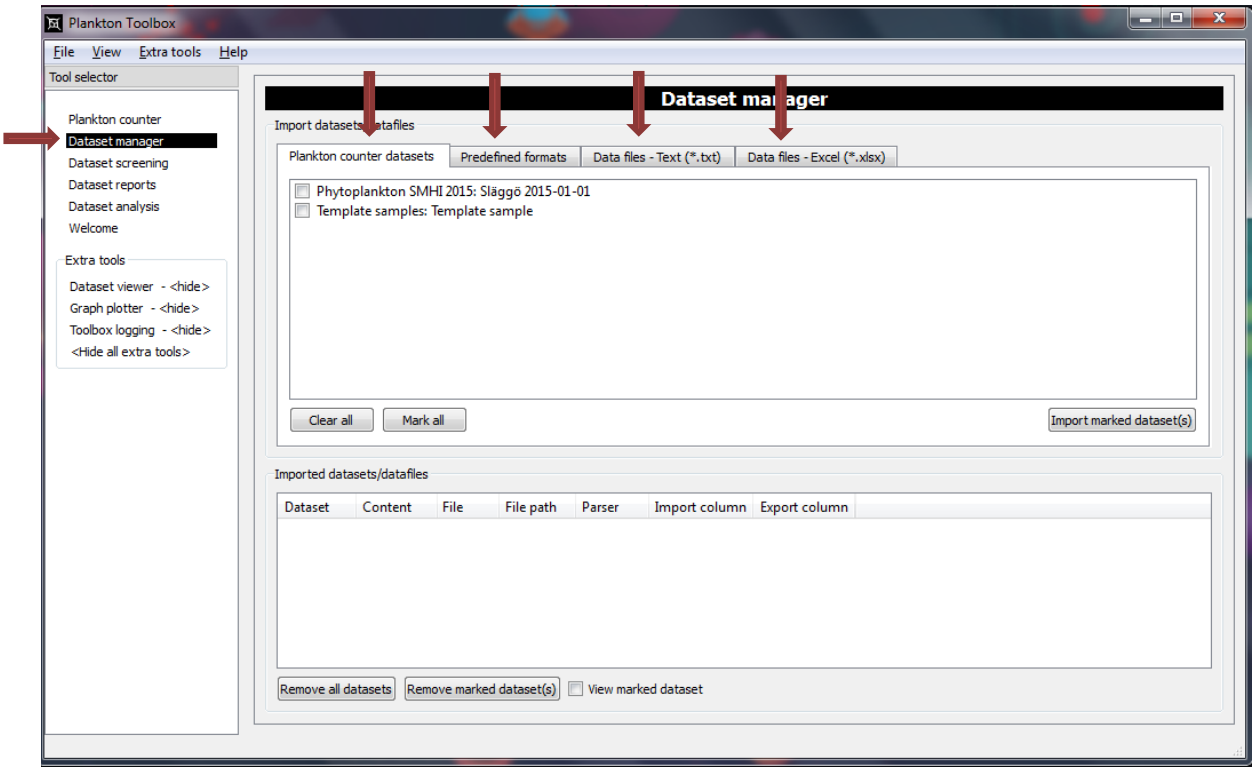



| | class | scientific_full_name | scientific_name | trophic_type | size_class | unit_type | counted_units | coefficient | abundance_units/l |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Nostocophyceae (Cyanophyceae) | Aphanocapsa incerta | Aphanocapsa incerta | | 1 | | 18 | 528 | 9504.0 |
| 2 | Nostocophyceae (Cyanophyceae) | Aphanocapsa incerta | Aphanocapsa incerta | | 2 | | 26 | 528 | 13728.0 |
| 3 | Nostocophyceae (Cyanophyceae) | Aphanocapsa incerta | Aphanocapsa incerta | | 3 | | 19 | 528 | 10032.0 |
| 4 | Nostocophyceae (Cyanophyceae) | Aphanocapsa reinboldii | Aphanocapsa reinboldii | | 1 | | 33 | 528 | 17424.0 |
| 5 | Nostocophyceae (Cyanophyceae) | Aphanocapsa reinboldii | Aphanocapsa reinboldii | | 2 | | 54 | 528 | 28512.0 |
| 6 | Dinophyceae | Dinophysis acuminata | Dinophysis acuminata | | 2 | | 8 | 1056 | 8448.0 |
| 7 | Dinophyceae | Dinophysis acuminata | Dinophysis acuminata | | 6 | | 9 | 1056 | 9504.0 |
| 8 | Dinophyceae | Dinophysis acuta | Dinophysis acuta | | 5 | | 3 | 528 | 1584.0 |
| 9 | Dinophyceae | Dinophysis norvegica | Dinophysis norvegica | | 1 | | 51 | 1056 | 53856.0 |
| 10 | Dinophyceae | Dinophysis norvegica | Dinophysis norvegica | | 2 | | 4 | 528 | 2112.0 |
| 11 | Dinophyceae | Dinophysis norvegica | Dinophysis norvegica | | 4 | | 3 | 528 | 1584.0 |
| 12 | Dinophyceae | Dinophysis tripos | Dinophysis tripos | | 1 | | 4 | 528 | 2112.0 |
| 13 | Dinophyceae | Dinophysis | Dinophysis | | 2 | | 5 | 528 | 2640.0 |

In the sample summary you get an overview of your count. If you detect mistakes you may change species or delete entire posts. Click Export sample (.xlsx)... and save your count wherever suitable.

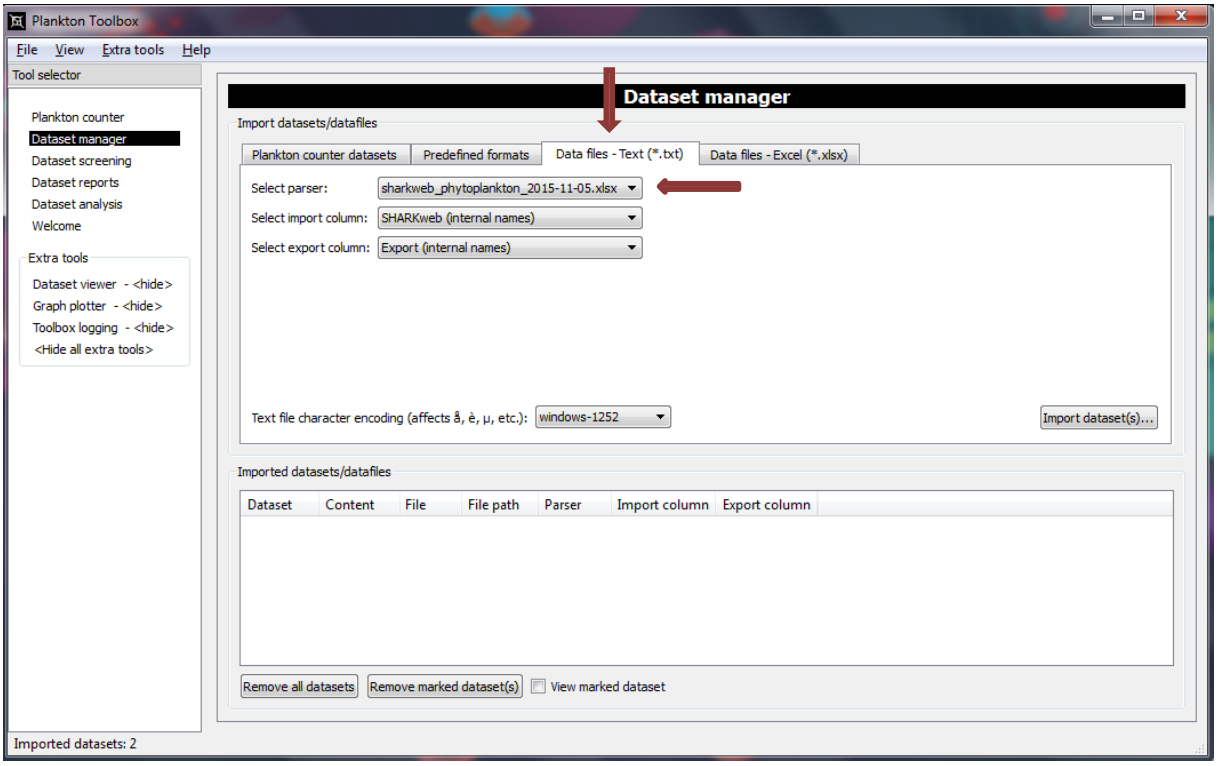
## Dataset manager

The plankton tool box offers a dataset manager where you can import data from different sources to manipulate in several ways.

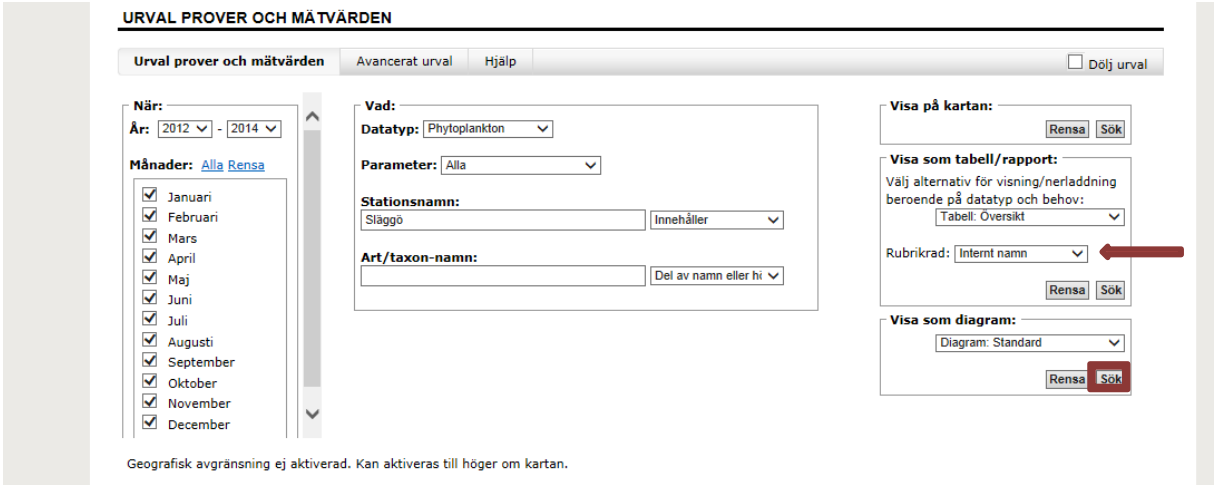



PTBX imports data in text (txt) and Microsoft Excel (xlsx) files in different formats. Since data formatting differs depending on the source of the data importing formats are user configurable through parsers. A useful function is that data from different sources, in different formats, can be combined by importing multiple files. The data can then be exported as one consistent dataset in txt or xlsx format by the user.

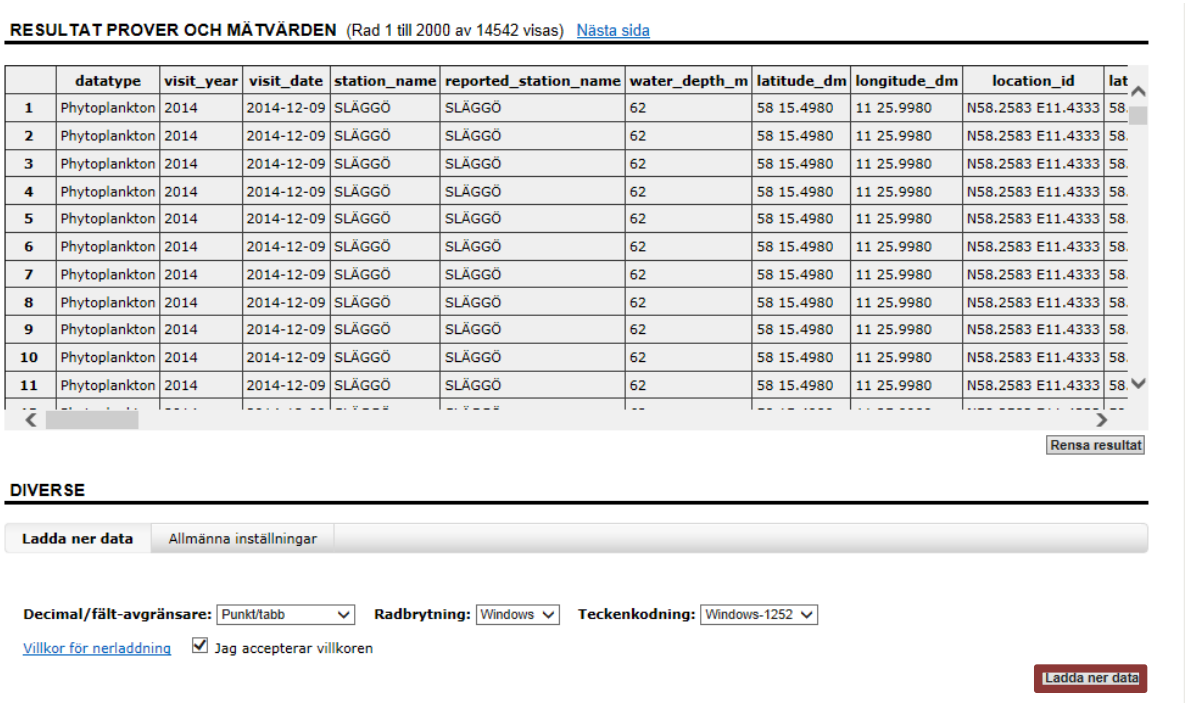
## Data files – Text(*.txt)



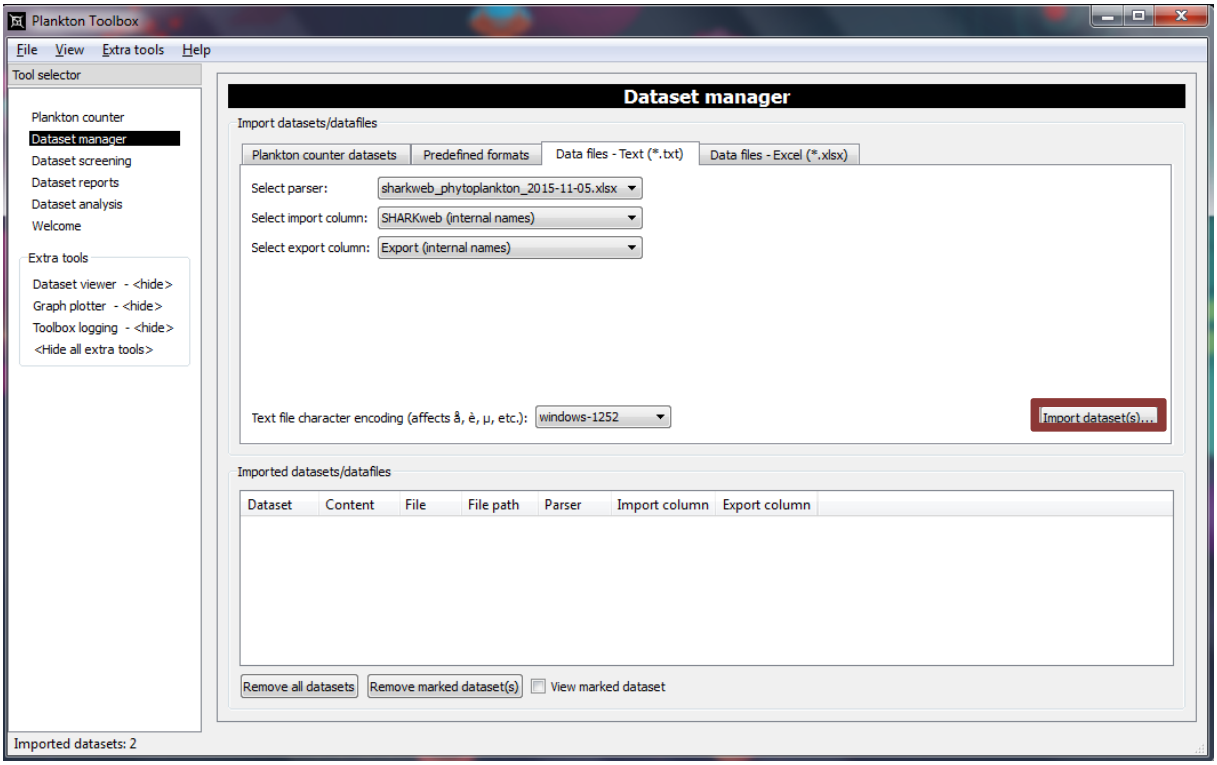

To work with text- or Excel files you may need a custom-made parser which SMHI can help you with. In the text files tab you can for instance manage data downloaded from the Sharkweb, http://www.smhi.se/klimatdata/oceanografi/havsmiljodata/marina-miljoovervakningsdata, unfortunately only available in Swedish still.



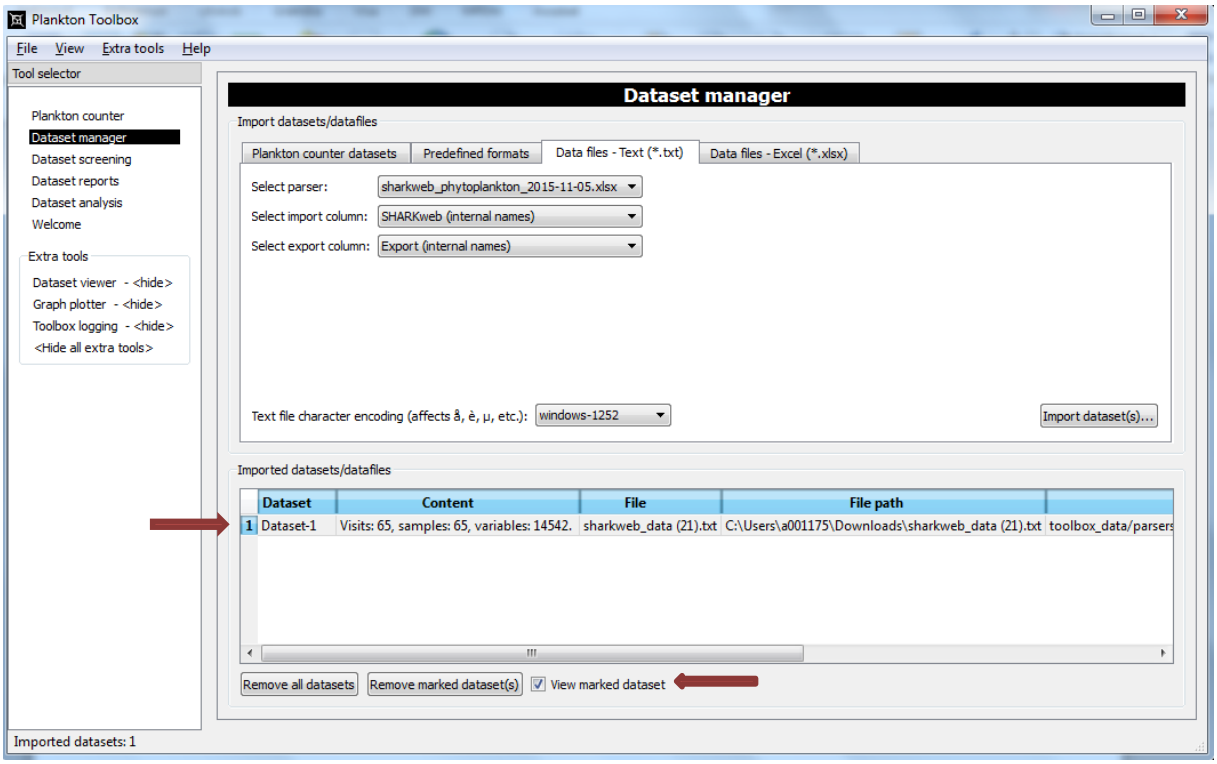

When downloading from the Sharkweb, make sure you choose “internt namn” (internal name), then click “sök” (search).



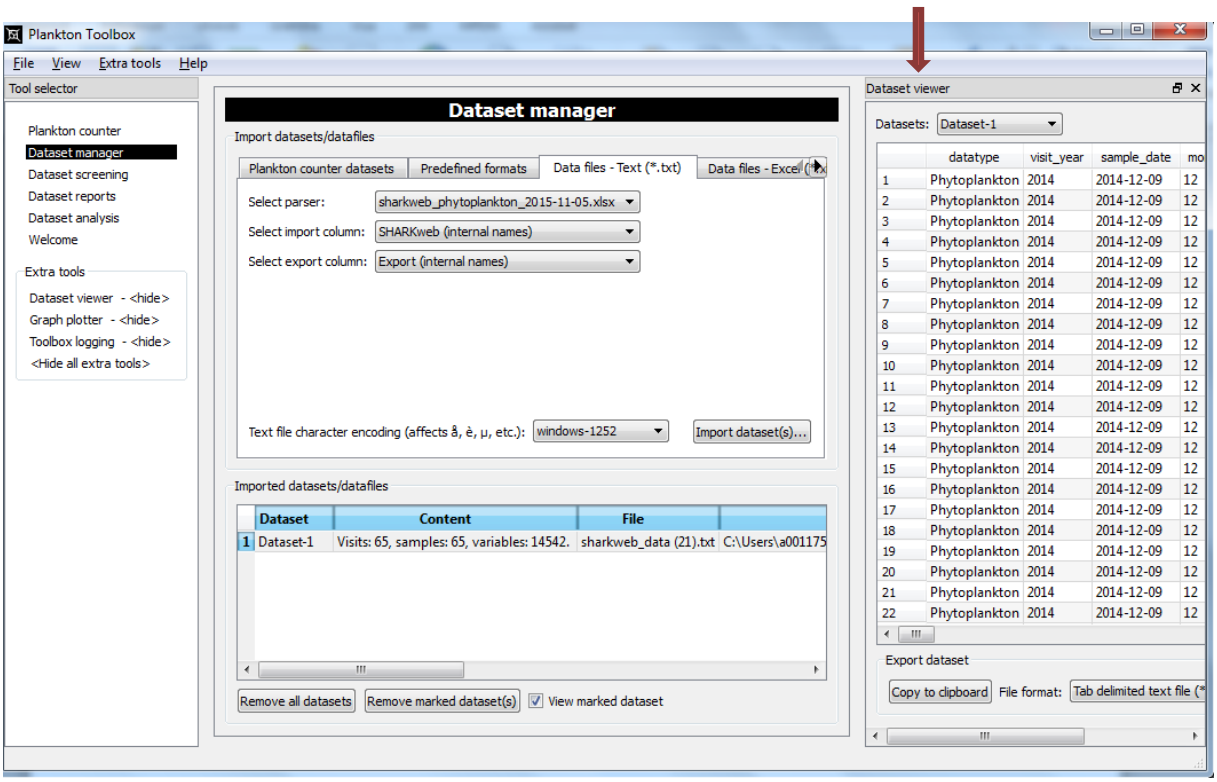

The results will appear, accept the downloading terms (jag accepterar villkoren), and click “ladda ner data” (download). Now your data is ready for the data manager.




Click the import dataset(s) to browse for required file(s).




Mark the data file and if you want a quick look, tick “view marked dataset”, then a window with the data will appear:

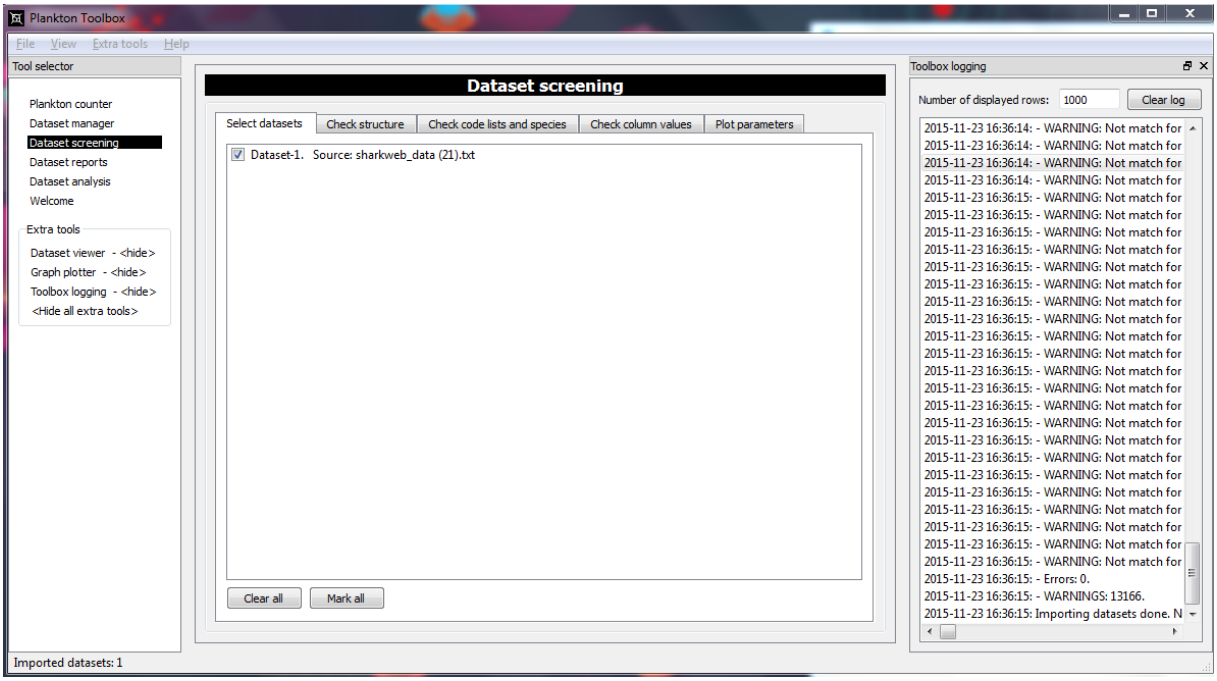



In the viewer, the data shows up, if it is satisfying already, choose file format and save. To hide the viewer, click <hide> in the Extra tools. For further analysis, go to Dataset analysis.

# Dataset screening

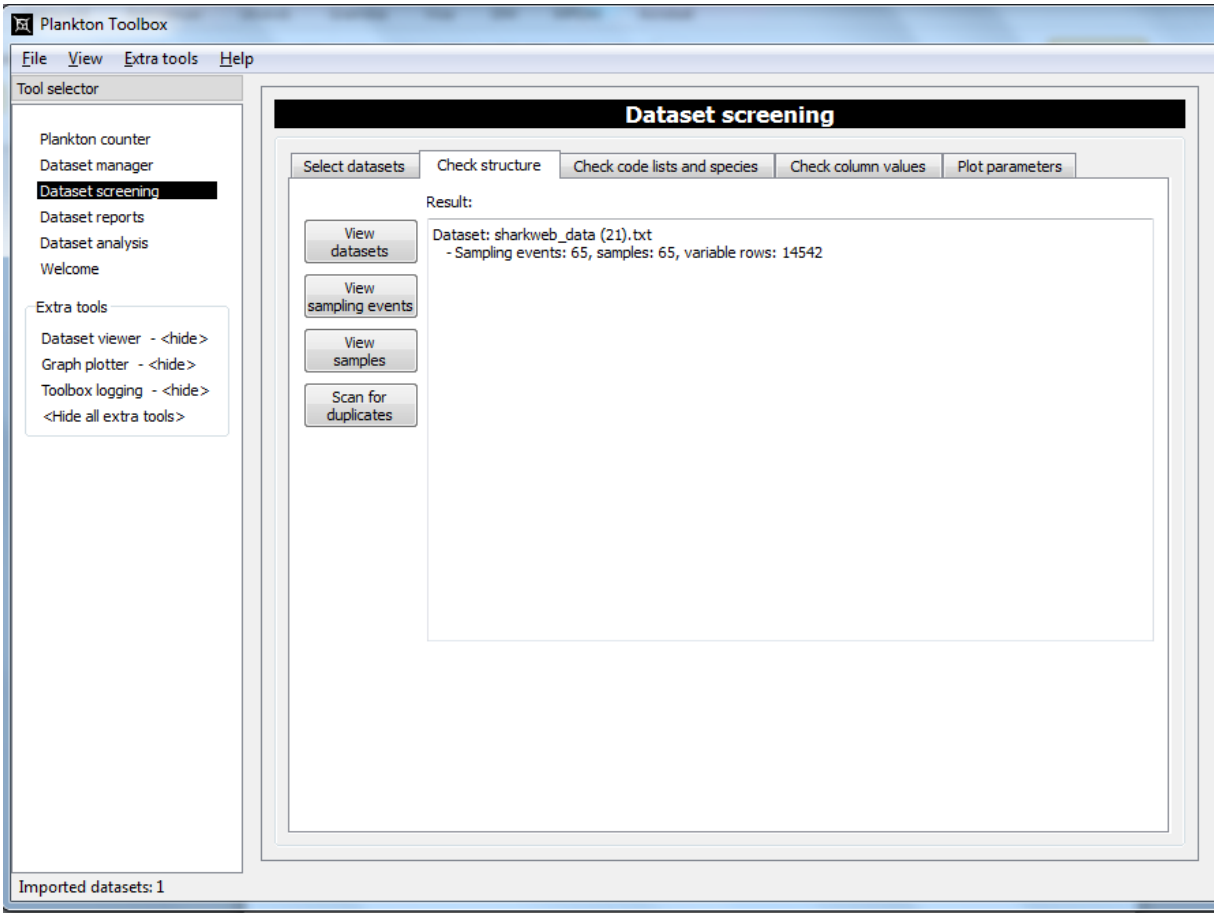
When the data has been imported the raw data can be screened in different ways to check for duplicate data, look for unrealistic dates, positions etc. There are also plotting tools to visualize the raw data.

## Select datasets



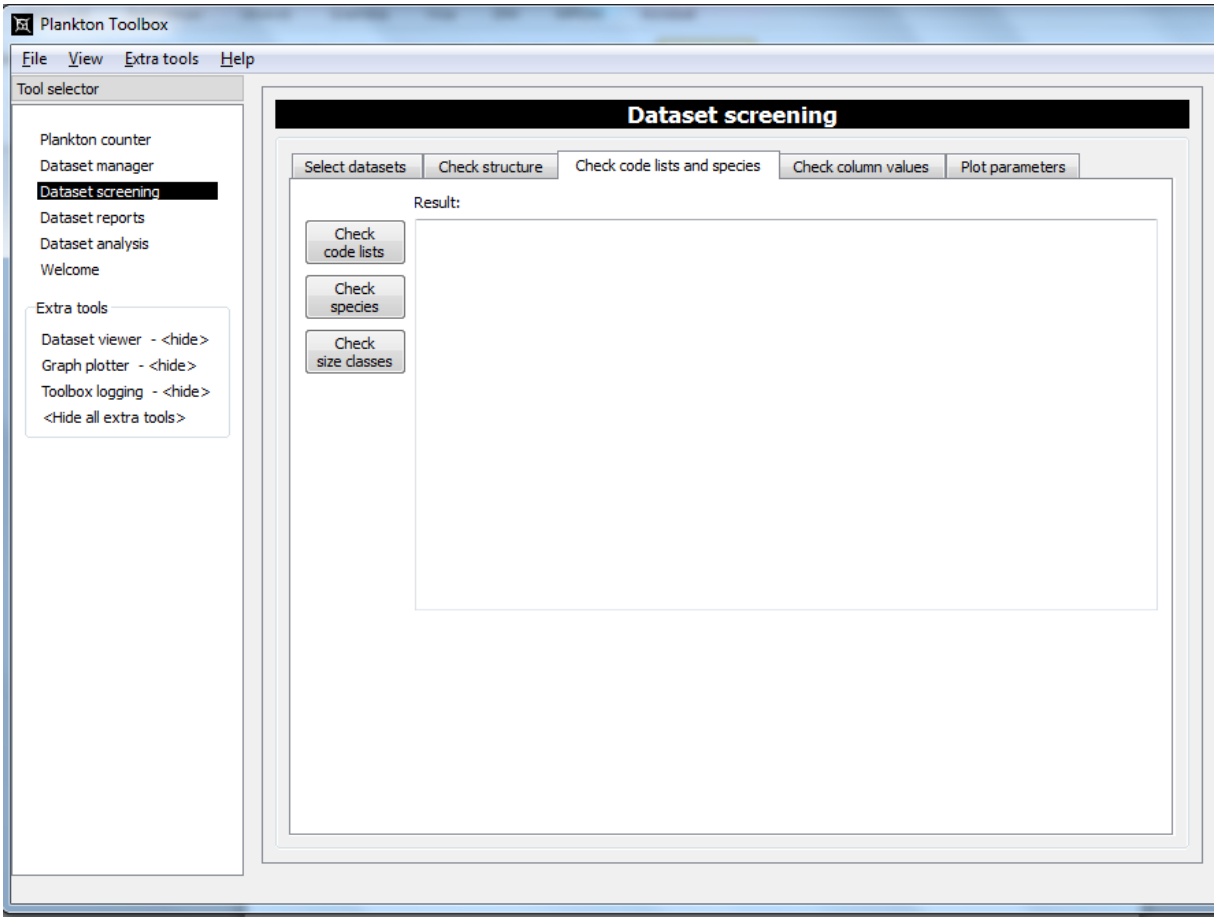

The dataset(s) imported in the data manager can be screened here, mark the one(s) to be screened.

## Check structure

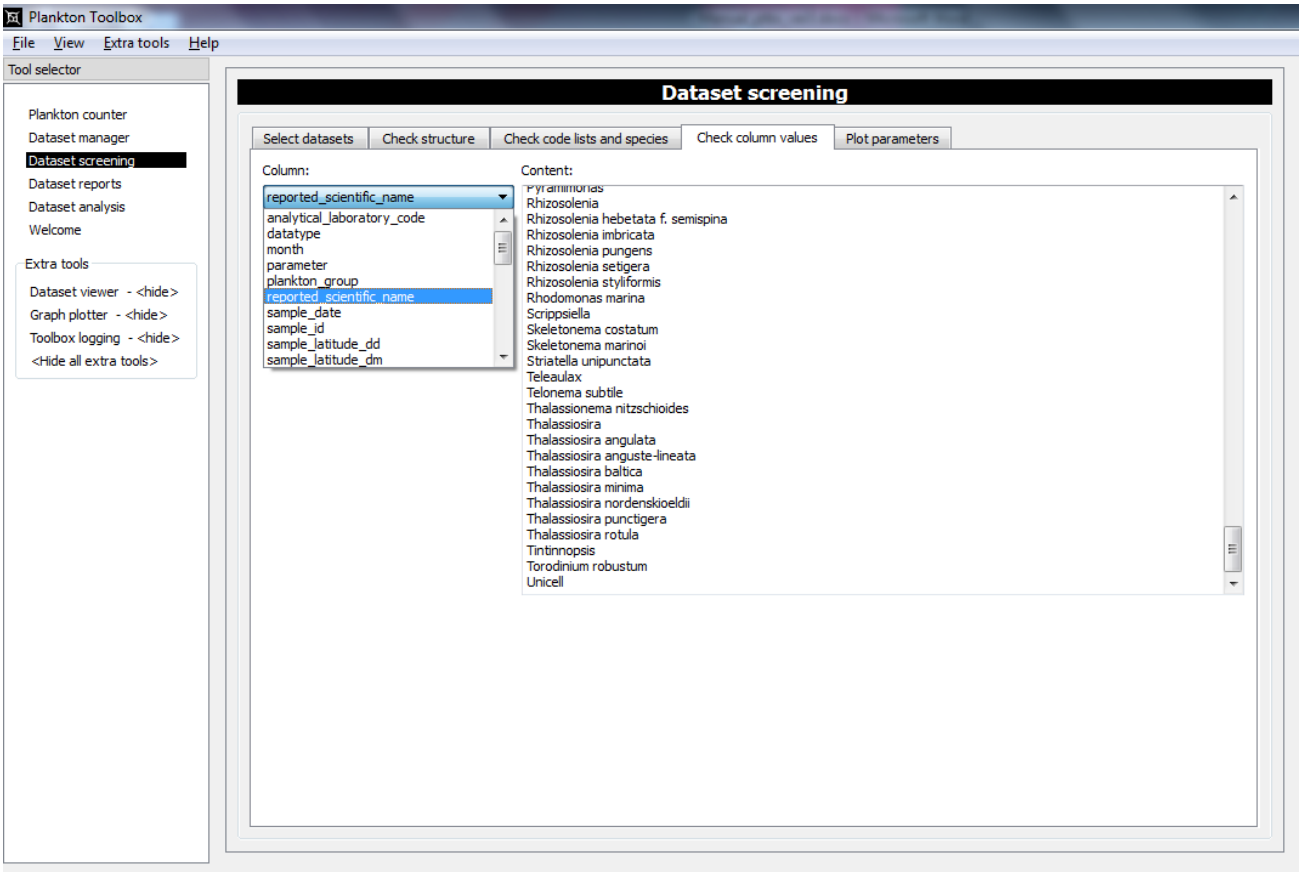



Check the data structure and also get a quality check by clicking View datasets, View sampling events, View samples and Scan for duplicates.

## Check code list and samples

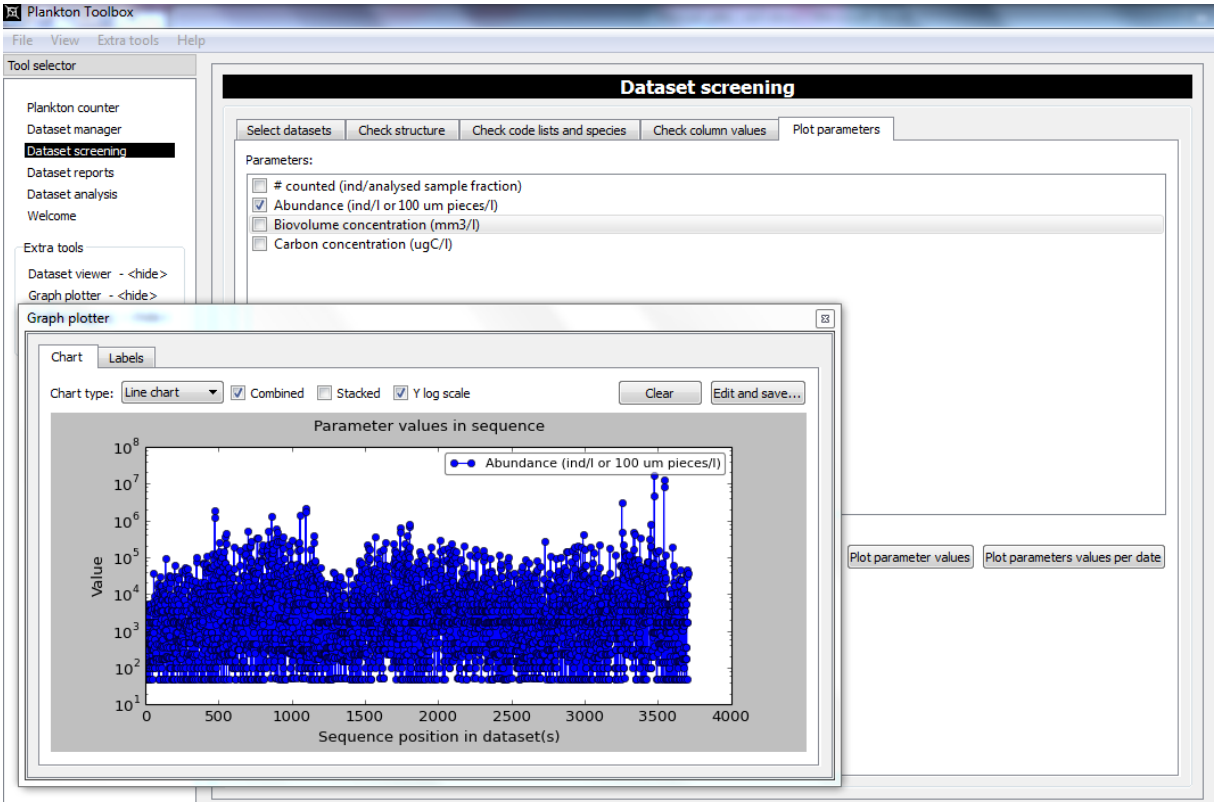


## Check column values



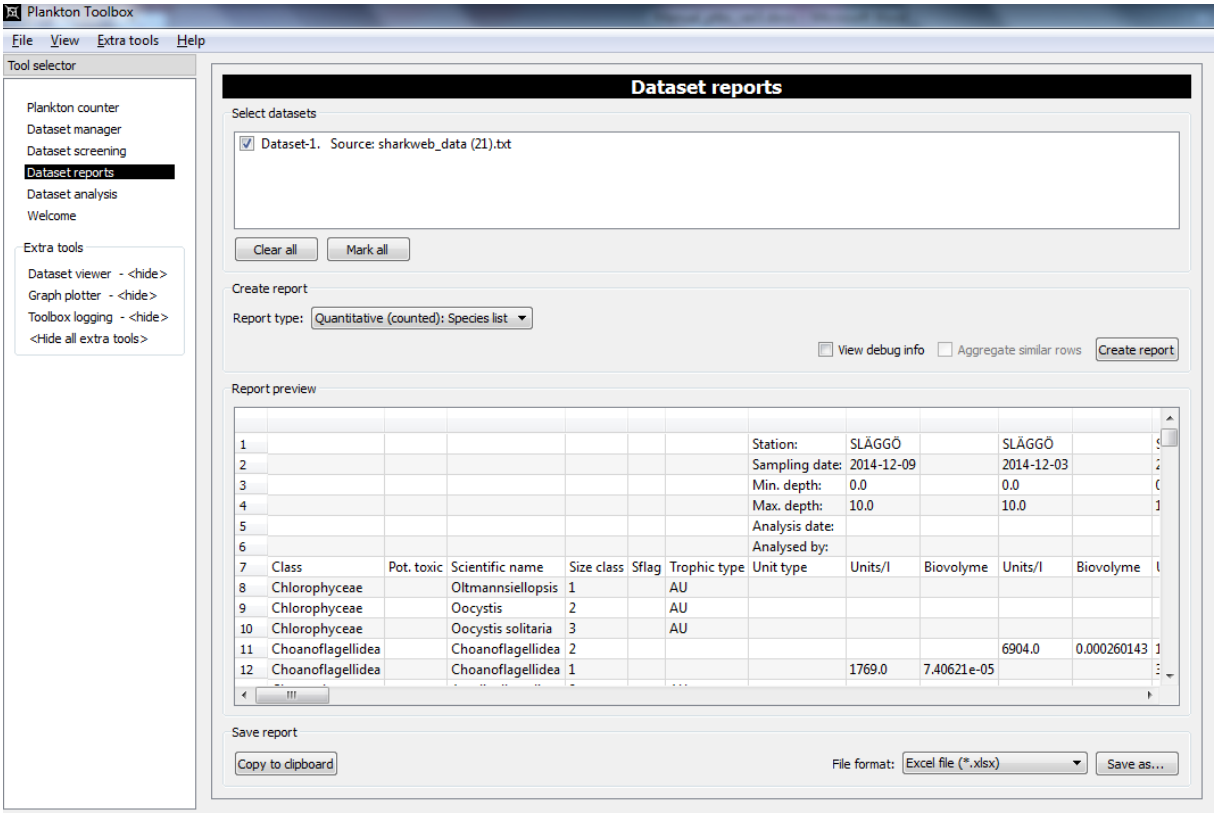

Each column may be marked in the drop down menu and checked.

## Plot parameters



Mark the parameter(s) you want to plot and click either Plot parameter values or Plot parameters values per date.

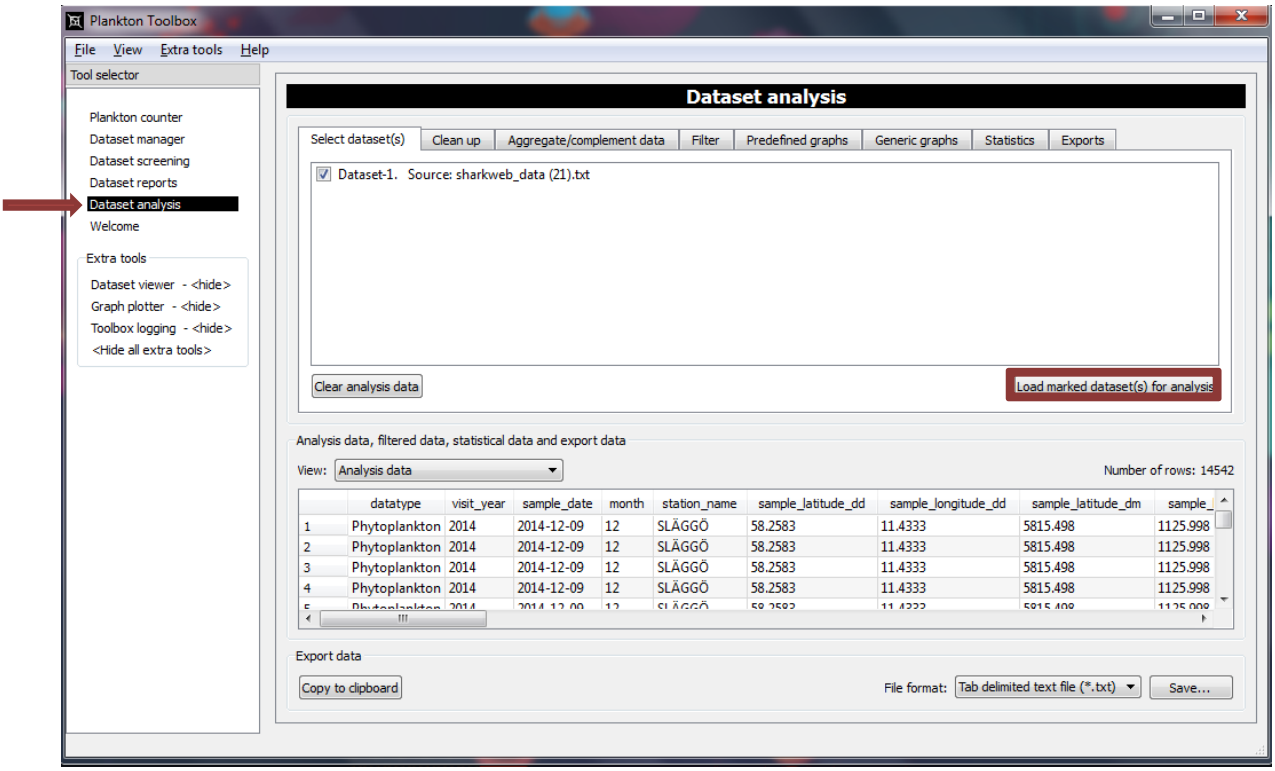
## Dataset reports




A handy way to format a dataset into a data report for delivery, for instance for data host format.

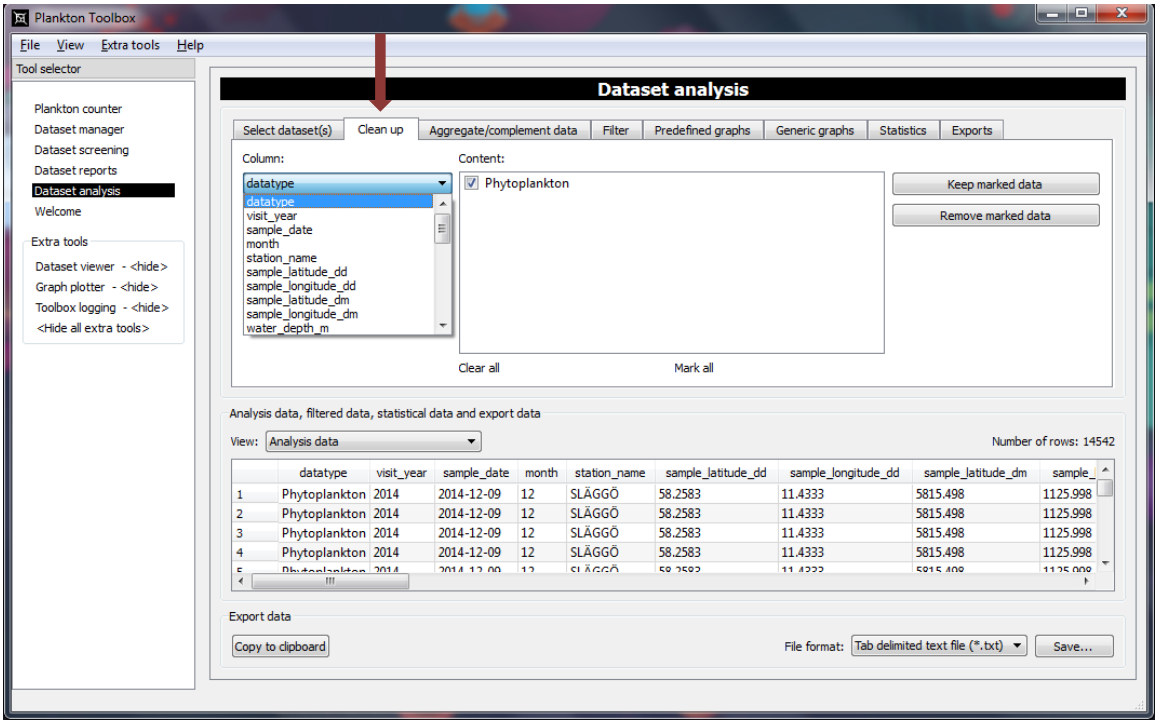
# Dataset analysis

## Select dataset(s)



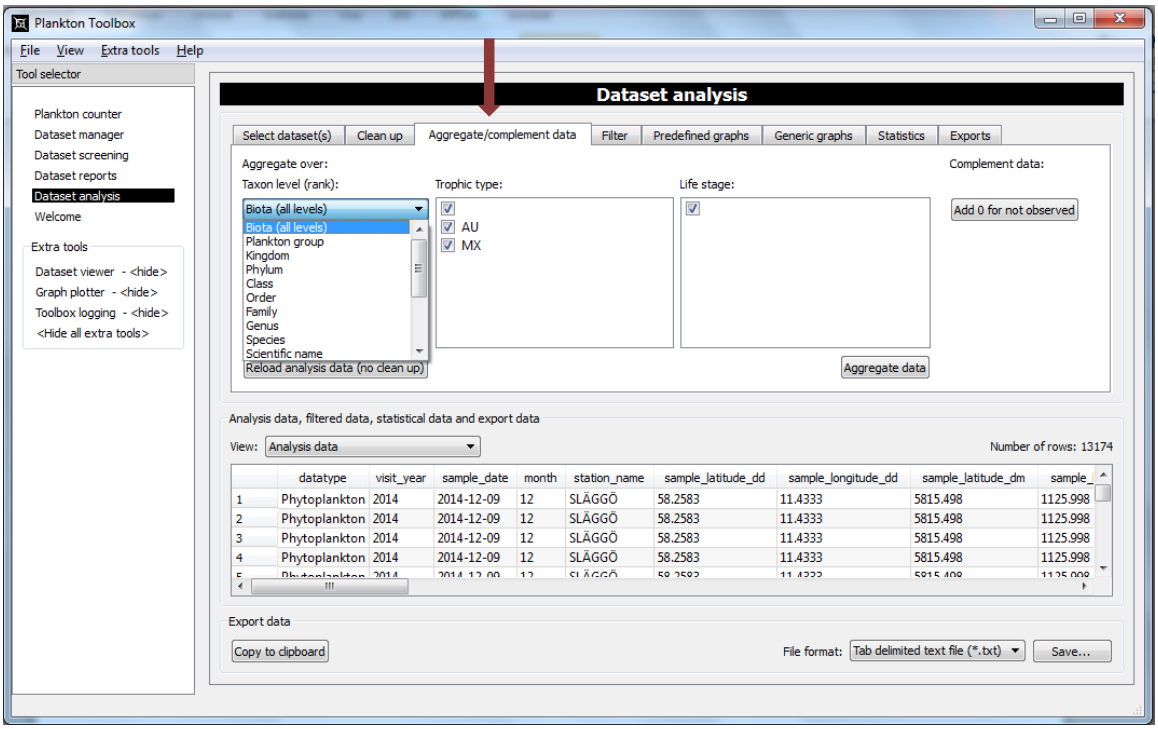

To analyse data, click Load marked dataset(s) for analysis, and the data will appear in the lower window. Observe that the data at any time can be saved in two different ways, either by clicking Copy to clipboard or Save..., remember to choose format.

## Clean up



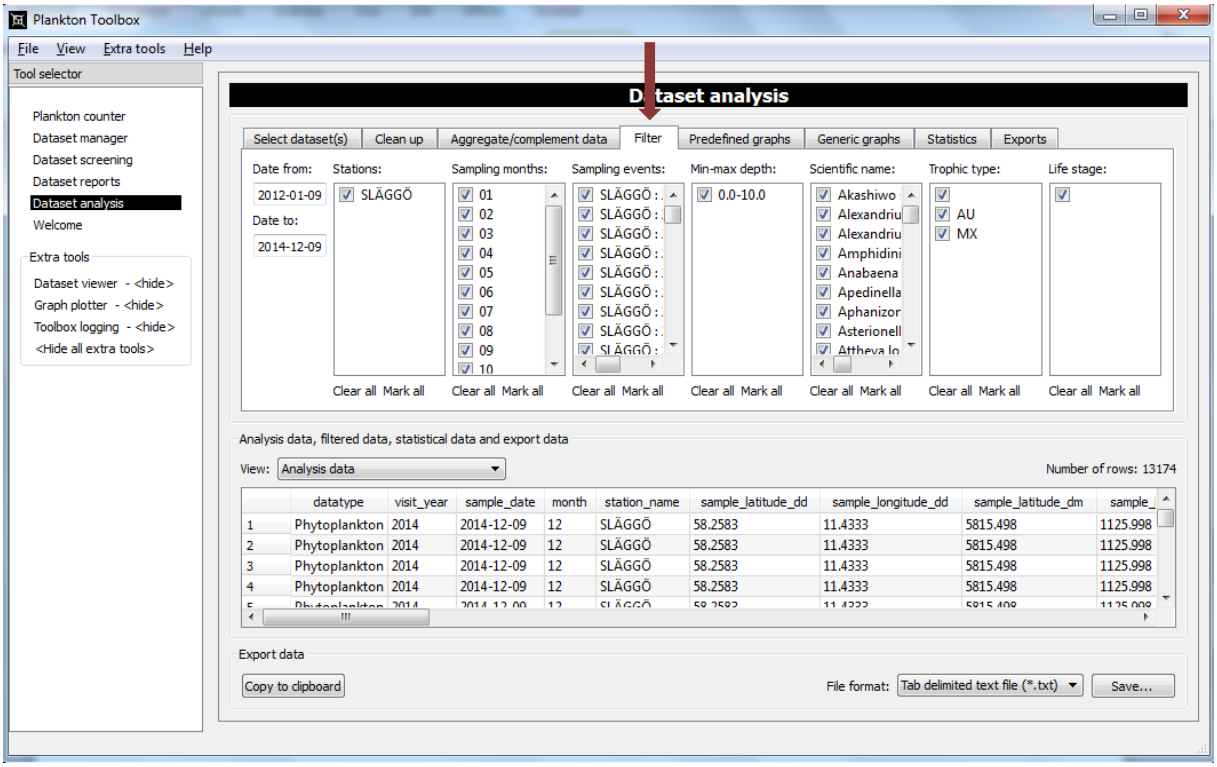
In the “clean up” step you can remove data you do not need, like certain dates, stations, depths, trophic types etc.

## Aggregate/complement data



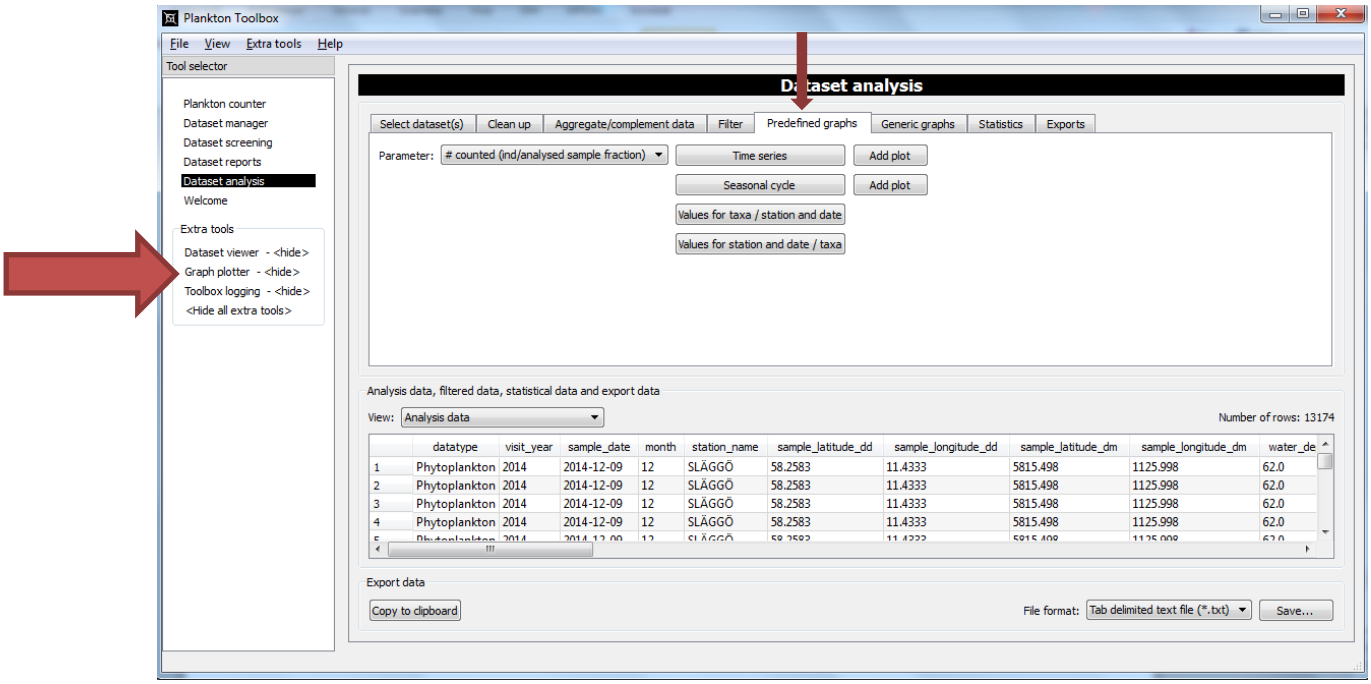

At this step data can be aggregated to different taxonomic levels, e.g. from species level to class level. Here also a function for adding zeros for organisms that are not observed in a sample is found. The software looks through all the sampling locations, dates and depths, creates a complete list of taxa observed in all the samples in the dataset, and adds zeros in abundance for a taxa that were not observed.

## Filter

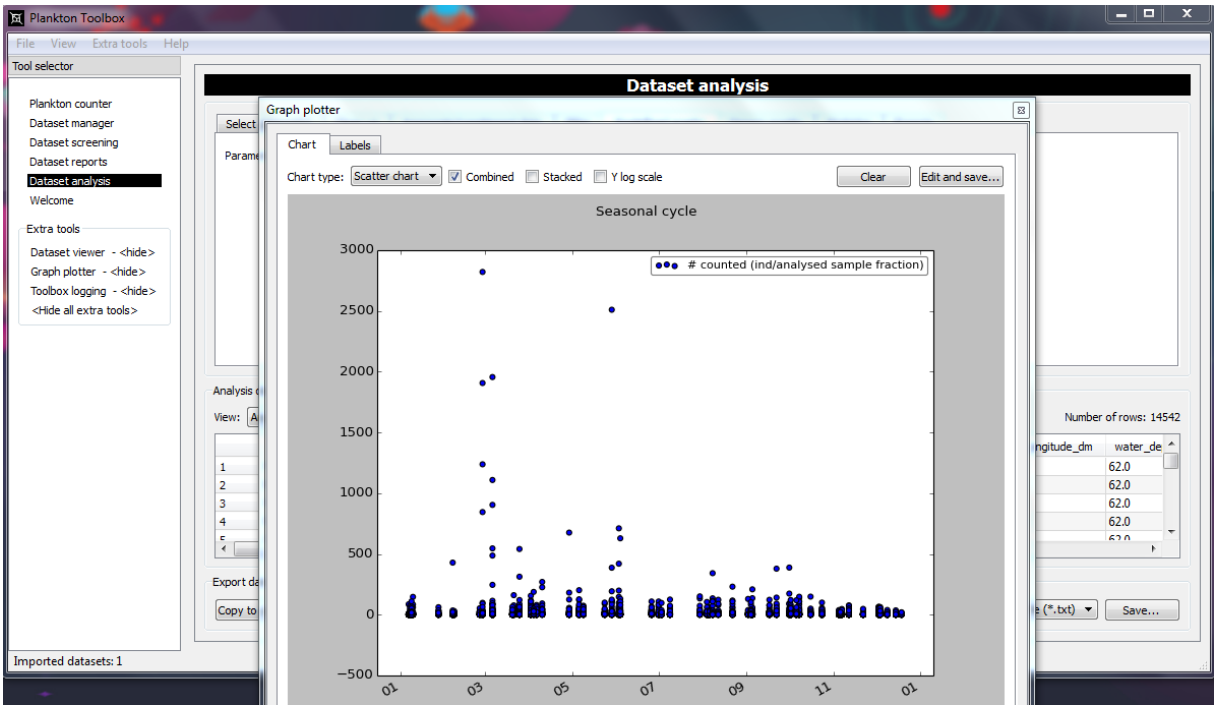



Here you can filter your data further, for example to exclude certain species or months.

## Predefined graphs

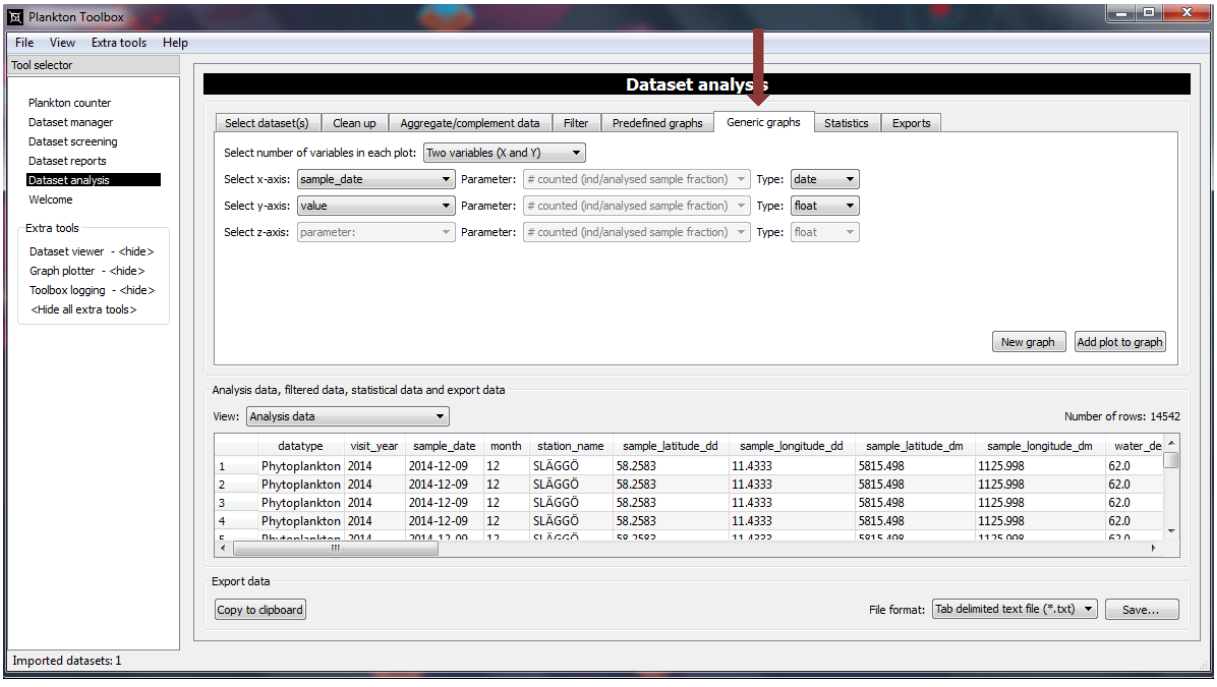



A few graphs are available for quality control and such. With the Extra tools in the left margin you can easily view/hide the dataset, graphs or loggings. The current aim of the plotting tools is to give the user the ability to produce fairly simple graphs. For publication quality output it is often necessary to export the data and use some graphical software package.



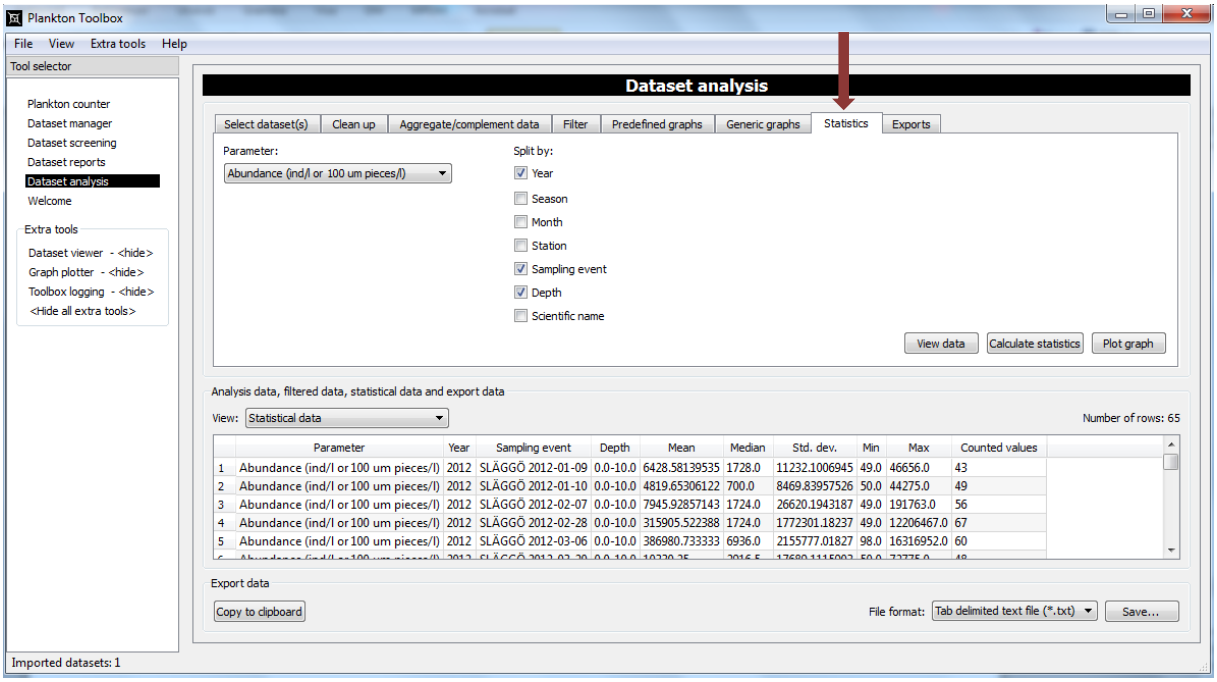

A seasonal cycle is one of the predefined graphs.

## Generic graphs



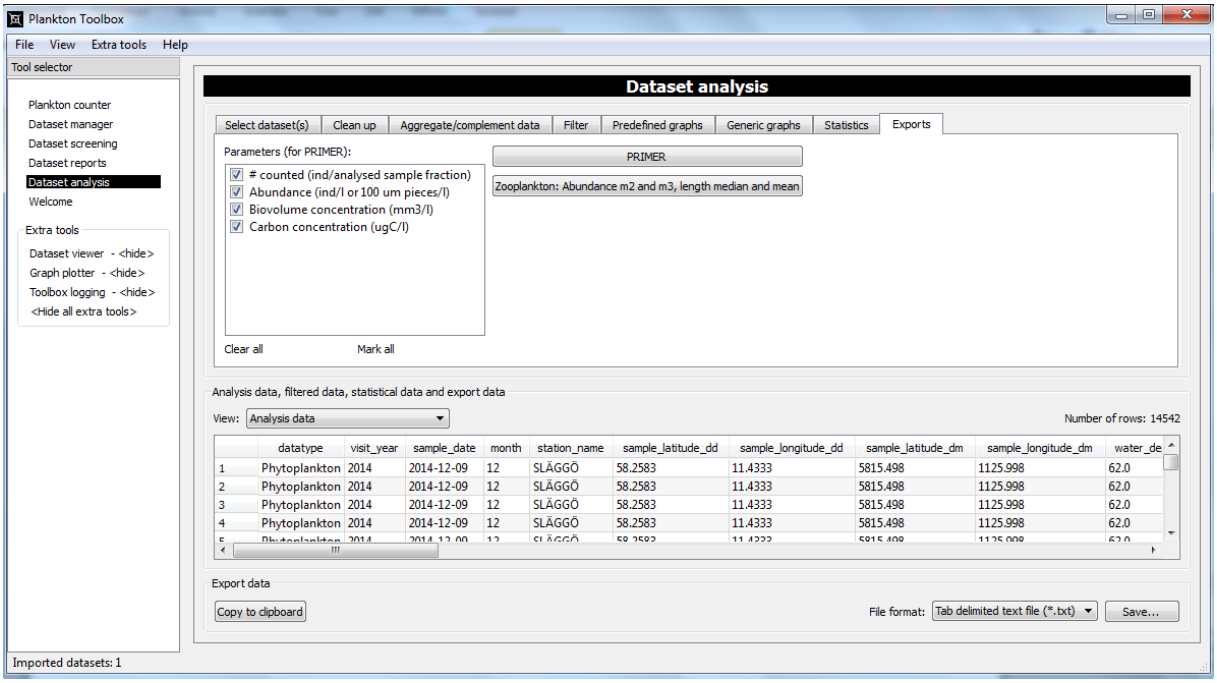

This tab offers more options in generating graphs.

## Statistics




Simple statistics may be performed and plotted. This part is planned to be developed further in future releases of the software.

## Exports




Arranges the data in for example PRIMER format, a statistical tool specialized for biological data.

At most steps in the data processing data can be copied to the clipboard and pasted into other software running on the computer used. Data may also be exported in txt or xlsx formats for further analyses or plotting using other software.

## Managing counting lists

When you count a sample you may click on *Save counting species list as* and save a list of the taxa and size classes you may want to re-use in the future. The counting list will show up in the drop down window to the left in the counting window pane. Keep in mind that the counting lists for counting samples always are subsets of the larger species lists. The subsets are found in the folder:

Plankton Toolbox -> toolbox_data\plankton_counter\config\counting_species_lists

You may change these lists as you please but make sure that you only include taxa and size groups that are found in the general species lists (see separate section on this topic).

### Managing general species lists

Plankton Toolbox provides species lists based on the HELCOM-PEG groups lists. You may also use other lists or create your own. Keep in mind that you need to include all organisms found in your sample or your data set in the species lists. Organisms missing in the taxonomic hierarchy will not be included when data is aggregated to different taxonomic levels.

The files with the species list and lists on taxonomic hierarchy are found in the folder Plankton Toolbox -> toolbox_data\species

The lists are imported into Plankton Toolbox when the software is started.

### Important lists

1. A list of taxa that you expect to find in the samples you analyze or in the dataset you work with
    a. The HELCOM-PEG 2015 list is provided. It includes a list of taxa, trophic type, cell shape, cell volume, carbon content per cell etc.
    b. An amendment to the list focusing on organisms in the Kattegat-Skagerrak may also be included.
    c. You may add taxa in the file user_taxa.xlsx file. Always include information on taxonomic hierarchy, e.g. rank and the name of the taxon higher in the taxonomic hierachy.
2. To be able to aggregate data to different taxonomic levels a taxonomic hierarchy is needed
    a. In the HELCOM-PEG 2015 list the following taxonomic levels are provided:
        i. Division
        ii. Class
        iii. Order
        iv. Scientific name (most often species)
    b. In the Nordic Microalgae list (it will be added at a later stage) the following taxonomic levels are provided:
        i. Biota
        ii. Phylum
        iii. Class
        iv. Order
        v. Genus
        vi. Species
        vii. Subspecies or Variety or Forma
    c. In the list taxa_user.xlsx you may add new taxa with information on taxonomic hierarchy
3. A list defining synonyms and translations from misspelled to correct names is also provided. The file name is translate_user.xlsx.

Keep in mind that the counting lists always are subsets of the larger general species lists. The subsets are found in the folder:

Plankton Toolbox -> toolbox_data\plankton_counter\config\counting_species_lists.

## Technical information for developers

The software Plankton Toolbox was developed by Arnold Andreasson using open source software, i.e. Python version 2.7. The code is free as defined by the MIT-license, the Open Source Initiative, http://opensource.org/licenses/mit-license.php.
The code is available at http://code.google.com/p/planktontoolbox/ (a move to GIThub is planned)
A version control system for keeping track of different versions of the code is used.

## Acknowledgements

The development of Plankton Toolbox was supported by the Swedish Research Council through the Swedish Lifewatch project. The effort by phytoplankton specialists who tested the software and suggested improvements is much appreciated.

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