# The MorFishJ User Manual

MorFishJ v0.0.1

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# Introduction

#### Status of the manual

Hi there! This manual is a work-in-progress.

MorFishJ is an open-source software package for traditional morphometric analysis of fish from side view images in ImageJ. With MorFishJ you can collect morphological trait data from fish images in a standardised and reproducible way through a semi-automated procedure, which accelerates data collection and increases data accuracy by reducing user bias.

### Motivation

While advances in modern morphometrics (landmarks configuration and outline analysis) have increasingly facilitated data acquisition and analysis, traditional morphometrics has not kept pace. Collection of traditional morphometric data from fish images is generally slow and not reproducible. A typical workflow in ImageJ is:

- 1. Open an image.
- 2. Adjust the image if necessary (or do this beforehand with another software).
- 3. Draw reference lines to help with trait measurement (Optional).
- 4. Measure traits one by one.
- 5. Save results.
- 6. Close the image (losing track of the work done).
- 7. Open the next image and repeat the procedure.

Additionally, traits are often defined and measured differently by different researchers as a standardised protocol does not exist yet, making it impractical to merge data obtained from multiple studies.

## The MorFishJ package

MorFishJ allows users to perform a complete morphometric characterisation in a limited time while recording and storing all steps, and saving results in a ready-to-use format suitable

for statistical analysis with multiple software. Analyses of large photographic databases are facilitated by allowing users to stop and restart their analyses without losing progress.

### **About this manual**

This manual is a step-by-step user guide to MorFishJ. It explains best practices for project organisation, walks through each implemented analysis, and provides detailed explanations and examples for specific steps.

# 1 Installation

ImageJ is an open source image processing program commonly used by researchers to extract morphometric data from fish images. As such, MorFishJ has been developed as an extension of this software and it can be used in both ImageJ 1.x and Fiji (ImageJ2) distribution.

Therefore, the installation of ImageJ (https://imagej.nih.gov/ij/download.html) or Fiji (https: //imagej.net/software/fiji/downloads) is required before installing MorFishJ.



Warning

MorFishJ depends on ImageJ 1.53e, thus, if ImageJ/Fiji was previously installed, first check the current ImageJ version below the toolbar. If it is older than 1.53e, to update ImageJ click Help -> Update ImageJ..., choose the latest version and click OK. Then ImageJ/Fiji must be restarted.

Once ImageJ/Fiji is installed/updated, proceed with installing MorFishJ following these steps:

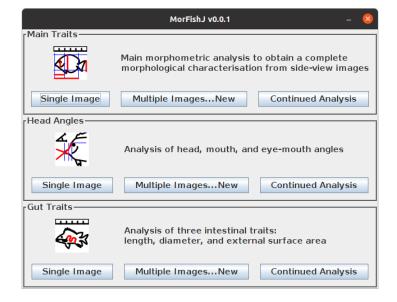
- 1. Download MorFishJ:
  - for people familiar with GitHub: clone or download the entire GitHub repository;
  - for people not familiar with GitHub: click here to go to the project's main page on GitHub, click on the green button Code and then click on Download ZIP.
- 2. Extract the content.
- 3. Copy the MorFishJ v0.0.1 folder in the ImageJ/plugins/ or Fiji.app/plugins/ directory.

Now MorFishJ is installed. Open ImageJ/FIJI and click Plugins -> MorFishJ v0.0.1 -> MorFishJ GUI. The following GUI appears in the upper left corner of the screen.

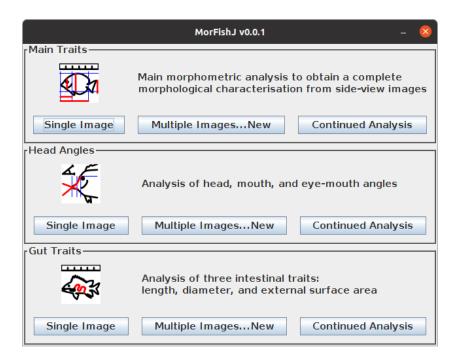


🕊 Tip

In Fiji the Plugins menu is often crowded, thus it may be easier to use the Search field under the toolbar to find and start MorFishJ.



# 2 GUI



The GUI of MorFishJ provides a simple and direct access to all the morphometric analyses. Each analysis can be started by clicking on the appropriate button.

## 2.1 Morphometric analyses

Three morphometric analyses are currently available in MorFishJ:



The workhorse of MorFishJ. Performs a complete morphometric analysis measuring 22 traits that cover all body parts visible from side view images, excluding dorsal, pelvic, and anal fins.



It allows to measure three head angles related to vision and feeding (Brandl and Bellwood 2013; Bellwood et al. 2014; Brandl, Robbins, and Bellwood 2015).



It allows to measure three intestinal traits related to fish diet (Ghilardi et al. 2021).

### 2.2 Single and multiple image analysis

Each analysis have three different buttons:

- Single Image: to analyse only one image (may be useful for beginners to get used to each step before analysing the entire image database).
- Multiple Images...New: to start a new analysis of an image database.
- Continued Analysis: to continue analysing an image database from where it was last left.

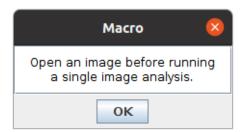
### 2.2.1 Single Image



Warning

An image must be opened before starting a single image analysis.

Clicking on the Single Image button of the desired analysis before opening an image will display the following message.



To open an image click on **File** -> **Open...** or use the shortcut **Ctrl+O** or drag the image over the ImageJ GUI.

Once the image is opened click on the Single Image button of the desired analysis. The image is duplicated and all the following steps will be performed on the duplicated image to avoid any unwanted modification of the raw image. The ROI Manager window is automatically opened and the first dialog box appears, to either set the scale or adjust the image, depending on the type of analysis selected.

The steps required to perform the selected analysis are explained in the following chapters.

### 2.2.2 Multiple Images

#### **New analysis**

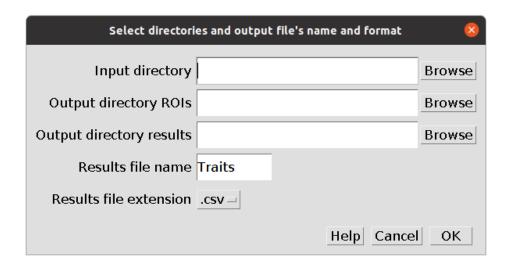
Before starting to analyse an image database the new project should have a clear folder structure. MorFishJ does not require a fixed folder structure, but only an input directory where the raw images are stored and two output directories, one to save the ROIs and one to save the results and log files (although the same output directory could be used for all outputs). Therefore, you can set your own folder structure. However, it is advisable that each project has its own directory with the following structure.

```
./ProjectName/
raw_images
img1.JPG
img2.JPG
img3.JPG
...
results
ROIs
```

There are three subdirectories: one containing the raw images, one to save the ROIs and processed images, and one to save the results and log files.

To start a new analysis click on the Multiple Images...New button of the desired analysis. The following dialog box appears.

Use the Browse button to add the path to the input directory where the raw images are stored, and the output directories where the ROIs and results will be saved. Alternatively, it is possible to drag the directories over the dialog box. By default the results file will be named **Traits** and will be saved in .csv format, but it is possible to change both the name and the extension, which can be changed to .txt through the drop down list.



Once all the fields are completed click OK. Two files are created in the selected directory for the results:

- a log file with the name TraitLog followed by the date of creation and including all the metadata required to restore the project.
- a results file with the selected name and extension. Results are appended to this file at the completion of the analysis of each image.

Important

Do not edit the TraitLog file by hand and do not change its directory!

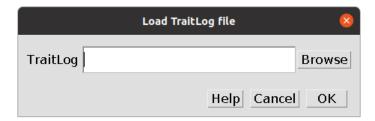
The first image in the input directory is automatically opened and duplicated. All the following steps will be performed on the duplicated image to avoid any unwanted modification of the raw image. The ROI Manager window is automatically opened and the first dialog box appears, to either set the scale or adjust the image, depending on the type of analysis selected.

The steps required to perform the selected analysis are explained in the following chapters.

#### Continued analysis

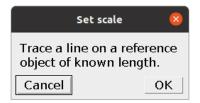
To continue a project from where it was last left, click on the Continued Analysis button of the desired analysis. The following dialog box appears.

Use the Browse button or drag the file over the dialog box to add the path to the TraitLog file of the project. Thus click OK. The project is restored and can be continued from the next image. The new results will be appended to the existing results file.



# 3 Set scale

Two analyses, Main Traits and Gut Traits, require to set the scale for the image. This is the first step of both analyses. The following dialog box appears.

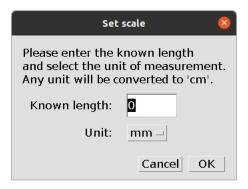


Trace a straight line on a reference object and click OK.



The reference object can also be the fish itself if its length (standard, fork, or total) is known. In this case trace a line over the fish to select the appropriate length.

The following dialog box appears.



Enter the known length of the traced line and select the unit from the drop down list. Three options are available (mm, cm, and inch), but any unit will then be converted to cm. Thus all linear measurements will be saved in cm and areas in  $cm^2$ .

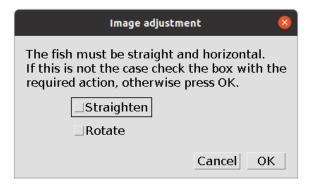
The traced line will be stored in the ROI Manager as px.cm and then saved with all other ROIs. A column px/cm will also be included in the results file indicating the scale of the images (pixels cm<sup>-1</sup>).

### ⚠ Warning

At the moment it is not possible to run the Main Traits and Gut Traits analyses on images without a reference object or known fish length as setting the scale is required. However, in the upcoming update setting the scale will become optional and all images can be analysed.

# 4 Straighten and rotate

Two analyses, Main Traits and Head Angles, require the fish to be in a straight and horizontal position. For such analyses the software allows to adjust the image if required. For the Main Traits analysis this is the second step, after setting the scale (as described in Chapter 3), whereas for the Head Angles analysis it is the first step. The following dialog box appears.



It allows to select one of two options:

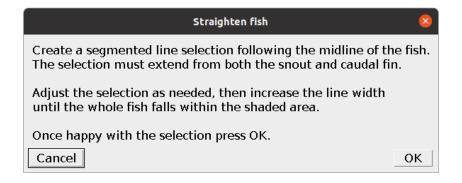
- Straighten: to straighten the fish if it is in a bent position.
- Rotate: to rotate the image and set the fish in an horizontal position.

Therefore, if the fish is not straight and horizontal, select the appropriate option and click OK. Otherwise, click OK without selecting any option and continue.

# Straighten

When the fish is bent, select Straighten and click OK. The following dialog box appears together with the Line Width window.

Follow the instructions given in the dialog box, create a segmented line selection, adjust the width and click OK. The fish will be straightened and in an horizontal position. The following figure shows an example of fish straightening.



### 4.0.0.1 Before



### 4.0.0.2 After



# Tip

Selecting Spline fit within the Line Width window can improve the selection and straightening of the fish.

### Warning

If the fish is overly bent, straightening can lead to deformation of the body which affects the measurement of the traits. Use this option with care.

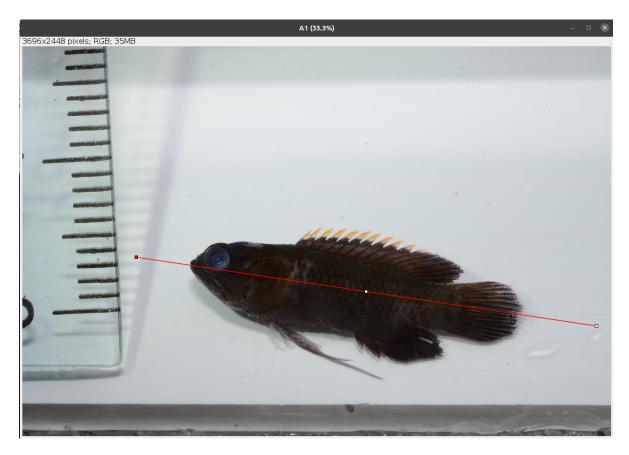
### Rotate

When the fish is straight but not horizontal, select Rotate and click OK. The following dialog box appears.

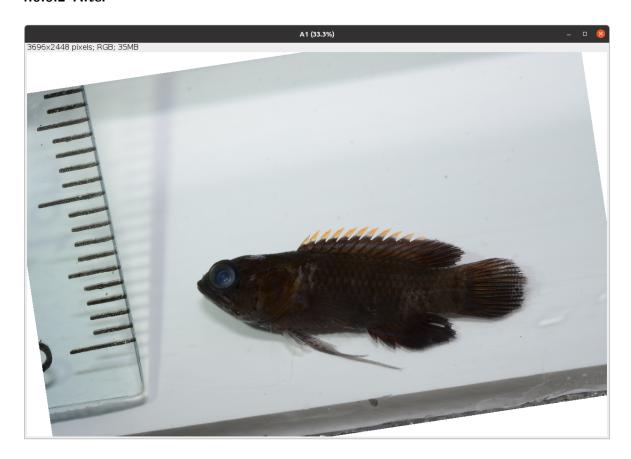


Follow the instructions given in the dialog box, trace a line and click OK. The image will be rotated and, if the line was placed well, the fish will be in an horizontal position. The following figure shows an example of image rotation.

# 4.0.0.1 Before



# 4.0.0.2 After



# References

- Bellwood, D. R., C. H. R. Goatley, S. J. Brandl, and O. Bellwood. 2014. "Fifty million years of herbivory on coral reefs: fossils, fish and functional innovations." *Proceedings of the Royal Society B: Biological Sciences* 281 (1781): 20133046. https://doi.org/10.1098/rspb. 2013.3046.
- Brandl, S. J., and D. R. Bellwood. 2013. "Morphology, sociality, and ecology: can morphology predict pairing behavior in coral reef fishes?" *Coral Reefs* 32 (3): 835–46. https://doi.org/10.1007/s00338-013-1042-0.
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