

# **The MorFishJ User Manual**

**MorFishJ v0.2.1**

Mattia Ghilardi

4/3/23

# Table of contents

<b>Introduction</b>	<b>4</b>
Motivation . . . . .	4
The MorFishJ package . . . . .	4
About this manual . . . . .	5
Versioning . . . . .	5
<b>1 Installation</b>	<b>6</b>
<b>2 GUI</b>	<b>7</b>
2.1 Morphometric analyses . . . . .	7
2.2 Single and multiple image analysis . . . . .	8
2.2.1 Single Image . . . . .	8
2.2.2 Multiple Images . . . . .	9
<b>3 Image scale</b>	<b>12</b>
3.1 Main Traits scale . . . . .	12
Scale through reference object . . . . .	12
Scale through fish length . . . . .	13
3.2 Gut Traits scale . . . . .	14
<b>4 Straighten and rotate</b>	<b>15</b>
Straighten . . . . .	15
Rotate . . . . .	17
<b>5 Main Traits</b>	<b>20</b>
5.1 Analysis . . . . .	24
5.2 Results . . . . .	33
<b>6 Head Angles</b>	<b>34</b>
6.1 Analysis . . . . .	36
6.2 Results . . . . .	38
<b>7 Gut Traits</b>	<b>41</b>
7.1 Analysis . . . . .	41
7.2 Results . . . . .	45



# Introduction

**MorFishJ** is an open-source software package for traditional morphometric analysis of fish 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.

To cite **MorFishJ** in publications use:

Mattia Ghilardi (2022). MorFishJ: A software package for fish traditional morphometrics (v0.2.1)

A BibTeX entry for LaTeX users is:

```
@software{,  
  author      = {Mattia Ghilardi},  
  title       = {{MorFishJ: A software package for fish traditional  
                 morphometrics}},  
  year        = 2022,  
  publisher   = {Zenodo},  
  version     = {v0.2.1 },  
  doi         = {10.5281/zenodo.6969273},  
  url         = {https://doi.org/10.5281/zenodo.6969273}  
}
```

## 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.

## Versioning

The manual follows the same versioning of MorFishJ. For each new release of MorFishJ this manual will be updated and will be given the same version number.

The latest version of the manual can always be accessed at [https://mattiaghilardi.github.io/MorFishJ\\_manual/](https://mattiaghilardi.github.io/MorFishJ_manual/).

Previous versions can be accessed at:

- [v0.2.0](#)
- [v0.1.0](#)

A list of changes made for each version of MorFishJ can be seen [here](#).

# 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 (instructions [here](#)) or Fiji (instructions [here](#)) is required before installing **MorFishJ**.

## Warning

**MorFishJ** depends on **ImageJ 1.53s**, thus, if ImageJ/Fiji was previously installed, first check the current ImageJ version below the toolbar. If it is older than 1.53s, 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:

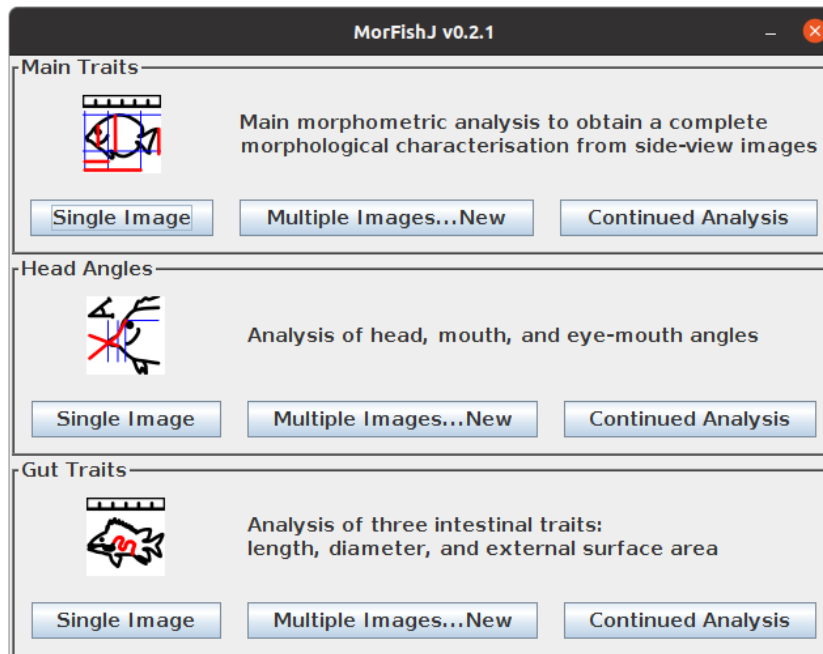
1. Download **MorFishJ v0.2.1** from [here](#) as a ZIP file.
2. Extract the content.
3. Copy the folder named **MorFishJ** (NOT the entire downloaded folder) in the `ImageJ/plugins/` or `Fiji.app/plugins/` directory.

Now **MorFishJ** is installed. Open ImageJ/FIJI and click **Plugins** → **MorFishJ** → **MorFishJ GUI**. The Graphical User Interface (GUI) (Chapter 2) of **MorFishJ** 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:

	<b>Main Traits</b>
<p>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.</p>	



### Head Angles

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).



### Gut Traits

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

## 2.2 Single and multiple image analysis

Each analysis has three different buttons:

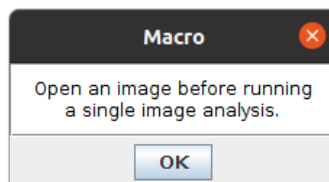
- **Single Image:** to analyse only one image (may be useful for beginners to get used to each step before analysing the entire image database).
- **Multiple Images...New:** to start a new analysis of an image database.
- **Continued Analysis:** to continue analysing an image database from where it was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



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:

- Chapter 5: **Main Traits**
- Chapter 6: **Head Angles**
- Chapter 7: **Gut Traits**

## 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 Regions Of Interest (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.

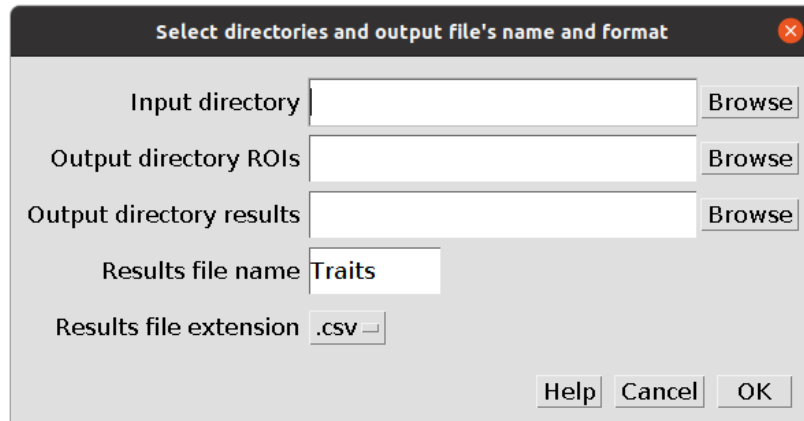
```
./ProjectName/  
  raw_images  
    img1.JPG  
    img2.JPG  
    img3.JPG  
    ...  
  results  
  ROIs
```

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.

#### ! Important

Do NOT use the same directory as both input and output! This would prompt an error when trying to continue the analysis loading the **TraitLog** file.

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 in **dd\_mm\_yyyy** format 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.

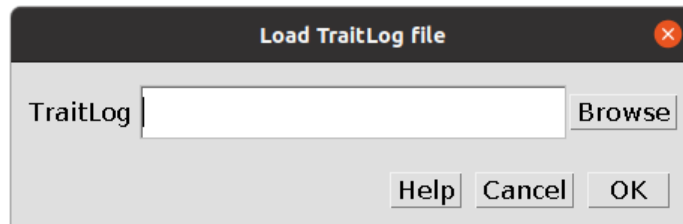
The steps required to perform the selected analysis are explained in:

- Chapter **5: Main Traits**
- Chapter **6: Head Angles**
- Chapter **7: Gut Traits**

## Continued analysis

It is possible to stop and restart each analysis without losing progress. Everything done until the last completed image is saved, thus, if the analysis is stopped before completing an image, only the steps performed on that image are lost.

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.



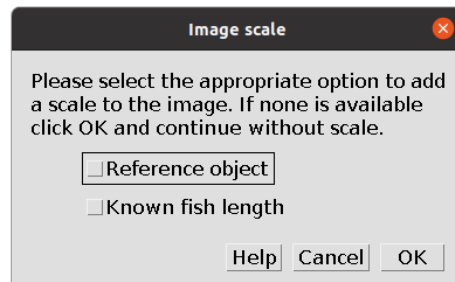
Use the **Browse** button or drag the file over the dialog box to add the path to the **TraitLog** file of the project. Thus click **OK**. The project is restored and can be continued from the next image. The new results will be appended to the existing results file.

## 3 Image scale

The first step of the **Main Traits** and **Gut Traits** analyses is to set the scale for the image. While for the **Main Traits** analysis this is optional, it is required for the **Gut Traits** analysis.

### 3.1 Main Traits scale

When starting the **Main Traits** analysis the following dialog box appears.



There are three options:

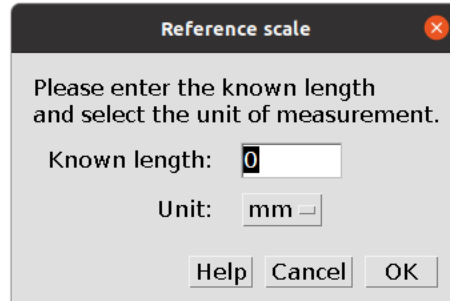
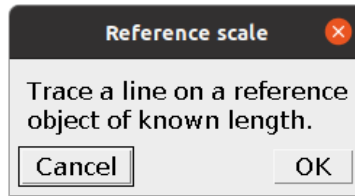
- Add a scale through a reference object in the image.
- Add a scale through the known length of the fish.
- Do not add a scale to the image.

Check the box of the appropriate option to add a scale, or leave the boxes empty and click **OK** to continue without adding a scale. If both options are selected, a reference object will be used.

#### Scale through reference object

After selecting **Reference object** the following dialog box appears.

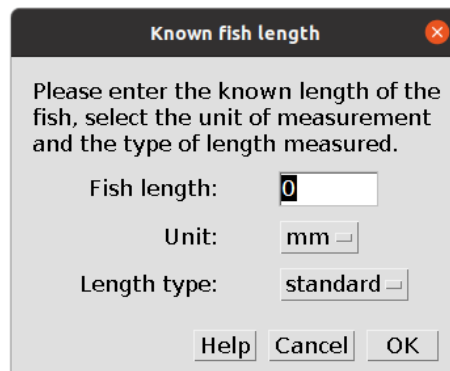
Trace a straight line on a reference object and click **OK**. The next dialog box appears.



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*. The traced line will be stored in the ROI Manager as *px.cm* and then saved with all other ROIs. The scale of the image, in *pixels/cm*, will be saved in the results file within the column *px.cm* to allow converting the results, which are saved in *pixels*, to *cm*, if preferred.

### Scale through fish length

After selecting **Known fish length** the following dialog box appears.

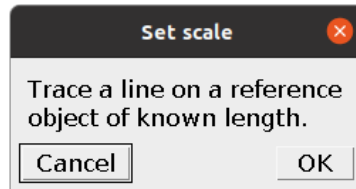


Enter the known length of the fish, select the unit from the drop-down list, and select the type of length, either **standard length** or **total length**, from the drop-down list. As for the reference object, the scale of the image, in *pixels/cm*, will be saved in the results file within the column *px.cm* to allow converting the results, which are saved in *pixels*, to *cm*, if preferred.

## 3.2 Gut Traits scale

The procedure to add a scale to the Gut Traits analysis is the same as using a reference scale in the Main Traits analysis.

When starting the Gut Traits analysis the following dialog box appears.

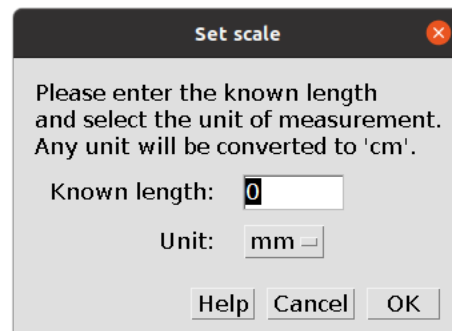


Trace a straight line on a reference object and click OK.

### Tip

For the Gut Traits analysis the reference object could also be the fish itself if it is in the image and its length (standard, fork, or total) is known. In this case trace a line over the fish to select the appropriate length.

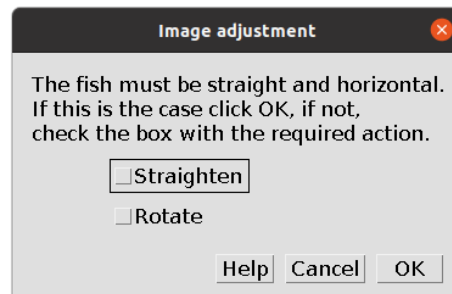
The following dialog box appears.



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*. The traced line will be stored in the ROI Manager as *px.cm* and then saved with all other ROIs. The scale of the image, in *pixels/cm*, will be saved in the results file within the column *px.cm* to allow converting the results, which are saved in *cm*, to the original *pixels*, if preferred.

## 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 adding 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:

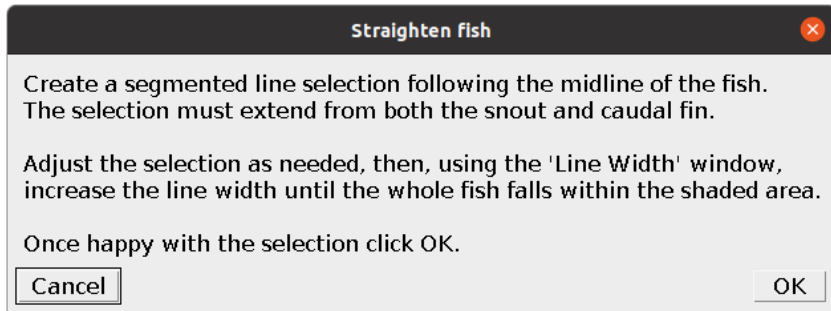
- **Straighten**: to straighten the fish if it is in a bent position.
- **Rotate**: to rotate the image and set the fish in an horizontal position.

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.

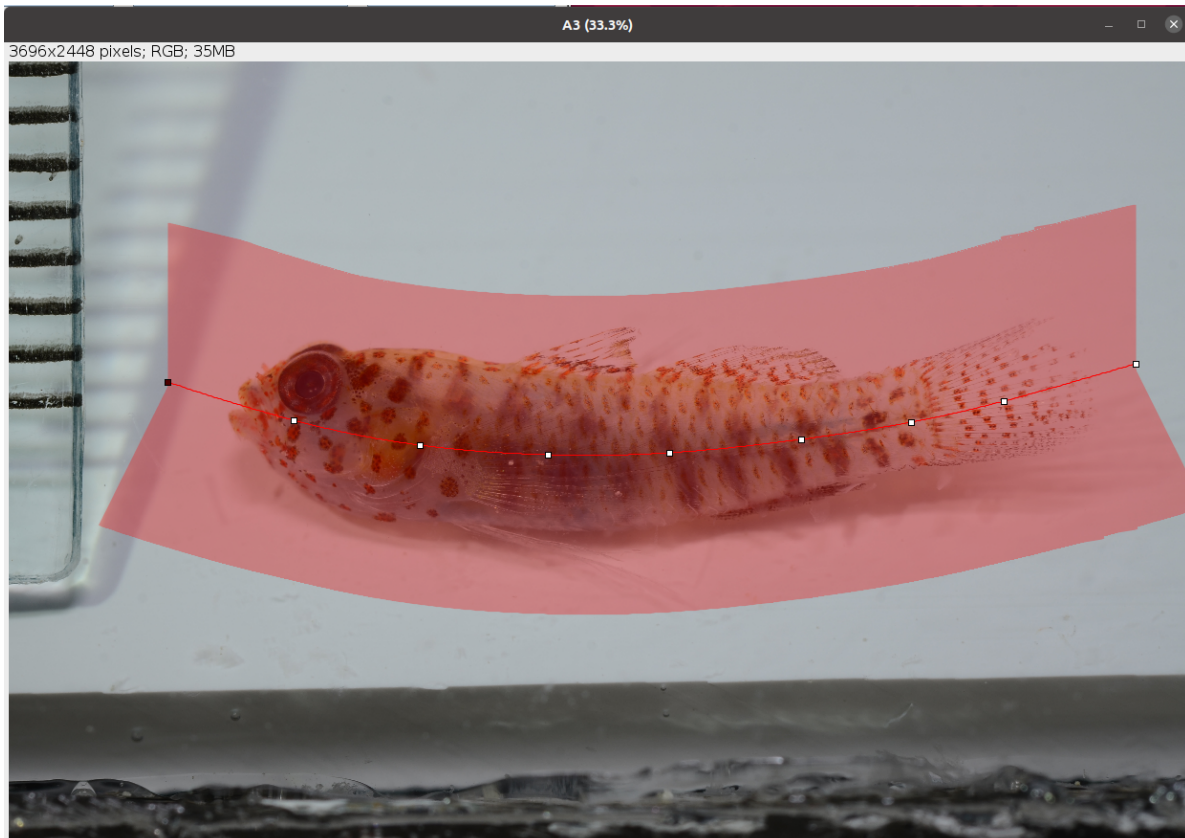
### Straighten

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, increase the line width until the whole fish falls within the shaded area 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

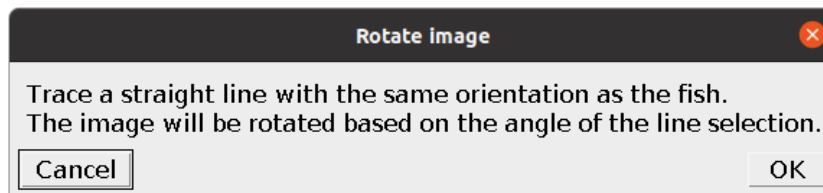
Selecting **Spline fit** within the **Line Width** window can improve the selection and straightening of the fish.

#### 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.

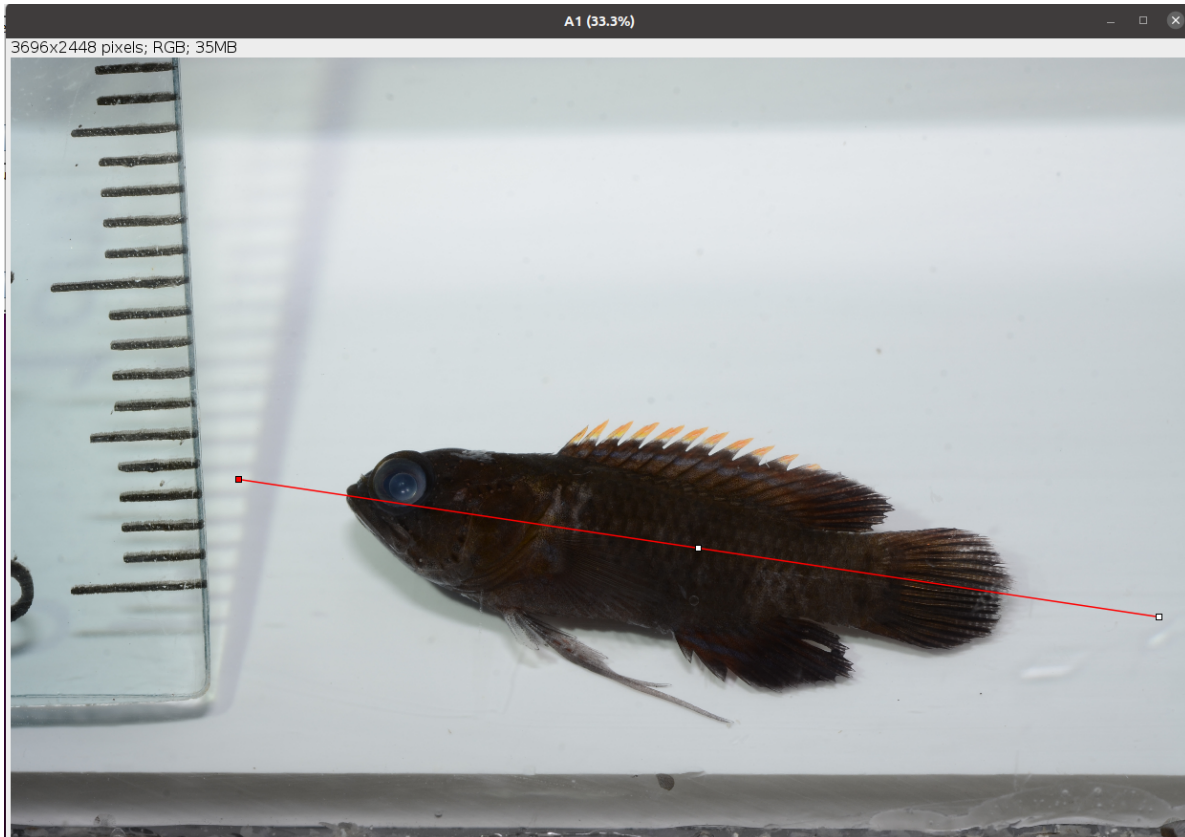
## Rotate

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

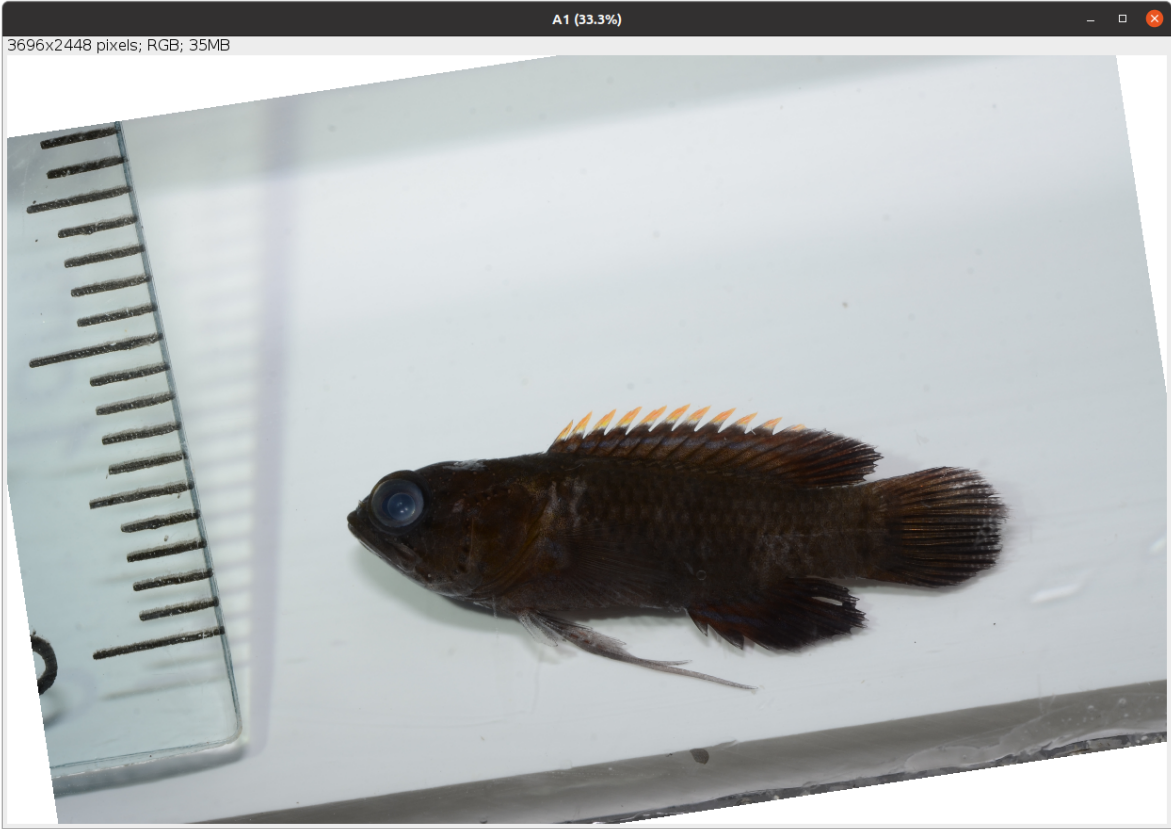


Follow the instructions given in the dialog box, trace a line and click **OK**. The image will be rotated and, if the line was placed well, the fish will be in an horizontal position. The following figure shows an example of image rotation.

#### 4.0.0.1 Before



4.0.0.2 After



## 5 Main Traits

The **Main Traits** analysis is the workhorse of **MorFishJ** as it performs a complete morphometric characterisation of fishes. It requires the user input to trace the outline of the body and pectoral fin, and to position some reference lines (Figure 5.1, Table 5.1) and landmark points. The coordinates of these lines and points allow to extract 22 morphometric measurements described in Figure 5.2 and Table 5.2.

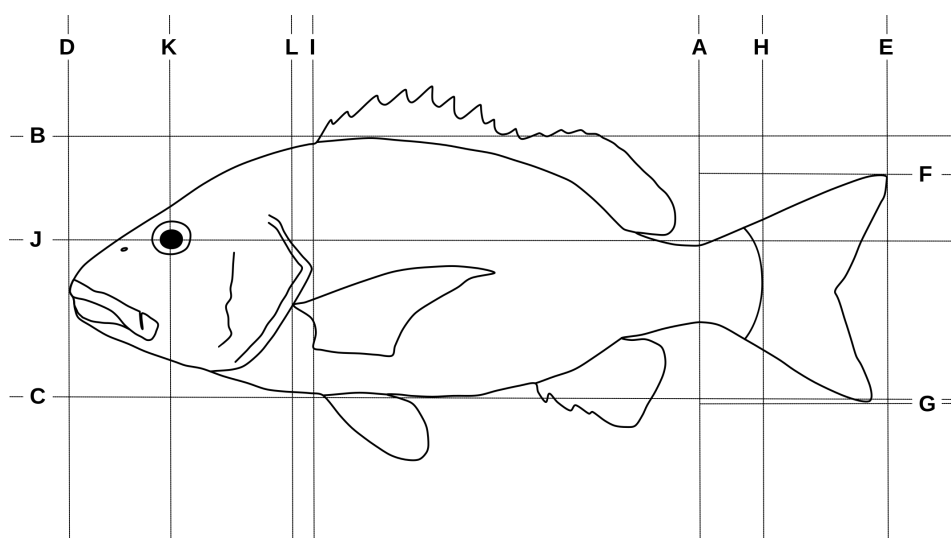


Figure 5.1: Reference lines traced on fish images for main traits analysis (see Table 5.1 for descriptions)

Table 5.1: Description of reference lines traced on fish images for main traits analysis

Reference line	Description	User input
A	Vertical line at the narrowest point of the caudal peduncle	Yes
B	Horizontal line touching the highest edge of the body (excluding fins)	No
C	Horizontal line touching the lowest edge of the body (excluding fins)	No

Reference line	Description	User input
D	Vertical line touching the most anterior tip of the body	No
E	Vertical line touching the most posterior tip of the caudal fin	No
F	Horizontal line touching the highest edge of the caudal fin	No
G	Horizontal line touching the lowest edge of the caudal fin	No
H	Vertical line at the base of the caudal fin (end of the vertebral column or posterior edge of the hypural plate). Parrotfishes (Labridae: Scarini) are an exception and the line should be placed between the last and second to last scale lying along the midline ( <a href="#">FishBase</a> )	Yes
I	Vertical line touching the posterior margin of the operculum	Yes
J	Horizontal line cutting the eye in halves	No <sup>1</sup>
K	Vertical line cutting the eye in halves	No
L	Vertical line at the pectoral fin insertion	Yes

Table 5.2: Description of morphometric parameters taken on fish images

Code	Trait	Description	Reference
TL	Total length	Length from the most anterior point of the body (D) to the most posterior point (E) (excluding the caudal filaments)	<a href="#">FishBase</a>
SL	Standard length	Length from most anterior point of the body (D) to the the base of the caudal fin (H)	<a href="#">FishBase</a>

<sup>1</sup>The user has to trace an ellipse around the eye and lines J and K will be automatically drawn from the coordinates of this selection.

Code	Trait	Description	Reference
MBd	Maximum body depth	Body depth at the deepest part of the body (excluding fins). Measured as the vertical distance between B and C	Bellwood et al. (2014)
Hl	Head length	Horizontal distance from most anterior tip of the head (D) to the posterior margin of the operculum (I)	Barnett, Bellwood, and Hoey (2006)
Hd	Head depth	Head depth measured at the vertical of the orbit centroid along K	Villéger et al. (2010)
Ed	Eye diameter	Internal diameter of the orbit measured along J	Bellwood et al. (2014)
Eh	Eye position	Vertical distance from the orbit centroid ( $J \times K$ ) to the bottom of the body ( $K \times C$ )	Toussaint et al. (2016)
Snl	Snout length	Horizontal distance from the anterior margin of the orbit to the tip of the snout (D)	Barnett, Bellwood, and Hoey (2006)
POC	Posterior of orbit centroid	Horizontal distance from the orbit centroid ( $J \times K$ ) to the posterior margin of the operculum ( $J \times I$ )	Bellwood et al. (2014)
AO	Anterior of orbit	Horizontal distance from the anterior margin of the orbit to the anterior margin of the body along J (AO may be close, or equal to, zero in species with very anterior eyes)	Bellwood et al. (2014)
EMd	Eye-mouth distance	Distance between the orbit centroid ( $J \times K$ ) to the anterior tip of the premaxilla	Bellwood et al. (2014)

Code	Trait	Description	Reference
EMa	Eye-mouth angle	Angle between EMd and a horizontal line intersecting the tip of the premaxilla	Bellwood et al. (2014)
Mo	Oral gape position	Vertical distance from the tip of the premaxilla to the bottom of the body (C)	Toussaint et al. (2016)
Jl	Maxillary jaw length	Length from the tip of the premaxilla to the intersection between the maxilla and the mandible (i.e. the corner of the mouth)	Toussaint et al. (2016)
Bs	Body surface area	Area of a polygon drawn following the contour of the body excluding fins, and up to the narrowest point of the caudal peduncle	Bellwood et al. (2014)
CPd	Caudal peduncle depth	Measured at the narrowest point of the caudal peduncle along A	Villéger et al. (2010)
CFd	Caudal fin depth	Maximum depth of the caudal fin	Villéger et al. (2010)
CFs	Caudal fin surface area	Area of a polygon drawn following the contour of the caudal fin, with the anterior margin marked by a straight vertical line through the narrowest point of the caudal peduncle	Villéger et al. (2010)
PFs	Pectoral fin surface area	Area of a polygon drawn following the contour of the pectoral fin	Villéger et al. (2010)
PF1	Pectoral fin length	Length of the longest ray of the pectoral fin	Toussaint et al. (2016)

Code	Trait	Description	Reference
PFi	Pectoral fin position	Vertical distance from the upper insertion of the pectoral fin to the bottom of the body (C)	Toussaint et al. (2016)
PFb	Body depth at level of pectoral fin insertion	Body depth measured along L	Villéger et al. (2010)

## 5.1 Analysis

Once the steps described in Section 2.2 are completed the screen will be populated with a number of windows:

- the ImageJ/Fiji main window
- the MorFishJ GUI
- a fish image (this is a duplicate of the raw image to prevent any modification)
- the ROI manager
- the `Image scale` dialog

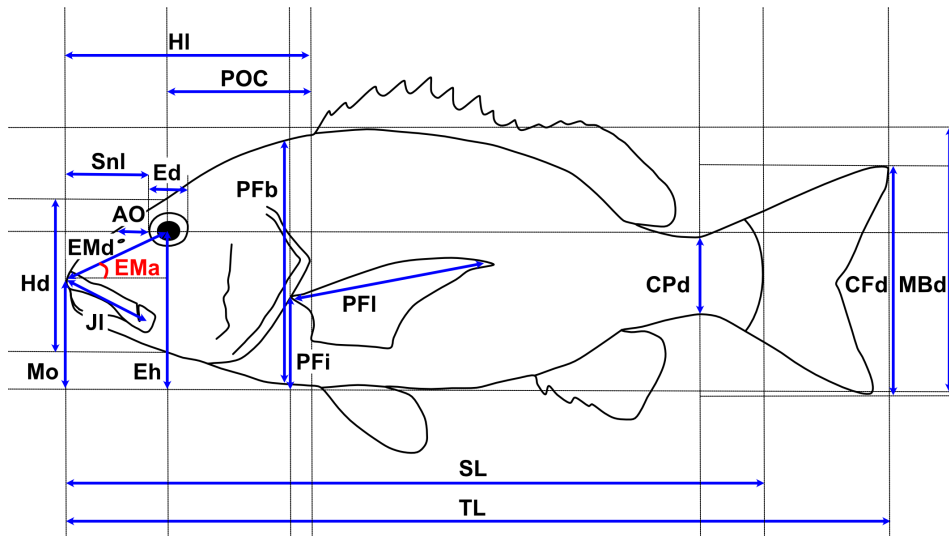
While the majority of the analysis is automated, there are a number of steps that require the user input:

1. If available, add a scale to the image as described in Chapter 3.
2. Adjust the image if necessary as described in Chapter 4.
3. Select the orientation of the fish (i.e., whether the fish is facing left or right) from a drop-down list. This step is important for the correct automatic placement of some reference lines and points.
4. Select the fish outline following the instruction in the dialog. Trace a polygon around the body and caudal fin, excluding the dorsal, pelvic, and anal fins as in the example below. Also avoid including any portion of pectoral fin protruding from the body area. Once the selection is completed it can be adjusted as needed.

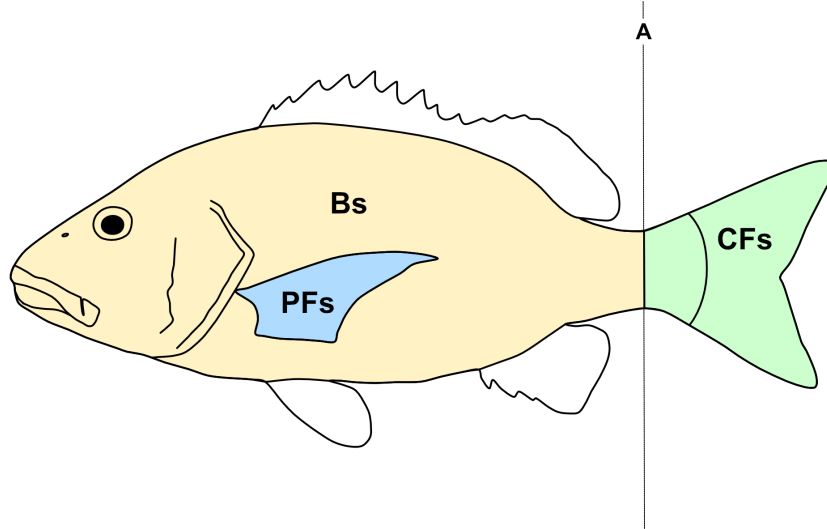
### Tip

The points that define the selection can be moved. To add a new point to the selection press **Shift** and click on an existing point. To remove one point press **Alt** and click on the point. See [Polygon Selection Tool](#).



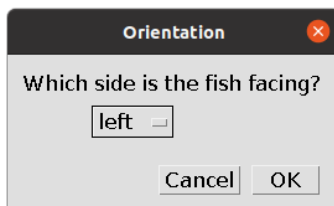


(a) Linear measurements



(b) Surface areas

Figure 5.2: Morphometric parameters taken on fish images (see Table 5.2 for descriptions)

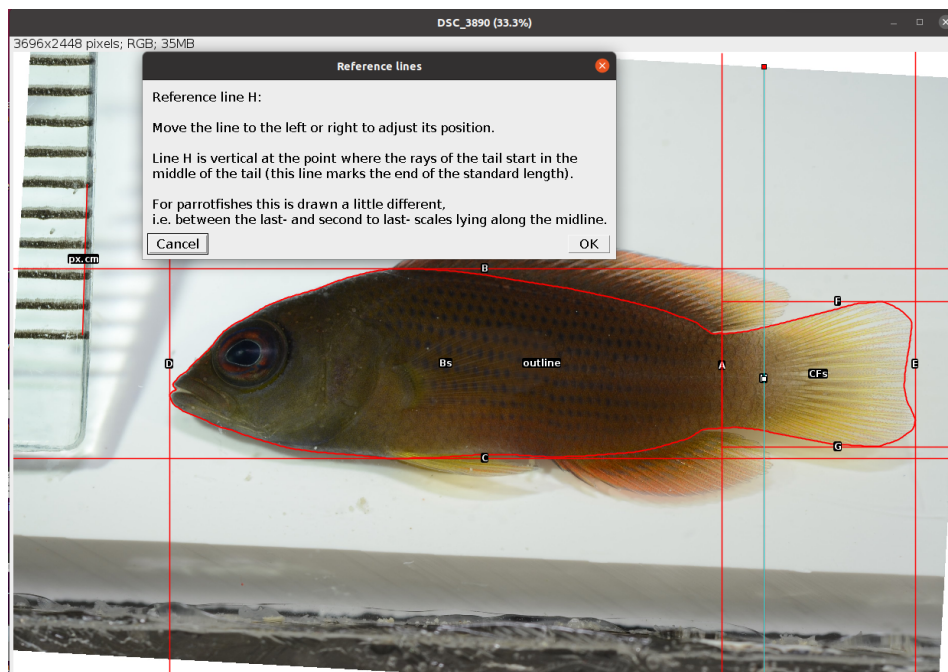
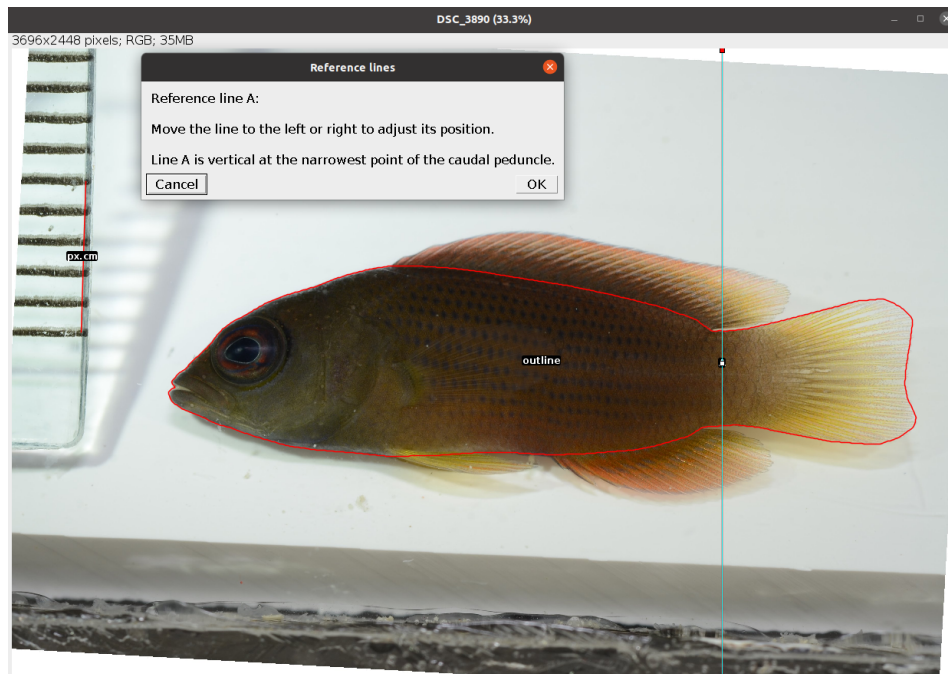


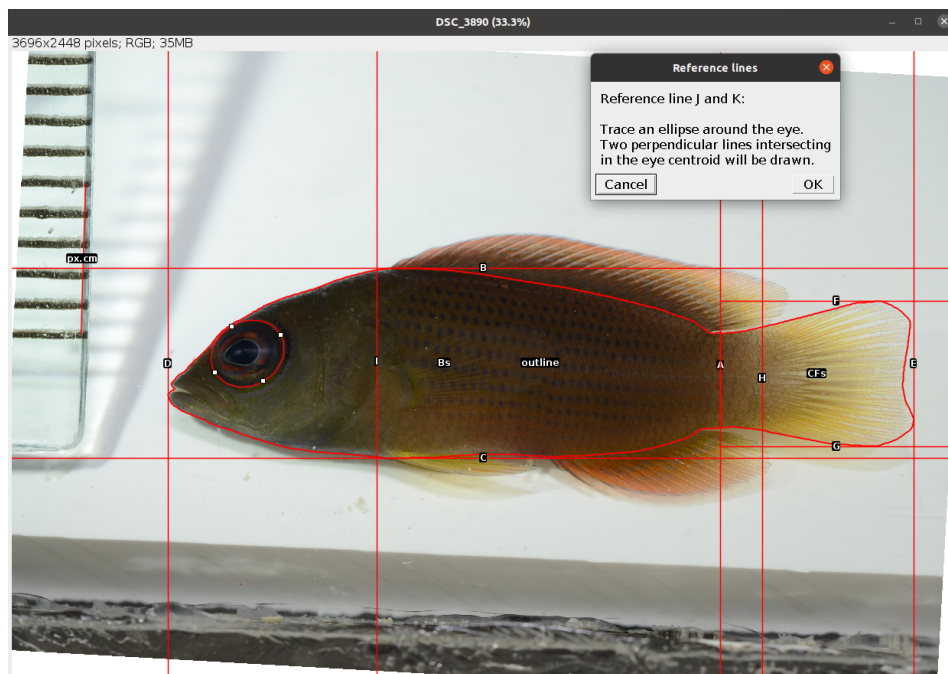
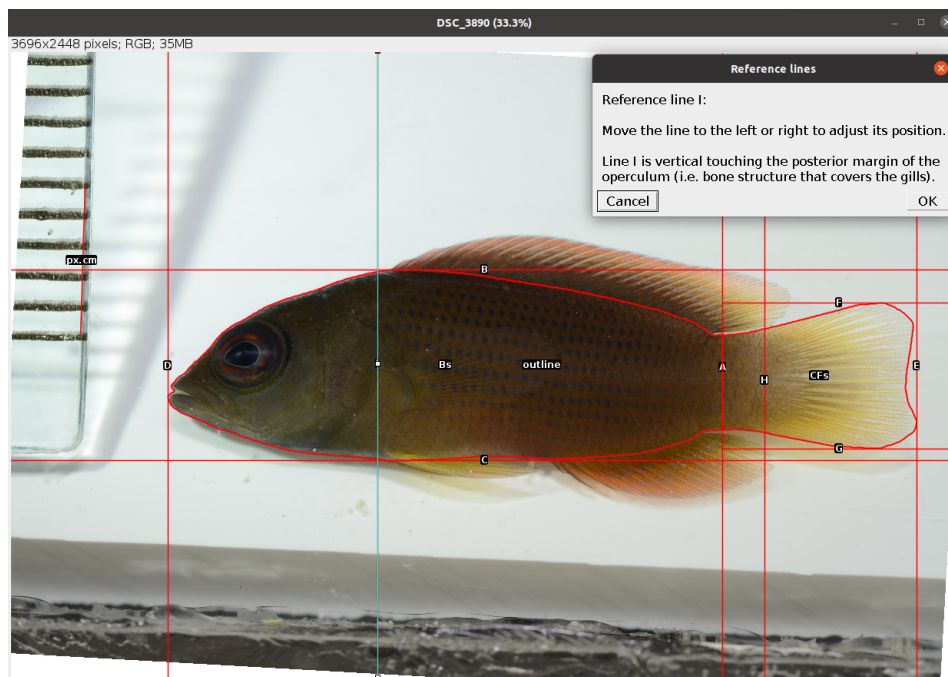


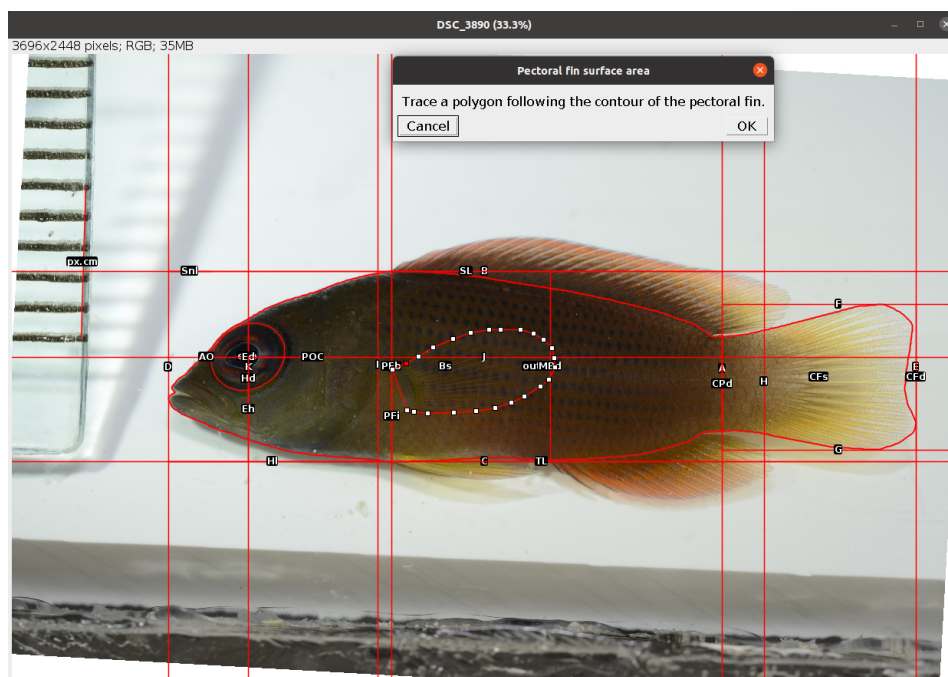
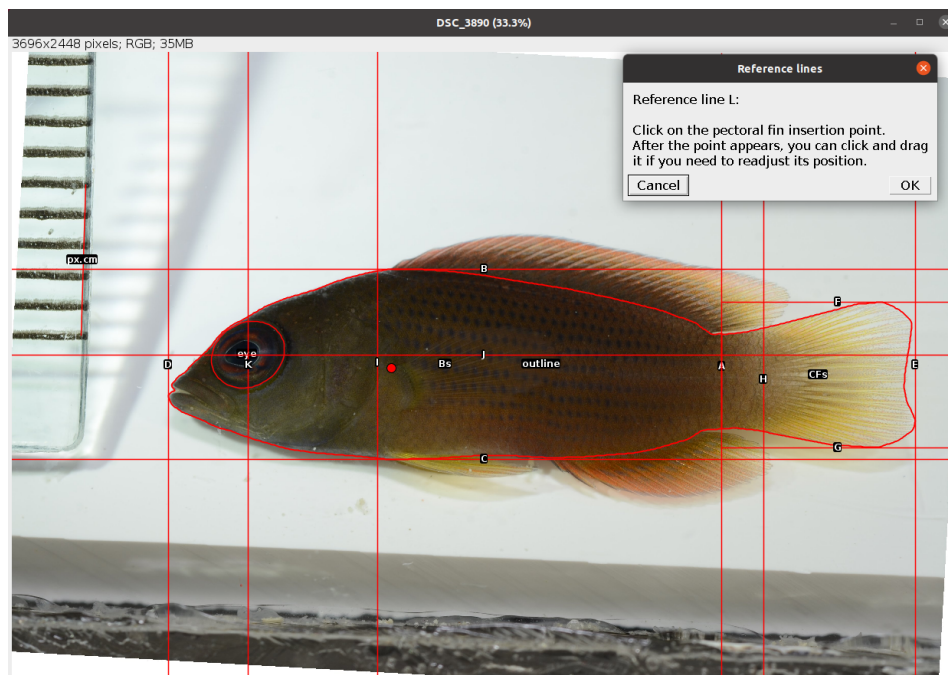
5. Position reference line A at the narrowest point of the caudal peduncle. After clicking OK several automatic steps will split the outline in two, the body area (Bs) and caudal fin area (CFs), and add reference lines B-G.
6. Position reference line H at the base of the caudal fin to mark the end of the standard length.
7. Position reference line I at the posterior margin of the operculum.
8. Trace an ellipse around the eye as in the example below (see [Elliptical Selection Tool](#) for specific instructions on how to use this tool). After clicking OK two new reference lines (J-K) are drawn perpendicular intersecting at the eye centroid.
9. Place a point at the insertion of the pectoral fin as in the example below.

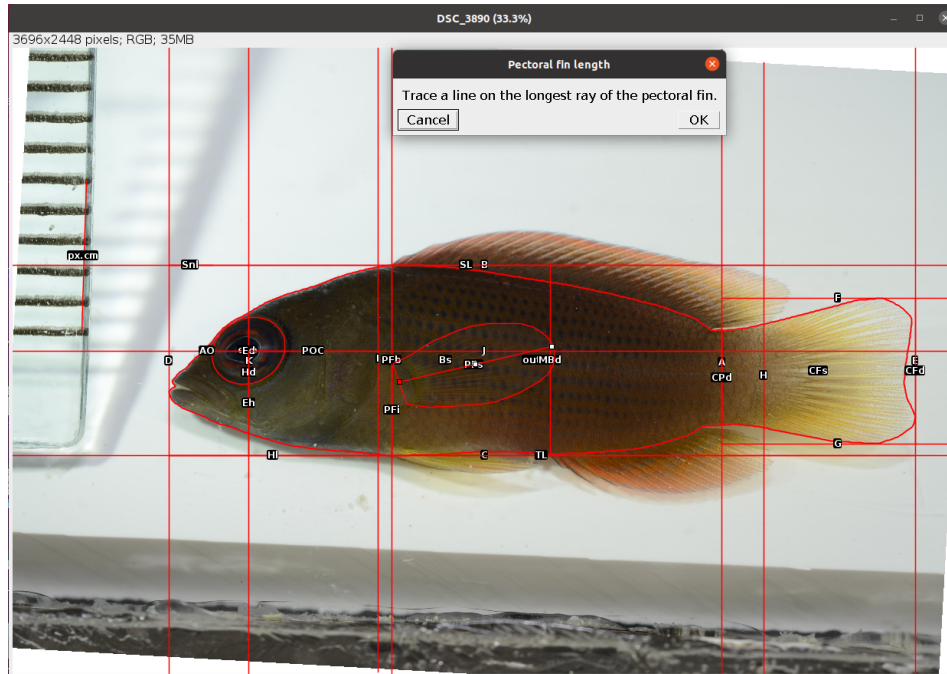
After clicking OK reference line L appears passing through the selected point and 14 new traits are drawn and added to the ROI manager together with all reference lines.

10. Select the outline of the pectoral fin as in the example below. After clicking OK this is saved in the ROI manager as PFs.
11. Trace a line on the longest ray of the pectoral fin. After clicking OK this is saved in the ROI manager as PFl.









12. Place a point at the tip of the premaxilla (upper jaw) as in the example below. After clicking OK a new trait (Mo) is drawn and saved in the ROI manager.

13. Place a point at the corner of the mouth.

**! Important**

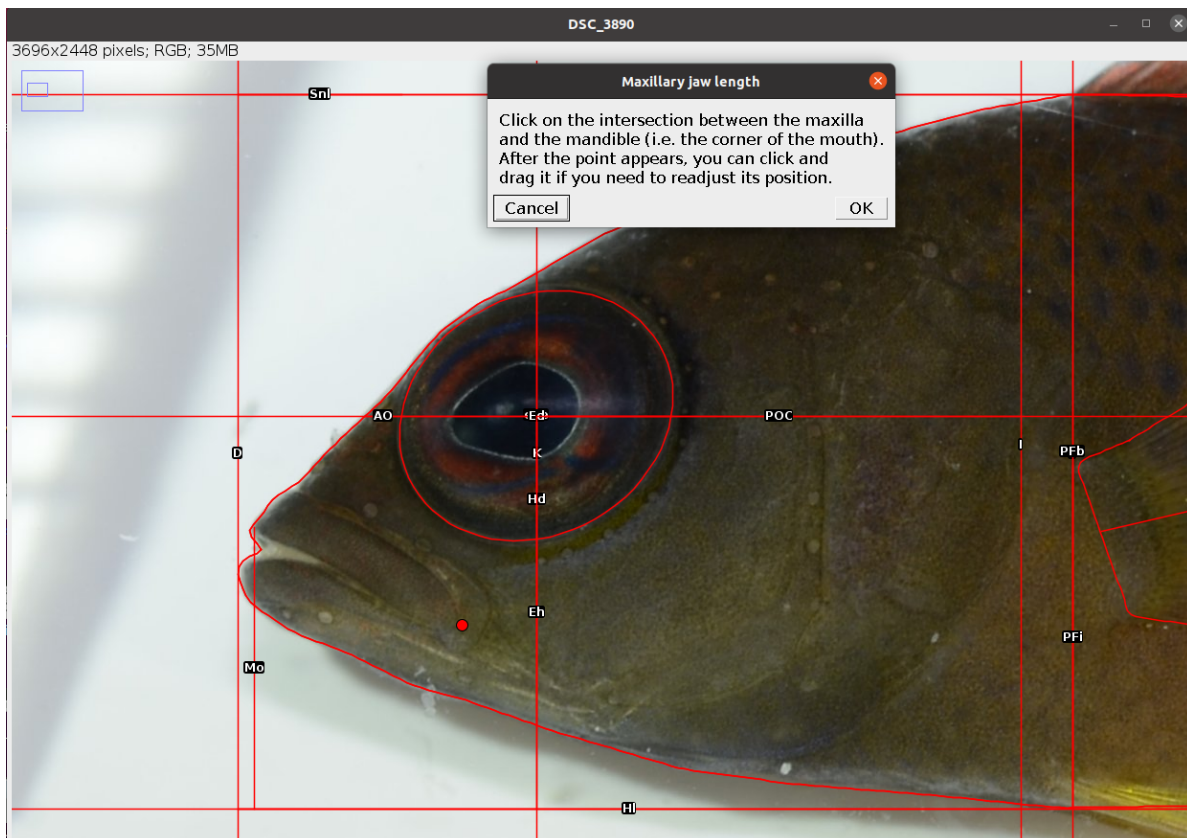
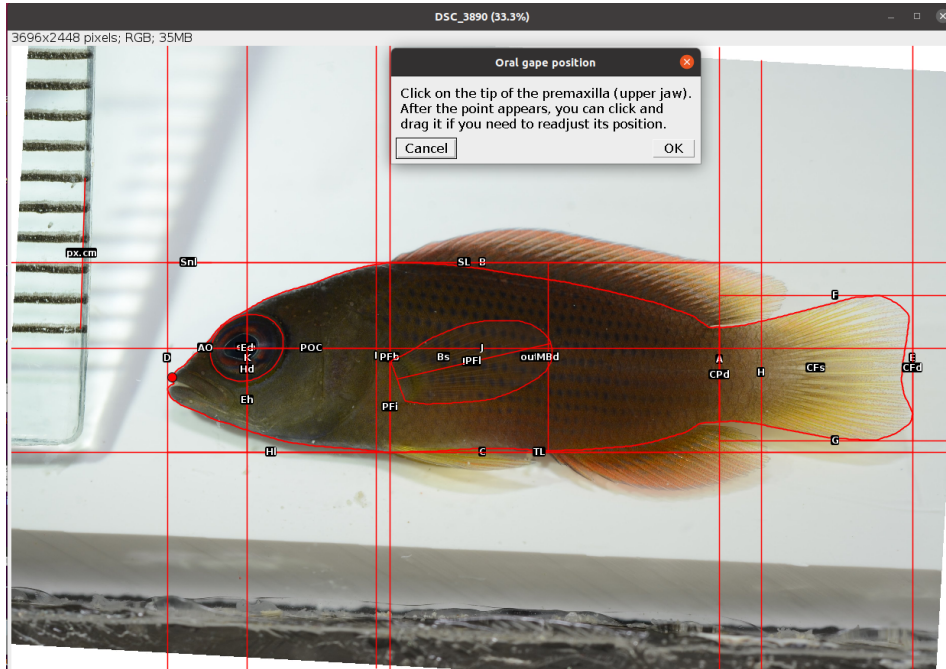
The point should be placed at the intersection between the maxilla and the lower jaw ([fish skull anatomy](#)), not where the flesh of the upper and lower jaw meet! See the example below.

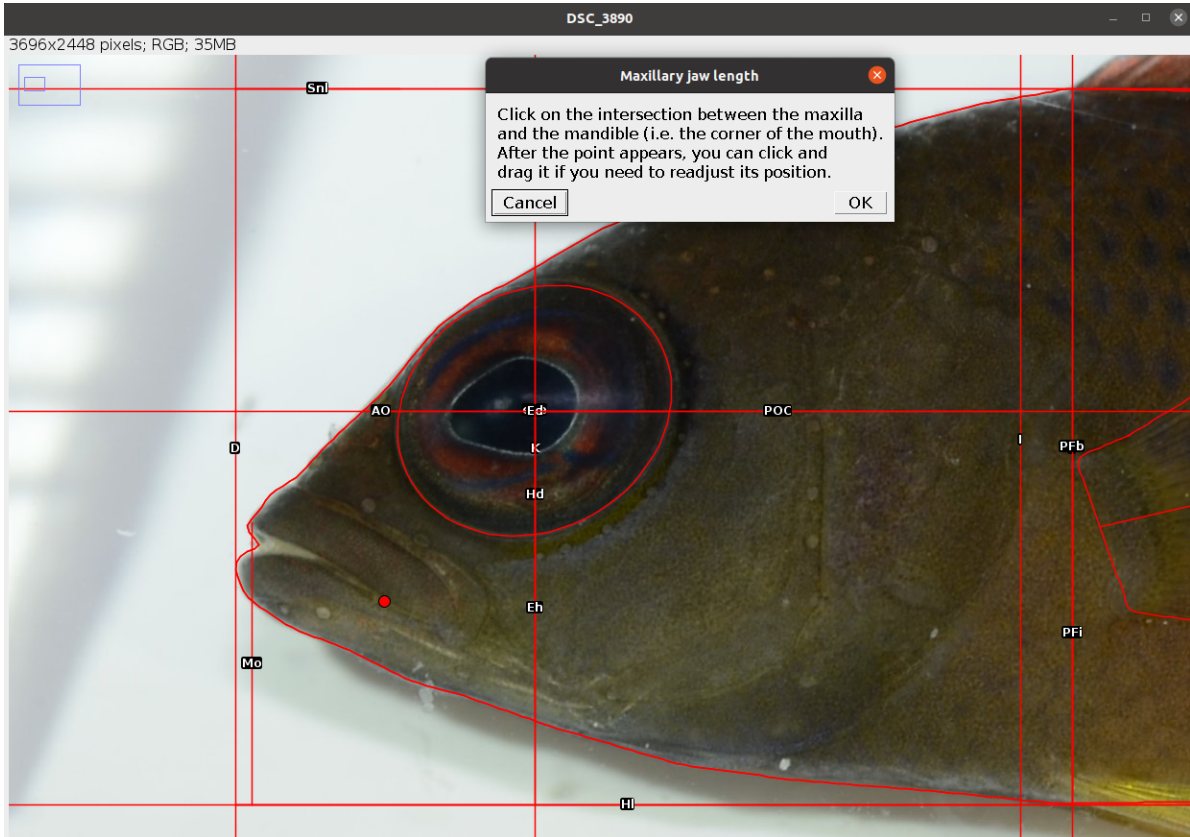
**5.1.0.1 Correct**

**5.1.0.2 Incorrect**

After clicking OK the last three traits are drawn and saved in the ROI manager.

The analysis of the image is completed. In case of single image analysis a window named **Traits** containing all the results appears. This can be saved by clicking on **File -> Save As...** All ROIs in the ROI manager can also be saved as a zip file by clicking on **More >>> -> Save....** In case of multiple image analysis a new row will be added to the results file, the ROIs are saved in their directory, where also the rotated or straightened images are saved







as .jpg files. The current image is closed and the next is opened. Repeat steps 1-13 for all images.

## 5.2 Results

The results file/table contains one row for each image and 25 columns. The first column, `image_id`, is the name of the image without extension. The second column, `px.cm`, is the scale of the image in *pixels/cm*. This will be NA for images without scale. The columns 3-24 are the morphometric measurements described in Table 5.2. All linear measurements are in *pixels* and all areas in *pixels<sup>2</sup>*. The angle `EMa` is in *degrees*. The last column, `time`, is the time spent to analyse the image (steps 1-13) in *seconds*.

## 6 Head Angles

The **Head Angles** analysis allows to extract three angles (Figure 6.1, Table 6.1) related to vision and feeding (Brandl and Bellwood 2013; Bellwood et al. 2014; Brandl, Robbins, and Bellwood 2015) from the coordinates of some reference lines and points (Figure 6.2, Table 6.2).

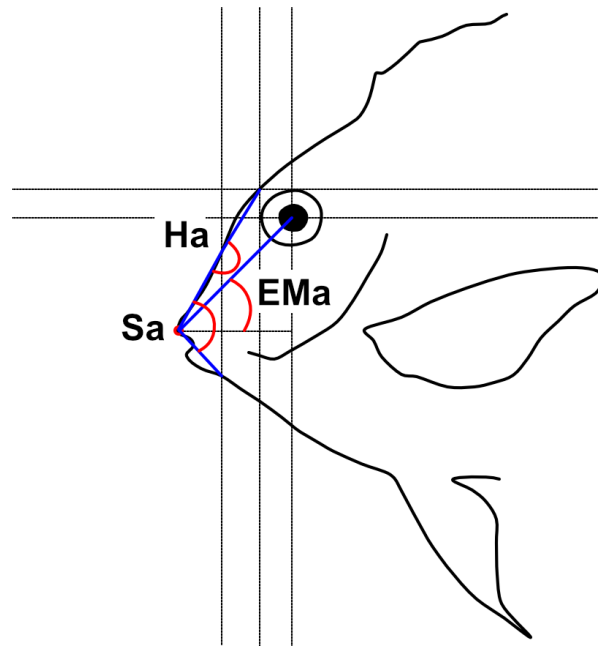


Figure 6.1: Angles measured on fish head (see Table 6.1 for descriptions)

Table 6.1: Angles measured on fish head

Code	Trait	Description	Reference
Ha	Head angle	The angle formed by a line connecting the tip of the premaxilla (P1) to the point where line L5 crosses the upper margin of the snout and a line connecting the latter point to the intersection between lines L3 and L4	Brandl and Bellwood (2013)
Sa	Snout angle	The angle formed by the two lines drawn to the tip of the premaxilla (P1) from the point where line L5 crosses the upper and lower margins of the snout	Brandl and Bellwood (2013)
EMa	Eye-mouth angle	The angle formed by a line connecting the tip of the premaxilla (P1) to the eye centroid and a horizontal line intersecting P1	Bellwood et al. (2014)

Table 6.2: Description of reference lines and points traced on fish images for head angles analysis

Reference line/point	Description	User input
P1	Anterior tip of the premaxilla (upper jaw)	Yes
L1	Horizontal line cutting the eye in halves	No <sup>1</sup>
L2	Vertical line cutting the eye in halves	No
L3	Vertical line touching the anterior margin of the eye	No
L4	Horizontal line touching the upper margin of the eye	No
L5	Vertical line half way between L3 and P1	No

<sup>1</sup>The user has to trace an ellipse around the eye and lines L1-L4 will be automatically drawn from the coordinates of this selection.

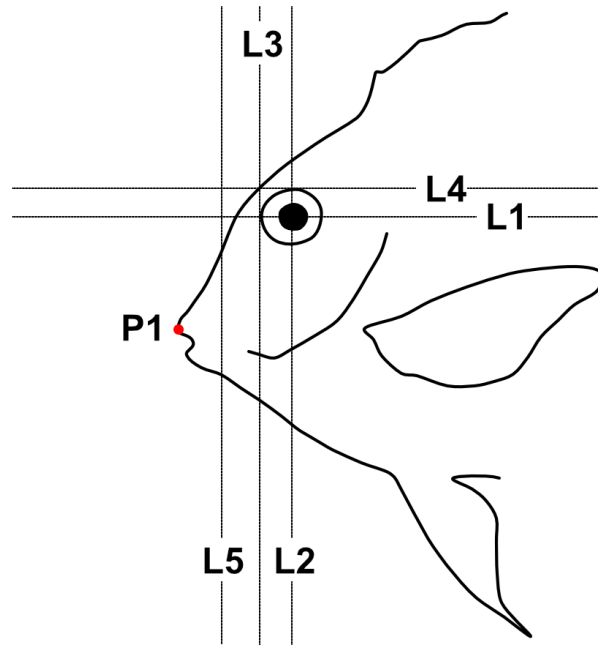


Figure 6.2: Reference lines and points traced on fish images for head angles analysis (see Table 6.2 for descriptions)

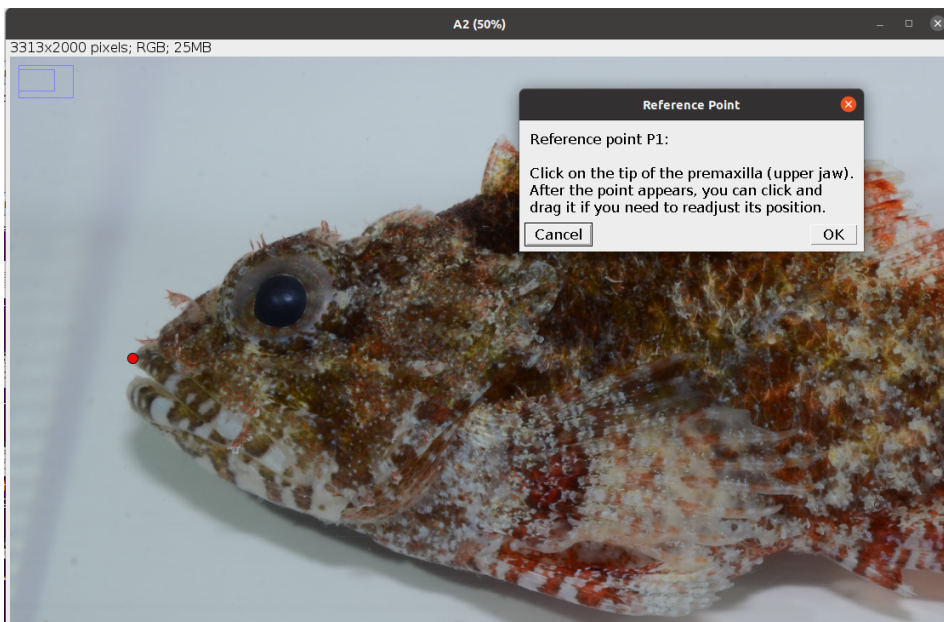
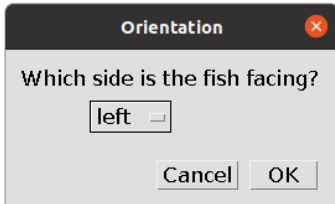
## 6.1 Analysis

Once the steps described in Section 2.2 are completed the screen will be populated with a number of windows:

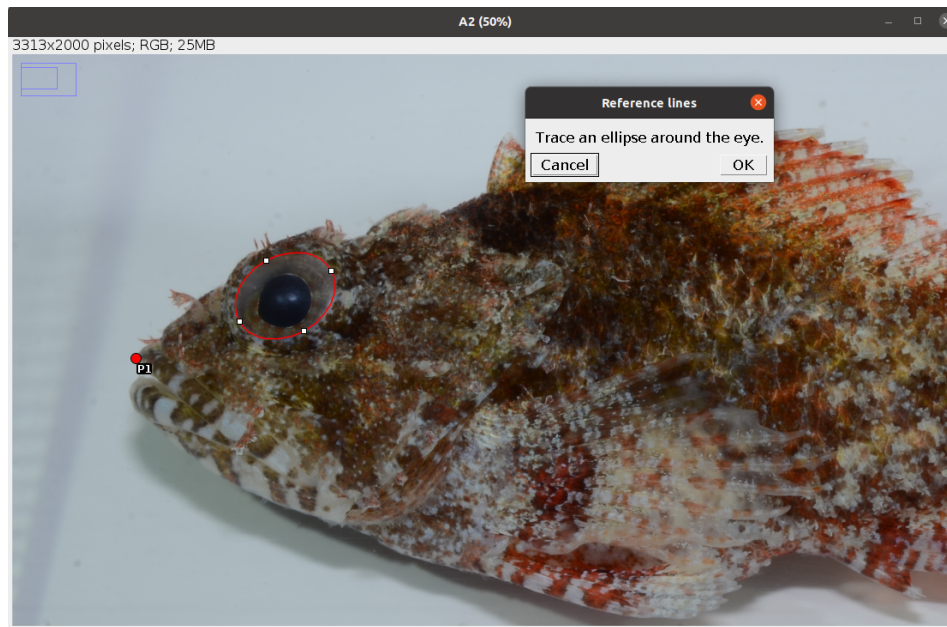
- the ImageJ/Fiji main window
- the MorFishJ GUI
- a fish image (this is a duplicate of the raw image to prevent any modification)
- the ROI manager
- the Image adjustment dialog

This analysis has six steps that require the user input:

1. Adjust the image if necessary as described in Chapter 4.
2. Select the orientation of the fish (i.e., whether the fish is facing left or right) from a drop-down list. This step is important for the correct automatic placement of the reference lines.
3. Place a point at the tip of the premaxilla (upper jaw) as shown below. After clicking OK this is saved in the ROI manager as P1.



- Trace an ellipse around the eye as shown below (see [Elliptical Selection Tool](#) for specific instructions on how to use this tool). After clicking OK five reference lines (L1-L5) are drawn and saved in the ROI manager together with the ellipse (eye).

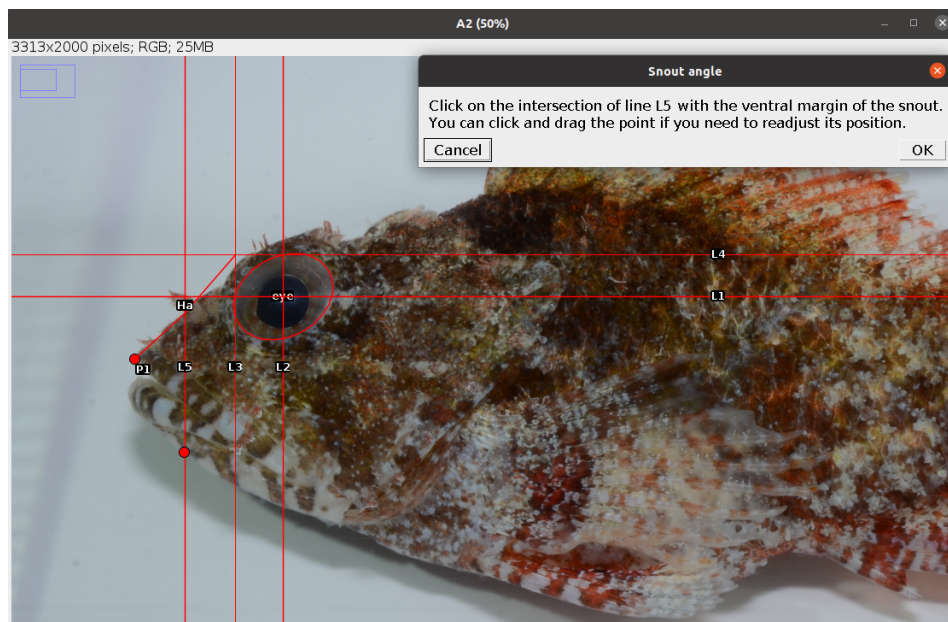
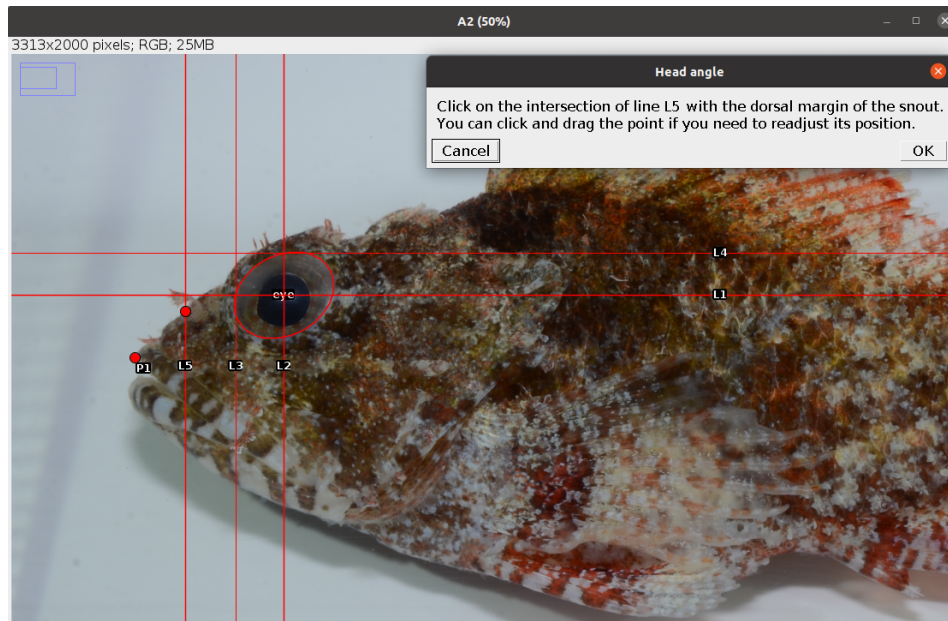


- Place a point at the intersection between line L5 and the dorsal margin of the snout as shown below. After clicking OK the first angle ( $H_a$ ) is drawn and saved in the ROI manager.
- Place a point at the intersection between line L5 and the ventral margin of the snout as shown below. After clicking OK the other two angles ( $S_a$ ,  $EM_a$ ) are drawn and saved in the ROI manager.

The analysis of the image is completed. In case of single image analysis a window named **Traits** containing all the results appears. This can be saved by clicking on **File** -> **Save As...**. All ROIs in the ROI manager can also be saved as a zip file by clicking on **More** >>> -> **Save...**. In case of multiple image analysis a new row will be added to the results file, the ROIs are saved in their directory, where also the rotated or straightened images are saved as .jpg files. The current image is closed and the next is opened. Repeat steps 1-6 for all images.

## 6.2 Results

The results file/table contains one row for each image and 5 columns. The first column, **image\_id**, is the name of the image without extension. The columns 2-4 are the angles



described in Table 6.1 in *degrees*. The last column, `time`, is the time spent to analyse the image (steps 1-6) in *seconds*.



# 7 Gut Traits

The **Gut Traits** analysis allows to measure three intestinal traits related to fish diet (Table 7.1).

Table 7.1: Morphometric parameters measured on fish intestine (see Figure 7.1)

Code	Trait	Description	Reference
GL	Gut length	Length of the intestine from the pyloric outlet to the anus in fishes with a stomach, or from the oesophagus to the anus in stomachless fishes	Ghilardi et al. (2021)
GD	Gut diameter	External diameter of the intestine (average of 10 measurements: GD1-GD10)	Ghilardi et al. (2021)
GS	Gut surface area	External surface area of the intestine calculated as $GL \times 2\pi(GD/2)$	Ghilardi et al. (2021)

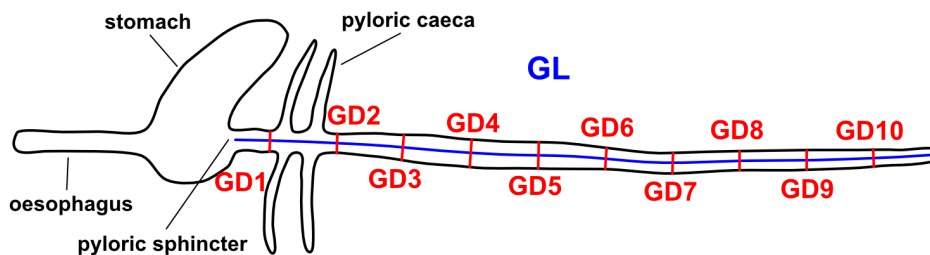


Figure 7.1: Morphometric parameters taken on fish intestine

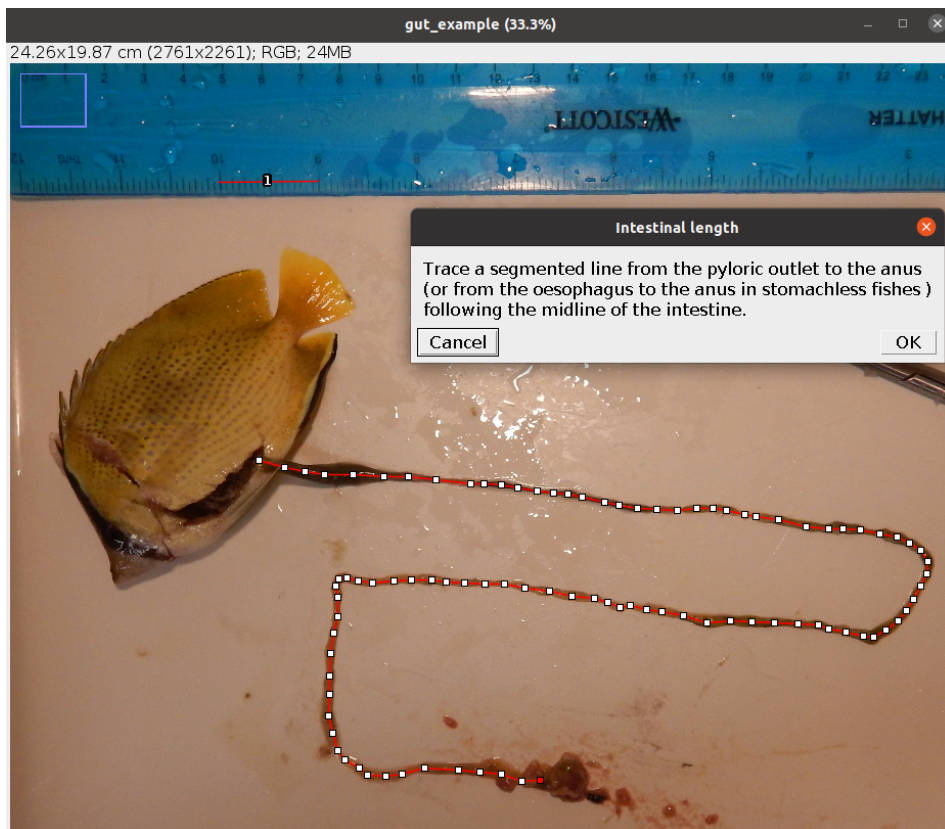
## 7.1 Analysis

Once the steps described in Section 2.2 are completed the screen will be populated with a number of windows:

- the ImageJ/Fiji main window
- the MorFishJ GUI
- a fish image (this is a duplicate of the raw image to prevent any modification)
- the ROI manager
- the `Set scale` dialog

This analysis has a few steps that require the user input:

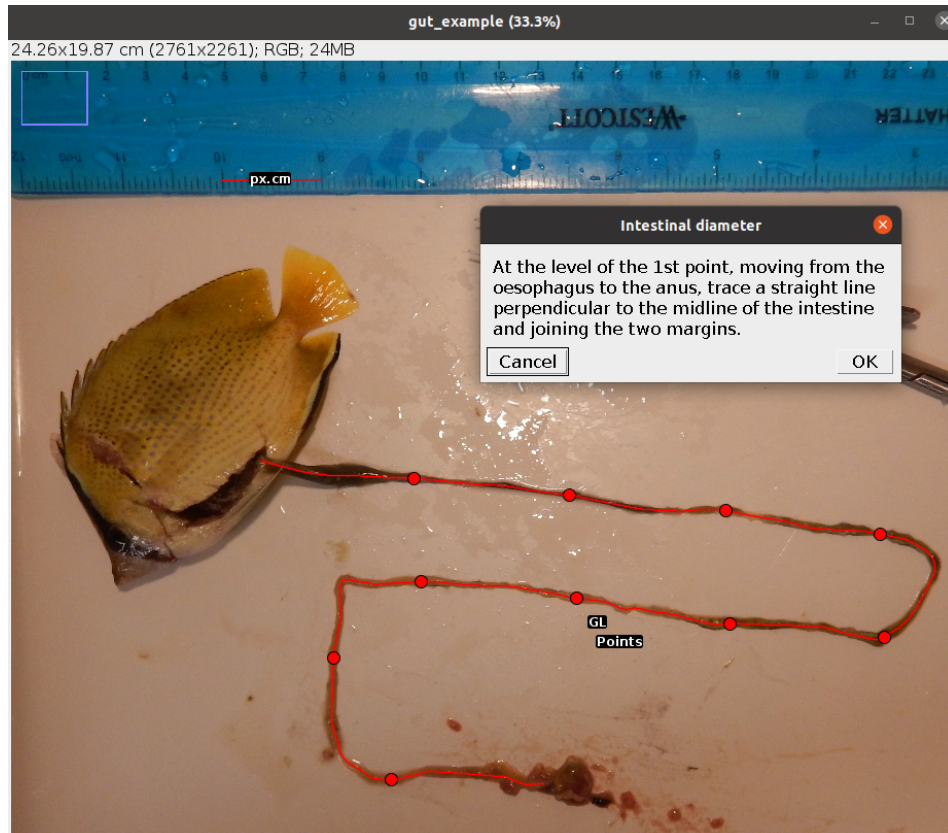
1. Set the scale for the image as described in Chapter 3.
2. Trace a segmented line following the midline of the intestine as shown below.



After clicking OK 10 points appear equally distributed along this line.

3. At the level of the point closest to the oesophagus, trace a straight line perpendicular to the midline traced in the previous step and joining the two margins of the intestine.
4. Repeat step 3 for each point in order moving towards the anus. See example below.

The analysis of the image is completed. In case of single image analysis a window named **Traits** containing all the results appears. This can be saved by clicking on **File -> Save**





As . . . . All ROIs in the ROI manager can also be saved as a zip file by clicking on **More >>>** -> **Save . . . .** In case of multiple image analysis a new row will be added to the results file and the ROIs are saved in their directory. The current image is closed and the next is opened. Repeat steps 1-4 for all images.

## 7.2 Results

The results file/table contains one row for each image and 16 columns. The first column, **image\_id**, is the name of the image without extension. The second column, **px.cm**, is the scale of the image in *pixels/cm*. The columns 3-5 are the traits described in Table 7.1 in *cm* ( $cm^2$  for GS). The columns 6-15 are the 10 raw measurements of the diameter (GD1-GD10) in order from the oesophagous to the anus. The last column, **time**, is the time spent to analyse the image (steps 1-4) in *seconds*.

## References

- Barnett, A., D. R. Bellwood, and A. S. Hoey. 2006. “Trophic Ecomorphology of Cardinalfish.” *Marine Ecology Progress Series* 322: 249–57. <https://doi.org/10.3354/meps322249>.
- 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>.
- Brandl, S. J., W. D. Robbins, and D. R. Bellwood. 2015. “Exploring the nature of ecological specialization in a coral reef fish community: morphology, diet and foraging microhabitat use.” *Proceedings of the Royal Society B: Biological Sciences* 282 (1815): 20151147. <https://doi.org/10.1098/rspb.2015.1147>.
- Ghilardi, M., N. M. D. Schiettekatte, J. M. Casey, S. J. Brandl, S. Degregori, A. Mercière, F. Morat, Y. Letourneur, S. Bejarano, and V. Parravicini. 2021. “Phylogeny, body morphology, and trophic level shape intestinal traits in coral reef fishes.” *Ecology and Evolution* 11 (19): 13218–31. <https://doi.org/10.1002/ece3.8045>.
- Toussaint, A., N. Charpin, S. Brosse, and S. Villéger. 2016. “Global Functional Diversity of Freshwater Fish Is Concentrated in the Neotropics While Functional Vulnerability Is Widespread.” *Scientific Reports* 6: 22125. <https://doi.org/10.1038/srep22125>.
- Villéger, S., J. R. Miranda, D. F. Hernández, and D. Mouillot. 2010. “Contrasting Changes in Taxonomic Vs. Functional Diversity of Tropical Fish Communities After Habitat Degradation.” *Ecological Applications* 20 (6): 1512–22. <https://doi.org/10.1890/09-1310.1>.