

# Counting amphibian eggs and larvae

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Special thanks to Jessica Seratt for her contribution to the computer analysis part of this document.

Techniques used to count eggs include the 'DISPLACEMENT METHOD', 'IMAGE ANALYSIS', and 'DIRECT COUNTING'.

**'DISPLACEMENT METHOD'** This method is fast, yields immediate results for large numbers of eggs, enables field counts with replacement of eggs, and is reasonably accurate. In the displacement method a sub-sample of several hundred eggs are counted and their volume measured (usually in a measuring cylinder).



The volume of the total egg mass (including the sub-sample) is then

measured and the total number of eggs calculated.

Inaccuracies with this method can occur through differences in the gel and egg size between different parts of the spawn mass. This is particularly the case in some experimental situations where spawn masses may be of low quality with variable gel and egg quality.



SN = sub sample number.

SV = sub sample volume.

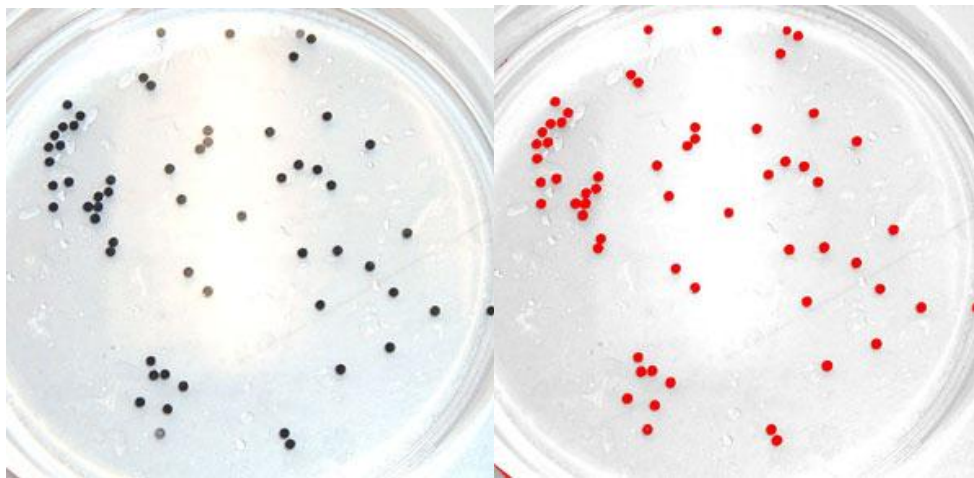
TV = total volume.

TN = total number

$$TN = TV/SV \times SN$$

**'IMAGE ANALYSIS'** counts eggs from an image of the spread eggs against a contrasting background. Inaccuracies with this method can occur through overlapping eggs making counting difficult. Overlapping eggs are a particular problem where the eggs are in a globular mass rather than in strings. To some extent image analysis can also be used for larvae if they are in shallow trays. The number of eggs can also be counted and marked off digital images by hand.

The eggs must be separate. Separation of eggs is easier with partial removal of the gel coat. see "Gel removal and extract". The use of the ImageJ™ program to automatically count eggs is described at the end of this document "Image analysis of object counts with ImageJ™"

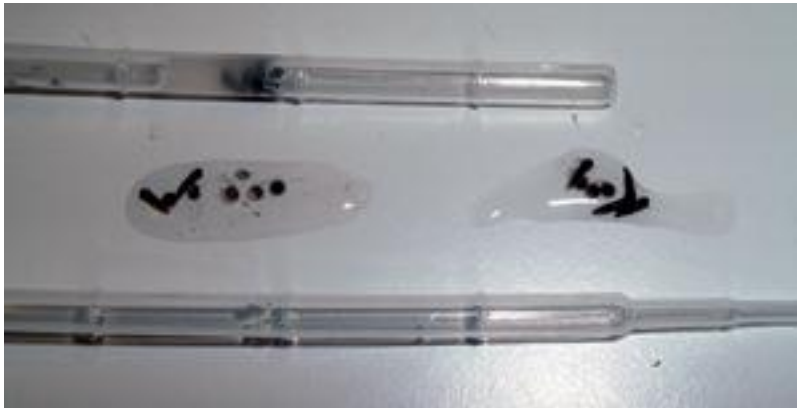


ImageJ™ program allows for easy counting of eggs. It automatically counts all objects it recognizes in the final red image (right).

See at documents end: Image analysis of object counts with ImageJ™

**'DIRECT COUNTING'** with pipettes by an experienced operator produces an exact count of eggs or larvae. However, direct counting can only be used when the eggs are dissociated (see **Reproduction technology > Gel removal and extract**), or when the larvae have separated from the gel naturally or naturally.

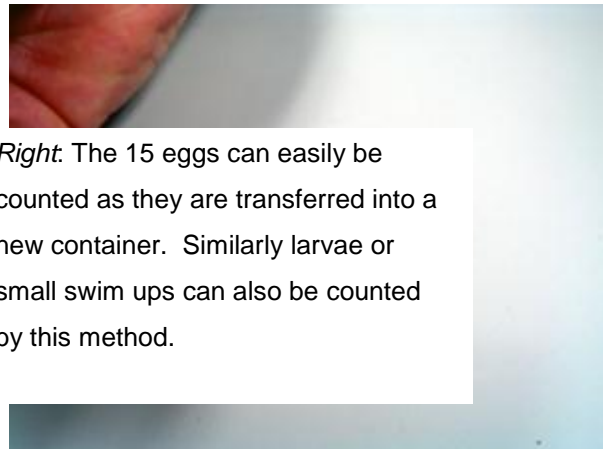
The end of a disposable plastic pipette is cut to leave the maximum open tube. Eggs or larvae - up to just below the diameter of the tube - can then be aspirated and counted and placed in a new container. This method is often used to count when separating larvae from infertile eggs to prevent poor water quality causing mortalities.



*Left:* A complete disposable pipette (below) and one with the end removed (above) with toad eggs (middle left) and larvae (middle right). Plastic disposable pipettes are graduated in diameter at the ends. The pipette is generally best cut off to give best adjusted to about 1.5 the diameter of the eggs or larvae. Toad eggs are shown in the middle left and larvae in the middle right.



*Left:* A petri dish of mainly infertile toad eggs and some larvae about 48hrs after spawning. As the larvae develop and hatch they excrete enzymes that dissolve the egg gel. This also enables the infertile eggs to release their yolk into the water creating an ideal environment for bacterial growth.



*Right:* The 15 eggs can easily be counted as they are transferred into a new container. Similarly larvae or small swim ups can also be counted by this method.



*Right:* To prevent hypoxic and unsanitary water the larvae must be moved to a new container with fresh water. This is easily and quickly accomplished using a disposable plastic pipette.

## Image analysis of object counts with ImageJ™

Special thanks to Jessica Seratt for her wonderful contribution to the computer analysis part of this document.

Using ADOBE Photoshop™ to get the best image for analysis.

1. Take an image with camera of eggs. White background for black eggs and black background for white eggs. The eggs must be separate. Separation of eggs is easier with partial removal of the gel coat. see “Gel removal and extract”
2. Open image in Adobe Photoshop™
3. Image > Mode > Greyscale
4. Image > Adjust > Brightness/Contrast. Adjust the contrast and brightness to maximize separation of the eggs from the background.
5. Delineate the area with eggs with the Elliptic or Rectangular marquee
6. > Cut
7. > Create new file > OK
8. Paste
9. Image > Levels - Adjust to distinguish eggs
10. Filter > Noise > Despeckle
11. Save as tif for analysis in image analysis program.

#### Analysis of image with Image J™

1. Open in” ImageJ™” 1.33u: Wayne Rasband, National Institutes of Health, USA;  
<http://rsb.info.nih.gov/ij/>
2. process > sharper
3. Process > noise > despeckle
4. Image > adjust threshold – image goes red
5. Analyse > particles
6. Show “masks” , “display results”, “summarise” > OK
7. The “Image J™” program then produces a count of objects and a summary of average size in pixels, and distribution of size. It also produces image of counted objects