

Photographing amphibian larvae for morphometric measurements

Tadpoles are photographed inside a Plexiglas cuvette mounted on a mirrored stage. This yields a photograph containing two images (side-view and bottom-view). Pictures and dimensions of the cuvette and photography stage are on the following pages.

Illuminating the subject is very important. The camera can focus more easily if you set up bright directional lights positioned to either side of the stage; fiber-optic light tubes are good. Currently, I get the sharpest images if I use the camera's built-in flash together with a slave flash aimed directly at the tadpole.

Make sure there is no bright overhead light. This can ruin the background of the ventral view.

Tadpoles are removed gently from the experiment using a net without a deep pouch. Place them into a small bin and take them to the room where the photography apparatus is set up.

Tadpoles should be processed promptly after removal from the mesocosms. When held outside the experiment, tadpoles lose 2% of their mass during the first 30 minutes and nearly 15% over 350 minutes (measured on stage 35 *Rana temporaria* tadpoles weighing about 650 mg at the outset).

Tadpoles are anaesthetized lightly in benzocaine just before photography. The stock solution is 10% benzocaine powder by weight with ethanol. About 3-5 drops of this in 200 ml water makes a solution that knocks tadpoles out in about 2-3 minutes. There is variation among species in their sensitivity to benzocaine, so do some testing if you are unsure.

A small piece of screen is used to place the anesthetized tadpole into the cuvette, and remove it after the photos have been taken. I use a small pointed object (blade of grass, for example) to manipulate the tadpole's position so that it is perpendicular to the camera, parallel to the bottom surface of the cuvette, and oriented vertically. Take care that all three of these are fulfilled, or you will regret your haste later on when making measurements.

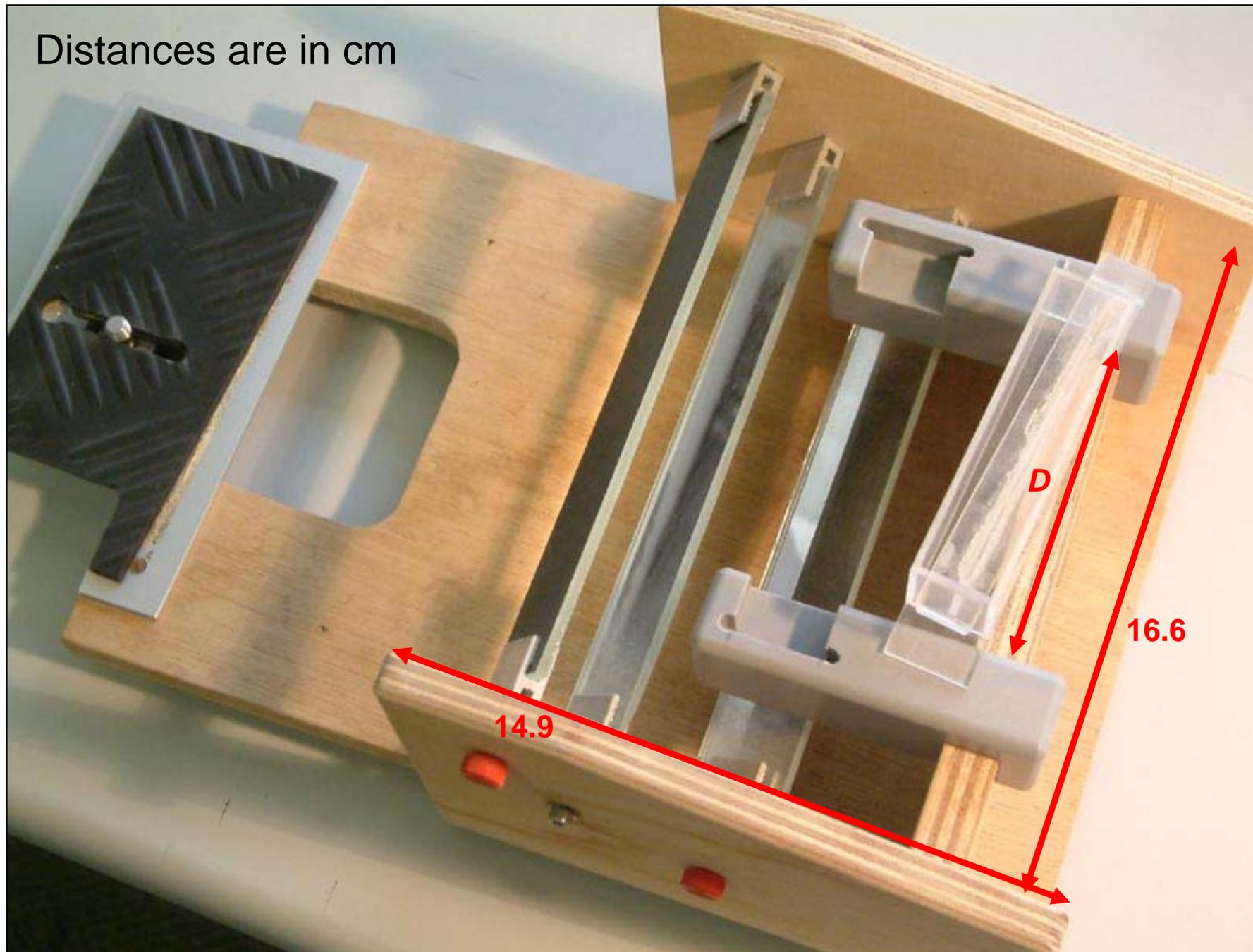
Estimates of repeatability indicate that presentation error is the greatest problem. It is not worth measuring an image twice, and it is not worth having more than one person measure each image. However, it is very worthwhile to take multiple independent images of each tadpole and measure all of them.

After photography, the tadpole is returned to its bin of fresh water. It usually regains consciousness within a minute.

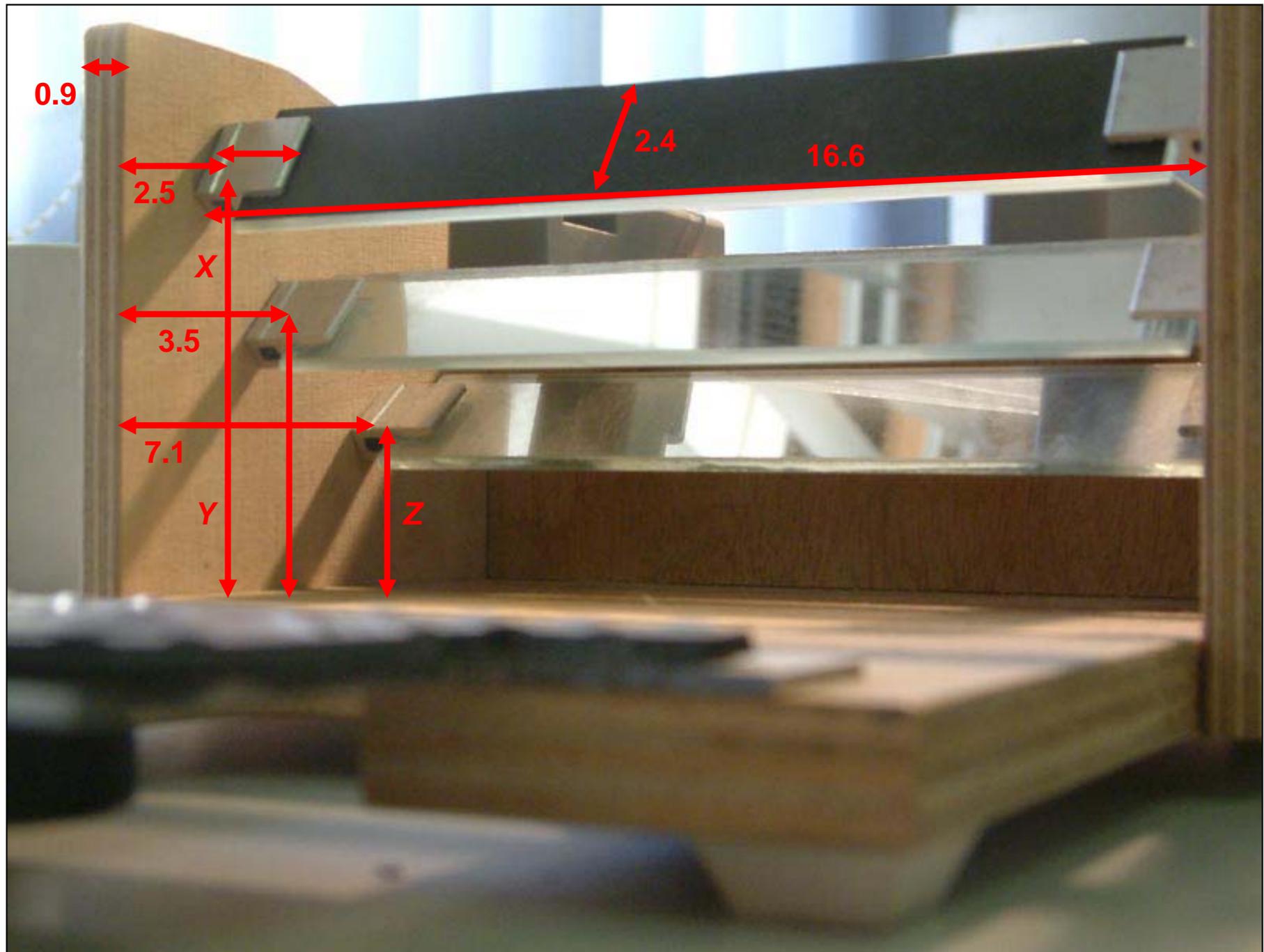
Calibration. The side and bottom of the cuvette have two small X's carved into them, and the exact distance between these X's is known. The thickness of the Plexiglas is known. The side-view indicates how far above the bottom of the cuvette the tadpole is positioned; the bottom-view indicates how far back the tadpole is from the front of the cuvette. All this information allows you to calculate a unique calibration for each image.

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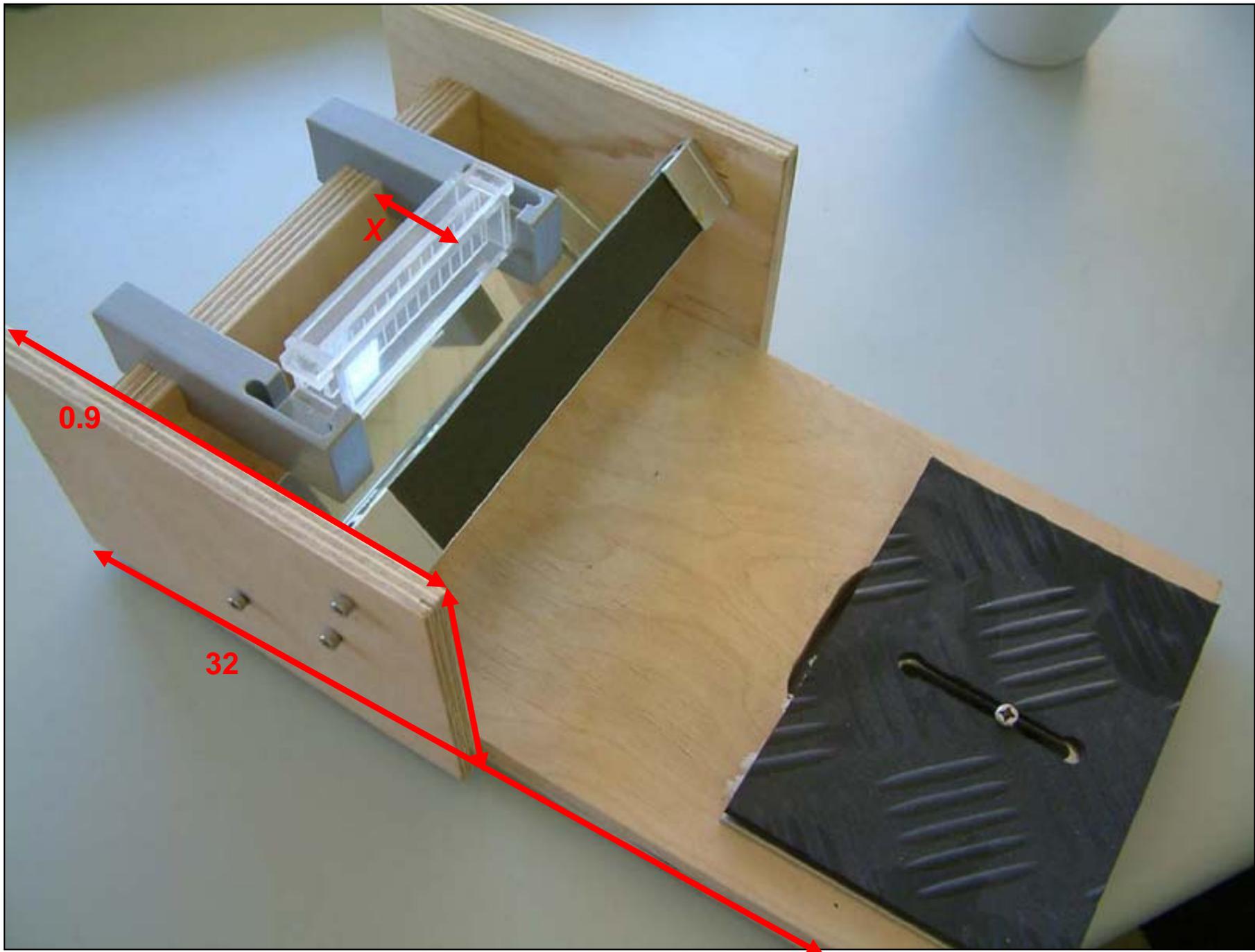
Distances are in cm



Distance D will depend on the cuvette that you build. I have several different cuvettes, so this distance is adjustable (i.e., the two grey plastic pieces can slide side-to-side).



Distances X, Y, and Z (the height of the mirrors) will depend on the camera you use. I designed mine for a Nikon FM2, but now it turns out to work pretty well with the Panasonic DMC-FZ18 and Sony DCS-HX1 that I currently use. The heights of the attachment points of the three mirrors, measuring from the bottom, are approximately 2.6, 4.5, and 6.9 cm. Distances from the front edge are to the attachment point of the mirror.



Distance X , from the wooden back wall to the front of the Plexiglas cuvette, is 5.0 cm.

I have a few different sizes of these cuvettes. The one with the measurements given below is good for *Rana temporaria* and even fairly large *Alytes*, but it's not big enough for the largest tadpoles or *Triturus cristatus* larvae.

