



Video Transcript How To Relax An Ascidian

Rosana M. Rocha

- Ascidians have strong muscles in their body walls, so it is important to relax them for fixing, otherwise you have animals too contracted for dissection and for the study of their internal anatomy.
- So, here we have a few animals, you can see that these specimens are opening and closing their siphons, so this is the time to start relaxing them.
- To relax an ascidian, we use mostly menthol.
- Menthol comes in two ways: These are crystals, and you only need to pour a little bit on the surface of the water.
- I am going to use crystal menthol in one of the colonies and these solitaries.
- And then, you cover your bowl and menthol will be concentrated in the seawater.
- You can also find menthol as an oil resin.
- Although it is oil, it will mix with seawater well and you have to mix them one day before using a bottle.
- To prepare the solution of sea water with menthol, you just need 1 or 2 caps of the resin in here and pour it together with sea water using this bottle size.
- Every time you are going to use it, shake it again to be sure the menthol completely mixes with the seawater.
- There is not a certain amount, just pour a little bit, and with practice, you are going to see how much the animals need.
- If you don't have any of these kinds of menthol, you can also use some menthol candy. The strongest ones will be better.
- Together with the menthol you can also use other procedures with animals that take more time to relax.
- Magnesium chloride is a possibility.
- You need an isotonic solution and you are going to pour the same volume of sea water that you already have in your sample.
- You can also take your bowls to the fridge and some animals will relax faster.
- After an hour, we are going to check our animals.
- Well, looks like they are still moving, not well relaxed yet, let's take this other one... yes, still moving, so we have to leave them a little bit longer in the menthol.
- With menthol, you have to check your animals after one hour to see if they are moving or still closing their siphons.
- If you are going to use the magnesium chloride or the fridge, you have to check them every 30 minutes.
- I am going to check again if the animal is already relaxed.

- The siphon is wide open, so it looks good, and we touch it gently... it is not moving anymore, so I think this animal is ready for fixation.
- I am going to fix the animal in formalin at 10%.
- I have already the formaldehyde in this jar; use gloves, because you know formaldehyde is toxic, and take the animal upside-down, so you can pour the liquid through the siphons but do not squeeze the animal, and be sure that the level of the formaldehyde is covering the whole animal.
- Next step is to label your jar.
- Remember, this is formaldehyde, so take your label with forceps and put it inside the jar. The label should have general information such as the point of collection, date of collection and your sample number.
- Also, notice that I fixed the whole animal with all the other tunicates on its tunic.
- It is better to fix them like this, and then try to separate the animals from each other after they are in formaldehyde for a few days.
- NOW WITH COLONIAL ASCIDIANS
- So, now let's check if the animal is ready for fixation.
- See, it is completely open and I will touch gently and see that the siphons do not close anymore.
- So it is ready, and I'm going to fix it in formaldehyde too, so this jar has formaldehyde already, and I am going to fix the colonial with the substrate.
- Pour the excess of sea water, and put the animal in the jar.
- Do not forget to label your sample.
- If the samples are going to be used in molecular studies, it is important to fix them in ethanol.
- You can fix the whole specimen or a piece of the colony for instance, and have a voucher of the rest of the colony in formaldehyde.
- Even for molecular studies, it is important to relax the animals in menthol before fixing them, this way the ethanol will penetrate better into the tissues.
- After 48 hours, it is a good idea to change the ethanol because it can be diluted in the liquids of the internal tissues of the animals.
- As a last tip, keep the animals, or keep the samples inside a closed cabinet because formaldehyde degrades in sunlight.

