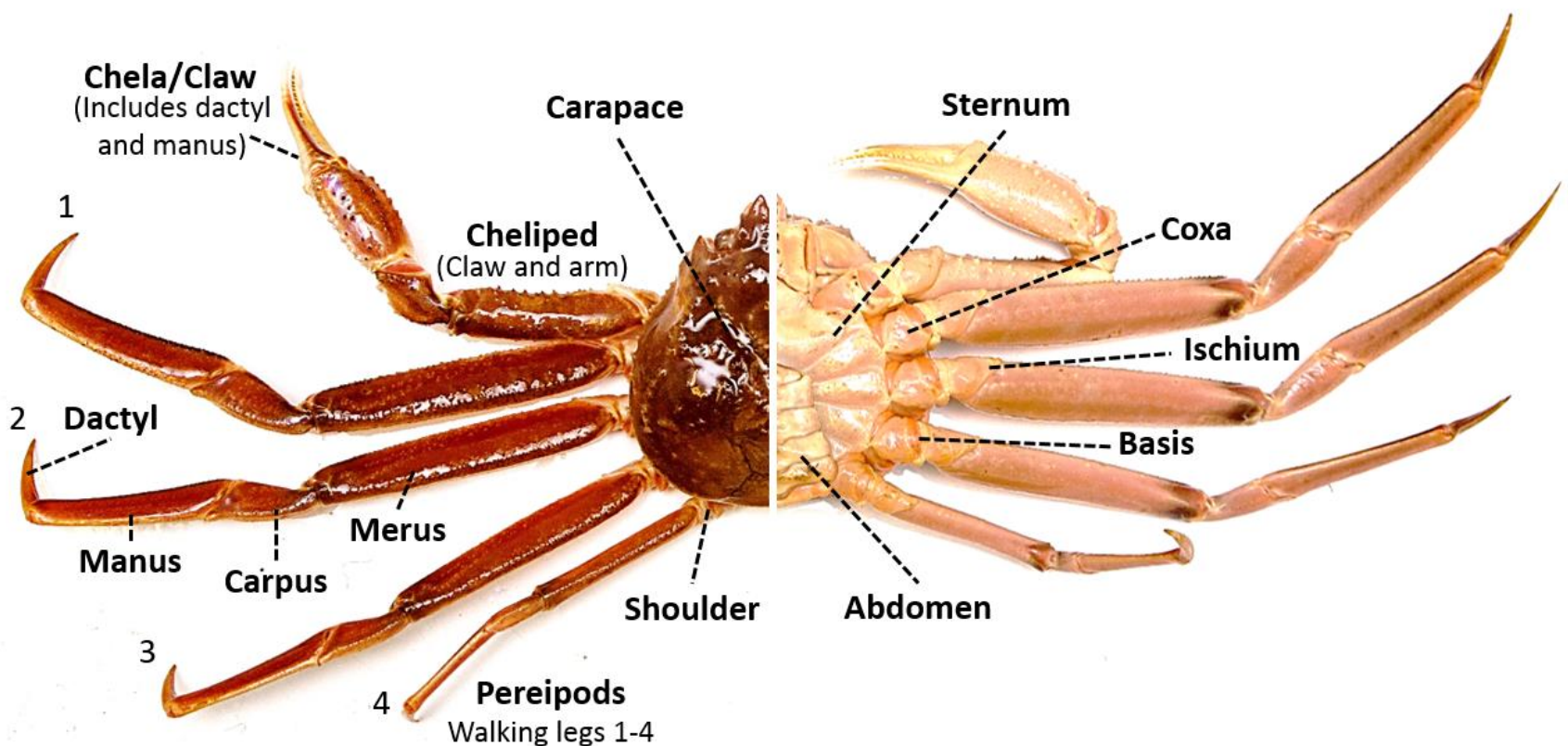


Protocol for sampling and dyeing of Snow crab hemolymph



This protocol is developed for easy detection of the parasite Hematodinium in Snow crab hemolymph.

Hematodinium is the cause “Bitter crab disease” in a wide range of crab species, including Snow crab.

Financing partner:



HEMOLYMPH SAMPLING

Equipment: Syringe (5ml), needle (0.4 x 19 mm)



1

Place the crab on its back.



2

Bend the two rear legs backwards to spread the shoulder joint.

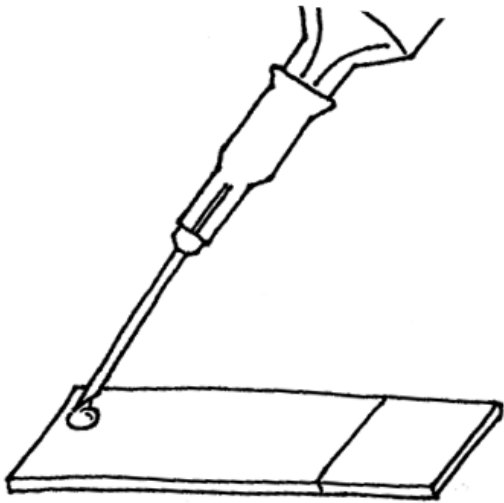


3

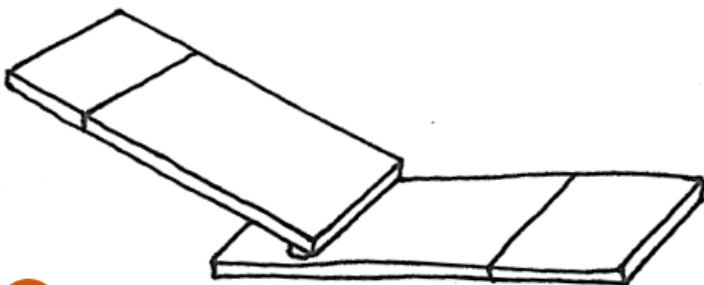
Insert the needle, parallel to the leg, half way in to the joint. Keep the needle steady and withdraw the sample (approximately 1 mL).

HEMOLYMPH SMEAR

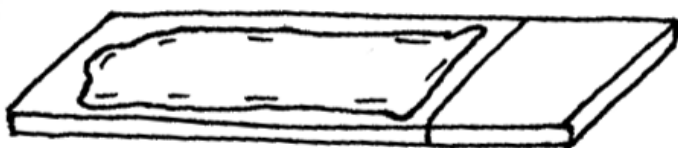
Equipment: Clean glass slides, methanol, collection tube (50 ml) and slide storage box



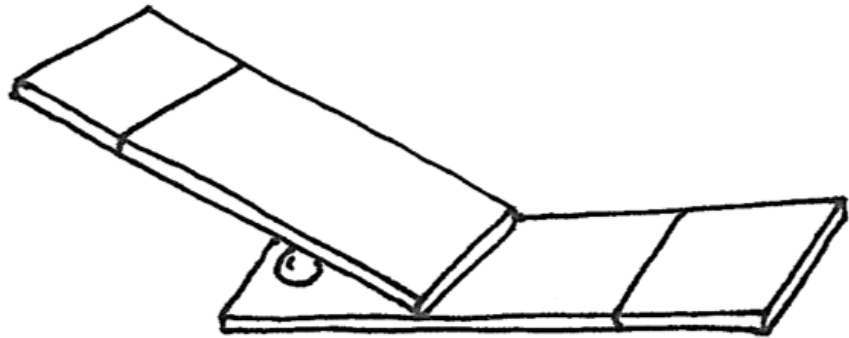
- 1 Place one drop (pinhead size) at the end of the glass slide.



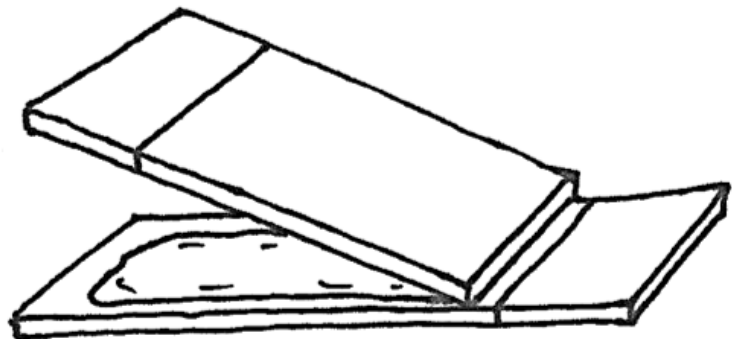
- 3 Draw the second slide backwards against the hemolymph droplet.



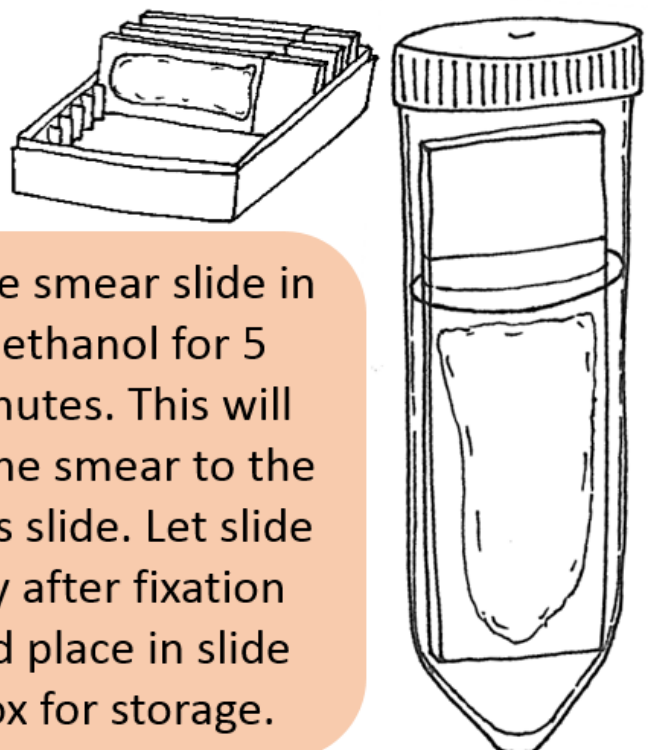
- 5 Remove second slide. Air dry the smear for approximately 10 minutes. Shake slide to decrease drying time.



- 2 Place the second slide in front of the drop. Hold the slide at 30-45° angle.



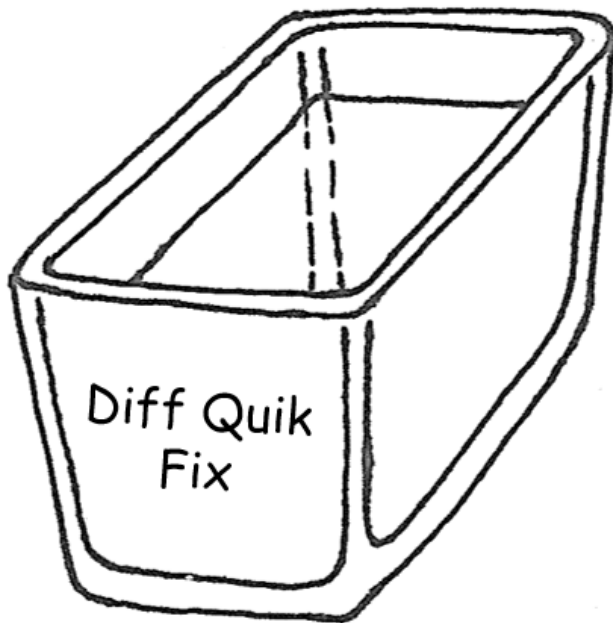
- 4 With a firm and steady pace, push the second slide forward to make the smear. End the smear ½ cm from the writing field. Maintain contact between the two slides.



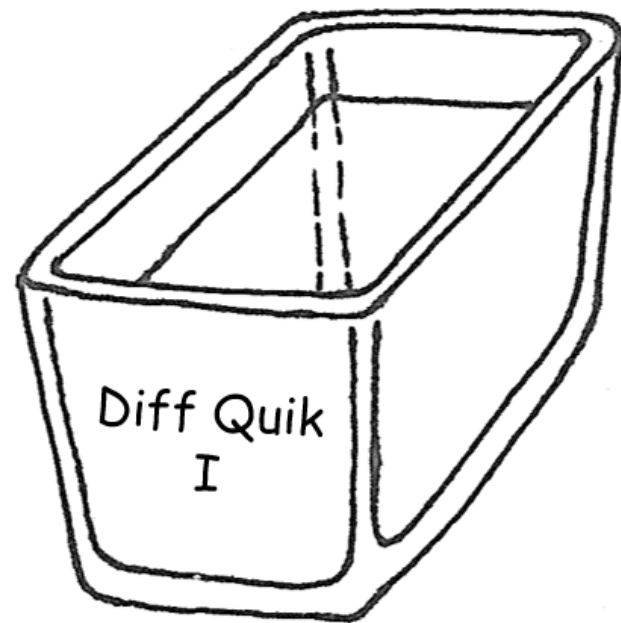
- 6 Place smear slide in methanol for 5 minutes. This will fix the smear to the glass slide. Let slide dry after fixation and place in slide box for storage.

STAINING HEMOLYMPH SMEAR

Equipment: Diff Quik staining solutions, staining jar and rack, distilled water

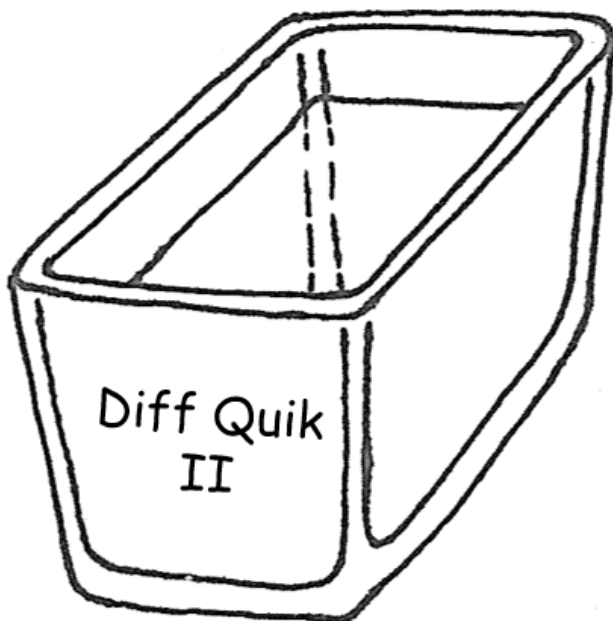


- 1 Dip smear slides 5 times for 1 second in Diff Quik fixative solution.



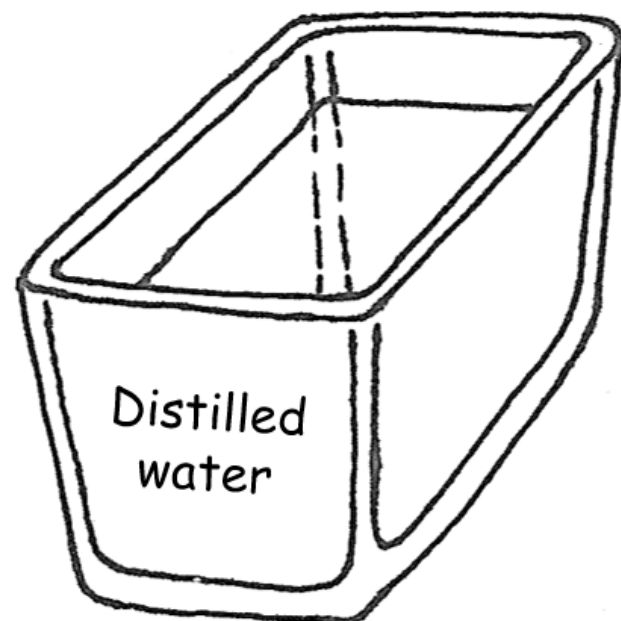
- 2 Dip smear slides 5 times for 1 second in Diff Quik Stain solution I.

Let excess solution drip off between each dip.



- 3 Dip smear slides 3 times for 1 second in Diff Quik Stain solution II.

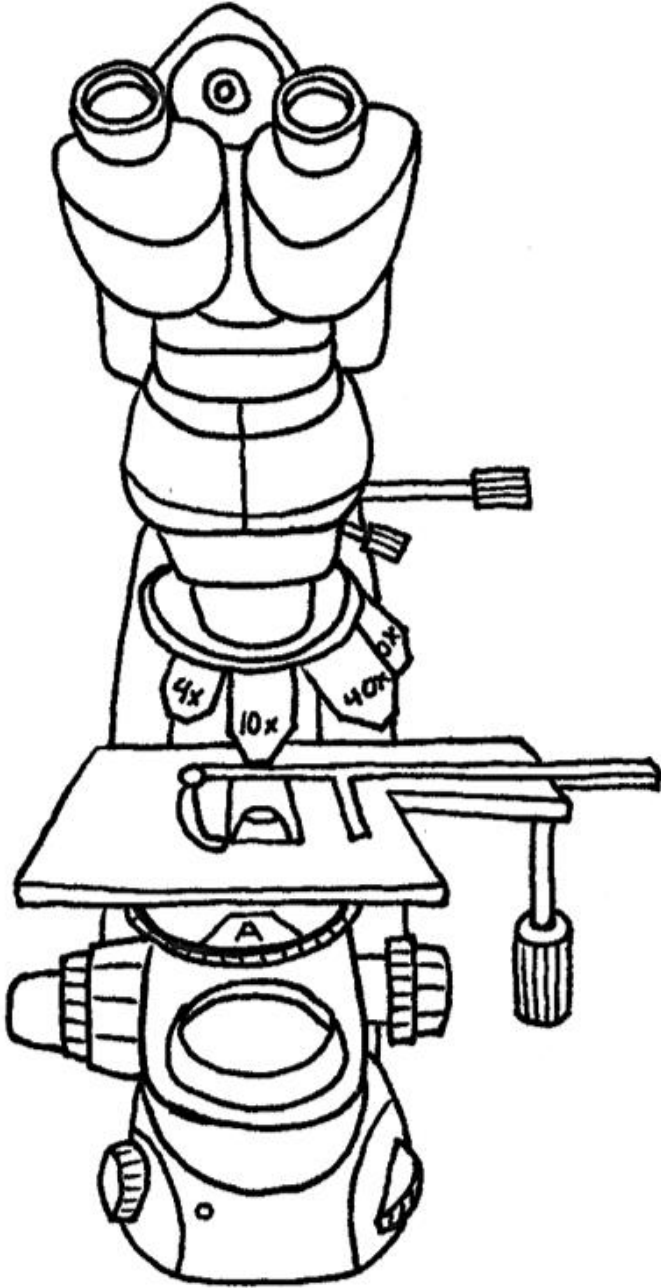
Let excess solution drip off between each dip.



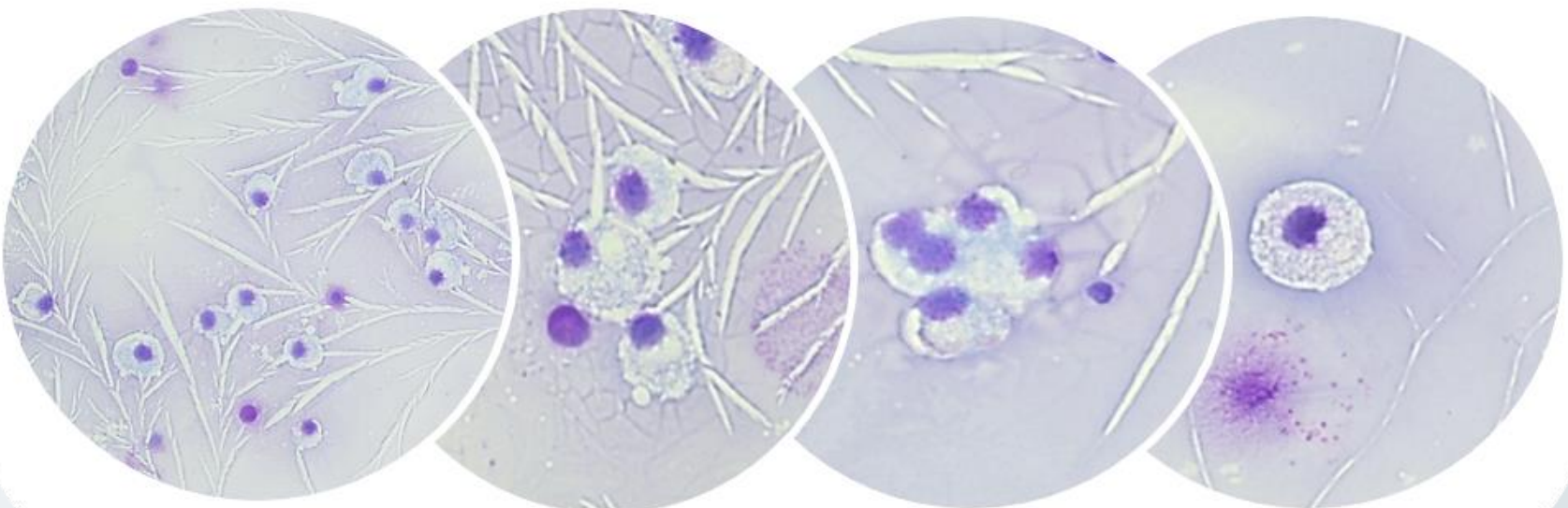
- 4 Rinse smear slides in distilled water and let slides dry. Slides are now ready to microscope.

MICROSCOPY

Equipment: Microscope and stained hemolymph smears



- 1 Adjust microscope according to your microscope guide lines.
- 2 Examine the smear on the microscope, first with low magnification and thereafter 40x to identify possible parasite life stages.
- 3 Hematodinium sp. has several life stages. We expect to find trophonts using this method. The parasite differs from the host cells with its foamy cytoplasm and clumped chromatin in the nuclei.



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