



TEST PROCEDURE



1

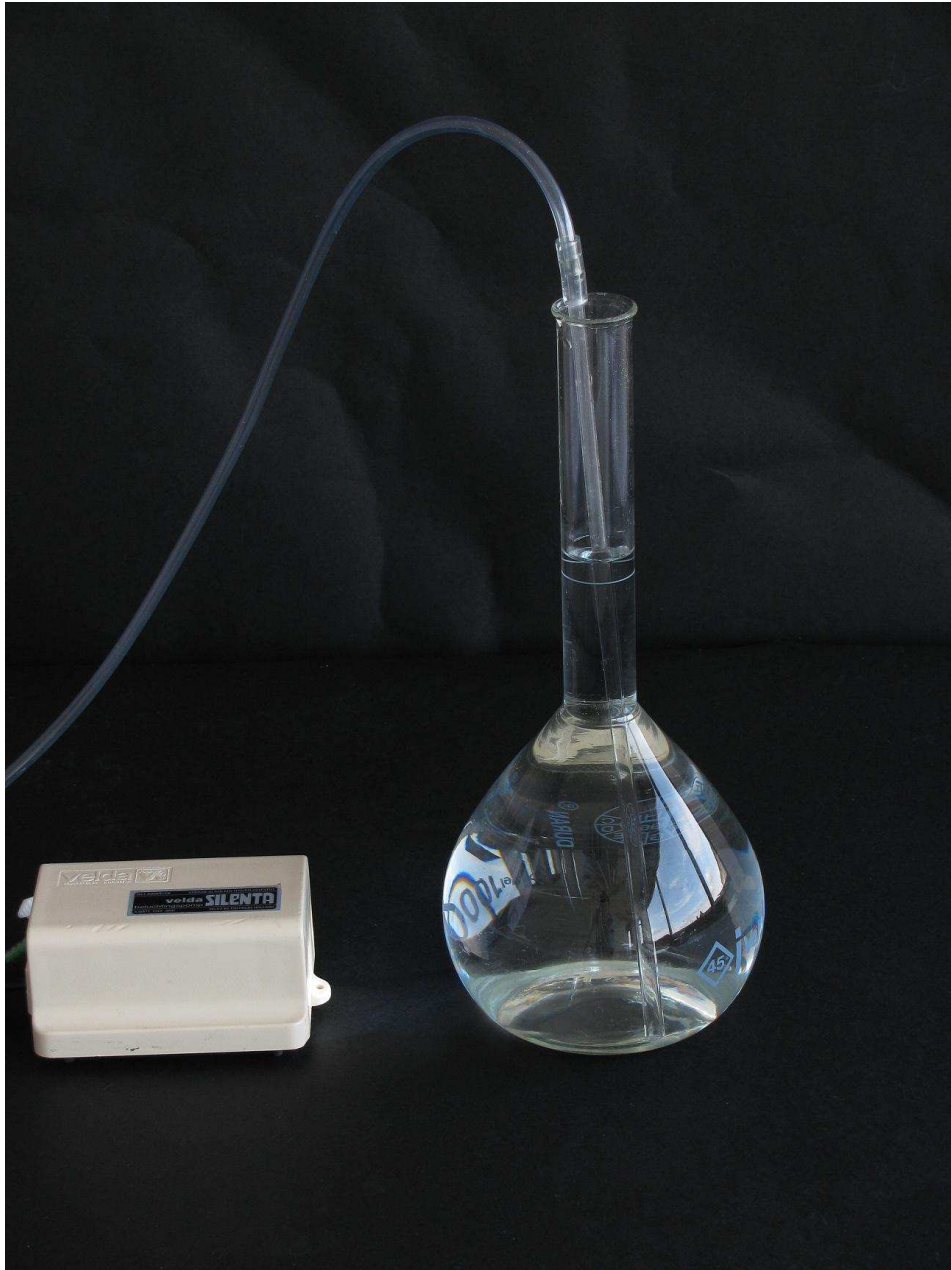
PREPARATION OF STANDARD FRESHWATER

- VOLUMETRIC FLASK (1 LITER)
- VIALS WITH SOLUTIONS OF
CONCENTRATED SALTS
- DISTILLED (or deionized) WATER



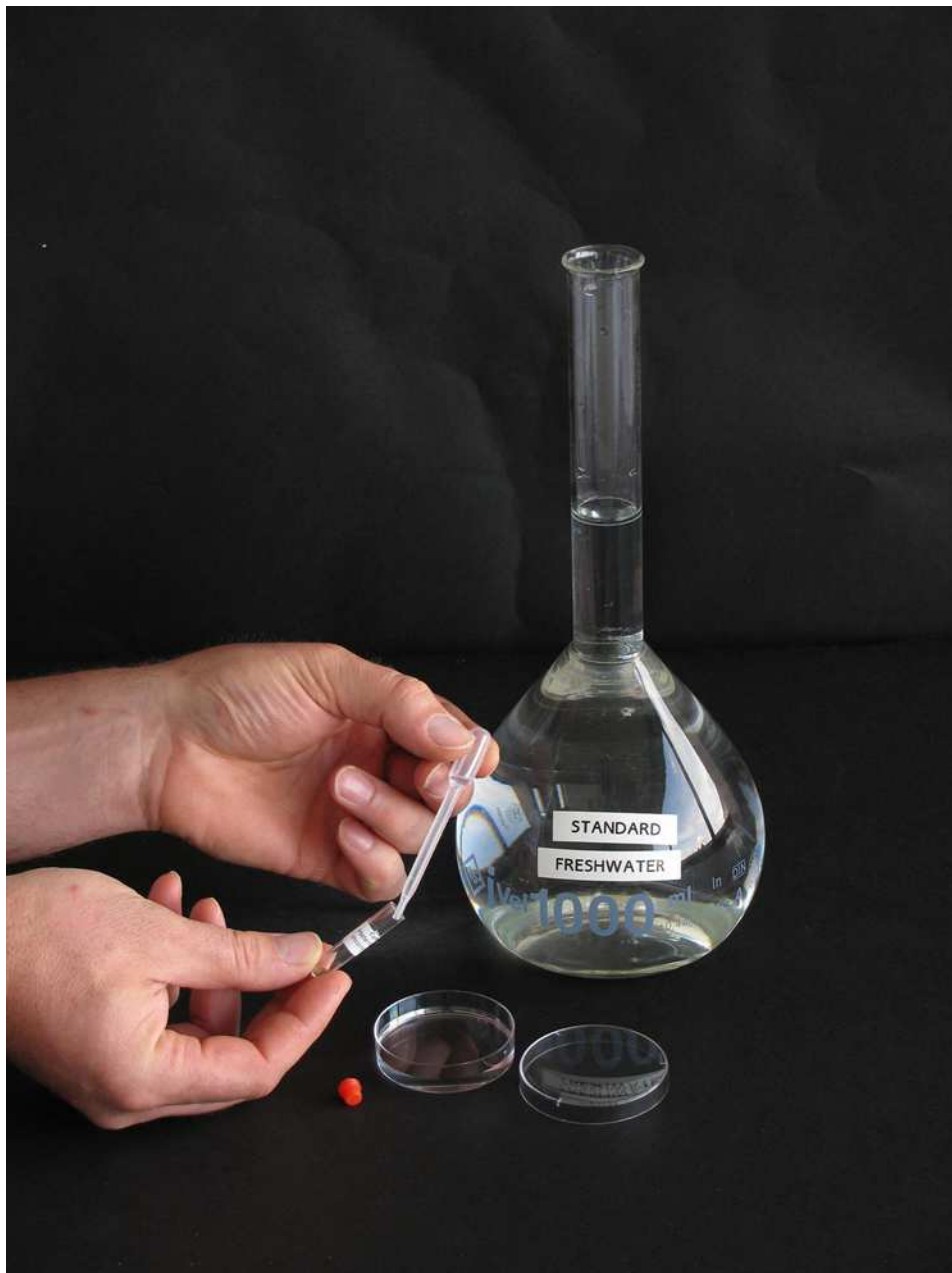
2

POUR THE 5 VIALS
WITH CONCENTRATED SALT SOLUTIONS
IN \pm 800 ML DISTILLED WATER,
IN THE 1 LITER VOLUMETRIC FLASK



3

- FILL THE FLASK TO THE 1 LITER MARK
- AERATE FOR AT LEAST 15 MINUTES



4

HATCHING OF OSTRACOD CYSTS

OPEN A TUBE WITH CYSTS AND FILL IT WITH 1 ML STANDARD FRESHWATER

STOPPER THE TUBE AND SHAKE IT

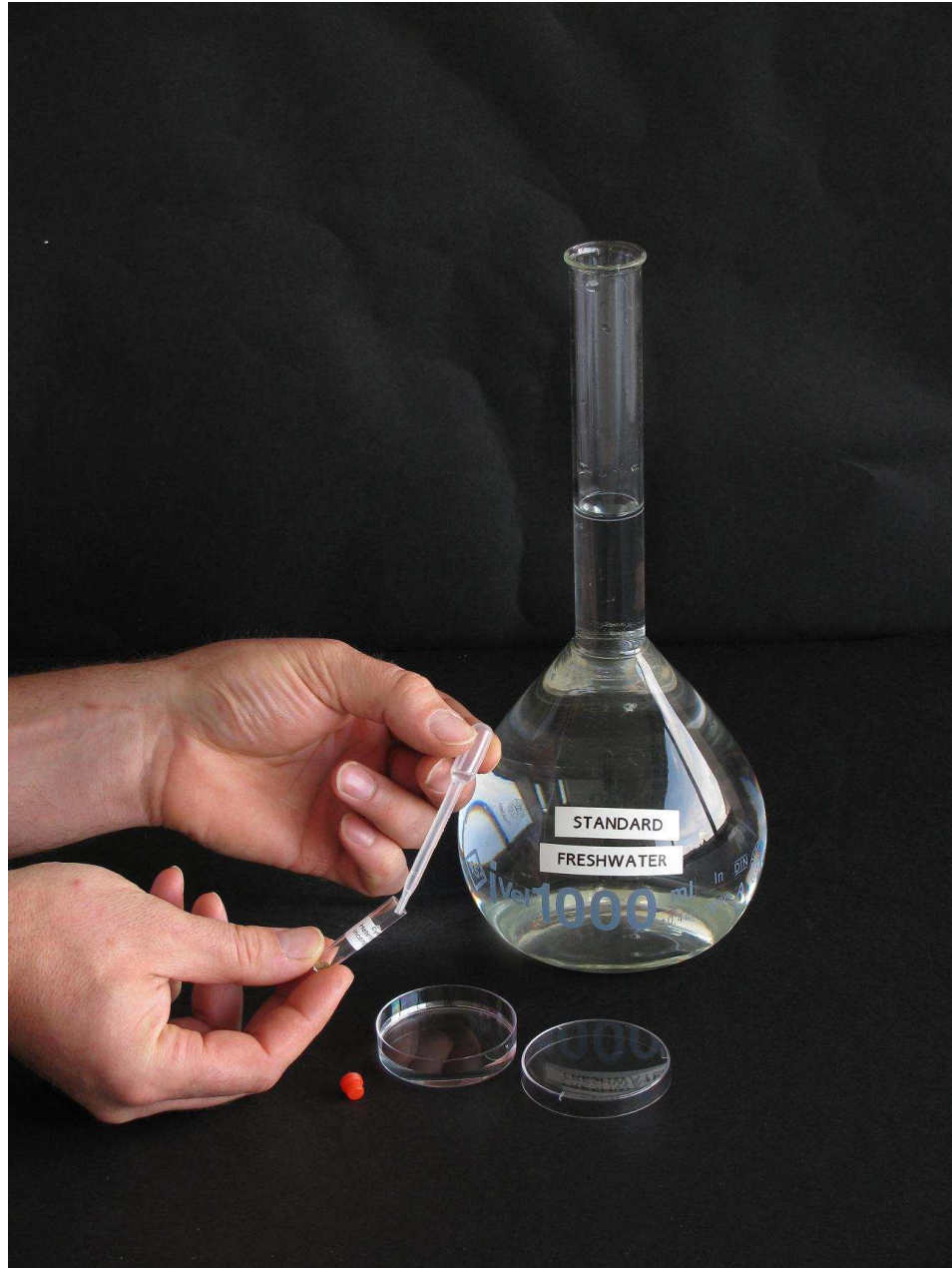


5

HATCHING OF OSTRACOD CYSTS

PUT 8 ML STANDARD FRESHWATER INTO
THE PETRI DISH

EMPTY THE CONTENTS OF THE VIAL
WITH CYSTS INTO THE PETRI DISH



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TO SECURE THE COMPLETE
TRANSFER OF THE CYSTS,
THE VIAL SHOULD BE RINSED
TWICE WITH 1 ML STANDARD
FRESHWATER

7



INCUBATION OF THE CYSTS

INCUBATE THE PETRI DISH
FOR 52 HOURS AT 25 °C
UNDER CONTINOUS ILLUMINATION
OF MIN. 3 000 – 4 000 LUX



8

4h PRE-FEEDING OF THE TEST ORGANISMS

TAKE ONE VIAL
WITH SPIRULINA POWDER AND
FILL IT WITH STANDARD
FRESHWATER



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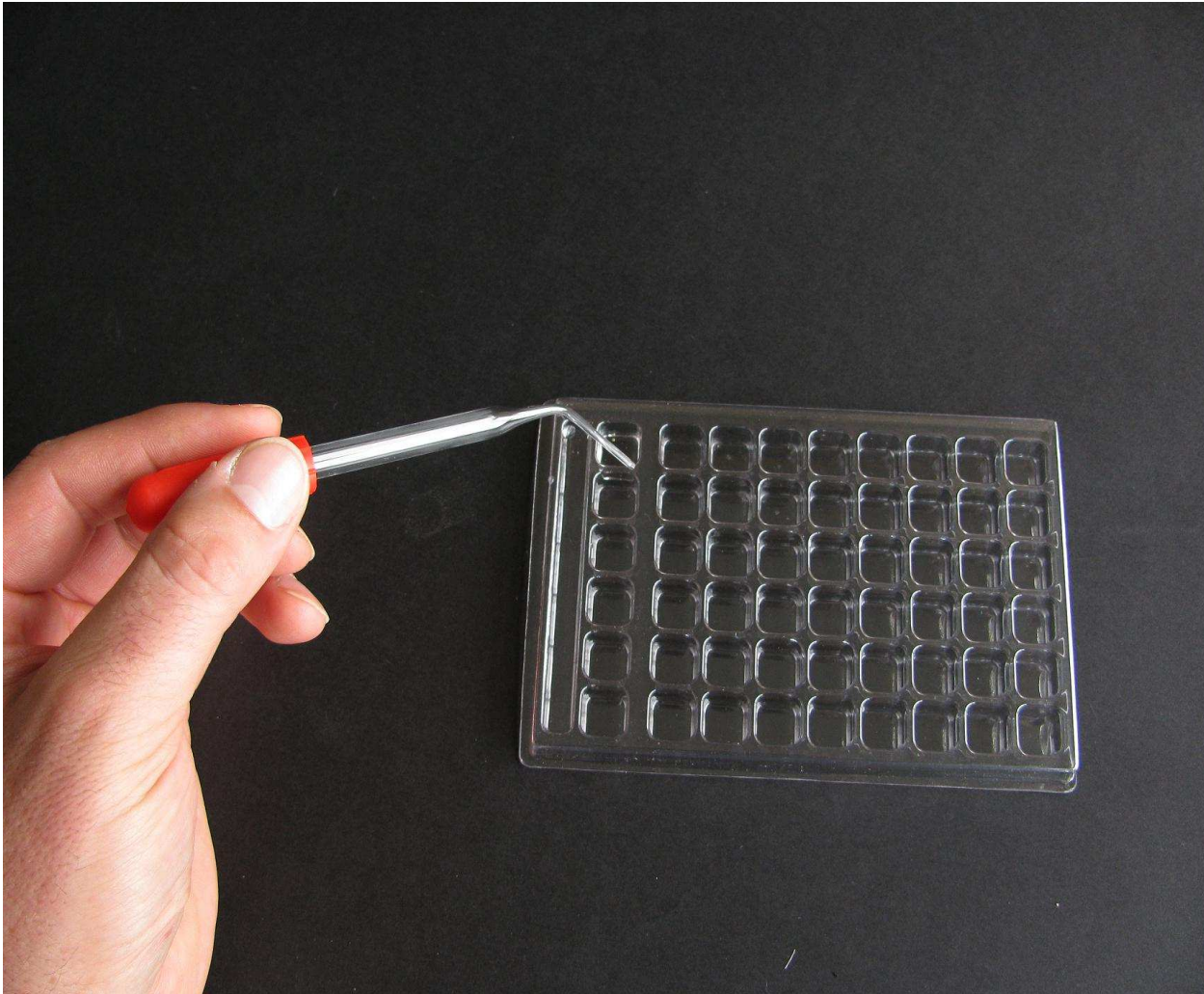
SHAKE THE VIAL WITH THE SPIRULINA SUSPENSION, POUR IT IN THE PETRI DISH WITH THE OSTRACODS AND SWIRL THE PETRI DISH GENTLY



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**LENGTH MEASUREMENT OF
FRESHLY HATCHED OSTRACODS**

PICK UP 10 OSTRACODS FROM THE
HATCHING PETRI DISH WITH A
GLASS MICROPIPETTE



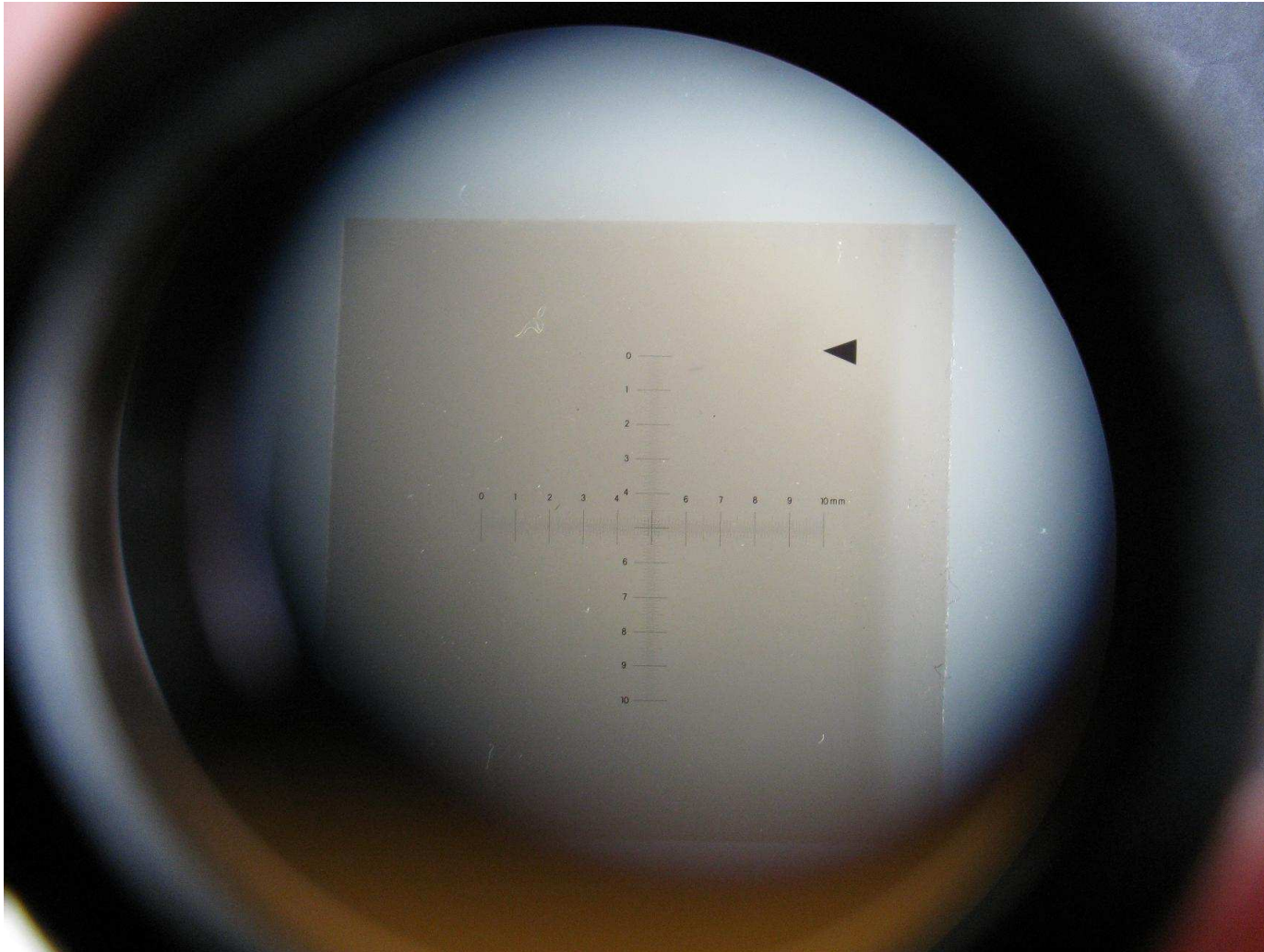
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TRANSFER THEM INTO
ONE CUP OF THE
MULTIWELL FOR "LENGTH
MEASUREMENT"



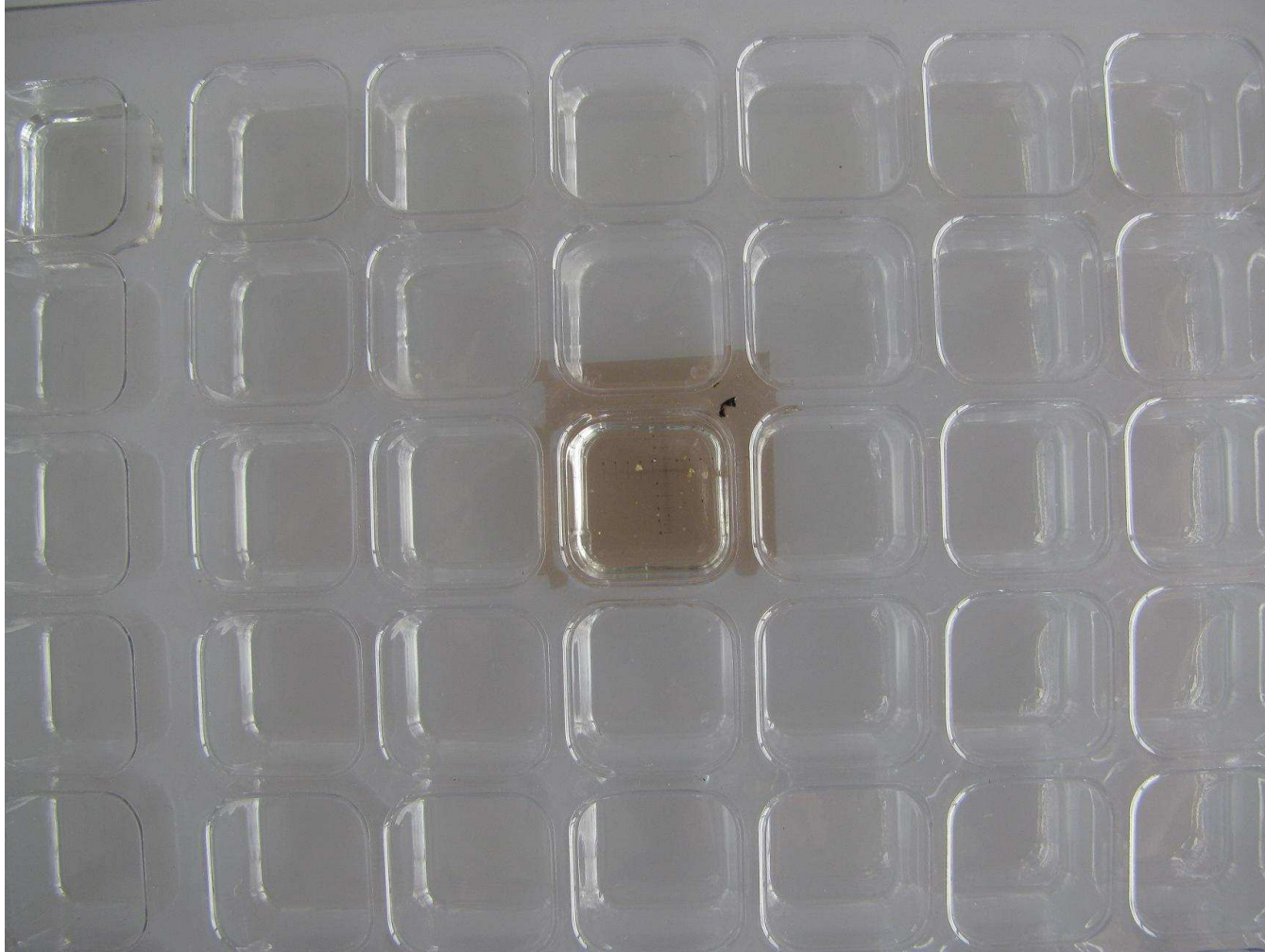
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ADD ONE DROP OF LUGOL FIXATIVE
TO THE CUP WITH THE OSTRACODS
AND WAIT UNTIL THE ORGANISMS ARE
COMPLETELY IMMOBILE



13

POSITION THE MICROMETER SLIP IN THE CENTRE OF VISUAL FIELD
OF THE DISSECTION MICROSCOPE



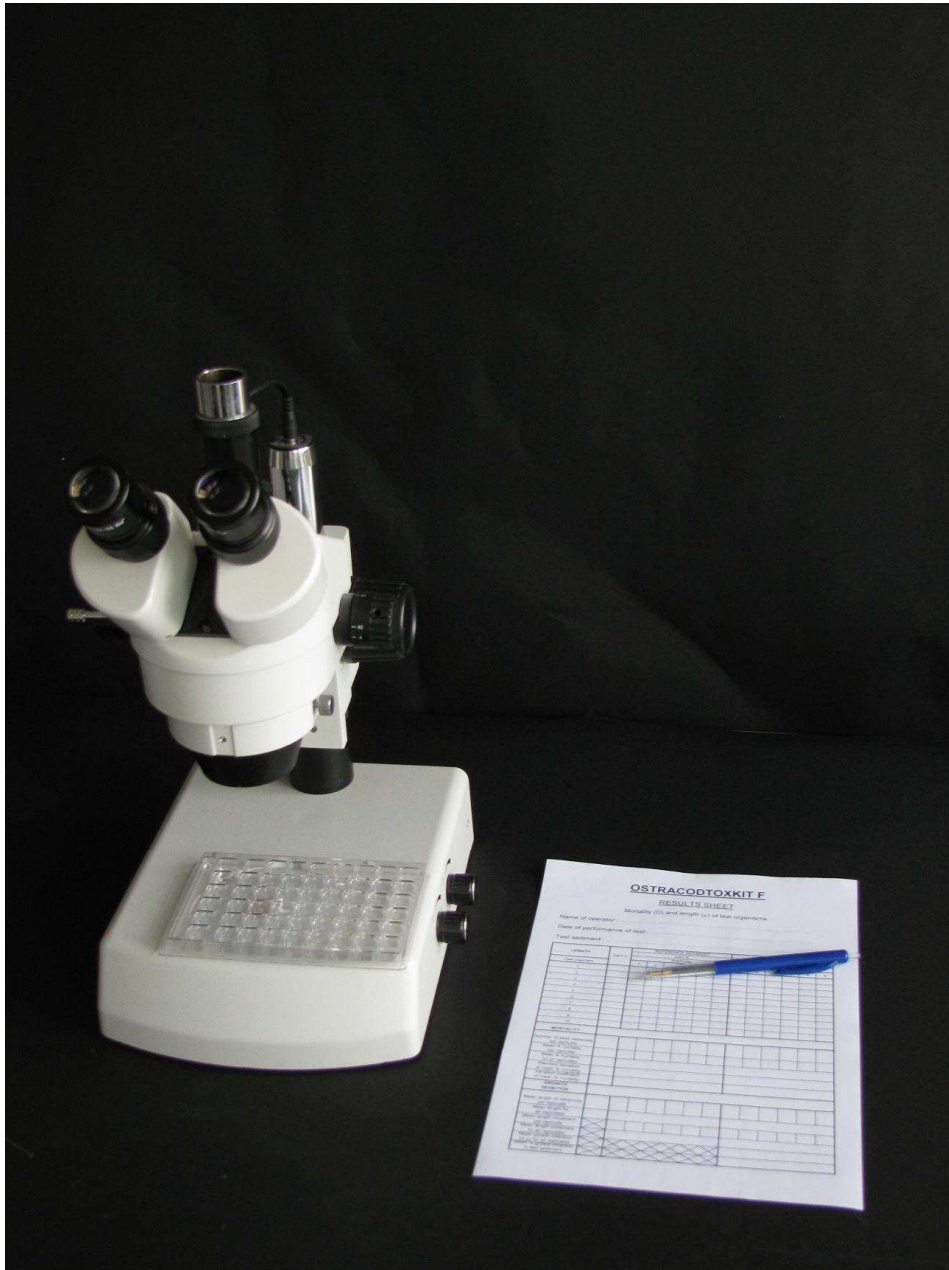
14

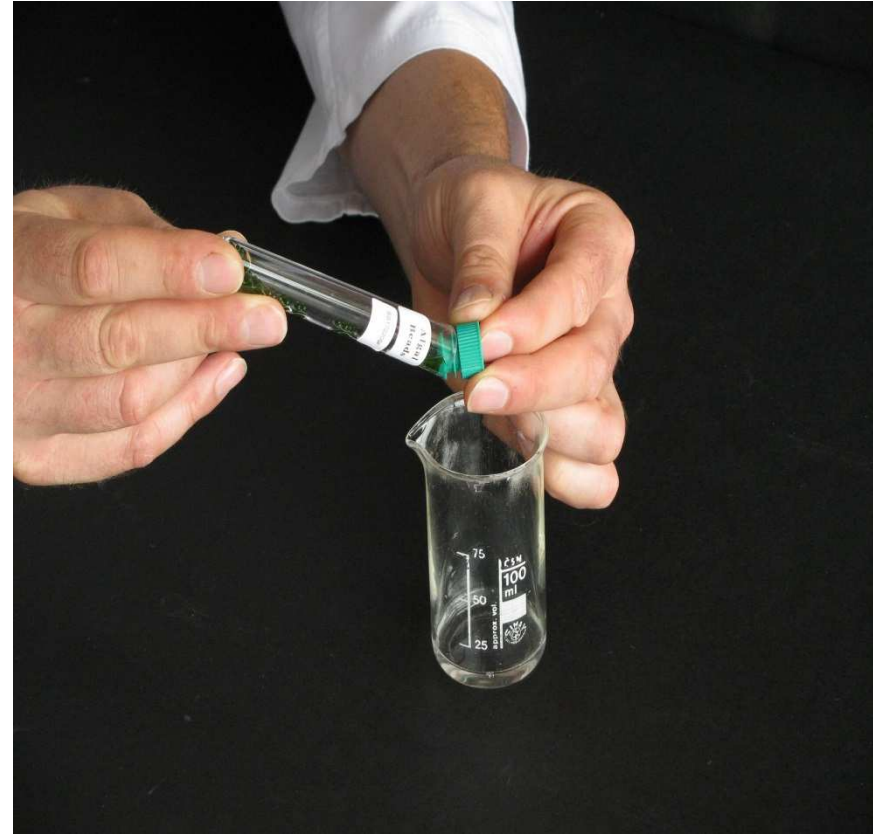
PUT THE MULTIWELL FOR LENGTH MEASUREMENT ON THE STAGE OF THE DISSECTION
MICROSCOPE, AND MEASURE THE LENGTH OF THE ORGANISMS

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SCORE THE LENGTH RESULTS ON THE
“RESULTS SHEET” (IN COLUMN DAY 0)

N.B. THE SMALLEST DIVISIONS OF THE
MICROMETER LINES ARE 50 μm
FRESHLY HATCHED OSTRACODS HAVE
A LENGTH OF ABOUT 200 μm

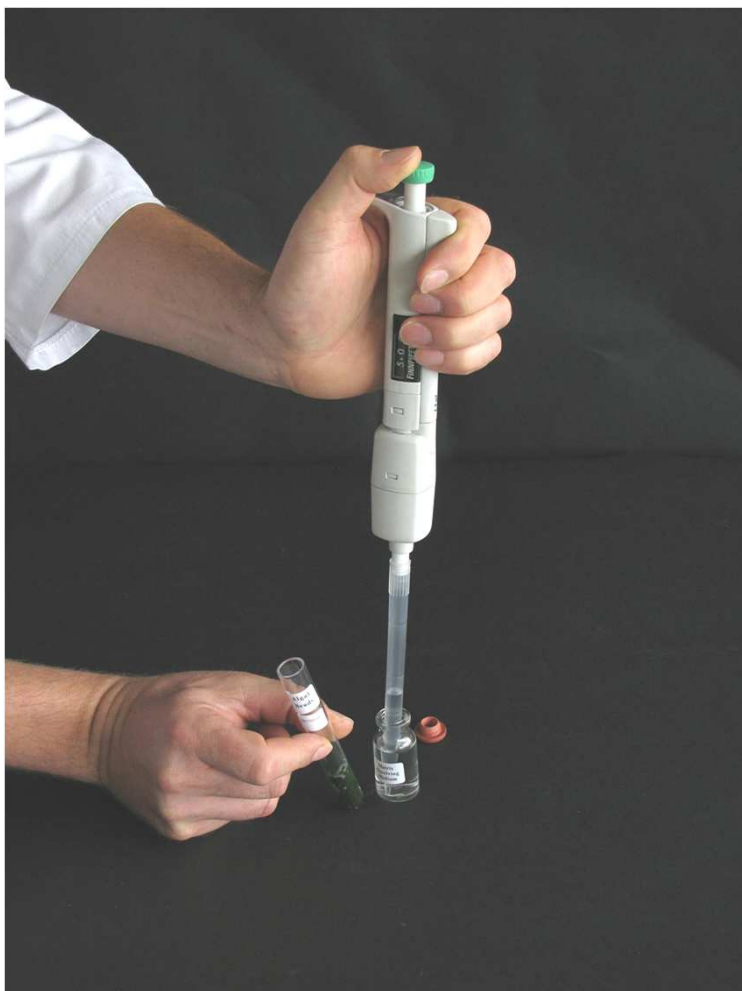




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PREPARATION OF THE ALGAL FOOD SUSPENSION

TAKE ONE TUBE WITH ALGAL BEADS AND POUR OUT THE STORAGE MEDIUM



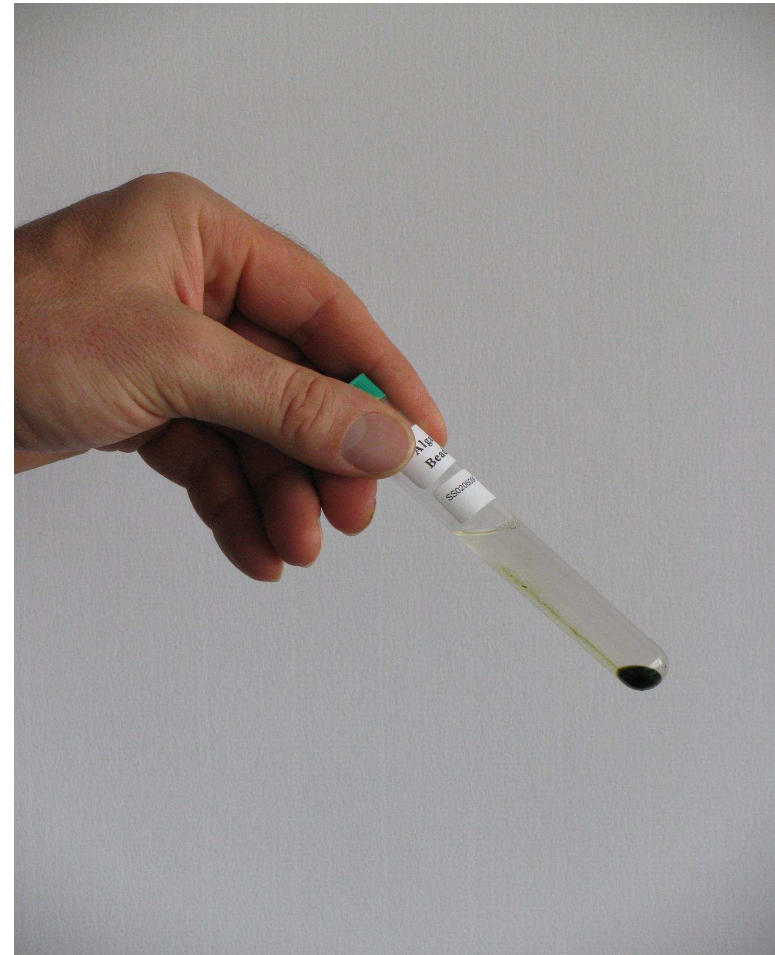
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ADD 7 ML MATRIX DISSOLVING MEDIUM AND CAP THE TUBE



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SHAKE THE TUBE ON A VORTEX UNTIL THE MATRIX SURROUNDING THE ALGAE HAS FULLY DISSOLVED AND THE MICROALGAE ARE TOTALLY SET FREE



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CENTRIFUGE THE TUBE FOR 10 MINUTES AT 3000 RPM IN A CONVENTIONAL LAB CENTRIFUGE



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POUR OUT THE SUPERNATANT FROM THE TUBE



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- ADD 10 ML DISTILLED WATER TO THE TUBE
- CAP AND SHAKE THE TUBE TO RESUSPEND THE ALGAE



22

CENTRIFUGE THE TUBE AGAIN AT 3000 RPM FOR 10 MINUTES
AND DECANT THE SUPERNATANT



23

- TRANSFER THE ALGAL PELLETT TO A 25 ML VOLUMETRIC FLASK
- ADD STANDARD FRESHWATER TO THE 25 ML MARK
- SHAKE TO OBTAIN A HOMOGENOUS ALGAL SUSPENSION



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**ADDITION OF SEDIMENT, ALGAL FOOD
AND OSTRACODS TO THE TEST PLATE**

ADD 2 ML OF STANDARD FRESHWATER
INTO EACH WELL OF A MULTIWELL TEST
PLATE



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REFERENCE SEDIMENT TEST PLATE

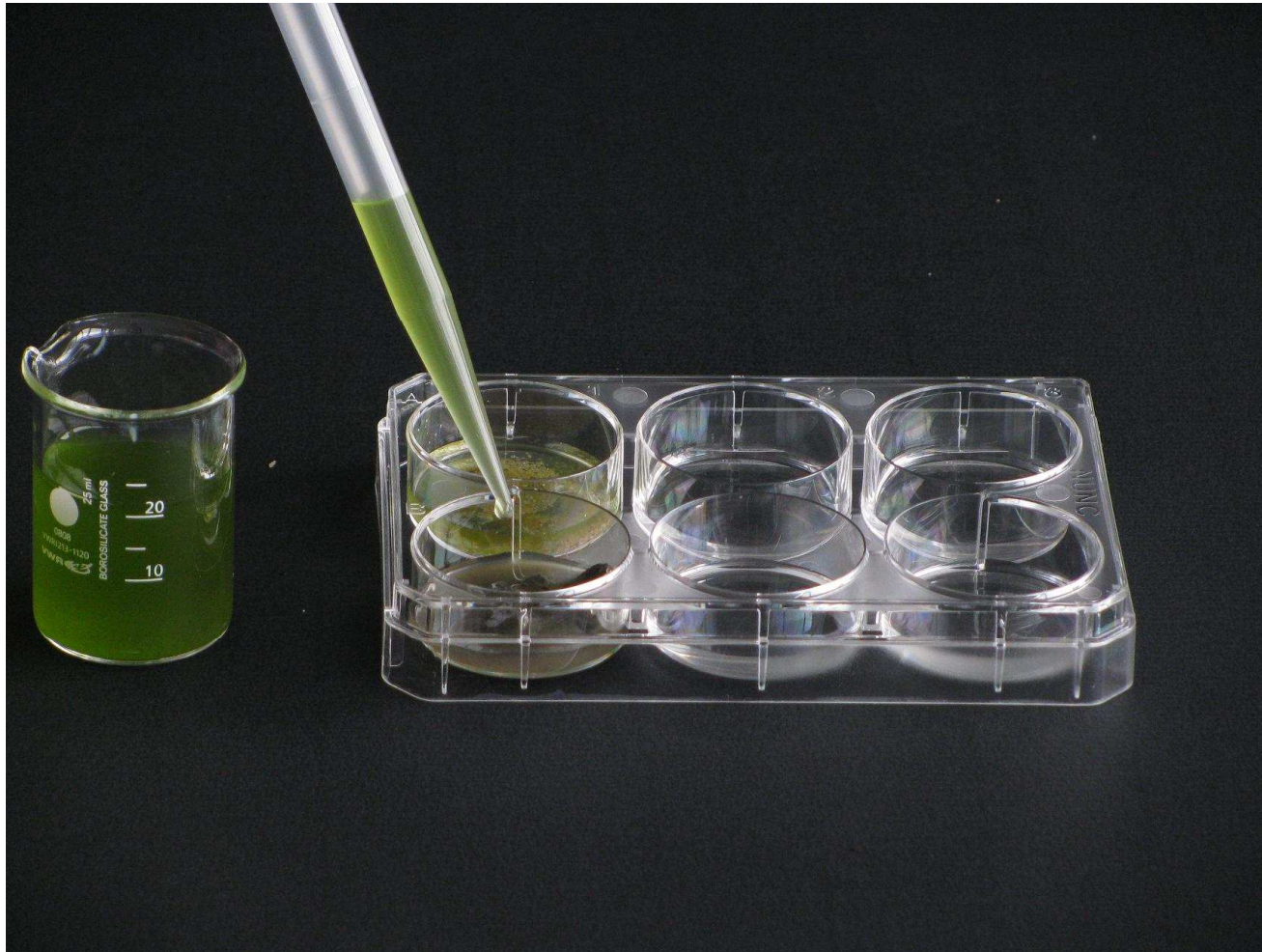
ADD 2 SPOONS OF 500 μ l EACH OF SEDIMENT INTO EACH WELL



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TEST SEDIMENT TEST PLATE

- ADD 2 SPOONS OF SEDIMENT INTO EACH WELL
- STRIKE OFF THE EXCESSIVE SEDIMENT FROM THE SPOON WITH THE PLASTIC SPATULA
- TRANSFER SEDIMENT INTO THE CUPS BY USING THE TIP OF THE SPATULA



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- TRANSFER THE ALGAL FOOD SUSPENSION INTO A BEAKER
- VERY GENTLY ADD 2 ML SUSPENSION INTO EACH WELL



28

FILL THE LID OF THE HATCHING
PETRI DISH WITH 10 ML STANDARD
FRESHWATER



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TRANSFER, WITH THE GLASS
MICROPIPETTE, A PART OF THE
OSTRACOD NEONATES FROM THE
HATCHING PETRI DISH INTO THE LID



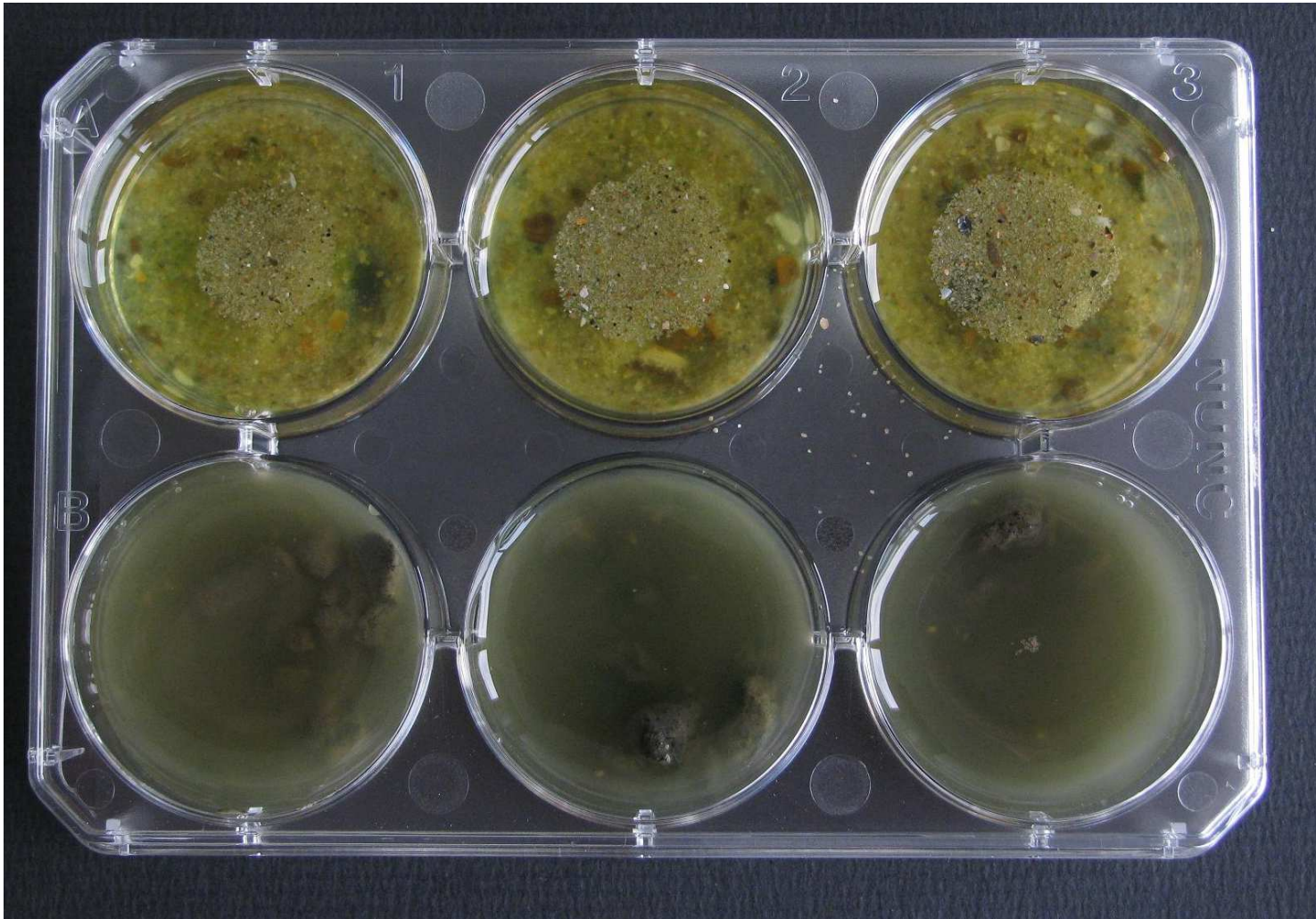
30

TRANSFER 10 OSTRACODS FROM THE LID INTO EACH WELL



31

- COVER THE MULTIWELL WITH A PIECE OF PARAFILM
- PUT THE LID ON TOP
- PUT THE MULTIWELL PLATE IN THE INCUBATOR AT 25 °C, IN DARKNESS, FOR 6 DAYS



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SCORING OF THE TEST

TRANSFER OF THE OSTRACODS INTO A PETRI DISH



33

A. SCORING OF THE REFERENCE SEDIMENT

- SUCK UP THE SEDIMENT SUSPENSION WITH A “LARGE MOUTH” MICROPIPETTE
- TRANSFER IT INTO THE MICROSIEVE



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- GENTLY RINSE THE CONTENTS OF THE MICROSIEVE UNTIL ALL THE FINE SEDIMENTS ARE WASHED OUT
- PROCEED FURTHER WITH THE STEPWISE TRANSFER OF THE SEDIMENT TO THE MICROSIEVE FOLLOWED BY RINSING, TILL MOST OF THE SEDIMENT HAS BEEN TRANSFERRED



35

- ADD A FEW ML STANDARD FRESHWATER TO THE CUP
- MIX IT WITH THE REMAINING SEDIMENT
- TRANSFER IT TO THE MICROSIEVE FOR RINSING.
- REPEAT THIS OPERATION, TO MAKE SURE THAT ALL THE SEDIMENT AND OSTRACODS HAS BEEN TRANSFERRED



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TURN THE MICROSIEVE UPSIDE DOWN AND RINSE THE CONTENTS INTO A
PETRI DISH WITH STANDARD FRESHWATER



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B. SCORING OF THE TEST SEDIMENT

REPEAT THE PROCEDURE PRESCRIBED FROM N° 32 TO 35 FOR REMOVING THE OSTRACODS FROM THE SEDIMENT TEST PLATE

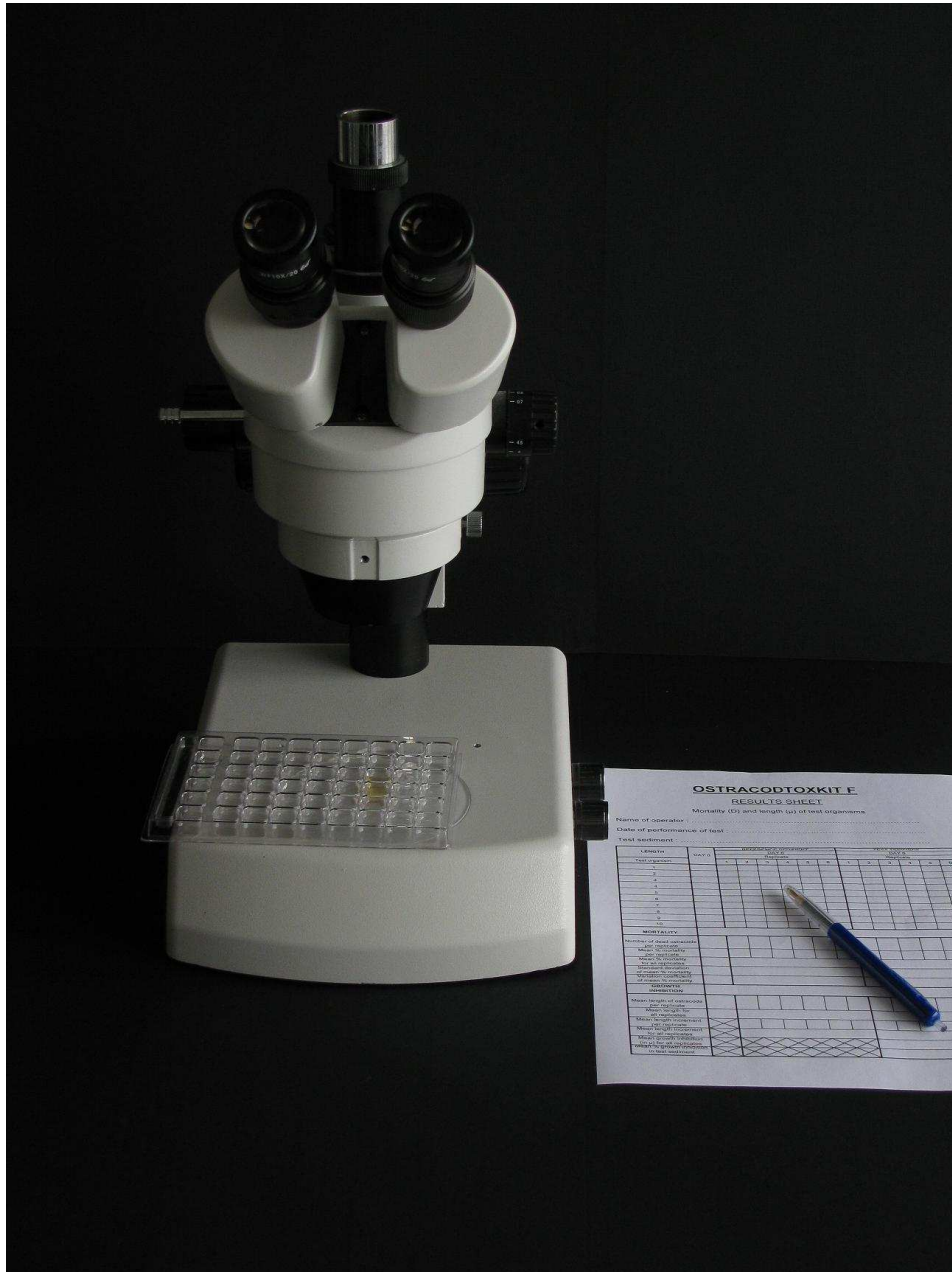


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SCORING OF THE RESULTS

A. MORTALITY SCORING

PICK UP ALL THE LIVE OSTRACODS WITH A GLASS MICROPIPETTE AND TRANSFER THEM INTO ONE CUP OF THE MULTIWELL PLATE FOR "LENGTH MEASUREMENT"



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SCORE THE NUMBER OF LIVE
OSTRACODS RESPECTIVELY FOUND
IN THE REFERENCE AND TEST
SEDIMENT ON THE “RESULTS SHEET”

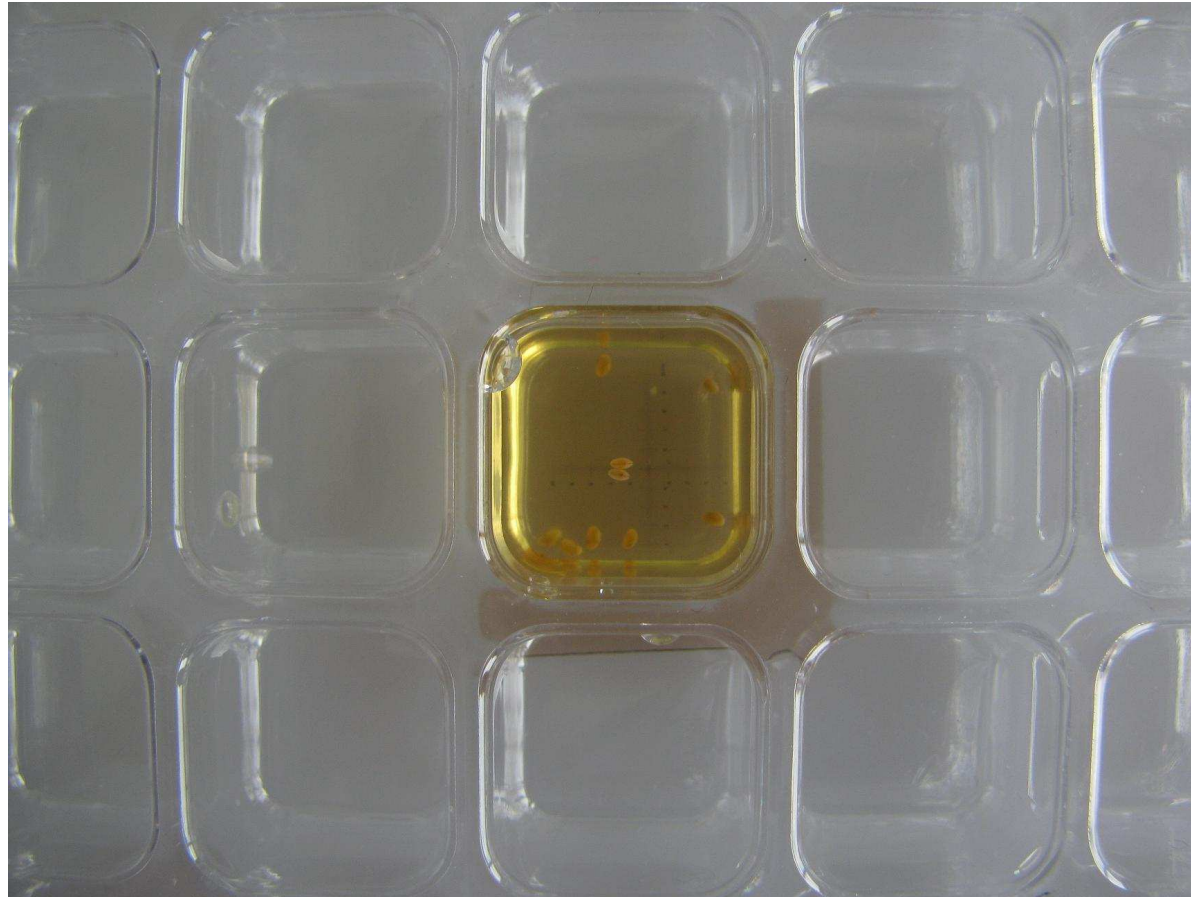


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B. LENGTH MEASUREMENT

IN SAMPLES IN WHICH THE MORTALITY IS LOWER THAN 30%, A SECOND SUBLETHAL EFFECT CRITERION (GROWTH INHIBITION) SHOW THE TOXICITY OF SEDIMENTS

AFTER THE LIVE OSTRACODS OF ALL THE TEST CUPS HAVE BEEN TRANSFERRED ADD ONE DROP OF LUGOL FIXATIVE TO EACH OF THESE CUPS



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- WAIT UNTIL THE OSTRACODS ARE IMMOBILE
- MEASURE THEIR LENGTH FOLLWING THE PROCEDURE INDICATED IN N° 13 & 14

OSTRACODTOXKIT F

RESULTS SHEET

Mortality (D) and length (μ) of test organisms

Name of operator : TAMINOL RIK

Date of performance of test : 16/07/2022

Test sediment : SAMPLE 314A

LENGTH	DAY 0	REFERENCE SEDIMENT						TEST SEDIMENT					
		DAY 6						DAY 6					
		Replicate						Replicate					
Test organism		1	2	3	4	5	6	1	2	3	4	5	6
1	200	1100	1100	1100	1100	1000	1100	850	850	800	850	800	850
2	200	1100	1100	1000	1100	1000	1100	850	850	800	800	800	850
3	200	1100	1000	1000	1000	1000	1100	850	600	800	800	800	700
4	200	1100	1000	1000	1000	1000	1000	850	600	800	800	700	700
5	200	1000	1000	1000	950	1000	1000	850	600	650	750	700	600
6	200	1000	950	1000	950	950	900	650	600	650	700	700	600
7	200	1000	950	900	950	950	900	650	600	650	700	700	550
8	200	900	900	900	850	950	850	500	600	600	650	650	550
9	200	900	900	900	850	900	850	500	M	600	650	650	M
10	200	900	850	M	850	900	M	500	M	550	550	600	M
MORTALITY													
Number of dead ostracods per replicate		0	0	1	0	0	1	0	2	0	0	0	2
Mean % mortality per replicate													
Mean % mortality for all replicates													
Standard deviation of mean % mortality													
Variation coefficient of mean % mortality													
GROWTH INHIBITION													
Mean length of ostracods per replicate													
Mean length for all replicates													
Mean length increment per replicate													
Mean length increment for all replicates													
Mean growth inhibition (in μ) for all replicates													
Mean % growth inhibition in test sediment													

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- SCORE THE LENTGH RESULTS ON THE “RESULTS SHEET”
- PERFORM THE DATA TREATMENT OF THE RESULTS WITH AN APPROPRIATE PROGRAM