PREPARATION OF STANDARD FRESHWATER

- VOLUMETRIC FLASK (1 liter)
- VIALS WITH SOLUTIONS OF CONCENTRATED SALTS
- DISTILLED (or deionized) WATER
POUR THE 5 VIALS WITH CONCENTRATED SALT SOLUTIONS IN ± 800 ML DISTILLED WATER, IN THE 1 LITER VOLUMETRIC FLASK
- Fill the flask to the 1 liter mark

- Aerate for at least 15 minutes
HATCHING OF OSTRACOD CYSTS

POUR THE CONTENTS OF ONE VIAL WITH CYSTS IN THE PETRI DISH
TO ENSURE THE TRANSFER OF ALL THE CYSTS, THE VIAL SHOULD BE RINSED TWICE WITH 1 ML STANDARD FRESHWATER
INCUBATION OF THE CYSTS

INCUBATE THE PETRI DISH FOR 48 HOURS AT 25 °C UNDER CONTINOUS ILLUMINATION OF MIN. 3 000 – 4 000 LUX
PRE-FEEDING OF THE TEST ORGANISMS

TAKE ONE VIAL WITH SPIRULINA POWDER AND FILL IT WITH STANDARD FRESHWATER
- SHAKE THE VIAL WITH THE SPIRULINA SUSPENSION

- POUR THE CONTENTS IN THE PETRI DISH CONTAINING THE HATCHED OSTRACODS AND SWIRL THE PETRI DISH GENTLY

- ALLOW THE OSTRACODS TO PRE- FEED FOR 4 HOURS
LENGTH MEASUREMENT OF FRESHLY HATCHED OSTRACODS

PICK UP 10 OSTRACODS FROM THE HATCHING PETRI DISH WITH A GLASS MICROPIPETTE
TRANSFER THE OSTRACODS INTO ONE CUP OF THE MULTIWELL FOR “LENGTH MEASUREMENTS”
ADD ONE DROP OF LUGOL FIXATIVE TO THE CUP CONTAINING THE OSTRACODS AND WAIT UNTIL THE ORGANISMS ARE COMPLETELY IMMOBILE
PUT THE MICROMETER SLIP ON THE GLASS STAGE OF THE DISSECTION MICROSCOPE, IN THE CENTRE OF THE VISUAL FIELD
PUT THE MULTIWELL FOR LENGTH MEASUREMENTS ON THE STAGE OF THE DISSECTION MICROSCOPE, AND MEASURE THE LENGTH OF THE OSTRACODS

N.B.: the smallest subdivisions of the micrometer slip are 50 µm.

FRESHLY HATCHED OSTRACODS HAVE A LENGTH OF ABOUT 200 µm.
SCORE THE LENGTH OF THE OSTRACODS ON THE “RESULTS SHEET” IN COLUMN “DAY 0”
PREPARATION OF THE ALGAL FOOD SUSPENSION

TAKE ONE TUBE WITH ALGAL BEADS AND POUR OUT THE STORAGE MEDIUM TAKING CARE NOT TO LOSE ANY BEAD DURING THE OPERATION.
ADD 7 ML MATRIX DISSOLVING MEDIUM TO THE TUBE WITH ALGAL BEADS AND CLOSE THE TUBE WITH THE CAP
SHAKE THE TUBE ON A VORTEX UNTIL THE MATRIX IN WHICH THE ALGAE ARE IMMObILISED IS TOTALLY DISSOLVED AND THE ALGAE ARE SET FREE
Centrifuge the tube for 10 minutes at 3000 rpm in a conventional lab centrifuge. Carefully pour out the supernatant from the tube.
ADD 10 ML DISTILLED WATER TO THE TUBE WITH THE ALGAL PELLET

CAP AND SHAKE THE TUBE TO RESUSPEND THE ALGAE
CENTRIFUGE THE TUBE AGAIN AT 3000 RPM FOR 10 MINUTES
AND POUR OUT THE SUPERNATANT
- Pour the concentrated algal suspension into a 25 mL volumetric flask
- Add standard freshwater to the 25 mL mark
- Cap the flask and shake to obtain a homogenous algal suspension
ADDICTION OF SEDIMENT, ALGAL FOOD AND OSTRACODS TO THE TEST PLATES

PUT 2 ML STANDARD FRESHWATER INTO EACH WELL OF TWO TEST PLATES (multiwell for reference sediment and multiwell for test sediment)
TEST PLATE FOR REFERENCE SEDIMENT

- Fill the spoon with reference sediment and strike off the excess sediment with the plastic spatula (the filled spoon then contains 500 µl sediment)
- Put 2 spoons (= 1000 µl) reference sediment into each well of the test plate
- Fill the spoon with test sediment and strike off the excess sediment with the plastic spatula (the filled spoon then contains 500 µl sediment)
- Put 2 spoons (= 1000 µl) of test sediment into each well of the test plate (use the tip of the spatula to perform the transfer)
- Pour the algal food suspension from the 25 ml flask into a beaker.
- Shake the beaker to distribute the algae evenly.
- Pipet 2 ml algal suspension into each well of the two test plates.
FILL THE LID OF THE HATCHING PETRI DISH WITH 10 ML STANDARD FRESHWATER
TRANSFER WITH THE GLASS MICROPIPETTE A NUMBER OF OSTRACOD NEONATES FROM THE HATCHING PETRI DISH INTO THE LID OF THIS DISH
TRANSFER 10 OSTRACODS FROM THE PETRI DISH LID INTO EACH WELL OF THE TWO TEST PLATES
INCUBATION OF THE TEST PLATES

- COVER THE TWO TEST PLATES WITH A SHEET OF PARAFILM AND PUT THE LID ON TOP
- INCUBATE THE TEST PLATES AT 25 °C, IN DARKNESS, FOR 6 DAYS
TEST SCORING – 1. TRANSFER OF THE OSTRACODS INTO A PETRI DISH

WITH THE AID OF THE “LARGE MOUTH” MICROPIPET, SUCK UP PART OF THE SEDIMENT SUSPENSION FROM ONE CUP OF THE TEST PLATE AND TRANSFER IT INTO THE MICROSIEVE
- GENTLY RINSE THE CONTENTS OF THE MICROSIEVE WITH TAPWATER TO WASH OUT THE FINE SEDIMENT
- PROCEED FURTHER WITH THE STEPWISE TRANSFER OF THE SEDIMENT SUSPENSION TO THE MICROSIEVE AND RINSE EACH TIME THE CONTENTS OF THE MICROSIEVE
- Add a few ml standard freshwater to the cup

- Mix the water with the remaining sediment and transfer it to the microsieve for rinsing.

- Repeat this operation until all the sediment and ostracods have been transferred into the microsieve
- TURN THE MICROSIENE UPSIDE DOWN AND WASH THE CONTENTS INTO A PETRI DISH, WITH THE AID OF A WASH BOTTLE CONTAINING TAPWATER
- REPEAT THE SEDIMENT TRANSFER AND RINSING OPERATIONS FOR ALL THE CUPS OF THE TWO TEST PLATES
PICK UP ALL THE LIVE OSTRACODS FROM THE PETRI DISH WITH A GLASS MICROPIPETTE AND TRANSFER THEM INTO ONE CUP OF THE “LENGTH MEASUREMENTS” MULTIWELL
- COUNT THE NUMBER OF LIVE OSTRACODS IN THE CUP

- SUBTRACT THIS NUMBER FROM 10 (i.e. from the original number of ostracods put in the cup)

- SCORE THE OUTCOME (i.e. the number of dead ostracods) ON THE RESULTS SHEET

- REPEAT THIS OPERATION FOR ALL THE CUPS OF THE TWO TEST PLATES

- CALCULATE AND SCORE THE MEAN % OSTRACOD MORTALITY FOR ALL THE CUPS
NB: only to be performed if the percentage mortality is < 30%

- Add one drop of Lugol fixative to each cup of the length measurements multiwell which contain the live ostracods from the two test plates.
- WAIT UNTIL THE OSTRACODS ARE IMMOBILE
- MEASURE THE LENGTH OF EACH OSTRACOD FOLLOWING THE PROCEDURE INDICATED IN STEPS 13 & 14
- SCORE THE RESULTS IN THE CORRESPONDING “LENGTH” BOXES OF THE RESULTS SHEET
- PERFORM THE DATA TREATMENT OF THE RESULTS WITH AN APPROPRIATE PROGRAMME