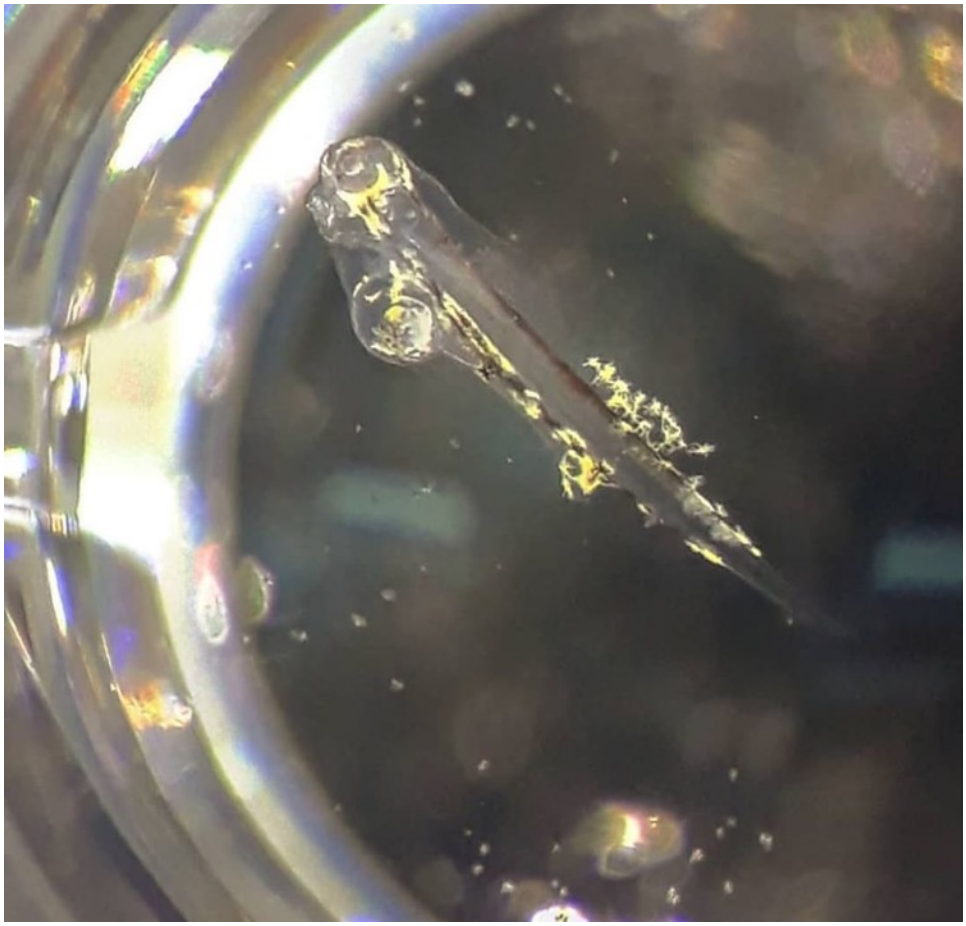
GWP Reproduction & Genetics
Protocol for the collection of eggs and evaluation of quality using a 48-well microtiter (mct or ELISA) plate



Objectives:

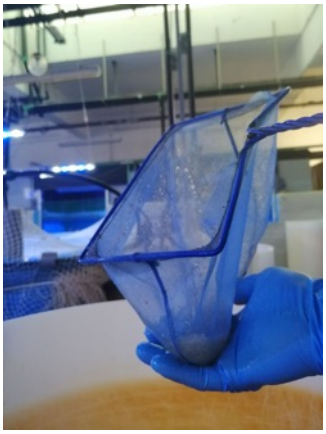
1. Choice of a method, which can be adopted for a large number of batches, for the estimation of the egg production parameters of greater amberjack (egg viability, fertilization rate and, larval survival after yolk sack absorption.
2. Using a reliable method for all the experiments with the same species and, if possible, with all species.

Egg collection from spawning tanks (Step 1):

Collect all the eggs from the overflow egg collector and pour them in a 10-l bucket (make a horizontal mark on the bucket at the 10-l level) as it follows:

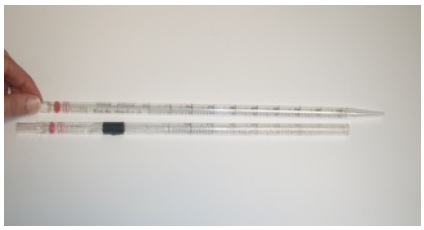
1. Fill the bucket with 5 l of water from the egg collector.
2. With a plankton dipnet, collect the eggs from the egg collector and strain them gently to remove water in excess. Pour the eggs into the bucket.
3. Repeat until all the eggs have been collected. Top up the bucket to the 10-l mark.

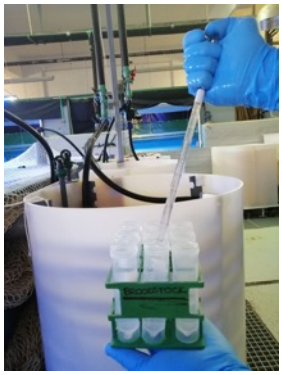


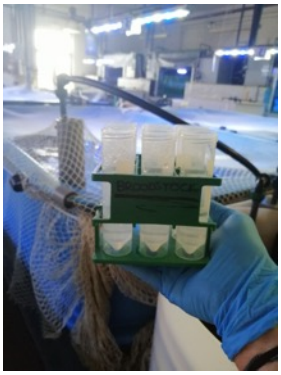



Egg sampling for fecundity and fertilization % evaluation (Step 2):

1. Take a 10-ml graduated plastic pipette (usually graded until 12) and cut off the conical tip, which starts at 9-ml mark. This allows for obtaining a homogeneous sample. Thus, to sample 10 ml will be sufficient to stop the water at the 11-ml mark.
2. Mix the water inside the 10-l bucket to disperse the eggs uniformly. Do this operation whit the pipette avoiding circular movements.
3. In a swift motion, sink the cut-off 10-ml pipette into the bucket until the pipette fills up to the 11 ml mark (for our buckets-pipettes, almost to the bottom of the bucket). Use your thumb to tap (clog) the top of the pipette, so that you will avoid overfilling or spilling the collected egg-water sample.
4. Place the sample into a 50-ml Falcon tube. The eggs contained in this sample should be 1/1000nd of the number of eggs spawned (contained in the 10-l bucket). To avoid difficulties during the count, if eggs in the bucket are > 1 million, it is preferable to do a sub-sample before counting. You can fill up the Falcon with more water (only water!) diluting the sample.
5. Repeat the step from 1 to 4 with the other egg collectors. When all the eggs are collected, you can proceed with the evaluation of fecudity and fertilization percentage (Step 4).

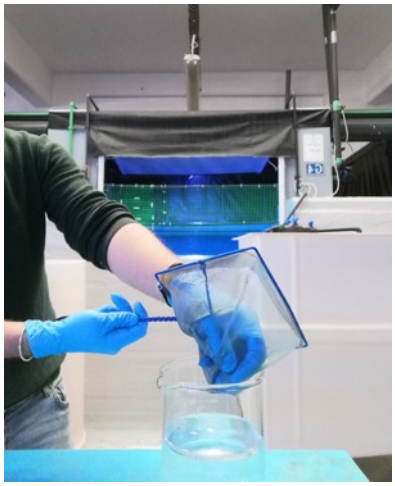


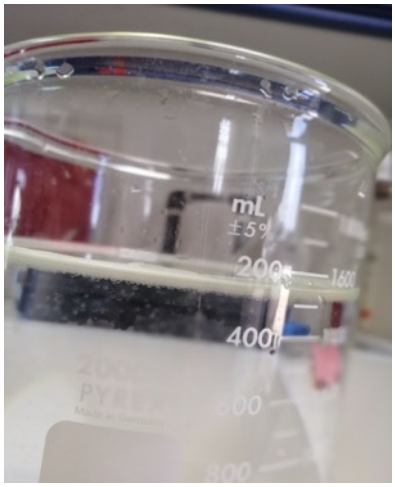




Egg sampling for microtiter plate loading (Step 3):

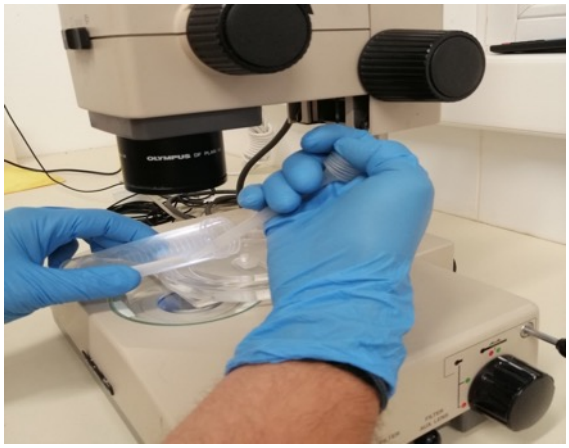
1. To get a sample for loading the microtiter (mct) plates, let the water and eggs in the 10-l bucket to calm down (5 min). Viable eggs float at the surface and, these are the only ones you need from now on. Most of the dead eggs will sink to the bottom and, excluding them will be easier.
2. Take a 2-l beaker and fill it to the 1.5-l mark with clean seawater from the broodstock tank.
3. Collect a corresponding amount of floating eggs, from the bucket, equal to ~ 2 soup spoonfuls and, put them in the beaker. Swirl gently.
4. Take this sample to the lab and store it in the controlled-temperature incubator until you are ready to clean the eggs with sterilized-autoclaved water (Step 5) and load the mct-plates (Step 6).

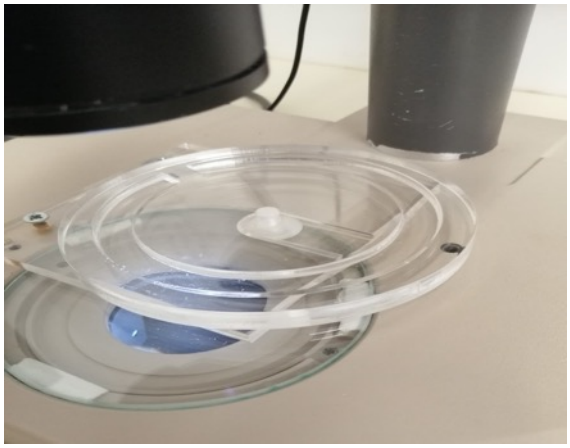


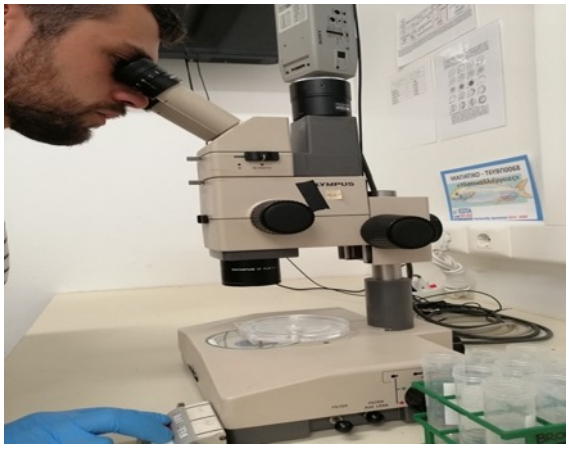


Evaluation of egg fecundity and fertilization success (Step 4):

1. Pour the 10-ml egg-water sample in a counting chamber (we use a circular one with a canal that has a dead end, so to avoid counting the same eggs.
2. Using a cell counter, count the alive (fertilized, L) and dead (D) eggs under the stereoscope.
3. The fecundity is equal the total number of eggs 1000 times. (L + D) x 1000.
4. Fertilization percentage is equal to the umber of dead eggs divided the total. L/(L + D).

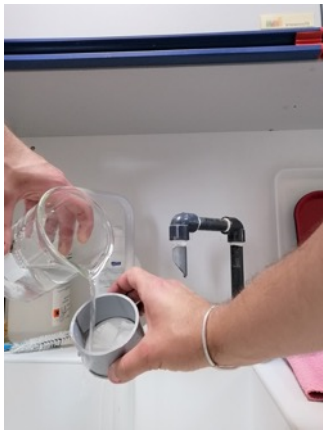






Eggs cleaning for microtiter plates loading (Step 5a):

1. Fill three 500-ml beakers with sterilized seawater. The sterilized water must be at the same temperature as the spawning tank.
2. With a small sieve (custom made with 500-µm mesh) take some eggs from the 2-l beaker, in which they were placed (Step 3). The eggs should be floating at the surface by now.
3. Wash the eggs thoroughly with sterilized seawater from one of the 500-ml beakers and pour them into the second 500-ml beaker. Add 35mg/L of Iodine to the eggs and stir gently. Let the eggs i cotact with iodine for 5 miutes.
4. After 5 minutes, remove the eggs and wash them with sterilized seawater to remove any residue of disinfectant. Pour them in the third 500-ml beaker.

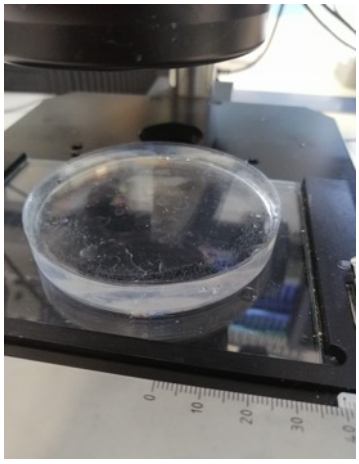
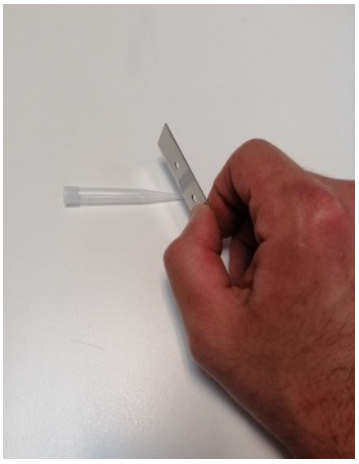





Eggs cleaning for microtiter plates loading (Step 5b):

1. Collect some (200-300) of the floating eggs with the small sieve, rinse them again with sterilized seawater and put them in a Petri dish with sterilized sea water, almost filling the petri dish.
2. Save the rest of the eggs and put the beaker back in the incubator, You may need to get more of them for loading the mct-plates.
3. Cut the top of a 1000-µl pipette tip, so that the opening will allow an egg to be aspirated inside. Set a 1-ml pipette to 200 µl.
4. Label 2 mct-plates with Tank #, date and replication (e.g., G5-20200615a & b)



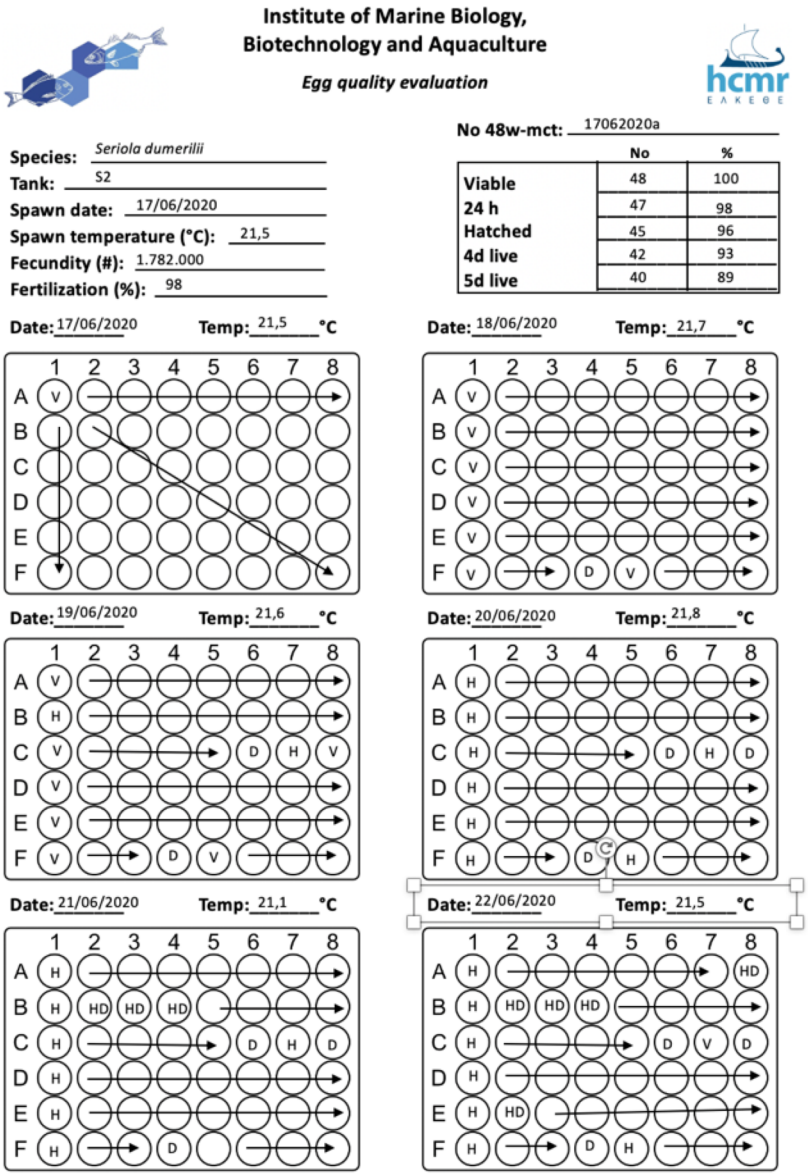

Mct-plates loading (Step 6):

1. Fill the 48 wells of the plate with 500 µl of sterilized seawater.
2. Using the 1-ml pipette, set to 200 µl, aspirate one (1) egg at a time. With a swift motion, place the pipette close to an egg and use the aspiration created by the pipette to suck it. Together with the egg, take up the settled volume of water.
3. Empty the tip inside a well (one egg each) of the mct-plate. Do not take any of the eggs at the bottom of the Petri, as they are probably dead. Take only the floating ones. Now the well must have 700 µl of water (500 + 200) and oe egg.
4. Refill the Petri with sterilized seawater when needed: the more water and fewer eggs, the easier it is to load the eggs with the pipette.
5. After the loading of the mct-plate is completed (48 eggs, 1 for each well), use the stereoscope to check all the eggs are alive.
6. When a well has a dead egg, remove it and replace it. Remove also eggs from wells if they contain more than one. Prepare two mct-plates for each spawn.
7. Cover the mct-plates with their lid and put them in the controlled temperature incubator at the spawning temperature of the water.

Warning!!! Do not leave the Petri dish on the microscope for too long with full light, as the water will warm up rapidly. Use low light intensity and be quick!!!

Microtiter plate monitoring sheet preparation (Step 7):

1. Fill up one data sheet for each plate with the following information:
    1. Species
    2. Tank
    3. Spawning date
    4. Spawning tank temperature
    5. Fecundity (from Step 4)
    6. Fertilization % (from Step 4)
    7. Number of viable eggs loaded (should be 48)



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Egg quality evaluation

No 48w-mct: 17062020a

Species: *Seriola dumerilii*
Tank: S2
Spawn date: 17/06/2020
Spawn temperature (°C): 21,5
Fecundity (#): 1.782.000
Fertilization (%): 98

	No	%
Viable	48	100
24 h	47	98
Hatched	45	96
4d live	42	93
5d live	40	89

Date: 17/06/2020 **Temp:** 21,5 °C

Date: 18/06/2020 **Temp:** 21,7 °C

Date: 19/06/2020 **Temp:** 21,6 °C

Date: 20/06/2020 **Temp:** 21,8 °C

Date: 21/06/2020 **Temp:** 21,1 °C

Date: 22/06/2020 **Temp:** 21,5 °C

Embryo development and larval survival monitoring (Step 8):

1. At our lab, mct-plates are usually examined at 1st (24 h embryo survival), 2nd and 3rd (hatching), 4th and 5th day after spawning (larval development and yolk sack absorption). During each monitoring day, the temperature of the incubator is also recorded.
2. Take the mct-plates out of the controlled temperature incubator and check them under the stereoscope (with the lids on). Score each well as follows (see also example in the next slide):
    1. V = viable, alive egg
    2. D = dead egg
    3. H = hatched egg, larva
    4. HD = dead larva (hatched, but it is now dead)
    5. More classifications are possible (e.g. S = skeletal deformity)

Warning!!! Do not leave the plates on the microscope for too long with full light, because the water will warm up rapidly. Use low light intensity and be quick!!!

Calculation of survival parameters (Step 9):

1. When data collection is completed (day 5), fill up the information at the top right of the data sheet, with the number (#) of eggs/larvae and survival % in the proper cells, as it follows:
    1. #24 h = number of viable eggs 1 day after spawning. The 24 h % = #24 h / #V (initially loaded)
    2. #Hatched = the total number of hatched eggs, both live (H) and dead (HD). The hatched % = #Hatched / #24 h
    3. #4d live = number of live larvae at 4 days after loading. The 4d live % = #4 d live / #Hatched
    4. #5d live = number of live larvae at 8 days after loading. The 5d live % = #5d live / #Hatched

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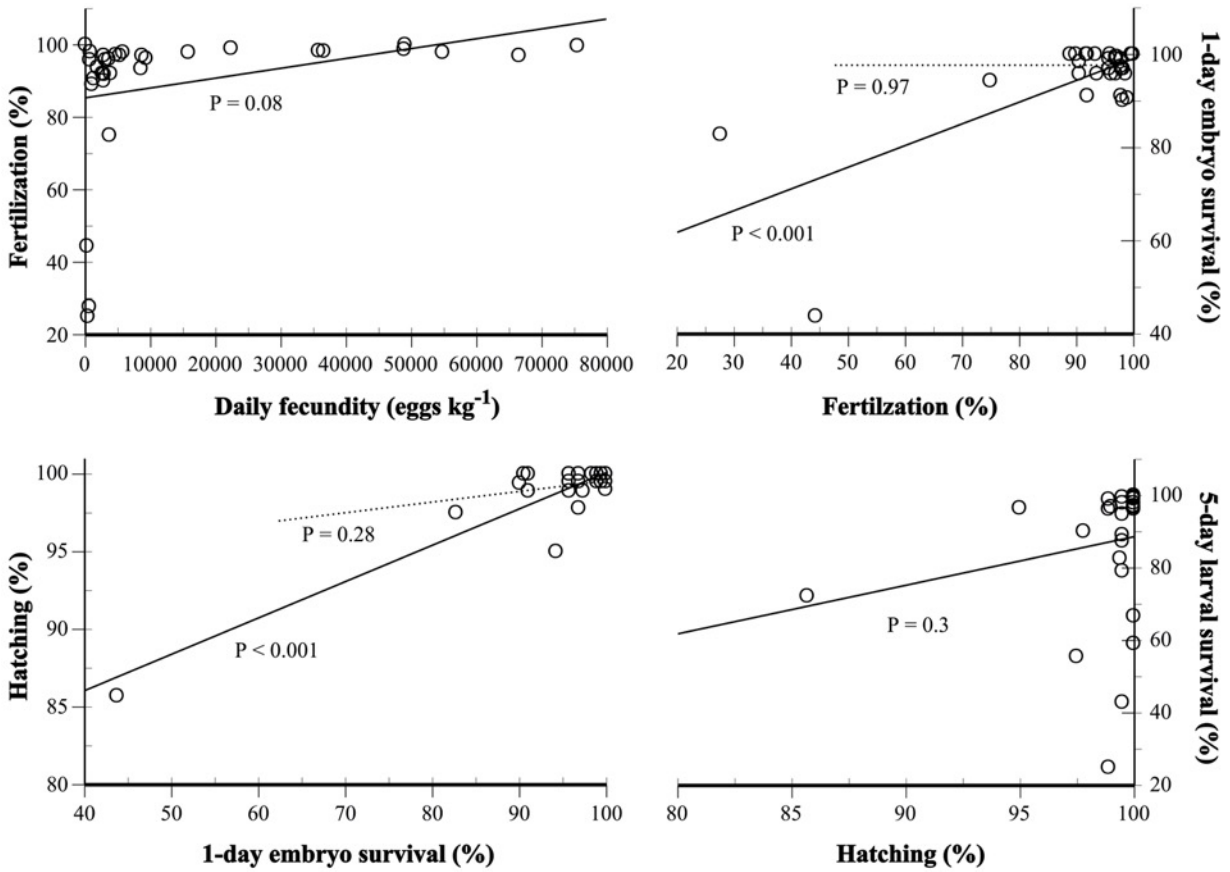
Calculation of survival parameters (Step 9, continued):
We consider estimating the percentage survival to the different stages by using in the denominator the number of individuals that survived to the previous developmental stage, as a more independent evaluation of survival within specific developmental stages, without the potential of a masking effect of the survival during the previous developmental stage.

In addition, one can develop egg and larval quality indicators that could be used in commercial hatcheries to predict the performance of a batch of eggs obtained, by examining the existence of correlations among fecundity, fertilization, embryo survival, hatching and larval survival.



Embryo development and larval survival monitoring with microtiter plate method - example data sheet

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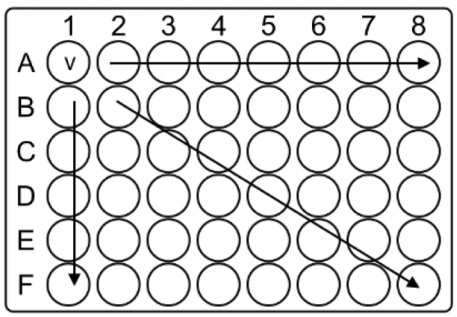
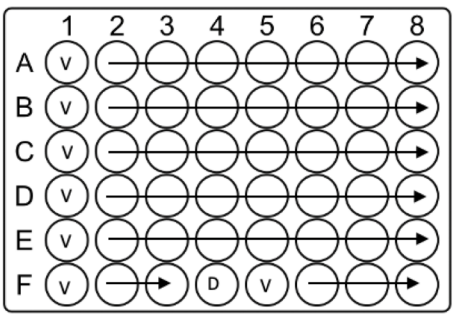
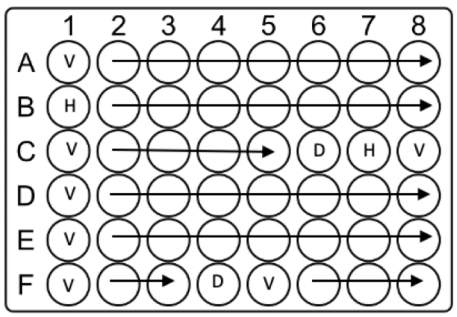


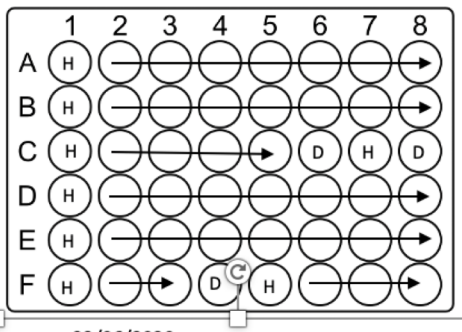
Date: 18/06/2020 **Temp:** 21,7 °C



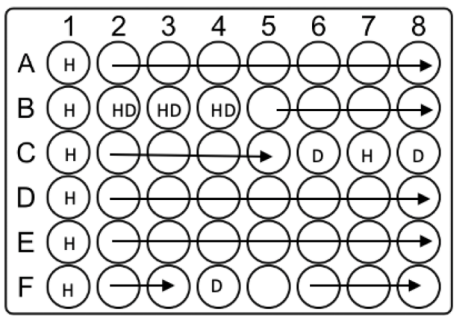
Date: 19/06/2020 **Temp:** 21,6 °C



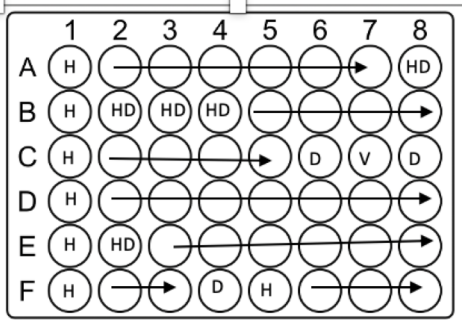
Date: 20/06/2020 **Temp:** 21,8 °C



Date: 21/06/2020 **Temp:** 21,1 °C



Date: 22/06/2020 **Temp:** 21,5 °C



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Tank: ____________________

Spawn date: ____________________

Spawn temperature (°C): ____________

Fecundity (#): ____________________

Fertilization (%): ____________________

	No	%
Viable		
24 h		
Hatched		
4d live		
5d live		

Date:________ **Temp:**________°C

	1	2	3	4	5	6	7	8
A								
B								
C								
D								
E								
F								

Date:________ **Temp:**________°C

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Date:________ **Temp:**________°C

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