OCEAN Appendix A1





Figure S1. Schematic drawing and a photograph of photobioreactors.

OCEAN Appendix A2



Figure S2. Chemical structure of some carotenoids.

OCEAN Appendix B



Spectrophotometer inside. A: Lamp housing. B: Cuvette holder. C: Lenses. D: Diffraction grating. E: Sensor array. F: USB connector.

OCEAN Appendix C1

Videos are placed on the desktop on the PC.

Introduction to Copepods.

Copepods (see figure C1) are small, free swimming creatures and are the most abundant animals in the world's oceans. They are considered as important 'grazers' of phytoplankton and serve as an important food source for many vertebrates, including fish and fish larvae. There are many different copepods species and they differ in size and shape, but also in behaviour. One important type of behaviour for its survival is escape behaviour from predators. We can investigate this behaviour to determine what copepod species would serve as a good prey for fish in a production system.



Figure C1. Copepod (Acartia tonsa) with mechanosensory hair on the antennae. The small hair can pick up disturbances in the water and in that way the copepod can perceive its environment, including detecting approaching.



Figure C2 Fish can perceive prey and start their attack at a certain distance, called the 'reaction distance', R.

Most fish use vision to remotely locate and catch their prey. The distance at which they can detect and start an attack, measured from the centre of their eyes, is called the 'reaction distance' or R (see figure2). Copepods do not use vision, but use 'hydrodynamic cues' to remotely detect their predators. They can perceive these hydrodynamic cues generated by their predators with their highly sensitive sensory setae on the antenna's (see figure 1).

There are differences between copepods in the distance at which they can perceive predators, but also in the velocity at which the copepod can escape from predators. Consequently, differences in 'detection distance' and 'escape velocity' predict differences in escape success between copepods and thus difference in suitability as a prey for fish. The worse the copepod is in escaping from predators, the better!

OCEAN Appendix C2

Experimental setup and filming setup.

To determine what copepod species is a good prey in an aquaculture system, we experimentally investigate the 'predator detection distance' and 'escape velocity' for two different copepod species. Because copepods escape at very high velocities, we use high-speed video filming. Our set-up (figure 3) consists of a high-speed camera facing a square aquarium, which contains a diagonally placed mirror. The frame rate we're filming at is 500 images per second.

Because of the mirror, we can observe the copepods movement in three dimensions. (This is equivalent to using two cameras viewing the aquarium from two directions at a right angle to each other, namely from the front and from the right side.) The 'real' copepod is on the right of the image and shows us the copepods movement in x- and y-direction (figure C4 part A). The mirror image of the copepod, on the left, shows us the copepods movement seen from the side. The movement in the horizontal direction of this mirror image gives us the movement of the copepod in the z-direction. The movement in the y-direction should be the same as for the direct image of the copepod.

For example, at time t=0 the location of the copepod in the aquarium is as in figure C4 part A. If the copepod moves upwards (in the y-direction) and to the left (x-direction), but not in the z-direction, the image could look like figure C4 part B. If the copepod would move towards or away from the camera, the movement in the x-direction of the mirror image would give us the movement in the z-direction as shown in figure C4 part C.





Figure C3 Filming set-up, top view.



Figure C4. Front view of the aquarium, as recorded by the high-speed camera. The two copepods seen is actually one individual and its mirror image. Part A shows the position of a copepod at t = 0. Part B shows movement in x-and y-direction at t = 1, and Part C shows movement of the copepod in the z-direction.

We do not put actual predators in the aquarium, but to simulate the hydrodynamic cues that are generated by fish we create a hydrodynamic cue by sucking water from the aquarium with a pipette. We define the 'predator detection distance' as the distance at which the copepod starts its escape jump, measured from the middle of the tip of the pipette and the middle of the copepod. Equation 1 describes the calculation of the 'predator detection distance'. By measuring changes in x-, y- and z-position of the copepod during an escape response over time, we can also calculate its' 'jump distance' and 'escape speed', see equation 2 and 3.

Equation 1: 'Predator detection distance'

$$d_{predator} = \sqrt{\left(x_{start} - x_{pipette}\right)^{2} + \left(y_{start} - y_{pipette}\right)^{2} + \left(z_{start} - z_{pipette}\right)^{2}}$$

Equation 2: 'Jump distance'

$$d_{jump} = \sqrt{(x_{start} - x_{end})^2 + (y_{start} - y_{end})^2 + (z_{start} - z_{end})^2}$$

Equation 3: 'Escape speed'

$$V_{escape} = \frac{d_{jump}}{\Delta t}$$

 x_{start} , y_{start} , z_{start} are respectively the x, y and z position of the copepod at the start of the jump, and x_{end} , y_{end} , z_{end} are the x, y and z position of the copepod at the end of the jump.

OCEAN Appendix D

List of physical constants

Specific heat capacity, water	C_{water}	4,18 ·10 ³	$J kg^{-1} K^{-1}$
Density, water at 0 °C	$ ho_{ m water}$	9,998 ·10 ²	kg m⁻³
Density, ice at 0 °C	$ ho_{ m ice}$	9,17 ·10 ²	kg m ⁻³
Density, air (1 atm, 20 °C)	$ ho_{ m air}$	1,22	kg m ⁻³
Velocity of light	С	3,00 ·10 ⁸	m s ⁻¹
Avogadro's number	N_A	6,022 ·10 ²³	mol ⁻¹
Planck's constant	Н	6,63 ·10 ⁻³⁴	Js
Electron charge	е	1,60 ·10 ⁻¹⁹	С
Gas constant	R	8,314	J mol ⁻¹ K ⁻¹
Boltzmann's constant	$k_{\scriptscriptstyle m B}$	1,38 ·10 ⁻²³	$J \ K^{-1}$
Proton mass	m_p	1,673·10 ⁻²⁷	kg
Neutron mass	m_n	1,675·10 ⁻²⁷	kg
Electron mass	m_e	9,11·10 ⁻³¹	kg
Atomic mass unit	u	1,661·10 ⁻²⁷	kg

OCEAN Appendix E

User manual for the Vernier spectrophotometer in absorbance mode.

- Log into the computer with the given username and password
- Double click on the "Logger Pro" icon
- Place the cuvette containing the blank solution in the spectrophotometer
- Click "Experiment" and under "Calibrate" click "Spectrophotometer: 1"
- Let the spectrophotometer warm up (90 s)
- Click "Finish Calibration"
- After 5 s, click "OK"
- Replace the cuvette containing the blank solution with a cuvette containing the solution you want to measure.
- Click the green button "Collect"
- Read off the absorbances at the wavelengths you need

Printing out from Logger Pro

- Choose "print" under File in Logger Pro.
- "Printing Options" will pop-up.
- Tick "Print Footer".
- Write your COUNTRY and TEAM (you will NOT get your print if there is no footer to identify it).
- In the figure below, you see an example for the spectrophotometer in emission mode. (The spectrum shown is from a different light source than used in the experiments).
- Tick "Print Visible Spectrum on Wavelength Graphs", if you want the coloured background in the graph.



<u>NB</u>: For the Video analyses you also need to have your COUNTRY and TEAM in the Footer (you will NOT get your print if there is no footer to identify it).

OCEAN Appendix F

User manual for Balancing of Centrifuges

See drawing below on how to balance tubes in a centrifuge. Balancing is important not to spoil the centrifuge – and to avoid accident. (The centrifuge has rapid rotation giving large centrifugal forces, which must be balanced.)

<u>Always balance the rotor before centrifugation.</u> Following are symmetrical loading of centrifuge tubes to rotor:



Incorrect method of loading tubes in centrifuge rotor:



NOTE: Incorrect method of loading tubes can lead to an accident.

Drawings and texts from: LLG Labware, Operating Manual Centrifuge.

Periodic table of the elements



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