**Protocol Parasite NP Experiment**

**Organisms & Material:**

* Two Daphnia clones (*Daphnia longispina* × *galeata* hybrids)
* Parasite *Metschnikowia bicuspidata* (1000 spores/ml)
* Three 100nm polystyrene NP treatments (0, 5 & 20 mg/L)
* 20 replicates for each Treatment -> 240 experimental units

**2 parasite/ no parasite X 3 NP X 2 clones X 20 rep. = 240 exp. units**

**Growing Food cultures:**

In exponential growth phase

*Scenedesmus obliquus* (green algae)

Medium: WC algal medium

Incubation temperature: 20°C, constant light (max 25 umol photons \*m-2\*s-1)

**Daphnia Diet:**

* Before Experiment Daphnia gets fed with 1mg C/L *S. obliquus*
* When transferred to individual jars (start of Experiment) diet changes to 0.5 mg C/L *S. obliquus* every day

**Preparation of Treatment media:**

* Use 3 x ISO flasks for preparation
* Prepare Media for no NP, low (5mg/L) and high (20mg/L) NP treatment by adding NPs stock solution to Daphnia culture Medium (concentration of 5 or 20mg/L, stock concentration 10 g/L -> volume needed for 550ml: 275 µl (5mg/L NP) or 1100 µl (20mg/L NP) from stock, volume needed for 850ml: 425 µl (5mg/L NP) or 1700 µl (20mg/L) from stock)
* Only for the first preparation of Media -> needed Volume 5ml per jar instead of 10ml (needed volume for each: 80 x 5ml = 400ml,

4 x 25ml = 100ml, volume to prepare: 550ml)

* For all other Media preparations -> needed volume for each of them: 80 x 10ml=800ml, volume to prepare = 850ml
* Transfer Media in experimental jars
* Measure optical density of the food source, calculate C-content and volume to add with the feeding protocol
* Add 0.5 mg C/L *S. obliquus* to each jar

**Preparation of parasite suspension:**

* Parasite dose: 1000 spores/ml
* A single *Metschnikowia bicuspidata* strain propagated on a laboratory‐reared *Daphnia magna* clone will be used
* Crushing the tissue from either infected or uninfected *D. magna* (control)

**Scaling-up of Daphnia clones (5 weeks 07.12-12.01)**

* Maintaining three *D. longispina* × *galeata* clones under standard conditions 12:12 light–dark photoperiod, fed daily with 1 mg C/L of *S. obliquus*, 19°C (two clones of them will be used in the experiment)
* Scaling up the Daphnia by splitting them up in new jars every week until January

**Synchronization of Daphnia (2-4 weeks 12.01-26.01/09.02):**

* Preparing 11 jars per clone and pipetting 20 Daphnia (as mothers) from the scale up for each jar -> prepare/label 33 jars, 660 Daphnia (2 mothers per juvenile needed -> 300 mothers per clone)
* 120 juveniles per clone (+ 5 extra juveniles per Treatment) -> 150 total
* Pipetting out the juveniles every second day for 2-4 weeks until the max. point of offspring is reached (120 juveniles per clone from the max offspring used as experimental animals: 240 total)
* Remove all adult/mother Daphnia (Day 0: 28.01)
* Transfer juveniles from two clones in experimental jars (Day 1: 29.01)
* Keep 5 extra juveniles per Treatment (60 juveniles) in separate jars (backup for early mortality in the first days before inoculation)

**Procedure/ Experiment (21 days starting between 26.01/09.02):**

**Day 0: (Thursday 28.01)**

* Collect juveniles for experiment by removing all mother Daphnia from synchronization jars (only two clones)
* **In the morning:** Prepare 3 ISO flasks with treatment media (Medium+HighNP, Medium+LowNP, Medium)
* Set up 240 labeled jars and transfer 5 ml treatment/control media in each jar (with electronic pipet)
* Set up 12 extra jars and transfer 25 ml treatment media in each jar
* Add 0.5 mg C/L *S. obliquus* to each jar

**Day 1: (Friday 29.01)**

* Transfer one Daphnia in each jar (120 + 30 extra Daphnia per clone)
* Transfer one clone first and then the second one

**Day 2: (Saturday 30.01)**

* Check Daphnia: remove and count dead ones and offspring (replace dead ones from extra juvenile jars)
* Feed Daphnia (0.5 mg C/L *S. obliquus)*

**Day 3: (Sunday 31.01)**

* Check Daphnia: remove and count dead ones and offspring (replace dead ones from extra juvenile jars)
* Inoculate 120 jars with parasite suspension (other 120 jars with uninfected suspension)
* No feeding!

**Day 4: (Monday 01.02)**

* Check Daphnia: remove and count dead ones and offspring
* **In the morning:** Prepare 3 ISO flasks with treatment media (Medium+HighNP, Medium+LowNP, Medium)
* Set up 240 labeled jars and transfer 10 ml treatment/control media in each jar (with electronic pipet)
* Add 0.5 mg C/L *S. obliquus* to each jar
* Feed Daphnia

**Day 5: (Tuesday 02.02)**

* Check Daphnia: remove and count dead ones and offspring
* Transfer Daphnia in new jars (120 Daphnia per clone)
* Transfer each label from old jars on the new jars while transferring Daphnia
* Clean the old jars

**Day 6: (Wednesday 03.02)**

* Check Daphnia: remove and count dead ones and offspring
* Feed Daphnia (0.5 mg C/L *S. obliquus)*

**Day 7: (Thursday 04.02)**

* Check Daphnia: remove and count dead ones and offspring
* Feed Daphnia (0.5 mg C/L *S. obliquus)*

**Day 8: (Friday 05.02)**

* Check Daphnia: remove and count dead ones and offspring
* **In the morning:** Prepare 3 ISO flasks with treatment media (Medium+HighNP, Medium+LowNP, Medium)
* Set up 240 labeled jars and transfer 10 ml treatment/control media in each jar (with electronic pipet)
* Add 0.5 mg C/L *S. obliquus* to each jar
* Feed Daphnia

**Day 9: (Saturday 06.02)**

* Check Daphnia: remove and count dead ones and offspring
* Transfer Daphnia in new jars (120 Daphnia per clone)
* Transfer each label from old jars on the new jars while transferring Daphnia
* Clean the old jars

**Day 10: (Sunday 07.02)**

* Check Daphnia: remove and count dead ones and offspring
* Feed Daphnia (0.5 mg C/L *S. obliquus)*

**Day 11: (Monday 08.02)**

* Check Daphnia: remove and count dead ones and offspring
* Transfer dead Daphnia from infected treatments into labelled Eppendorf to fix them (how?)
* Feed Daphnia (0.5 mg C/L *S. obliquus)*

**Day 12: (Tuesday 09.02)**

* Check Daphnia: remove and count dead ones and offspring
* **In the morning:** Prepare 3 ISO flasks with treatment media (Medium+HighNP, Medium+LowNP, Medium)
* Set up 240 labeled jars and transfer 10 ml treatment/control media in each jar (with electronic pipet)
* Add 0.5 mg C/L *S. obliquus* to each jar
* Feed Daphnia

**Day 13: (Wednesday 10.02)**

* Check Daphnia: remove and count dead ones and offspring
* Transfer Daphnia in new jars (120 Daphnia per clone)
* Transfer each label from old jars on the new jars while transferring Daphnia
* Clean the old jars

**Day 14: (Friday 11.02)**

* Check Daphnia: remove and count dead ones and offspring
* Feed Daphnia (0.5 mg C/L *S. obliquus)*

**Day 15: (Saturday 12.02)**

* Check Daphnia: remove and count dead ones and offspring
* Feed Daphnia (0.5 mg C/L *S. obliquus)*

**Day 16: (Sunday 13.02)**

* Check Daphnia: remove and count dead ones and offspring
* **In the morning:** Prepare 3 ISO flasks with treatment media (Medium+HighNP, Medium+LowNP, Medium)
* Set up 240 labeled jars and transfer 10 ml treatment/control media in each jar (with electronic pipet)
* Add 0.5 mg C/L *S. obliquus* to each jar
* Feed Daphnia

**Day 17: (Monday 14.02)**

* Check Daphnia: remove and count dead ones and offspring
* Transfer Daphnia in new jars (120 Daphnia per clone)
* Transfer each label from old jars on the new jars while transferring Daphnia
* Clean the old jars

**Day 18: (Tuesday 15.02)**

* Check Daphnia: remove and count dead ones and offspring
* Feed Daphnia (0.5 mg C/L *S. obliquus)*

**Day 19: (Wednesday 16.02)**

* Check Daphnia: remove and count dead ones and offspring
* Feed Daphnia (0.5 mg C/L *S. obliquus)*

**Day 20: (Thursday 17.02)**

* Check Daphnia: remove and count dead ones and offspring
* **In the morning:** Prepare 3 ISO flasks with treatment media (Medium+HighNP, Medium+LowNP, Medium)
* Set up 240 labeled jars and transfer 10 ml treatment/control media in each jar (with electronic pipet)
* Add 0.5 mg C/L *S. obliquus* to each jar
* Feed Daphnia

**Day 21: (Friday 18.02)**

* Check Daphnia: remove and count dead ones and offspring
* Transfer Daphnia in new jars (120 Daphnia per clone)
* Transfer each label from old jars on the new jars while transferring Daphnia
* Clean the old jars

Measured Factors:

Host fitness:

* Check Daphnia every 24hours (for 3 clutches/21 days) for: number of survived/dead, time until first offspring, number of neonates to calculate fecundity, population growth (offspring per day)

Parasite fitness:

* Checking fixed animals under microscope for parasite infectivity (presence of parasite spores)
* Estimation of parasite reproduction (number of spores produced until host death) by using a counting chamber