

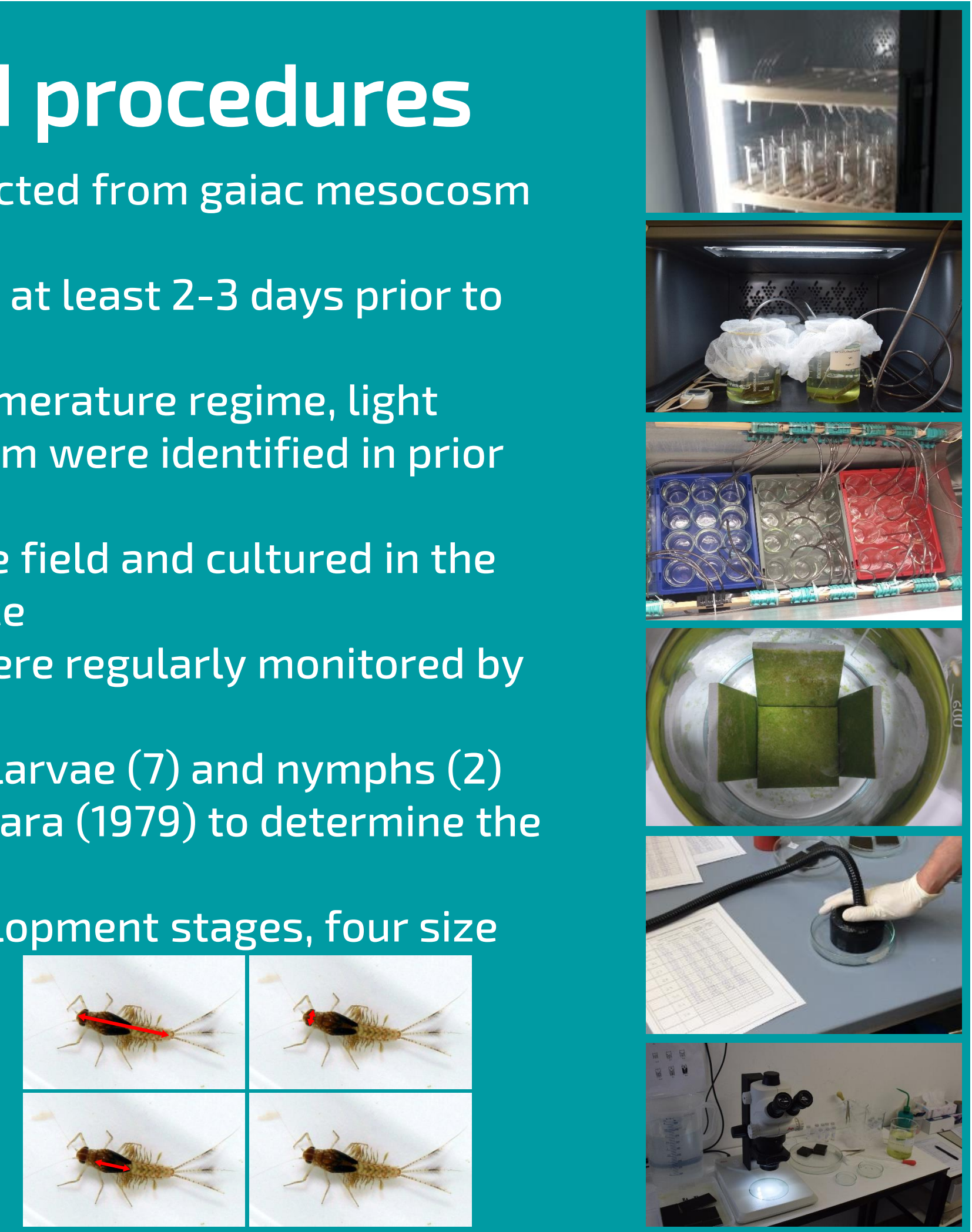
Why mayflies?

- Mayflies belong to the EPT taxa which are considered to be very sensitive against the exposure to several pesticides. Thus, they are of increasing interest in environmental risk assessment during the last decade.
- So far, no standardized test system is available for species belonging to the EPT taxa.
- In the last years some effort has been made to develop test systems for acute and chronic toxicity of mayflies.
- The species *Cloeon dipterum* turned out as the most promising species for pesticide risk assessment because (1) it has been proven as highly sensitive species in aquatic mesocosm studies and (2) it can be collected from mesocosm test facilities almost throughout the whole year.
- We developed a robust test systems for *Cloeon dipterum* to obtain reliable data on acute and chronic lethal effects of chemicals and sublethal endpoints like the larval development rate. At the moment, we are working on an optimal test design focusing on the endpoint feeding inhibition.



Test conditions and procedures

- Cloeon dipterum* larvae were collected from gaiac mesocosm ponds
- Larvae were cultured in the lab for at least 2-3 days prior to use in the experiments
- Optimal test conditions such as temperature regime, light conditions and suitable test medium were identified in prior experiments
- Natural periphyton collected in the field and cultured in the laboratory was used as food source
- Periphyton quantity and quality were regularly monitored by fluorescence measurements
- Different development stages for larvae (7) and nymphs (2) were identified according to Cianciara (1979) to determine the speed of larval development
- To characterize the different development stages, four size measures were used:
 - Body length (without cerci)
 - Width of the head capsule
 - Length of the wing pad
 - Dry weight

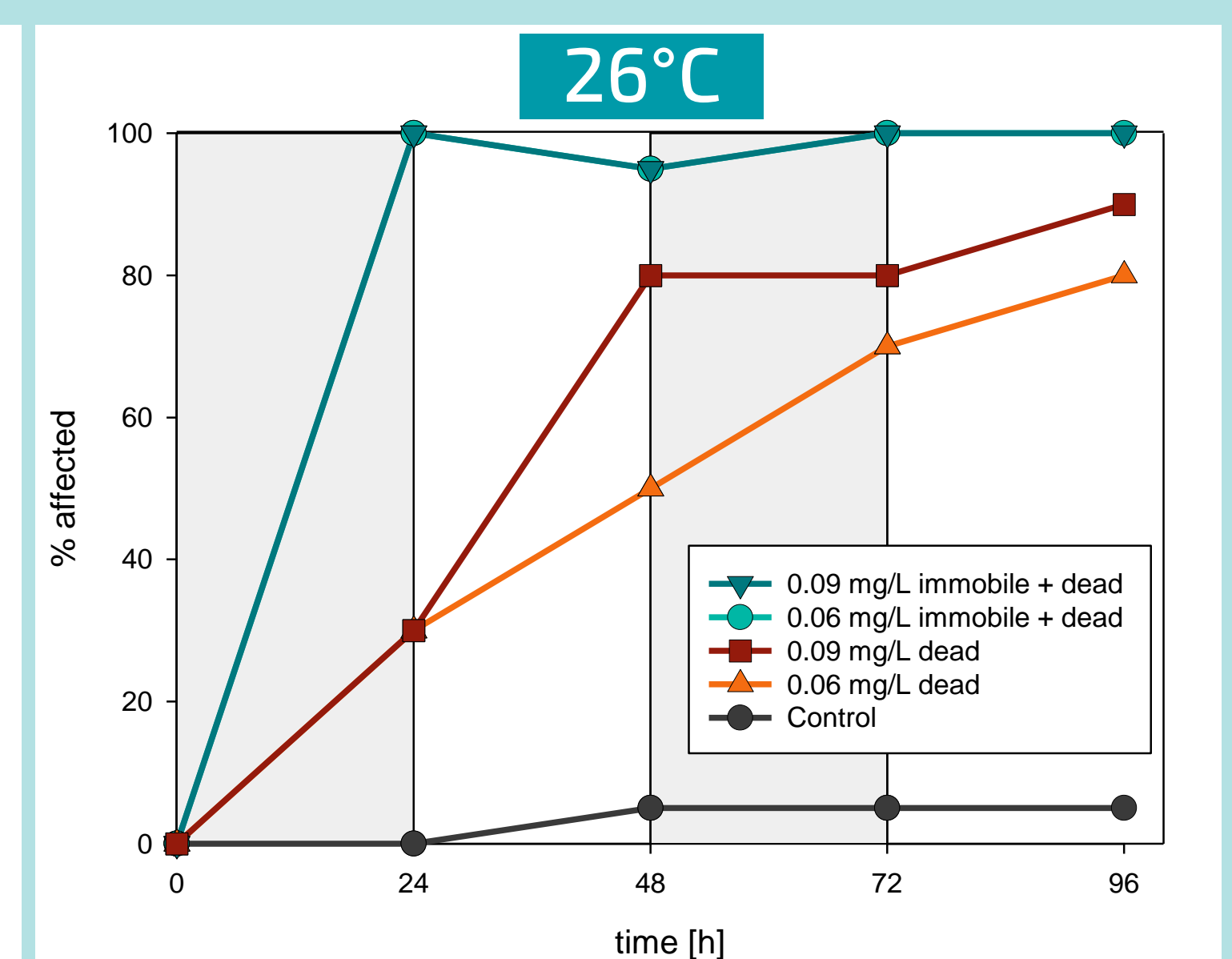
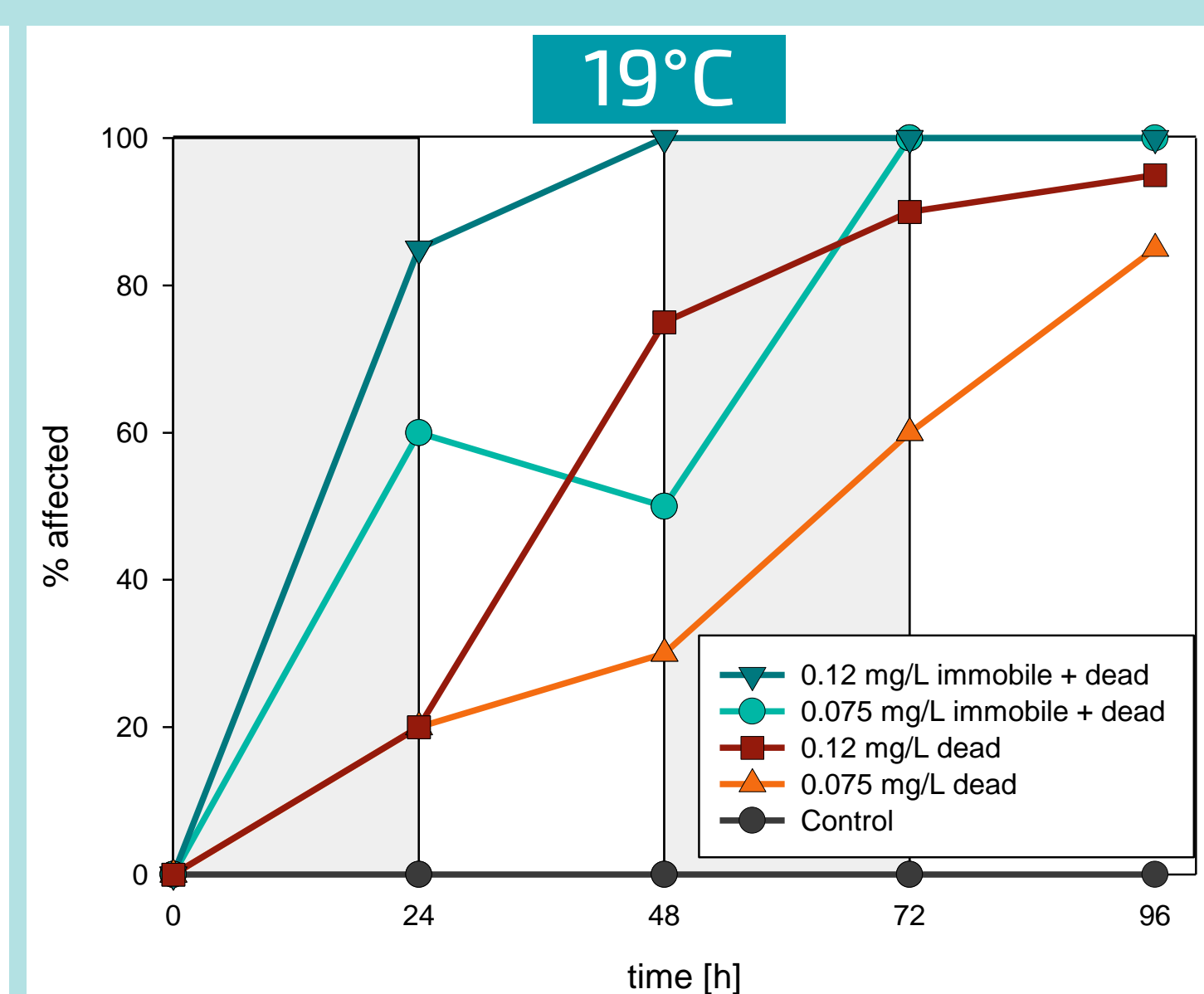
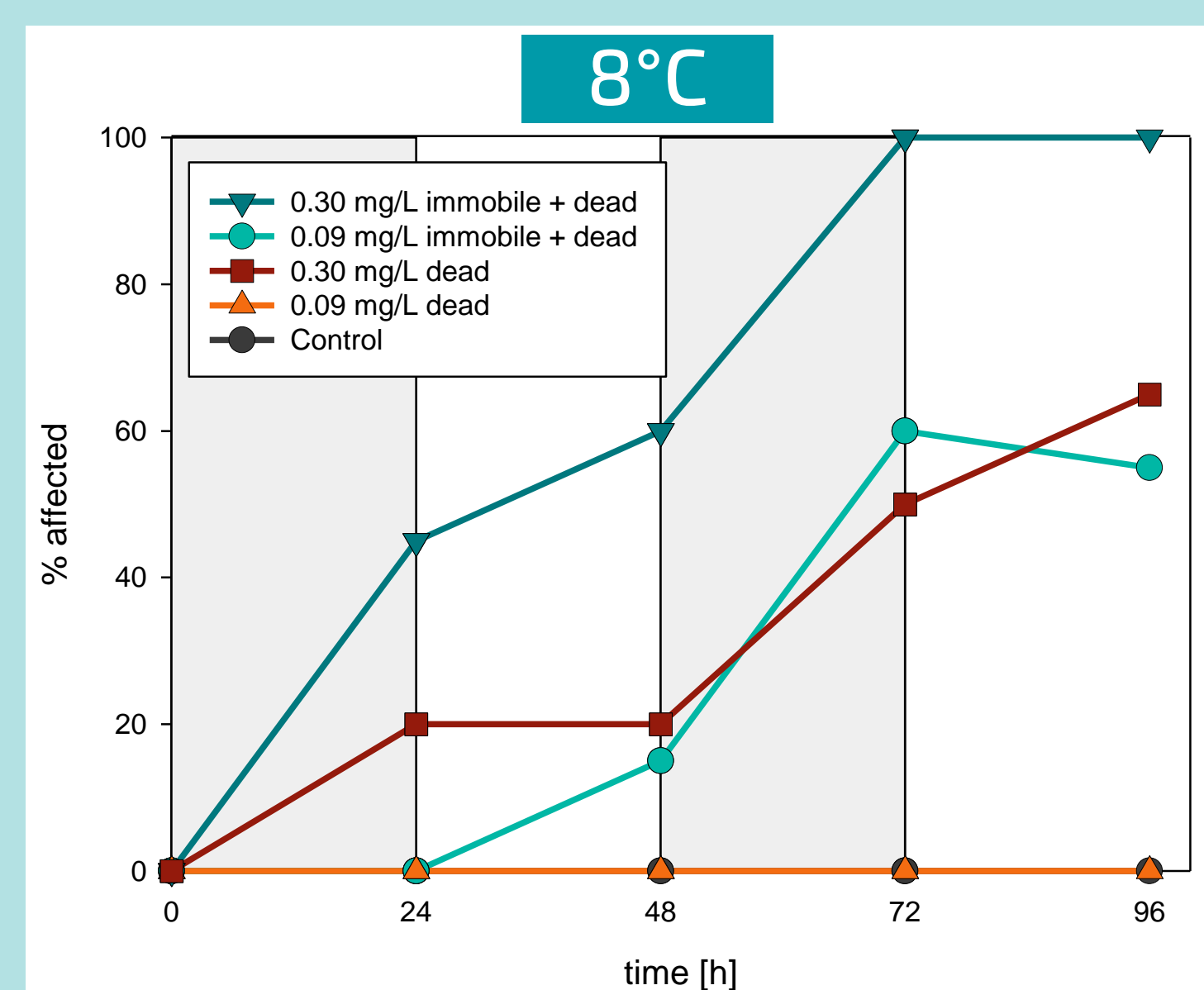


Acute tests with Imidacloprid >>> Low control mortality | Increasing sensitivity with increasing temperature

Endpoints: mortality & immobility

Test design:

- L4 larvae from mesocosm ponds
- 50 ml M4 medium in glass vessels
- 4 replicates with 5 individual larvae each
- Test duration 96h
- Observation every 24h
- No addition of food
- Gentle aeration
- 16h:8h light-dark cycle (diffuse light)
- Different temperatures
- Control and 2 test concentrations

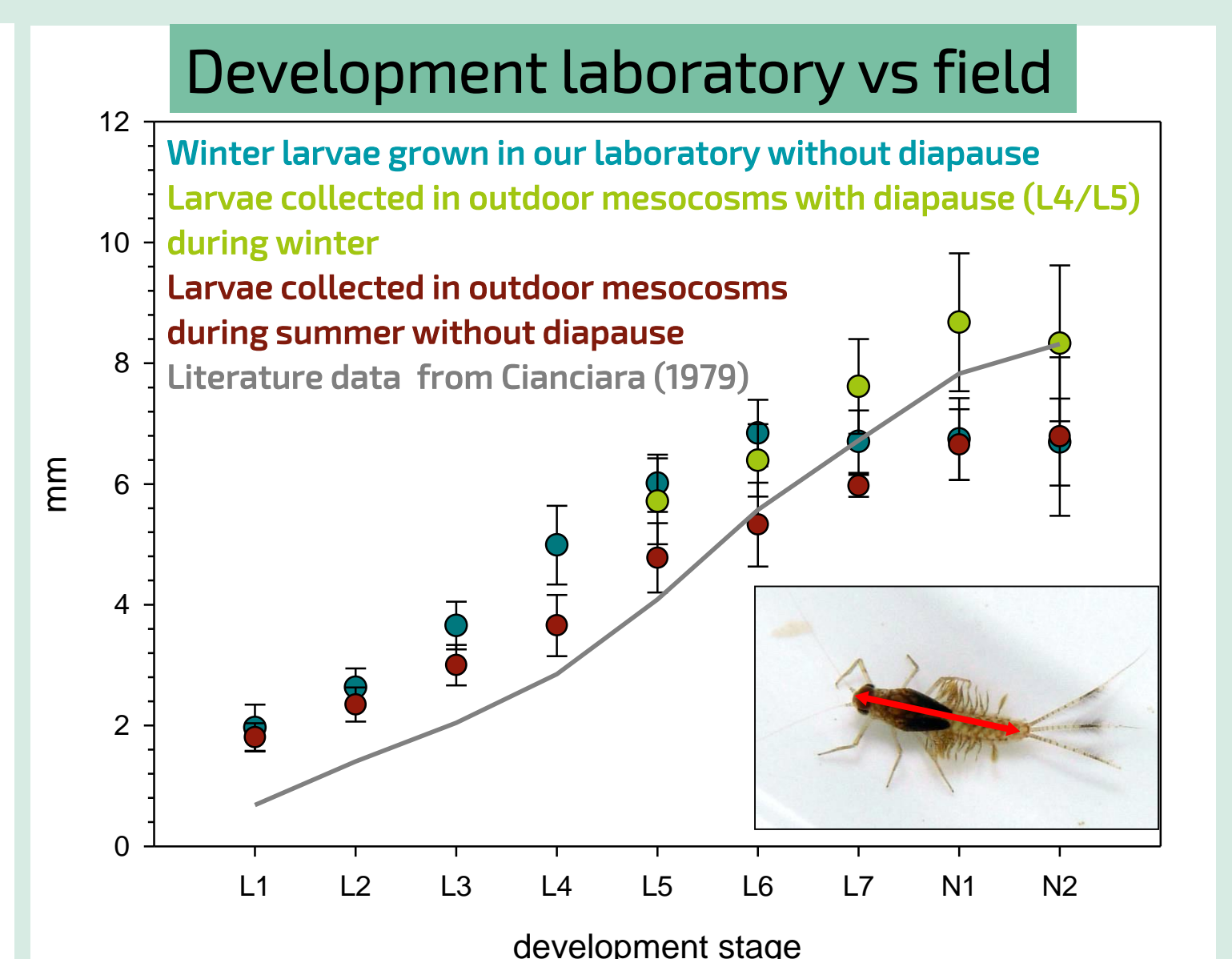
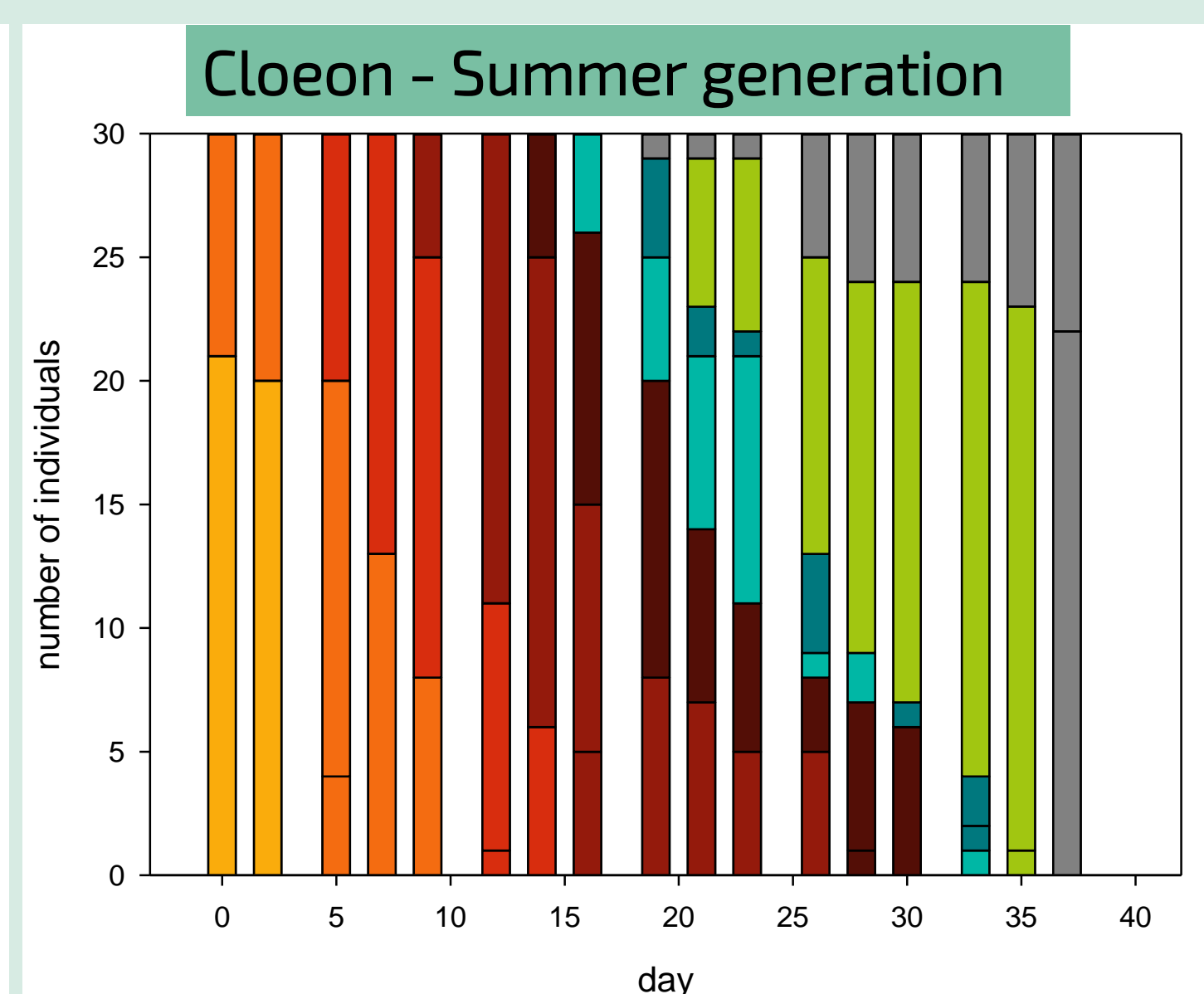
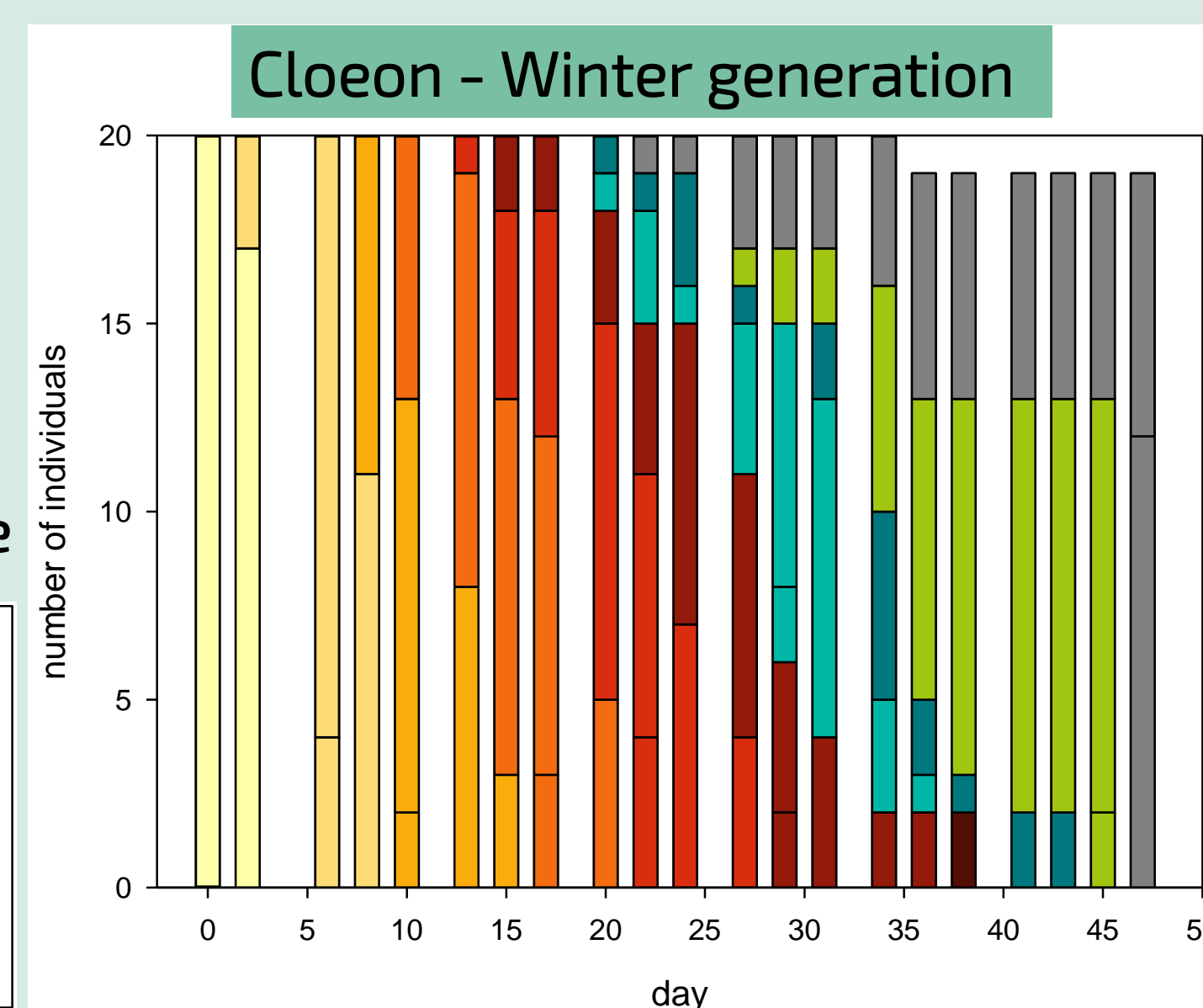


Chronic tests – Growth experiments >>> Comparable development rate of 0.03 and 0.04/day Comparable emergence ratio of 63 and 73% for winter and summer generation

Endpoint: mortality & development (size, weight, duration of larval stages)

Test design:

- L1 or L2/L3 larvae from mesocosm ponds
- M4 medium in 1L glass beakers
- 4 replicates with 5 individual larvae each
- Food: Natural periphyton from lab culture
- Test duration: until emergence
- Temperature: 18°C
- Gentle aeration
- 16h:8h light-dark cycle
- No treatment tested yet

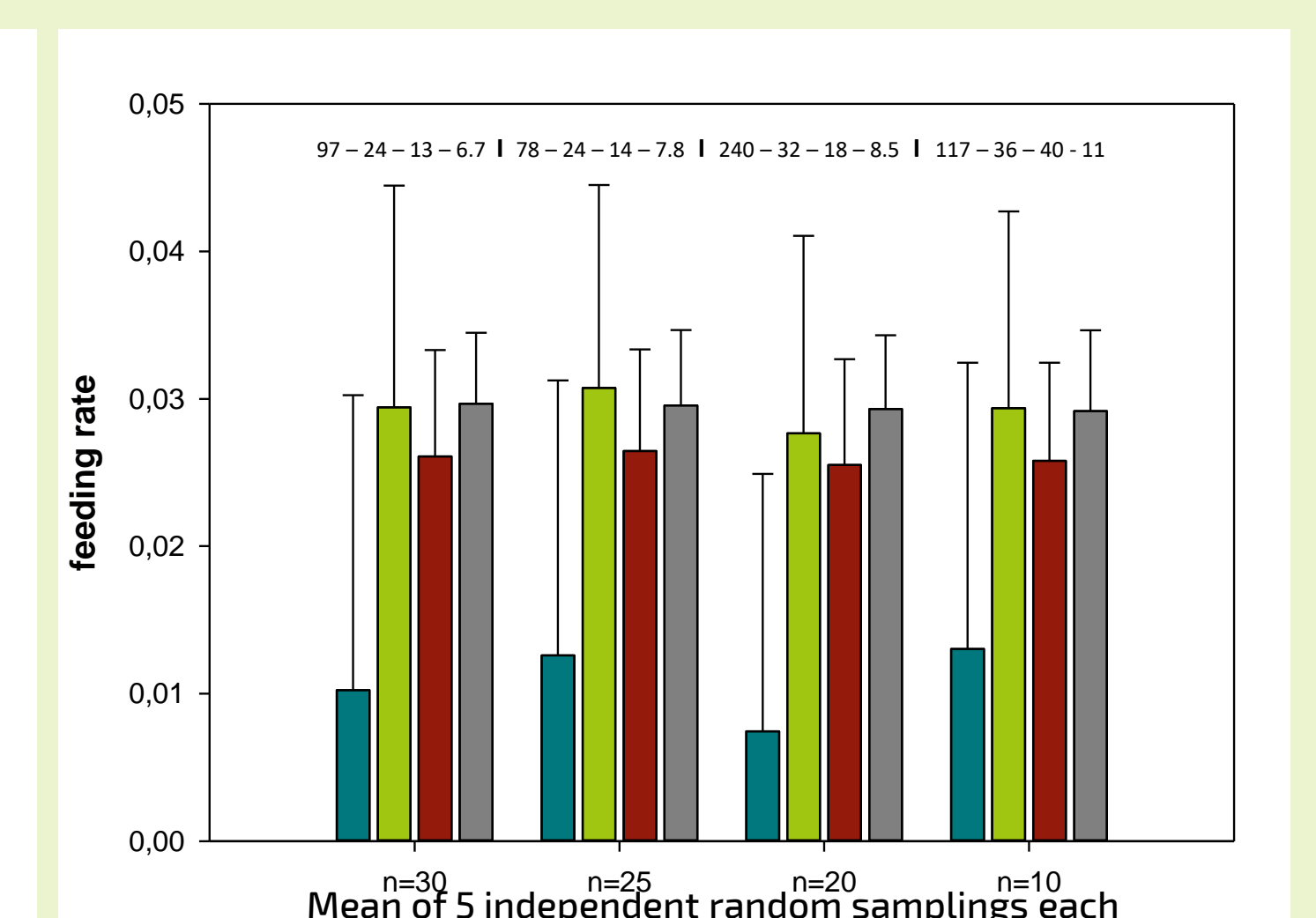
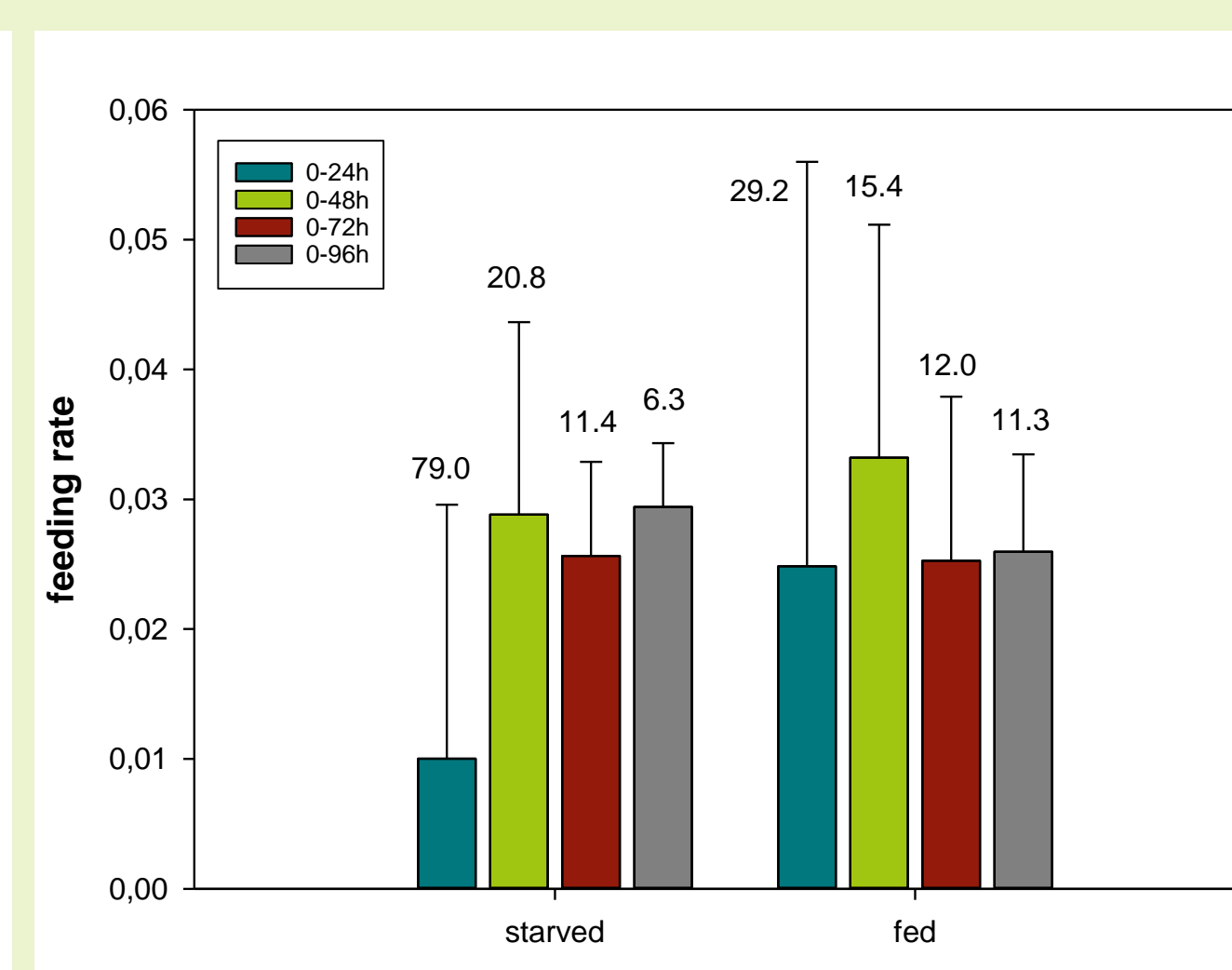
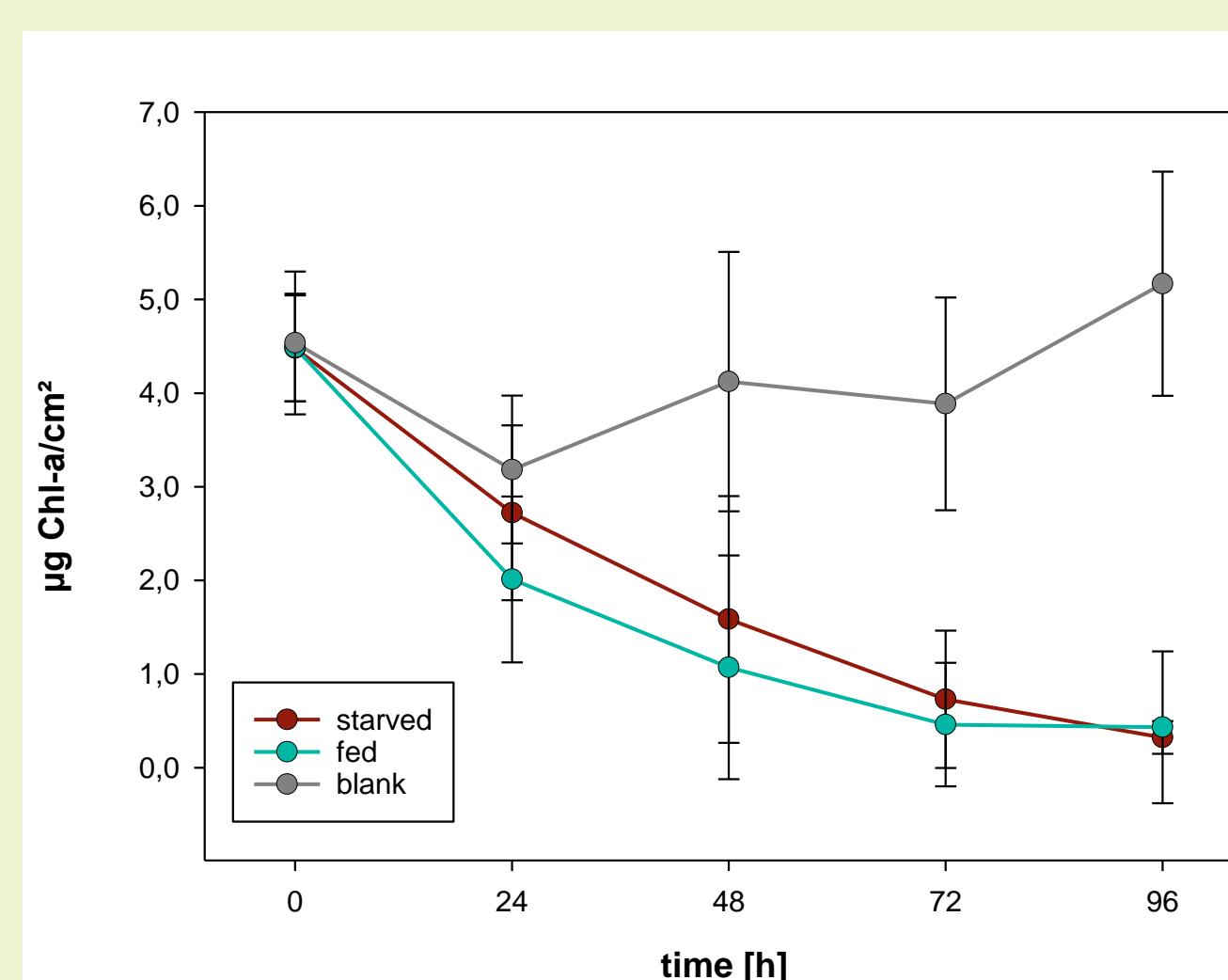


Feeding inhibition tests >>> Food uptake is well quantifiable every 24h without major disturbance of the test system A 72h preincubation period without food before test start will not influence the control feeding rate Variability of the feeding rate is decreasing with increasing test duration (%MDD indicated above the bars)

Endpoint: feeding rate

Test design:

- L5 larvae held individually, 35 replicates
- 200 ml M4 medium in glass vessels
- 20°C, no aeration
- 16h:8h light-dark cycle (diffuse light)
- Duration: 96 h
- Food source: unglazed tile with natural periphyton, cultured in the lab
- Measured endpoints: Chl-a fluorescence of periphyton, dry weight of *Cloeon* larvae
- Feeding rate according to Maltby et al. 2002
- No treatment tested yet



Conclusions

- Acute tests with *Cloeon dipterum* show low control mortality (<5%), high reproducibility and increased sensitivity with increasing temperature.
- Chronic tests pointed out that similar development rates and emergence ratios for summer and winter generation were found. In the lab test we reached development times as well as larvae sizes comparable to field and literature data.
- First experiments on feeding inhibition are promising and reveal that variability can be sufficiently decreased to detect treatment effects.

1.02P.10
Poster presentation at

SETAC SciCon
SETAC Europe 30th Annual Meeting
Open Science for Enhanced Global Environmental Protection

For more information on a standardized lab test with *Cloeon dipterum* see also poster 2.02P.23