

Impact of temperature and salinity on the morphological variability of lab-grown *Cyprideis torosa* (Jones, 1850) (Ostracoda)

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Introduction & Aim

Ostracods are tiny crustaceans, enclosed of a calcitic carapace to protect their soft body from harsh environments. Their valves are often used as proxies for palaeoenvironmental and climatic reconstructions. *Cyprideis torosa* is a widespread brackish water ostracod-species, tolerating a wide range of oxygen, salinity as well as temperature. Due to the high morphological variability of the carapace, the species is a well-suited study object relating environmental conditions to its morphology. But since they live in habitats with great spatio-temporal variability like estuaries or lagoons, it is necessary to study their phenotypic adaptations to different conditions in well monitored and controlled lab-cultures. The main focus of this study is to assess the differences in morphology between the female adults of the initial starter group and the female adults of the first generation, developed in artificial settings, whether temperature, specifically between 20°C and 30°C, affect the size and ornamentation within the same salinity and whether salinity plays a minor role in influencing morphology, too.



Fig.1: Overview Italy, data from word-administrative-boundaries

Material & Methods

- Location: **Marano Lagoon (northern Italy)** in June 2024
- Collection of local water (5 l) & sediment (~700 ml volume) samples
- Samples provided three levels of salinity: **PSU 7.7, 16.1 & 29.6**
- Samples were used to start experimental cultures - each salinity setting incubated at four different constant temperatures - 15°C, 20°C, 30°C & 35°C and outside with fluctuating temperatures (called NAT)
- **Experiment setup:**
 - 20-25g wet sediment and ~ 150 mL water
 - 12 adult female & 8 adult male specimens (ostracods from PSU 7.7) for all cultures --> served as initial population for the first generation
 - Adult ostracods were stored in 96% ethanol glass vials, valves separated & soft body dissected under the microscope
 - Pictures of valves & mandibles under Keyence VHX-1000 digital microscope
 - Describing parameters: **length & height** were measured using complementary program
 - Semi-quantitative assessment of ornamentation
 - Collection of an additional ecological samples (MLD4)
 - 130 ml sediment volume

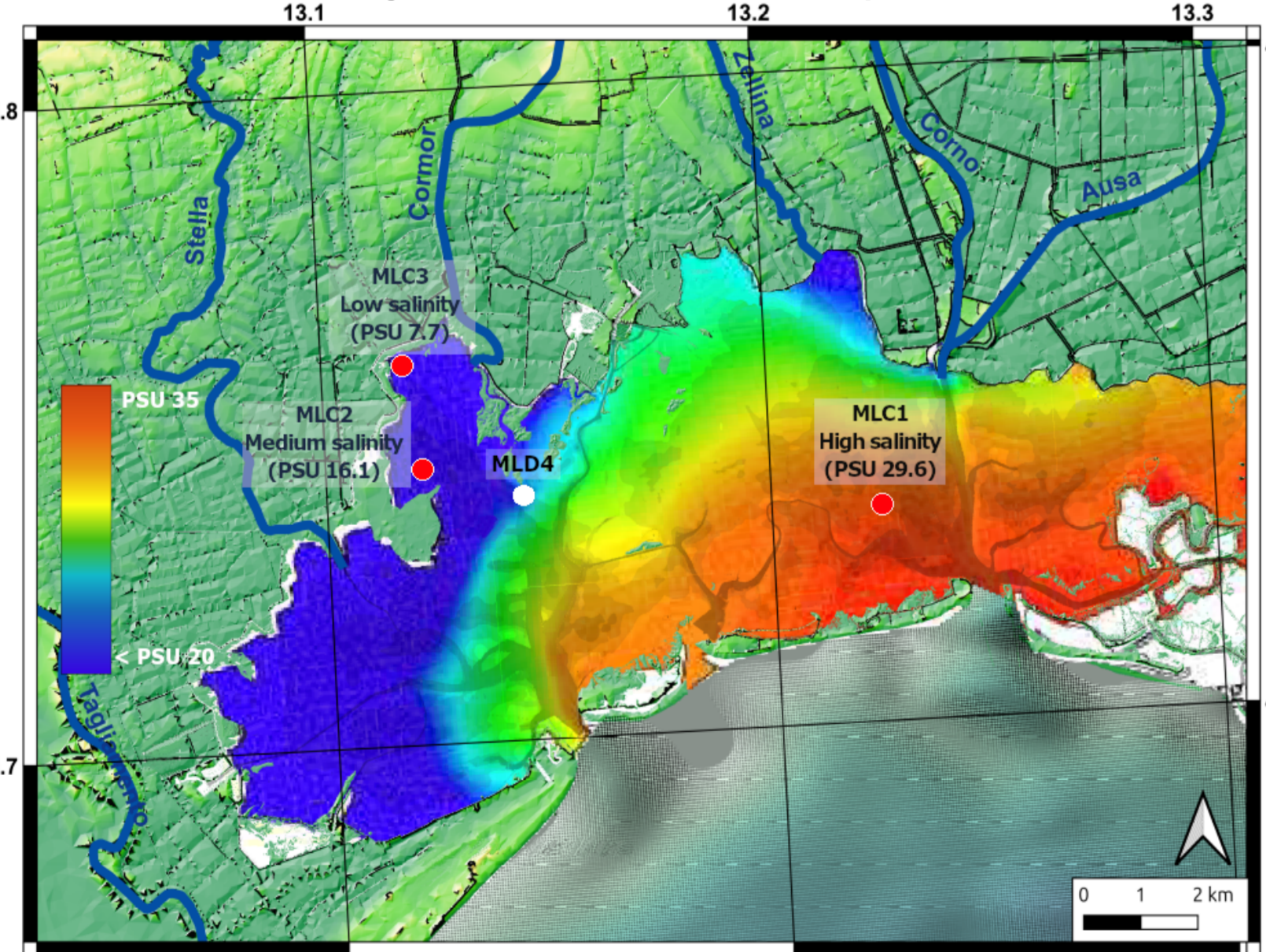


Fig.2: Location of the samples in the Marano Lagoon with salinity data TINITALY-DEM from Tarquini et al. (2023) & salinity data from Ferrarin et al. (2010); from Berndt et al. (2024); modified by Christopher Berndt and Paulina Kukacka

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Initial population (starter) for each culture:

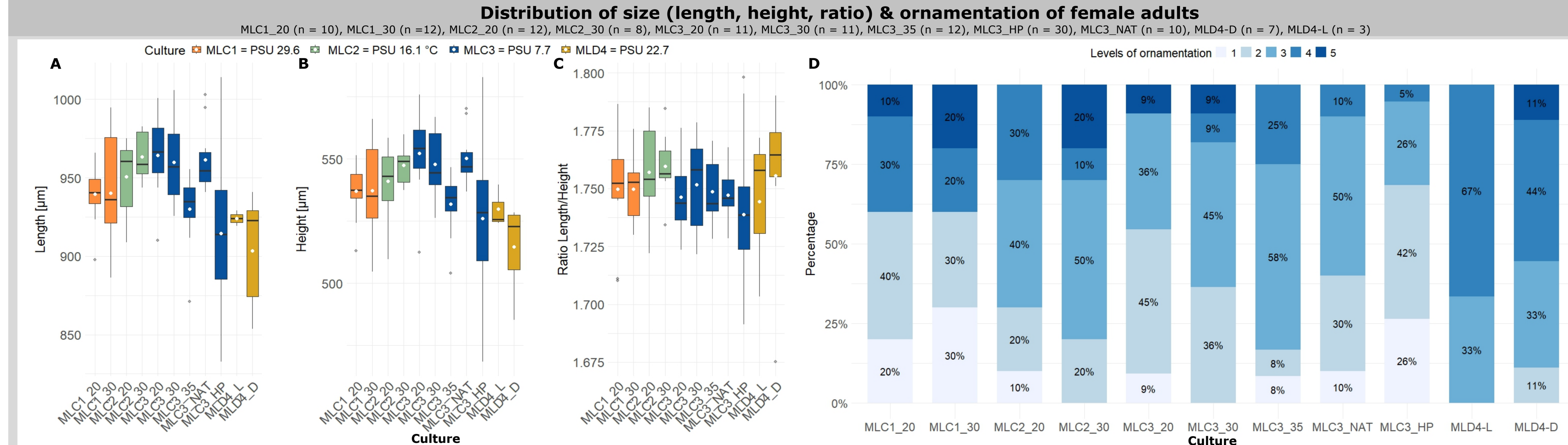


Fig.3: Distribution of length (A), height (B), L/H ratio (C) and ornamentation (D) of the initial population between the cultures; MLC3 cultures were set up first on 09.07.2024, followed by MLC2 (26.07.2024) and MLC1 (01.08.2024). For MLC3 at 35°C starters at 20°C were reestablished a few times (last one 10.10.2024) due to its high mortality. At lower salinity, starters incubated at 35°C differ significantly in length & height from those selected for 20°C. Culture names & abbreviations: MLC = Marano Lagoon Culture; MLC3_NAT = culture incubated at salinity of PSU 7.7 & located outside (exposed by varying temperatures: 8-28°C); MLC3_HP = Permanent Culture, individuals directly collected from the Marano Lagoon (experienced PSU 7.7 & ~ 26°C); MLD4 = Marano Lagoon location 4, L = Living (alive at the time of sampling), D = Dead (already dead at the time of sampling)

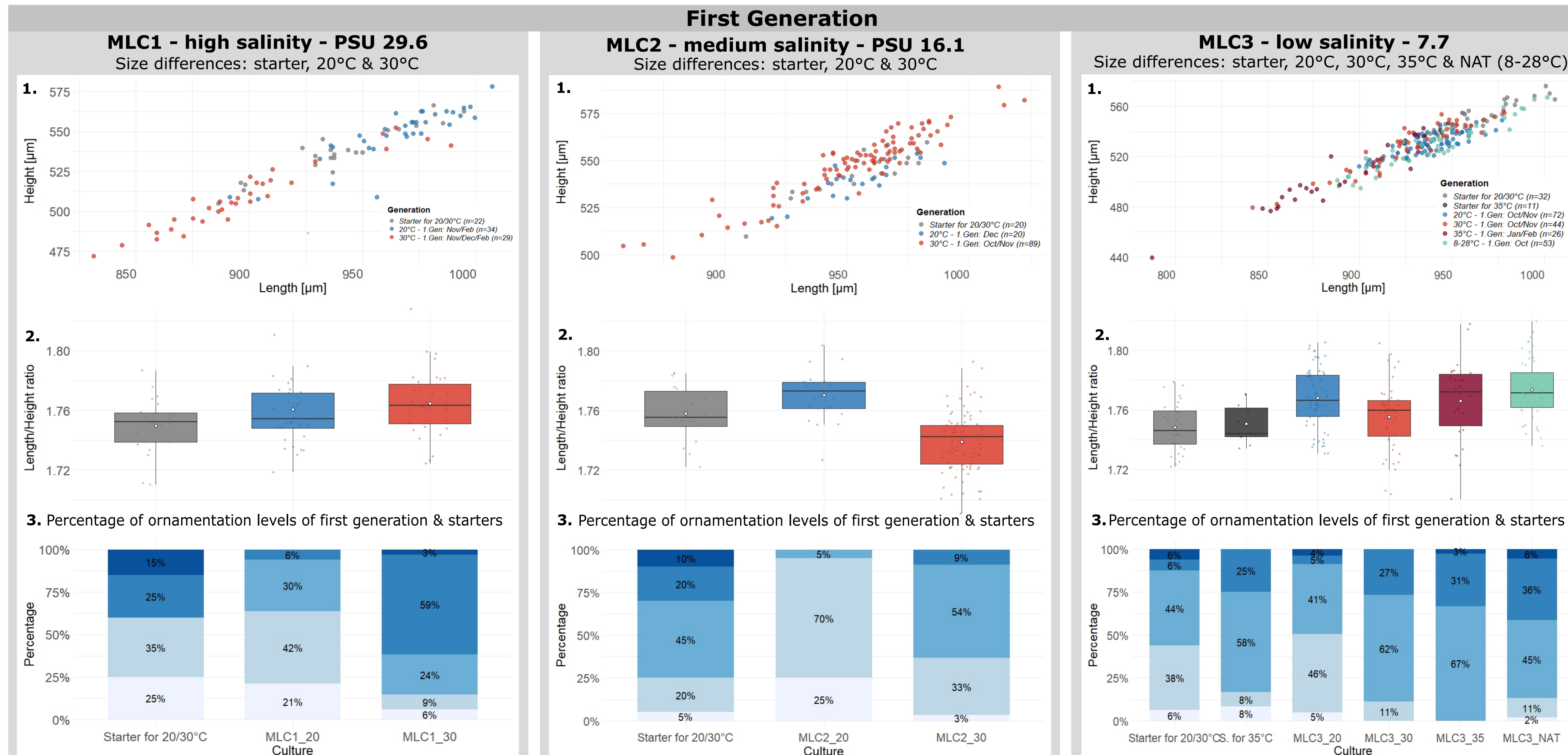
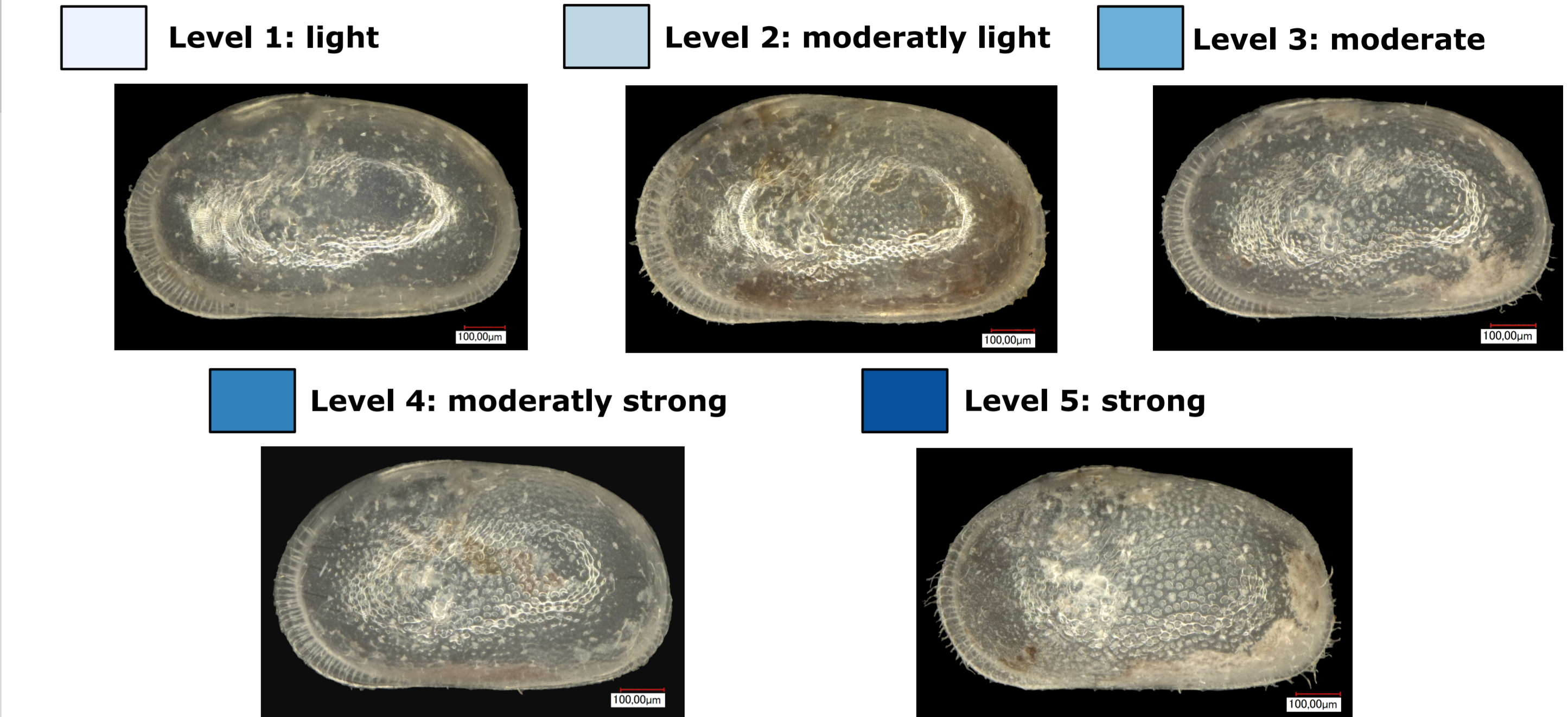


Fig.4: MLC1: 1. scatter plot (length vs. height), 2. boxplot (L/H ratio), 3. bar chart (ornamentation)
 Fig.5: MLC2: 1. scatter plot (length vs. height), 2. boxplot (L/H ratio), 3. bar chart (ornamentation)
 Fig.6: MLC3: 1. scatter plot (length vs. height), 2. boxplot (L/H ratio), 3. bar chart (ornamentation) with shown separated starter between 20/30°C and 35°C

Ornamentation levels of ostracods: 1 (light) to 5 (strong)



Differences in size & form



Fig. 7: left valves of species *Cyprideis torosa*; Nr. 48 from culture MLC3_30 (A), Nr. 27 from MLC2_20 (B), Nr. 25 from MLC1_20 (C); with length range between 787.3 µm (MLC3_35) to 1022,53 µm (MLC2_30) & height range between 439.8 µm (MLC3_35) to 589.2 µm (MLC2_30)

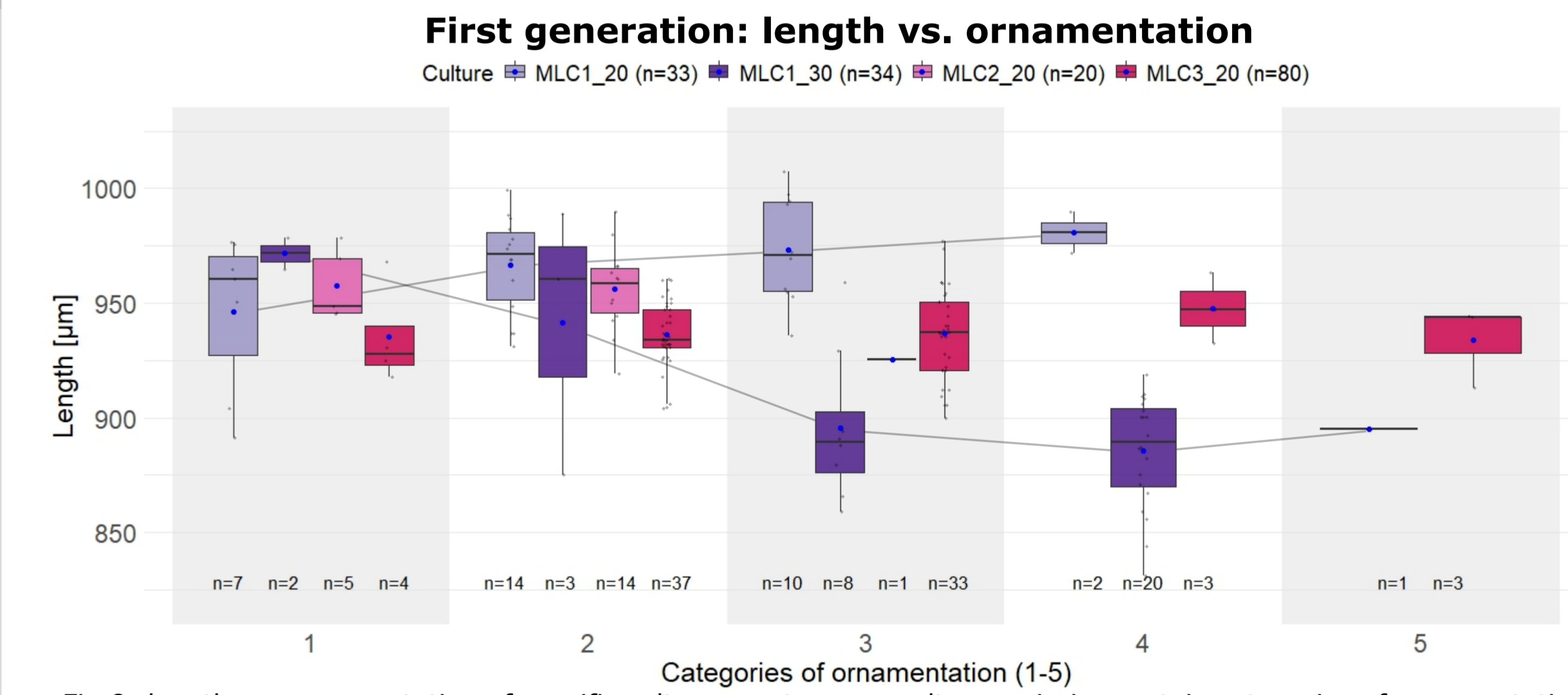


Fig.8: length vs. ornamentation of specific cultures; note some cultures missing certain categories of ornamentation

Take home message

- variation in experiment set-up times for different salinities, may be additional responsible for differences of the first generation between salinities
 - MLC1: bimodal distribution of starters reflects first generation (at 30°C) - no change in size distribution
 - Ratio of first generation get higher compared to starters (except for: MLC2_30)
 - stronger influence of salinity between temperatures, especially at higher salinities
 - higher temperatures leading to significantly smaller carapace except at moderate salinity
 - Smoother ornamentation at cooler temperature and higher salinity
 - Culture with fluctuating temperature (MLC3_NAT)
 - average size correlates best with MLC3_20 --> size may be unaffected to diurnal changes
 - MLD4: length & height resemble best permanent culture of MLC3 & ratio with MLC2 at 30°C & ornamentation with ~ MLC3 varying temperature or ~ MLC1 at 30°C
 - too small dataset for meaningful comparison
 - reproduction temperature vs. growth/molting temperature (calcification)
- > More morphological analysis are required to separate temperature & salinity influence on the size distribution of cultured specimen of *C. torosa*!**