Effects of chronic and acute salinity changes on thermal tolerance in the tidepool copepod (*Tigriopus californicus)*

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Background and Objective

- The copepod *Tigriopus californicus* inhabits dynamic, high-intertidal pools where multiple environmental factors vary in both predictable and unpredictable fashion.
- In particular, salinity and temperature can vary over a wide range, and they do so following different temporal patterns.
- *Tigriopus californicus* can serve as a model system for examining species' responses to variation in more than one type of stressor.
- **To quantify interactions between variation in salinity and acute thermal tolerance, we conducted three related experiments in which the timing and/or intensity of salinity change varied.**

Experiment # 1: Females reared to maturity at constant salinity and temperature

Methods

- Mature, egg-bearing females were placed in seawater at 33 PSU or 60 PSU, in each of two different temperatures (15 and 23°C).
- As they developed, mature, egg-bearing female offspring were subjected to acute heat stress at a range of temperatures in a PCR machine (Fig. 1).
- Survival at 3 days was used to estimate the lethal temperature (LT_{50}) in each treatment.

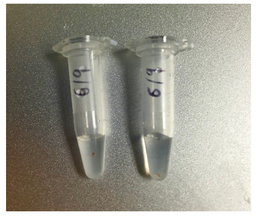

Figure 1. Microcentrifuge tubes with a single female copepod in each and 1ml of salt water, before exposure to a heat ramp in a PCR machine.

Results

- Rearing juveniles for their entire life from eggs to adult, egg-bearing females at two different constant temperatures had no effect on LT_{50} – it was ~38°C in both groups raised at 33 PSU (Table 1).
- Sample size was limited due to lower reproductive performance at elevated salinity, but egg-bearing females reared at 60 PSU in either 15 or 23°C exhibited 100% survival after 38°C heat ramp (n=6 for each temperature)
- Therefore, chronic developmental exposure to high salinity had more of an effect on acute thermal tolerance than variation in chronic temperature exposure.

Table 1. Median lethal temperature (LT_{50}) of adult female copepods reared at two temperatures and two salinities.

	33PSU	**60PSU**
15°C	37.8 ± 0.2°C	> 38.0°C
23°C	38.0 ± 0.2°C	> 38.0°C

Experiment #2: Adults exposed to acute salinity change following constant salinity acclimation

Methods

- Copepods collected from Hopkins Marine Station were acclimated in 60 PSU or 30 PSU seawater at constant temperature of 22°C (room temperature) for two weeks.
- Individuals were acutely transferred to one of six test salinities (5, 20, 35, 60, 80, or 100 PSU).
- Each source x acute salinity group was then tested in each of seven acute temperature ramps in a PCR machine, with 5 animals per tube (n = 10-20 at 22, 34, 36, 37, 38, 40, and 42°C).

Results

- Thermal tolerance (LT_{50}) varied dramatically between the two acclimation salinities when transferred to high acute salinities (Fig. 2):
  - 100 PSU: 9.3°C difference
  - 80 PSU: 4.8°C difference

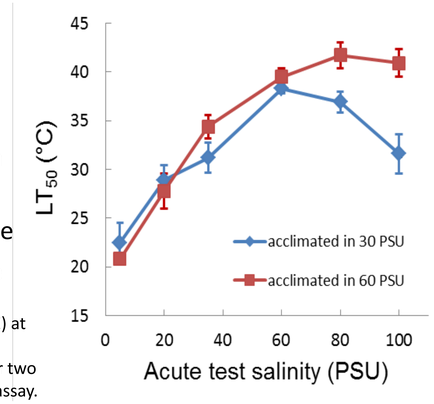

Figure 2. Lethal temperature (LT_{50} ± SE) at each test salinity for adult copepods acclimated to each of two salinities for two weeks prior to the thermal tolerance assay. LT_{50} was estimated from a binomial GLM.

- Animals acclimated to 60 PSU outperformed those from 30 PSU only when exposed to thermal stress at even higher salinity.
- We hypothesize that this physiological "cross-talk" occurs via accumulation of osmolytes that are also thermoprotectants.

Experiment # 3: Adults exposed to natural variation in salinity and temperature

Methods

- Mature, egg-bearing female copepods from a tidepool at Hopkins Marine Station (Fig. 3) were opportunistically sampled for thermal tolerance, before and after a natural wave event caused a dramatic decline in salinity.
- Acute thermal tolerance was quantified as in Experiments 1 & 2.

Figure 3. Twice daily water quality monitoring of the Hopkins Marine Station tidepool. The white temperature datalogger is also visible.

Results

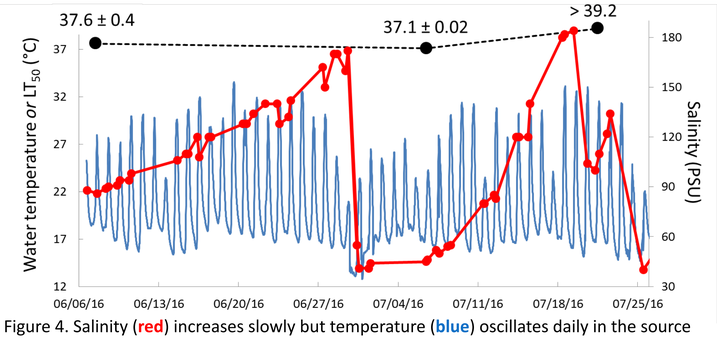

Figure 4. Salinity (**red**) increases slowly but temperature (**blue**) oscillates daily in the source tidepool. Lethal temperature (LT_{50} ± SE) of mature females was determined on three dates. NOTE: LT_{50} on 7/21 is an underestimate, because all females survived at this temperature.

- Temporal patterns of thermal tolerance in egg-bearing females reflected changes in ambient salinity in the tidepool.
- Specifically, LT_{50} decreased from 37.6 at 86 PSU to 37.1°C after the salinity decreased to 37 PSU in a stochastic, overnight wave event.
- The LT_{50} then increased by more than 2°C after the salinity naturally increased to 110 PSU over several weeks.

Conclusions and Future Directions

- Altogether, copepods exposed to a higher salinity – both chronically and acutely – exhibit compensatory physiological responses that enhance survivability of acute temperature stress.
- Such interactions between covarying environmental parameters add complexity to biological forecasts.
- Future laboratory studies will covary these environmental parameters in a more realistic fashion (Fig. 4):

  1) Diel fluctuations of temperature; 2) "Sawtooth" patterns of salinity driven by evaporative concentration, followed by wave inundation; 3) Salinity and temperature variation will be superimposed on realistic, diel fluctuations of oxygen (Fig. 5) and pH/pCO_2.

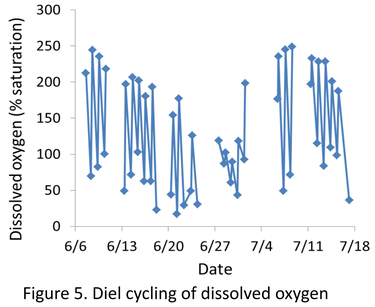

Figure 5. Diel cycling of dissolved oxygen percentages over a 6-week period in the HMS tidepool. Measurements were taken twice daily, just after sunrise and in mid-afternoon.

Acknowledgements

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