The Effects of Crowding on Behavioral and Physiological Stress Markers in *Hemigrapsus oregonensis*

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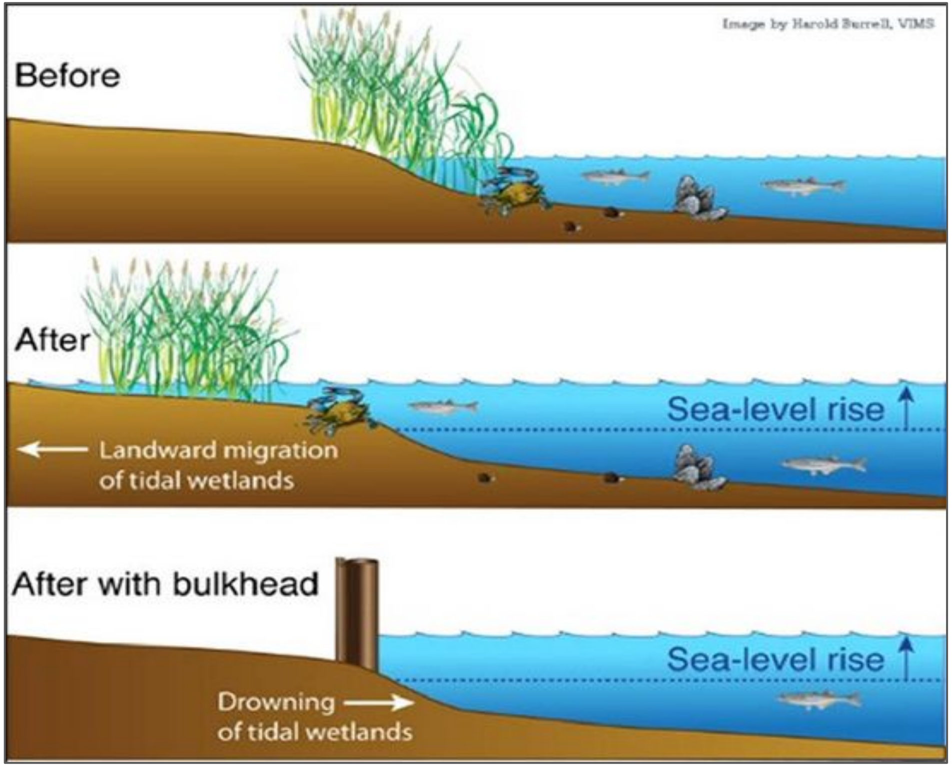
Background- Drivers of Habitat Loss

Shoreline Armoring & Coastal Squeeze

- 25% of Puget Sound coastline and 92% of Seattle coastline is armored (Boonzaier, 2018)
- Armoring structures (seawalls, rocks, sandbags) reduce intertidal habitat (Pontee, 2013; Long et al., 2011)
- Sea level rise compresses coastal habitats against fixed barriers → *Coastal squeeze*

Ecological Consequences

- Shoreline development alters sediment quality and reduces habitat complexity (Jackson & McIlvenny, 2011)
- Burrow density of *Ocypode quadrata* increases with moderate erosion and coastal development (Pombo et al., 2023)
- Intertidal crustaceans face rising competition and crowding pressures under future sea level rise (Jackson & McIlvenny, 2011)



Background

Physical crowding, aggression & metabolic activity

- Higher density increases physical force and defensive displays in hermit crabs (Hazlett, 1968).
- Repeated exposure to intruder conspecifics elevated baseline heart rate in the european shore crab (Rovero et al, 2000).
- Respiration increases during and after agonistic interactions in swimming crabs (Smith & Taylor, 1993).
- Longer fights → greater oxygen demand (Smith & Taylor, 1993).

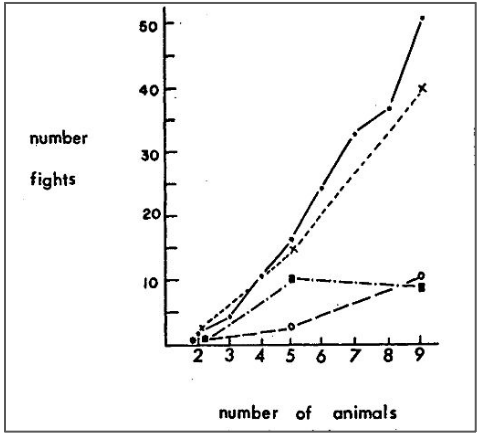


Fig 1: Number of agonistic encounters as a function of density in hermit crabs (Hazlett, 1968).

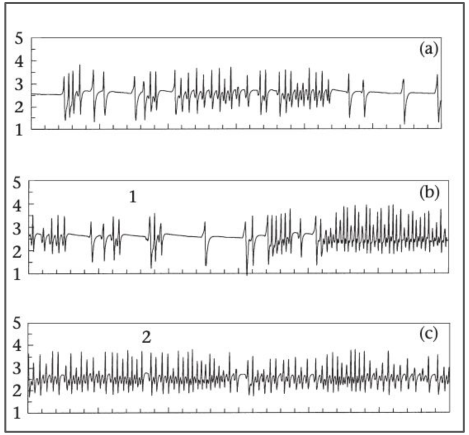


Fig 2: Mean heartbeat rate of *Carcinus maenas* during fights between resident and intruder (a) undisturbed, (b) intruder introduced (C) fighting (Rovero et al., 2000).

Background

Water Chemistry, crowding & metabolic activity

- Conspecific chemical cues change the duration and intensity of agonistic interactions in swimming crabs (Huntingford et al., 1995).
- Shore crab chemical cues can signal mating, predators, and dominance (Hardege, 2002; Trussell, 2003).
- Crowding-related changes in water chemistry can reduce survival and growth in shrimp (Nga et al, 2005).

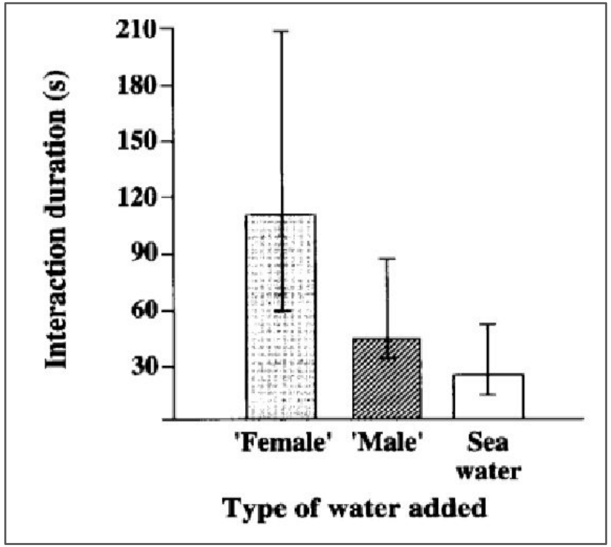


Fig 4: The mean durations of interactions between *N. puher* exposed to seawater (control) or to water conditioned by females and males. Error bars represent 95% confidence intervals) (Huntingford et al., 1995).

Background

Hairy Shore Crab (*Hemigrapsus oregonensis*)

- Model species for intertidal crab studies
- Range: Resurrection Bay to Alaska to Baja California (Visser et al. 2004)
- Habitats: Common in intertidal areas like mudflats, bays, estuaries, seagrass beds, open beaches (Visser et al. 2004)

Behavioral Traits & Density

- Typically remain in or near their individual burrows and display aggressive behavior toward other crabs during encounters, especially when foraging outside burrows (Visser et al. 2004)
- Reported densities (Jensen et al. 2002):
  – Avg. 472 crabs/m² under boulders (Puget Sound, WA)
  – Max 624 crabs/m² under oyster shells (Bodega Bay, CA)

Research Questions

How does crowding influence the metabolic activity of *H. oregonensis*?

How do crowding-induced chemical cues from crowded conditions influence the metabolic activity of *H. oregonensis?*

Physical Crowding Hypotheses

Null Hypothesis (H_0): Physical crowding does not affect the metabolic activity of *H. oregonensis*.

Alternative Hypothesis (H_1): Physical crowding effects the metabolic activity of *H. oregonensis*.

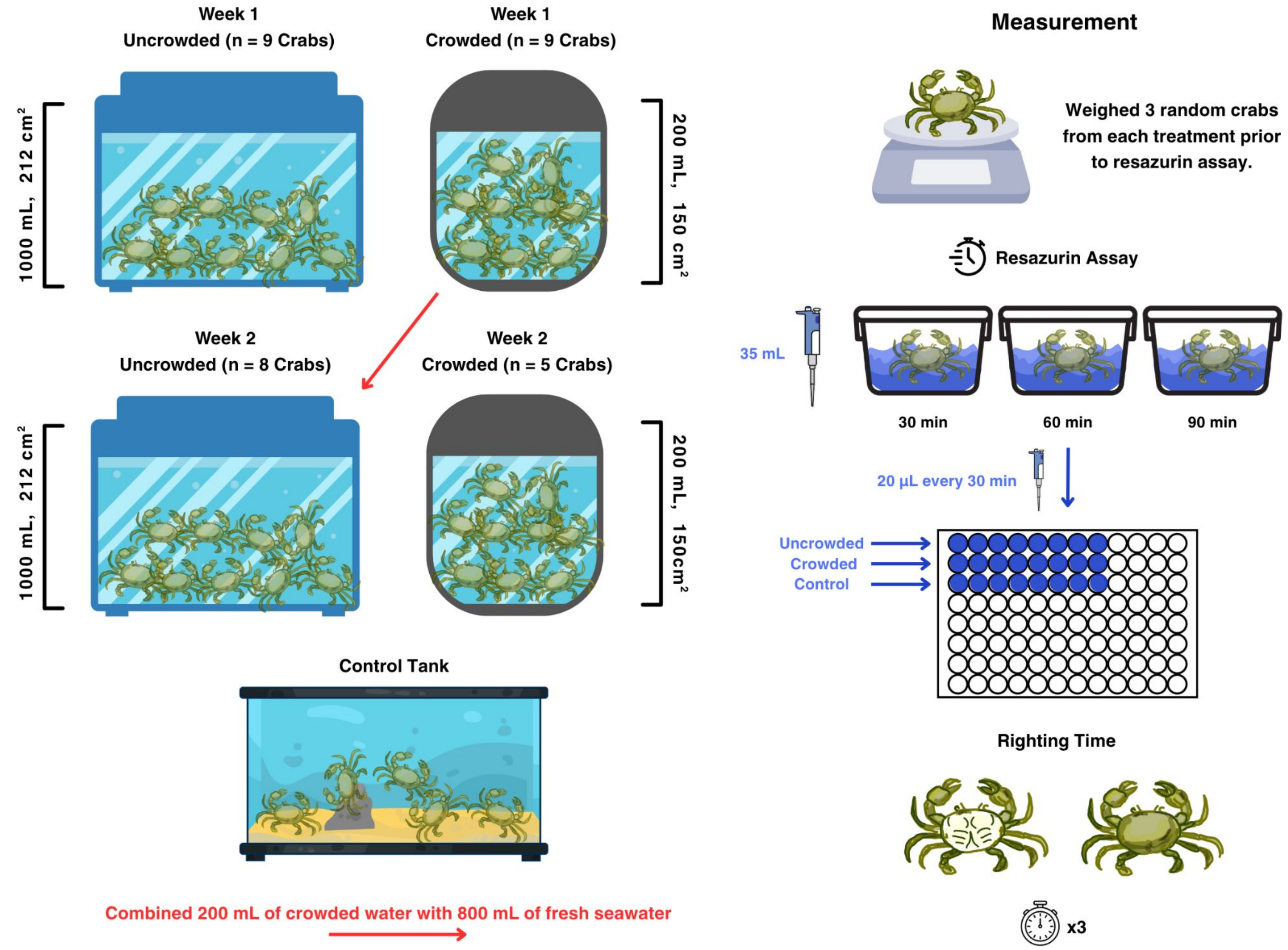
> Alternative Hypothesis (H_{1a}): Physical crowding increases the metabolic activity of *H. oregonensis*.
>
> Alternative Hypothesis (H_{1b}): Physical crowding decreases the metabolic activity of *H. oregonensis*.

Crowding-Induced Chemical Cues Hypotheses

Null Hypothesis (H_0): Chemical cues from crowded conditions have no effect on the metabolic activity of *H. oregonensis.*

Alternative Hypothesis (H_1): Chemical cues from crowded conditions have an effect on the metabolic activity of *H. oregonensis*.

> Alternative Hypothesis (H_{1a}): Chemical cues from crowded conditions increase the metabolic activity of *H. oregonensis*.
>
> Alternative Hypothesis (H_{1b}): Chemical cues from crowded conditions decrease the metabolic activity of *H. oregonensis*.



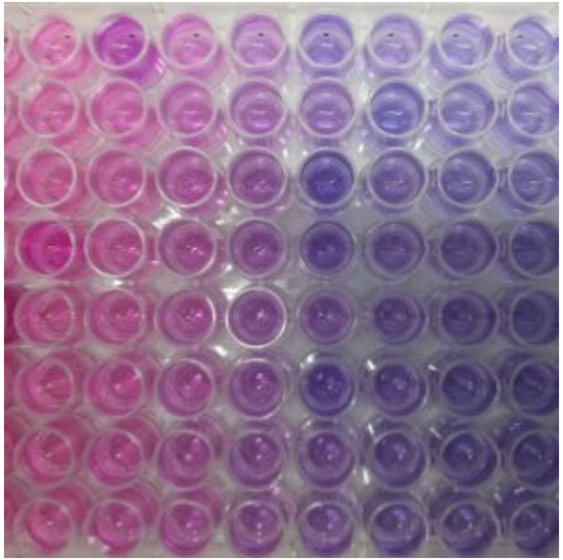
Week 1
Uncrowded (n = 9 Crabs)
Week 1
Crowded (n = 9 Crabs)
1000 mL, 212 cm²
200 mL, 150 cm²
Week 2
Uncrowded (n = 8 Crabs)
Week 2
Crowded (n = 5 Crabs)
1000 mL, 212 cm²
200 mL, 150cm²
Control Tank
Combined 200 mL of crowded water with 800 mL of fresh seawater
Measurement
Weighed 3 random crabs
from each treatment prior
to resazurin assay.
Resazurin Assay
35 mL
30 min
60 min
90 min
20 µL every 30 min
Uncrowded
Crowded
Control
Righting Time
x3

Why these metrics?

- Metabolic rate is a well established indicator of physiological stress and energy consumption in organisms

- Resazurin was used to quantify oxygen consumption which is directly related to metabolic rate.

- Righting behavior is a reflex that requires muscle coordination and neurological control, so a decline in righting ability can signal a decline in well-being and health (Wilson et al., 2022).



Observed Physical Effects of Treatments

Treatment	Mortality	Limb Loss	Escape
Crowded x2	3	2	3
Uncrowded	0	0	0
Uncrowded + Crowded Water	0	0	0
Control	0	0	0

Figure 1. Number of mortalities, limb loss and escape events recorded for each condition.

Respiration Rates Over Time

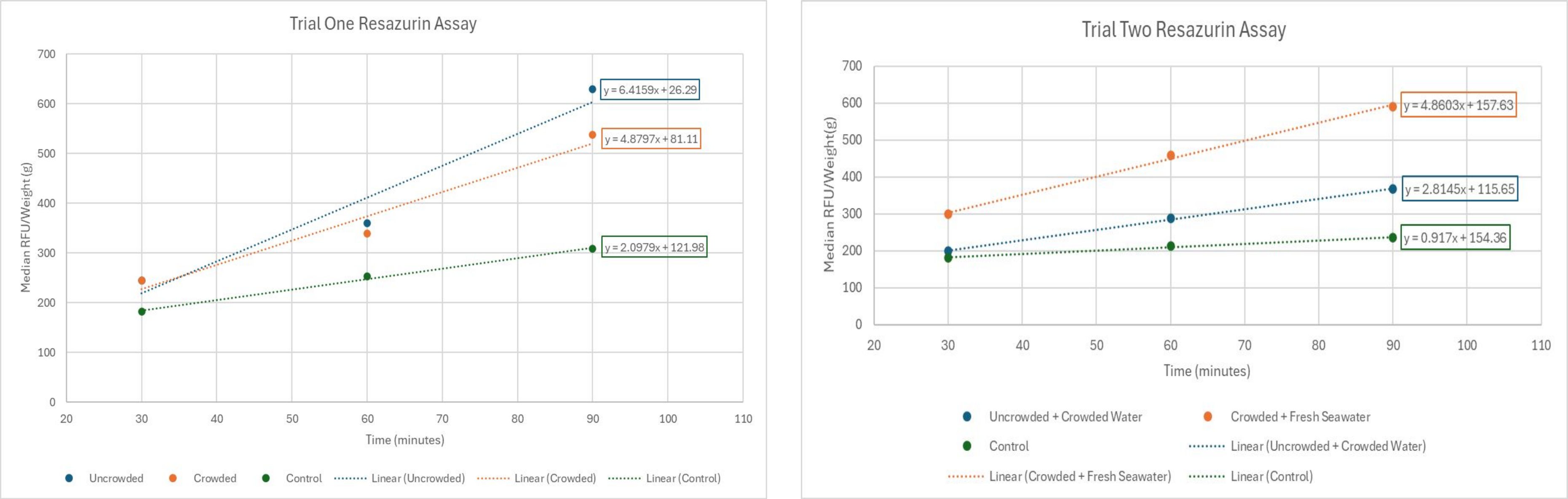


Figure 8. Resazurin assays were used to observe oxygen consumption every 30 minutes over a 90-minute period (Left; trial one, Right; trial two). Median RFU, normalized by individual weight, is shown for uncrowded (n=3, blue), crowded (n=3, orange), as well as control treatments (n=2 for trial one, n=3 for trial two, grey). Dashed lines represent linear fit for each treatment across sampled time points. Equations indicate slope value and y-intercept of each corresponding regression line.

Results

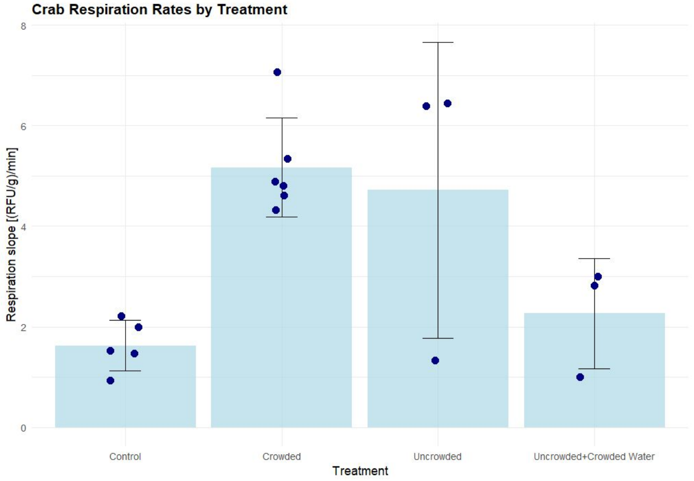


Figure 5. Crab respiration rates [(RFU/g)/min] for each experimental condition (control, crowded, uncrowded and uncrowded + crowded water) are plotted individually (blue points). Bar graphs represent the mean values for each condition, and error bars show standard deviation.

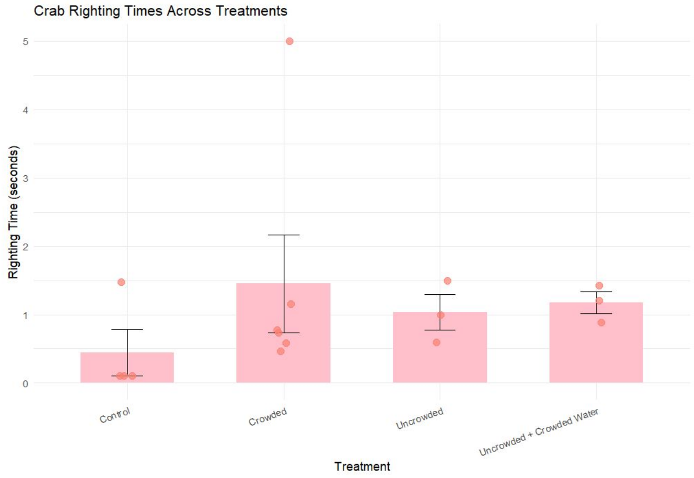


Figure 6. Crab righting time across treatment conditions. Bars represent the average righting time and error bars indicate the standard deviation. Points represent individual crab righting times.

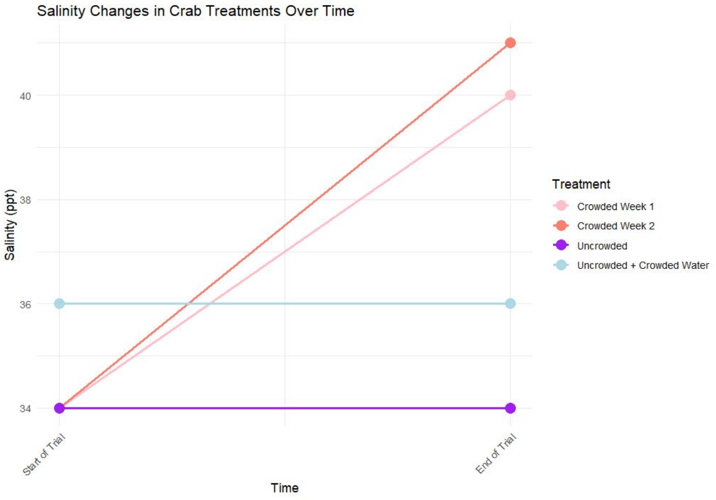


Figure 7. Salinity changes over time in each crab treatment condition. Lines represent the four different treatments.

Interpretations/Conclusions

- Physical crowding triggered the strongest stress responses (RFU, righting time, mortality, limb loss), likely due to aggression and conspecific intrusion (Smith & Taylor, 1993; Rovero et al., 2000)

- High salinity in crowded tanks may have elevated osmotic stress and respiration (Lin et al., 2022), while in UC + CW, it may have mitigated ammonia toxicity (Lin & Chen, 2001).

- Lower RFU in UC + CW may reflect reduced activity from chemical cue exposure (Hazlett, 2010): handling and mortality may have confounded crowded and UC week one results

Future Work

- Future research should assess ammonia and pH effects on crab stress responses and standardize trials by sex, age, and size (Schembri, 1981; Penn & Brockman, 1995).
- Crowding stress from increased shoreline armoring may harm H. oregonensis and other species like Cancer magister.
- Green shoreline infrastructure could reduce erosion while supporting intertidal habitat function
- Ethical concerns arise from high mortality and low replication, especially as decapods are now recognized as sentient (Wilson et al., 2022).

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