High post-release survival of spot prawns (*Pandalus platyceros*) declines with increasing air exposure and temperature­

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*Running headline: Spot prawn survival declines with air exposure and high temperature*

**ABSTRACT**

*Prawns like to be cold*

*Release them lickety split*

*Back to life at depth*

**KEY WORDS**

fisheries management, spot prawn, post-release survival, population dynamics

**INTRODUCTION**

* Big picture background on the increasing focus on evaluating the effectiveness of fisheries management interventions.
* Specific background on post-release survival evaluation.
* Life history & local fishery context of spot prawns in BC.
* Knowledge gap and framing of research question and approach.

**MATERIALS AND METHODS**

We conducted 23 experimental trials between May XX, 2022 and June XX, 2022 in the Broughton Archipelago, British Columbia, Canada (Fig. X). A given experimental trial consisted of three components conducted over three days: setting traps for field collection, collecting prawns and conducting the experimental treatment, and processing prawns at the end of the experiment to assess survival and condition. Each trial took 3-4 days to complete (the string of traps to initially collect prawns soaked for 24-48 hours).

EXPERIMENT SET-UP

To collect prawns for a given trial, we set a string of 10 prawn traps (insert technical specs) baited with pellets (insert technical specs) within a target depth range (55-110 meters) that aligns with the approximate depth range targeted by commercial and recreational fisheries. Depending on weather conditions and logistics, the string of traps soaked for 24-48 hours before we hauled the traps and began the experimental trial. On the day the trial began, at the trap setting site, we collected air temperature, water temperature (at 0 m and 10 m depths), and water salinity (at 0 m and 10 m depths) using a YSI (insert technical details). During some of the trials, the YSI was broken, and we collected temperature and salinity data using a thermometer and refractometer respectively. We hauled the string of traps using a hydraulic pot hauler (insert technical details). During trap hauling, as each trap came on the boat, we removed any bycatch and emptied the remaining prawns into a small square white bin (10 L, insert dimensions) with drilled holes that allowed water to flow through. We placed each white bin in a large fish tote (insert technical specs) filled with seawater (XX L). This method ensured that until the trial began, prawns experienced minimal air exposure (10-15 seconds as trap was emptied into white bin). After we finished hauling all traps, we assessed how many prawns to assign to each treatment (minimum 35 per treatment, maximum 70 per treatment to minimise density-dependent effects). We haphazardly assigned prawns to one of four or five treatments: ‘immediate release’ or air exposures of 30, 60, 90, or 120 minutes. In circumstances where numbers of prawns was a limiting factor, we did not include the 120 minute treatment. To implement the ‘immediate release’ treatment in an experimentally tractable manner, we hung prawns off the boat in a mesh drawstring bag (insert mesh specs) approximately 20 m below the surface of the water. This mimicked quick release (insert evidence that it is reasonable to assume 10 fathoms is similar to bottom depth conditions) while still allowing for the experimental ‘release’ of prawns from all treatments together in one string of traps. To minimise air exposure, we emptied the prawns of one of the white bins into a solid white bin filled with seawater. We counted out the appropriate number of individuals, using a forceps to place a coloured orthodontic elastic band (insert technical specs) on the base of the rostrum (Figure X), and placing each prawn in the mesh bag which was submerged in a 20 L bucket of seawater. Once all prawns had been banded for the ‘immediate release’ treatment, we cinched the mesh bag and attached it to a weighted line hanging off the boat to a depth of 20 m. To begin the air exposure treatments, we removed the remaining white bins from the fish tote at the same time such that all prawns hit the air together. We started a timer for the first treatment (30 minutes) and began to distribute the appropriate number of prawns to each bin, distributing haphazardly by size. For trials with fewer prawns, we allotted one bin for treatment (e.g., ~35 prawns per bin) and for trials with more prawns, we allotted two bins per treatment (e.g., two bins, each with ~35 prawns). For the duration of the treatment time, we kept the bins under the canopy of the boat such that they received no direct sun exposure or direct precipitation. The spatial arrangement of the bins was haphazard with respect to treatment. Choosing different colours for different treatments (the exact colour varied trial to trial), we applied coloured bands to the rostrum of each individual prawn. As the timer for a given treatment went off, we emptied the prawns of a certain band colour into a weighted mesh bag and clipped it to the hanging line such that it descended to hang with the other treatment bags at ~20 m. At the end of the final treatment (90 minutes or 120 minutes), we placed the final group of prawns in a mesh bag hung off the side of the boat such that all treatments experienced the process of being lowered and raised in a mesh bag. Finally, we raised all the bags at the same time and distributed the prawns from all treatments across six baited prawn traps with the tunnels tied shut such that prawns could not escape. To avoid confounding treatment effect with trap effect, we distributed some prawns from each treatment to each trap such that traps contained a mix of all treatments (the coloured bands facilitated mixing prawns without losing information on treatment). Once prawns had been distributed, we closed the traps and reset the string of six traps in the same location and depth they had been hauled from initially. For each trial, we timed the length of time it took from the trial ending (mesh bags coming out of water) to the final trap hitting the water during re-setting.

EXPERIMENT WRAP-UP

Approximately 24 hours after we conducted the experimental treatment, we returned to the re-set string of traps to end the trial. We followed the same procedure as at the beginning of the experiment, collecting temperature and salinity measurements before re-hauling the string and placing the contents of each trap into a white square bin which we kept in the seawater-filled fish tote.

After re-hauling the string, we emptied one square bin (i.e., one trap’s worth of prawns) at a time into a sampling tray and collected the end-of-trial data. For each individual prawn, we recorded their band colour, stage (juvenile, male, transitional, female, egged female, or spent female), and their carapace length as well as whether they were alive, dead, or scavenged. We considered a prawn dead if their gill filaments were not moving at all (i.e., the individual was no longer breathing). A ‘scavenged’ prawn referred to an individual that was dead and missing some body parts. We returned dead and scavenged prawns to the ocean. As they were counted and measured, alive prawns were transferred from the sampling tray to a mesh bag submerged in a 20 L bucket of seawater. After processing a single trap, the mesh bag of live prawns was hung off the boat at 20 m to maintain the prawns as close to their initial condition as possible.

After collecting survival data for all traps, we assay each live prawn for a suite of ten reflex behaviours, based on the approach outlined in Stoner (2012) that developed a set of ten reflex behaviours in spot prawns that cumulatively predicted long term survival in the lab. Processing one trap’s worth of prawns at a time, we assessed each prawn for how many of the reflex behaviours they displayed, resulting in a cumulative score that could range from 0-10. Here, a score of zero indicates a prawn that is alive but displays no other behaviours (poor condition) and a score of ten indicates a prawn that is alive and displays all assessed reflex behaviours (good condition). After we finish assessing the reflexes of the live prawns, we remove their nose band and return them to the ocean.

STATISTICAL ANALYSIS

To evaluate the influence of time out of water, air temperature, and carapace length on the post-release survival of *P. platyceros* captured in trap fisheries, we used generalized linear mixed-effects models (GLMMs) with a binomial error structure to model the probability of survival. The models included random effects to account for the hierarchical structure of the experiment.

Prior to statistical analysis, we excluded some prawns (488 of 5053) for which either treatment group or carapace length was unknown. A small portion of the prawns (273) lost their coloured band during the release stage of the experiment (Table 1). As the band colour denoted treatment group, prawns that lost their band could not be assigned to a treatment. We considered the possibility that small prawns may have been more likely to lose their band. To ensure we would not confound our results, we compared the size distribution of these prawns to that of the prawns that retained their band (Appendix 1). There was a statistically significant difference between the two groups (T=3.25, *p*=0.0013), however the difference was very small (1 mm, 3%) (Figure 1). We therefore excluded these individuals from the final dataset. We excluded an additional 215 prawns that had damage on their carapace such that we could not measure length accurately. Although there appeared to be a correlation between carapace damage and treatment group (Figure 2), we assessed the influence of this potential bias and found it was minor.

We also considered how loss of prawns may have influenced results. We lost prawns in two ways: through the mesh of the bags used during the treatment stage, or through the mesh of the traps during the release stage of the trial. We could not determine whether these individuals survived the treatment or not. To investigate whether there was a bias in prawn loss (i.e. if either dead or living prawns were more likely to be lost), we evaluated the percentage of prawns lost in each treatment. We found that slightly more prawns were lost at longer treatments times (Figure 3). To evaluate the influence of the potential bias in prawn loss, we simulated four scenarios for prawn loss: we lost no prawns; we lost only dead prawns; we lost only living prawns; we lost dead and living prawns with equal frequency. We evaluated the difference in survival estimates among the four scenarios to address whether loss of prawns could confound our interpretation of how survival did or did not differ across treatment groups. This analysis showed that for a typical percentage of prawns lost (20%) (Figure 3), effect on the estimated percentage of prawns that survived was minor (maximum 6% for most trials) (Figure 4) even if we lost living or dead prawns more frequently.

Before conducting statistical analysis, we also considered how the varying salinity of the water to which we exposed prawns in the treatment stage may have influenced the results. Fortunately, salinity varied little among trials (Table 1). However, salinity could not be collected for trial 11 due to broken equipment, therefore, we excluded trial 11 to avoid underestimating survival.

We took a model selection approach to evaluate the relative importance of three fixed effects and their two-way interactions: time out of water, air temperature, and carapace length. We did not include the three-way interaction term because it is difficult to interpret. In total, we considered a suite of 18 candidate models (Table 2) to predict prawn survival. All models included a normally distributed random effect on the intercept. This accounts for variation in survival caused by the trial and trap that a prawn was in. As there were 21 trials, and 6 traps in most trials (Table 1), there were 123 levels of the random effect. We expected survival may vary by trial and trap because location, time, and orientation on the ground varied between trial and trap. We conducted all analyses in R (R core team 2023). For completeness, we fit the models in two ways: Gaussian Quadrature (10 points) with lme4 (Bates et al. 2015), and Laplace approximation with glmmTMB (Brooks et al. 2017). To prioritise simplicity and interpretability, we compared models using Bayesian Information Criterion (BIC) (Table 1).

**RESULTS**

Leaving prawns out of water for periods between 0 and 120 minutes resulted in a significant number of dead prawns (2149/4598, 47% mortality). Prawns that were left out of water for a longer period of time died far more often than those released more quickly (Figure 5). Temperature also influenced survival; the individuals left out of water on hot days died more often than those on cool days (Figure 6). Although we expected short prawns to experience a lower survival rate than long prawns due to their higher surface area to volume ratio, short prawns survived slightly better than long prawns (Figure 7). Very small prawns (<29 mm), which were mostly juveniles (Figure 8), experienced the highest survival rates, although that may have been caused by the trials in which they were treated (Table 1). For mid-sized prawns (29-38 mm), which were primarily males and transitionals, there were slightly more living prawns than dead prawns. The biggest prawns (>38 mm), transitionals and females, died at the highest rate.

To determine wheth­er our estimates of mortality were accurate and not right-censored (i.e. whether prawns died due to treatment after the experiment), we assessed the surviving prawns for a suite of reflex behaviours. Surviving prawns retained most of their reflexes (Figure 9), indicating that the treatment did not severely damage them. Stoner et al. (2009) exposed prawns to different types of stress, recorded how many reflexes each prawn had lost (impairment score), and monitored their survival for a month in a laboratory setting. They found that impairment score is a strong predictor of mortality during that period. They created a model which relates impairment score to the probability that a prawn will die within a month. Using this model, along with the impairment scores recorded for each of our treatments, we calculated the number of prawns expected to die within a month after the experiment, for each treatment (Figure 10). Across treatments, the predicted post-experiment mortality ranged from 6-14%; it was higher for shorter treatments, due to the number of surviving prawns. The reflex scores show that most of the prawns that survived sustained little physiological damage and that we slightly overestimated survival due to right-censoring.

During the model selection, BIC did not select a single best model but instead scored five models similarly (Table 2). The five ‘best’ models all included treatment time and air temperature as main effects and as an interaction; four of the top five models included length as well. We performed model averaging based on BIC scores and compared the averaged model against the top model (BIC=0) and a model with only main effects (Table 3). The averaged and best models predict very similarly; the largest deviance between the probability of survival predicted by the two models was 4.6%. The main-effects-only model also predicted similarly to the averaged model, with a maximum deviance of 5.1% from the averaged model. The accuracy was also very similar for the three models, all within 78-80%. The coefficients in all three models were similar. Because the average model and top model predict similarly, we decided to present results based on the latter for simplicity.

The top model included two interaction terms, treatment time x temperature and temperature x length, and three main effects: treatment time, temperature, and length. Treatment time had the biggest effect on prawn survival (Table 3) and the effect increased with temperature (Figure 11). The top model predicted longer prawns will have lower survival rates at low temperatures, compared to smaller prawns, however as temperature increases a greater proportion of longer prawns will survive. The effects of length and the temperature x length interaction are both relatively small (Figure 12).

**DISCUSSION**

* Discussion of the physiological mechanisms for death and how we expect air exposure/temperature to affect that process
  + Acknowledge lack of consideration of density-dependence
  + Discuss possible dynamics re: temperature differential (per Dylan’s comments, cite sea lice research?)
* Discussion of limitations & considerations
  + Lost prawns
  + Issues comparing to ‘real world’ (if anything, we might be underestimating survival in some respects)
  + Accounting for predation (is there literature on this?)
* Discussion of implications for management:
  + Commercial license requirements and enforcement
  + Informing recreational fishers
  + Evidence that it is worth making the effort
  + Temperature matters

**CONTRIBUTIONS**

**REFERENCES**

Figure 1 <- Unbanded vs banded length violin plot

Figure 2<- Length NAs per treatment

Figure 3 <- prawn loss per treatment

Figure 4 <- Loss thought experiment

Figure 5 <- Treatment Survival histogram

Figure 6 <- Temp survival

Figure 7 <- Length survival

Figure 8 <- Stage Dist?

Figure 9 <- Reflex Dist

Figure 10 <- predicted release mortality stacked barplot---- discussion?

Figure 11<- 3 panel Survival curves by temperature with temperature-binned survival average points.

Figure 12<- Survival 9 curves, showing relative influence of length and temperature

Table 1 <- Trial summary table

Table 2 <- BIC Table

Table 3 <- Model Comparison table

Appendix 1 <- multi-page trial summary

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