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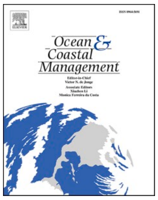

Relating capture and physiological conditions to viability and survival of Pacific halibut discarded from commercial longline gear

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ABSTRACT

Mortality of fish discarded from commercial fisheries is often estimated by using viability keys to parse fish into different categories with unique estimated survival outcomes. In this study we examined the relationships of environmental and physiological parameters and viability classifications of Pacific halibut (*Hippoglossus stenolepis*) released from commercial longline gear using three distinct hook-release methods. Our results indicate that the hook release method used strongly influenced viability, with careful shake and gangion cut resulting in minimal injuries leading to discarded fish characterized as having excellent viability. In contrast hook stripping resulted in most released fish having moderate or poor viability. Physiological parameters (lactate, hematocrit) were strong indicators of Pacific halibut assigned to the dead viability category. In addition to being captured at greater depth and lower sea bottom temperature, dead categorized fish were associated with high plasma lactate levels and lower hematocrit, attributed to sand flea intrusion. Reducing the use of hook strippers and limiting soak times in areas of known sand flea activity are likely to improve viability outcomes of Pacific halibut released from commercial longline gear. Discard survival and understanding of the factors affecting it remain critical to successful long-term management of Pacific halibut, as sustainable fishing levels are adjusted yearly for estimated mortality consistent with recent fishing discards.

1. Introduction

While the practice of discarding of live unwanted or protected fish back into the water is as old as the practice of fishing (Zeller et al., 2018), understanding and quantifying the survival outcomes or mortality of discarded fish has recently become an area of intense investigation (Madsen et al., 2022). Regulatory requirements to account for all sources of mortality in fishery stock assessments (e.g., Magnuson-Stevens Act) and public pressure on the fishing industry to reduce waste and improve fish welfare, incentivize these efforts (Madsen et al., 2022). The cascading set of physical and physiological events that ultimately determine survival outcomes of discarded fish are complex (ICES 2014). The mechanistic linkages between physical trauma associated with capture by enclosure or hooking and the concomitant physiological responses are difficult to untangle and as such remain poorly understood for most fishery-species combinations (Baker et al., 2013). Uncertainty in post-release survival estimates can have substantial impacts on estimates of fishery yield, perceived mechanisms underpinning stock trends and appropriate management responses (Baker et al., 2014; Coggins et al., 2007).

Pacific halibut (*Hippoglossus stenolepis*) supports a large and economically valuable commercial fishery producing 10,643 mt of catch with an estimated economic impact of US$ 170.3 M in 2019 (IPHC 2020; IPHC 2022a). The directed commercial fishery in US and Canadian waters is limited to capture using benthic longlines with a requirement that all commercially captured Pacific halibut smaller than 81.3 cm fork length must be returned to the sea with a minimum of injury. Regulatory discarding is estimated to comprise about 5% of total estimated Pacific halibut mortality within the directed commercial fishery annually (approximately 619 mt net weight over the past decade; 2011–2020; IPHC 2022b). Mortality resulting from the discard process is included in stock assessments via the use of discard mortality rates (DMRs; i.e., proportional estimates of fish that are projected to die after release) applied to estimates of discarded fish. DMRs are based on the

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classification of fish into viability categories (e.g., excellent/moderate/poor/dead) using qualitative assessments of physical condition. The techniques and protocols currently employed for these viability assessments are based on experiments that characterized the relationship between injuries associated with the hooking and hook removal processes and survival (Kaimmer 1994; Kaimmer and Trumble 1998; Peltonen 1969). Through this work, researchers (Kaimmer and Trumble 1998) noted that when classified into viability categories using the extent of hook removal injuries and other visually available descriptors of condition (e.g., bleeding, gill color, evidence of sand flea (a parasitic amphipod) predation, muscle tone, etc.), fish in the excellent category had higher survival rates (97%) than fish characterized as poor (76%) or dead (26%), and consistent with Loher et al. (2022). Despite their demonstrated utility in characterizing potential mortality, the qualitative nature of these assessments yields discrete, rather than continuous results which, in turn, represents a concomitant source of poorly quantified uncertainty in the estimation of total mortality within current stock assessment models. This uncertainty can bias the output of the stock assessment including stock status, reference points, and available yield in the directed fisheries, by over- or under-estimating past mortality resulting in over- or under-estimates of stock productivity. Given current low Pacific halibut yields relative to yields experienced in the last few decades, even small catch limit reductions can place undue hardship on individual fishery sectors particularly those that may be constrained by Pacific halibut bycatch caps.

Refining our understanding of the relationship between the discard process and post-release survival can reduce uncertainty in viability assessments (made by fisheries observers) either through more clearly defined viability categories or more precise mortality rates for a category, thereby improving DMR estimates (Davis 2002) and reducing associated uncertainty in stock assessment models with more accurate estimates of fishing mortality. During the discard process, fish are captured (by hooking in the case of the longline fishery), handled by fishers, identified as discards, and released back into the ocean. The process of capturing, handling and releasing Pacific halibut exposes individual fish to injuries from hooking, hauling, hook removal and handling, as well as additional potential stress and damage resulting from oxygen deprivation, desiccation, or rapid changes in temperature or pressure (Broadhurst et al., 2006). The potential effects of these experiences on post-release survival are dependent upon a) variability in the type, magnitude, and duration of physical and environmental stressors encountered during the capture and discard process, and b) variability in the biological characteristics of individual fish (both prior to and resulting from the current capture/handling/release event) (Benoit et al., 2013).

Previous studies exploring the relationships between environmental changes experienced during the catch and release process and survival likelihood in Pacific halibut have shown that exposure to rapid increases in temperature (water and air) after hooking can result in increased post release mortality (Davis and Olla 2001). While this result is comparable to that observed for released fish of other species (review of 21 families and 52 species, Gale et al., 2013; bluegill, *Lepomis macrochirus*, Gingerich et al., 2007), the impact of prolonged (i.e., 30 min) air exposure on the long-term ($\geq$10 days) post-release survival of line caught Pacific halibut has been shown to be minimal (Haukenes and Buck 2006). This is in contrast to trawl caught Pacific halibut where it has been shown that increased air exposure significantly decreases survival outcomes (Rose et al., 2019).

The physiological condition of individual fish may also influence their susceptibility to the stressors associated with capture and handling events (Baker et al., 2013; Benoit et al., 2013). Further, different capture and handling procedures can elicit different physiological responses in captured fish. Consequently, understanding both the landscape of individual condition in captured fish and the potential relationships between individual morphology and physiology, injury, and viability has the potential to inform our understanding of the mechanisms influencing post-release survival (Baker et al., 2013). Studies focusing on the individual condition of fish suggest that incorporating information on the physiological characteristics of captured fish can improve predictions of post-release survival (Davis 2010; ICES 2014). Previous research has measured levels of stress hormones (e.g., cortisol) and/or metabolites associated with the secondary stress response (e.g., glucose, lactic acid) to examine the relationships between the stress response, metabolism, osmoregulation, body condition, the immune response, growth, and reproductive success in a variety of marine and freshwater fish species (Baker et al., 2013; Barton 2002; Haukenes and Buck 2006; Hosoya et al., 2007; Hur et al., 2007; Fast et al., 2008). While information specific to Pacific halibut is limited, previous studies have shown elevated levels of cortisol, glucose, lactate, sodium, and potassium in the plasma of Pacific halibut subjected to external stressors including handling (Oddsson et al., 1994), air exposure, and increased temperature (Davis and Schreck 2005; Haukenes and Buck 2006). However, although a previous study concluded that plasma constituents are generally not good predictors of mortality in Pacific halibut in captive experiments (Davis and Schreck 2005), no studies to date have investigated the effects of capture and handling techniques on physiological stress indicators or physiological condition nor their relationship with post-release survival in Pacific halibut captured in the field. In addition, no studies to date have investigated other commonly employed metrics of fish condition, including Fulton's Condition Index (Bolger and Connolly 1989), fat and associated energy content (Crossin and Hinch 2005), or gross morphology (e.g., length and weight) as covariates to post-release survival in Pacific halibut.

The present study is part of a larger exploration of discard mortality of Pacific halibut commercially caught and released in situ. The first completed part of this study had as its main objective to, for the first time, directly estimate the survival outcomes of individual released Pacific halibut following capture on longline gear using acceleration-logging pop-up archival transmitting (PAT) tags. This approach was selected to avoid some of the challenges identified in previous studies reporting on mortality rates of Pacific halibut caught by longlines (e.g., uncontrolled environmental effects, abrasion effects and injuries in fish confined in net pens (Peltonen 1969), and bias due to variable tag loss and reporting rates inherent to mark-recapture studies (Kaimmer 1994; Kaimmer and Trumble 1998; Leaman and Stewart 2016)). The results from this first part of the study indicated that the DMR of Pacific halibut categorized as having excellent viability was in the range of 4.2–8.4% (Loher et al., 2022) at 96 days post-release, consistent with the 3.5% reported by Kaimmer and Trumble (1998). The second part of this study that is presented here investigates the potential influences of capture and handling conditions including physiological and environmental parameters not previously investigated with the goal of identifying variables that might better define post-release survival categories or aid in more accurate classification of Pacific halibut into these categories. Specifically, we explored relationships among 1) viability category as assessed using the current qualitative method; 2) injury type and severity; 3) morphological and physiological characteristics of individual fish – including body condition, blood stress indicators, etc.; and 4) a suite of physical and environmental stressors encountered by Pacific halibut during the catch and discard process – including hook release method, time on deck, change in temperature, etc. In this analysis, we adopted a hierarchical approach in which we examined the distributions of injury types within viability categories and distributions of physical, environmental, morphological, and physiological characteristics within both injury types and viability categories.

By exploring potential correlations between individual effects, physical and environmental effects, injury, and viability and by drawing mechanistic links between the hierarchical levels of our analysis, we provide the first attempt at a holistic perspective on the suite of factors that can potentially influence post-release survival in Pacific halibut. Importantly, this approach not only serves to quantitatively characterize the effects of the capture and release process on Pacific halibut, but it also provides new insights into discard mortality mitigation practices. Improved understanding of survival predictors is critical as discard mortality rates directly affect not only the stock, but also the fisheries reliant on them, as sustainable fishing levels are adjusted downwards each year to account for projected discard mortality consistent with recent fishing activity (Stewart et al., 2023).

2. Materials and methods

2.1. Study area, fishing gear, and sampling

Field sampling was conducted aboard a commercial longline fishing vessel chartered for the project. Vessel configuration and fishing practices were selected to mirror those used in the Pacific halibut commercial fishery. The vessel fished eight skates of conventional fixed longline gear per set. Each skate was configured with one hundred circle hooks (#3 (16/0) Mustad model 39965) threaded through the front of the hook eye, spaced 5.5 m apart, with a 2.3 kg–4.5 kg groundline weight at each skate junction. Bait consisted of 0.11–0.15 kg of chum salmon (*Oncorhynchus keta*) per hook. Two fishing trips consisting of six fishing days per trip were conducted between October 18th and November 2nd, 2017. Each day, two or three sets of gear were fished. Soak times averaged approximately 6 h (range 3–10 h). The vessel was configured with a secondary roller with an automatic hook-removal unit (hook stripper) inboard of the gunwale roller. An aluminum ramp was employed to redirect fish from their 'release' point to an onboard holding area for sampling and handling.

Fishing was conducted in the Gulf of Alaska, near Chignik (Supplementary Fig. S1) in IPHC Regulatory Area 3B. This area was selected based on its potential to provide Pacific halibut catch of both legal and sublegal sizes at rates sufficient to support the study design. The commercial Pacific halibut fleet does not physically measure each fish prior to discarding and is believed to err on the side of caution by discarding fish below approximately 84 cm to avoid landing fish that could appear to be sublegal post-icing owing to shrinkage. Therefore, in this study, "discarded or sublegal fish" differed from the regulatory definition, and were considered to be all fish under a fork length of 84 cm. This study generally followed the sampling protocols of the IPHC's fishery-independent setline survey (IPHC 2022c), with all legal sized fish subject to terminal sampling following guidelines for the euthanasia of finfish from the American Veterinary Medical Association. All sublegal fish (≤84 cm) were subject to tagging (the majority with a simple wire opercular tag, and a subsample of 80 fish were tagged with a survival pop-off archival tag as described in Loher et al. (2022)) and release post sampling with minimal harm. This study was conducted under LOA 2017-19 from the Alaska Fisheries Science Center – NOAA Fisheries.

2.2. Viability and injury assessments

Previous studies (Kaimmer 1994; Kaimmer and Trumble 1998; Trumble et al., 2000; Leaman and Stewart 2016) and the national fisheries observer programs (AFSC 2023) have utilized a series of classifications for predicting discard mortalities in Pacific halibut with similar injuries or states of injury. Early studies (Peltonen 1969; Kaimmer 1994; Kaimmer and Trumble 1998) used three terms "Excellent", "Poor", and "Dead" to define release 'condition' of the fish, more recent publications (Trumble et al., 2000) used four terms focused on injuries of "Minor, Moderate, Severe, Dead", and some have used these terms interchangeably (Trumble et al., 2000; Leaman and Stewart 2016). In this study, all captured Pacific halibut were assigned one of four viability codes (Table S1) that describe the predicted survival likelihood of the released fish (excellent, moderate, poor, and dead) following the protocols used by the National Marine Fisheries Service observer program (AFSC 2023) and mapping to their injury code category terminology of (minor, moderate, severe, and dead). The term 'viability codes' is used here to distinguish from 'condition codes' previously used by Kaimmer and Trumble (1998), to align with terminology commonly used in observer programs and to prevent confusion when discussing the influence of fitness (condition) on survival in this study. The presence of sand fleas was noted if they had penetrated the fish's body. Hook injuries sustained by each fish were classified into 14 different categories (i.e., injury codes; Table 1) following the classification criteria initially outlined by Kaimmer (1994) and expanded by Kaimmer and Trumble (1998).

Hook release techniques used included: 1) careful shaking, a "hands on" technique (described in Kaimmer and Trumble 1998) in which a gaff is used to twist the hook while gently shaking off the fish, 2) gangion cutting, releasing the fish by cutting the gangion while leaving the hook and a trailing portion of the gangion embedded in the fish, and 3) hook-stripping, an automated "hands-off" process in which the groundline is pulled between a pair of rollers, and the hook is mechanically removed from the fish by force. It is common for longlines to be hauled through hook strippers to remove any bait still on the hooks in preparation for the next set. We note that while IPHC Regulations (IPHC 2021) currently prohibit the use of hook-stripping to release Pacific halibut, it was nonetheless included in this study because the rate at which hook-stripping occurs (prior to the fisher removing the hook or cutting the gangion) in both directed and non-directed longline fisheries is currently unknown. However, patterns associated with the occurrence of prior-hooking injuries (Dykstra 2016) suggest that hook-stripping may be more prevalent than is currently assumed. Hook release treatments were randomly assigned to entire skates of gear in an unbalanced design based loosely on their expected frequency of use in the fishery, while ensuring meaningful sample sizes: one skate of gangion cutting, two skates of hook stripping, and five skates of careful shaking per set. Only Pacific halibut were brought aboard during this study and all other fish species were released using the careful shake method.

2.3. Biological sampling

In addition to viability and hook injury assessment, the fork length (±1.0 cm) and round weight (±20 g less than 60 kg, ±50 g greater than 60 kg) of all Pacific halibut were recorded and used to calculate Fulton's K as round weight (g)/fork length (cm)^3 * 100 (Froese, 2006). A small fin tissue sample (5 mm × 5 mm) was obtained from one corner of the caudal fin and stored on moisture absorbent filter paper for subsequent genotypic sex identification (Drinan et al., 2018) in the laboratory. For sublegal fish, somatic fat content was assessed using a hand-held microwave-based device (Distell Fish Fatmeter, model 692, Distell, West Lothian, Scotland) applied directly onto the skin of the fish midway between the lateral line arch and the dorsal fin insertion. This is a non-invasive method for determining energy levels from live fish

Table 1
Pacific halibut injury codes and their descriptions.

Injury Code	Description
NO	No apparent injury.
CO	Cheek only (not through skin).
JO	Jaw only (but not clear through the jaw).
TL	Torn lip (skin covering external portion of jaw), cheek not punctured.
TC	Torn cheek, small hole through cheek only.
TJ	Torn jaw, either side. Little or no tearing in cheek.
CJ	Cheek and jaw. Tear in cheek extending through jaw.
EY	Hook penetrated eye.
TF	Torn face. Torn though cheek and jaw, like above, but large flap of side of head is ripped/missing.
SJ	Split jaw. Lower jaw is split laterally.
JB	Jig body. Fish snagged by hook somewhere on body other than head.
JH	Jig head. Fish snagged by hook in the head (not through mouth).
TS	Torn snout. Upper jaw is split laterally, usually tearing through the snout as well.
UN	Injury unknown or unrecorded.

(Donaldson et al., 2010; Van Sang et al., 2009), and the unit was previously calibrated for Pacific halibut (Dykstra, unpublished data). Surface body temperature was recorded for each sublegal fish immediately prior to release with the use of a handheld infrared thermometer (Ceenwes, Model 550), and the total elapsed time on deck between hook removal and release overboard was recorded for a systematic random selection of sublegal fish. Hook removal occurs within seconds of the fish exiting the water and, therefore, time on deck represents the duration of air exposure.

Blood samples (approximately 1 mL) were collected from every second sublegal fish via caudal puncture using heparinized hypodermic (1 mL) syringes and 21-gauge needles. Whole blood hematocrit was determined by centrifugation in glass capillary tubes. Blood samples were centrifuged at 1500×*g* for 30 min to separate plasma, which was then removed via pipetting and stored frozen at – 80 °C for cortisol, lactate, and glucose analyses. Plasma cortisol levels were measured by enzyme linked immunosorbent assay (ELISA; Cortisol ELISA Kit, Cayman Chemical, USA). Plasma lactate and glucose levels were measured directly in the plasma samples by standard commercial colorimetric assay kits (Lactate Assay Kit, Cell Biolabs, Inc, USA; Glucose Colorimetric Detection Kit, Invitrogen, USA).

2.4. *Environmental sampling*

Air temperature at the time of sampling was recorded using a Tifictour Fridge Thermometer (DTH-94). Average bottom sea water temperature beginning at 4 min post-temperature stabilization and ending 4 min prior to haul was recorded for each haul using a Vemco (Minilog–II–T, Bedford, Nova Scotia, Canada) Temperature Logger attached to the bottom anchor. Finally, sea surface conditions were assessed (Beaufort Sea State (WMO 1970)) once during each haul.

2.5. *Data analysis*

While the primary scope of this study was to identify and summarize the relationships that release viability has with physiological, morphological, and environmental covariates, an equally important aim was to understand the prevalence of specific injuries sustained and their association with individual characteristics (i.e., morphology). This is especially relevant given this study's focus on commercially caught Pacific halibut in the longline fishery and the role of our results in informing effective longline halibut fishery management. To assess the proportions observed for both the viability and release method groups, a Bayesian inference on binomial proportions was used to estimate credible intervals (CI) at the 0.95 confidence level. Here, Jeffreys prior [e.g., a Beta (0.5, 0.5) distribution] was used to construct the posterior credible intervals for each comparison (Brown et al., 2001). Proportional comparisons between injury types by viability group were also performed using this Bayesian binomial inference. Group comparisons between release viabilities by physiological characteristics and environmental conditions were performed using Kruskal-Wallis rank sum tests (chi-squared H statistic reported), followed by Dunn's pairwise comparison tests (Z statistic reported) using Bonferroni p-value adjustments for multiple comparisons (Dunn 1961). From a preliminary random forest analysis (Breiman 2001), performed to identify potentially significant covariates, fish weight was found to have a strong association with injury type. Random forest model diagnostics are available in Table S2. Subsequently, multinomial logistic regression modeling (Tabachnick and Fidell 2001) was performed on the five most commonly observed injuries (torn cheek, torn jaw, cheek and jaw, eye, torn face) by release method in relation to fish weight. The multinomial regression model was defined as: (InjuryType ~ RoundWeight) and, via a bootstrap approach with 5000 iterations, 95% confidence intervals were constructed around the predicted estimates (Efron and Tibshirani 1986). Auxiliary relationships between key continuous variables of interest were also examined using Pearson's correlation coefficients. All statistical analyses and graphical outputs were performed using R Statistical Software (v4.3.1; R Core Team 2023). Confidence intervals for binomial proportions, using Bayesian inference, were calculated using the 'binom' R package (v1.1; Dorai-Raj 2022). Random forest models were developed using the 'randomForest' R package (v4.5.1.1; Liaw and Wiener 2002). Multinomial logistic regression was performed using the 'nnet' R package (v7.3.2; Venables and Ripley 2002). Dunn's tests of multiple comparisons were performed using the 'dunn.test' R package (v 1.3.1; Dinno 2017).

3. Results

3.1. *Effort and treatments*

Thirty-eight fishing sets were completed over 14 fishing days and during this period 2408 Pacific halibut were captured, of which 1139 (47%) had fork lengths ≤84 cm (sublegal). Legal fish ranged in fork length from 85 to 180 cm (mean ($\bar{x}$) = 102.6, standard deviation (sd) ± 15.1 cm) and in weight from 6.75 to 71.90 kg ($\bar{x}$ = 15.7, sd ± 8.2 kg) with sublegal fish ranging in fork length from 45 to 84 cm ($\bar{x}$ = 70.7, sd ± 8.4 cm) and in weight from 1.15 to 8.92 kg ($\bar{x}$ = 4.8, sd ± 1.7 kg). Of the sublegal fish, 619 were female, 447 were male, and 73 were undetermined or no fin clip was obtained. For legal fish, 783 (62%) were released by careful shake, 315 (25%) by hook stripper, and 171 (13%) by gangion cutting. For sublegal fish, 707 (62%) were released by careful shake, 278 (24%) by hook stripper, and 154 (14%) by gangion cutting.

3.2. *Viabilities*

When scored for viability, 822 (65%) of legal fish were categorized as excellent, 329 (26%) as moderate, 107 (8%) as poor, 7 (1%) as dead, and 4 unassigned during the field effort (Fig. 1A). Of the sublegal fish, 688 (60%) were categorized as excellent, 234 (21%) as moderate, 181 (16%) as poor, and 35 (3%) as dead and 1 unassigned (Fig. 1A). The careful shake and gangion cutting release treatments resulted in very similar viability outcomes, as shown by the Bayesian CI, regardless of fish size, with both treatments producing >75% of fish in the excellent viability category, with far fewer fish in the moderate (15–19%), poor (4–6%), and dead (1–4%) viability categories (Fig. 1B). In contrast, the hook stripper treatment resulted in significantly more moderate and poor viability outcomes for legal sizes (excellent = 26%, moderate = 55%, poor = 18%, and dead =<1%), and even worse outcomes for the smaller sublegal fish (excellent = 9%, moderate = 36%, poor = 52%, and dead = 3%) as shown by the Bayesian CI (Fig. 1B). For all fish, those in the poor and dead viability categories had significantly ($p < 0.001$) smaller fork length and round weight, two highly correlated variables, than fish in the excellent and moderate categories (Supplementary Fig. S2).

3.3. *Injuries*

The distribution of injury types varied considerably among viability categories with little difference between legal and sublegal fish as shown by the Bayesian CI (Fig. 2). For fish in the excellent and dead viability categories, torn cheek was the dominant injury (>80%). Higher variability in injury types was observed in fish in the moderate viability category, with torn cheek, torn jaw, cheek and jaw, and eye injuries as the most common. Poor viability fish tend to have sustained the most severe tearing injuries, with torn face making up 28% of legal sized fish and 57% of sublegal fish in this category (Fig. 2).

When examined by release method, torn cheek was the predominant injury observed in fish released by careful shake and gangion cut (>75%), with both treatments showing very similar injury profiles for legal and sublegal size fish (Supplementary Fig. S3). In contrast, fish released by the hook stripper sustained more severe injuries, including

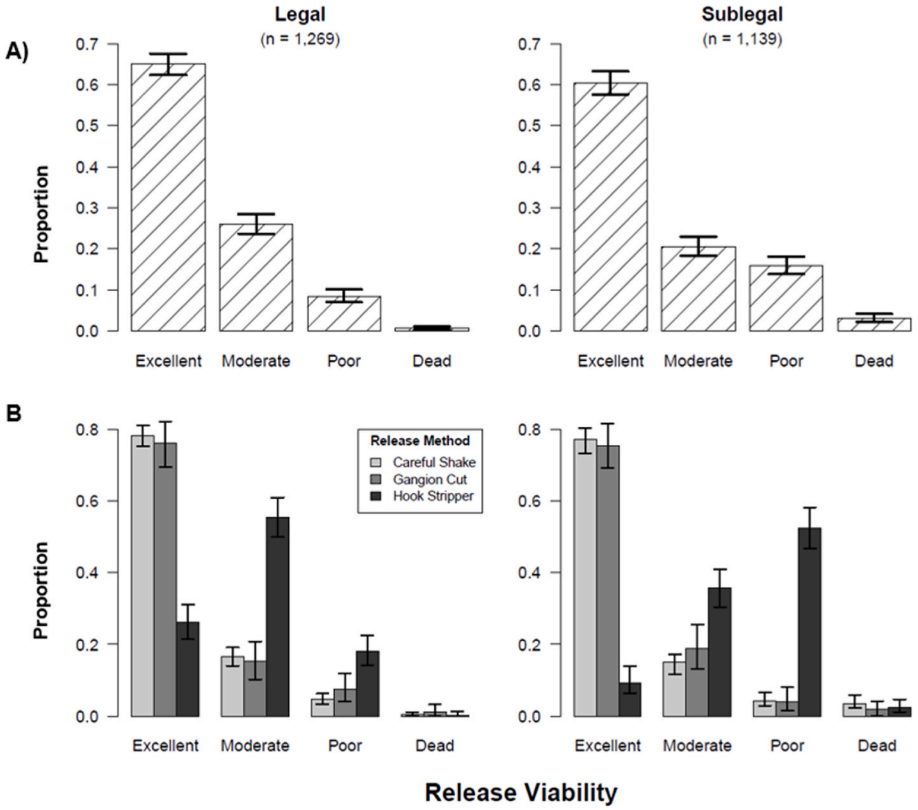

Fig. 1. Proportions of Pacific halibut assigned to viability category (A) for legal (upper left) and sublegal (≤84 cm) size fish (upper right). The lower panel (B) shows the proportions of viability assignments for Pacific halibut resulting from different release method treatments for legal fish (lower left) and for sublegal (≤84 cm) fish (lower right). Bars represent the mean ± 95% credible intervals as derived by Bayesian binomial inference.

torn jaw, cheek and jaw, and torn face which was most frequent (≥44%) in sublegal fish (Supplementary Fig. S3). The multinomial logistic regression model predictions of injury type based on round weight, for fish released by the hook stripper, demonstrated that lighter fish (<10 kg, 90 cm) have a higher probability of sustaining more severe injuries (e.g., torn face, cheek and jaw, torn jaw) than heavier fish (Fig. 3). However, this relationship disappeared when restricting the analysis to sublegal fish, as these were all below the 10 kg threshold.

3.4. *Environmental influences*

Pacific halibut characterized as having excellent viability were captured during significantly ($Z = 7.02$, p.adj< 0.05) calmer sea surface conditions (Fig. 4A), at shallower depth ($Z = 9.34$, p.adj<0.05), and at higher sea bottom (Supplementary Fig. S4, $Z = -9.88$, p.adj<0.05) and air temperature (Supplementary Fig. S5, $Z = -5.22$, p.adj<0.05) than fish classified in the dead viability category. Further, fish surface temperature was also significantly ($H = 57.09$, $df = 3$, $p < 0.05$) higher in fish in the excellent viability category than in fish in all other viability categories (Supplementary Fig. S5). Differences between air temperature and sea bottom temperature were significantly ($H = 14.29$, $df = 3$, $p < 0.05$) smaller in fish in excellent ($Z = 3.27$, p.adj<0.05) and moderate ($Z = 2.81$, p.adj $= 0.01$) viability categories than in fish in the dead viability category (Supplementary Fig. S6A). Similarly, the temperature differences between fish surface and sea bottom were significantly ($H = 35.38$, $df = 3$, $p < 0.05$) smaller in fish in the excellent and moderate viability categories than in fish in the poor ($Z = -2.21$, p.adj<0.05) and dead ($Z = 5.40$, p.adj<0.05) viability categories (Supplementary Fig. S6B). Fish from all four viability categories were exposed to similar fish surface: air temperature differences (Supplementary Fig. S6C).

We found no strong relationships between soak time, time on deck, and fish viability. Although fish characterized as having excellent viability generally came from sets with shorter soak times compared to fish characterized as having moderate and dead viability, this difference was not statistically significant (Fig. 4B). However, fish in the moderate viability category experienced significantly ($p < 0.02$) longer soak times than fish in the poor ($Z = 2.95$, p.adj $= 0.02$) viability category (Fig. 4B). Fish in the excellent, poor and dead viability categories did not experience significantly different times on deck. However, fish in the moderate viability category experienced significantly longer time on deck than fish in the excellent ($Z = -4.2$, $p < 0.05$) and dead ($Z = -2.95$, $p < 0.05$) viability categories (Fig. 4C). For all sizes of fish, 89 % of fish in the dead viability category experienced sand flea penetration (data not shown).

3.5. *Physiological variables*

We observed large individual variation in the plasma levels of three physiological stress indicators: the stress hormone cortisol and the metabolites glucose and lactate (Fig. 5). Cortisol plasma levels did not differ among fish ($H = 3.48$, $df = 3$, $p = 0.32$) in the four viability categories (Fig. 5A). Glucose plasma levels were lower in fish in the dead viability category than in fish in all other categories, although this relationship was only statistically significant ($Z = -2.89$, $p < 0.02$) when compared to fish in the poor viability category (Fig. 5B). Lactate plasma levels were significantly ($H = 22.36$, $df = 3$, $p < 0.05$) higher in fish in the dead viability category than in fish in all other categories (Fig. 5C).

No significant differences in cortisol ($H = 10.74$, $df = 13$, $p = 0.63$), glucose ($H = 19.48$, $df = 13$, $p = 0.11$) or lactate ($H = 16.23$, $df = 13$, $p = 0.24$) plasma levels were observed among fish with different injury types (Supplementary Fig. S7). Correlation analyses between environmental and capture conditions (air, bottom, and fish temperature, depth of capture, time on deck, and soak time) and the levels of physiological stress indicators showed no significant relationships (Pearson's correlation coefficients all ≤0.31; data not shown). Hematocrit was

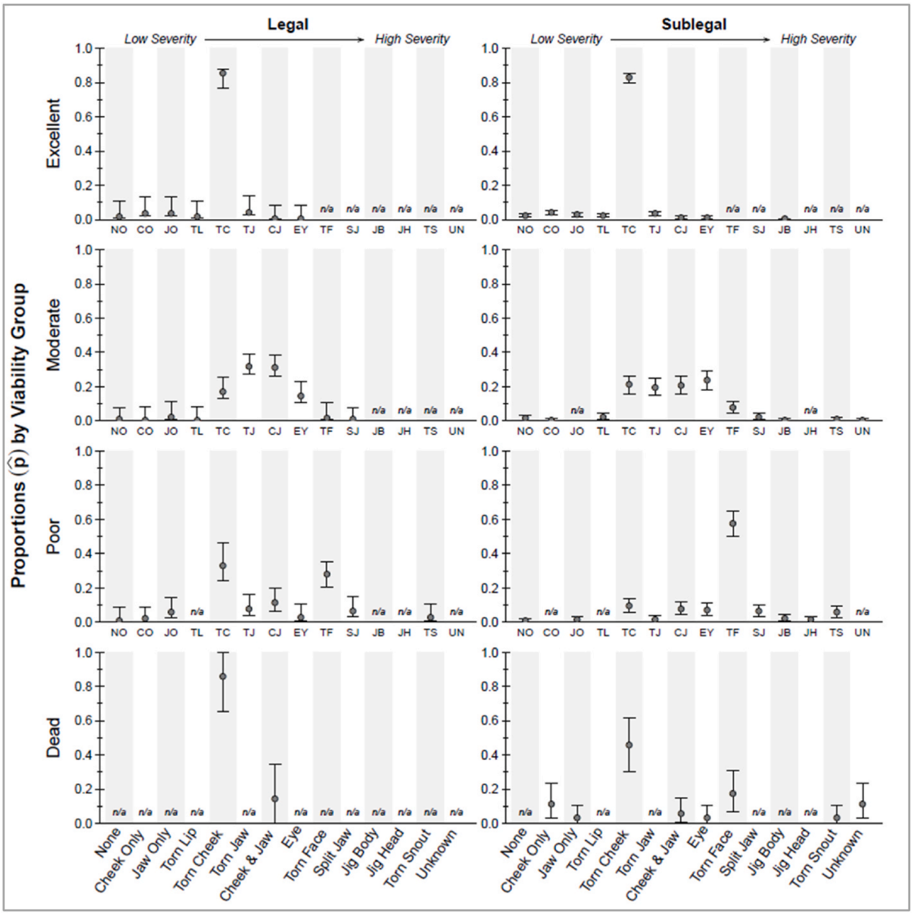

Fig. 2. Injury proportions ($\hat{p}$) for Pacific halibut by viability category assignment for legal (left column) and sublegal ($\leq$84 cm) size fish in the right column. Points represent the mean proportion ($\hat{p}$), and the bars represent the 95% credible intervals as derived by Bayesian binomial inference. Injuries are arranged from left to right by increasing perceived injury severity.

significantly ($H = 8.89$, $df = 3$, $p = 0.03$) lower in dead viability fish than in all other categories (Fig. 6A). In contrast, somatic fat content was significantly ($H = 14.27$, $df = 3$, $p < 0.05$) higher in fish in the dead viability category than in fish in all other viability categories (Fig. 6B). Fulton's condition factor did not show statistically significant differences ($H = 1.12$, $df = 3$, $p = 0.77$) among fish in the different viability categories (Fig. 6C). Further, no significant differences in hematocrit, somatic fat content or Fulton's condition factor were observed among fish with different injury types (data not shown). In addition, there was no effect of sex on any of the measured physiological or condition variables by viability category or injury type (data not shown).

Examples of four key random forest models predicting release viability and their results are presented in Supplementary Table S1. Release method was the most influential variable in predicting viability, generally followed by round weight, or capture depth in importance. Overall, the random forest analysis had poor predictive value (Out of bag error (OOB) error estimates >20% overall, and percent accuracy for any fish not in the excellent or dead viability categories were often <50%).

4. Discussion

In this study we conducted the first investigation into the relationships among capture conditions, physiological status, release injuries and resultant viability outcomes in wild captured Pacific halibut from longline gear. Our results show that the type of hook release method used had a strong influence on the viability category assigned to the released fish. Indeed, the fish released by careful shaking and gangion cutting had similar injury profiles, predominantly suffering from a simple torn cheek, fundamentally coincident to the capture or hooking injury, and resulted in 75–77% of the fish being assigned to the excellent viability category. As we previously reported, the estimated survival rate of Pacific halibut assessed as having excellent viability from this same study population that were discarded by either careful shaking or gangion cutting was between 92 and 96% (Loher et al., 2022), further demonstrating that minimal injuries lead to excellent viability with corresponding high survival outcomes. Careful release techniques led to a relatively small (~15–19%) proportion of fish assessed as having moderate viability and even lower (~4%) as having poor viability, comparable to observations from previous studies on viability assessment in Pacific halibut by Kaimmer and Trumble (1998) and Trumble et al. (2000). Conversely, using the hook stripper to release Pacific halibut from the hook resulted in the great majority (88%) of fish being classified in the moderate and poor viability categories. Fish in the moderate and poor viability categories had usually sustained injuries of medium and high severity, respectively, that affected their jaws and face (torn jaw, cheek and jaw, eye, and torn face), likely impairing their ability to locate and acquire prey and, thereby, reducing their survival outcome. While previous tagging studies have shown that fish characterized as having moderate and poor viability have poorer outcomes than fish characterized as having excellent viability (reviewed in Leaman and Stewart 2016), there is evidence that Pacific halibut can survive extensive jaw and facial injuries, with up to 4% of the Pacific halibut catch in certain areas showing evidence of prior hooking injuries consistent with fish released with moderate or poor viability classifications (IPHC 2022d).

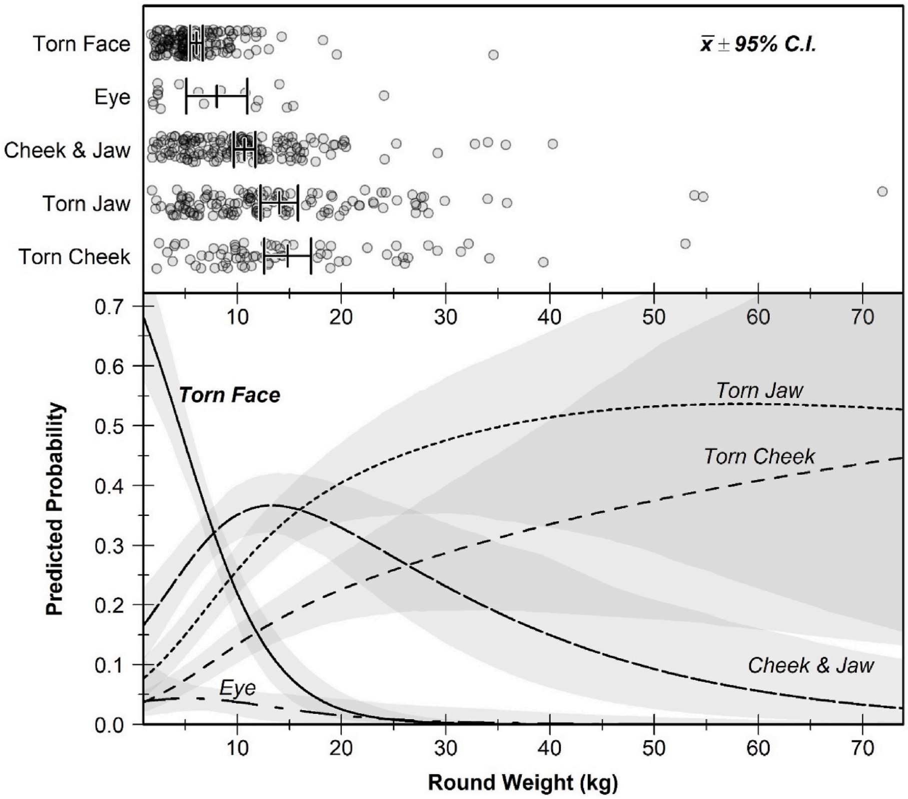

Fig. 3. Upper panel: Raw data results for the five most common injuries incurred in Pacific halibut from hook stripper release by weight (kg). Bars represent the mean ± 95% confidence interval. Lower panel: Multinomial Logistic Regression probabilities of injury type in relation to round weight (kg) for the five most common injuries resulting from release by hook stripper. Error bands represent the mean ± 95% confidence intervals.

The hook stripper caused more severe injuries, and smaller fish (less than 10 kg or 90 cm) were most affected. The relationship between fish size and injury severity reflects differences in the relative strengths and positions of anatomical structures between smaller and larger fish. As fish grow, their cartilage and bone tissues change from highly elastic with low bone strength to becoming more mineralized and denser, with corresponding peak bone strength exhibited in older and larger sized fish (Hamilton et al., 1981). Studies utilizing a technique that compares the distance that a hook penetrates into the mouth relative to the fish's total length, known as relative hooking depth (Dunmall et al., 2001), have shown that as the relative hooking distance increases, injury severity also increases (Cooke and Suski 2004; Pálsson et al., 2003). While hook penetration depth was not assessed in this study, the observation that smaller fish suffered more severe injuries when released by the hook stripper is consistent with lower facial structural strength and higher relative hooking depths in smaller fish. Similar evidence of more severe effects in smaller fish have been found in Atlantic cod (Milliken et al., 1999), and smaller fish were shown to have higher mortality rates in trawl caught Pacific halibut (Rose et al., 2019) as well as in a study conducted on both trawl and longline caught Atlantic halibut (*Hippoglossus hippoglossus*; Neilson et al., 1989). This further reinforces the value of restricting removal methods to careful means (IPHC 2021) and provides support to encourage longline fleets targeting species other than Pacific halibut to avoid using hook strippers when unhooking unwanted Pacific halibut bycatch to minimize damage to these fish.

Given that capture and handling can potentially impact the physiology and survival outcome of discarded fish (Davis 2002), we initially focused our attention to indicators of primary and secondary stress responses in plasma never before investigated in Pacific halibut caught by longlines. The plasma levels of cortisol, widely used as a primary stress response indicator in fish (Baker and Vynne 2014; Sopinka et al., 2016), did not show differences among fish with different viability or injury severity. While cortisol responses to acute stressors in fish are known to be fast (within minutes) (Sopinka et al., 2016), previous studies on Pacific halibut have demonstrated that cortisol responses to stressors can take 2–4 h to peak in plasma (Davis and Schreck 2005; Kroska et al., 2021). The lack of differences in cortisol plasma levels across viabilities are not surprising given that, even though the exact duration of the hooking event was unknown, the soak times used in this study were longer (6.4 h in average) than the expected plasma response, to mirror those used in the Pacific halibut commercial fishery. Therefore, it is possible that cortisol plasma levels may have already reached a plateau (i.e., maximum levels) when fish were unhooked and sampled on board, preventing the detection of early primary stress responses. Although the cortisol plasma levels observed in this study were higher and showed more inter-individual variability than in previous studies conducted on wild-caught Pacific halibut (Haukenes and Buck 2006) they were similar to control levels reported in laboratory studies with Pacific halibut (Davis and Schreck 2005; Kroska et al., 2021) and in studies on wild-caught sablefish (*Anoplopoma fimbria*; Davis et al., 2001) and Atlantic cod (*Gadus morhua*; Mandelman et al., 2012). Furthermore, this study is consistent with previous laboratory studies on juvenile Pacific halibut (Davis and Schreck 2005) and sablefish (Davis et al., 2001) that found no correspondence between cortisol plasma levels and mortality caused by exposure to different stressors.

In contrast to cortisol-mediated primary stress responses, secondary stress responses, characterized by changes in the plasma levels of glucose, mobilized from internal glycogen stores through the action of cortisol, and lactate (Schreck et al., 2016), produced by anaerobic metabolism during exhaustive exercise, were observed in fish in the dead viability category. In addition, fish in the dead viability category had lower hematocrit and higher somatic fat content, were smaller in size and were caught at greater depths with lower bottom sea

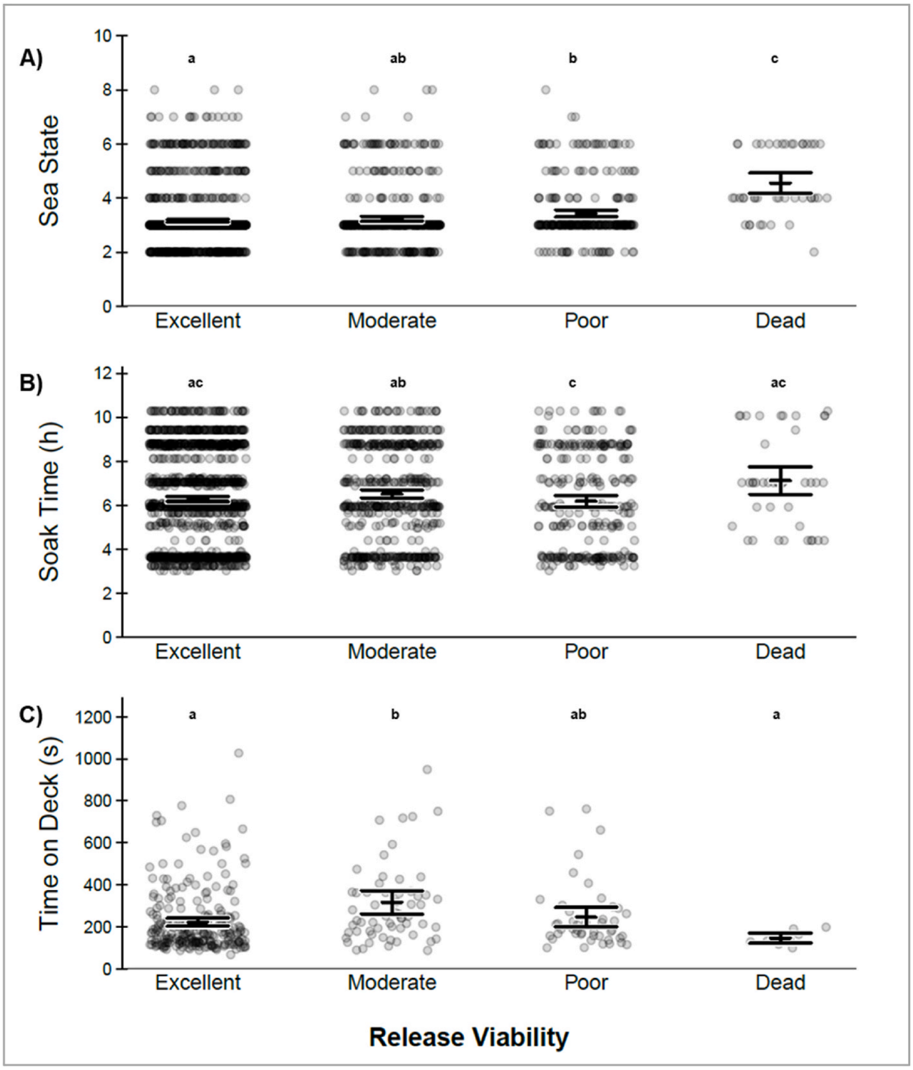

Fig. 4. Sea state (A) during hauling measured on Beaufort Scale (2: Light breeze with wavelets, 3: gentle wind with large wavelets, 4: moderate wind with small waves, 5: fresh wind with moderate waves, 6: strong wind with large waves and spray, 7: near gale with sea heaps and white foam, 8: gale with moderate long wave crests and well-marked wind streaking), soak time in hours (B), and time on deck in seconds (C) experienced by discarded sublegal (≤84 cm) Pacific halibut in different viability categories. Bars represent the mean ± 95% confidence interval. Variables denoted by the same letter are not significantly different, while variables with different letters indicate significant differences as determined by Kruskal-Wallis at $p < 0.05$.

temperatures than fish in other viability categories. Further, unlike fish in other categories, fish in the dead viability category experienced sand flea intrusion. Stressors from the current capture and release techniques should have no influence on somatic fat content within the experimental time frame, and as fat meter readings can be influenced by water content, it is possible that an unobserved intracellular disturbance of water balance may have resulted in higher fat readings for dead viability category fish. The lower hematocrit and higher lactate plasma levels in fish in the dead viability category are particularly clear indications of physiological disturbance, and we hypothesize that this may have occurred primarily due to the presence of sand flea ectoparasites. Although no previous studies have reported the stress-related effects of sand flea intrusion, it is possible that elevated lactate levels in Pacific halibut in the dead viability category were due to lactate build-up after physical exhaustion in their attempt to escape or dislodge sand fleas while alive, similar to the well-known increase in plasma lactate after exhaustive swimming in other species (reviewed in Schreck et al., 2016). Studies on discard scavenging have shown that amphipods can arrive at baits within 50–90 min of arrival at the bottom (Bozzano and Sardà 2002; Jones et al., 1998) and, therefore, sand fleas are likely often present when Pacific halibut are hooked, or soon after. The lack of a relationship between lactate levels and injury types strongly suggests that lactate was not elevated due to injury but rather to other factors such as sand fleas. In support of this, the injury profile of fish in the dead viability category was more closely aligned to the injury profile of excellent fish, with the majority exhibiting a simple torn cheek or cheek only (combined 60%) as the predominant injury with only 18% with a more severe torn face or torn snout injury. Lactate has previously been shown by Davis and Schreck (2005) to be a possible predictor of mortality in captive juvenile Pacific halibut exposed to high temperatures. Furthermore, severe plasma acidosis, in part due to elevations in lactate plasma levels, in addition to depletion of energy stores and elevated energy consumption rates (which could explain the lower glucose plasma levels in fish in the dead viability category), have been linked to higher post-exercise mortality in fish (reviewed in Rodnick and Planas 2016). Hematocrit was also lower in dead category fish, likely indicative of hemorrhagic anemia, a pathological condition resulting from the loss of red blood cells due to bleeding (Roberts and Rodger 2012), caused by blood consumption by sand fleas while fish were alive. In support of this hypothesis, blood-feeding gill ectoparasites, have been reported to decrease the hematocrit in other fish species, including the blackeye thicklip (Labridae) (Jones and Grutter 2005) and the starry flounder

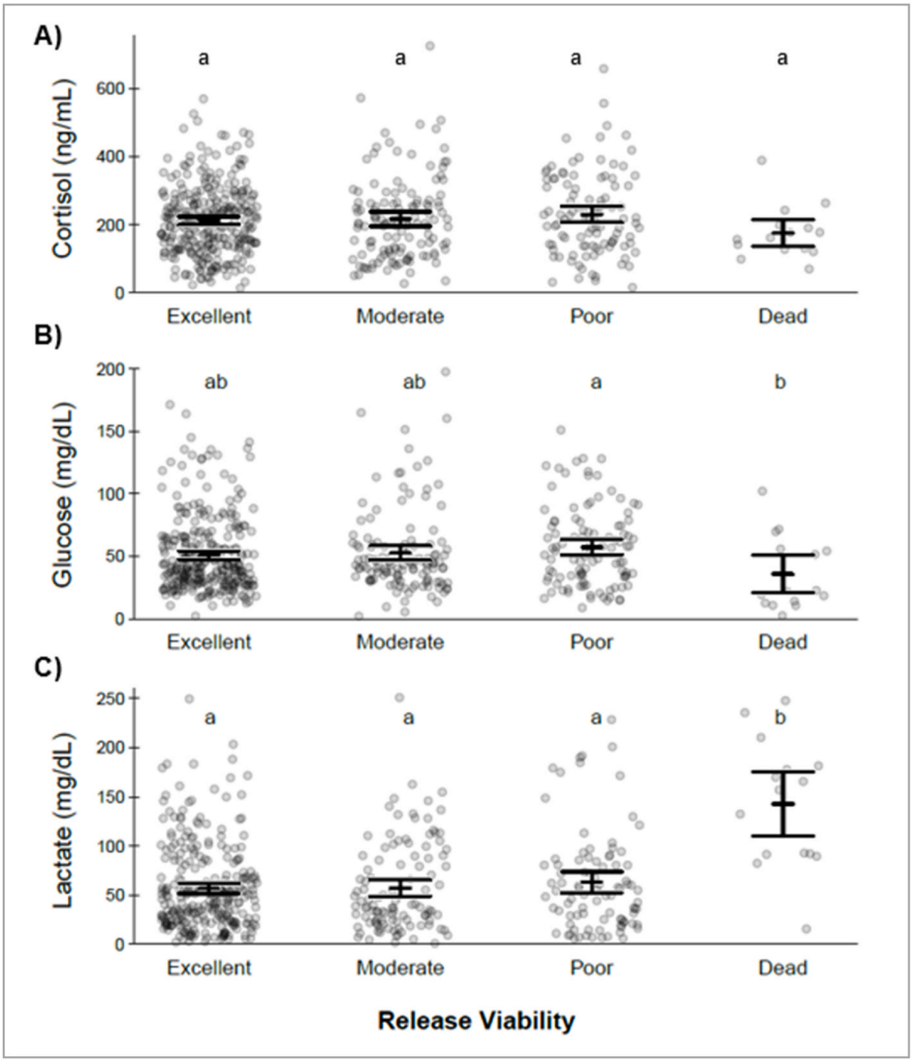

Fig. 5. Blood plasma levels of cortisol (ng/mL) (A), glucose (mg/dL) (B), and lactate (mg/dL) (C) by release viability for sublegal (≤84 cm) Pacific halibut. Bars represent the mean ± 95% confidence interval. Variables denoted by the same letter are not significantly different, while variables with different letters indicate significant differences as determined by Kruskal-Wallis at $p < 0.05$.

(Wood et al., 1979). Although it cannot be ruled out if mortality of Pacific halibut by sand flea intrusion was due to high lactate-induced plasma acidosis, severe anemia, energy depletion or a combination of these factors, sand flea predation is a known cause of fish mortality (Stachura et al., 2012) including in Pacific halibut (Trumble et al., 2000). Overall, our findings are consistent with intrusion of sand fleas being a negative predictor of discard survival in Pacific halibut.

Plasma glucose and lactate levels observed in the present study were, on average, similar to those reported in previous studies of captive (Davis and Schreck 2005) and wild-caught (Haukenes and Buck 2006) Pacific halibut and, like other blood parameters measured, showed large inter-individual variability. However, given the quick processing time between hook removal and blood sampling, it is unlikely that variability in the observed values of blood parameters was associated with the hook release method used or with any other aspect of the experience on deck of the captured fish. Instead, it is more likely that the broad inter-individual variability observed in the present study was the result of individual differences in physiological condition as well as differences in the environmental conditions and length of time experienced by fish while on the hook. In addition to the presence of sand fleas, environmental temperature has been shown to exert a strong influence in the physiology and resultant mortality, particularly for smaller fish (Davis and Schreck 2005; Davis et al., 2001; Veldhuizen et al., 2018). As this study was completed in the fall, temperature was generally low and differences between air and sea bottom temperatures were generally small and likely had a minimal impact on the results obtained. Future studies should include a broader range of the thermal environments to which Pacific halibut may be exposed during warmer and colder months of the year to fully capture the potential impact of temperature and change in temperature from bottom to surface. This may be of particular relevance to Pacific halibut released during the summer months when weather allows the majority of fishing to occur, and when summer air and water temperatures continue to reach new highs (Ren et al., 2023). Furthermore, as previous studies (Rose et al., 2019) have shown the importance of the duration of stress exposure on Pacific halibut discard mortality, future studies should endeavor to capture the time of the initial catch stressor (i.e., hooking) through the use of hook timers to provide further resolution to the influence of physiological stressors on true discard mortality outcomes and enable refinement of the current qualitative viability classification criteria.

5. Conclusions

This is the first large scale field study investigating physical, environmental, and physiological influences on viability classification driving survival outcomes in Pacific halibut. Fish released in excellent

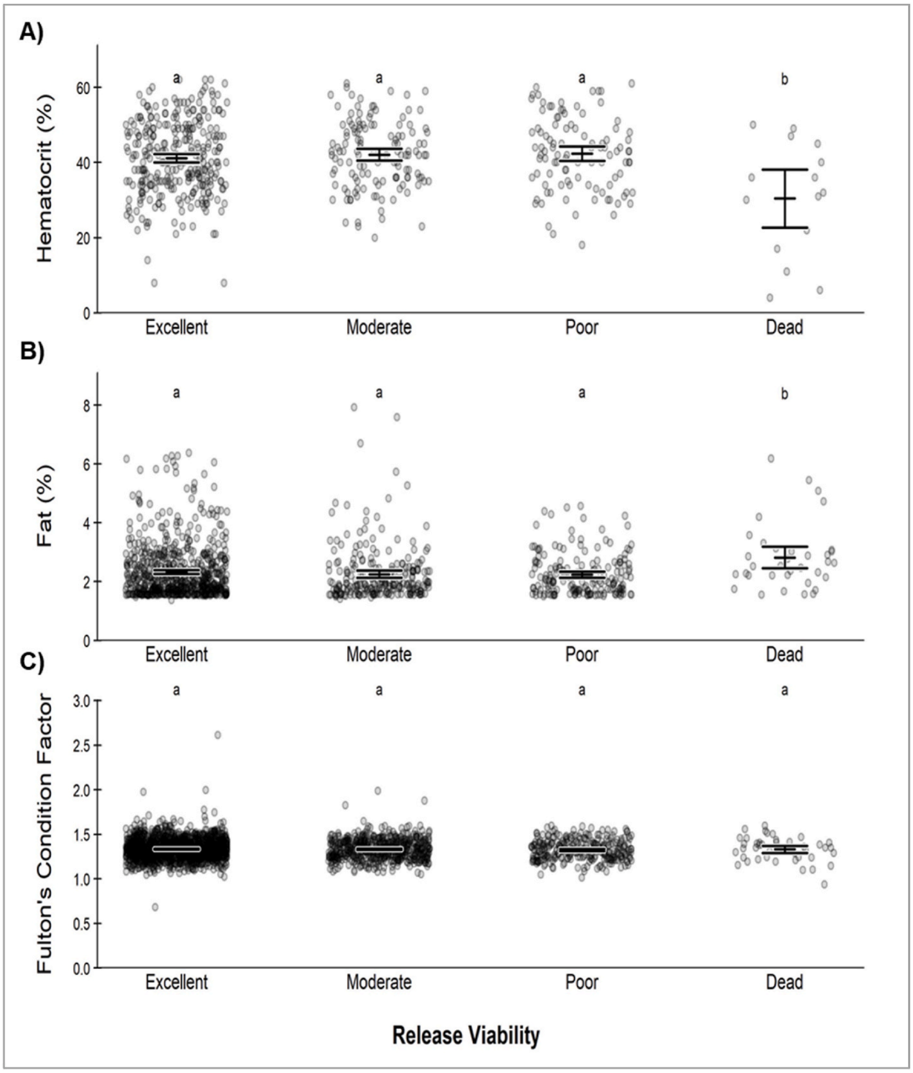

Fig. 6. Hematocrit (%) (A), somatic fat content (%) (B), and Fulton's condition factor (C) by release viability for sublegal (≤84 cm) Pacific halibut. Bars represent the mean ± 95% confidence interval. Variables denoted by the same letter are not significantly different, while variables with different letters indicate significant differences as determined by Kruskal-Wallis at $p < 0.05$.

viability are generally associated with careful release methods resulting in minimal injuries, largely coincident to the hooking event itself, and fish of excellent viability generally have very high survival outcomes (Loher et al., 2022). In contrast, Pacific halibut in the dead viability category were predominantly smaller fish that were caught on deep, cold stations, and had lower hematocrit and higher lactate levels that are attributed to the effects of sand flea predation/intrusion during the soak period. Therefore, avoiding long soaks in deep locations could mitigate poor survival outcomes for Pacific halibut and would be a useful practice in the directed fishery. This work highlights potential approaches for reducing discard mortality for better understanding the mortality that does occur. Specifically, both careful release techniques were found to be effective in minimizing injuries to Pacific halibut as the use of hook strippers results in significantly more complex injuries and reduced viability outcomes, particularly in smaller fish, and should be minimized in non-directed fisheries to optimize survival of discarded Pacific halibut. Second, this work suggests that estimates of viability might be improved by considering fish size in tandem with release method, whereas all current approaches apply similar criteria to all sizes of Pacific halibut. The IPHC includes estimates of discard mortality for all fisheries when assessing the trend and status of the Pacific halibut resource (Stewart et al., 2023). In addition to lost yield that could be harvested and utilized, discard mortality is only indirectly estimated; where these estimates may be biased, due to incomplete understanding of the factors affecting discard mortality, the stock assessment results may also be biased. The successful management of Pacific halibut relies on minimizing discard mortality, and precisely estimating what cannot be avoided. Thus, understanding of the physiological basis and key environmental factors influencing survival during and after the capture process remain critical under rapidly changing climatic and fishery conditions.

CRediT authorship contribution statement

Claude L. Dykstra: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Nathan Wolf:** Formal analysis, Writing – original draft, Writing – review & editing. **Bradley Harris:** Formal analysis, Writing – original draft, Writing – review & editing. **Ian J. Stewart:** Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing, Formal analysis. **Allan C. Hicks:** Methodology, Writing – original draft, Writing – review & editing, Conceptualization, Formal analysis. **Felipe Restrepo:** Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Josep V. Planas:** Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ocecoaman.2024.107018.

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