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Final Report

Evaluating the Condition and Discard Mortality of Skates Following Capture and Handling in the Sea Scallop Dredge Fishery.

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1. Introduction

In the U.S. commercial fishing industry (marine) approximately 12.7% (~ 607 million lbs.) of all landings are discarded annually (NMFS 2014). However, the fate (dead or alive) of these fish has typically gone unaccounted for in population assessments (Alverson et al., 1994; Hall et al., 2000) because of the numerous factors and species-specific differences that effect discard survival and make data inherently difficult to collect (Davis, 2002). Due to this lack of information the ability to provide accurate post-release mortality (PRM) estimates of discarded fish has become of the most significant issues affecting current marine fisheries management (Davis, 2002).

In the limited studies to date, a common approach to investigate PRM has been to capture animals and then monitor their short-term survival with seafloor holding pens and on-board aquaria (Benoît et al., 2010, 2012; Mandelman et al., 2013; Depestele et al., 2014). While such studies can provide management with short-term survival estimates and best-practice frameworks that reflect the fishery, they are frequently laden with caveats (Benoît et al., 2010, 2012; Mandelman et al., 2013, Depestele et al., 2014). For example, this approach can both inflate (i.e. due to stress from crowding/confinement, forced thermal changes and recompression, lack of foraging ability) and deflate (i.e. via shielding from predation from birds and other marine species) mortality risk, thus biasing study results relative to what might occur under non-experimental conditions (i.e. Weltersbach and Strehlow, 2013). Moreover, confinement in cages or aquaria can only account for the initial few days post-release, despite the strong likelihood that additional fatalities would occur beyond that monitoring window. In addition, the number of animals that can be evaluated (given the logistics of containment dimensions and cost of at-sea operation) in these studies can limit the statistical power and make it difficult to account for all possible factors influencing survival (Benoît et al. 2010). To overcome these limitations, rapidly assessed, semi-quantitative indices used as proxies for survival can be applied to both discarded animals and subsamples kept for holding periods, to integrate a wider-range of fishing conditions that can affect discards (e.g. Barkley and Cadrin, 2012; Mandelman et al., 2013; Depestele et al., 2014; Benoît et al., 2015). These quantitative indices are most effective as proxies when their design includes various predictors of survival, such as overt physical trauma and reflex impairment (van Beek et

al., 1990; Benoît et al., 2010, 2012; Depestele et al., 2014). Using the probability of a given index score (conditional on capture factors) and the probability of survival (conditional on the quantitative proxy), this type of study is able to obtain representative estimates of PRM (Benoît et al., 2015).

The order Rajiformese (i.e. skates) is in particular need of PRM estimates given the 47.9% increase in U.S. landings over the past 10 years (~ 11.4 million lbs. captured in 2013; FAO 2014) and the significantly high levels of bycatch, which can be nearly six times greater than total landings (~ 72.3 million lbs. discarded in 2010; NMFS 2014). In addition to these high discard rates, concern for skate populations is exacerbated by their K-selected life history strategy (e.g. slow development, low fecundity, long longevity, and late age-at-maturity) that makes them vulnerable to PRM as bycatch in the commercial fishing industry (e.g. Waring, 1984; Hoening and Gruber, 1990; Sulikowski et al., 2003). Despite these issues there is still a lack of PRM information in the U.S, with the majority of research taking place in other geographical regions and with different species. Reports of short-term mortality from these studies vary from 3 to 59% and can be species-specific and dependent upon gear-type and other relevant technical (e.g. tow duration and catch biomass), biological (e.g. size), and environmental (e.g. air temperature) factors (e.g. Benoît, 2006; Enever et al., 2009; Benoît et al., 2010, 2012; Mandelman et al., 2013; Depestele et al., 2014). The sole investigation of skate PRM in the U.S. was performed in the Northwest Atlantic Ocean (NWAO) and was focused on the commercial otter trawl fishery (Mandelman et al., 2013).

Fisheries in the U.S. NWAO are responsible for nearly 96% (~ 69 million lbs.) of the total U.S. skate bycatch. Of these fisheries, the New England (NE) scallop dredge fishery has the second highest discard rate (~ 26 million lbs. of skate discarded annually; NMFS 2014), making this gear-type a critical PRM concern for management and conservation. This concern is amplified given the need for effective management in the most valuable fishery in the U.S. NWAO, with an estimated ex-vessel value of over 467 million U.S. dollars in the 2013 fishing year. Thus, the primary objective of this study was to quantify PRM rates of the three most commonly captured skate species (little skate, *Leucoraja erinacea*; winter skate, *Leucoraja ocellata*; barndoor skate, *Dipturus laevis*) using extended holding periods in a novel on-deck tank system equipped with a

refrigerated seawater system (Knotek et al., 2015). The secondary objective of this study was to evaluate the variety of factors (e.g. tow duration, depth, catch biomass, sex, size) that could influence PRM and formulate a best practice framework to reduce mortality in the fishery.

2. Methods

2.1. Study overview

PRM trials were performed aboard four commercial scallop-fishing vessels (Fishing Vessels: F/Vs “Weatherly”, “Ranger”, “Araho”, and “Resolution”) that ranged in length from 95 to 118 ft. Data were collected over six seven-day cruises on Georges Bank and waters offshore of Long Island, New York, USA (NW Atlantic). These research trips occurred from May to September in 2012 and 2013. Fishing was conducted under normal fishing conditions and practices, using standard 4-inch ring commercial scallop dredges with a dredge width of 15 to 15.5 ft and 10-inch square mesh twine tops. Bottom seawater and air temperatures ranged from 8.3 to 13.0°C and 8.7 to 23.6°C, respectively. The gradient between bottom seawater and air temperatures ranged from 0.4 to 12.5°C. Fishing was conducted on soft (i.e. sand) substrate.

2.2. Fishing research protocol

A total of 295 tows were conducted during the six research cruises. Tow speed was approximately 4.5 to 5.0 knots. During each trip, tow duration (10 – 90 min) was randomized to account for variations in tow length (minimum and maximum) that are typically observed in the scallop fishery. Bottom seawater and air temperature profiles were recorded with HOBO temperature loggers (Onset Computer Corporation, Bourne, MA, USA) fastened to the scallop dredge and positioned on-deck. Catch biomass was recorded as the number of scallop bushels collected by deck hands (i.e. harvesting scallops).

2.3. Post-release mortality experiment

All catch from experimental tows was deposited on deck and culled by commercial fishermen for harvestable scallops, after which a subsample of the remaining skates (i.e. discards) were sampled. The exception was for skates that were captured in short duration tows (<10 min), which were sampled immediately and quickly placed in refrigerated seawater once the dredge was recovered such that they could serve as minimally harmed experimental “controls”.

To account for variations in air exposure, exposure times for skates other than the control subject were varied from 0 to 30 min (maximum duration seen in fishery). Exposure time was the time the dredge was removed from the water to the time skates were sampled and either released to the sea or to the deck tanks (explained further in Section 2.3.2). Sampling of individual skates involved an assessment of reflex impairment and physical injury (see methods Section in 2.3.1), recording of the species, sex, and total length (TL, cm) and tagging with a coded dart tag (Floy Tag & Mfg. Inc., Seattle, WA, USA)

2.3.1. Health indicators

Skates were scored based on reflex impairment and physical trauma indices (i.e. health indicators). Five reflexes were selected for this study using a modified approach from Davis (2007). To validate the use of individual reflexes, preliminary testing was conducted on control skates (i.e. healthy and unstressed) at the University of New England Marine Science Center under the protocol described by Davis (2010). These skates were kept in a 186 × 104 × 63 cm holding tank equipped with a flow-through (38 L min⁻¹ turnover rate) seawater system. Skates were fed daily *ad libitum* throughout the experiment. To determine the appropriate reflexes (i.e. consistently present in control skates), skates were tested for the same ten reflexes every seven days, throughout July of 2012. The five most consistent reflexes (Table 1) were then chosen for the reflex impairment index that would be used in the field PRM trials.

At-sea, each reflex was tested individually and conservatively scored as present or absent, such that the reflex in question was considered absent when any doubt in the response existed (reducing any observer biases). Reflex impairment was evaluated immediately upon handling and took approximately 30 seconds per animal. Each skate

was then assessed using a categorical injury code (1-3) developed by Mandelman et al. (2013) to determine the degree of overt physical trauma (Table 2, Fig. 1).

2.3.2. *Holding tank trials*

A subsample of skates (representative of the ranges of air exposure and health indicators) were transferred into one of six on-deck holding tanks (Promens Saint John Inc., Saint John, New Brunswick, Canada; Model: MS1400; internal dimensions: 110 cm × 96 cm × 80 cm) following evaluation (described in Section 2.3) and held for 2 to 118 hours (Fig. 2). Tanks were equipped with a flow-through, refrigerated seawater system (Knotek et al., *in review*) that cooled surface intake water to mimic bottom water temperatures. Although species and number of skate varied within each tank, biomass was kept constant (i.e. ~8-10 smaller individuals; ~2-4 larger individuals). Tanks were monitored every six-hours and if a mortality was observed, the individual was removed and replaced with a skate from subsequent tows. Following 72-hour trials, mortality was recorded and living skates were re-evaluated using the aforementioned health indicators, before being released.

2.4. *Analysis*

The data recorded for each skate consisted of tow-specific fishing conditions and practices, initial health indicator scores, and the elapsed time until mortality (i.e. uncensored) or live release (i.e. right-censored) if the skate was selected for holding tank trials. The later form of longitudinal data (i.e. time interval) was considered right-censored because we are unable to make inferences regarding their survival beyond the observation period in holding tanks. A survival analysis was therefore used in this study for its ability to accommodate our longitudinal data and model the probability of survival as a function of time (e.g. Cox and Oakes, 1984; Benoît et al., 2012, 2015). In addition, survival was modeled specific to health indicators, given our belief that injury and reflex scores have the ability to integrate the various fishing conditions and practices, and individual traits that affect survival (Anderson and Phillips, 1981; Agresti, 2002, pp. 277-279; Wood et al., 1983; Davis, 2002; Broadhurst et al., 2006). The effect of these factors

on health indicators was therefore considered in separate analyses. All analyses were performed separately for each species and

2.4.1. Health indicators ability to predict survival

The data recorded for each skate consisted of the initial health indicator scores and the elapsed time until mortality (i.e. uncensored) or live release from holding tanks (i.e. right-censored). The later form of longitudinal data was considered right-censored because we are unable to make inferences regarding their survival beyond the observation period in holding tanks. A survival analysis was therefore used in this study for its ability to accommodate our longitudinal data and model the probability of survival as a function of time (e.g. Cox and Oakes 1984; Benoît et al. 2012, 2015).

To validate reflex impairment and injury code as suitable predictors for intraspecific short-term survival, we used a mixed-effects Cox proportional-hazards model (CPHM; Cox, 1972; Therneau and Grambsch, 2000). This model is defined as:

$$\hat{h}(t) = h_0(t)\exp(X'\beta + Z'b) \quad (1)$$

where $\hat{h}(t)$ is the estimated hazard function, instantaneous probability of mortality at time t , which is a function of a baseline hazard function $h_0(t)$, a vector of covariates X' (i.e. five reflexes and injury code) and a Gaussian random effect Z' (i.e. tow-specific). This class of survival analysis is semi-parametric in that it makes no assumption about the shape of $\hat{h}(t)$ (i.e. baseline hazard function), but assumes that the ratio of hazards for two individuals is constant over time and is a function of covariates. The CPHM was therefore first used to validate which health indicators were suitable for each species (i.e. best set of covariates). Partial maximum likelihood was used to estimate parameters (Cox, 1972; Ripatti and Palmgren, 2000).

Model building began by fitting a fully saturated (i.e. all relevant covariates) mixed-effect CPHM (Eq. (1)). To determine if the random effect was appropriate, a likelihood ratio statistic was computed against a chi-squared distribution and resulting P-value corrected for boundary testing. If the random effect was not significant ($P < 0.05$), the model was built using fixed-effects CPHM, whereas mixed-effects CPHMs were used

in other scenarios. To find the most parsimonious set of health indicators, model selection followed protocol from Benoît et al. (2010). Based on this protocol the covariates were incrementally added to the intercept-only model and compared using Akaike's Information Criterion corrected for small sample sizes (AICc; Burnham and Anderson, 2002). An additional covariate to the candidate model was only retained if the model $\Delta\text{AICc} \geq 3$ unit reduction. For models that had an equal amount of covariates but different compositions, if the ΔAICc was ≤ 3 , the models were considered equally plausible and both were retained. Following this analysis we performed formal log-rank tests to assess the differences in survival curves as a function of the selected health indicator scores, and in cases where there was no statistical difference between curves ($P < 0.05$), the data was combined for the purpose of our survival analysis. All data were analyzed using R 3.1.0 (R-Core Development, 2014).

2.4.2. Predicting short-term survival based on health indicators

To estimate the survival of each species as function of time for a given response level (i.e. based on the combination of health indicators selected previously in Section 2.4.1) we used CPHMs. Model fits were then compared to the empirical Kaplan-Meier survival curve (KM; Kaplan and Meier, 1958), which is a solely a function of the data as it follows the proportion of individuals alive within each time interval during the holding tank trials. For KM curves, the probability of survival within each time interval i is defined as:

$$\hat{S}(t) = \prod_{t_i \leq t} \left(\frac{n_i - m_i}{n_i} \right) \quad (2)$$

where $\hat{S}(t)$ is the probability of survival at time t , n_i is the number of skates at risk of mortality within the time interval i , and m_i is the number of mortalities during i .

2.4.3. Estimating short-term using fishery-scale observations

To estimate the species-specific short-term survival for the larger sample of skate discarded during the study (i.e. more representative of fishery-scale conditions) we combined the injury and reflex impairment scores selected in Section 2.4.1 with survival

rates estimated with CPHMs (Eq. (1)). Predictions of survival were made up until 72 hours and Monte Carlo simulations based on bootstrapping were used to estimate the variation in survival rate (Efron and Tibshirani, 1993, Benoît et al., 2012). In each individual Monte Carlo iteration, tows were sampled with replacement from the data to capture uncertainties of selecting each tow for observation. Furthermore the individuals within each selected tow were sampled with replacement, to also account for uncertainties of selecting an individual for health indicator assessment. See Benoît et al. (2012) for additional details.

2.4.4. Factors potentially influencing mortality

The effect of fishing conditions and practices and individual traits (Table 3) on injury and reflex indices were estimated using different modeling techniques because of the varying nature in ranking of the data and belief that both indices likely affect mortality via different underlying mechanisms (i.e. physical trauma vs. physiological stress). The inherent ranking within ordinal injury scores led to our choice of a multinomial proportional-odds model (POM) based on cumulative logits (e.g. Benoît et al., 2010). In addition, a random effect term was included to account for subjectivity in scoring by different researchers and possible within-tow correlations (e.g. Pinheiro and Bates, 2000; Hartzel et al., 2001; Sheu, 2002; Zuur et al., 2009) due to the clustered nature of the data (i.e. large numbers of each species of skate within a single tow). This model is defined as:

$$\text{logit}[P(Y_{ij} \leq v | X'_{ij}, u_i)] = \alpha_v + X'_{ij}\beta + u_i, \quad \text{where } u_i \sim N(0, \sigma^2) \quad (6)$$

where Y_{ij} is the observed injury code for a skate j captured in tow i , v is the injury score, α_v is intercept for each level of injury, X'_{ij} is the covariate design matrix, β is the vector of parameters values for the covariates, and u_i is the random effect drawn from a normal distribution with a mean of zero and variance σ^2 (Agresti, 2002, pp. 275-277; Carrière and Bouyer, 2006). For α_v , only two values can be estimated because $P(Y_j \leq 3)$ must equal one, and therefore only two distinct probabilities can exist. $P(Y_{ij} \leq v | X'_{ij}, u_i)$ is the probability that the observed injury score for a skate is less than or equal to the injury score v , conditional on the covariates and random effect. The model was fit using the

adaptive Gauss-Hermite quadrature approximation to integrate across the random effects (Pinheiro and Bates, 1995; Hartzel et al., 2001) and the Newton-Raphson method was used to maximize the likelihood and find estimates of the parameters.

Model building started with fitting a fully saturated model with all relevant fixed effects (i.e. those that had the potential to influence physical trauma), which included tow duration, depth, catch biomass, sex, and TL. To determine if the random effect was appropriate we used the procedure previously described for CPHMs, and subsequently to find the most parsimonious set of covariates to explain the variation in injury scores, we used a forward selection process based on AICc as described above (refer to Section 2.4.1). The predicted responses from POMs are based on the ordering of injury-level specific intercepts α_v , while the effect of the covariate is based upon a common slope across injury levels. To determine if separate slopes for the effects of covariates on each injury score were more appropriate than common slopes, we also fit generalized logits models (Carrière and Bouyer, 2006) for the covariates retained in the best-fit POM following the approach used by Benoît et al. (2010). Generalized logits and proportional odds models were compared using AICc.

To explain the variation in impairment for the reflexes pertinent to each species survival (i.e. those retained in CPHMs from Section 2.4.1.), we first combined these reflex responses into a single code containing all the possible present or absent combinations. Next we choose to fit the data to a baseline-category logits model (BCLM; Agresti 2002. pp.267-274), given the multinomial response of the reflex code lacked any inherent ranking. For this model, let $\pi_j(x) = P(Y = j|X')$ be the probability of a skate Y demonstrating a given combination of reflex response j conditional on a set of covariates and with $\sum_j \pi_j(x) = 1$. The model is defined as:

$$\log \frac{\pi_j(x)}{\pi_J(x)} = \alpha_v + X'_j \beta, \quad j = 1, \dots, J - 1 \quad (7)$$

where J is the number of potential reflex response combinations (e.g. two reflexes = 4 potential present/absent combinations) and X' and β are the vectors of covariates and parameters, respectively. The response term in Eq. (7) is the logit for the reflex combination j compared with a baseline response J , which for the purpose of this analysis

was unimpaired (i.e. all reflexes present) reflex combination. A random effect was not considered in this analysis because we felt the nature of the data (i.e. reflexes scored on a present or absent scale) would reduce bias and produce a very small observer effect. This is in contrast to injury code, where there subjectivity could exist in choosing between adjacent categories (i.e. a more pronounced observer effect). Any remaining random effect in this analysis would therefore be due to data clustering and unobserved factors. This can be important when the number of observations in a cluster (i.e. within each tow) is relatively large, while the number of cluster is modest. However in this study the number of skates sampled in each tow was modest compared to the much larger number of tows, which suggests that random effect is less important for this analysis. Maximum likelihood estimation was used to fit the BLCM (Agresti, 2002, pp. 272-274).

A forward selection process based on AICc values was used to find a parsimonious set of covariates to explain variation in reflex responses. The set of covariates considered in the BLCM consisted of tow duration, depth, catch biomass, air exposure duration, temperature differential, sex, and TL, all of which were considered to have potential to comprise the physiological state of a skate (i.e. influencing reflex responses; Raby et al., 2012). To evaluate the fit of our final model we compared the predicted response probabilities with field observations of reflex responses binned into six bins of equal size (based on the appropriate covariate). Predictions of reflex response probabilities were obtained via the logit-transformation (Agresti, 2002, pp. 271-272) of Eq. (7):

$$\pi_j(x) = \frac{\exp(\beta'_j X)}{\sum_{h=1}^J \exp(\beta'_h X)}, \quad \text{where } \beta_j = 0 \text{ and } j = 1, \dots, J \quad (8)$$

To obtain confidence intervals for the predicted probabilities from Eq. (8) we performed an empirical bootstrap (Efron and Tibshirani, 1993) with tows sampled with replacement from the population.

3. Results

In this study a total of 4216 skates were evaluated (little skate: $n = 2634$; winter skate: $n = 1313$; and barndoor skate: $n = 269$), with a subsample of 334 skates (little skate: $n =$

179; winter skate: $n = 116$; and barndoor skate: $n = 39$) held in holding tanks for PRM trials.

3.1. *Post-release mortality with respect to health indicators*

3.1.1. *Health indicators ability to predict short-term post-release mortality*

The best-fit CPHMs for little and winter skate retained the same set of health indicators: injury code and back fly and gag reflex impairments (Table 5). Injury scores 1 and 2 for both species were combined for the remainder the survival analysis, given there were little to no statistical difference in survivorship (little skate: $\chi^2 = 5.3$, $df = 1$, $p = 0.02$, winter skate: $\chi^2 = 1.6$, $df = 1$, $p = 0.206$) or hazard ratios between each level (little skate: $p = 0.26$; winter skate: $p = 0.31$; Table 5). No health indicators were retained in the best-fit model for barndoor skate (i.e. intercept-only model supported) and therefore we were unable to predict PRM as function with health indicators for this species.

3.1.2. *Post-release mortality during holding tank trials*

The CPHM survival estimates varied between both little and winter skate and for initial health indicator scores (Fig. 3). Estimates of survival ranged from 82.9 to 6.1% (Fig. 3A, C) and 95.9 to 4.9% (Fig. 3B, D) for little and winter skate, respectively, with increasing physical trauma and added reflex impairment negatively impacting the probability of survival. For these species, CPHMs fit the KM survival curves reasonably well for larger sample sizes (i.e. $n > 10$), but when sample sizes for a given health indicator combination were low it was difficult to compare the model fit because the KM curves are an inverse function of sample size. Barndoor skate survival was analyzed using only the empirical KM survival curve (i.e. since health indicators were not appropriate predictors) and resulted in an overall 11.2% probability of survival (Fig. 4).

3.1.3. *Estimating short-term PRM with fishery-scale observations of health indicators*

The overall trend in the proportion of skate observed in each injury code was similar for each species (Table 6), such that lowest proportions were seen for little to no injury (code 1) and highest for moderate (code 2). For reflex impairment, little and winter skate were found to have a comparable trend with impaired back fly and unimpaired gag reflex as

the most common, followed by both reflexes unimpaired (Table 6). Whereas barndoor skate observations displayed highest proportions for complete reflex impairment and an impaired back fly and unimpaired gag reflex (Table 6).

The average estimated short-term survival rate (up to 72-hours) for skate was species-specific. Winter skate had a higher rate of survival than little skate ($65.2\% \pm 0.05$ and $49.1\% \pm 0.03$, respectively; Table 7) and although we were unable to estimate PRM for barndoor based on health indicators, the higher observed proportion of impaired individuals (Table 6) suggests that mortality would likely be low.

3.2. Factors potentially influencing health indicators and post-release mortality

3.2.1. Barndoor-specific

No covariates (including the random effect term) produced a reduction in AICc large enough for selection in the final CPHM for barndoor skate (Table 8). This is likely due to an overall small sample size for this species ($n = 39$). Barndoor were not included in the following results because injury code was not selected in the plausible CPHM (Section 3.1.1).

3.2.2. Injury code

The random effect term was not significant for either little or winter skate in the fully saturated covariate POMs. The final POMs for these skate species indicated that tow duration had a significant effect on the level of injury, while the POM for little skate also retained the depth covariate (Table 9). Tow duration had the largest effect size (relative risk) for winter skate injury, whereas depth was more influential for little skate. Tow duration was found to have a negative impact on the injury code (i.e. extended tow durations increased the probability of having a higher degree of physical trauma) for both little and winter skate (Table 9; Fig. 5A-D), while depth had a positive impact on little skate injury (Table 9; Fig. 5A-C) with greater physical trauma observed at shallower depths (e.g. Fig. 5A) than at depth (e.g. Fig. 5C). POMs for both species were considered to fit the data well based upon comparisons of predicted probabilities (contingent on final POMs and selected covariates) and observed proportions of skate with each injury code for each species (little skate: Fig. 5A-C; winter skate: Fig. 5D). There was no evidence to

support generalized logits models (i.e. separate slopes specific to each injury code) for any covariate in final models for either species (Table 9).

3.2.3. *Reflex impairment*

The final BCLMs for little and winter skate both suggested that tow duration had a significant effect on reflex impairment. In addition, temperature gradient and tow depth were retained for the little skate model, while the winter skate BCLM selected for deck duration. Tow duration had an overall similar effect on both species (regardless of varying levels of other covariate effects; Fig. 6, 7) with an increase in tow duration leading to further reflex impairment (i.e. from unimpaired to completely impaired). In addition, the reflex combination of an unimpaired back fly and impaired gag response was the least likely for both species (Fig. 6, 7), regardless of the scenario. For little skate, increases in temperature gradient led to a lower probability of a completely unimpaired individual, a higher probability of an impaired back fly and present gag reflex, and relatively no change in the likelihood of the other reflex combinations (Fig. 6A-C). Moreover for little skate, an increase in tow depth causes a higher probability for an impaired back fly and present gag reflex, while the odds of complete impairment decrease, and there is little to no effect on the other combinations (Fig. 6D-F). In the winter skate BCLM, extended bouts of aerial exposure led to an increased overall reflex impairment, while the trend of the combination present back fly and impaired gag response (i.e. as tow duration lengthens there is an increase followed by a decrease in probability) shifted towards shorter tow durations (Fig. 7A-C). The unimpaired back fly and absent gag reflex combination was relatively unaffected by variations in air exposure. BCLMs for both species fit the data reasonably well based on comparisons of predicted and observed probabilities of a skate exhibiting a certain reflex combination (little skate: Fig. 6, winter skate: Fig. 7), however low sample sizes within given bins of covariates led to some uncertainty. Correlations between predicted and observed combinations for both species models were intermediate (little skate: 0.39; winter skate: 0.41).

4. Discussion

4.1. *Post-release mortality of discarded skates*

The use of health indicators (e.g. vitality, condition, injury, and reflex impairment indices) on fish to prior to release has become increasingly well established in literature as an effective tool for predicting PRM of discards in both teleosts and elasmobranchs (Laptikhovsky, 2004; Enever et al., 2009; Humborstad et al., 2009; Barkley and Cadrin, 2012; Benoît et al., 2012; Mandelman et al. 2013; Raby et al. 2012; Depestele et al., 2014; Gallagher et al., 2014). The advantage to this technique is that once proven for a given species and fishery (via tagging or extended holding trials) it becomes a simple and inexpensive way to expand research efforts across broader scales of time and space, to better understand how the various fishing conditions and practices affect survival (Benoît et al., 2010, 2012).

In this study we determined that injury and reflex impairment indices were appropriate for modeling the survival of discarded skate. Previous studies have shown that injury codes based on overt physical trauma can effectively predict skate survival (Mandelman et al., 2013; Depestele et al., 2014), while other studies have used pseudo-reflex impairments (e.g. levels of overall body movement) incorporated into a vitality index (Benoît et al., 2010) as another effective measure. The present study, however, is the first occasion where individual reflexes have shown a proven relationship with PRM for skate. By combining these two health indicators we are able to evaluate mortality from a physical trauma (i.e. injury code) and physiological standpoint (i.e. reflex code), given that reflex impairment serves as a proxy for a comprised physiological state (Raby et al., 2012).

Estimates of short-term survival (via rates associated health indicators) applied to field observations suggest that species-specific differences in PRM exist in the scallop dredge fishery. Barndoor skate are likely most susceptible to capture and handling (PRM upwards of 90%, however this is based only upon the KM method), while little skate have a moderate capacity to deal with these fishing pressures (PRM of 51%), and winter skate are the most resilient (PRM of 35%). Species-specific survival rates for skates (Rajidae) within a given fishery have also been reported from demersal trawlers in the

Bristol Channel (Eneever et al., 2009), as well as for little and winter skate captured by otter-trawl in the NW Atlantic (Mandelman et al., 2013). In the later study, winter skate were also found to be more robust to the rigors of capture than little skate (PRM of 9 and 22%, respectively). However, little and winter skate exhibit nearly a two and four-fold increase in mortality when captured in the scallop dredge fishery. It is possible that the inflation in PRM is an artifact of increased deck durations during sampling (otter-trawl: 10 min; scallop dredge: 10-30 min) that have also been attributed to more dramatic rises in mortality for winter skate captured in the Canadian trawl fishery (PRM of 50%, deck duration: 1-2 hours; Benoît, 2006). Discard PRM has also been reported as highly dependent upon gear type (Chopin and Arimoto, 1995; Davis, 2002) and in this scenario we can directly compare the physical trauma in the otter-trawl and scallop dredge, because the injury code used for these study was identical. As such, the proportion of individuals in worse condition (i.e. injury codes 2 and 3) nearly doubles in the scallop dredge fishery for both little (.35 to .78) and winter (.39 to .81) skate. Therefore, the increase in physical trauma (and subsequent inflation of mortality) may be attributed to the steel infrastructure and metal rings of the dredge, as opposed to the softer and less abrasive twine of the otter-trawl gear.

This study also revealed differences in survivorship in little and winter skate across varying levels of injury and reflex impairment (Fig. 3A-D). For example, both species experienced increases in PRM as physical condition deteriorated (i.e. higher injury codes). This trend has been well documented for skates (Benoît et al., 2012; Mandelman et al. 2013; Depestele et al., 2014) and other elasmobranchs (Hueter and Manire, 1994; Campana et al., 2009; Braccinni et al., 2012). Mortality was also shown to increase for little and winter skate as reflexes impairment increased (with an absent gag reflex associated with a higher PRM than an impaired back fly). As mentioned earlier, the relationship between reflexes and survival has not yet been reported for skates in any fishery, however other studies have shown that added impairment to reflexes in elasmobranchs can lead to lower survivorship (Gallagher et al., 2014).

4.2. Factors influencing health indicators and survival

The impact of fishing conditions and practices and individual traits on the health indicators for skate was evaluated across a wide range of conditions (Table 3) that were typical to the standard scallop dredge fishing operations. The relative impact of these factors on injury and reflex impairment is therefore providing a more realistic view of the mechanisms driving differences in PRM.

Tow duration had the most consistent influence on little and winter skate health indicators, however because we were unable to pinpoint the exact time skate entered the dredge and therefore the duration each animal was exposed to the capture event, the actual impact is inherently limited (Neilson et al., 1989). Despite this limitation the overall effect of tow duration was consistent for both species, with extended tows producing higher injury codes (Fig. 5), added reflex impairment (Fig. 6), and increased mortality. This positive relationship has also been reported for little and winter skate in the NW Atlantic otter-trawl fishery (Mandelman et al. 2013), as well as for other species of skate (Cedrola et al., 2005; Enever et al., 2009). The impact of tow duration on observed injuries for little skate also appeared to vary by depth (most pronounced impact in shallowest waters; Fig. 5A). Increases in fishing depth were also associated with less physical damage and reflex impairment, and lower mortality rates in little skate. For fish with physoclistous swim bladders such as Atlantic cod, *Gadus morhua* (e.g. Benoît et al., 2010), rapid changes in depth that occur during capture can lead to barotrauma and potentially mortality, however, skate lack a swim bladder and are therefore not likely to succumb to this scenario (e.g. Milliken et al., 1999; Mandelman and Farrington, 2007; Mandelman et al. 2013). The impact of depth on little skate observed in this study requires further investigation to understand the mechanism, but it is possible that fishing a shallower depth alters the behavior of the dredge in such a way that it intensifies the physical interaction and physiological stress of a captured animal.

Reflex impairment for little skate was also influenced by changes in temperature gradient (between bottom seawater and air temperatures experienced on deck), such that steeper gradients led to additional impairment and consequent mortality. This relationship characterized previously by Cicia et al. (2012) suggests that acute temperature increases from water to air ($\Delta T = + 9^{\circ}\text{C}$) accompanied by bouts of aerial exposure can lead to physiological perturbations, inhibiting reflexes (Davis, 2007; Raby et al., 2012), and

inflating PRM. The temperature gradient observed for little skate in this study range from 0.5 to 12.5°C, corroborating with the findings of Cicia et al. (2012). Mandelman et al. (2013) found a similar trend in temperature change for winter skate in the otter-trawl fishery. Extended periods of time left on deck were also shown to increase reflex impairment in winter skate. This relationship is frequently observed for teleosts and elasmobranchs and is a product of the gill lamellae collapsing during aerial exposure, which inhibits gas exchange (Ferguson and Tufts, 1992) and leads to physiological disturbances that are additive with time, disrupting normal reflex response, and increasing the probability of delayed mortality (Gingerich et al., 2007). Other influential factors that have been reported for skate species in other fisheries include sex (winter skate, Mandelman et al., 2013); catch biomass (skate (Rajidae), Benoît et al., 2010); and length (skate (Rajidae), Depestele et al., 2014).

4.3. Holding tank limitations and considerations

The effective estimation of discard mortality of skate using holding periods in on-deck aquaria (such as this study) is an appropriate and commonly used technique in commercial fisheries, however careful consideration must be involved in study design and interpretation of results. For example, confinement within holding tanks can exacerbate post-capture and handling stress when the experiment is unable to best mimic natural conditions (e.g. temperature, pressure, or stocking densities), which can then lead to artificial mortality (Broadhurst et al., 2006; Portz et al. 2006). To validate the use of our holding tank and technique, Knotek et al. (2015) reported that the tank system was effectively able to mimic bottom temperature regimes and that the flow-through configuration of the system ensured appropriate water conditions throughout holding trials (e.g. dissolved oxygen). Furthermore, while some flow-through systems violate the assumption of independence between replicates because of a single-flow design throughout multiple tanks (van Beek et al., 1990; Kaiser and Spencer, 1995), our system has separate flow-through for each of the six tanks (i.e. providing a replicate tank design). Factors that we cannot account for within this study design include the added risks of predation and disease upon discarding.

4.4. Implications for management and conclusions

Based upon the various factors that influence survival for skate captured in the scallop dredge fishery, limitations on extended tow durations (upwards of 90 min) would likely be the best candidate to consider if put into a best-practice framework for reducing overall skate discard mortality. If additional concern were to be placed on little skate, fishing restricted to times of the year when the water column is well mixed and the temperature gradient is minimal, would likely further decrease mortality for this species. Reducing deck duration by prioritizing both culling of the pile and the immediate return of discards would also increase survival of winter skate. More research (i.e. increased sample size) must be performed on barndoor skate, to better understand the mechanisms driving what appears to be the highest PRM of any of the captured skate species in the NW Atlantic.

5. Communication of Results

5.1. Presentations

Benoît HP, Capizzano CW, Knotek RJ, Dean MJ, Goldman KJ, Mandelman JW, Rudders DB, Sulikowski JA. 2014. A generalized model for longitudinal short and long-term mortality data for commercial fishery discards and recreational fishery catch-and-releases. ICES Workshop on Methods for Estimating Discard Survival. Copenhagen, Denmark.

Knotek RJ, Rudders DB, Mandelman JA, Benoît HP, Sulikowski JA. 2014. The survival of rajids discarded in the New England scallop dredge fishery. In: Annual Meeting of American Fisheries Society; August 17 – August 21; Quebec, QC, Canada.

Knotek RJ, Rudders DB, Mandelman JA, Benoît HP, Sulikowski JA. 2014. The survival of rajids discarded in the New England scallop dredge fishery. In: Joint Meeting of Ichthyologists and Herpetologists; July 30 – August 3; Chattanooga, TN, USA.

Knotek RJ, Rudders DB, Mandelman JA, Benoît HP, Sulikowski JA. 2014. The survival of rajids discarded in the New England scallop dredge fishery. In: Northeast Fish and Wildlife Conference; April 13 - April 15; Portland, ME, USA.

Knotek RJ, Rudders DB, Mandelman JA, Benoît HP, Sulikowski JA. 2014. The survival of rajids discarded in the New England scallop dredge fishery. In: Annual Meeting of American Fisheries Society Southern New England Division; January 29; Hadley, MA, USA.

5.2. Published works

Benoît HP, Capizzano CW, Knotek RJ, Rudders DB, Sulikowski JA, Dean MJ, Hoffman WS, Zemeckis DR, Mandelman JW. 2015. A generalized model for longitudinal short and long-term mortality data for commercial fishery discards and recreational fishery catch-and-releases. *ICES Journal of Marine Science*.

Knotek RJ, Gill SM, Rudders DB, Mandelman JW, Benoît HP, Sulikowski JA. 2015. The development of a refrigerated flow-through seawater system for at-sea operation and estimation of post-release mortality. In press: *Fisheries Research Journal*

Knotek RJ, Rudders DB, Mandelman JA, Benoît HP, Sulikowski JA. The survival of rajids discarded in the New England scallop dredge fishery. In prep.

5.3. Non-published works

Knotek RJ, Rudders DB, Mandelman JW, Sulikowski JA. September 2013. Fisherman, scientists investigate skate mortality in scallop dredges. *Commercial Fisheries News* 41(1): 10 & 38.

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Table 1. Description of stimulus and response for each reflex tested in the field. Reflex impairment were conservatively assessed such that any doubt in response was considered an impaired response.

Reflex	Stimulus	Response
Back fly	Skate lifted by the base of tail	Dorsal contraction of body and wings
Body flex	Skate positioned dorsal side up and restrained with a hand on each pectoral wing	Active escape (e.g. muscle tension)
Gag	Skate positioned ventral side up and 1/8" wooden dowel inserted into back of mouth	Extrusion of mouth forcing out probe
Spinal	Skate positioned ventral side up and the abdominal cavity is massaged	Ventral contraction of wings and tail
Righting	Skate is positioned ventral side up and released into holding tank	Actively righting itself within 10 seconds

Table 2. Description of injury code (based on overt physical trauma) developed by Mandelman et al. (2012) that was used to assess pre-discarded skates.

Injury code	Description
1	Little to no physical trauma (< 10 mm lacerations, no hemorrhaging or internal bleeding)
2	Moderate physical trauma (11-20 mm lacerations, slight to moderate hemorrhaging or internal bleeding)
3	Extensive physical trauma (>20 mm lacerations, extensive hemorrhaging or internal bleeding)

Table 3. Mean [minimum, maximum] values for potential explanatory variables collected during each cruise the subsample of skates that were kept in holding tanks for mortality trials.

Variable	May 20 – May 25	Jun 23 – Jun 31	Jul 23 – Jul 31	Aug 27 – Sept 3
Tow duration (min)	36.5 [5, 85]	39.5 [5, 90]	45.2 [4, 94]	50.2 [5, 105]
Tow depth (m)	58.4 [48.5, 70.4]	69.7 [54.5, 86.5]	75.2 [61.3, 87.8]	42.4 [33.8, 55.8]
Bushels (harvestable scallops)	6.2 [1.5, 12]	3.6 [0, 11.5]	5.2 [0, 18]	4.5 [0.1, 26]
Air duration (min)	13.0 [1.2, 30]	8.8 [1.2, 28.4]	9.7 [0.9, 25.6]	14.3 [1.7, 29]
Bottom seawater temp (°C)	8.29	11.04	11.08	12.99
Surface seawater temp (°C)	10.0 [8.8, 11.3]	14.4 [12.3, 16.5]	20.4 [15.3, 23.6]	21.1 [19.8, 23.1]
Seawater gradient temp (°C)	1.7 [0.5, 3]	3.4 [1.3, 5.5]	9.4 [4.2, 12.5]	8.1 [6.8, 10.1]
Air temp (°C)	15.0 [12.3, 17.2]	18.0 [14.8, 21.3]	22.4 [18.8, 28]	22.2 [20.1, 27.8]

Table 4. Mean [minimum, maximum] values for potential explanatory variables collected in this study for all skates that were releases or kept in holding tanks for mortality trials.

Variable	Release	Hold
Tow duration (min)	48.7 [4.0, 105.0]	44.8 [4.0, 105.0]
Tow depth (m)	64.9 [33.8, 90.2]	63.1 [33.8, 87.8]
Bushels (harvestable scallops)	6.5 [0.0, 26.0]	4.2 [0.0, 26.0]
Air duration (min)	15.0 [0.3, 168.5]	11.7 [0.9, 30.0]
Temperature (°C)		
Bottom seawater	11.0 [8.3, 13.0]	11.3 [8.3, 13.0]
Surface seawater	16.8 [8.7, 23.2]	17.5 [8.8, 23.6]
Seawater gradient	5.7 [0.4, 11.5]	6.20 [0.5, 12.5]
Air	19.4 [11.4, 30.8]	20.1 [12.3, 28.0]

Table 5. Health indicators affecting little and winter skate PRM: parameter estimates and standard errors (SE) for the final Cox proportional-hazards model. Significant p-values (< 0.05) are denoted with an asterisk.

Species	Parameter	Exp (estimate)	SE	p-value
Little	Injury = 2	1.479	0.349	0.2626
	Injury = 3	3.091	0.347	0.0011*
	Back = Present	0.321	0.444	0.0106*
	Gag = Present	0.567	0.237	0.0167*
Winter	Injury = 2	2.247	0.803	0.3131
	Injury = 3	6.854	0.756	0.0108*
	Back = Present	0.144	0.618	0.0017*
	Gag = Present	0.384	0.403	0.0177*

Table 6. Mean proportion and standard error of observed injury and reflex combinations for little, winter, and barndoor skate.

Health indicators			Mean proportion		
Injury	Back	Gag	Little skate	Winter skate	Barndoor skate
1	Present	Present	0.08 (0.01)	0.09 (0.01)	0.01 (0.00)
	Present	Absent	0.01 (0.00)	0.01 (0.00)	0.02 (0.01)
	Absent	Present	0.12 (0.01)	0.08 (0.01)	0.04 (0.01)
	Absent	Absent	0.01 (0.00)	0.01 (0.01)	0.04 (0.02)
2	Present	Present	0.10 (0.01)	0.17 (0.02)	0.04 (0.02)
	Present	Absent	0.02 (0.00)	0.03 (0.01)	0.04 (0.02)
	Absent	Present	0.31 (0.01)	0.26 (0.02)	0.28 (0.04)
	Absent	Absent	0.06 (0.01)	0.06 (0.01)	0.22 (0.03)
3	Present	Present	0.02 (0.00)	0.04 (0.01)	0.01 (0.00)
	Present	Absent	0.01 (0.00)	0.01 (0.01)	0.00 (0.00)
	Absent	Present	0.17 (0.01)	0.15 (0.02)	0.11 (0.03)
	Absent	Absent	0.09 (0.01)	0.09 (0.01)	0.19 (0.04)

Table 7. Estimated short-term discard survival rate (%; up to 72 hours) and standard error for little, winter, and barndoor skate. Little and winter skate estimates are based on the Cox proportional-hazards model with survival as function of injury and back and gag reflexes.

Species	Survival	
	Mean	Std. Error
Little	49.1	0.03
Winter	65.2	0.05

Table 8. Single-covariate Cox proportional-hazards models during the forward selection process (first step) for barndoor skate, and the ΔAICc relative to the intercept-only model.

Parameter	ΔAICc
Tow duration	-1.89
Depth	+1.84
Bushels of scallops	+1.25
Air exposure	+1.95
Temperature gradient	+1.49
Sex	+1.62
TL	+1.41

Table 9. Factors affecting little and winter skate injury code: parameter estimates, standard errors (SE), p-values, and relative risk (RR) for the final multinomial proportional-odds model. Significant p-values (< 0.05) are denoted with an asterisk. ΔAICc denotes the difference in AICc values between the proportional-odds and generalized logits model for the respective covariate.

Species	Parameter	Estimate	SE	p-value	RR	ΔAICc
Little	Tow duration	0.016	0.006	0.0040*	1.92	1.80
	Depth	-0.041	0.010	$<0.0001^*$	2.55	-2.32
Winter	Tow duration	0.038	0.010	$<0.0001^*$	3.88	-2.32

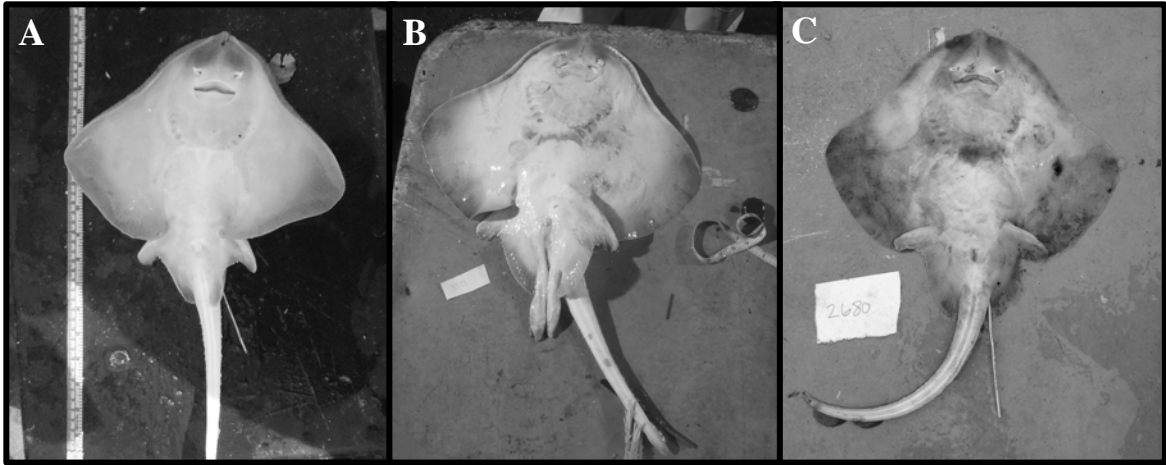


Figure 1. Examples of the increased physical trauma associated with each level of the injury code (A: minor; B: moderate; C: severe).

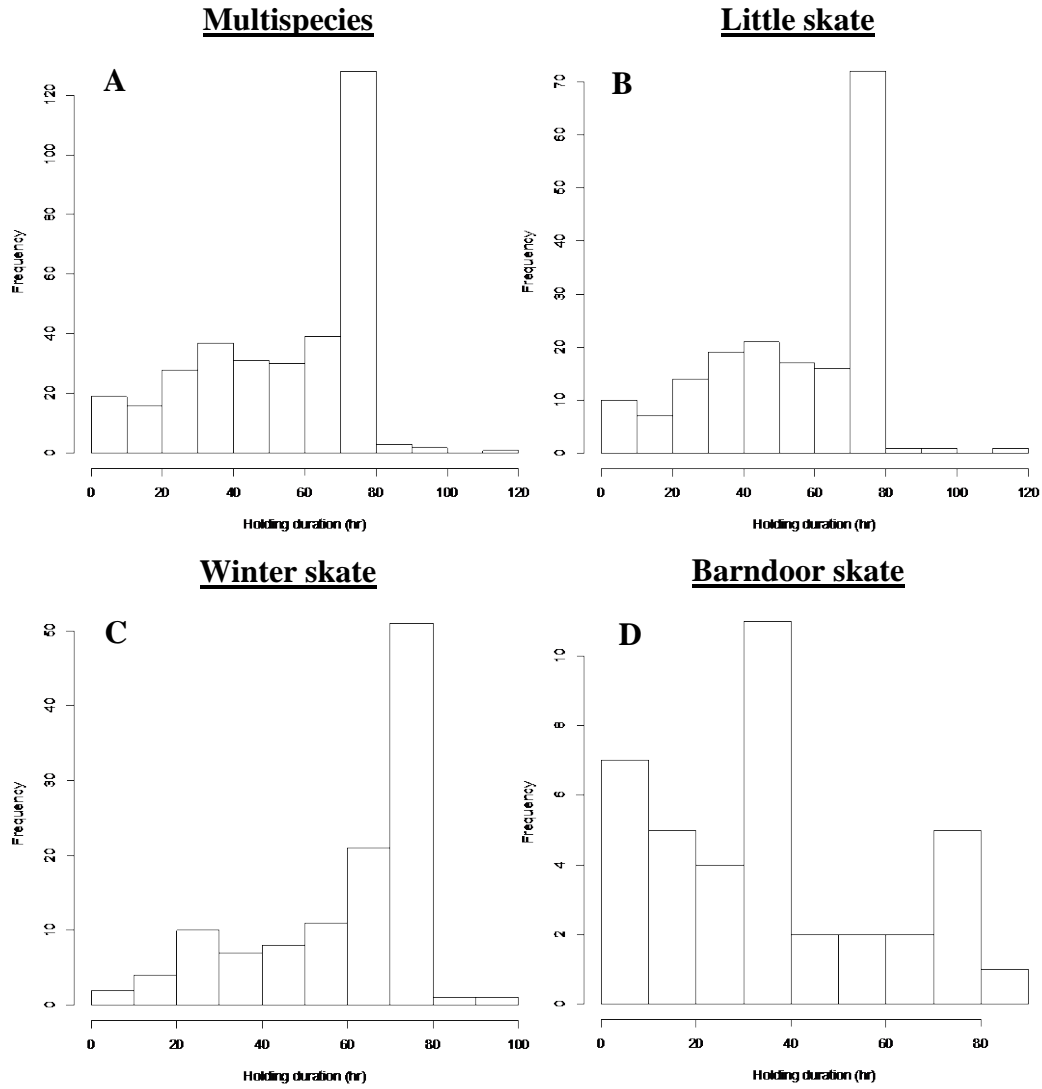


Figure 2. Frequency distribution of the duration of holding period for all species (A), little skate (B), winter skate (C), and barndoor skate (D). Post-holding period, all live skates were released overboard and considered a censored observation during the analysis.

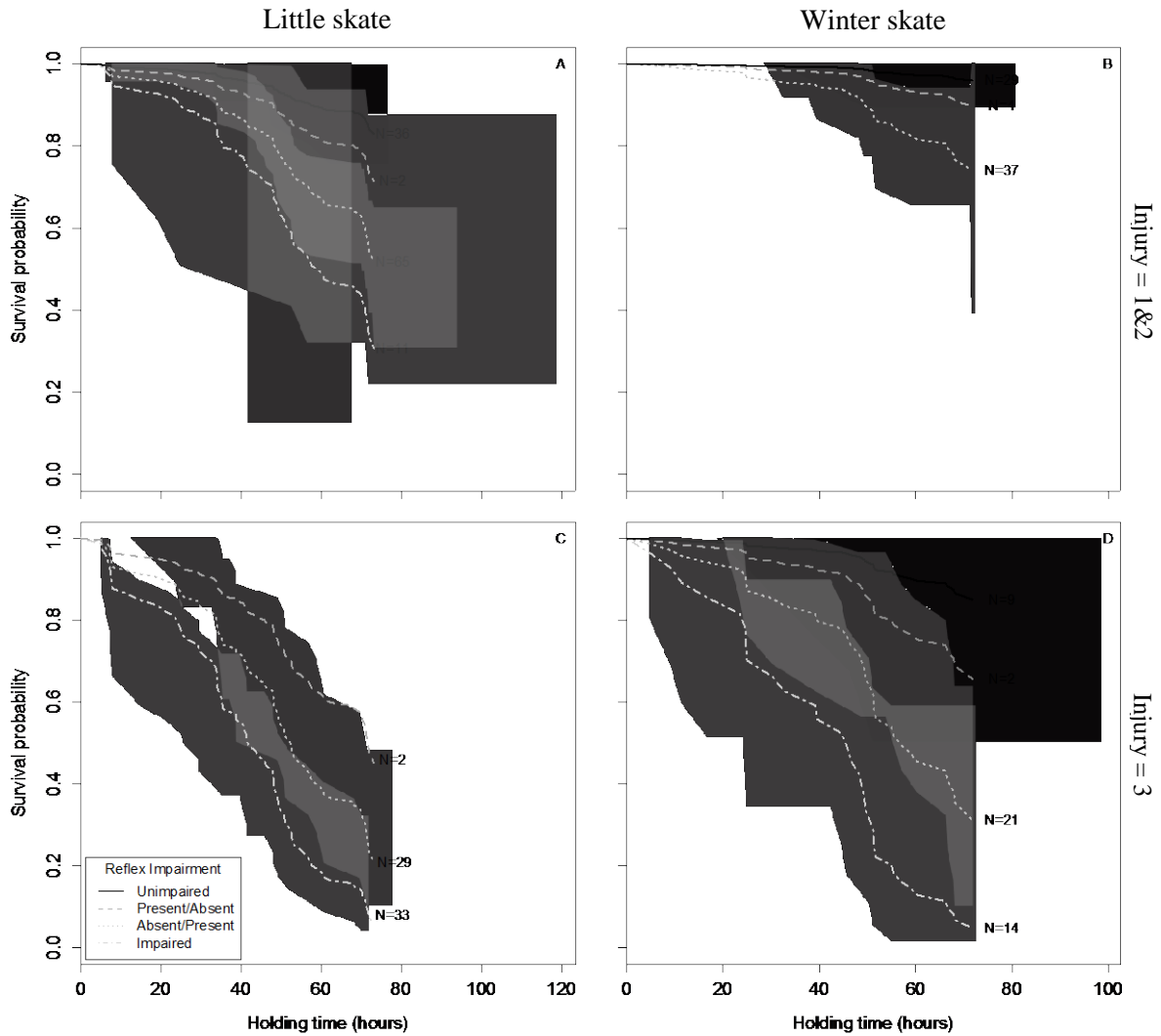


Figure 3. Probability of survival over time for little (left panels) and winter (right panels) skate with respect to initial injury (top panels: codes 1 and 2, bottom panels: code 3) and reflex impairment scores (colors and line types). Shaded areas represent 95% confidence intervals from the empirical Kaplan-Meier survival curve and are plotted up until the last observation for the respective combination of health indicator scores. The lines represent the Cox proportional-hazards model fits. Number of observations for a given combination of health indicators is shown next to the model fit line.

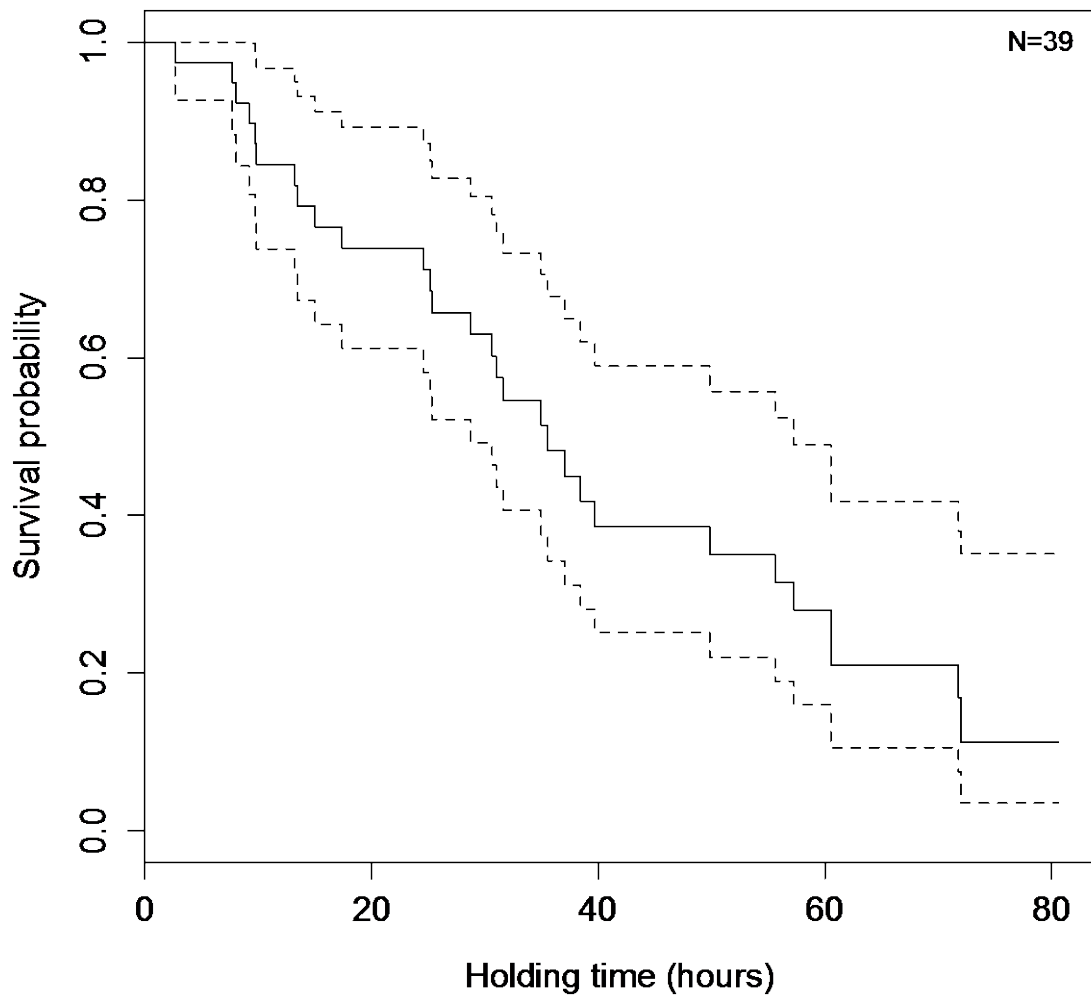


Figure 4. Kaplan-Meier survival estimate for barndoor skate during holding tank trials. Dashed lines indicate the 95% confidence intervals and sample size is given in the top right corner of the figure.

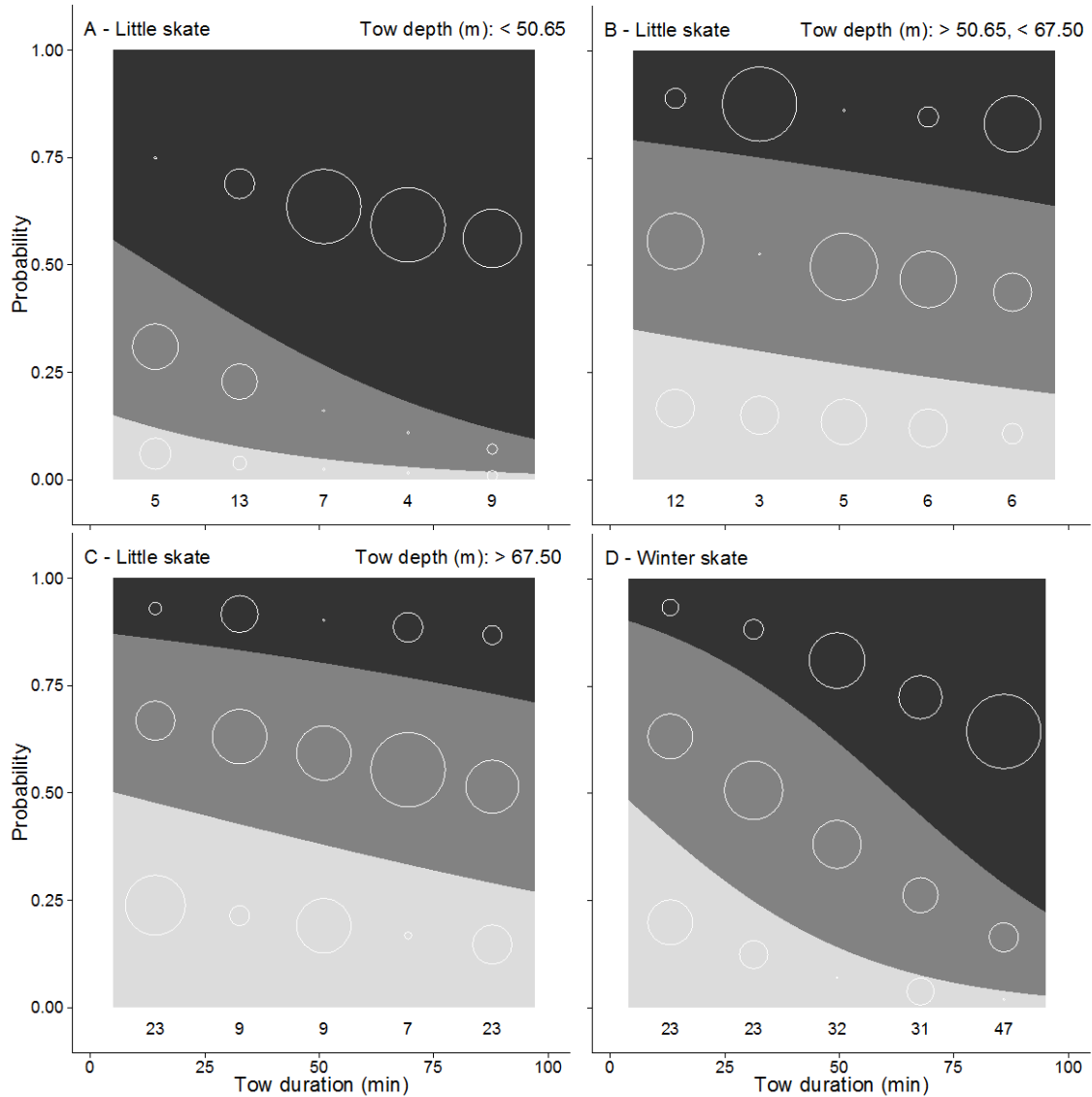


Figure 5. Predicted probabilities of a skate belonging to a certain injury code (shaded area) using proportional odds model with separate slopes for each injury code. Probabilities for little skate are a function of tow duration (continuous), while tow depth was binned into three different levels (equal and based on observed range; A-C), and for winter skate, a function of tow duration (continuous). Injury code shading is as follows: code 1 (light grey), code 2 (dark grey), and code 3 (black). Expanding circles represent the relative proportion of individuals observed within each injury code in given bins of the covariate.

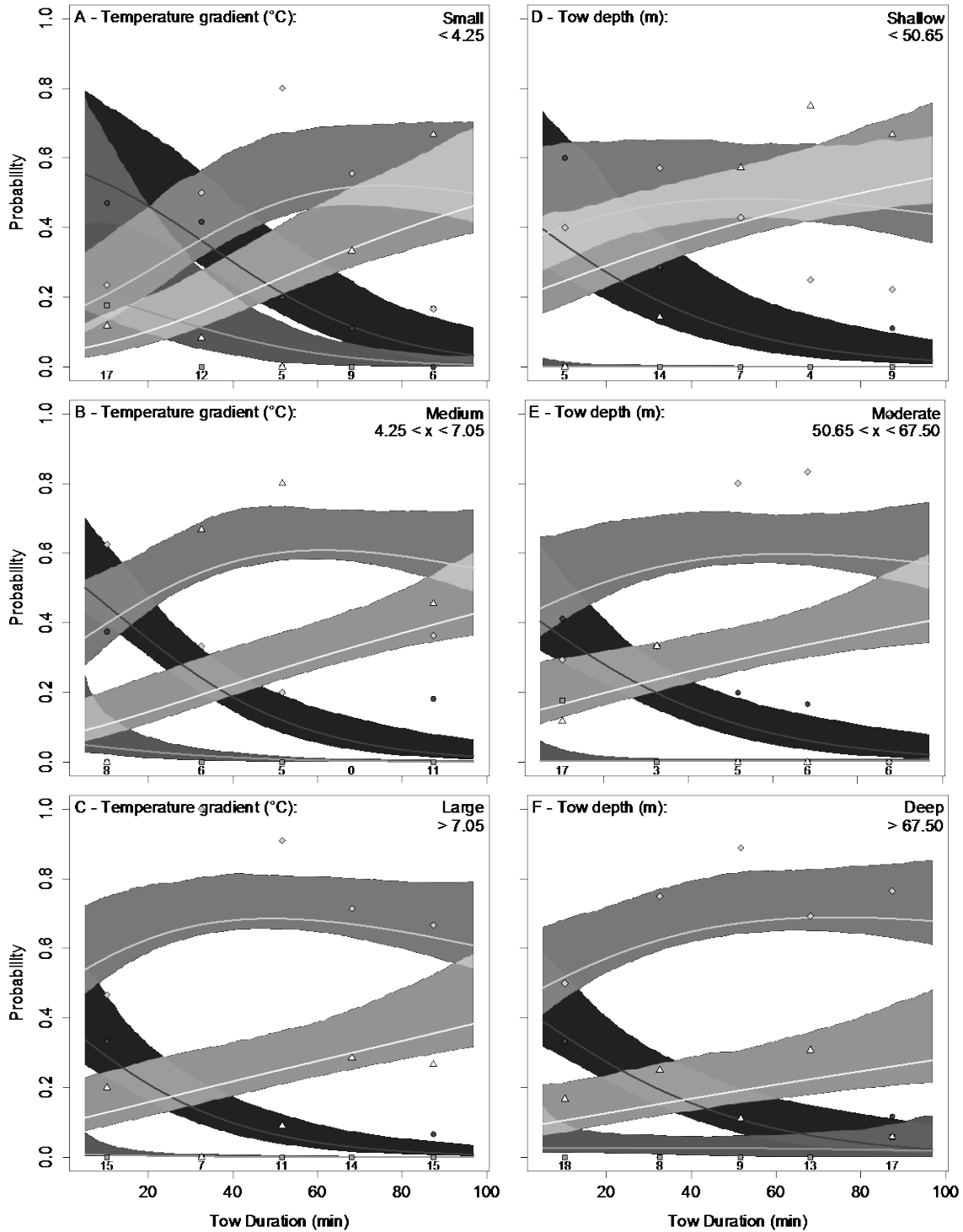


Figure 6. Predicted probabilities of a little skate having a certain combination of reflexes using baseline categories logits model (lines, with shaded areas denoting 95% confidence bands). Probabilities are a function of tow duration (continuous), while temperature gradient was binned into three different levels (equal and based on observed range) and

tow depth held at the mean (A-C), or while temperature gradient was held at the mean and tow depth was similarly binned into three different levels (D-F). Reflex code coloring is as follows: Present-present (dark grey), present-absent (dark-medium grey), absent-present (medium-light grey), and absent-absent (light grey). Symbols represent the relative proportion of individuals observed within each reflex code in the given bins of the covariate. Observed skate sample sizes for each bin are indicated on the x-axis of each plot.

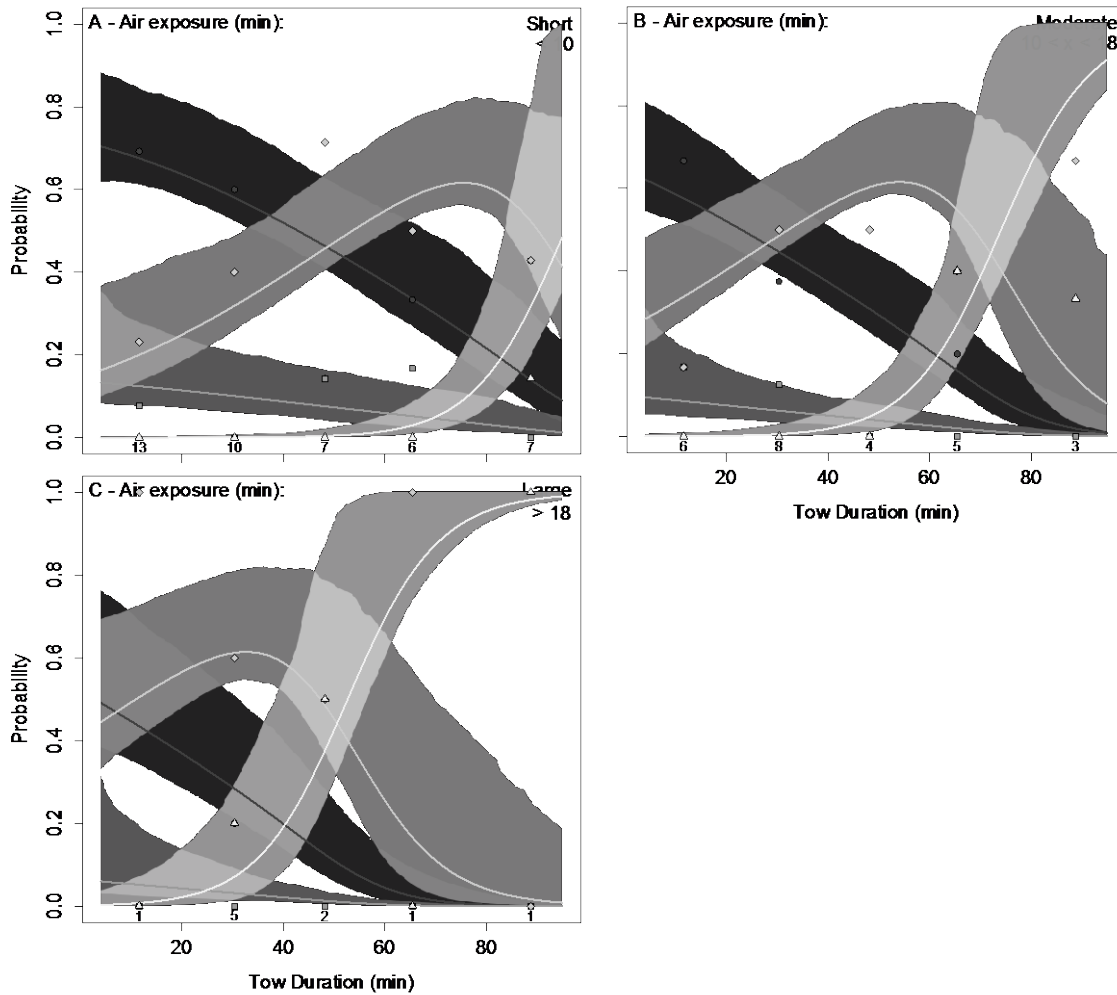


Figure 7. Predicted probabilities of a winter skate having a certain combination of reflexes using baseline categories logits model (lines, with shaded areas denoting 95% confidence bands). Probabilities are a function of tow duration (continuous), while air exposure was binned into three different levels (equal and based on observed range; A-C). Reflex code coloring is as follows: Present-present (dark grey), present-absent (dark-medium grey), absent-present (medium-light grey), and absent-absent (light grey). Symbols represent the relative proportion of individuals observed within each reflex code in the given bins of the covariate. Observed skate sample sizes for each bin are indicated on the x-axis of each plot.