Discard survival of thornback ray caught by flyshoot in the Eastern English Channel (7d)

Final report

Requested by FROM Nord and elaborated by ILVO.

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This report cannot be published or disseminated publicly without the approval of the authors.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

GLOSSARY OF ABBREVIATIONS

Abbreviation	**Meaning**
AIC	Akaike Information Criteria
CI	Coefficient Interval
DO	Dissolved Oxygen
FROM Nord	Fisheries producer organization (PO)
GLMER	Generalized Linear Mixed-Effect Model
GTN	Gill nets
INJ	Injury score
LO	Landing obligation
MLS	Minimum Landing Size
PO	Producer Organization
RJC	*Raja clavata* (thornback ray)
RAMP	Reflex Action Mortality Predictor
SSC	Scottish Seine / Flyshoot
SUMARiS	Sustainable Management of Rays and Skates
WMR	Wageningen Marine Research

SUMMARY

Despite the rapid growth in popularity of flyshoot commercial fishing in the English Channel and the North Sea, discard survival estimates for rays and skates caught with this gear have yet to be published. This research was conducted on request of the producer organization FROM Nord, to specifically examine the discard survival of thornback rays caught by flyshooters in the Eastern English Channel (7.d), to provide survival estimates for fisheries management (i.e. landing obligation exemption) and within the framework of the FIP (Fishery Improvement Project) on thornback rays in 7d. In the period from June to September 2022, from four commercial sea trips fishing with flyshoot, 460 thornback rays of the catch were measured, sexed, and scored for injuries, reflex impairments (RAMP), and vitality. Only two rays (0.43%) were scored as being in an excellent condition (vitality class "A"), while the majority were found to be in "poor" conditions (vitality class "C", 70.43%) or "good" conditions (vitality class "B", 24.57%). Finally, 21 (4.57%) thornback rays were found dead when landed onboard, resulting in an immediate survival of 95.43%. This value falls close to the estimates for active and passive gears (93.56-100%) reported during the SUMARiS project (van Bogaert et al. 2020).

In addition, from these 460 rays, 80 were sampled (representing each vitality class) and selected to be monitored onboard until the end of the fishing trip, after which the rays were brought to captive holding facilities (Nausicaá) for delayed survival monitoring (n=67) for 21 days. A total of 24 rays died during the monitoring period, of which 13 died onboard of the vessel before reaching the aquaria, whilst 11 died throughout the 21 days of monitoring. Due to the high onboard mortality, estimations of delayed and total survival were performed following two methodologies: (1) including all 24 mortality events or (2) censoring the mortality events occurring at sea. Considering this, delayed survival was found to vary significantly between trips, ranging from 47.33 to 87.49% when considering all mortality events, and between 77.78 and 100% when censoring onboard mortalities. The third and fourth trips were found to register comparatively lower survival. This variation between trips was likely to be influenced to some degree by the temperature differences to which rays were exposed when transported from their natural habitat to the aquaria. The vitality class was found to significantly affect the rays' probability to survive, with rays in worse conditions (i.e. vitality class "C") being significantly more likely to face delayed mortality. Considering these factors, the delayed survival was estimated using a generalized linear mixed-effect model. Including all 24 mortality events, the delayed survival was estimated at **73.06% (CI: 56.5-85%)**, which situates flyshoot discard survival for thornbacks below the estimates reported for trammel netters (93.5%), above beam trawl (56.9%) and very close to otter trawls (76.5%) (van Bogaert et al. 2020). However, when censoring the 13 onboard mortalities, delayed survival for flyshoot finds itself closer to trammel netters than other active gears, with an estimated **91.57% (CI: 69.1-98.14)** delayed survival. Considering the calculated immediate and delayed survival presented above, the total survival is estimated at **69.73% (CI: 53.92-81.12%)**. or **87.40% (CI: 65.95-93.66)** when including all mortalities or censoring onboard mortalities respectively.

Finally technical (i.e., exposure time, landed weight), environmental (i.e., water depth, water temperature, sea state, and substrate type), and individual variables (i.e., length, sex, and fish condition) were analysed for significance as predictors of the immediate and delayed survival status. The probability of immediate survival was found to be significantly affected by the length and injury score of each fish, as well as by the total landed weight and sea state of the haul in which they were caught. Whilst, the probability of delayed survival was only found to be significantly affected by the injury score. These findings provide evidence on how in-situ monitored variables (i.e., injury score) could be used as a proxy for the delayed survival of thornback rays caught by flyshooters.

1 INTRODUCTION

In the last two decades, several discard survival estimates for rays and skates have been calculated for specific metiers and species. Overall, these studies have reported comparatively high immediate/at-vessel survival, independently of the species and gear analysed (STECF, 2022). This initial, at the vessel, survival is however not always proportional to the long-term (delayed) survival of fish monitored for a longer period (e.g., 21 days) (e.g., van Bogaert et al. 2020). Delayed survival has been shown to vary significantly between gears (van Bogaert et al. 2020; STECF, 2022) and therefore requires gear-specific studies to accurately estimate survival rates. In 2022, the Scientific Technical and Economic Committee for Fisheries (STECF) summarized all available discard survival estimates published for rays and skates fishing in the Northeast Atlantic, while also highlighting the lack of data for specific gears, species, and areas (STECF, 2022). For instance, despite being a commonly studied species (in terms of discard survival) with commercial importance, thornback ray's discard survival rates for specific gears and areas of its distribution are still unknown/unpublished (i.e. flyshoot in the English Channel and North Sea). Although, providing estimates for every possible species and gear combination might not be the most efficient path for survival research, having a better understanding of the discard mortality/survival across a wide range of different gears and the factors influencing these rates remain relevant for fisheries management (i.e., landings obligation exemption and stock assessments).

Flyshoot is a relatively new fishing technique used mainly in the North Sea and the English Channel., which after experiencing rapid growth is now stabilizing. This gear is used by a mixed demersal fishery fleet targeting mainly squid (*Loligo vulgaris*), striped red mullet (*Mullus surmuletus*), dab (*Limanda limanda*), and gurnards (*Chelidonichtys* sp.), but can result in the bycatch of undersized commercial species, such as rays and skates (van Opstal & Soetaert, 2023; van Overzee et al., 2019). Previously described as a combination of anchored seining and demersal trawls, flyshoot, in comparison to other demersal trawls (i.e. beam trawl), leads to shorter (~40mins) hauls and a reduced exposition to mechanical disturbance. During typical hauls, fish are herded by the dragged cables with no direct physical contact and are only exposed to mechanical injuries at the end of the fishing operation (i.e. last 10 minutes), when they enter the net, are trawled and lifted onboard (van Overzee et al., 2019). This relatively new gear and its operational differences with gears for which discard survival has already been published, makes our capacity to extrapolate survival rate from previous estimates quite limited. Although it might be logical to assume that discard survival in flyshoot would be higher compared to typical demersal trawls, so far no estimates have been published to confirm this hypothesis. Only a precautionary proxy (i.e., based on estimates for plaice and assuming high survival due to short air exposure) for starry rays has been proposed, setting a delayed survival rate at 80% (van Opstal & Soetaert, 2023; van Overzee et al., 2019).

Considering the relevance of the gear in the English Channel and North Sea, there is a growing need to fill in these relevant data gaps. To fill in the existing knowledge gap about the survival of rays caught with flyshoot, this study analysed the survival of discarded thornback rays caught by French flyshooters in the Eastern English Channel (7.d). Despite not being the only ray species caught by flyshooters, thornback rays are one of the best-studied species and are comparatively one of the most abundantly caught species, hence representing an interesting model species for discard survival estimates. This allows for comparability with other studies targeting the same species (e.g. Schram and Molenaar 2018, Ellis et al. 2018, van Bogaert et al. 2020) and could be used to extrapolate flyshoot survival estimates to other species in the future. This study aimed (1) to analyse the conditions and immediate survival of thornback rays caught by flyshooters in the Eastern English Channel, (2) to analyse their delayed survival onboard and in captive holding facilities (Nausicaá) during 21 days, and finally (3) to analyse the factors (i.e. individual, technical and environmental) affecting the probability of thornback rays to be found dead/alive when landed on deck (immediate survival) and after being discarded (delayed survival).

2 MATERIALS AND METHODS

2.1 GENERAL INFORMATION

This project aimed to increase the available knowledge on the survival of discarded rays, looking specifically at thornback rays (RJC) caught with a flyshooting vessel (SSC, Scottish Seine, cod-end 80-110 mm) in the Eastern English Channel (27.7d). Data was collected through four flyshoot fishing trips which took place in the Eastern English Channel (7.d), between June and September 2022. These trips accounted for a total of 161 fished hauls (Figure 1), with average fishing times of 37.5 minutes (range: 33-50 min) and average landings of 314.8 kg (range: 20-1860kg) of fish per haul.

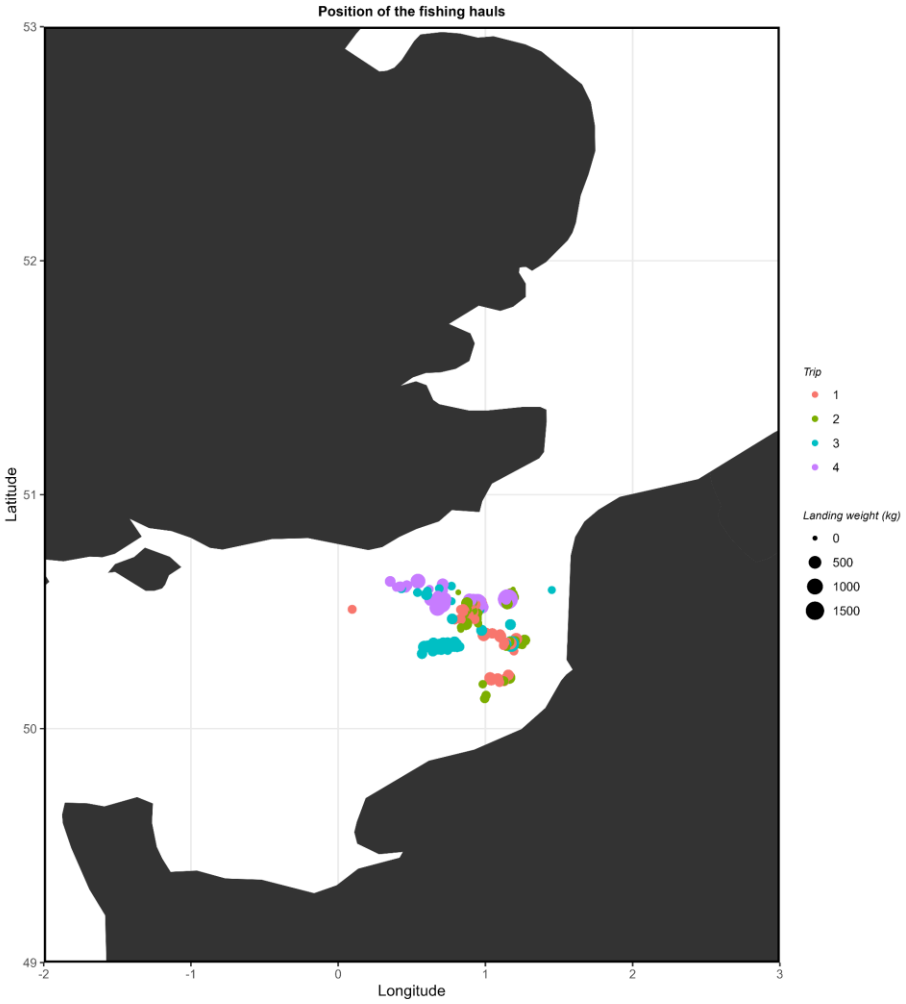

Figure 1: Position of the flyshoot fishing hauls in the Eastern English Channel during the four trips carried out between June and September 2022. The size of the dot is relative to the total landed weight for each haul.

During each trip, the observer sampled as many hauls as logistically feasible to describe the overall catch composition, and presence of rays and to analyse their discard survival (Figure 2). In addition, technical (haul duration and landed weight) and environmental (water temperature (°C), sea state[1] , depth (meters), and substrate type (soft (sand), medium (both sand and stones) and hard (stones)) parameters were

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[1] Sea state was estimated using 10 point a categorical scale: 0 = calm (0 Beaufort), 1: = calm (~ 1 Beaufort), 2= smooth (~2 Beaufort), 3= slight (~3-4 Beaufort), 4= moderate (~5-6 Beaufort), 5= rough (~7 Beaufort), 6= very rough, 7= high, 8= very high, 9= phenomenal

monitored for all hauls. From the 161 hauls carried out during the four trips, 16 were sampled in detail to collect catch data (i.e., number of thornback rays and number at length) and 50 hauls (31%) to specifically analyse the vitality and delayed survival of the caught thornback rays, in addition to the general catch data detailed in the 16 catch hauls. Data on injuries, reflexes, vitality, and immediate survival was collected onboard following the SUMARiS protocol (van Bogaert et al., 2020). Finally, a stratified sample of rays was taken from the survival hauls, for monitoring delayed survival onboard of the vessel and 21 days in captive holding facilities (i.e., aquaria). This stratified sampling consisted in selecting a number of rays for delayed survival monitoring from each vitality class to obtain a sub-sample with a similar to equal vitality class proportion. However, this was not always possible, therefore a correction for a mismatching proportion of vitality classes is included in the analysis and calculation of delayed survival.

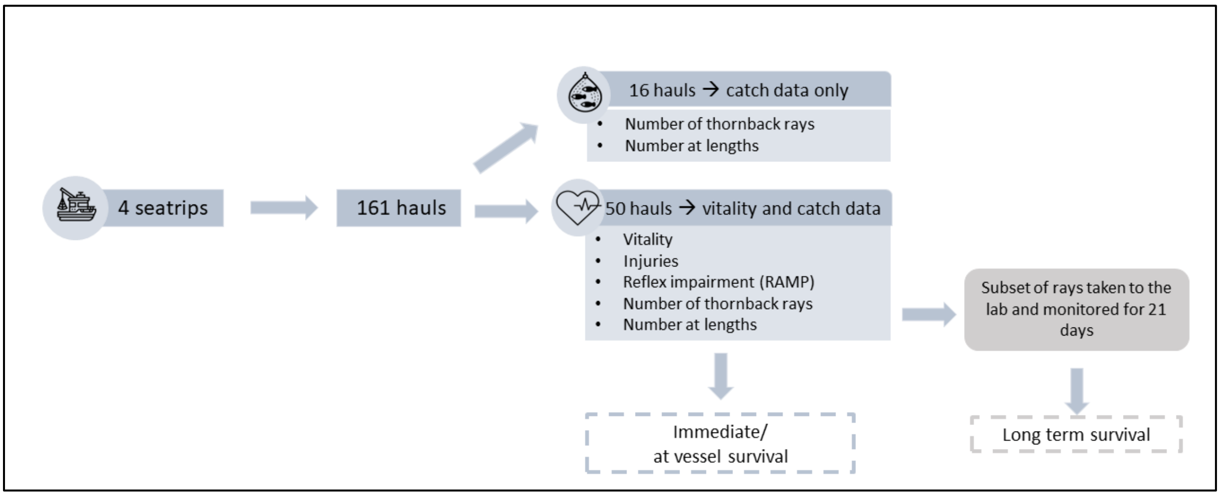

Figure 2: Overview of the trips, hauls, and variables monitored in the discard survival study

2.2 CATCH HAULS

Of the 161 hauls, 16 hauls were used to analyse and describe the overall thornback ray catches (total number of thornback rays and number at length) in flyshoot fishing activities. These hauls provide valuable information but are unlikely to be analysed independently from the survival hauls, as the latter also provide similar information. The results on the number of rays and length distribution of thornback rays caught during flyshoot fishing activities in the Eastern English Channel presented below are therefore based on both catch and survival hauls.

2.3 SURVIVAL HAULS

Of the same 161 hauls, 50 hauls focused on data collection on survival and were used to compile data on individual fish (sex, length), vitality (injuries, reflex impairment, and vitality score), immediate mortality, and delayed mortality. Sorting began as soon as the catch was retrieved from the water and landed on deck. The observer logged the time and started to sample rays for reflex impairment, injury, and vitality score following the protocol described below. The time exposed to air for each ray was calculated by taking the difference between the time at which the haul was landed on board and the time at which the ray was scored for vitality and would have been discarded (i.e. in real fishing conditions). All hauls contained a relatively small number of rays (8 or less) making it possible to score all caught rays. As detailed above, from these, a stratified sample (~10% of each vitality class when possible) was sampled for delayed survival monitoring.

2.3.1 PROTOCOL

The SUMARiS protocol (van Bogaert et al., 2020) for scoring reflex impairment, injury, and vitality score was followed to allow comparability between studies and survival estimates. Details on the methodology for each score are presented below.

2.3.1.1 VITALITY AND IMMEDIATE SURVIVAL

A vitality score was assigned to the rays according to a 4-point categorical scale (Table 1) (Benoit, Hurlbut, & Chasse, 2010). Rays scored as vitality class “D” and unresponsive to any of the reflex tests when landed on deck were reported as dead and therefore considered in the calculation of the immediate/at-vessel mortality. The immediate survival rate is then simply the proportion of fish alive from the total of fish scored.

Table 1: Vitality of rays categories and description.

Score	State	Description
A	Excellent	Vigorous body movement; no or minor[a] external injuries only
B	Good	Weak body movement; responds to touching/prodding; minor[a] external injuries
C	Poor	No body movement but can move spiracle opening; minor[a] or major[b] external injuries
D	Dead	No movement of body or spiracle opening (no response to touching or prodding)

a: Minor injuries were defined as ‘minor bleeding, or minor tear of mouthparts or wing (≤10% of the diameter), or minor surface abrasion. b: Major injuries were defined as ‘major bleeding, or major tear of mouthparts or wing, or major surface abrasion.

2.3.1.2 REFLEX IMPAIRMENT AND INJURY SCORES

The reflex impairment was assessed using the reflex action mortality predictor (RAMP) method (Davis, 2005, 2010; Davis and Ottmar, 2006). The RAMP score is calculated as the mean of all individual reflexes (tailgrab, spiracles, startle touch, bodyflex; 0 = absent, 1 = present, Table 2).

Table 2: Description of the four reflexes score to obtain the RAMP score. *The tailgrab reflex was tested in seawater, the other reflexes were not.

Reflex	Description	Unimpaired response
Tailgrab*	Gently grab the ray by the tip of the tail between thumb and index finger (watch out for any spines)	Actively struggles free and swims away
Spiracles	Look at the opening and closing of the valves inside the spiracles	The spiracles actively open and close
Startle touch	Tap gently but firmly behind the eyes and spiracles using a fingertip	Actively closes and retracts its eyes
Bodyflex	Hold the ray by the anterior end of its disc in a horizontal, plane position, one hand on either side of the mid-line (dorsal side facing up); larger specimens may be supported also by their posterior end	Actively moving its pectoral fins, tail, and body

Similarly, the injury score is the mean of the scores given to each of the five injury types assessed (Table 3). For each injury type, a score from 0 to 3 was given based on the surface coverage of the injury. For bleeding injuries, absence of discoloration was scored as "0", less than 10% discoloration as "1", between 10% and 50% as "2" and more than 50% as "3". Fin damage (i.e., splits) was assessed for the outer edge of the body wings. Open wounds were scored separately for head, body, and tail along the same categorical scale while looking at both the ventral and dorsal sides of the body.

Table 3 Description of the five types of injuries selected for scoring.

Injury type	Description
Bleeding head	Point bleeding and/or bruising of the head
Bleeding body	Point bleeding and/or bruising of the body
Bleeding tail	Point bleeding and/or bruising of the tail
Open wounds	Areas where the skin was removed and underlying tissue can be observed
Fin damage	Areas of the fin that were damaged and/or split

In addition to the two individual indices, an integrative RAMPINJ (RAMP+INJ) score was calculated by taking the mean of all reflex and injury scores reported for one individual fish.

2.3.1.3 DELAYED SURVIVAL

To assess the delayed survival of the discarded thornback rays, a stratified subset of scored and alive individuals was sampled from each vitality class (~10% per vitality class) for onboard delayed survival in monitoring boxes (~134L per individual box). These rays were tagged and linked to the specific haul in which they were caught. The stratified sampling strategy ensures the long-term monitoring of each vitality class, as survival is likely to differ between them. Fish were kept and checked for survival (i.e. three times per day) in monitoring boxes onboard until the end of the fishing trip (almost 4 days on average). The water temperature, dissolved oxygen, and salinity were monitored in each monitoring box over this period. Fish that were found dead before reaching the port was replaced to maximize the monitoring efficiency (i.e., all monitoring boxes filled with fish) throughout each trip. At the end of the fishing trip all rays were transported by road to the captive holding facilities at the Nausicaá aquarium (Boulogne-Sur-Mer, France) for their delayed survival monitoring for 21 days.

At Nausicaá, all rays were monitored twice a day for their survival. A mix of defrosted salmon, herring, smelt, sardines, squid, shrimps, and mackerel was daily fed to the rays (*ad libitum,* 5% of their body mass). When dead individuals were observed, they were removed immediately. Post-mortem necropsies were performed to identify the possible causes of death. In all water-filled units where rays were kept, water quality parameters (dissolved oxygen, salinity, temperature) were measured at regular intervals. The aquaria were kept constant at 10°C throughout the study (four trips and monitoring periods), irrespective of the temperature at sea. This led to differences in the temperature deltas, between at-sea and aquaria temperatures for each trip.

2.3.1.3.1 CONTROLS

To determine the level of experimentally induced mortality, 12 control (3 per trip) thornback rays were monitored in captivity alongside the rays that were caught and discarded (delayed survival monitoring- 21 days). Ten of these were caught with gillnets (GTN) during an experimental trip, looked vital (strong reflexes responses, none or minor injuries), and were kept at Nausicaá previous to the start of this study. Only one of these rays died throughout the monitoring period, at the end of the fourth trip during transport between the boat and captive holding facilities.

However, after the third trip and monitoring period, only one ray caught with gillnets was available to be used as a control in trip four. For this reason, the remaining two controls were two rays collected in trip three, which survived the monitoring period of the third trip and were subsequently used as controls in trip four. Coincidently, these two rays (ID= 2544 and 2570) were found to die on day nine of the monitoring period. Reviewal of the physical state of the fish on arrival at the monitoring facilities (Figure 3: Picture of control (ID: 2544) for the 4th trip on arrival at the monitoring facilities (Nausicaa), showing clear ventral injuries. and Figure 4: Picture of control (ID: 2570) for the 4th trip on arrival at the monitoring facilities (Nausicaá), showing sign of haemorrhages on the ventral side of the wings.) showed that severe signs of injuries/hemorrhages were present before the monitoring commenced. It was therefore decided to exclude the two controls from the analysis as the injuries would make it unlikely to provide insights on the experimentally induced mortality. Considering this, only 1 of the 10 effective controls died throughout the experimental study resulting in a 10% experimentally induced mortality, which was considered acceptable for this study.

Figure 3: Picture of control (ID: 2544) for the 4th trip on arrival at the monitoring facilities (Nausicaa), showing clear ventral injuries.

Figure 4: Picture of control (ID: 2570) for the 4th trip on arrival at the monitoring facilities (Nausicaá), showing sign of haemorrhages on the ventral side of the wings.

2.4 ETHICAL STATEMENT

During this study, experimental work was in accordance with the scientific permits of Nausicaá. The relevant maritime authorities of France issued further permits to keep undersized alive fish onboard and bring a subset onshore. The treatment of the fish was following the French animal experimentation act.

2.5 DATA ANALYSIS

Before any statistical analysis, an exploratory analysis was performed to identify outliers, visualize variation, and identify correlation among explanatory variables.

2.5.1 IMMEDIATE, DELAYED, AND TOTAL SURVIVAL

The immediate survival was calculated as the proportion of living (vitality class “A”, “B” and “C”) fish from the total of fish scored for vitality onboard of the fishing vessel. Calculations for delayed survival are more complex and are represented by the estimated marginal means of a mixed-effect logistic regression model. This model includes a random effect on the trip code to account for inevitable differences between different trips of the project. The delayed survival estimates also include a correction to account for differences in the vitality class proportions between all fish scored onboard of the vessel and the delayed survival subsample which was taken for monitoring at the lab. For instance, a delayed survival subsample with a proportionally larger representation of vitality class C (compared to the proportion in the catch) would result in an underestimated delayed survival estimate, as these are more likely to die than classes A and B.

Throughout the study a total of 24 mortality events occurred, however, 13 of these occurred while fish were still onboard of the vessel in the monitoring boxes and were replaced by another living fish. Due to this, the result of the delayed survival estimations are presented following two approaches: (1) including all 24 mortality events and (2) censoring the mortality events occurring at sea and keeping only the 11 mortalities during the monitoring period at Nausicaá.

Nonparametric Kaplan-Meier (Kaplan and Meier, 2012) curves were produced to visualize the overall, trip-specific, and vitality class-specific delayed survival across the monitoring period.

Finally, the total survival was calculated as the complement to one of the sum of immediate and delayed mortality of the remaining proportion of fish alive. The total survival is therefore equal to:

$$Total\ survival\ = 1 - (Immediate\ mortality\ + (1 - \ Immediate\ mortality) * \ Delayed\ Mortality)$$

2.5.2 FACTOR ANALYSIS

Mixed-effect logistic regression models were fitted to the immediate and delayed survival status (0 =alive, 1= dead) for different technical (i.e., air exposure time, landed weight), environmental (i.e., water depth, water temperature, sea state, and substrate type) and individual variables (i.e., length and sex), with the inclusion of a random effect on trip. These models were used to explore which of the variables lead to significant differences in the probability of immediate and delayed survival of the sampled and monitored thornback rays. For this analysis, all 24 mortality events (onboard and at the monitoring facilities) are considered.

Similarly, the effect of the time on board (in the monitoring box), and water conditions of the monitoring boxes onboard of the vessel were explored to determine, if these were significant in the occurrence of onboard delayed survival. This was done to explore whether there are any indications of mortality induced by the conditions in which the fish were kept on board.

Finally, a forward model selection using the Akaike Information Criterion (AIC) and significance levels of individual variables (p-value) was followed to identify the “best” model to predict the probability of immediate and delayed survival. This was done by forward model selection, as new variables would be included step by step into the model.

All plotting, exploratory and statistical analysis were performed in R.-4.2.1 (R Core Team, 2022)

3 RESULTS

3.1 DATA COLLECTION

The sampling of the 161 hauls resulted in length and vitality data for 460 individual thornback rays (124, 100, 175, and 61 rays in trips 1, 2, 3, and 4 respectively), more specifically 238 females and 222 males were sampled. From these, a total of 80 rays were sampled and monitored for onboard delayed survival. However, over the four trips, 13 individuals died onboard before being landed and transported to the monitoring facilities at Nausicaá. Therefore 67 individuals effectively arrived at the monitoring facilities and were monitored for long-term survival for 21 days.

3.2 LENGTH DISTRIBUTIONS

The size distribution of the thornback rays caught during the four flyshoot trips was found to be fairly constant between the different fishing trips, with lengths ranging between 25-97 cm (mean: 53.1cm, median; 50cm). However, most thornback rays appear to have lengths ranging from 30 to 50 cm (Figure 5: Overview of lengths measured as measured at sea, for the 460 thornback rays measured over the project.).

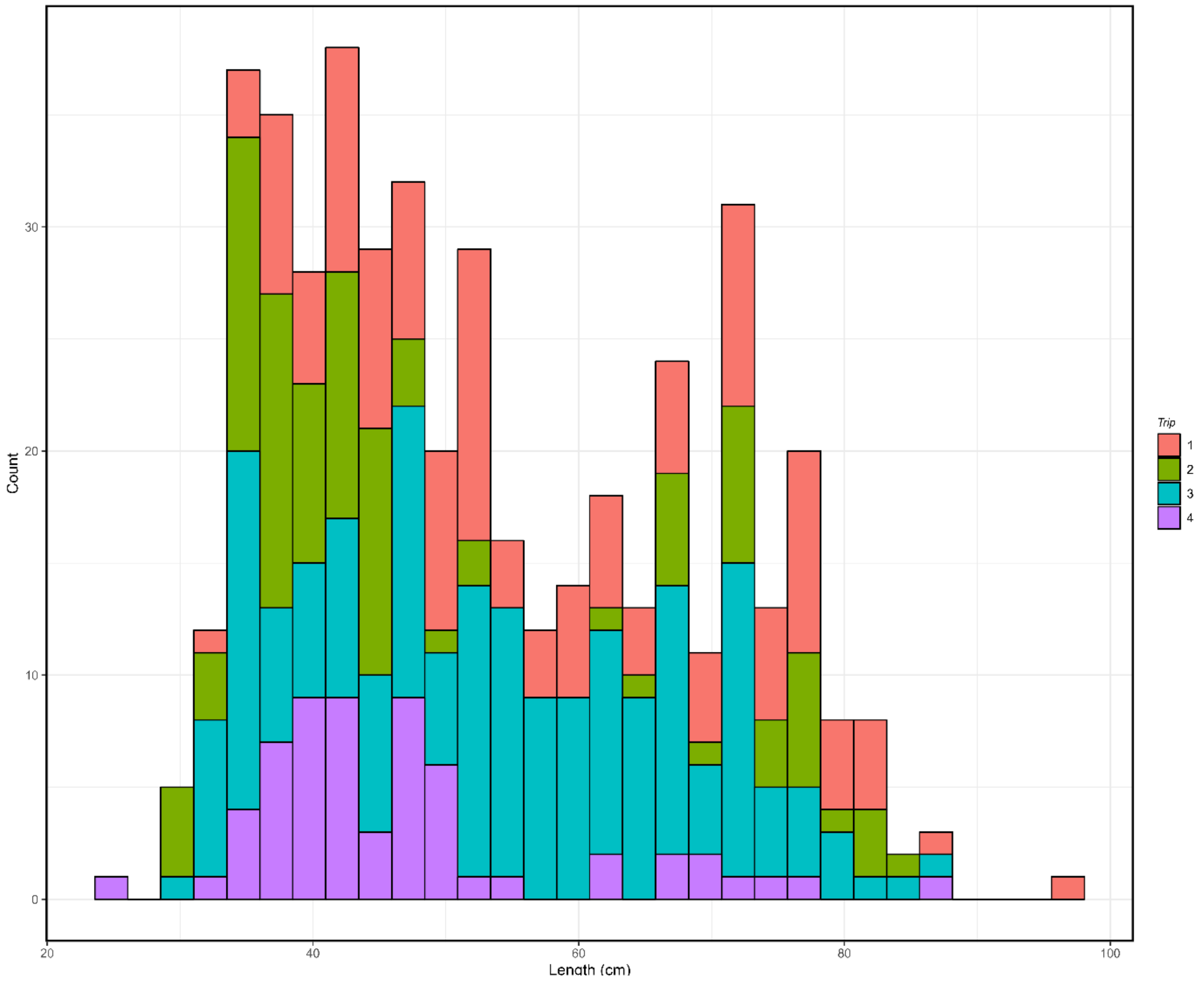

Figure 5: Overview of lengths measured as measured at sea, for the 460 thornback rays measured over the project.

The thornback rays monitored at the holding facilities for delayed survival follow a slightly smaller, but very similar length distribution. These rays ranged from 31.5 to 74 cm with a mean and median of 47.08 and 44 cm respectively (Figure 6: Overview of lengths, measured on the thornback rays that were brought to the captive holding facilities for survival monitoring.). Again, the size distribution is consistent between different trips.

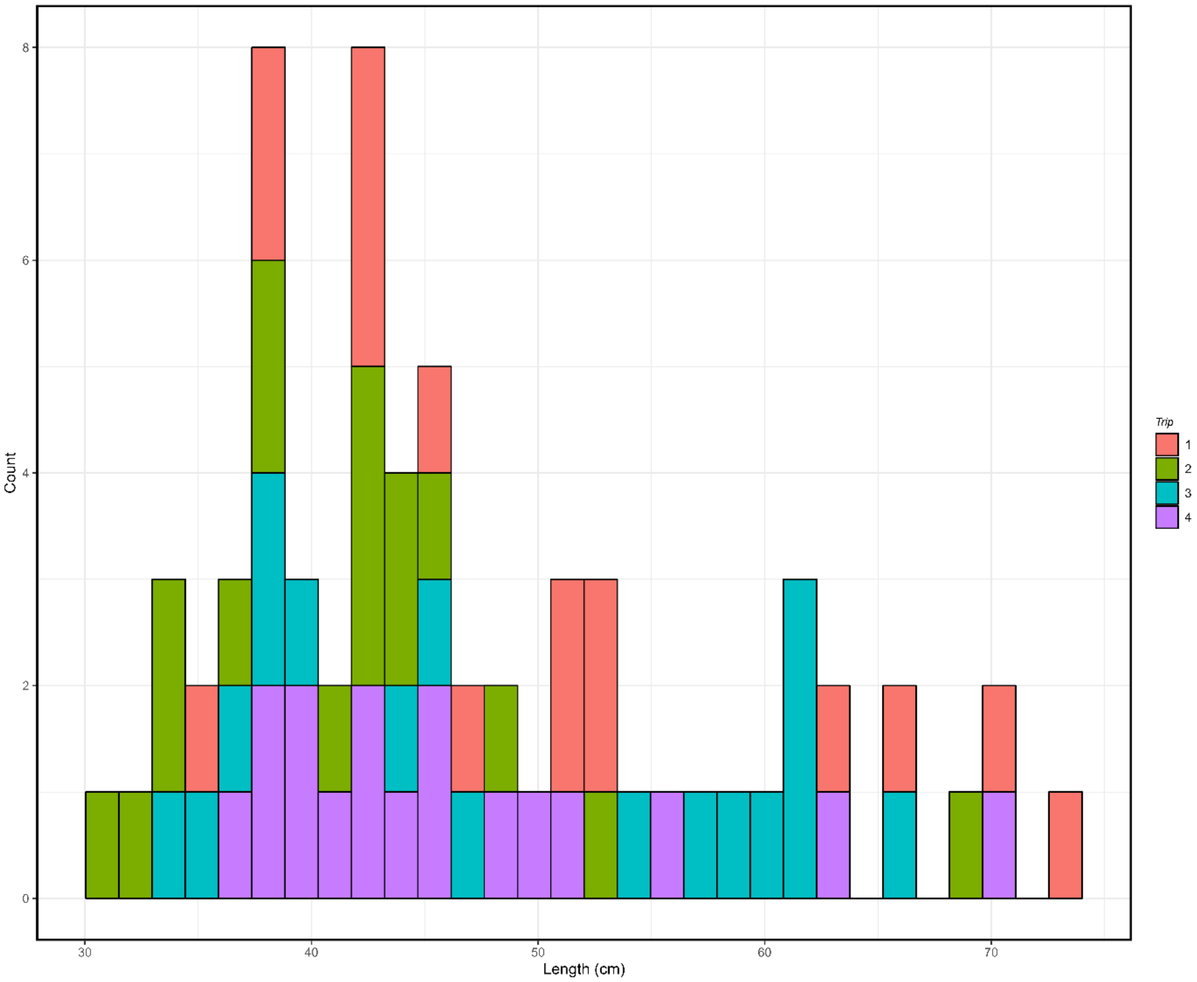

Figure 6: Overview of lengths, measured on the thornback rays that were brought to the captive holding facilities for survival monitoring.

3.3 VITALITY CLASSES AND IMMEDIATE SURVIVAL

Throughout the four fishing trips, most thornback rays were found to be in “poor” (vitality class “C”) conditions when landed on deck. These accounted for a total of 324 rays or 70.43% of all rays caught, followed by vitality class “B” with 113 rays, or 24.57% (Figure 7: Overview of vitality score proportion of all scored thornback rays caught during the four trips.). Only two rays (0.43%) were scored with vitality class “A”, representing fish in “excellent” conditions. Lastly, **4.57%** (21 fish) were found to be dead (vitality class “D”) when landed onboard and therefore represent the calculated immediate mortality for thornback rays caught with flyshoot in the Eastern English Channel.

The proportion of rays in each vitality class was found to vary between trips. For instance, the first two trips were found to have a higher proportion of rays with a vitality score “B” in comparison to the two last trips, where catches were almost completely dominated by vitality class “C”. These differences are later considered in the delayed survival estimation, as it is logical to think that rays with different vitality scores are not equally likely to die. On another hand, the proportion of fish found dead (vitality class “D”) is fairly consistent between trips (3.43 to 7%).

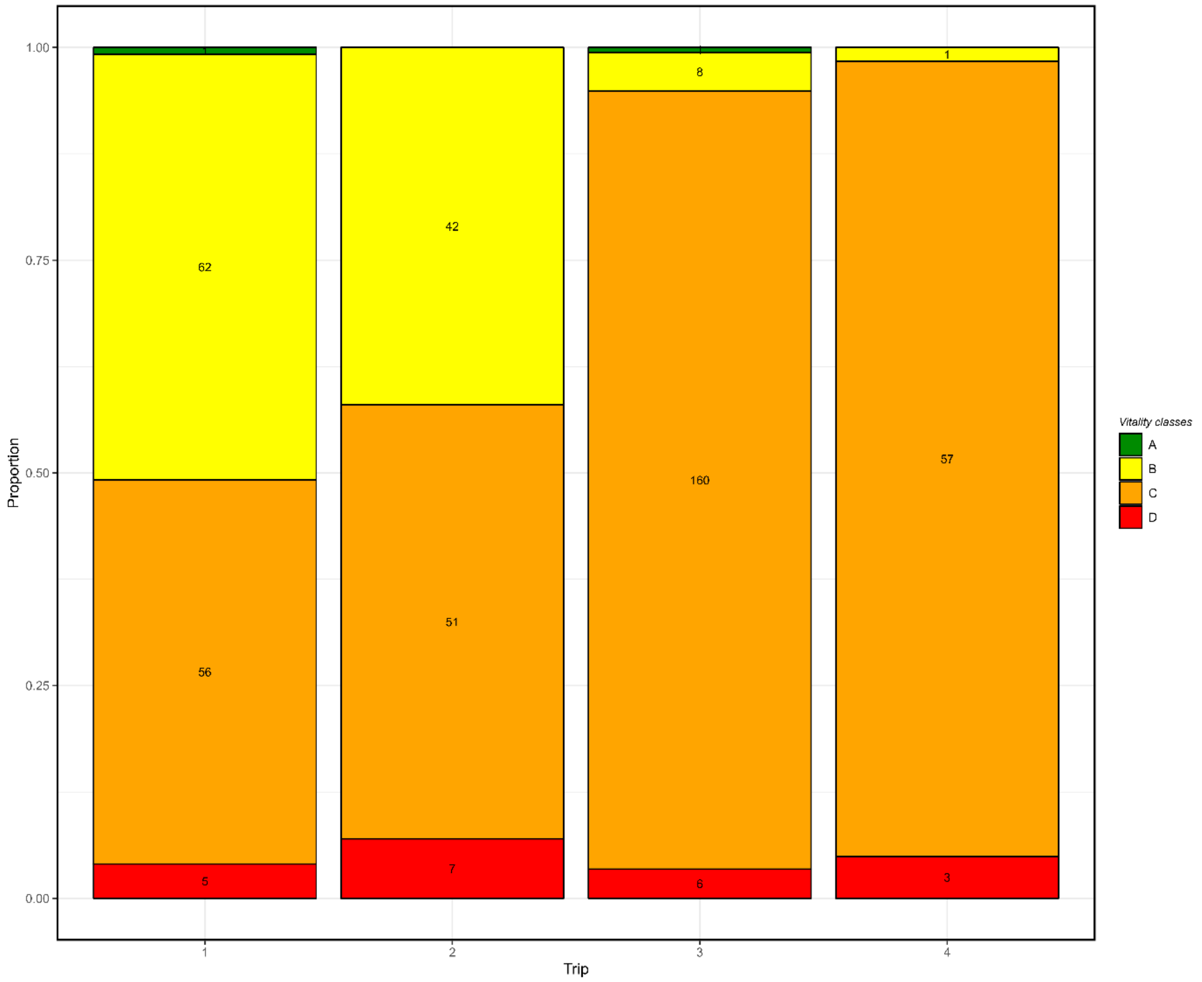

Figure 7: Overview of vitality score proportion of all scored thornback rays caught during the four trips.

The proportions of vitality scores sampled for delayed survival monitoring are a representative stratified sample of the overall catches, with vitality class “C” representing 70% (56 rays) of the total, followed by vitality classes “B” and “A” with 28.7% (23 rays) and 1.25% (1 ray) respectively. As reported for the overall catches, trips 3 and 4 were found to have a larger proportion of rays scored as in “poor” conditions compared to trips 1 and 2 (Figure 8: Overview of vitality score proportion of thornback rays monitored onboard and in captive holding facilities for delayed survival.).

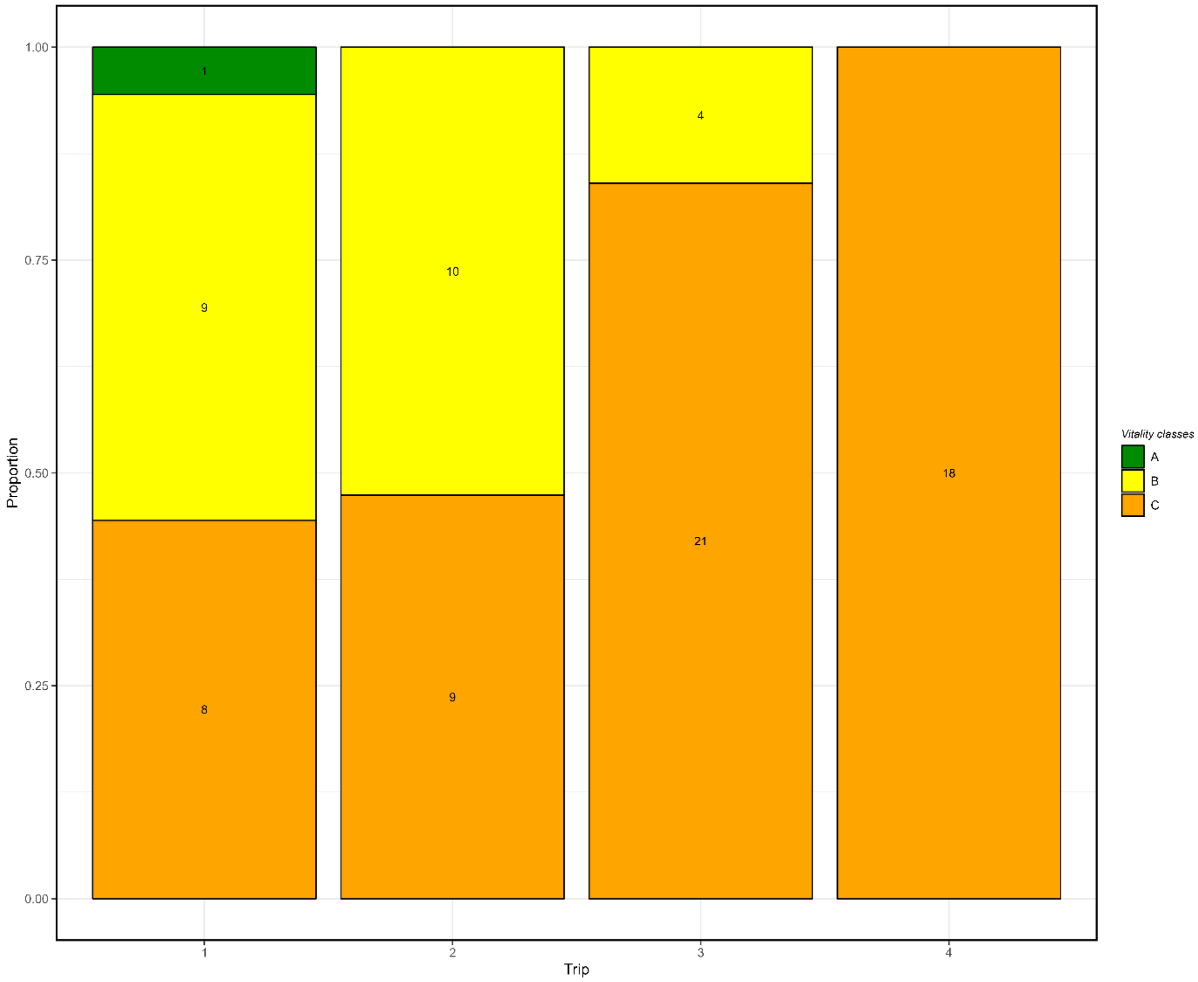

Figure 8: Overview of vitality score proportion of thornback rays monitored onboard and in captive holding facilities for delayed survival.

3.4 DELAYED SURVIVAL

From the 80 individuals monitored for delayed survival (onboard and at holding monitoring facilities), 24 rays died throughout the monitoring period. Thirteen of these died onboard the vessel in their monitoring boxes before reaching the monitoring facilities, while the rest (n=11) died during the 21-day monitoring period at Nausicaá. Due to this and as detailed before, the delayed survival estimation presented later is performed in two ways: (1) including all 24 mortality events and (2) censoring the mortality events occurring at sea (n=13).

Height of the thirteen (61.54%) observed onboard mortality events, occurred on the third trip. However, since none of the controls allocated to this trip died and no evidence of experimentally induced mortality was found, these were considered as fishing-induced mortality events. For instance, the oxygen and water temperatures in the monitoring boxes in which fish died did not differ from the rest of the monitoring boxes throughout the four trips (Figure 9 and Figure 10). Furthermore, no correlation or significant effect was found between the time the fish were kept in the monitoring boxes and their likeliness to die onboard.

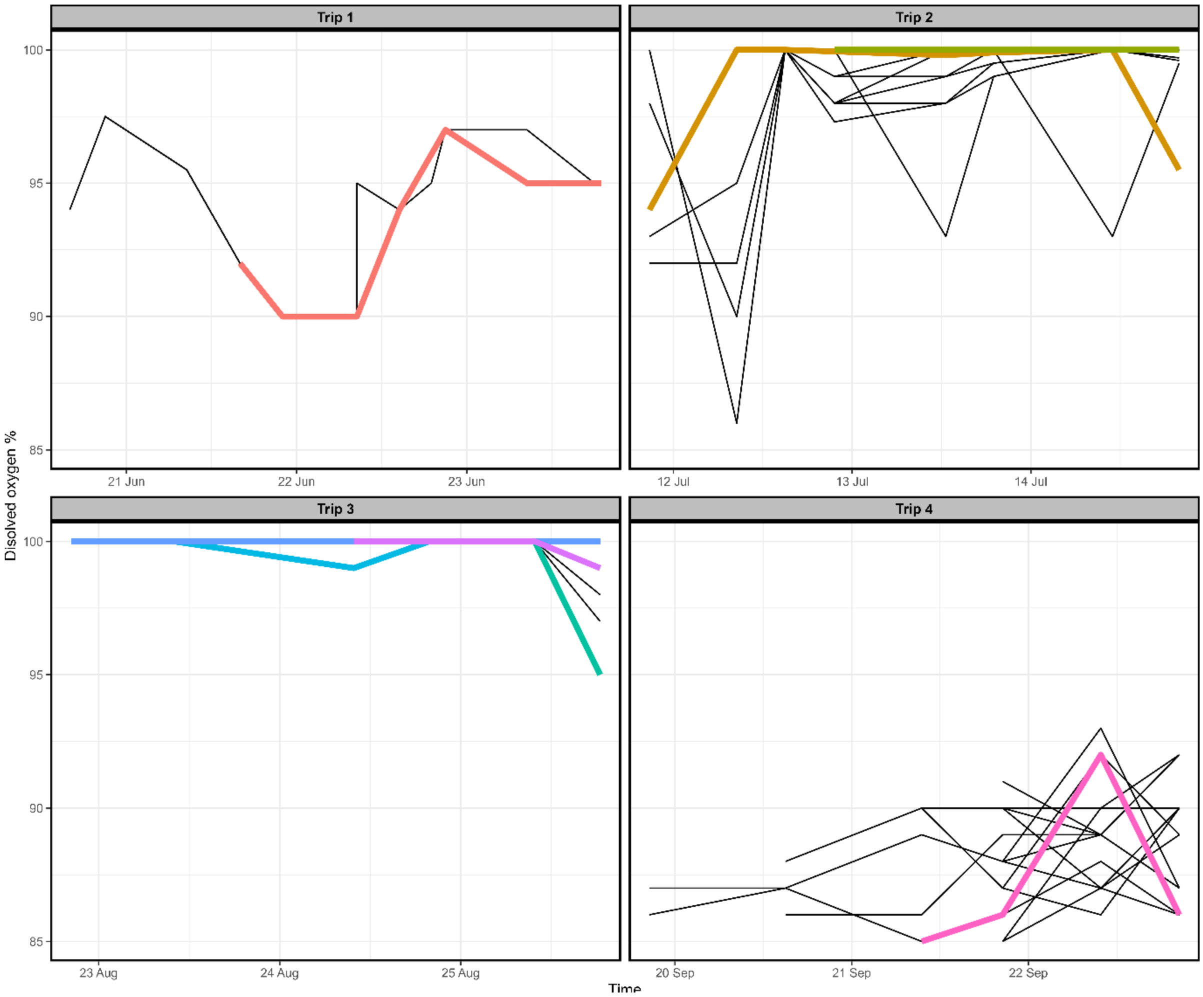

Figure 9: Overview of the dissolved oxygen (%) in the monitoring boxes used on board for each of the four trips carried between June and September 2022. Each black line represents one monitoring box over its monitoring period. Lines in colour represent boxes in which onboard delayed mortality events occurred.

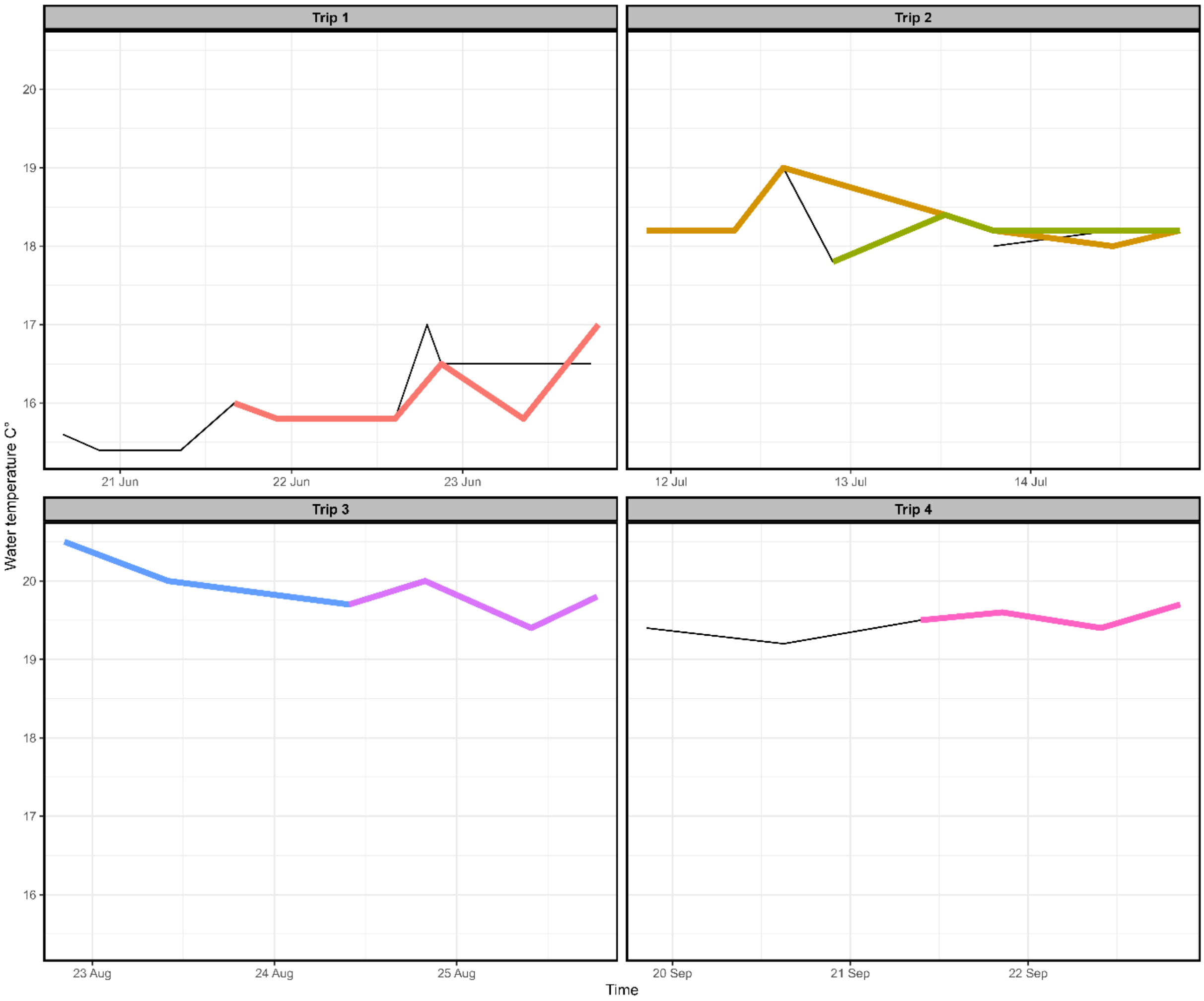

Figure 10: Overview of the water temperature (C°) in the monitoring boxes used on board each of the four carried between June and September 2022. Each black line represents one monitoring box over its monitoring period. Lines in colour represent boxes in which onboard mortality events occurred.

From the 24 mortality events registered throughout the monitoring period 2, 4, 13, and 5 occurred from the 1st, 2nd, 3rd. and 4th trips respectively, leading to delayed survival ranging between 47.33 and 87.49% across different trips. On another hand, when censoring the 13 onboard mortality events only 0, 2, 5, and 4 fish died from the 2nd, 3rd, and 4th trip, resulting in delayed survival estimates of 100, 88.57, 79.50, and 77.78% respectively. This rather large deviance between estimates in different trips shows the need to include different trips in the calculation of the delayed survival estimate and to consider these differences through a random effect. Furthermore, the large differences in survival rates between thornbacks of different vitality scores (Figure 11) indicates the need to also consider these proportion in the delayed mortality prediction and estimation.

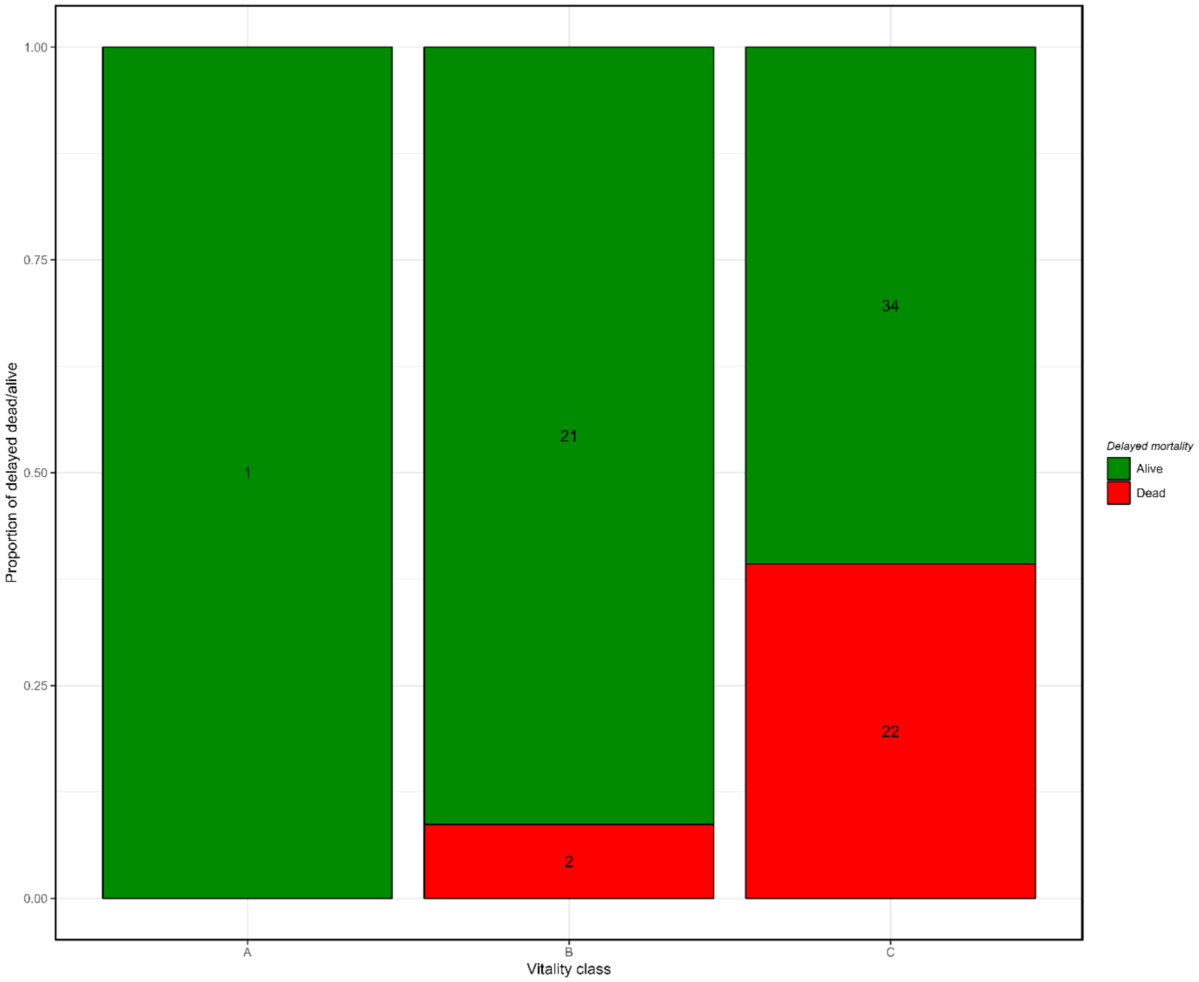

Figure 11: Overview of the relation between vitality class and delayed mortality events (onboard and during the 21-day monitoring period).

The delayed survival estimate for thornback rays caught by flyshooters was derived from a mixed-effect logistic regression model in which the trip code was used as a random effect. In addition, a correction (weighting) was performed to balance the unequal distribution of individuals over vitality classes for the rays caught and monitored (delayed survival) sample sizes (n). From this, the delayed survival for thornback rays caught by flyshoot in the Eastern English Channel is estimated at **73.06% (CI: 56.5-85%)**. However, the censoring of onboard mortality events increases survival to **91.57% (CI: 69.1-98.14)**. The rather large CI around these estimates is caused by the large variation in delayed survival observed between trips. This might be related to different technical or environmental conditions and is explored later in the factor analysis section.

When considering all 24 mortality events in the analysis, these occurred mostly within the first 100 hours (~4 days) after being caught (Figure 12). Comparatively, when censoring the deaths reported at sea, no sudden large mortality event can be outlined clearly from the curve (Figure 13). Despite this difference, both Kaplan-Meier curves show that the long-term monitoring period of 21 days allowed mortalities to reach asymptote until all discard-related mortalities had been observed.

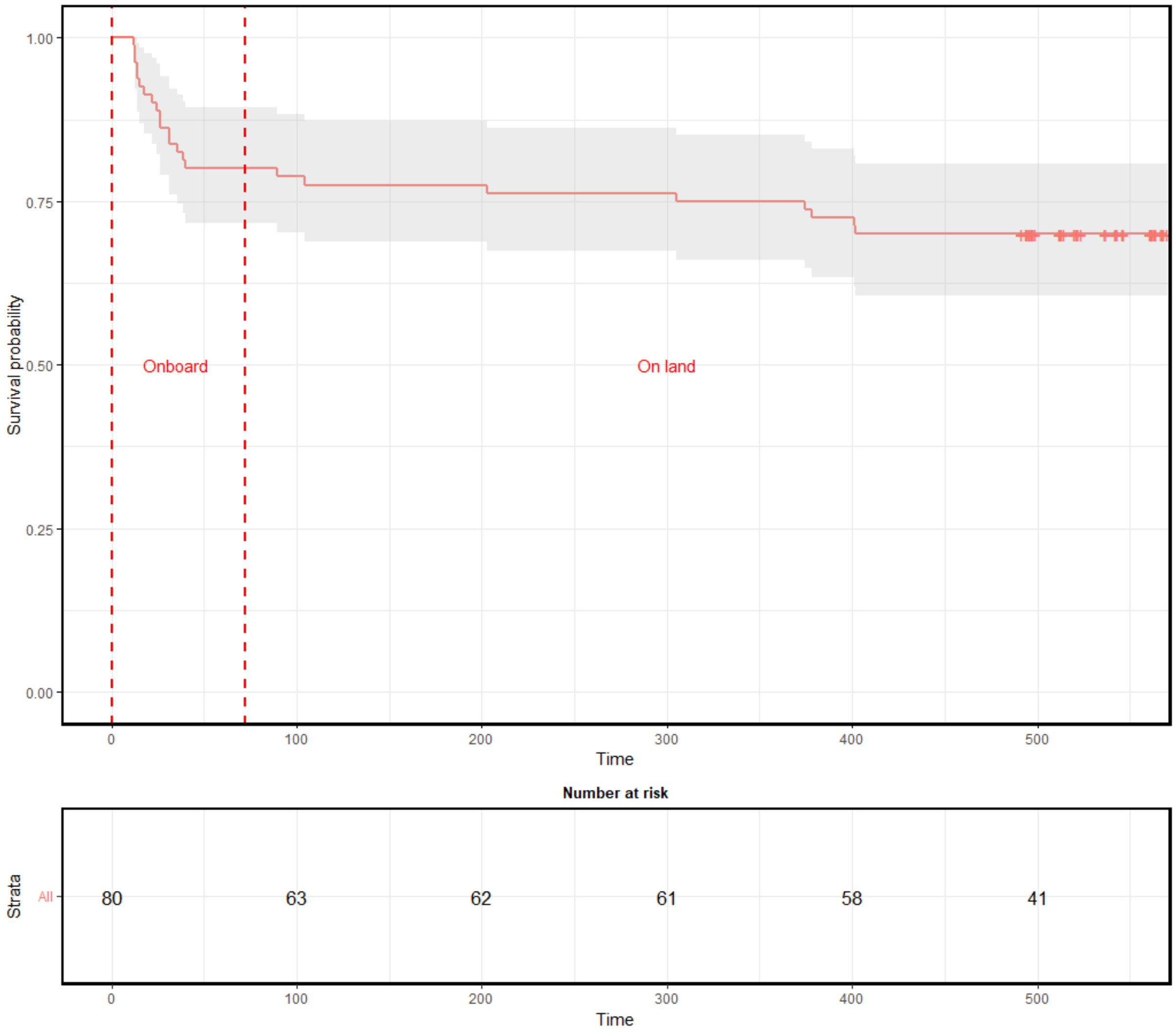

Figure 12: Nonparametric Kaplan Meier survival curves of thornback ray (RJC) over the hours of monitoring, considering all 24 mortality events. Pooled across all vitality classes and trips. The red dotted lines represent the start and end of the onboard monitoring. Crosses represent censoring (end of monitoring period while fish is still alive). The shaded area represents the 95% confidence interval around the estimated survival probability at each time-step.

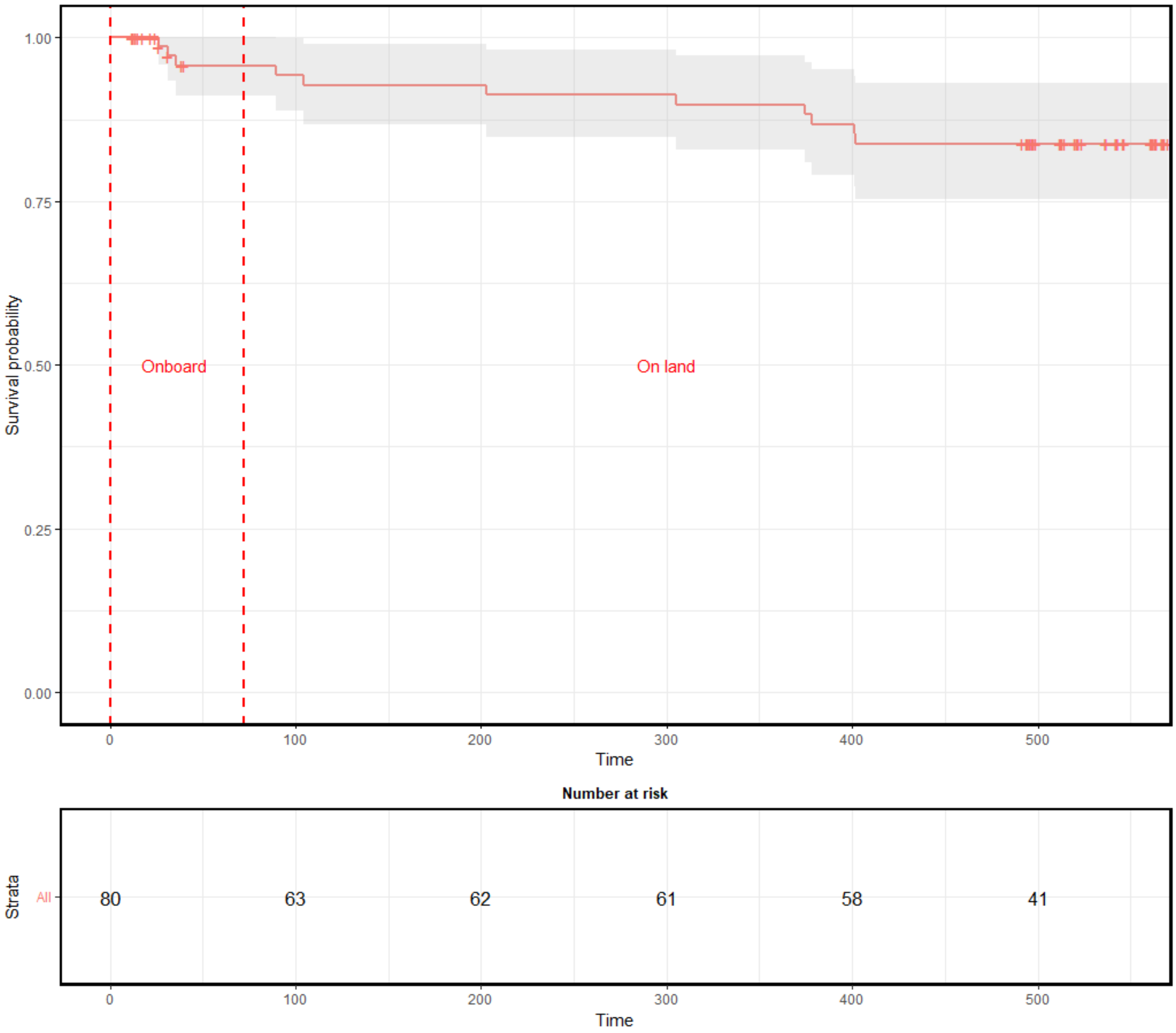

Figure 13: Nonparametric Kaplan Meier survival curves of thornback ray (RJC) over the hours of monitoring, considering onboard mortality events as censored. Pooled across all vitality classes and trips. The red dotted lines represent the start and end of the onboard monitoring. Crosses represent censoring (end of monitoring period while fish is still alive). The shaded area represents the 95% confidence interval around the estimated survival probability at each time-step.

Similarly, Kaplan-Meier curves by trip and vitality class, also show that mortalities have reached an asymptote before the end of the monitoring period (No censoring: Figure 14 and Figure 16, with onboard mortalities censored: Figure 15 and Figure 17). These curves also highlight the clear differences in delayed survival registered between different trips and vitality classes, and the need to include these factors in the delayed survival estimation.

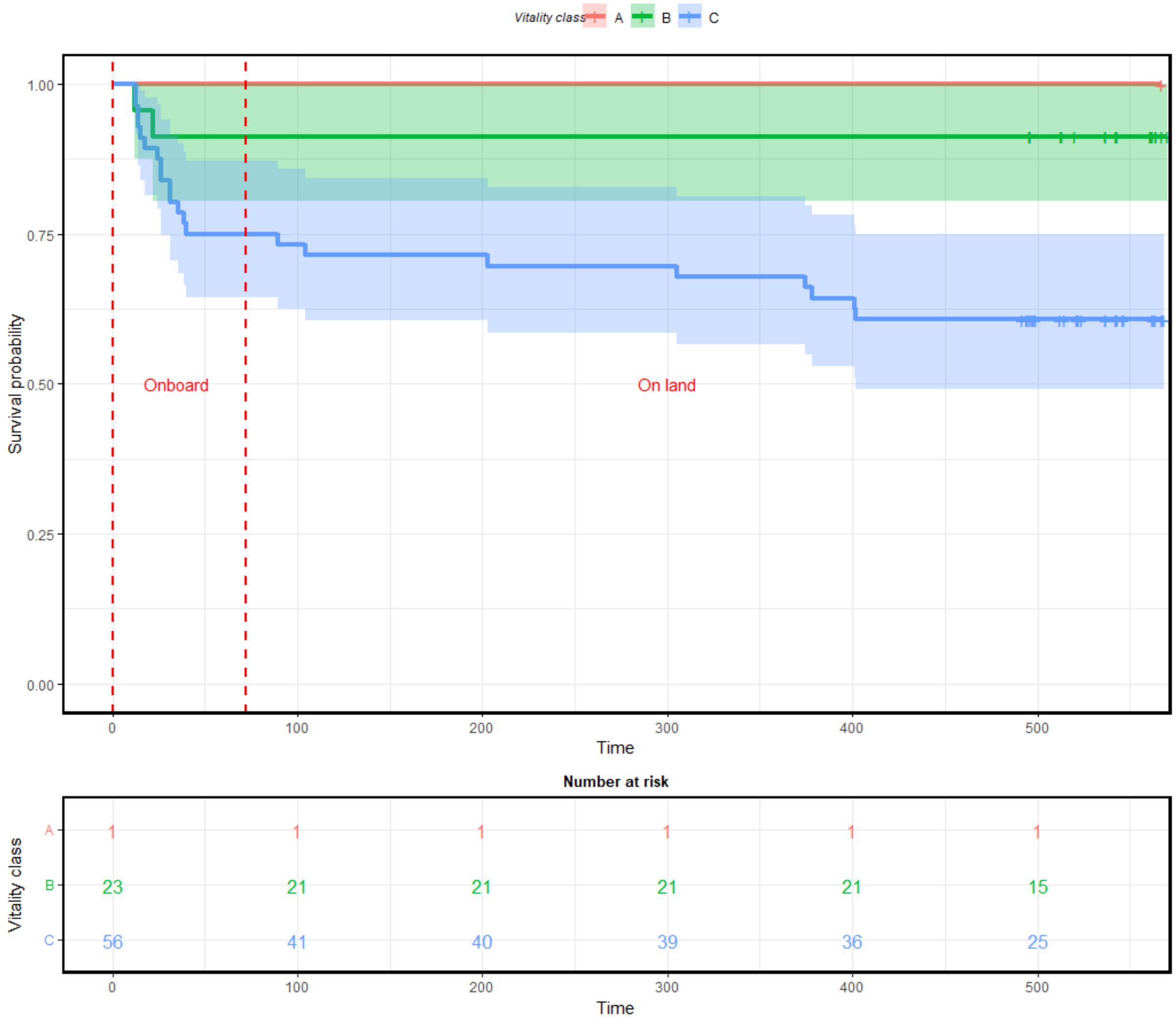

Figure 14: Nonparametric Kaplan Meier survival curves of thornback rays (RJC) over the hours of monitoring for each vitality class, considering all 24 mortality events. Pooled across all trips. The red dotted lines represent the start and end of the onboard monitoring. Crosses represent censoring (end of monitoring period while fish is still alive). The shaded area represents the 95% confidence interval around the estimated survival probability at each time-step.

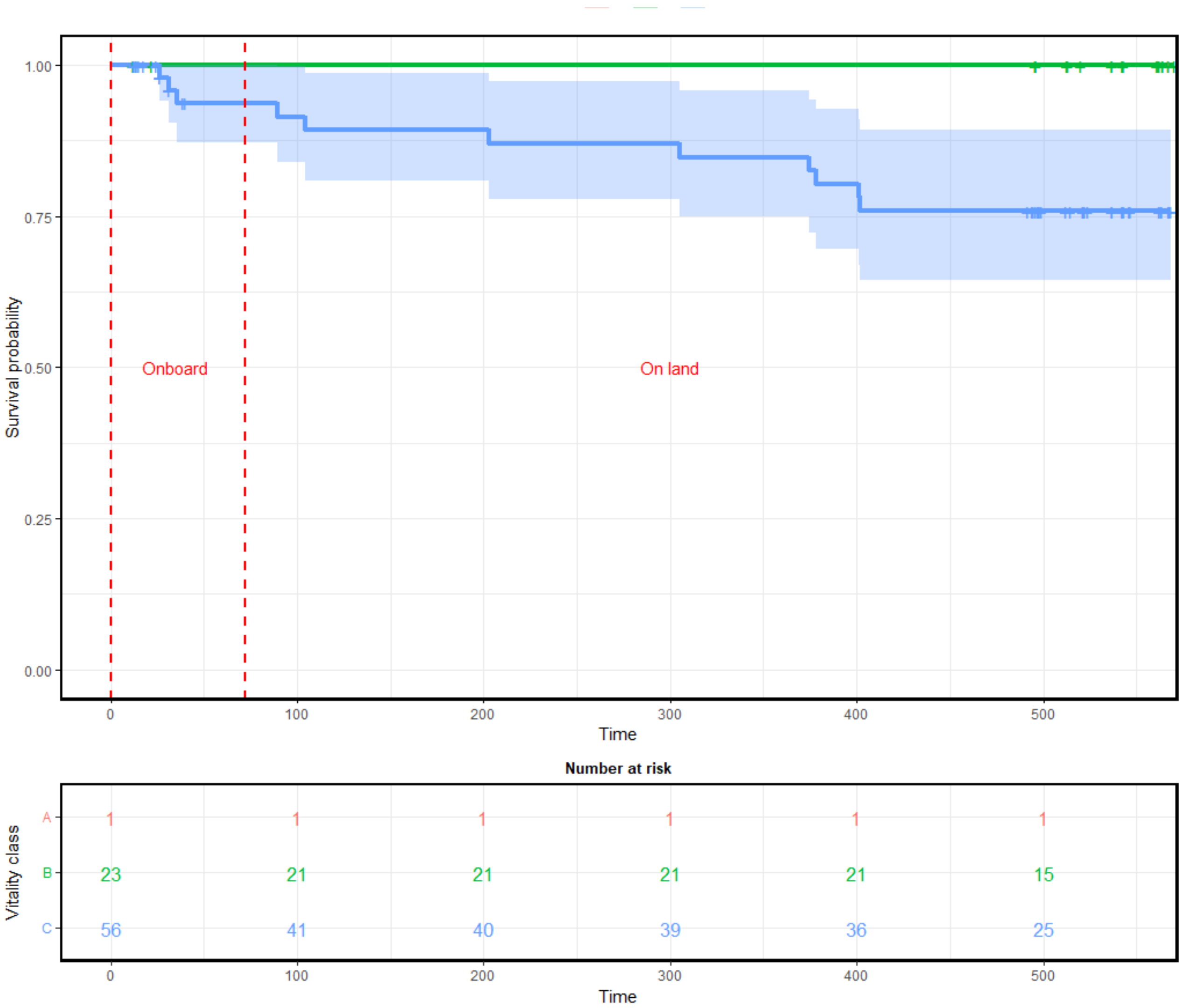

Figure 15: Nonparametric Kaplan Meier survival curves of thornback rays (RJC) over the hours of monitoring for each vitality class, considering onboard mortality events as censored. Pooled across all trips. The red dotted lines represent the start and end of the onboard monitoring. Crosses represent censoring (end of monitoring period while fish is still alive). The shaded area represents the 95% confidence interval around the estimated survival probability at each time-step.

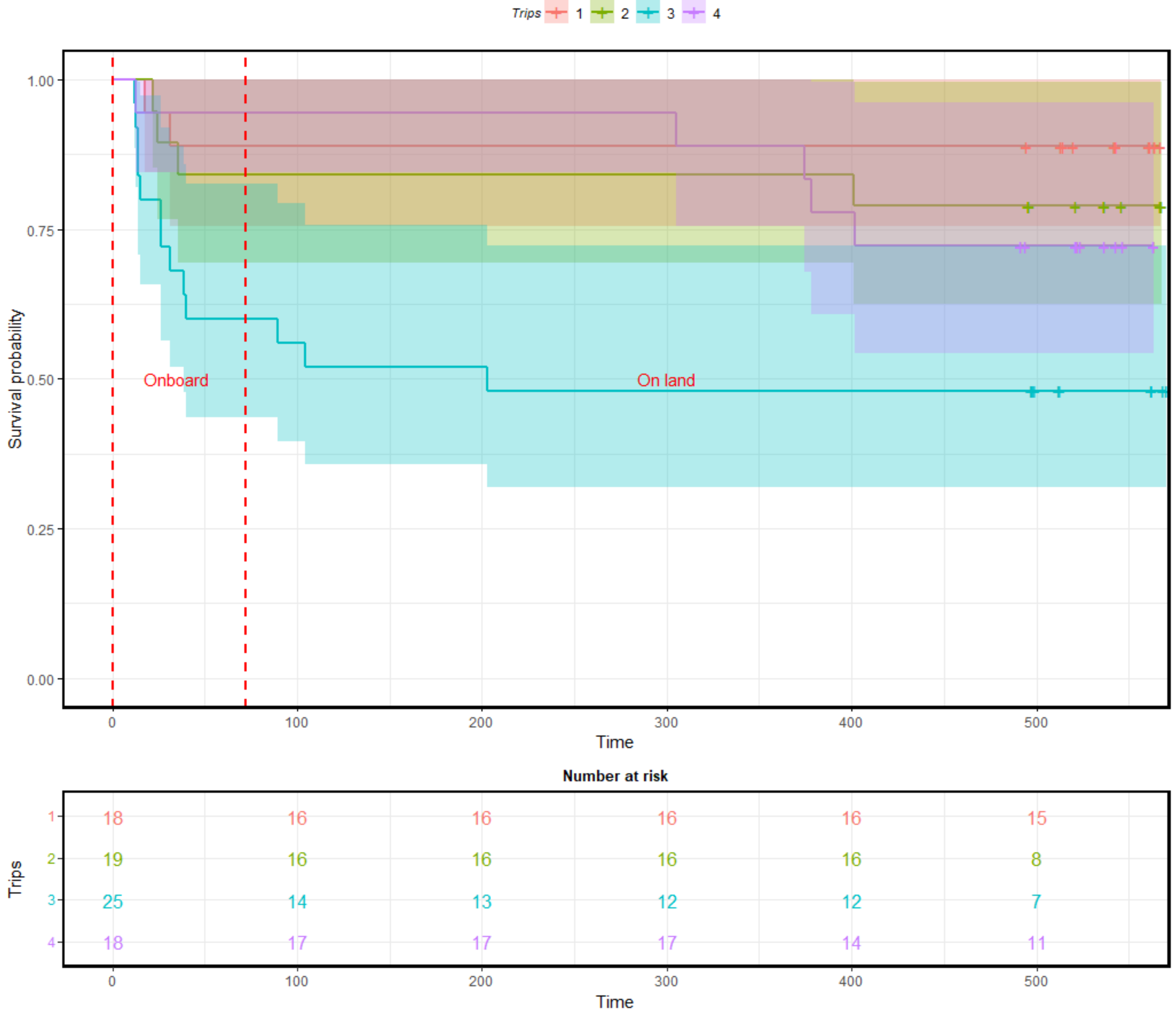

Figure 16: Nonparametric Kaplan Meier survival curves of thornback rays (RJC) over the hours of monitoring for each of the four trips, considering all 24 mortality events. Pooled across all vitality classes. The red dotted lines represent the start and end of the onboard monitoring. Crosses represent censoring (end of monitoring period while fish is still alive). The shaded area represents the 95% confidence interval around the estimated survival probability at each time-step.

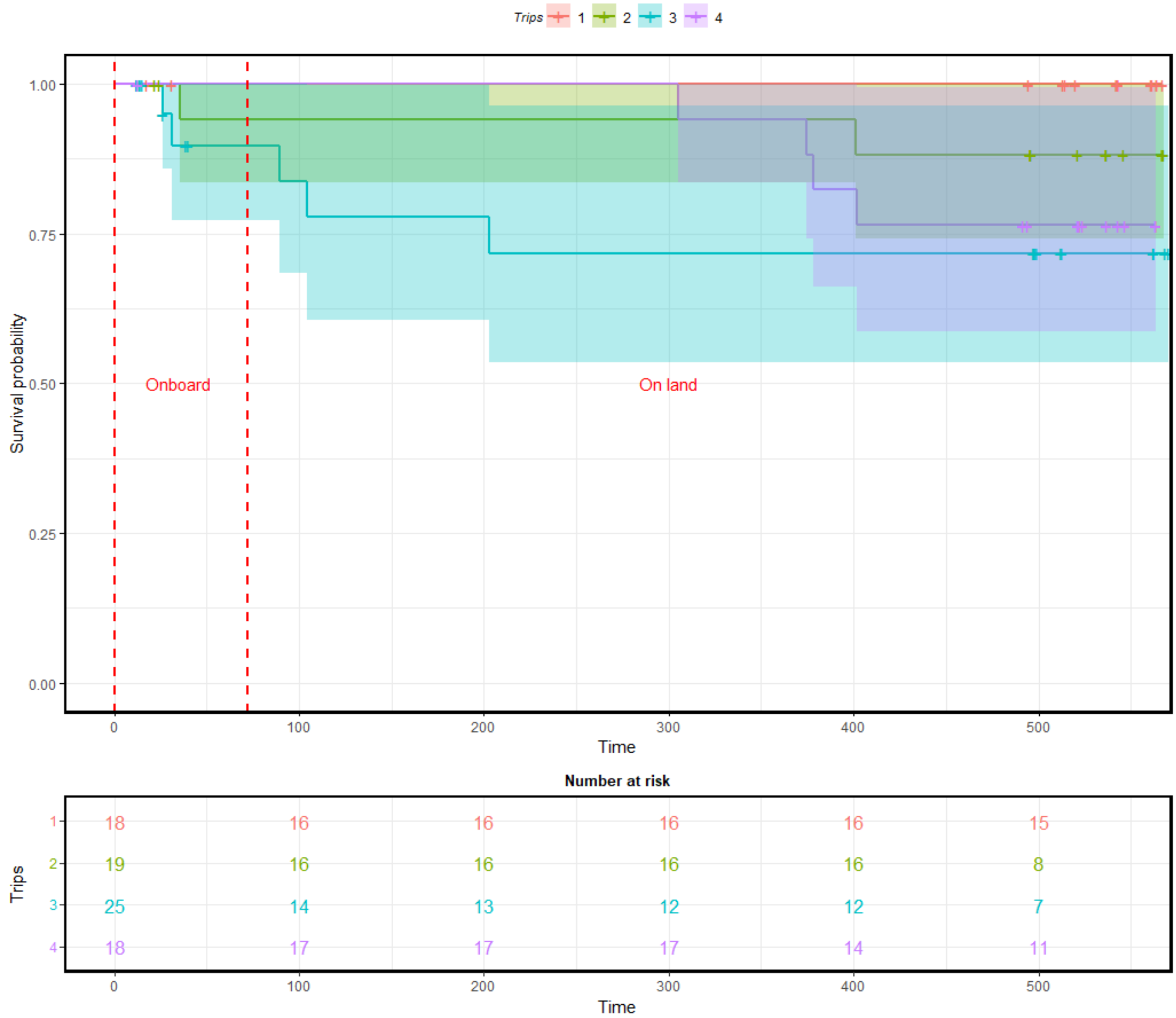

Figure 17: Nonparametric Kaplan Meier survival curves of thornback rays (RJC) over the hours of monitoring for each of the four trips, considering onboard mortality events as censored. Pooled across all vitality classes. The red dotted lines represent the start and end of the onboard monitoring. Crosses represent censoring (end of monitoring period while fish is still alive). The shaded area represents the 95% confidence interval around the estimated survival probability at each time-step.

3.5 TOTAL SURVIVAL

The total survival was calculated as the complement to one of the sum of the immediate mortality reported on board and the estimated mortality reported for the remaining fish alive. This resulted in an estimated total discard survival of **69.73% (CI: 53.92-81.12%)**. when considering all mortality events or **87.40% (CI: 65.95-93.66)** when censoring the thirteen onboard mortalities.

3.6 FACTOR ANALYSIS AND MODEL BUILDING

Before starting with the factor analysis and model-building procedure, the correlation between variables was analysed.

3.6.1 CORRELATION EXPLORATION

The correlation between all variables measured for all thornbacks caught in the fishing trips was analysed (Figure 18). As expected, a correlation between the vitality score and the RAMP, INJ, and immediate mortality events was registered. No high correlation was found between any other pair of variables. The injury score and water depth were found to be slightly positively correlated (corr: 0.44), indicating a tendency to find that rays with more injuries when fished in deeper waters.

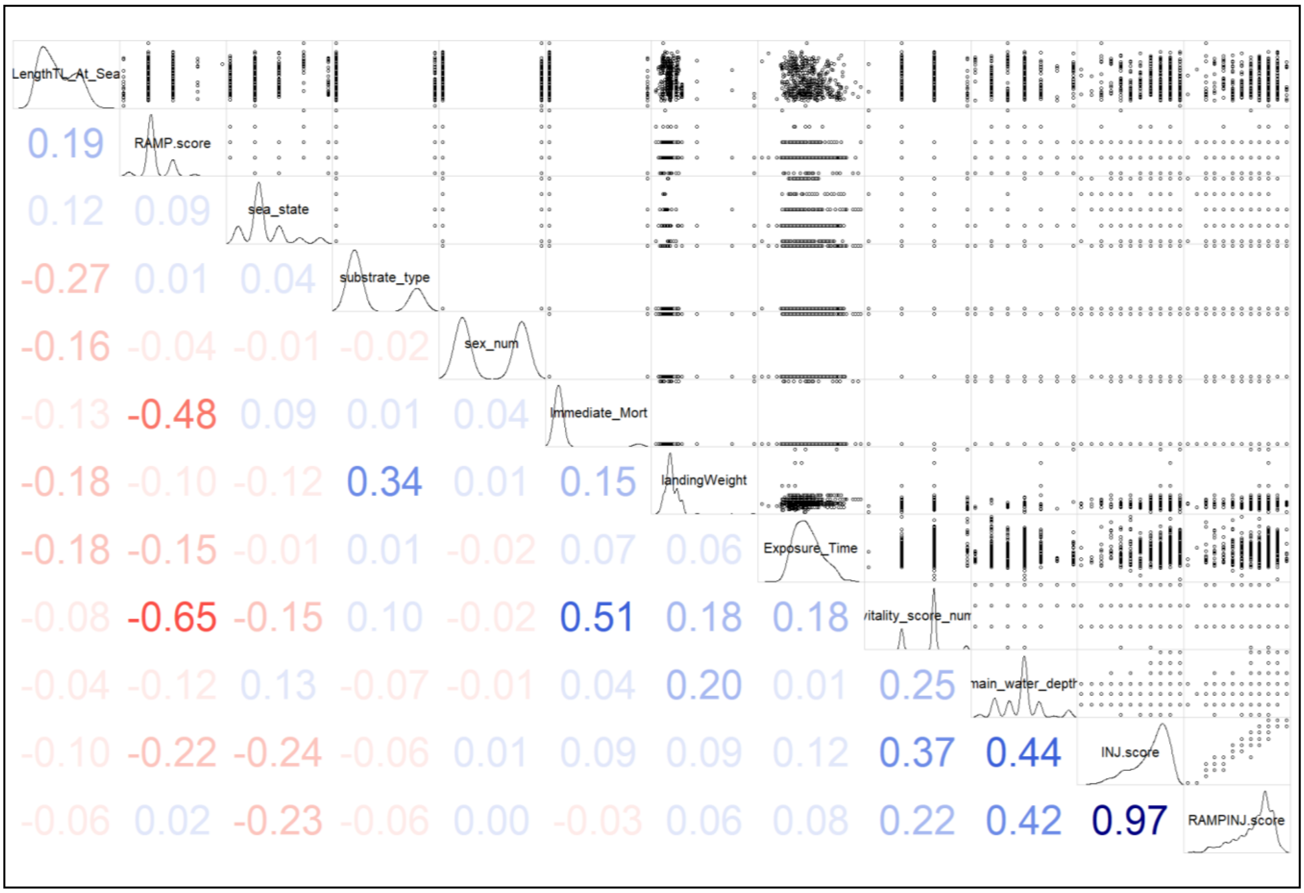

Figure 18: Correlation plot for all fish caught between immediate mortality status (Immediate_Mort), vitality (vitality_score_num), injury (INJ.score), reflex impairement (RAMP.score), RAMPINJ.score, length (LengthTL_at_Sea), total landed weight per haul (landingWeight), substrate type (substrate_type), sea state and depth (main_water_depth).

Similar correlations are found when looking at the rays monitored for delayed survival (Figure 19). Again, the injury, RAMP, RAMPINJ, vitality score, and delayed mortality are quite closely correlated. Again fish caught in deeper water appear to present more injuries. In addition, a negative correlation is reported between the sea state and injury score (corr: -0.56), there is however no logical explanation on why better fishing conditions (better sea state) would lead to fish in worse conditions. This correlation is likely an artifact caused by a rather small number of fish caught in bad fishing conditions (i.e., sea state 3 or 4) compared to better conditions.

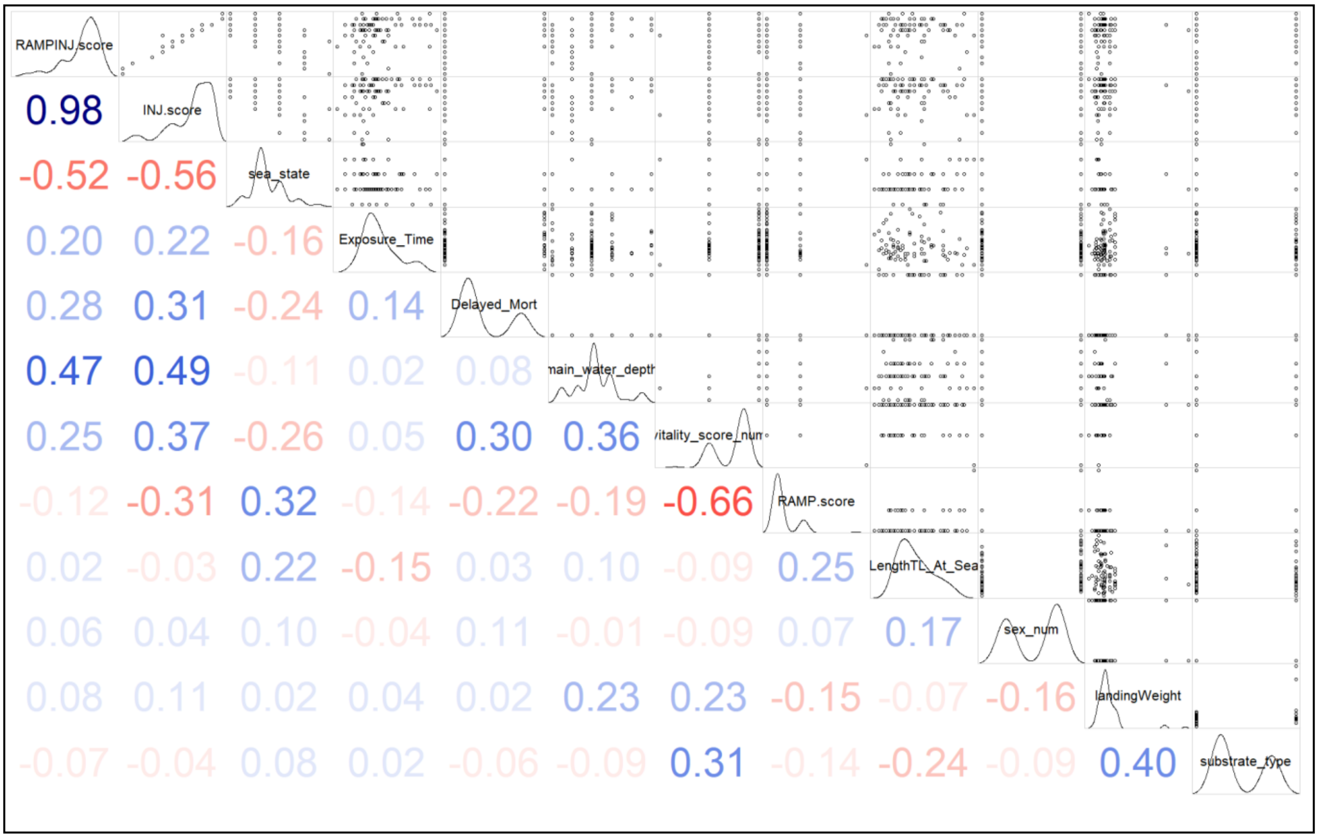

Figure 19: Correlation plot for rays monitored for delayed survival between delayed mortality status (Delayed_Mort), vitality (vitality_score_num), injury (INJ.score), reflex impairement (RAMP.score), RAMPINJ.score, length (LengthTL_at_Sea), total landed weight per haul (landingWeight), substrate type (substrate_type), sea state and depth (main_water_depth).

3.6.2 SINGLE FACTOR ANALYSIS

Using a generalized linear mixed-effect model, the significance of individual (i.e., length, sex, and vitality), technical (i.e., landed weight), and environmental (i.e., depth, sea state, substrate type) variables in predicting the immediate and delayed (after 21 days) mortality status (0=alive, 1=dead) were tested.

3.6.2.1 IMMEDIATE MORTALITY

From this analysis, it was found that larger (total length) thornback rays are less likely to die when caught and discarded (p-value = 0.00889, Figure 20). Subsequently, rays caught in larger hauls (with higher landed weight) were found to have a significantly (p-value = 0.00644) higher immediate mortality probability. However, it is unclear whether this is a true effect or rather an artifact of the small sample size of hauls with larger landed weights (Figure 21).

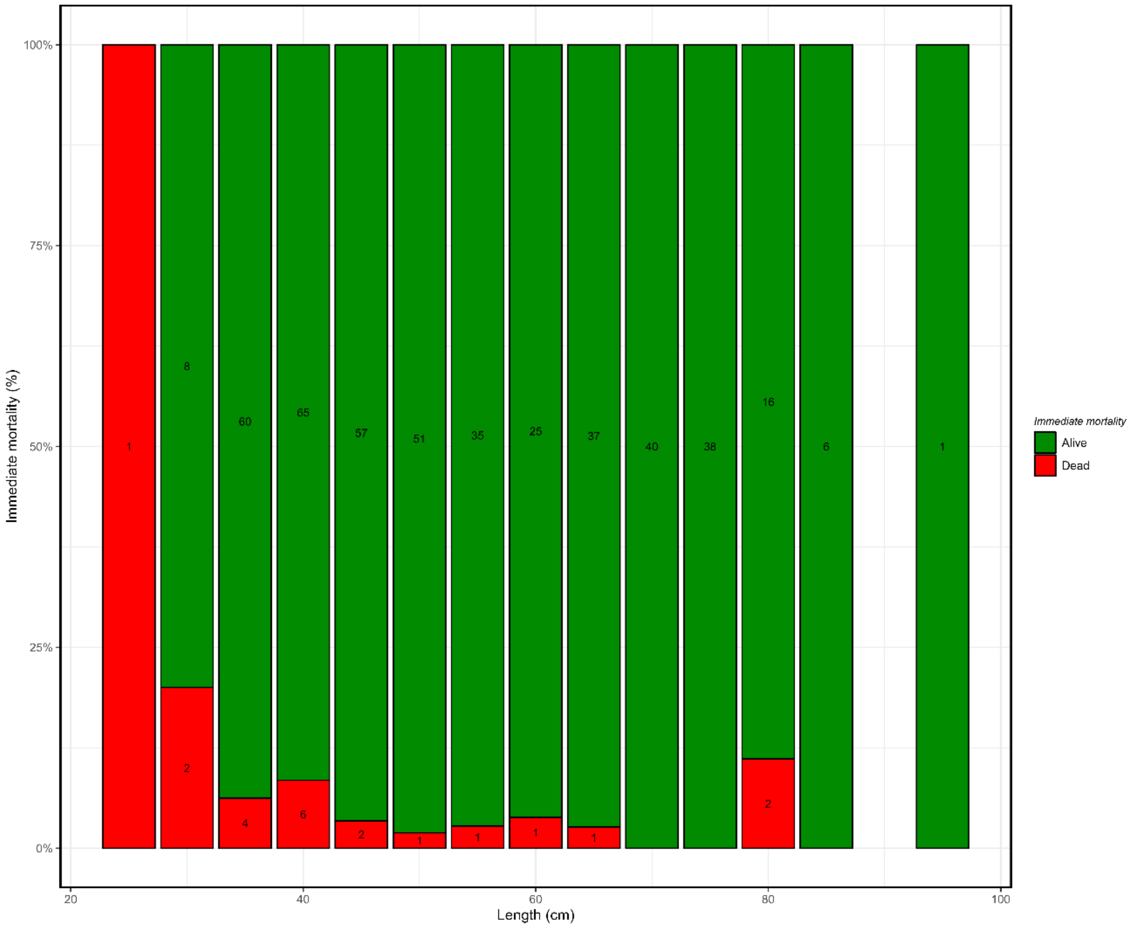

Figure 20: Overview of the effect of length on the immediate mortality of individual rays.

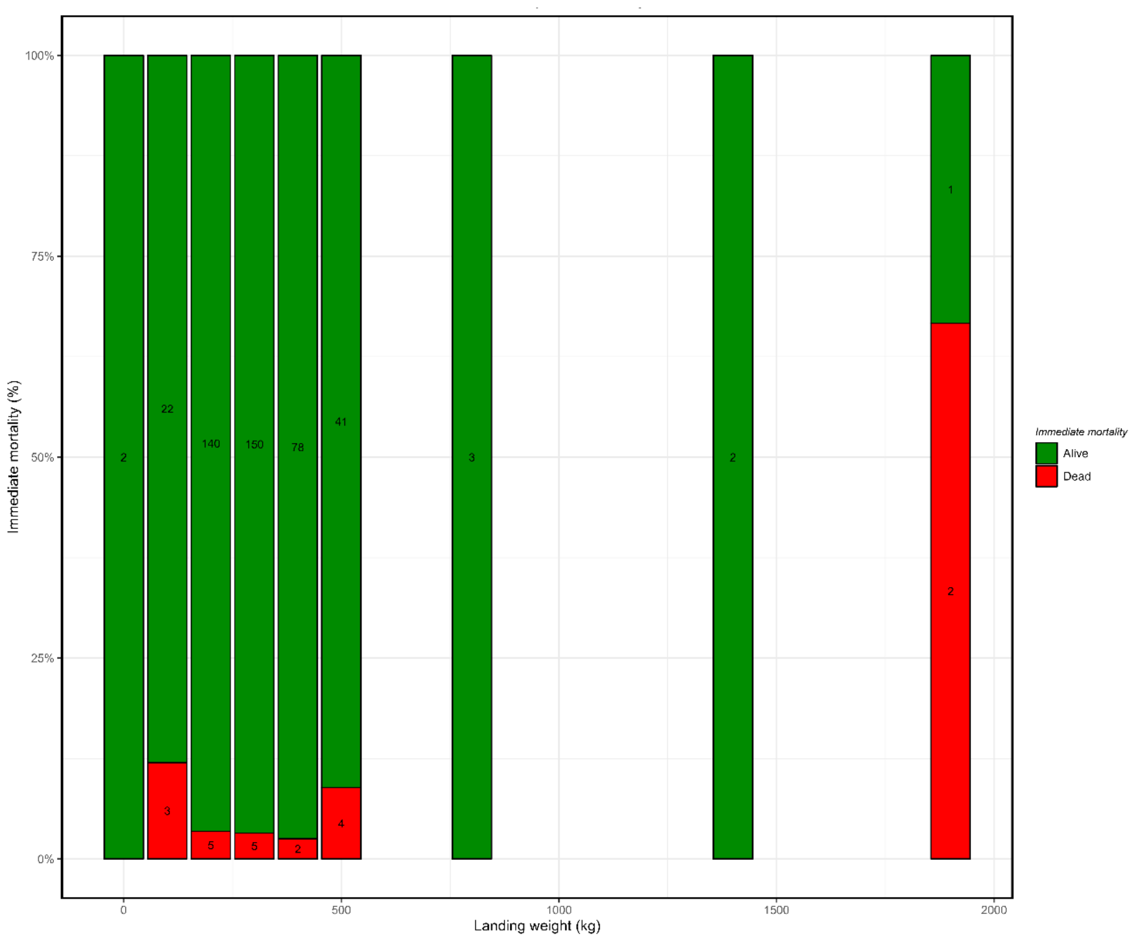

Figure 21: Overview of the effect of total landed weight of the haul on the immediate mortality.

3.6.2.2 DELAYED MORTALITY

In the case of the delayed mortality status, no significant effect was found for any of the technical and environmental variables. Nonetheless, in terms of individual variables, the fish's injury score (p-value = 0. 0.00845) was found to lead to significant differences in its delayed survival probability. In addition, the combined score (RAMPINJ) was found to be significant as well (p-value = 0.01572), while the RAMP score was marginally not significant (p-value = 0.055). In all cases and as already detailed above, fish in worse conditions were more likely to be death by the end of the delayed survival monitoring. (Figure 11: Overview of the relation between vitality class and delayed mortality events (onboard and during the 21-day monitoring period). Figure 22, Figure 23, and Figure 24)

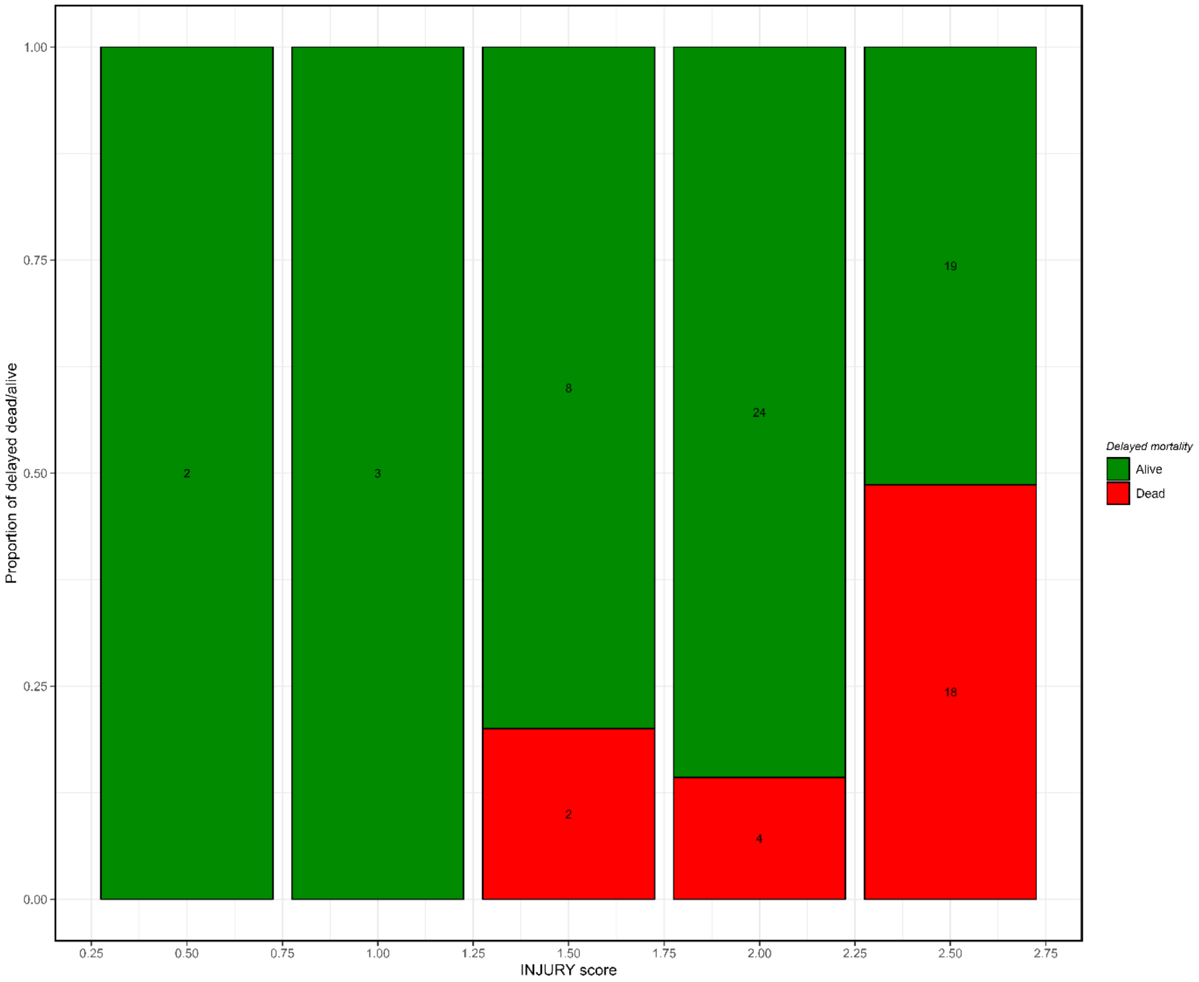

Figure 22: Overview of the effect of injury score on the delayed mortality.

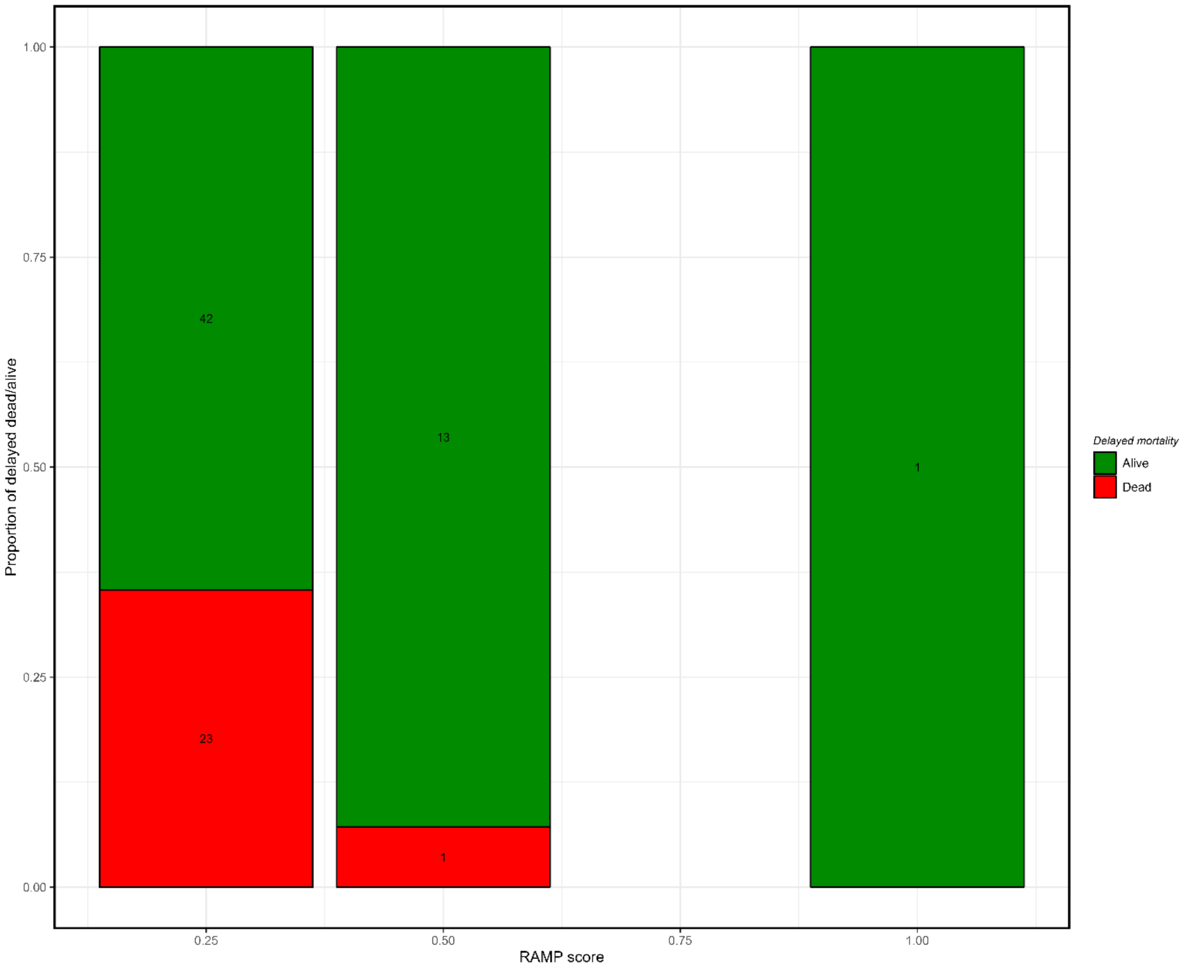

Figure 23: Overview of the effect of the RAMP- score on the delayed mortality.

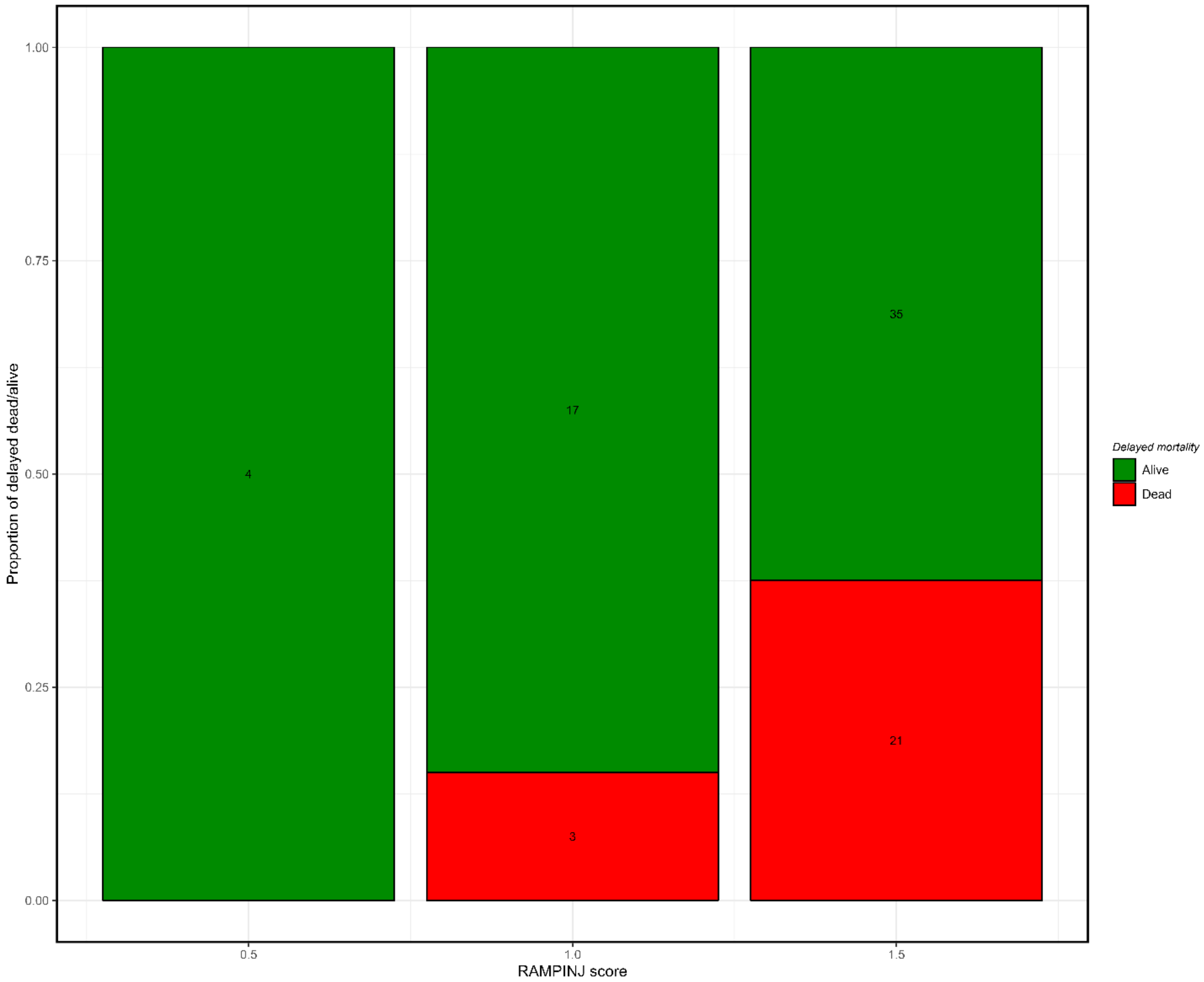

Figure 24: Overview of the effect of the RAMPINJ score on the delayed mortality.

3.6.3 MODEL BUILDING

A forward model-building procedure using the significance levels and Akaike Information Criterion as the selection criterion was used to identify the most "parsimonious" model to predict immediate and delayed survival. The most parsimonious model (a model that achieves a certain level of explanation or prediction with as few explanatory variables as possible) for the probability of immediate survival included the individual length and injury score of each fish as predictors, in addition to the sea state and landing weight of the haul in which those were caught (Table 4). In this sense, larger fish are less likely to die, whilst injuries, worse sea state, and larger haul (weight) will increase the probability of fish experiencing immediate mortality.

The delayed survival most parsimonious model was found to be relatively simple compared to the model for immediate survival and included only one variable: fish in worse conditions, specifically with more injuries (higher injury score) are more likely to experience delayed mortality (Table 4 and Figure 22).

Table 4: Overview of the most "parsimonious" models obtained using forward model building to predict the immediate and delayed mortality status of an individual fish.

Model	AIC	Variables	Estimate	p-values
Immediate mortality status ~Length+ Sea state+ Injury score+ Landing weight+ (1|Trip)	160.1905	Length	-0.477	0.01743 *
		Sea state	0.67	0.00334 **
		Injury score	1.39	0.04198 *
		Landing Weight	0.001	0.03374 *
Delayed Mortality status ~ Injury score+ (1| Trip)	92.96251	Injury score	2.3147	0.00845 *

4 DISCUSSIONS

Flyshoot fishing operations are relatively short (~35 min) compared to otter and beam trawl operations (~92 min) (van Bogaert et al. 2020). Furthermore, it is only during the last 10 minutes of the fishing operation, when fish enter the trawl, that they are exposed to mechanical injuries caused by the trawl (van Overzee et al., 2019). With these differences, it would be expected that fish caught by flyshooters would tend to be in better conditions and more likely to survive than when caught by trawlers, but in worse conditions and less likely to survive compared to passive gears (i.e. gill/trammel nets). However, this study shows that thornback rays caught by flyshoot were mostly found in worse conditions compared to the ones caught by beam and otter trawls (Ellis et al., 2018; Randall et al., 2018; van Bogaert et al., 2020). This might be explained, by the faster pace of the fishing activity, causing a short traumatic shock (which is still present at the time of the scoring on board) from which fish are most likely to recover. Barotrauma in fish is intrinsically linked to the depth and speed of ascent (Carlson, 2012), therefore the differences in the mean fishing depth between studies are also likely to lead to differences in the proportion of fish in excellent/good/bad conditions. On another hand, by repeating the vitality scoring on the same individuals on deck and after a few hours (3-24h), it was observed that most individuals that were scored as vitality class “C” or “B” when arriving on deck, were often scored as being in an excellent (vitality class “A”) condition after a few hours. This shows that their initial vitality class might be the result of “non-serious” trauma and “stress” from which most rays can recover rather quickly. This divergence also shows that using vitality as a proxy for delayed survival might not be straightforward and that more specifications on the gear and fishing activity are needed to make such assumptions. It is also very important to consider the subjective nature of scoring a fish’s vitality when comparing results from different studies and projects. Different observers, protocols, and methods are likely to influence these proportions, which can reduce the comparability of vitality assessments between studies (Benoit, Hurlbut, & Chasse, 2010).

Despite the reported differences in vitality scores (in comparison to other studies) detailed above, the estimated immediate discard survival for flyshoot (95.43%) falls within the range of values estimated for others gears in the past (STECF, 2022). Gears that lead to “theoretically” larger sources of disturbance and exposure to mechanical traumas have reported similar immediate survival rates (i.e., 95.71% for beam trawl, 93.56% for otter trawl) (Randall et al., 2018; van Bogaert et al., 2020). As expected, flyshoot does seem to lead to slightly lower immediate survival compared to passive gears such as trammel (96%), gill (100%) (van Bogaert et al., 2020), and tangle nets (97.4-98%) (Ellis et al. 2018). Although immediate survival seems to vary “logically” between gears, differences are relatively small and continue to show a generally high immediate survival for the species (93.56-100%) across all gears analysed so far.

This generally high immediate survival might indicate that other factors (i.e. intrinsic to the fish or its habitat) are more influential in shaping a ray’s destiny in the net. The individual length has repeatedly been identified as a significant factor influencing the immediate mortality probability (Ellis et al., 2018; van Bogaert et al., 2020), such as in this study. Other factors such as the injury score and total haul landed weight have also been found to be significant in the SUMARiS project (van Bogaert et al., 2020) and this study. In contrast to the SUMARiS project, in this study, the substrate type was not found to explain significantly the immediate survival probability. The sand and stone and benthos proportion in the catch could not be included in the factor analysis due to a complete homogeneity of the sampled hauls. Further research should focus on increasing the trip and haul sample sizes (through additional sampling and/or meta-analysis) to consider these variables in future analysis.

In contrast to the small differences between the immediate survival estimates of different gears, the delayed survival has been found to vary largely and significantly between gear types, with passive gears reporting higher survival rates (e.g. 93.35% for trammel nets) compared to active gears (e.g. 56.9 for beam trawls respectively) (van Bogaert et al., 2020). The estimated delayed mortality of 73.06% (i.e. without censoring) for thornback rays in flyshoot fisheries falls between both gear categories (i.e. passive and active gears), which relates to the active nature of the gear but apparent shorter exposure to traumas and shocks (i.e., short hauls and no dragging of the net). However, it is lower than the estimated delayed survival for thornbacks (81%, Schram et al. 2023) and proposed for starry rays (80%; van Overzee et al., 2019) caught by the Dutch flyshoot fleet in the southern North Sea (4.c) and Eastern English Channel (7.d). However, when censoring the onboard mortalities, the estimated delayed survival increases to 91.57%, situating delayed discard survival for flyshoot in this study closer to estimates obtained for passive gears (i.e. trammel nets), and comparatively more distant (higher) to discard survival rates for active gears (i.e. otter and beam trawls) and to the estimates for thornbacks caught by the Dutch flyshoot fleet (Schram et al. 2023). Despite this relatively large difference, in both cases, flyshoot shows a comparatively large survival rate for thornback rays. It is also relevant to emphasize that a relatively large uncertainty was reported around these estimates, which is caused by high variability in delayed survival between different trips. This, together with a clear difference in the proportion of vitality classes between different trips indicates that other factors, beyond the gear itself, are affecting the delayed survival of discarded thornback rays.

Sea water temperatures in August (mean = 19.8°C) and September, (mean = 19.6°C), when the 3rd and 4th trips occurred were comparatively higher than in June (mean = 15.8°C) and July (mean = 18.2°C), when the first two trips took place, which directly or indirectly might explain the higher mortality rates reported in these (52 and 27.8% respectively). Higher temperatures have already been linked to higher base metabolism and therefore lower tolerance to disturbance (Clarke & Johnston, 1999); for example, plaice (*Pleuronectes platessa*) caught and discarded by Belgian beam trawlers were found to be more likely to survive in colder sea water during winter (Uhlmann et al., 2021). However, in this study, the water temperature was not found to be significant to predict immediate or delayed survival mortality probability. Apart from that, the water temperature at Nausicaá was kept constant throughout the study at around 10°C, meaning that rays caught during the warmer months were exposed to a stronger thermal stress when being transferred from the onboard monitoring boxes to the aquaria facilities. Similarly, the vitality class and immediate survival for thornback and blonde rays caught by the Belgian beam trawl fleet were found to be significantly affected by the water temperature difference (bottom vs. air) (Lemey et al., unpublished data). However, the water temperature difference was not found to be significant in this study. Rather than not being relevant for the fish, it is possible that the limited sample size of temperature differences, caused by the rather homogeneous seawater temperature registered in hauls of the same trips, prevented the detection of a significant effect.

Finally, the delayed mortality probability was found to be significantly affected by the injury scores and therefore could be used as a viable proxy to extrapolate/predict delayed discard survival estimates in the future without the need for long-term monitoring. Since the effectiveness and power of such predictor models is highly dependent on the amount of data available, it would be ideal to perform an integrated meta-analysis of all available data (from this and other studies) to provide more accurate and precise predictions of delayed mortality and identify key factors to predict discard survival in the future. Although this would require the integration and homogenization of different scoring methodologies, the outputs of such predictive models are of interest for stock assessments, ecosystem modeling, and decision-making (STECF, 2022).

5 CONCLUSIONS

Flyshoot fishing operations are short, with brief exposure to mechanical injuries, leading to a relatively high discard survival for thornback rays compared to other active gears. Immediate survival for thornback rays caught by flyshooters in the Eastern English Channel was estimated at 95.43%. Delayed survival was found to vary significantly between trips and vitality classes and was estimated at **73.06% (CI: 56.5-85%)** when considering all mortality events, but at **91.57% (CI: 69.1-98.14)** when the onboard mortalities were censored. Despite this difference, it is key to note that the confidence intervals of both estimates overlap largely and in both cases represent a comparatively (to other active gears) high delayed survival. Similarly, the total mortality was estimated at **69.73% (CI: 53.92-81.12%)** or **87.40% (CI: 65.95-93.66)** without and with onboard mortalities censored respectively.

The individual length and injury score, together with the weight of the catch and sea state of a specific haul were found to lead to significant differences in immediate mortality probabilities. On the other hand, only the vitality and injury scores were found to be significant for the delayed mortality probability. These variables could, in the future and in the form of a meta-analysis, be used as proxies and predictors of delayed survival. Hence reducing/avoiding the need of realizing costly, time and effort-intensive long-term monitoring studies.

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