

## Discard survival estimates of commercially caught skates of the North Sea and English Channel

Work Package 2

Output O 5.1

**Version nr. 2**

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### 3 Summary

In the North East Atlantic, rays and skates are caught as bycatch in otter- and beam-trawl fisheries or targeted with gillnets, trammel nets, longlines and by recreational anglers. Within the INTERREG 2-Seas SUMARiS (Sustainable Management of Rays and Skates) project, the goal of Work Package (WP2) was to quantify vitality, reflex impairment, injury and survival probability of skates discarded in the English Channel and the North Sea after being captured by commercial active (beam trawl - TBB, otter trawl - OTB) or passive (gillnets - GTN, trammelnets - GTR) fishing gears. This was achieved by combining on-board vitality assessments with monitoring observations of skates held in captivity (min. 21 days). The focus was on four commercially important skates of the North Sea (ICES-area 4c) and English Channel (ICES-area 7d), i.e. thornback ray (*Raja clavata*, L.), blonde ray (*Raja brachyura*, L.), spotted ray (*Raja montagui*, Fowler) and undulate ray (*Raja undulata*, Lacepède).

Thirty-one trips were organized on-board of French, English and Belgian commercial vessels between July 2018 and January 2020. During these trips, biological parameters (e.g., length, sex, maturity, amongst others) were collected on-board and the condition of randomly selected individuals of skate species scored for their reflex responsiveness and visible bleeding injury ('vitality assessments'). Skates were picked from the beginning, mid- and end part of the catch sorting process on deck. Vitality assessments comprised out of attributing a generic vitality score (A = "excellent", B = "good", C = "poor" or D = "dead") to randomly selected individuals from the catch and scoring these for four reflexes and six types of injuries. Using the proportion of "dead" (vitality score D) individual, the immediate survival was calculated. Trips were spread out over the year to incorporate potential seasonal effects on discard survival. For French and Belgian trips, a subset of the vitality-scored rays was kept on-board and transported to onshore holding facilities for further discard survival monitoring during a period of 3 weeks (21 days). On-board of the vessel and during transport to the holding facility, rays were kept alive in custom-built monitoring units with recirculating seawater. During the monitoring period, dead rays were recorded and removed on a daily basis. During transport and monitoring, control rays were exposed to identical conditions as test rays to account for mortality caused by experimental procedures and holding conditions. At the end of the monitoring period, discard survival probability estimates were calculated based on the counts of surviving rays.

During UK trips on-board of gillnetters, only thornback and spotted ray were sampled. Of these, 54% were scored as "excellent" (vitality score A), 33% as "good" (vitality score B), 8% as "poor" (vitality score C) and 4% was dead at the time of assessment (vitality score D).

These rays were not monitored for discard survival onshore and hence no empirical discard survival estimates are available. For trammelnetters, 79% of all sampled skates was in “excellent” condition (vitality score A) and 21% was in a “good” condition (vitality score B). A marginal 0.4% was in a “poor” state (vitality score C) and none were dead at the time of assessment (vitality score D). The resulting average discard survival percentages were very high (>99% for all skates). The percentages sampled per vitality score for otter trawlers were: 48% for vitality score A (excellent), 28% for vitality score B (good), 19% for vitality score C (poor) and 5% for vitality score D (dead). Skates caught by beam trawlers were most often found in a “good” state (36% vitality score B), followed by individuals in a “poor” state (30%, vitality class C). Twenty-nine percent was scored as “excellent” (vitality score A) and 6% was dead (vitality score D). Hence, the two passive gears resulted in higher proportions (>54%) of skates in an excellent or good condition, compared to the active gears.

Total discard survival probability, calculated by using both the mean immediate and delayed survival estimates, for thornback and blonde rays discarded by beam trawlers were 54% and 67% respectively. For otter trawlers total survival estimates for thornback ray and blonde ray were 72% and 86%, respectively. For spotted ray and undulate ray caught by beam trawlers, total survival was 27% and 58% respectively. Sample sizes for spotted rays and undulate rays caught by otter trawlers and trammel netters were too low ( $n < 10$ ) to produce reliable survival ranges. Hence for these species-gear combinations, discard survival estimates should be interpreted with care, as these are based on limited numbers of observations per species. In conclusion, the results of the survival tests show that blonde ray survived best of all four species tested. Additionally, passive gears resulted in much higher survival rates compared to active gears.

For all four species tested, immediate and delayed discard survival seemed most strongly affected by fish condition (i.e. the combination of injury and reflex scores). Individual fish length also appeared to be an important factor for explaining immediate survival: larger skates have a bigger chance of immediate survival. Another important factor that contributed to immediate survival was sorting time. Hence, increasing the condition of caught rays and skates onboard could be achieved by technical (e.g. shorter sorting process) modifications. Finally, according to observer experiences, survival of skates could further be improved by a correct handling on board and a prompt release back to the sea when being discarded.

## 4 Introduction

In the North Sea, about ten skate species occur, as well as about ten demersal shark species (Daan et al., 2005). Rays and skates belong to the subclass of the Elasmobranchs (class *Chondrichthyes*) that are highly vulnerable to fishing and slow to recover from population depletion (Ellis et al. 2012). This is because their rate of reproduction is slow and their large size and aggregating nature makes them susceptible to capture (Ellis et al. 2012).

Most skates in the North East Atlantic region are primarily caught as bycatch in otter and beam trawl fisheries and are targeted with gillnets, trammel nets, longlines and by recreational anglers (ICES 2019). Managing skate stocks in this region is challenging, mainly for the following reasons:

- Many stocks are data-limited, meaning that landing and discard statistic are uncertain (ICES 2019). Therefore, stock advice is currently mostly based on analyses of survey trends and qualitative information;
- Different skates are pooled under one generic multi-species Total Allowable Catch (TAC), along with prohibitions for severely depleted, endangered and threatened species (ICES 2019). This TAC ignores, however, species-specific biological traits and comprises often species that may have very different vulnerabilities to exploitation. Furthermore, these TACs alone may not adequately protect these stocks as restrictive TACs may lead to high discarding;
- Landings of different species of skates are often misidentified by fishers and/or lumped together under one category (ICES 2019). Anecdotal evidence suggest that also landed and discarded skates are misidentified by seagoing observers.

Skates were phased in under the European landing obligation (LO) on January 1st, 2019 and given the disparity in quota and actual landings, it was expected that at least some species would become “choke” species in certain fisheries. This would result in an early depletion of the quota for skates, forcing fisheries to stop. As stated in STECF 2014 “Article 15 paragraph 2(b)” exemptions from the LO are possible for species for which "scientific evidence demonstrates high survival rates". This means that if robust scientific data demonstrates a high survival for a specific species, the species may get exempted from the LO.

In 2018, the EC approved a Joint Recommendation that was written by the advisory councils of the North Sea (NSAC) and the North Western Waters (NWWAC) to request a temporal exemption for skates based on preliminary discard survival data and estimates from the literature. This exemption was approved for a period of three years (starting January 2019, Commission Delegated Regulation (EU) 2018/2033).

As part of these temporal exemptions, the EC demanded concrete steps that would improve the current scientific knowledge of skates and rays. These steps were outlined in a document named “Roadmap for rays and skates”<sup>1</sup> including three main areas of work:

- Advancement of data, research and knowledge of discard survival for different ray and skate species, by métier and area by member states in cooperation with scientific bodies and institutions;
- Coordination of a program of measures to reduce discards and improve survival by the Advisory Councils (AC) of the regional groups;
- Coordination of work and following progress by the chairs of the regional groups, including taking of initiatives to ensure progress and facilitating cooperation and delivering of results.

Since its launch in 2017, the INTERREG 2-Seas SUMARiS (Sustainable Management of rays and skates) project has anticipated potential implications of the LO for rays and skates. The ultimate aim of the SUMARiS project is to propose a more sustainable and cross-border management strategy for rays and skates stocks. The second half of the SUMARiS project coincided with the timing of the Roadmap for rays and skates (2019-2021). Therefore, the project was well-suited to fill in important data gaps with regards to ray and skate discard survival. SUMARiS Work Package 2 (WP2) aimed to fill in some of these data gaps. The main goal of this work package was to quantify vitality, reflex impairment, injury and survival probability of four ray species discarded by English Channel and North Sea active and passive gear fisheries. To achieve this, the RAMP method was used (Davis, 2005, 2010; Davis and Ottmar, 2006). The RAMP method involves scoring for the presence or absence of reflexes and scoring the severity of different injury types to generate an index, which is then correlated with the observed survival probability of the same individual. Hence, a RAMP relationship links mortality to reflex impairment and injury (Davis, 2005, 2010). For sampling and scoring a protocol was developed taking into consideration guidelines of the ICES (International Council for the Exploration of the Sea) working group on Methods to Estimate Discard Survival (WGMEDS, ICES 2018a,b), as well as harmonizing as much as possible previously established assessment protocols for rays (P. Molenaar, pers. com.; D. Kopp, pers. com.; Catchpole, T.; pers.com.; Catchpole et al. 2017; Ellis et al. 2017). Besides these sources, this study took into account five guidance criteria required to produce a fishery representative estimate of discard survival (Catchpole et al. 2017), as well as best practices to undertake survival studies defined by STECF (STECF 14-19, STECF 17-16).

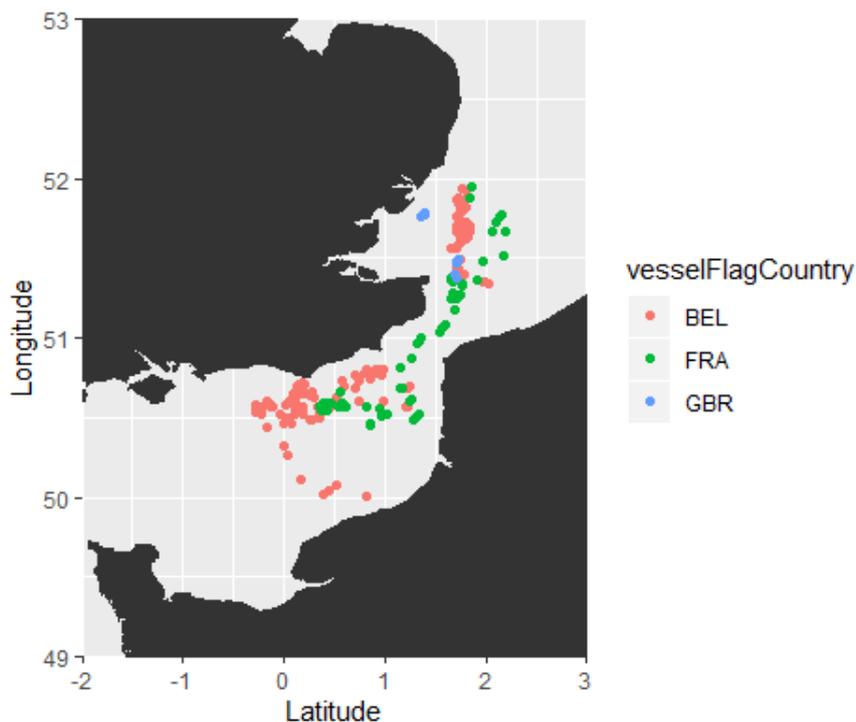
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<sup>1</sup> Version “DRAFT: 11 October 2018”. Available here: <http://nsrac.org/wp-content/uploads/2018/07/Paper-4.1-Draft-Roadmap-Skates-and-Rays-v1-For-Info.pdf>

## 5 Material and methods

### 5.1 Selection of vessels and hauls

Between July 2018 and January 2020, 31 commercial fishing trips were monitored in the English Channel (ICES sub-Division 7d) and Southern North Sea (4c, Figure 1) using conventionally configured otter trawlers (OTB; 1 participating Belgian and 1 French vessel), beam trawlers (TBB; 5 Belgian vessels), trammelnetters (GTR, 1 French vessel) and gillnetters (GTN, 3 UK vessels) (Appendix Table 1). The selection of vessels was opportunistic and dependent on availability and co-operation by the fishing industry. Trips were spread out over different months of the year to incorporate potential seasonal effects on discard survival. During each trip a trained observer collected data. Observers of each participating country were given an identical training<sup>2</sup> to sample, identify and score rays according to the SUMARiS protocol.



**Figure 1.** Geographic locations of sampled hauls trips by flag country of participating vessels: BEL = Belgium, FRA = France, GBR = Great Britain/UK.

During each trip, observers sampled as many hauls as logistically feasible. While from 1 out of every 3 hauls, rays were scored following the protocol outlined below (5.2.1 *Immediate survival assessment*), from the other 2 out of the 3 hauls, the observer measured the catch composition for all rays, including an estimation of the percentage sand and stones in the catch.

<sup>2</sup> See: SUMARiS Reflex Training tutorial on YouTube: [https://youtu.be/IDXZdhVnL\\_I](https://youtu.be/IDXZdhVnL_I)

## **5.2 Data collection and sampling protocol**

To quantify on-board conditions of captured skates (immediate survival assessment, see below “5.2.1 Immediate survival assessment”) and monitor their fate after release (in captivity; see below “5.2.2 Delayed, post-release survival assessment”), a sampling protocol was devised and seagoing observers instructed and trained on how to use it (SUMARiS Deliverable D 2.1.1). All data was recorded on fixed data sheet templates (i.e. “SUMARiS forms”), mirroring the same data entry fields that were devised for the SUMARiS database ([www.sumaris.net](http://www.sumaris.net), <https://github.com/sumaris-net/sumaris-app>).

Belgian observers also used electronic measuring boards linked to toughbooks according to their standard operating procedures<sup>3</sup>. Upon arrival at ILVO, this data was electronically transferred to the SmartFish database and consequently uploaded after checking into the SUMARiS database. French and UK observers used printed SUMARiS forms to manually input their observations from each trip. This data was consequently manually transcribed into the SUMARiS database upon arrival. A shared SUMARiS database<sup>4</sup> with fisheries biological data (WP1) and SUMARiS vitality and survival data (WP2) was developed as one of the outputs of WP1 (O 2.1). The database contains all data from SUMARiS trips, uploaded by each country (France, UK, Belgium). Data entry was done by an observer and/or scientist of each country. Consequently each trip was checked and validated by a second person in charge. By means of a data extraction module, all relevant data could be extracted in various formats for data-analyses.

### *5.2.1 Immediate survival assessment*

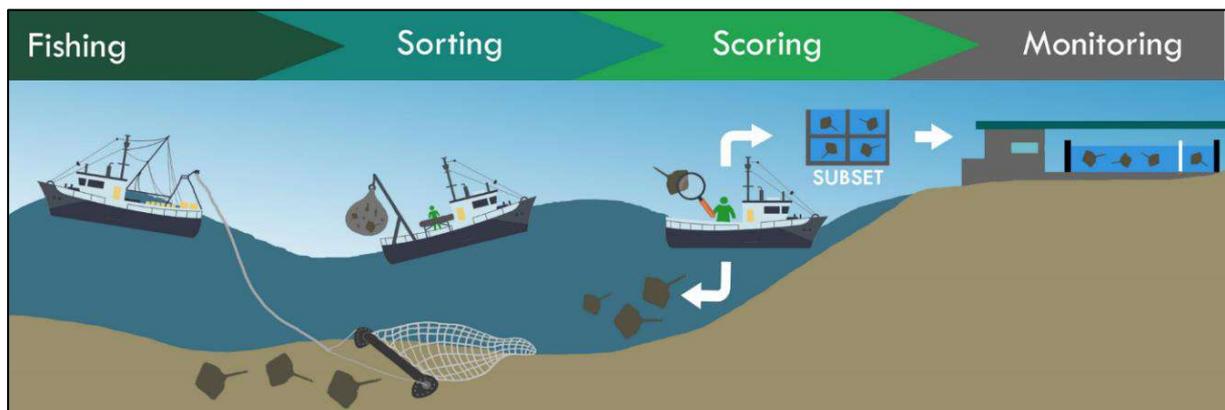
The immediate survival assessment on-board a vessel was divided into three steps: fishing (Step 1); sorting (Step 2); scoring (Step 3); which was then followed by monitoring for any delayed, post-release survival (Step 4, Figure 2, see section 5.2.2 ). Rays were caught using conventional fishing practices on-board of commercial vessels (Figure 2 - Step 1, Appendix Table 1). As soon as the catch was retrieved from the water and landed on deck, the sorting began. The observer logged the time and decided on a sampling strategy for (sub)sampling the catch as follows. The minimum number of individuals to sample for reflex responsiveness and injury (vitality) was 10 per haul for trawls and 5 per net for netters (if the catch allowed for this). During the sorting of the catch on-board of otter-and beam trawlers, when crew members separated marketable from unwanted catches (Figure 2 - Step 2), the observer evaluated the size of the catch of rays to decide on a sampling strategy.

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<sup>3</sup> More information: <http://www.smartfisheries.be/>

<sup>4</sup> More info: <https://github.com/sumaris-net/sumaris-doc/blob/master/user-manual/table-of-contents.md>

If the catch contained more than 20 skates (per haul), the observer randomly sub-sampled the catch, by picking a batch of between 5 and 10 skates and put them into dry baskets during the beginning, mid- and end phase of catch sorting. If the catch contained less than 20 rays, then the observer picked every individual for the immediate survival assessment. Before scoring each individual, the observer noted the time, so that the time each individual was exposed to air on deck could be calculated afterwards.



**Figure 2.** Schematic overview illustrating the different steps of data collection comprising of fishing, sorting, scoring on-board commercial fishing vessels and monitoring at shore-based monitoring facilities. Commercially caught-and-discarded skates were scored on-board for their vitality, reflex impairment, injuries and a subset of alive individuals (representing different species and vitality classes) was transported to shore to derive their post-release survival probability (Illustration credit Rens Hensgens & Laura Lemey).

For the immediate survival assessment, the number of dead rays was determined when landed on deck. A dead ray was unresponsive to any of the reflex tests and categorized as “D” for its vitality class (Table 1). Skates were scored for their responsiveness to reflex stimuli and any visible injury (Figure 2 - Step 3). The observer allocated each individual ray to a “vitality class” (Table 1), and subsequently scored the following four reflex responses: “Tailgrab”; “Startle touch”; “Spiracles”; and “Bodyflex” (Table 2); and six injuries (Table 3): bleeding injuries to the head, body and tail, open wounds and fin damage. A response to a reflex stimulus was scored as present (unimpaired, 0) when clearly visible, or absent (impaired, 1) when not visible, weak or in doubt, within 5 seconds of observation. The corresponding reflex impairment score (R&I index, see: 5.4 *Data analyses*) for each fish was calculated as the mean score of impaired reflexes and present injuries (score on a 4-point categorical scale) (Davis 2010, Uhlmann et al. 2016). Previously established reflexes<sup>5</sup> were evaluated and tested on different captive-held rays during a pilot-study (Appendix Figure 1). Based on these tests, four easy and unambiguous reflexes were selected (Table 2).

<sup>5</sup> Acquired from reports by CEFAS (2017, Tom Catchpole), IFREMER (Sonia Méhault, Dorothee Kopp, personal communication) and WMR (Edward Schram, Pieke Molenaar, personal communication).

**Table 1.** Vitality of rays was categorized on-board by using four ordinal scoring classes, ranging from “excellent” (= A) condition to “dead” (= D; following Benoît et al., 2010 Catchpole et al., 2017).

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

<sup>a</sup> Minor injuries were defined as ‘minor bleeding, or minor tear of mouthparts or wing ( $\leq 10\%$  of the diameter), or minor surface abrasion. <sup>b</sup> Major injuries were defined as ‘major bleeding, or major tear of mouthparts or wing, or major surface abrasion.

**Table 2.** Description of the four reflexes selected for scoring. These reflexes were first tested and confirmed among a different sample of captive-held rays to test whether each reflex shows a consistent and unambiguous response among rays that were not acutely stressed from prior fishing capture (Appendix Figure 1).

Reflex	Description	Unimpaired response
Tailgrab*	Gently grab 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 its 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

\*The reflex “Tailgrab” was tested on the ray in a seawater-filled reflex test box. The other reflexes were tested above the water.

**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 skin was removed and underlying tissue can be observed
Fin Damage	Areas of the fin that were damaged and/or split

For the injury assessment, the amount of surface coverage (% discoloration) of bleeding injury was scored along a four-point, categorical scale from 0 to 3 (following Uhlmann et al., 2016; Table 3). Absent discoloration was scored as “0”, <10% as “1”, between 10% and 50% as “2” and > 50% as “3”. Fin damage (any splits, where the tissue between fin rays was torn in) was assessed for the outer edge of the body wings (using the same 4-point categorical scale as above). Open wounds were scored separately from head body and tail along the same categorical scale, while looking at both the ventral and dorsal side of the body. Important biological data, such as length, sex and maturity were recorded as well.

### 5.2.2 *Delayed, post-release survival assessment*

Following on from the immediate survival assessment, a subset of scored and still alive rays was stratified randomly (to represent each species and vitality class) for (on-board) monitoring of any delayed survival in custom-built and water-filled monitoring units (~ 134 L per individual box ; Figure 3).



**Figure 3.** Left: design of the custom-built monitoring units used for Belgian trips (Illustration credit: Rens Hensgens and Laura Lemey); Right: monitoring units used in France (Nausicaa).

This sampling strategy was selected to make sure that individuals of all vitality classes were included for monitoring. However, the number of rays retained for monitoring was dependent on the available space on-board of the vessel to set-up monitoring units without compromising vessel and crew safety. A subset of ~10% of the vitality-scored rays were kept in individual monitoring units on-board of the vessel and transported to ILVO (Ostend, Belgium) or Nausicaa (Boulogne-sur-mer, France) for discard survival monitoring.

After a vessel arrived back in port, these rays were transported on the road (<1 hour) and transferred to captive holding facilities (i.e. aquaria) and monitored daily for 21 days. Aeration (and/or oxygen tablets) were used during road transport while keeping rays in stagnant (= no flow-through) water tanks. If rays were transferred from individual, on-board tanks to a larger tank in the van and/or shore-based facility, they were tagged individually. Tag-IDs were written down and linked to the numbered on-board monitoring units. Throughout the transport, extra care was taken to avoid any thermal shocks when transferring rays from unit to another and minimize any water spillage.

On-board and at the shore-based monitoring facilities, all rays were checked daily for their survival. Rays were fed daily *ad libitum* 5% of their body mass a mix of defrosted brown shrimp, worms, whiting and squid. Any dead individuals were removed immediately. In all water-filled units where rays were kept at any stage, water quality parameters (dissolved oxygen, salinity, temperature) were measured at regular intervals using a handheld YSI (Pro-2030) logger (*YSI-Xylem*) and ammonia was measured using PRODACTest kits for NH<sub>3</sub>/NH<sub>4</sub> (*Prodac International*). Holding conditions were designed and maintained to mimic the natural habitat of the skates as much as possible (e.g. water temperature, provision of sand on the bottom).

### 5.2.3 Controls

For the Belgian trips, thornback rays were collected during the Belgian beam-trawl-survey (September 2018) to determine the level of experimentally induced mortality. These rays were monitored in captivity alongside the rays that were caught-and-discarded. Rays that looked vital (strong reflex responses, none or minor injuries) were collected and taken ILVO for a 6-week acclimatisation period. During this time, no mortalities occurred. For the French trips, no control rays were used during the first trip in July 2018. Three surviving test (thornback) rays were used as control rays for the second trip in October 2018. Control rays were held in the same holding tank as the test rays during 21 days of captive observation and subjected to the same procedures as the test rays. For each new (Belgian or French) trip three to five control rays were used. Controls were replaced in between trips or when dead.

## 5.3 **Ethical statement**

During this study all experimental work was in accordance to scientific permits of Nausicaa and ILVO. The relevant maritime authorities of France and Belgium issued further permits to keep undersized alive fish on-board and bring a subset onshore. The treatment of the fish was in accordance with the Belgian and French animal experimentation act and was approved by the ILVO ethical committee (ILVO-EC License number: 2018 323).

## 5.4 Data analyses

For the statistical analyses, data from all uploaded trips was downloaded from the database as csv-files at different level of aggregation per trip, haul and individual ray:

- 1) *Trip data* (TR): contained technical parameters about each trip (e.g., vessel, departure-and arrival times, amongst others);
- 2) *Haul data* (HH): technical parameters related to each haul;
- 3) *Individual ray data* (ST): vitality, injury and reflex scores and survival parameters of each assessed, individual ray.

TR, HH and ST files were merged in R statistical software ([www.r-project.org](http://www.r-project.org)). An exploratory analysis was done first following the protocol of Zuur et al. (2007) to check for any outliers, contrasts in the explanatory variables, confounding effects of research-related handling and any correlative relationships among explanatory variables.

Three quantitative indices were calculated from the individual reflex and injury scores for each individual ray: a reflex impairment index (R-index), injury index (I-index) and reflex impairment and injury index (R&I-index). The R-index was calculated as the mean of all impaired reflexes of each individual ray. The same was done for the injuries (I-index), which had first been converted from a four-point scale to binary scores (0 and 1). The R&I-index was the mean of impaired reflexes and present injuries together. The relationship between vitality class and R, I and R&I- indices was explored statistically. Immediate survival (per species and gear) was calculated by summing up the number of individuals assessed as dead (vitality score =D) over the total number of vitality-assessed individuals per haul. Delayed, post-release survival was calculated using the estimated marginal means (“emmeans” function in R) of a mixed logistic regression model (“glmer” function in R) with “trip-ID” as a random factor and “gear type” x “species” interaction. Delayed survival was corrected for differences in vitality class (A-D) distributions between the immediate mortality sample size (i.e., all individuals assessed onboard) and delayed survival sample size (i.e. the subset of monitored individuals). This was done because proportionately more excellent fish (vitality score A) were monitored than present with the population of fish.

Survival curves, showing species-specific delayed survival over the duration of the monitoring period (21 days or more) were estimated using the non-parametric Kaplan-Meier estimator (Kaplan and Meier, 2012) using the function “survfit” from the R-package “survival”.

Total survival was calculated by taking the mean immediate and (weighed) delayed survival estimates of all trips into account using the following formula:

$$\text{Total survival} = 1 - \text{Total mortality} = \text{Immediate mortality} + (1 - \text{Immediate mortality}) * \text{Delayed mortality}$$

To explore which variables contributed significantly to both immediate and delayed survival estimates (uncorrected for the high proportion of monitored fish in excellent condition), mixed logistic regression models were fitted to immediate and delayed survival status for different technical (e.g. sorting time), environmental (e.g. water depth), and biological variables (e.g. individual length), with “trip” as a random effect. The selection of these variables (Appendix Table 3) was based on contributing factors from previous studies (Depestele et al. 2014, Schram & Molenaar 2018) as well as inspection of the completeness and accuracy of the available collected data for each variable. In a first step, explanatory variables were checked for multicollinearity via a correlation matrix (correlograms, “corrgram” package in R). In a second step, boxplots were made for immediate and delayed survival per explanatory variable to explore relationships. Together with this, different mixed logistic regression models for each explanatory variable were run with or without “species” as interaction, for immediate and delayed mortality as response variables. If interactions were not significant, based on the Type-III p-values from Wald Chi-square test (“Anova” function, “car” package), then the interaction effect was left out. In a third step, different mixed logistic regression models were tested for immediate and delayed mortality, by adding all the significant explanatory variables from the previous exercise in the equation. Via a backward selection procedure using the Akaike Information Criterion (AIC), non-significant variables were excluded at each step. Given a set of candidate models for the data, the preferred model was the one with the lowest AIC value (Zuur et al., 2007).

## 6 Results

### 6.1 Description of the fisheries

Cooperating commercial fishing vessels varied considerably between countries in general characteristics (e.g. vessel length, engine power and overall tonnage, Appendix Table 1). The Belgian otter trawler and beam trawlers in this study used a standard mesh size of 80mm. The French otter trawler and trammelnetter deployed nets with mesh sizes of 80mm and 90mm, respectively (Appendix Table 1). All three UK gillnetters used a mesh size of 100mm (Appendix Table 1).

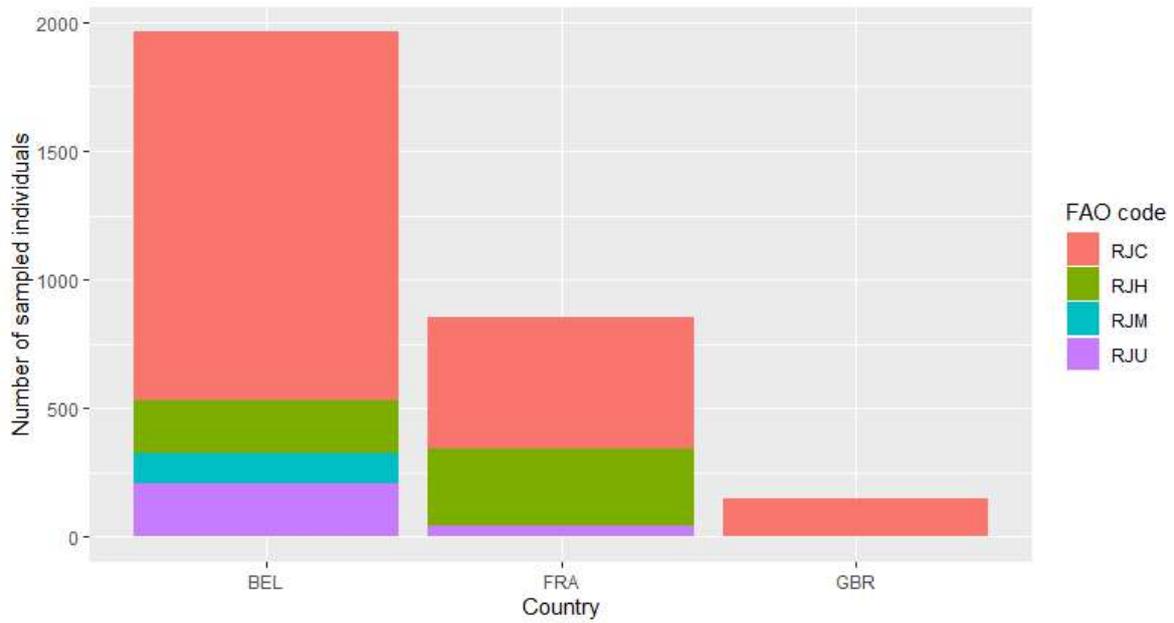
Out of the total of 31 trips, 10, 13 and 8 trips were done by Belgian, French and British observers, respectively (Table 1-2, Appendix). French and British observers scored on average 120, 66, 24 rays on-board and registered an average landing weight per trip of 114, 258, and 34kg respectively. For passive gears (GTR and GTN), the average sorting time was  $47 \pm 27$  minutes. For active gears (TBB and OTB), average sorting time was lower  $23 \pm 12$  (Table 2, Appendix). Average fishing time (or soaking time), defined as the time during which the fishing gear was in the water, was calculated as the difference between the start time of hauling and the start time of shooting the fishing gear. Average fishing time was highest for trammelnetters (1031 min  $\sim$  17h), followed by otter trawlers (179 min  $\sim$  3h), beam trawlers (104 min  $\sim$  1h45) and gillnetters (76min  $\sim$  1h15).

### 6.2 Immediate survival assessment

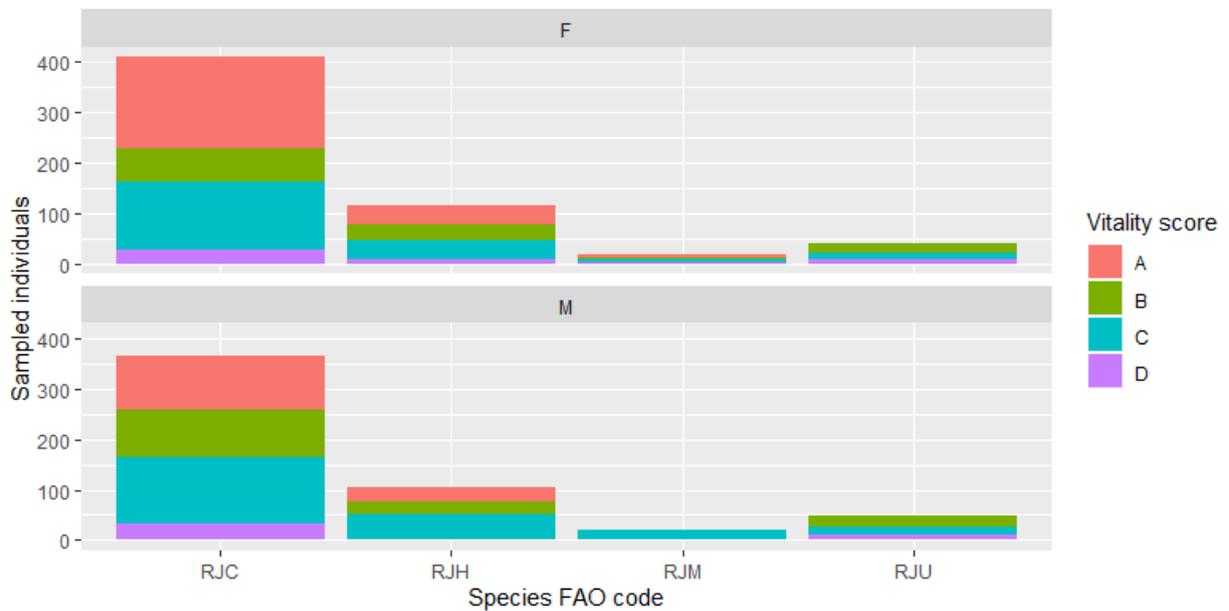
#### 6.2.1 *General observations*

During all monitored trips, a total of 3006 skates were assessed on-board for vitality, reflexes and injury. Of the four species, thornback ray was the most sampled overall and within each country (BEL: 73.1%, FRA: 60%, UK: 98%, Figure 4). The second most abundant sampled species was blonde ray (16.7%), followed by undulate ray (8.3%) and spotted ray (4.1%). Overall, the majority of all rays sampled were in excellent condition (vitality class A, Figure 4) with similar distributions over the four vitality classes for both sexes (Figure 5).

Table 4 shows the distribution of individuals over the four vitality classes (A, B, C, D) among the four gears and species. When taking all species into account, Table 4 shows that most individuals caught by trammel-and gillnetters and otter trawlers are in “excellent” condition (>48%). Beam trawlers have the highest percentages of individuals in classes C (“poor”) and D (“dead”) compared to the other gears.



**Figure 4.** Total number of vitality-scored individuals per species summed across all trips done per partner country. FAO-codes: thornback ray – RJC; blonde ray – RJH; spotted ray – RJM; undulate ray - RJU; and country BEL = Belgium, FRA = France, GBR = Great Britain/UK.



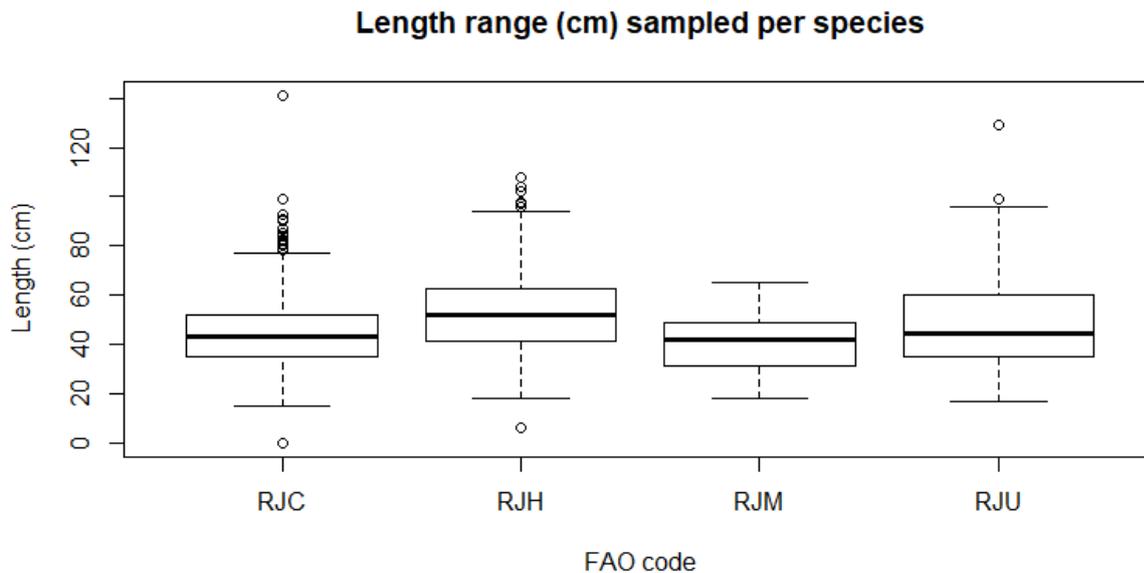
**Figure 5.** Distribution of vitality classes (A, B, C, D) among all scored individuals per species pooled across country.

**Table 4:** Percentage (%) of sampled individuals per vitality class (A, B, C, D), species and gear. n = sample size.

NA = none sampled.

Species	Gear	% "Excellent" (A)	% "Good" (B)	% "Poor" (C)	% "Dead" (D)
RJC	GTR	55 (n=171)	33 (n=48)	8 (n=1)	4 (n=1)
	OTB	46 (n=490)	28 (n=300)	20 (n=212)	6 (n=9)
	TBB	27 (n=177)	36 (n=233)	33 (n=214)	4 (n=28)
	GTN	78 (n=104)	22 (n=62)	0 (n=16)	0 (n=8)
RJH	GTR	83 (n=19)	17 (n=4)	NA	NA
	OTB	53 (n=149)	31 (n=87)	16 (n=44)	1 (n=2)
	TBB	30 (n=58)	37 (n=73)	28 (n=54)	6 (n=11)
	GTN	NA	NA	NA	NA
RJM	GTR	100 (n=1)	NA	NA	NA
	OTB	52 (n=11)	29 (n=6)	19 (n=4)	NA
	TBB	37 (n=36)	30 (n=29)	31 (n=30)	3 (n=3)
	GTN	33 (n=1)	0.67 (n=2)	NA	NA
RJU	GTR	100 (n=7)	NA	NA	NA
	OTB	62 (n=21)	21 (n=7)	18 (n=6)	NA
	TBB	28 (n=58)	41 (n=85)	21 (n=44)	10 (n=21)
	GTN	NA	NA	NA	NA
All species pooled	GTR	<b>78.9 (n=198)</b>	<b>20.7 (n=52)</b>	<b>0.4 (n=1)</b>	<b>NA</b>
	OTB	<b>47.7 (n=671)</b>	<b>28.4 (n=400)</b>	<b>18.9 (n=266)</b>	<b>5.1 (n=71)</b>
	TBB	<b>28.5 (n=329)</b>	<b>36.4 (n=420)</b>	<b>29.6 (n=342)</b>	<b>5.2 (n=63)</b>
	GTN	<b>54.4 (n=105)</b>	<b>33.2 (n=64)</b>	<b>8.3 (n=16)</b>	<b>4.2 (n=8)</b>

Average lengths of sampled individuals ranged around the minimum landing sizes of the three countries involved (MLS; in France = 45cm, Belgium= 50 cm, UK = 40cm). Blonde rays were on average the largest sampled species (53±16.7) followed by undulate ray (49.3±23.5cm), thornback ray (45±13.6cm). Sampled spotted rays had the lowest average length (40.2±10.8cm, Figure 6).



**Figure 6.** Length (in cm) of all vitality-tested rays per species (pooled across countries) between the 10th and 90th percentile (FAO-codes: RJC, RJH, RJM, RJU). Line in middle is the median.

Overall, immediate survival estimates were very high (>90%) for all species and gear combinations (Table 5). Sample sizes to determine immediate survival for each species and gear combination vary significantly (“n”, Table 5). In some cases sample sizes were too low (e.g. for RJM x GTR and RJU x GTR) to provide a robust estimate.

**Table 5.** Overview of the proportion of individuals (immediate survival) of skates landed alive on deck after gear retrieval. Immediate survival expressed in % on-board for each ray species (FAO-codes: RJC, RJH, RJM, RJU) and gear (GTN, GTR, OTB, TBB) combination, n = sample size.

FAO-code	Gear	n	Immediate survival (%)
RJC	GTN	190	95.79
	GTR	220	100
	OTB	1071	93.56
	TBB	652	95.71
RJH	GTR	23	100
	OTB	282	99.29
	TBB	196	94.39
RJM	GTN	3	100
	GTR	1	100
	OTB	21	100
	TBB	98	96.94
RJU	GTR	7	100
	OTB	34	100
	TBB	208	89.9

The relationship between the quantitative vitality indices (R-index, I-index and R&I index) and vitality class (A, B, C, D) was explored through boxplots (e.g. Figure 2-3 Appendix). From these boxplots, strong patterns could be observed: skates that had been classified as less vital or moribund (vitality classes C and D respectively) showed more impaired reflexes and injury (Figure 3 Appendix).

### 6.2.2 UK

A total of 190 thornback rays (average size 44.43 cm  $\pm$  14.80 SD) and 3 spotted rays (average size 40.67 cm  $\pm$  4.51 SD) were vitality-assessed during eight UK trips with gillnetters in the period August 2018- December 2019. Of both species, 54% were scored as “excellent” (vitality class A), 33% as “good” (vitality class B), 8% as “poor” (vitality class C) and 4% was dead at the time of assessment (vitality class D). Hence, immediate survival on-board of English gillnetters was 96%, meaning that the majority of the sampled thornback rays was alive at the time of assessment on-board. Skates were not further monitored for discard survival.

### 6.2.3 France

A total of 852 skates were assessed during French trips, of which 510 thornback rays (average length 54.06  $\pm$  15.07 SD), 299 blonde rays (60 cm  $\pm$  15 cm SD), 3 spotted rays (48 cm  $\pm$  5 cm SD), 41 undulate rays (45 cm  $\pm$  8 cm SD). Among these 55% were found in excellent condition (vitality class A) and 28% in good condition (vitality class B). Fifteen percent was scored as “poor” (vitality class C) and overall immediate survival was 98%. However, there was a visible difference between the two types of gears used (i.e. trammel nets vs otter trawling). The large majority of rays caught by trammel netters were found in excellent condition (vitality class A, 79%), 21% were in “good” condition (vitality class B). Only 0.4% of the picked individuals were scored as “poor” (vitality class C) and none were dead (vitality class D). For otter trawlers, percentages of “poor” and “dead” individuals were higher, i.e. 21% and 3%, respectively. A total of 161 rays were brought to shore-based aquaria of Nausicaa (Boulogne-sur-mer, France). The majority of the monitored individuals were thornback rays (67%) followed by blonde ray (25%) and only occasionally of undulate and spotted ray.

### 6.2.4 Belgium

A total of 1961 skates were assessed for vitality, injuries and reflexes on-board by Belgian observers, of which 1433 thornback rays (average size 41.78  $\pm$  11.14 SD), 203 blonde rays (average size 42.46  $\pm$  12.83 SD), 117 spotted rays (average size 39.97  $\pm$  10.92 SD), 208 undulate rays (average size 50.82  $\pm$  25.36 SD). Hence, thornback ray was the most represented species accounting for 73.1% of the total number of vitality-assessed rays, followed by blonde and undulate ray in equal percentages (~10% each). Only 6% of the total sampled population consisted out of spotted rays.

Among the scored rays belonging to the four focus species, 37.2% were found in excellent condition (vitality class A) and 32.13% in good condition (vitality class B). Twenty-five % belonged to vitality class C (poor condition) and overall immediate mortality was 6% for both gear types. For beam trawlers, there were less individuals in “excellent” condition (vitality class A, 28.5%) and more individuals in “good” condition (vitality class B, 36.4%) compared to otter trawlers. Almost 30% was in a “poor” state (vitality class C) and 5.5% was found dead (vitality class D). A total of 143 rays were brought onshore for a monitoring period of 21 days at the aquaria facilities of ILVO (Ostend, Belgium). Most (44.1%) of the monitored individuals were thornback rays, followed by spotted ray and undulate ray (both 19.58%) and blonde ray (16.78%).

### 6.3 Delayed, post-release survival assessment

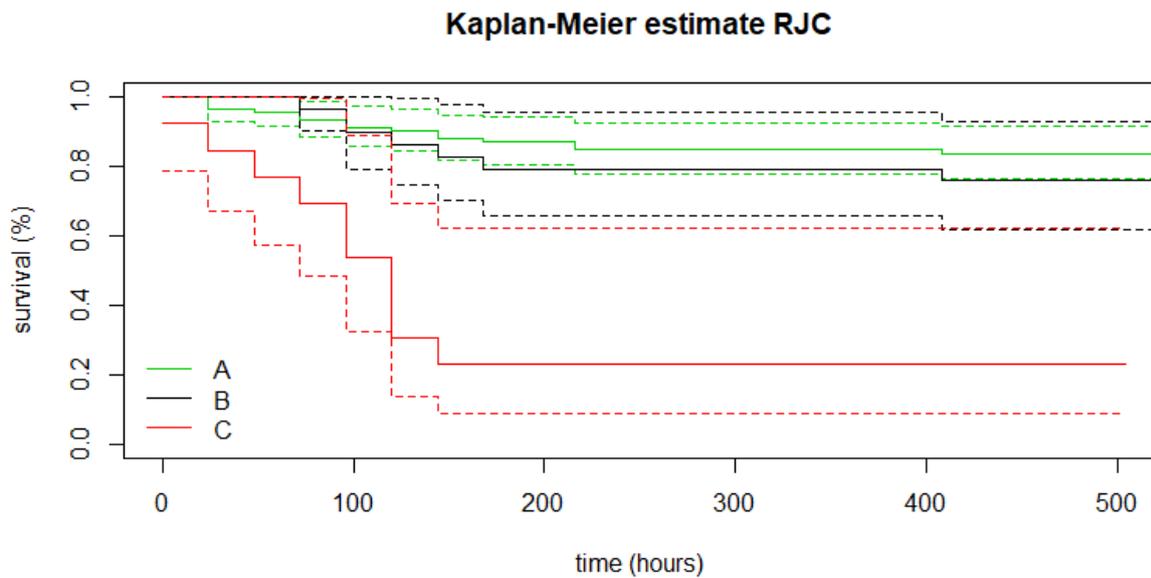
Delayed survival was defined as the number of monitored individuals that survived the monitoring period of 3 weeks (21 days ~ 504 hours). Delayed survival estimates (Table 6) were derived from a mixed logistic regression model in which vitality class and gear type were used as factors. A correction (weighing) was made for the unequal distribution of individuals over vitality classes for immediate and delayed mortality sample sizes (n). Sample sizes of individuals that were monitored for delayed mortality deviate for the different species and gear combinations (Table 6). For spotted ray and undulate ray caught by trammel netters and otter trawlers sample sizes were too low to provide a robust estimate (Table 6).

**Table 6.** Weighed delayed survival estimates (in %) after 3 weeks of monitoring for each ray species (FAO-codes: RJC, RJH, RJM, RJU) and gear (GTN, GTR, OTB, TBB) combination, n = sample size. \* Low sample sizes.

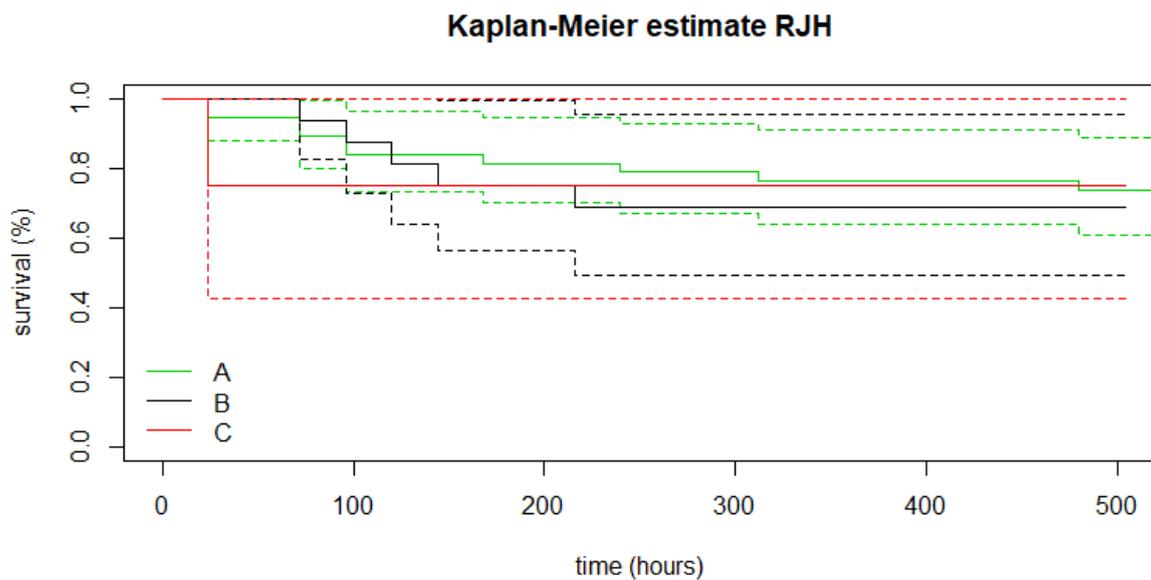
FAO-code	Gear	n	(Weighed) Delayed survival (%)
RJC	GTR	42	93.35
	OTB	71	76.5
	TBB	21	56.9
RJH	GTR	1	100*
	OTB	37	87
	TBB	20	70.5
RJM	GTR	1	94.75*
	OTB	2	100*
	TBB	25	27.4
RJU	GTR	5	98.85*
	OTB	5	26.4*
	TBB	27	64.4

As Kaplan-Meier curves for thornback and blonde ray show (Figure 7 and 8, respectively), the long-term monitoring period of three weeks allowed mortalities to reach asymptote, until all discard related mortalities had been observed.

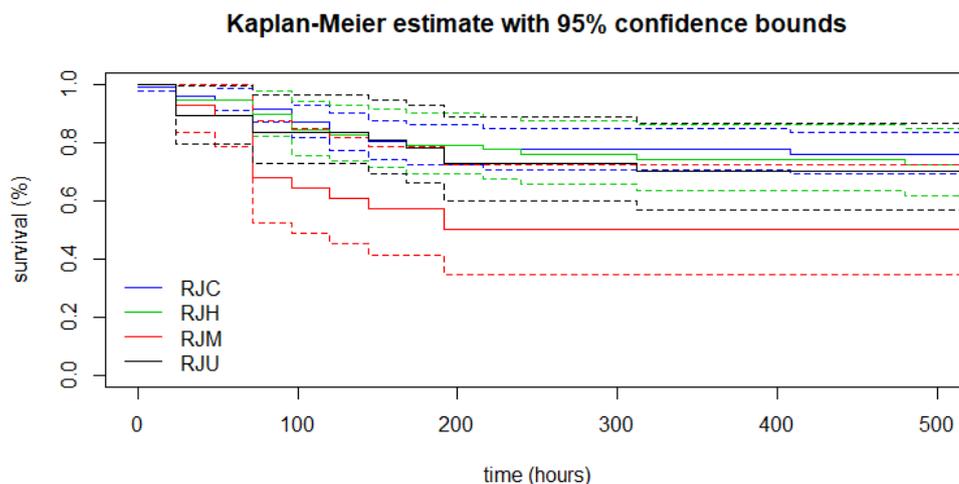
In some cases rays were monitored for longer times (> 504 hours) before their release, depending of available space in the aquaria facilities. For thornback ray, the Kaplan-Meier curves show a lower survival for vitality class C, compared to A and B (Figure 7-8). When comparing the four species, blonde ray achieved the highest survival, followed by thornback ray, undulate ray and lastly spotted ray (Figure 9).



**Figure 7.** Non-parametric Kaplan Meier survival curves of thornback ray (RJC) over hours of monitoring per vitality class (A = green, B = black, C = red). Pooled across all gears and countries.



**Figure 8.** Non-parametric Kaplan Meier survival curves of blonde ray (RJH) over hours of monitoring per vitality class (A = green, B = black, C = red). Pooled across all gears and countries.



**Figure 9.** Non-parametric Kaplan Meier survival curves of thornback ray (RJC, blue), blonde ray (RJH, green), spotted ray (RJM, red) and undulate ray (RJU, black) within 95% confidence limits.

#### 6.4 Total survival

Total survival estimates for all species tested were extremely high for trammel netters (GTR, Table 7) resulting in total survival estimates of 99-100%. For active gears (OTB, TBB), average total mortalities vary significantly according to species and gear combination. For thornback ray caught by active gears (TBB and OTB), total survival ranged between 54% (TBB) and 72% (OTB, Table 7). Blonde ray had an even higher survival range of 67-86% (Table 7). For spotted ray and undulate ray caught by beam trawlers, total survival was 27% and 58% respectively.

**Table 7.** Total survival (in %) after 3 weeks of monitoring for each ray species (FAO-codes: RJC, RJH, RJM, RJU) and gear (GTN, GTR, OTB, TBB) combination, n(imm.) = all vitality-assessed individuals, n(del.)= monitored individuals. \* Low sample sizes.

FAO-code	Gear	n(imm.)	n(del.)	Total survival (%)
RJC	GTR	220	42	99.34
	OTB	1071	71	71.56
	TBB	652	21	54.46
RJH	GTR	23	1	100.00*
	OTB	282	37	86.36
	TBB	196	20	66.58
RJM	GTR	1	1	100.00*
	OTB	21	2	100.00*
	TBB	98	25	26.55
RJU	GTR	7	5	100.00*
	OTB	34	5	92.64*
	TBB	208	27	57.86

## 6.5 Controls

Survival of control rays ranged between 88.5% and 90% for individuals maintained at Nausicaa and ILVO, respectively (Table 8). Of these fish, only 10% died at ILVO and 11.5% at Nausicaa. About ten opportunistic post-mortem analyses were conducted on rays that died during the monitoring period at the Nausicaa facilities. The majority of these dead rays showed various injuries (haemorrhages) around the head, body, tail parts of the body. For some rays other observations were noted, such as a loss of weight, infections and a buildup of fluid in the abdomen.

**Table 8.** Overview of the number of control rays per trip for France and Belgium.

Country	TripCode	Arrival date	FAO-codes	Total per trip	Mortality
France	1	NA	NA	0	NA
	2, 3	28/09/2018	RJC	3	0
	4, 5	19/10/2019	RJC	3	0
	6	27/11/2019	NA	0	NA
	102	15/02/2019	RJC	3	2
	110	5/04/2019	RJC, RJH	3	0
	111	17/04/2019	RJC	3	0
	113	3/05/2019	RJC, RJH	3	0
	114	14/05/2019	RJC	2	1
	152	12/06/2019	RJC	3	0
	153	21/06/2019	RJC	3	0
			<b>Total</b>	<b>26</b>	
			<b>Survival (%)</b>	<b>88.5</b>	
Belgium	53	16/10/2018	RJC	5	0
	54	16/12/2018	RJC	5	0
	57	18/1/2019	RJC	5	0
	63	3/3/2019	RJC, RJH	5	2
	108	28/3/2019	RJC, RJH, RJM	5	0
	109	6/6/2019	RJC, RJH, RJM, RJU	5	1
	203	22/10/2019	NA	0	NA
	204	8/11/2019	NA	0	NA
	205	14/11/2019	NA	0	NA
	206	14/01/2020	NA	0	NA
			<b>Total</b>	<b>30</b>	
		<b>Survival (%)</b>	<b>90</b>		

## 6.6 Factors influencing survival

Explanatory variables (Table 3 Appendix) were checked for collinearity via a correlation matrix. From this matrix, it appeared that all vitality indices (vitality class, R-score, I-score and R&I score), as well as the variables “sorting time” and “air exposure time”, were highly correlated to one another. Multiple variables came forward as important (i.e. significant p-value) after running boxplots and mixed logistic regression models per variable, including or without species as an interaction factor (Table 4 Appendix). In a next step, this selection of variables was used as an input for a new series of logistic regression models for immediate and delayed mortality. Via a backward selection procedure using AIC, non-significant variables were excluded at each step. The models that were applied for delayed mortality as a response variable were based on delayed mortality estimates that had *not* been corrected (weighed) for differences in vitality class (A,B,C,D) distribution of the population of individuals for which immediate mortality was assessed.

The most parsimonious models for immediate and delayed mortality are shown in Table 9. For immediate mortality, the variables “R&I score” and “Total Length” and “Sorting Time” were most significant ( $p$ -values  $< 0.001$ , Type III Wald Chi-square tests, Table 9). The model predicted a negative estimate for total length which means that larger individuals have a bigger chance of immediate survival compared to small individuals. In the model for delayed mortality only R&I score was significant ( $p < 0.05$ ): an individual with a higher R&I score had a higher chance of dying (Figure 10).

**Table 9.** Model equations for immediate and delayed mortality including their respective AIC’s and p-values. \* = significant ( $p < 0.05$ ), \*\* = very significant ( $p < 0.001$ )

Model	AIC	p-values	
Imm. Mort. ~ glmer(substrateType + R&I index + lengthTotalCm + landingWeight + sortingTime (1   tripCode)), family=binomial)	351.88	Substrate type	0.2397
		R&I score	$< 2.2 \cdot 10^{-16}$ **
		Total length	$1.54 \cdot 10^{-10}$ **
		Landing weight	0.9768
		Sorting time	0.0007 **
Del. Mort. ~ glmer(Gear category+ R&I index + sorting time +(1   tripCode)), family = binomial)	163.01	Only R&I-score significant (0.01873*)	



**Figure 10.** Relationship between R&I-score (RAMPINJ.score, y-axis) and delayed mortality (0 = alive or 1 = dead).

## 7 Discussion & conclusions

Previous estimates of discard survival of rays and skates of the North-East Atlantic covered only a limited number of métiers, areas and species. Additionally, factors that influence survival are poorly understood, making extrapolation across species, fisheries and areas challenging (STECF 2017). While it has been assumed that discards survival for rays and skates could be quite high, specific estimates for different species and gears are still lacking (STECF 2017). To fill in some of these important knowledge gaps, this study aimed to provide results on the discard survival of four ray species: thornback ray (*Raja clavata*), blonde ray (*Raja brachyura*), spotted ray (*Raja montagui*) and undulate ray (*Raja undulata*) caught and discarded by commercial fishing vessels in the North Sea (4c) and English Channel (7d). Immediate survival (on-board) and delayed survival (after 3 weeks of monitoring in a captive environment) were determined to calculate total survival. Additionally, different environmental, biological and technical parameters were collected during the trips to determine factors that might possibly influence the survival of rays.

Immediate survival rates varied depending on the ray species and gear between 96-100% for passive gears and between 90-100% for active gears. Previously aggregated data for all skate species also indicated low immediate mortalities, ranging around 0-2.35% for otter trawlers and between 0-6.16% for netters (Ellis et al. 2018). A study by Mandelman et al. (2013) showed negligible immediate mortalities (<1%) for *Rajidae* caught by otter trawlers in the West North Atlantic fishery.

While immediate survival was generally high for all gear and species combinations in the SUMARIS study area, delayed and total survival estimates are much lower. Our study showed survival probabilities for thornback ray caught by active gears (i.e. beam trawlers and otter trawlers) between 54% and 72%, respectively. These estimates are in line with earlier estimates from Catchpole et al. (2017; 57-79%) and Schram & Molenaar (2018; 53%). Depestele et al. (2014) observed higher survival estimates for *Rajidae* caught during scientific beam trawl surveys (fisheries independent surveys) in the North Sea, i.e. 72-77%. However, survival may have been overestimated due to the shorter haul durations (average = 92min ± 12min) and reduced monitoring period (~80 hours, Depestele et al. 2014).

In this study, blonde rays exhibited the highest survival: 67% in the case of beam trawl and up to 86% for otter trawl. Survival estimates for blonde ray caught by beam trawlers in the Western English Channel range between 21-67% (Ellis et al. 2012) and modelled results point to 41-44% survival (Catchpole et al. 2017). Estimates for blonde rays caught with fishing methods designed to minimize stress onboard of otter trawls in the Bristol Channel were 92%, however, these are likely to be overestimated (Catchpole et al. 2017).

Another study reported survival estimates between 55-67% for blonde ray caught by otter trawlers (Enever et al. 2009), although for the latter study it should be noted that discard survival was not monitored until asymptote (Rihan et al. 2019).

Within the SUMARiS project, undulate rays caught by beam trawlers survived quite well (58%). Previously reported estimates for undulate ray caught by beam trawlers are high (~80%) (Ellis et al. 2012; Bird et al. 2018; Randall et al. 2018). In the case of otter trawlers and trammelnetters, insufficient numbers of undulate ray were caught and monitored to reliably estimate longer-term survival. The second underrepresented species within the SUMARiS project, spotted ray, showed a lower survival rate after being caught by beam trawlers (27%) compared to the other three species. Earlier estimates of survival for spotted ray range between 40-67% for beam trawlers in the Western English Channel (Ellis et al. 2012) and 21-67% for spotted rays caught by pulse trawlers in the North Sea (Schram & Molenaar, 2018). It is recommended to collect more data for spotted rays in the future in order to narrow down their survival ranges and provide more precise estimates for survival probability.

In conclusion, within the SUMARiS-project, the highest survival percentages were obtained for blonde ray, followed by thornback ray and undulate ray, and finally by spotted ray. Previous literature indicated thornback ray as the species with the highest probability of survival, because of the physical protection offered by the more accentuated spinulose skin (Ellis 2018). Additionally, beam trawlers have been suggested one of the most impacting gears because of the use of tickler chains or chainmats, resulting in a worse condition of the caught fish (Ellis et al. 2017, van Beek et al. 1990). This is confirmed in our results, where beam trawlers result in lower survival percentages compared to all other gears.

As the Kaplan-Meier curves show, the long monitoring period of three weeks allowed mortalities to reach asymptote, until all discard related mortalities have been observed. However, captive observations did not include the effect of predation or other causes of “natural mortality” (e.g. infections). Therefore, the discard mortality rates presented in this study are likely underestimated (Enever et al. 2009, Catchpole et al. 2017). To prove that observed discard mortalities occurred because of the fishing process, rather than confinement effects, control rays were used that underwent similar conditions as the test fish. Of these control population, a survival of 90% and 88.5% was obtained at ILVO and Nausicaa, respectively. This corresponds to average survival percentages of control fish found in the discard survival study by Schram & Molenaar (2018), i.e. >90%.

Logistic regression models were used to investigate the role of technical, environmental and biological variables in determining discard survival. In concordance with previous studies, the results of our models highlight the strong relationship between fish condition (the combination of injuries and reflexes i.e. R&I index) and delayed mortality.

Similar to previous studies (Depestele et al. 2014) the individual length of the fish appeared to play an important role in immediate survival. In the study by Depestele et al. (2014) the most parsimonious model contained length and injuries as variables, showing that survival probability was significantly higher for large and less injured skates. Additionally, Ellis et al. (2018) reported that skates smaller than 50 cm were more likely to die immediately after arrival on board. Furthermore, the variables “haul duration” and “air exposure” time have been put forward as significant factors in explaining survivorship in previous research with rays and skates (Enever et al. 2009, Mandelman et al. 2013). These factors, however, did not turn out to be significant during our modelling exercises. For immediate survival of skates, sorting time was important as well. Shortening the sorting procedure may therefore result in a better immediate survival of skates.

At the end of all SUMARiS trips, observers were asked to fill in some general questions about their personal experiences on-board of commercial vessels through a small questionnaire. The questionnaire showed that most observers find thornback ray, followed by blonde ray, to be the most abundantly caught species in the English Channel (7d) and southern North Sea (4c). Observers indicate thornback ray as the “most robust” species (i.e. with the highest chance of surviving the discarding process). To increase the survival of rays, observers recommend using shorter tow durations, selectivity devices or benthos release panels in the nets (incl. the removal of stones, sand or benthos), water provision during the sorting process and a quick release when being discarded. It was commonly agreed that it remains important to further encourage good practice on fish handling onboard to maximally increase the discard survival of skates.

## 8 Acknowledgements

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10 Appendix

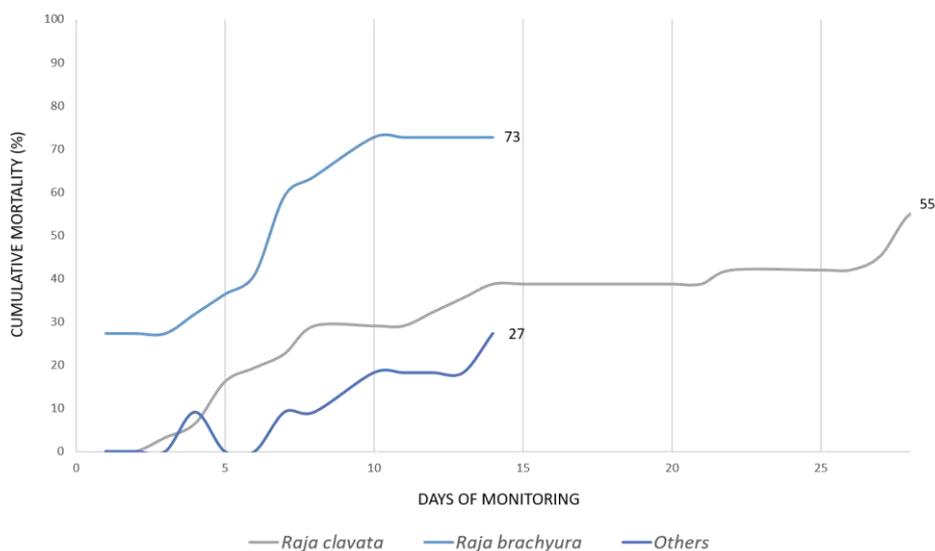
## 10.1 Overview vessel characteristics per country

**Table Appendix 1:** Main characteristics of anonymized participating Belgian, French and UK vessels. Gear types: OTB = otter trawler, GTN = gillnetter, GTR = trammelnetter, TBB = beam trawler.

	Country									
	Belgium					France		UK		
Vessel code	BE1	BE2	BE3	BE4	BE5	FR1	FR2	UK1	UK2	UK3
(Arrival) harbour	Ostend (Belgium)					Boulogne-sur-mer (France)		Ramsgate (UK)		
Gear	OTB SOL	TBB	TBB	TBB	TBB	GTR	OTB	GTN	GTN	GTN
Vessel length (m)	25.9	38	24	24	32.5	11.92	22.5	9.9	9.82	9.9
Engine power (kW)	518	960	221	221	772	162	552	127	89	112
Overall tonnage	207	385	130	130	223.62	19.6	102.49	10.13	7.1	7.85
Mesh size (mm)	80	80	80	80	80	90	80	100	100	100

## 10.2 Establishing candidate reflexes

To test and confirm previously established candidate reflexes obtained (Davis 2010, Catchpole et al. 2017; D. Kopp, pers. comm.; P. Molenaar, pers. comm.) 63 rays (mean length:  $45.81 \pm 8.62$  cm) belonging to different species were caught with a beam trawl during an ILVO-scientific survey in the North Sea in February. The catch was composed of 31 thornback rays (*R. clavata*), 22 blonde rays (*R. brachyura*), 6 spotted rays (*R. montagui*) and 4 small-eyed rays (*R. microcellata*) (Figure 3). All rays were kept in monitoring tanks with seawater recirculation at ambient temperature. Mortality, together with water quality, was checked on a daily basis. The rays were first tested for reflexes after 10 days of acclimatization. The goal was to identify at least three to six easy and unambiguous responses that were consistently presented by rays after stimulation. The reflexes were recorded as “unimpaired/present” (= scored as 1) if there was no doubt about the fish response, and “impaired” if absent, weak or when unsure (=scored as 0). The final selection of reflexes based on this pilot test are presented in Table 2. The mean reflex impairment score was low (0.13), indicating that most rays responded to each stimulated reflex. Additionally; rays were in general in good condition, with injury coverages ranging from 0 to 10% on average. At the end of the monitoring period of 29 days, 55% of the initial test population of thornback rays died (Figure 3). The protracted mortality of rays observed in Figure 3, illustrates the importance of a longer-term monitoring period and ideal holding conditions in independent tanks.



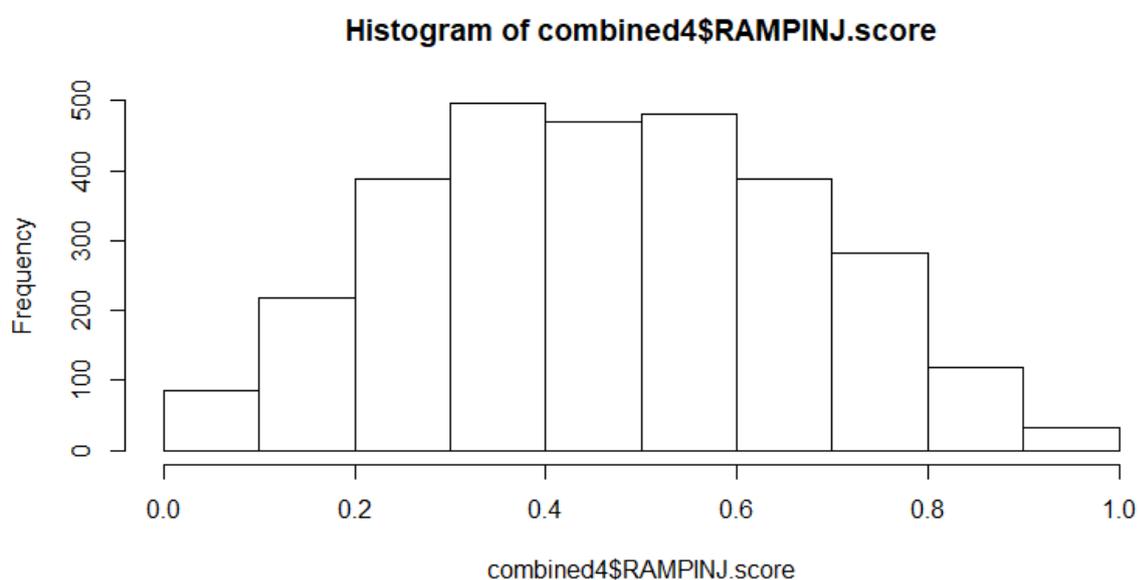
**Appendix Figure 1:** Cumulative mortality curve (%) for *R. clavata*, *R. brachyura* and “Others” (= *R. montagui* and *R. microcellata*) during a pilot-experiment.

### 10.3 Overview of technical and environmental parameters per trip

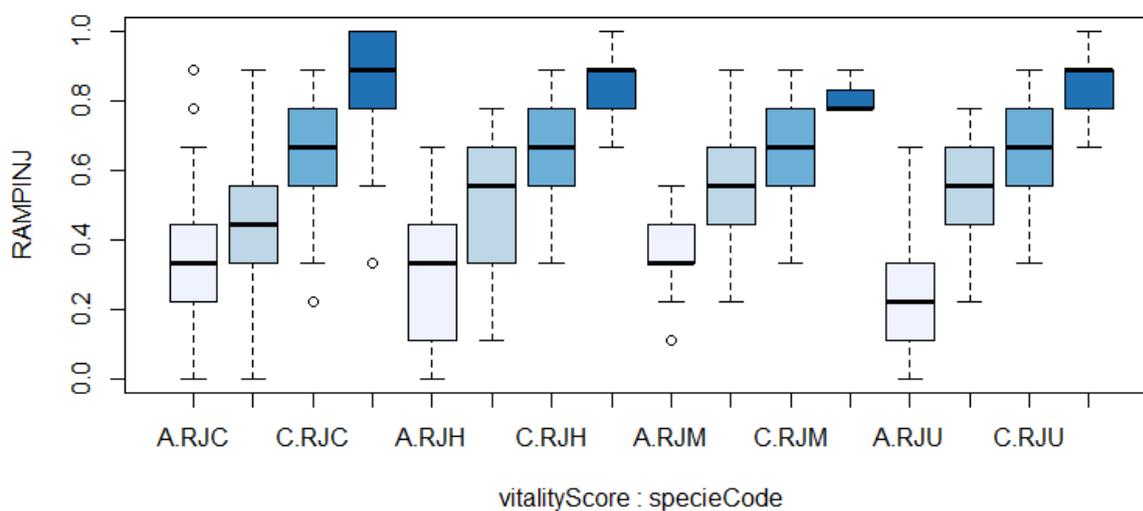
**Appendix Table 2:** Overview of the thirty-one monitored SUMARIS trips between July 2018 and January 2020. “Sampled species”= FAO-codes of the species that were sampled for vitality, injury and reflexes, “Sample size”= number of individuals that were scored for vitality, injury and reflexes.

Country	Tripcode	Month	Date	Gear	Sampled species	Sample size	Mean fishing time	Mean landing weight	Mean water depth
France	1	July	9/7/2018 -10/7/2018	GTR	RJH RJC RJM RJU	46	328.7	120.8	11.0
	2	Sep	24/9/2018 - 26/9/2018	OTB	RJH RJC	37	184.2	340.9	24.4
	3	Sep	26/9/2018 - 28/9/2018	GTR	RJC	42	1489.5	78.3	16.0
	4	Oct	16/10/2018 - 19/10/2018	OTB	RJH RJC	41	178.9	632.7	28.8
	5	Oct	15/10/2018 - 16/10/2018	OTB	RJC	30	215.4	450.7	38.2
	6	Nov	26/11/2018 - 27/11/2018	OTB	RJC RJH	51	184.4	235.8	13.6
	102	Feb	11/2/2019 - 15/2/2019	OTB	RJH RJC RJU	129	187.6	260.1	43.6
	110	Apr	1/4/2019 - 5/4/2019	OTB	RJH RJC RJU RJM	166	179.3	423.4	34.9
	111	Apr	17/4/2019 - 17/4/2019	GTR	RJC RJU RJH	50	1440.0	33.1	14.7
	113	Apr	29/4/2019 - 5/5/2019	OTB	RJC RJH RJM	89	194.6	284.6	23.1
	114	May	14/5/2019 - 14/5/2019	GTR	RJC RJH	51	1440.0	31.3	15.8
	152	Jun	11/6/2019 - 12/6/2019	GTR	RJC RJH RJU	62	457.5	47.4	21.2
153	Jun	16/6/2019 - 21/6/2019	OTB	RJC	58	180.0	419.5	18.5	
Belgium	53	Oct	11/10/2018 - 16/10/2018	OTB	RJC RJM	221	156.0	116.9	23.2
	54	Dec	8/12/2018 - 16/12/2018	TTB	RJH RJC RJU RJM	219	158.5	461.5	33.0
	57	Jan	14/1/2019 - 18/1/2019	TTB	RJH RJM RJC	119	88.8	81.3	20.2
	63	Feb	26/2/2019 - 3/3/2019	OTB	RJC RJH RJM	236	158.3	82.8	17.8
	108	Mar	25/3/2019 - 28/3/2019	TTB	RJM RJU RJC RJH	18	92.3	62.1	32.8
	109	Jun	3/6/2019 - 6/6/2019	TTB	RJC RJM RJU RJH	173	83.6	126.1	29.1
	203	Oct	17/10/2019 - 22/10/2019	OTB	RJC RJH RJM	350	153.6	134.0	24.7
	204	Nov	4/11/2019 - 8/11/2019	TTB	RJH RJC RJM RJU	148	88.8	74.2	21.1
	205	Nov	11/11/2019 - 14/11/2019	TTB	RJC RJU RJM RJH	101	88.0	69.8	28.8
	206	Jan	6/01/2020 - 14/01/2020	TTB	RJU RJH RJC RJM	376	126.7	NA	44.8
UK	55	Aug	29/8/2018 - 29/8/2018	GTN	RJC	18	104.0	17.0	20.0
	56	Sep	27/9/2018 - 27/9/2018	GTN	RJC	17	48.0	23.5	16.0
	59	Oct	10/10/2018 - 10/10/2018	GTN	RJC	36	85.5	38.2	19.5
	60	Oct	25/10/2018 - 16/10/2018	GTN	RJC	25	61.6	31.3	2.6
	61	Nov	23/11/2018 - 23/11/2018	GTN	RJC	37	82.3	32.0	19.0
	62	Feb	22/2/2019 - 22/2/2019	GTN	RJC	17	78.3	24.1	23.0
	352	Nov	13/11/2019 - 13/11/2019	GTN	RJC RJM	33	67.30	98.68	33.97
	353	Dec	28/12/2019 - 28/12/2019	GTN	RJC RJM	10	83.50	9.80	48.00

## 10.4 Relationship R, I and R&I indices



**Appendix Figure 2:** Histogram of R&I (RAMPINJ) index.



**Appendix Figure 3:** Relationship between R&I (RAMPINJ) index (x-axis and vitality class (A, B, C, D) per species (FAO-code) on the y-axis.

## 10.5 Overview of response and explanatory variables

**Appendix Table 3:** Type, name and description of all response and explanatory variables that were used in logistic regression models.

	Variable	Description	Type
<b>Response</b>	Delayed mortality	Status (dead/alive) of a ray after 21 d of monitoring	Continuous (Binary)
	Immediate mortality	Status (dead/alive) of a ray on deck	Continuous (Binary)
<b>Explanatory</b>	Vitality score	A (excellent), B (good), C (poor), D (dead)	Categorical
	R-index	Mean score of impaired reflexes (sum of impaired reflex scores divided by the total number of tested reflexes)	Continuous (Binary)
	I-index	Mean score of present injury (sum of present injury scores divided by the total number of tested injury types)	Continuous (Binary)
	R&I index	Mean score of impaired reflexes and present injury (sum of impaired reflex and present injury scores divided by the total number of tested reflexes and injury types)	Continuous (Binary)
	LengthTotal	Length (in mm) of the individual (head to end of tail)	Continuous
	Fishing time	Time of fishing (minutes)	Continuous
	Main Water Depth	Mean depth at which fishing occurred (in m)	Continuous
	Landing weight	Landing weight (in kg)	Continuous
	Sorting time	End time sorting – start time of the sorting procedure (in minutes)	Continuous
	Air exposure	Time in min spent on deck after being emptied from the codend and until being sampled.	Continuous
	Sex	Sex of the individual (male or female)	Categorical
	Gear type	Gear type used during the trip (OTB, TBB, GTR or GTN)	Categorical
	Substrate type	1=soft (sand), 2 = medium (both sand and stones), 3= hard (stones only)	Categorical
	Sea state	0 = calm (glassy) (0 beaufort), 1 = calm (rippled) (~1 beaufort), 2 = smooth (wavelets) (~2 beauforts), 3 = slight (~3-4 beauforts), 4 = moderate (~5-6 beauforts), 5 = rough (~7 beauforts), 6 = very rough, 7 = high	Categorical
	Sand and stones weight range	Weight categories: 0 = none, 1 <=10% of the total catch weight, 2= 10-50%, 3 >= 50%	Categorical
	Benthos weight range	Weight categories: 0 = none, 1 <=10% of the total catch weight, 2= 10-50%, 3 >= 50%	Categorical
Gear category	“Active” = TBB and OTB, “Passive”=GTR and GTN	Categorical	

## 10.6 Model selection

**Appendix Table 4:** Overview of p-values for all variables with/without species interaction. NS = not significant, \* = significant ( $p < 0.05$ ), \*\* = very significant ( $p < 0.001$ )

Response variable	Explanatory variables	Type	Response ~ variable * species + (1   trip)	Response ~ variable + species + (1   trip)	Response ~ variable + (1   trip)
Immediate mortality	Sex	Categorical	NS	< 2.2e-16**	NS
	Gear type		0.04158*	0.02168*	NS
	Substrate type		NS	< 2.2e-16**	NS
	Sea state		NS	0.01901*	NS
	Sand and stones weight range		NS	0.01826*	NS
	Benthos weight range		NS	0.02155*	NS
	Gear category		NS	0.016739*	NS
	Vitality score	Continuous	NS	NS	NS
	RAMP-score		NS	NS	NS
	Injury- score		0.02445*	0.001374*	NS
	RAMP-Injury score		0.04225*	0.04509 *	NS
	Vitality score		NS	NS	NS
	LengthTotal (cm)		< 2.2e-16**	< 2.2e-16**	NS
	Fishing time (minutes)		0.006575*	< 2.2e-16**	NS
	Main Water Depth (m)		NS	< 2.2e-16**	NS
	Landing weight (kg)		NS	0.04313*	NS
	Sorting time (minutes)		< 2.2e-16**	< 2.2e-16**	NS
Air exposure (minutes)	0.0001132*	0.0087691*	NS		
Delayed mortality	Picking time	Categorical	NS	NS	NS
	Sex		NS	NS	NS
	Gear type		NS	NS	0.0027404 *
	Substrate type		NS	NS	NS
	Sea state		NS	NS	NS
	Sand and stones weight range		NS	NS	NS
	Benthos weight range		NS	NS	NS
	Gear category	Continuous	NS	NS	0.0018574 **
	Vitality score		NS	NS	0.0003905 **
	RAMP-score		< 2.2e-16***	NS	0.000285 **
	Injury-score		NS	NS	<2e-16 **
	RAMP-Injury score		NS	NS	3.724e-07 **
	Vitality score		NS	NS	0.0003905 **
	LengthTotal (cm)		NS	NS	NS
	Fishing time (minutes)		NS	NS	0.01483 *
	Main Water Depth (m)		NS	NS	NS
	Landing weight (kg)		NS	NS	NS
Sorting time (minutes)	NS	NS	0.0005691**		
Air exposure (minutes)	NS	NS	NS		

