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# Initial experiments to assess short-term survival of discarded plaice (Pleuronectes platessa) caught in trammel nets during winter season 

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## A R T I C L E I N F O

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Catch-Damage-Index (CDI)
Discard survival
Reflex Action Mortality Predictor (RAMP)
Set-net
Temperature
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#### Abstract

European plaice (Pleuronectes platessa) is a key species in commercial fisheries in the North Sea, Skagerrak, Kattegat, and Baltic Sea. The reformed European Union Common Fisheries Policy includes the possibility of exemptions from the landing obligation for "species for which scientific evidence demonstrates high survival rates". Discard survival from set-net fisheries is poorly studied. Trials were conducted on two commercial fishing vessels over seven trips from November to February 2017-2018 in the Baltic Sea. The nylon trammel nets had a nominal bar size for the inner/outer wall of $75 / 350 \mathrm{~mm}$ and $85 / 400 \mathrm{~mm}$. Soaking time was $23-47 \mathrm{~h}$, water depth $7-18 \mathrm{~m}$, and deck temperature was $-0.1-6.0^{\circ} \mathrm{C}$. Following commercial practice, the trammel nets were hauled back onto the vessel, after which netting and fish passed through a net hauler onto a steel sorting table where the entire fish catch were manually untangled by the fishers and plaice collected by scientists. We used a storage system for housing the captured fish inside fishing harbours during observations. Catch-damage-index (CDI) and Reflex Action Mortality Predictor (RAMP) scores were used to assess fish condition immediately after capture and at the end of observation periods. All plaice below 40 cm were sampled with a total number of 118 individuals from 13 fleets (several nets joined together). The fish were assessed for short-term survival for 4-10 days with full survival ( $100 \%$ ). The majority of fish exhibited no reflex impairments. Minor bruises, fraying, and net marks were frequently observed on captured fish. The overall condition of the fish did not change during observation periods.


## 1. Introduction

The European Union (EU) has enacted a landing obligation in the Common Fisheries Policy (CFP), prohibiting the discard of quota regulated fish species (EU regulation, 1380/2013). However, the discarding of fish with high survival rates would benefit maintaining the populations (Condie et al., 2014a, 2014b; Guillen et al., 2014). The EU regulation, therefore, includes the possibility of an exemption for "species for which scientific evidence demonstrates high survival rates" (EU regulation, 1380/2013, Article 15, paragraph 4b).

Demersal set-net fisheries are passive fishing methods where the nets are placed on the seabed to target demersal species. Trammel nets and gill nets are used in European fisheries targeting commercial important
demersal species like gadoids and flatfish. A gill net consists of a single wall of netting in which fish are retained by the gills (i.e., gilling), or the body as a result of struggling (He and Pol, 2010; He et al., 2021). By comparison, a trammel net consists of three walls of netting tied together on the float line and the lead line: a slack inner wall with a smaller mesh size, sandwiched between two outer walls with larger mesh sizes (Hovgård and Lassen, 2000; He and Pol, 2010; He et al., 2021). In trammel nets, fish pass through one of the outer walls and encounter the inner wall but are retained as they become entangled in a pocket of the smaller mesh (see detailed description in He et al., 2021). In contrast to single-walled gill nets, which catch a narrow size range of fish, trammel nets generally catch a wider variety of fish sizes and species (Stergiou et al., 2002; He and Pol, 2010). As a result, they are preferred by Danish

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vessels fishing with set-nets (often termed gillnetters).
Set-net fishing is widely used in commercial Danish fisheries in the North Sea, Skagerrak, Kattegat and Baltic Sea, and plaice (Pleuronectes platessa) is a key species in these fisheries. Set-nets targeting flatfish are usually soaked overnight ( $<24 \mathrm{~h}$ ) because fish dying in the nets have low value and are often eaten by crabs and other invertebrates. Furthermore, the net will have reduced efficiency with increasing soaking time because of catches, debris, and invertebrates. In set-net fisheries, plaice are usually fished at shallow waters $(\sim 5-30 \mathrm{~m})$. The sorting practice is relatively uniform on gillnetters where it is a common practice to sort the net immediately on a sorting table below the net hauler.

Plaice is one of the most studied species in relation to discard survival in European fisheries, with studies covering a range of demersal towed gears including beam trawls (Revill et al., 2013; Depestele et al., 2014; Uhlmann et al., 2016; Van der Reijden et al., 2017), trawls (Methling et al., 2017; Morfin et al., 2017; Eskelund et al., 2019; Kraak et al., 2018; Savina et al., 2019; Noack et al., 2020) and Danish seine (Noack et al., 2020). Survival rates in these studies range from 89\% (Methling et al., 2017) to $15 \%$ (Eskelund et al., 2019). There is a lack of knowledge on demersal set-net fisheries, where the fishing practice (soak time, handling, etc.) differs substantially from towed gears.

In this study we focused on the Baltic Sea where discard rates of plaice from passive gears are relatively high, being $38 \%$ (weight) for 2018 (ICES, 2018a, 2018b). The landing obligation causes additional sorting work and there are no buyer systems of plaice below MRCS (minimum conservation reference sizes) in most of the smaller harbours (can be sold for non-human consumption). We focused on trammel nets because it is the most widely used set-net. The data were collected during the winter season where higher survival rates would be expected because of lower temperatures (Methling et al., 2017; Savina et al., 2019; Noack et al., 2020) and because legislation makes seasonally separation for plaice ( 1 November-30 April) in exemptions for plaice (STECF, 2019).

The main objectives of this study are to assess short-term survival and catch related traumas of discarded plaice from trammel nets, fished in the Baltic Sea at wintertime.

## 2. Materials and methods

### 2.1. Ethical statement

The experiments followed legislations of the European Union (EU Directive 2010/63/EU) and Denmark (c.f. Danish law on animal experimentation LBK no 474 of 15/05/2014), which are based on the 3 R principles (Replacement, Reduction, and Refinement). Our experiments and protocols were approved by The Danish Animal Experiments Inspectorate (Permit Number: 2017-15-0201-01297 and 2020-15-0201-00486).

### 2.2. Experimental trials

Experimental trials were conducted in the Western Baltic Sea in ICES subdivisions 23 (The Sound) and 22 (Belt Sea). Fish were collected from 13 fleets over seven fishing days between the 25th of November 2017 and the 10th of February 2018. A detailed summary of fishing conditions is provided in Table 1. Two commercial set-net fishing vessels, with a length of 9.8 and 12.6 m , were used for sea trials to account for potential differences of vessel type, fishing area, fish sorting, net handling, and soak time on discard survival. The vessels own commercial nylon trammel nets were used. For trials 1-10, net length (float/lead line) was $50 / 59 \mathrm{~m}$ and nominal bar size (half full mesh size) for inner and outer wall was $75 / 350 \mathrm{~mm}$. For trials $11-13$, net length (float/lead line) was $60 / 76 \mathrm{~m}$ and nominal bar size (inner/outer wall) was $85 / 400 \mathrm{~mm}$. Detailed information on vessels and nets is provided in the supplementary material (Table S1). During trials, we only collected the captured plaice. The remaining catch included cod (Gadus morhua), brill (Scophthalmus rhombus), turbot (Scophthalmus maximus), European flounder (Platichthys flesus), common dab (Limanda limanda) and sole (Solea solea).

### 2.3. Fish sampling

The trammel nets were hauled back onto the vessel by a net hauler (NET-OP 125), netting and fish passed between two net drums (pressured together by 10 kg tension in each drum) onto a steel sorting table where the fish were manually untangled by the fishers. Following general commercial practice, before the discard ban, discard fish would immediately be released back into the sea after untangling. The commercial part of the catch is placed in fish boxes on the deck. The captured plaice were manually untangled by the fishers, collected by the scientists and placed into a 45 L plastic tank. Here the fish were assessed for reflex impairments and injuries (detailed in 2.4). Following this, the TL (total length) was measured, the fish was tagged in the dorsal fin with a 1 cm plastic tag, and moved to a 300 L lidded holding tank with aerated seawater. The holding tank was compartmentalized with three stacked 90 L boxes to prevent the individual fish from covering each other. The 90 L boxes were perfused, allowing water to move between the three compartments. A fifth of the volume in the 300 L holding tank was exchanged with seawater 1-2 times per hour. The discarded fraction of plaice can cover all length classes because some fishing nations have no quota for plaice (ICES, 2018b). All captured plaice $\leq 40 \mathrm{~cm}$ TL were collected and used for experimentation.

During the first trials (fleet 1-4, Tables 1 and 2), the captured plaice were moved from the fishing vessel onto a small motorboat for further handling. The aim was to test the conceptual idea of being able to conduct experiments from smaller gillnetters because working and storing space can be limited on these types of vessels. The small

Table 1
Summary of fishing conditions in the Western Baltic Sea in ICES subsquare 23 (The Sound) (Trip 1-5) and 22 (Belt Sea) (fleet 11-13). Temp (temperature) is air temperature on the deck. Fish length (total) is indicated with standard deviation and range (min-max). The first 10 fleets is set from the vessel Fuglen (H 32) and the last 3 with the vessel Duddi Krog (NF 76).

| Fleet no. | Fishing date | Depth (m) | Soaking time (hrs) | Temp on deck ( ${ }^{\circ} \mathrm{C}$ ) | Seabed temp ( ${ }^{\circ} \mathrm{C}$ ) | Fish length (cm) | No. of plaice |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | 25.11.17 | 7 | 23 | 4.0 | 7.1 | $32.8 \pm 4.2(30-40)$ | 5 |
| 2 | 25.11.17 | 9 | 26 | 4.0 | 7.0 | $31.3 \pm 2.0$ (28-35) | 10 |
| 3 | 26.11.17 | 7 | 24 | 5.0 | 7.0 | $33.8 \pm 1.8$ (31-37) | 8 |
| 4 | 26.11 .17 | 7 | 25 | 5.0 | 7.0 | $32.3 \pm 7.3$ (22-39) | 4 |
| 5 | 18.01.18 | 11 | 24 | 6.0 | 3.8 | $35.0 \pm 0.0(35-35)$ | 3 |
| 6 | 18.01.18 | 11 | 25 | 6.0 | 4.1 | $33.0 \pm 4.3$ (25-37) | 10 |
| 7 | 19.01.18 | 9 | 23 | 4.0 | 3.7 | $34.5 \pm 4.4(25-40)$ | 11 |
| 8 | 19.01.18 | 11 | 24 | 4.0 | 4.0 | $31.8 \pm 3.2(26-35)$ | 9 |
| 9 | 20.01.18 | 8 | 24 | 4.7 | 3.4 | $33.5 \pm 2.8(28-36)$ | 6 |
| 10 | 20.01.18 | 10 | 24 | 4.7 | 3.6 | $33.0 \pm 4.4(27-39)$ | 5 |
| 11 | 09.02.18 | 16 | 46 | 0.0 | 2.3 | $33.1 \pm 1.9$ (30-38) | 16 |
| 12 | 09.02.18 | 17 | 47 | 0.0 | 2.1 | $33.9 \pm 1.7(31-37)$ | 13 |
| 13 | 10.02.18 | 18 | 19 | -0.1 | 2.3 | $35.4 \pm 3.2$ (30-40) | 18 |

Table 2
Summary of plaice survival and water parameters in the Flex Boxes during the observation periods, including the start date and the duration of the observation periods. Values for water parameters are mean ( $\pm 1 \mathrm{SD}$ ) and range (min-max).

| Fleet no. | Start date (dd.mm.yy) | Duration (days) | Temperature ( ${ }^{\circ} \mathrm{C}$ ) | Dissolved oxygen (\%) | Salinity (ppt) | Survival (\%) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | 25.11.17 | 10 | $6.1 \pm 0.1(5.4-7.0)$ | $91.8 \pm 0.4(87.0-97.0)$ | $10.7 \pm 0.1(9.8-11.9)$ | 100 |
| 2 | 25.11.17 | 10 |  |  |  | 100 |
| 3 | 26.11 .17 | 10 |  |  |  | 100 |
| 4 | 26.11.17 | 10 |  |  |  | 100 |
| 5 | 18.01.18 | 7 | $3.1 \pm 0.1(3.0-3.2)$ | $93.9 \pm 0.1(92.0-94.0)$ | $12.2 \pm 0.1(12.0-12.4)$ | 100 |
| 6 | 18.01.18 | 7 |  |  |  | 100 |
| 7 | 19.01.18 | 6 |  |  |  | 100 |
| 8 | 19.01.18 | 6 |  |  |  | 100 |
| 9 | 20.01.18 | 5 |  |  |  | 100 |
| 10 | 20.01.18 | 5 |  |  |  | 100 |
| 11 | 09.02.18 | 5 | $1.0 \pm 0.1(0.5-1.5)$ | $96.5 \pm 0.1(95.2-97.4)$ | $10.5 \pm 0.1(9.7-11.1)$ | 100 |
| 12 | 09.02.18 | 5 |  |  |  | 100 |
| 13 | 10.02.18 | 4 |  |  |  | 100 |

motorboat also allowed faster transportation of the captured fish to storing facilities. During these trials, the untangled fish (discards) were immediately placed by the fisherman into a knotless rubber landing net (made for catch and release recreational angling) and transferred to the small motorboat. On the small motorboat, each fish was assessed immediately for reflex impairments in a 45 L plastic tank and subsequently tagged. They were then moved to a 160 L holding tank on the small motorboat that was compartmentalized with three stacked 45 L perfused boxes.

All fish were transported to shore within 3 h of being placed into the holding tank. Within 30 min of arriving at the harbour, each fish was moved to an individual 45 L plastic tank on the dock for assessment of injuries before being placed in the Flex Boxes suspended in the harbour water (see details in Section 2.5). This was done to be able to conduct the assessment more carefully than on the vessel (light conditions, sea movements and time pressure). Previous discard survival studies on plaice do not report any additional damages on individuals transported in similar holding tanks (Methling et al., 2017; Eskelund et al., 2019).

Water temperature and salinity at the fishing sites were measured using a Star-Oddi DST CTD (Star-Oddi, Skeidaras 12, 210 Gardabaer, Iceland). The measurements were taken before the fishers began pulling the net aboard the vessel, using a sink on a line. Depth at the fishing site was taken from the vessel's echo sounder. Water salinity in the holding tank was measured using an EC300 Conductivity Meter (VWR, 1, 100 Matsonford Rd \#200, Radnor, PA 19087, USA). Water temperature and dissolved oxygen in the holding tank were measured using a MULTI 3420 D.O. Meter (WTW, Dr.-Karl-Slevogt-Straße 1, 82362 Weilheim, Germany).

Time on deck was less than one minute for all plaice. We made a more precise estimate from video recordings (GoPro). Recordings were analysed for the time from a netted fish exiting the sea, passed through the net hauler, and was untangled from the net on the sorting table. Video footage from the first trial $(\mathrm{N}=48)$ showed the time from netted fish exited the sea until they entered the net hauler was $4.2 \pm 0.2 \mathrm{~s}$, and the time from the fish exited the net hauler until they were untangled from the net on the sorting table was $10.8 \pm 1.0 \mathrm{~s}$. None of the video recordings from the subsequent trials was of sufficient quality for analysis.

### 2.4. Fish vitality

Vitality assessment is a widely recognized methodology (Benoît et al., 2010, 2013; Davis, 2010; Breen and Catchpole, 2021) that can be used to indirectly predict species-specific discard survival from validated vitality indicators (Breen and Catchpole, 2021). Vitality indicators include reflex impairments and injuries, which can be assessed using the reflex action mortality predictors (RAMP) method (Davis and Ottmar, 2006; Davis, 2007, 2010) and the catch damage index (CDI) (Esaiassen et al., 2013), respectively. Our RAMP and CDI vitality indicators are
described in Table 3. The RAMP indicators were selected from candidates validated by demonstrating a high correlation $\left(R^{2}>0.97\right)$ with survival rate in recent discard survival studies on plaice from Danish fisheries (Methling et al., 2017; Eskelund et al., 2019) (Fig. 1S in supplementary material). The individual RAMP and CDI indicators were chosen based on the ease with which they could be quickly, accurately, and objectively assessed on the deck of a fishing vessel (Methling et al., 2017; Eskelund et al., 2019). For individual reflex impairments and injuries, the fraction of fish exhibiting each variable was calculated by dividing the number of fish with a score of 1 with the total number of fish captured. For individual fish, RAMP scores ranging from 0 to 3 and CDI scores ranging from 0 to 11 were calculated by adding scores for reflex impairment and injury variables. The fraction of fish with RAMP scores $0-3$ and CDI scores $0-11$ was calculated for each score by dividing the number of fish with that score with the total number of fish captured (i.e., 118). Finally, an overall RAMP score for all fish was calculated by dividing the total number of reflex impairments (342) by the total number of assessed reflexes (354).

### 2.5. Observations

We developed a fish housing concept (named 'Flex Box') for observation of short-term discard survival rate (Fig. 1). The Flex Box can be used in remote areas without land based storing facilities, where set-net fisheries are often conducted. The Flex Box is developed to be transportable, relatively cheap to build, and to provide good conditions for the stored fish. Each Flex Box was constructed out of planed wood ( $360 \mathrm{~L}, \mathrm{~W} 120 \times \mathrm{D} 80 \times \mathrm{H} 38 \mathrm{~cm}$ ), using two wooden pallet collars as sides. This design allowed the Flex Boxes to be completely collapsed

Table 3
Stimulus and responses of reflexes, and description of injuries in the plaice. For reflex impairment individuals was scored 0 if the response was completed with 5 s of the stimulus, or 1 if the response was not completed within 5 s (i.e., impaired). For injuries, individuals were scored 0 if the damage was absent, or 1 if the damage was present.

| Reflex | Stimulus and responses |
| :---: | :---: |
| Righting | Righting itself when turned upside down under water |
| Evasion | Swims toward the bottom when released at the surface |
| Tail grab | Struggle or tries to escape when tail is held between two fingers |
| Injury | Description |
| $\begin{aligned} & \text { Abrasions ( }<10 \% / 10-50 \% / \\ & >50 \% \text { ) } \end{aligned}$ | Areas with discoloration or scale loss |
| Fin fraying | Shredding of the thin skin between the fins |
| Minor wounds (head / body) | Shallow cuts or punctured skin |
| Deep wounds (head / body) | Deep cuts or punctured skin, often with bleeding |
| Intestine | Intestines visible through the anus |
| Net marks | String cuts from net contact |



Fig. 1. . (a) assembled Flex Box, (b) disassembled Flex Box, (c) four deployed Flex Boxes, and (d) plaice with net marks.
during transport between study sites. All sides had holes to facilitate continuous water exchange between the Flex Boxes and the surrounding water. The bottom of the Flex Boxes was covered by a $1-\mathrm{cm}$ layer of sand to simulate the natural environment of the fish.

A maximum of 10 fish were assigned to each of the Flex Boxes used in the study. Food was withheld for the duration of observation periods to avoid production of biowaste products and reduced water quality, and most aquatic species can survive several weeks without food (Breen and Catchpole, 2021). A similar practice was used by Methling et al. (2017) where no control fish morality was observed for plaice housed 10 days. Fish were inspected individually by removing the lid on the Flex boxes. Individual fish would be identified as dead if they exhibited a lack of visible operculum movement, loss of equilibrium, or were unresponsive to a gentle nudge on the caudal peduncle (following the guidelines of in our animal experimental permit). Once a day, water temperature, dissolved oxygen, and salinity were measured in the Flex Boxes, and in the surrounding water. Salinity was measured using an EC300 Conductivity Meter (VWR, 1, 100 Matsonford Rd \#200, Radnor, PA 19087, USA). Temperature and dissolved oxygen were measured using a MULTI 3420 D.O. Meter (WTW, Dr.-Karl-Slevogt-Straße 1, 82362 Weilheim, Germany). Water temperature in the harbour during the observation periods was $0.5-7.0^{\circ} \mathrm{C}$. Dissolved oxygen was $87-97 \%$ and salinity was 9.7-12.4 ppt (Table 2). Differences in water parameters (temperature, dissolved oxygen, and salinity) between the inside of the Flex Boxes and the sounding water was virtually zero (Table 2).

At the end of observation periods, the individual fish were transferred from Fish Boxes to 45 L tanks on the pier and assessed a second time for reflex impairments and injuries. Subsequently, the fish were euthanized in 2-phenoxyethanol, and sacrificed by spinal transection as specified in our animal experimental permission.

We systematically reduced days from a 10-day to a 4-day observation period without mortalities (i.e., a 4-10-day asymptote), since it is expected that discard survival typically is lowest during the first few days after capture (see discussion). The maximum observation period allowed by our Animal Experiments permission is 10 days. The end of our observation period was supported by vitality assessment, and the possibility to continue in case of lowered vitality (particularly RAMP). This approach enabled a higher number of soaks and an additional trial for the resources available (trials are a substantial and limiting part of the budget).

### 2.6. Evaluation of uncertainty of our estimates

We assessed the uncertainty of our survival estimates. Since there is
no variation in our data it is impossible to compute empirical confidence intervals. We calculated the probability of our observations (all fish surviving) with increasing number of fish if the expected survival probability was $90 \%, 80 \%$, and $70 \%$, respectively, using Eq. 1 :
$\mathrm{P}_{\mathrm{S}_{\text {all }}}=(\mathrm{Ps})^{\mathrm{n}_{1}} \times(\mathrm{Ps})^{\mathrm{n}_{2}} \quad \times \ldots \times(\mathrm{Ps})^{\mathrm{n}_{\mathrm{x}}} \leftrightarrow \quad(\mathrm{Ps})^{\left(\mathrm{n}_{1}+\mathrm{n}_{2}+\ldots+\mathrm{n}_{\mathrm{x}}\right)}$
in an experiment with $x$ replicates $(1,2, \ldots, x)$ and $n$ observations in each replicate $\left(n_{1}, n_{2}, \ldots, n_{x}\right)$ the probability that all fish survive (probability of all fish surviving, $\mathrm{P}_{\mathrm{Sall}}$ ) at a given survival rate (probability of survival, Ps) under the assumption that the individual observations do not affect each other (i.e., are statistically independent). For example, if probability of expected survival for one fish is $\mathrm{Ps}=0.7$ (70\%), then the chance that 10 fish will survive simultaneously is $0.7^{10} \approx 0.03$ or $3 \%$.

## 3. Results

### 3.1. Fishing conditions and catches

A total of 118 plaice from 13 fleets were assessed during the seven trials (Table 1). All 118 fish survived for the duration of the observation periods (Table 2). During trials, water depth was $7-18 \mathrm{~m}$ and deck temperature was $-0.1-6.0^{\circ} \mathrm{C}$. Mean ( $\pm$ standard deviation (SD)) net soak time was $24 \pm 2 \mathrm{~h}$ except for 2 fleets, which had soak times of 46 and 47 h , respectively (delayed retrievement because of weather conditions). Total length (TL) of plaice was $22-40 \mathrm{~cm}$ with a mean ( $\pm \mathrm{SD}$ ) TL of $33.4 \pm 3.3 \mathrm{~cm}$.

### 3.2. Reflex Action Mortality Predictor (RAMP) and Catch-Damage-Index (CDI) scores

Reflex impairments and injuries after capture and after observation are presented in Fig. 2. RAMP and CDI scores after capture and after observation are presented in Fig. 3. Less than $10 \%$ of the fish exhibited any reflex impairments after capture, and less than $2 \%$ exhibited reflex impairments after the observation period. In terms of injuries, the proportion of fish with $<10 \%$ abrasions increased slightly from 0.66 after capture to 0.72 after observation. In contrast, the proportion of fish with $10-50 \%$ abrasions decreased from 0.31 after capture to 0.22 after observation. Less than $5 \%$ were recorded with $>50 \%$ abrasions. In addition to $<10 \%$ abrasions, fin fraying and net marks (Fig. 1D) were the two most prominent injuries after capture and after observation, with $40-50 \%$ of fish displaying these injuries. Body wounds were observed on $10-12 \%$ of the fish and less than $3 \%$ of the fish had minor head wounds, deep head wounds, or exposed intestines.


Fig. 2. Proportion of plaice with (a) reflex impairments and (b) injuries (b) after capture (white) and at the end of the observation periods (grey).


Fig. 3. Distribution of the (a) Reflex Action Mortality Predictor (RAMP) and (b) Catch-Damage-Index (CDI) scores in plaice after capture (white) and after observation (grey).

A total of 108 (92\%) fish exhibited no change in RAMP score after capture and RAMP score after observation, while 7 (6\%) fish exhibited a change of -1 in RAMP score from after capture to after observation. The overall RAMP score was 0.03 after capture and 0.01 after observation. A total of 72 ( $61 \%$ ) fish exhibited no change in CDI score after capture and after observation. A total of 15 (13\%) fish had a change in score of +1 and 27 ( $23 \%$ ) fish had a change in score of -1 .

### 3.3. Evaluation of uncertainty

The theoretical calculations (Fig. 4) shows the probability of observing all fish surviving with an increasing number of fish, if expected survival rate was $90 \%, 80 \%$ or $70 \%$. The probability that the survival rate was $90 \%$ is less than $1 \%$ when the number of captured fish exceeds 50 and with 118 fish (captured in this study) it is negligible (0.0003\%).

## 4. Discussion

The Flex Boxes used to house the fish during observation provided a mobile alternative to the traditional land-based holding facilities often used in discard survival studies (Catchpole et al., 2015; Van der Reijden


Fig. 4. The plot shows the probability of observing all fish surviving as a function of captured fish when the expected survival probability is $0.9,0.8$, and 0.7 , respectively (Eq. 1 ).
et al., 2017; Eskelund et al., 2019; Savina et al., 2019; Noack et al., 2020). This mobility is useful when studying small-scale fisheries, such as set-net fisheries, which are often spread over a larger number of smaller harbours located in relatively remote areas. When using the Flex Boxes in harbours it is important to be aware of the water quality and ensure a good water exchange in the box.

Our use of a small motorboat allowed sampling of fish from smaller fishing vessels with insufficient working and storing space. The small motorboat also allowed us to transport the sampled fish faster to the harbour. Capturing controls for set-net studies constitute a challenge. Attempts at catching control plaice with fyke nets and baited hooks were not successful. Furthermore, short-duration soaks with trammel nets might injure the fish during the capture process. However, in hindsight the $100 \%$ survival observed in this study eliminated the need for control fish for estimating survival.

Discard mortality studies on plaice demonstrates high variability in captive observation periods, within and between studies, with periods ranging from 1 to 4 days (Revill et al., 2013; Depestele et al., 2014; Morfin et al., 2017), 5-10 days (Methling et al., 2017; Morfin et al., 2017; Eskelund et al., 2019) and 14-34 days (Uhlmann et al., 2016; Van der Reijden et al., 2017; Savina et al., 2019; Noack et al., 2020). An ICES working group for estimating discard survival (WGMEDS) do not specify a specific observation period duration when assessing discard survival but recommend that monitoring is continued until mortality approaches an asymptote (Breen and Catchpole, 2021). Discard survival is typically lowest during the first few days after capture (Benoît et al., 2010, 2013; ICES 2016; Breen and Catchpole, 2021). This trend is also observed in most plaice studies (Depestele et al., 2014; Revill et al., 2013; Catchpole et al., 2015; Uhlmann et al., 2016; Methling et al., 2017; Morfin et al., 2017; Van der Reijden et al., 2017; Eskelund et al., 2019; Savina et al., 2019; Noack et al., 2020; Madsen et al., 2022). Experiments with longer observation periods does not report any substantial changes in survival rates after a 10-day period or when reaching 3 days with full survival (Uhlmann et al., 2016; Van der Reijden et al., 2017). An atypical continuously decline in survival rate, observed in some studies (Savina et al., 2019; Noack et al., 2020), would have been detected by our experimental approach since the maximum period without mortality is less than 3 days. Other plaice survival studies conducted at wintertime demonstrate a lower period without mortality (1-3 days) (Depestele et al., 2014; Methling et al., 2017; Morfin et al., 2017; Van der Reijden et al., 2017; Revill et al., 2013; Savina et al., 2019), than the present study (4-10 days). We observed a high RAMP score at the end of the observation period which gives relatively strong indications of survival beyond the 4-10-day observation periods.

The 100\% survival rate and high RAMP score in the assessed fish are likely because fish from set-net fisheries are sorted while the net is being pulled aboard the vessel. This practice results in a short duration of air exposure during the capture and handling process when compared to fish caught with towed gears where all the caught fish are moved from the sea onto the deck of the fish vessel and sorted thereafter. Furthermore, the mechanical impact might be lower.

Fin fraying was observed in about $50 \%$ of the assessed plaice. This fraction is lower than the $\sim 70 \%$ reported in plaice caught with Nephrops trawls (Eskelund et al., 2019) and higher than the $\sim 30 \%$ reported in plaice caught with a fish trawl (Methling et al., 2017). The fraction of fish with fin fraying did not change during the observation periods and we would not expect it to affect long term mortality beyond the duration of the observation periods. Net marks were also observed in about 50\% of the assessed plaice and did also not appear to worsen during the observation periods. Net marks may cause infections which could affect the survival rate, beyond the observation period in this study. It would, therefore, be relevant to assess the effect of net marks on long term survival by developing a methodology for such studies.

After observing full survival and high RAMP score, we prioritised to increase variability by several trials, employing one additional fishing vessel fishing in another area, with different nets, longer soaking times,
deeper water and lower temperatures. It is of importance to increase focus further on detecting any potential variability. RAMP assessment is a useful tool that could increase coverage substantially by being cheaper and less time-consuming than survival observation studies. This is also in line with the 3 R principle in the EU animal welfare directive (EU Directive 2010/63/EU), since we experience increasingly restrictions on captative experiments (animal experimental permissions). Further validation of our used RAMPs (there is no contrast in this study observing full survival) and the potential use of additional RAMP candidates should be considered. Detecting of potentially low RAMP scores should be supported by captive experiments.

Prolonged air exposure during capture and handling is one of the greatest contributors to discard mortality within and among fish species (Benoît et al., 2013), including plaice (Methling et al., 2017). Consequently, the release of discarded fish back into the sea immediately after capture is a condition for the exemption from the landing obligation in the Common Fisheries Policy (EU regulation 2019/2238). Air exposure, handling time and other procedures might change with higher catch rates than observed in this study and it would be relevant to investigate this further. Net soak time in this study is, to our judgement, above the average soak time for Danish set-net fisheries targeting plaice (our information from fishermen) and if any concern (also considering fishing practice in other countries) this could be addressed in a potential exemption.

Fisheries have been granted an exception from the EU landing obligation based on discard survival estimates from $46 \%$ to $90 \%$ survival (Noack et al., 2020). The exemption from the EU landing obligation for towed gear targeting plaice is based on several studies of plaice captured during both summer and winter seasons. The results of the present study indicates that it is relevant to conduct similar studies in set-net fisheries. Consequently, future studies should also focus on the summer season where higher water temperatures and the occurrence of low-oxygen zones are likely to reduce survival rates in fish from set-net and gill-net fisheries.

## CRediT authorship contribution statement

Rasmus Ern: Conceptualization, Writing - review \& editing, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing - original draft. Katrine Molbo: Data curation, Investigation. Trine H. Jensen: Methodology, Data curation. Sergey V. Kucheryavskiy: Methodology, Formal analysis, Writing original draft. Peter R. Møller: Conceptualization, Methodology, Project administration, Funding acquisition. Niels Madsen: Conceptualization, Writing - review \& editing, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Visualization.

## Declaration of Competing Interest

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

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fishres.2022.106308.

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