Seasonal variation in the survival of discarded *Nephrops norvegicus* in a NW Mediterranean bottom-trawl fishery

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Abstract: The landing obligation in the revised European Union Common Fisheries Policy allows for exemptions to obligatory landing of the entire catch for species for which "high survival" of discards can be demonstrated. *Nephrops* norvegicus is an important target species in many fisheries across Europe in the Mediterranean Sea, NE Atlantic Ocean and North Sea. Historically, Mediterranean fisheries have had a high discard rate of small-sized *Nephrops*, and it is suspected that this unwanted component of the catch may have a high survival potential that is comparable to those of other EU fisheries, where survival rates of up to 0.56 have been demonstrated. However, to date, no investigations have confirmed a high discard survival rate for *Nephrops* in the Mediterranean Sea. Furthermore, the environmental, technical and biological characteristics that could affect *Nephrops* survival have been shown to be substantially different from those in the survival assessments conducted in the NE Atlantic and the North Sea. To address this knowledge gap, this study was conducted to determine the survival of *Nephrops* discarded from trawls in the Mediterranean Sea. The survival and vitality status of the discarded *Nephrops* removed from trawl catches were monitored onboard and for 14 days in the laboratory. The results showed seasonality in survival, with the highest survival rate in winter (0.74; CI: 0.7−0.78), lower survival in spring (0.36; CI: 0.31−0.41) and the lowest survival in summer (0.06; CI: 0.04−0.09). Survival was monitored to the asymptote in all cases, and season and vitality status were shown to have statistically significant relationships with survival.

Keywords: Survival, Discards, *Nephrops norvegicus,* Vitality assessment, Landing obligation

1. Introduction

Norway lobster (*Nephrops norvegicus*) is a commercially important species that is widely distributed throughout Europe fisheries from the Mediterranean Sea and the NE Atlantic to the northern North Sea and Baltic Sea (Vasilakopoulos and Maravelias, 2016). The total discard rates in the trawl fishery targeting Mediterranean *Nephrops* can reach 30 % of the total catch, comprising a high proportion of undersized (i.e., below the minimum conservation reference size (MCRS) specimens of *Nephrops* (García -de-Vinuesa et al., 2018).

The introduction of the landing obligation (LO) in the European Union's (EU) Common Fisheries Policy aims to shift harvesting patterns in EU fisheries by reducing unwanted catches by banning discarding practices and encouraging more selective capture methods (EU Reg., 1380/, 2013). Currently, the LO applies to regulated species, that is, species for which there is a quota or MCRS; it stipulates that no unwanted catches of regulated species can be discarded: they must be landed in port and not used for human consumption ((EC) No 850/ 1998). However, there are situations (exemptions) in which animals may be legitimately released (discarded) from commercial fishing catches, such as those with high survival – when the survival of a species from a particular fishery has been demonstrated to be sufficiently high to justify its release (Art. 15 of EU Reg., 1380/, 2013). For the high survival exemption to be implemented, fishery-specific evidence must be presented to the European Commission, which will consider the merits of an exemption to the LO on a case-by-case basis (Rihan et al., 2019).

Technical measures have been in place for decades in an attempt to avoid catching undersized animals in the Mediterranean *Nephrops* fisheries, including an MCRS of 20 mm in carapace length (CL) and minimum trawl cod-end mesh sizes of 40 mm (square-mesh) or 50 mm (diamond) (Council Regulation (EC) No., 1967/, 2006; GFCM/29/ 2005/1). However, these control measures have not been not fully effective because substantial numbers of undersized *Nephrops* between 15 and 20 mm CL continue to be caught and discarded, contravening the LO that has been in place since 1st January 2019 (García -de-Vinuesa et al., 2018). Furthermore, the size of maturity for *Nephrops* in the Mediterranean Sea is between 30 and 36 mm in CL (Orsi Relini et al., 1998), which is much larger than the MCRS. As such, immature individuals between 20 and 30 cm are routinely caught legally, which makes this management strategy questionable.

Several studies carried out in Atlantic coastal waters have demonstrated that *Nephrops* is likely to have a high discard survival rate (Mehault et al., 2016; Merillet et al., 2018). In 2016, we carried out preliminary tests to evaluate whether *Nephrops* or *Parapenaeus longirostris* were good candidates for a study that could demonstrate a high discard survival rate in Mediterranean crustacean fisheries. It was concluded that only *Nephrops* was a good candidate, and these results were published by Demestre et al. (2018). However, these tests were limited to vitality assessment on deck, and it became evident that a more robust methodology and larger sampling efforts were needed to eventually demonstrate a high discard survival rate for *Nephrops*. To date, no investigations have confirmed a high discard survival rate for *Nephrops* in the Mediterranean Sea despite its commercial importance and the repeated realization that technical regulatory measures have proven ineffective in reducing unwanted catch.

Several factors are thought to potentially affect the survival of discarded *Nephrops*, including technical, biological and environmental characteristics (Giomi et al., 2008; ICES-WKMEDS, 2014; Mehault et al., 2016). Specifically, *Nephrops* survival varied seasonally in assessments conducted in the Atlantic (Castro et al., 2003; Albalat et al., 2010), and this may be related to biological factors such as the period of maturation and reproduction (Orsi Relini et al., 1998). Survival may also be affected by handling practices on deck (Bergmann et al., 2001; Macbeth et al., 2006) and the duration of air exposure (Davis and Olla, 2002: Broadhurst et al., 2006; Benoît et al., 2010, 2012). Rapid and abrupt changes in salinity and temperature have also been shown to negatively affect the survival of *Nephrops* (Harris and Ulmenstrand, 2004) and other crustaceans (Giomi et al., 2008).

One of the main differences between the Atlantic and Mediterranean *Nephrops* fisheries is the depth of the fishing grounds. In the North Sea and in areas close to the Iberian Peninsula, such as the northern Bay of Biscay, *Nephrops* are fished from 50 to 80 m (Ungfors et al., 2013), whereas in the western Mediterranean Sea, *Nephrops* populations are mainly located in deep water on the continental slope from 300 to 600 m (Maynou and Sardà, 1997; Maynou et al., 1998; Abello et al., 2002). This depth difference has important implications for several aspects related to the survival of discarded *Nephrops* catches. At a technical level, deep water fishing in the Mediterranean generally entails a single 6−7 hour haul per day, compared to areas near Scotland where 2 hauls of 3 or 4 h are generally carried out (Johnson et al., 2013). Therefore, captured individuals are likely under stress for longer periods. The fishing depths also create a large temperature differential between the nearly constant 13 °C year-round bottom temperature in the Mediterranean deep sea (Hopkins, 1985) and the warm to hot air temperatures to which catches are exposed once hauled on deck. In summer, air temperatures higher than 30 °C are common, and surface water temperatures can be 26 °C or higher in July and August (Spanish National Meteorological Office, AEMET). Sudden seasonal changes in temperature may affect the survival of discarded animals differently in the Mediterranean and Atlantic since the temperature difference to which *Nephrops* catches are exposed in the Atlantic is generally lower.

Three methods for assessing the survival of discarded animals have been described by the ICES Workshop on Methods for Estimating Discard Survival (WKMEDS): captive observation, vitality assessments (i.e., indicators of survival potential) and tagging/biotelemetry (ICES, 2014). In isolation, each method has limitations that can restrict the usefulness of the produced survival estimates. However, when two or more of these methods are combined, there is clear potential for considerable synergistic benefits, including reduced resource requirements and improved accuracy and precision of survival estimates. (ICES, 2014; ICES CRR, 2020).

The study presented here aimed to assess the survival of discarded *Nephrops* from a Mediterranean trawl fishery on the Catalan Coast, NE Spain. It used captive observations and vitality assessments to determine the seasonal variability in survival rates as well as likely causes of mortality. The results may be used to improve the sustainable management of this fishery by better informing decisions about the most appropriate measures for promoting the survival of released *Nephrops*.

2. Material and methods

2.1 Study area and fishing Characteristics

The animals included in this study were sampled from 10 hauls, which were carried out in three seasonal blocks between 2016 and 2017 (spring from 24 May to 14 June; summer from 6 to 20 September; winter from 21 December to 12 February) on the "Malica" fishing grounds, which are adjacent to Blanes on the Catalan coast (Fig. 1). Malica was chosen as the study area because it has characteristics typical of Mediterranean *Nephrops* fishing grounds with regard to both the commercial importance and discard rates of *Nephrops* (García - deVinuesa et al., 2018). The sampling was done onboard a commercial trawler (20.6 m length, 600 HP and 64.91 GT). The cod-end nominal mesh was 50 mm diamond. The towing speed of the trawl was between 2.3 and 2.8 knots. The tow durations of the hauls were between 127 and 376 min (Table 1), with a maximum fishing depth of 408 m. The catch weight was measured through data extrapolation of subsamples taken on board and varied, by haul, between 44.1 and 248.8 kg. The air temperature was measured onboard and ranged between 5 °C in winter and 25 °C in summer. The surface water temperature was captured from official data published by the L'Estartit weather station (operated by the Catalonia Meteorological Service) near the study area and ranged between 13 °C in winter and 24 °C in summer. The temperature of the black plastic non-slip surface where the catch was sorted was between 30 and 37 °C when measured in the summer of 2019. Additionally, more cloud cover was observed in summer than in spring and winter.

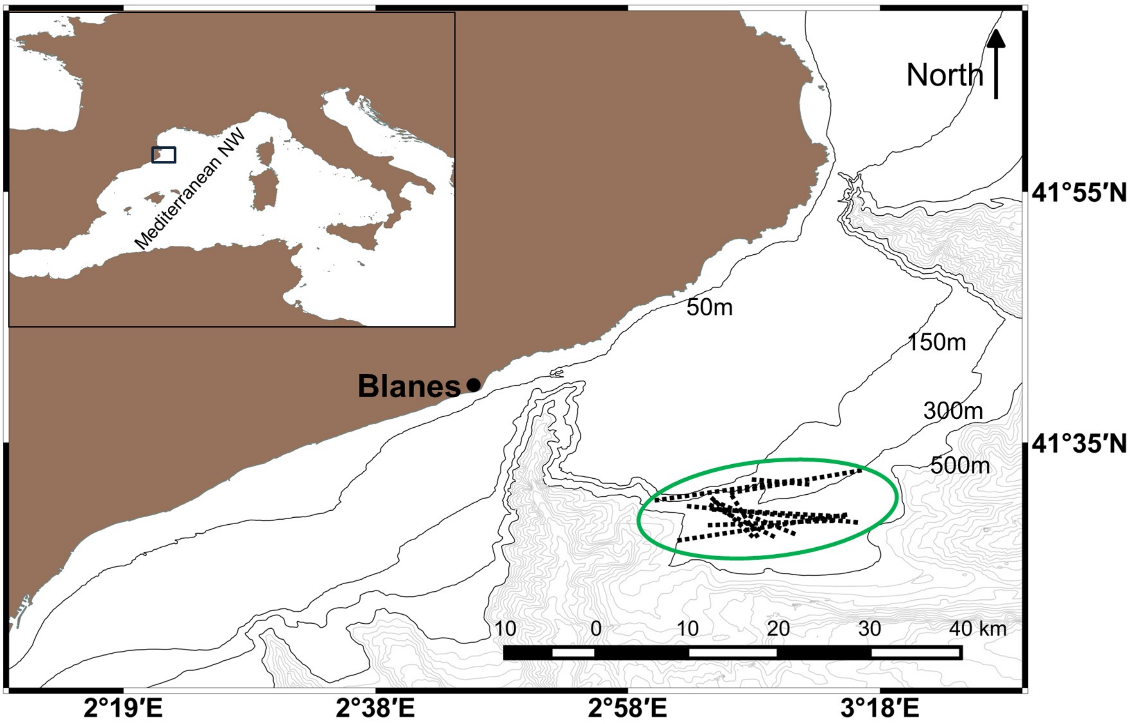

Fig. 1: Catalan Coast study area and haul locations: the "Malica" fishing grounds are shown by a green ellipse adjacent to the port of Blanes, and the 10 selected hauls for the study are indicated by dashed black lines.

Table 1: Characteristics of each haul used for the captivity experiment: Rep ID indicates the experiment replicas in chronological order taking into account the seasons (Spr (spring), Sum (summer), Win (winter)), sample size (n), cloudiness from 1 (no clouds) to 8 (totally cloudy) (Cld (1-8)), air temperature (AT), surface water temperature (WT), haul depth (Dph), haul duration (HT) and catch weight (CW).

Rep ID	n	Cld (1-8)	AT (°C)	WT (°C)	Dph (m)	HT (min)	C.W (kg)
Spr1	141	1	20	16	311	350	248.8
Spr2	87	1	18	20	362	210	158.5
Spr3	111	2	23	21	364	132	106
Sum1	112	3	22	23	320	376	100.9
Sum2	100	7	25	23	307	127	44.1
Sum3	101	8	23	24	320	188	86.9
Win1	109	1	7	14	275	180	91.5
Win2	108	1	12	13	309	120	112.55
Win3	115	2	7	15	408	315	180
Win4	116	1	5	15	247	345	118.7

2.2. Vitality assessment

The vitality status of each sampled *Nephrops* was assessed using the categorical vitality assessment (CVA) method (ICES CRR, 2020) and to avoid stressing the specimens, the evaluations were carried out as quickly as possible. This assessment method used both behavioural indicators and the presence of injuries to determine the vitality status of each animal with respect to one of four categories: 1 (excellent), 2 (good), 3 (poor) or 4 (dying or dead) (Table 2). In the event of a contradiction between the behaviour and injuries, the most negative assessment prevailed.

Table 2: Criteria for the Categorical Vitality Assessment (CVA) for *Nephrops norvegicus* in a western Mediterranean trawl fishery.

Vitality status	Code	Behavioural	Injuries
Excellent	1	Spasmodic body movements, aggressive posture	No external injury
Good	2	Continuous body movements, responds to contact	Superficial injury or loss of some pereiopods
Poor	3	Weak body movements, can move antennas, pereiopods or maxillipeds	Loss of some chelipeds or cuts
Dying or dead	4	No movement, does not respond to repeated contact	Deep cuts, crushed or punctured carapace

2.3. Sampling and survival experiment

To determine the seasonal variation in survival, a total of 10 replicate treatment hauls were carried out: 4 replicates in winter, 3 in spring and 3 in summer. A total of 1100 discarded *Nephrops* (< 27 mm CL) were sampled from the catch and were briefly held aboard the fishing vessel before transfer to a shore-based aquarium for monitoring, including a 2−3 hour transit time to port. During the 2-week monitoring period, a total of 13 CVAs were carried out (T0…T12), either onboard the vessel, following transit or during captivity in the landbased aquaria (Table 3). Individuals classified in the excellent vitality category (Table 2) at time T0 were taken as pseudo-controls for each haul, as per WKMEDS guidelines (ICES CRR, 2020).

Table 3: Location and timing of vitality status assessments from the beginning of the experiment (T0=0.5 hours) to the end (T12= 2 weeks).

Places/vitality	T0	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
assesment in time	0.5H	4H	16H	28H	40H	52H	64H	76H	88H	94H	1 WEEK	1.5 WEEK	2 WEEK
On-board	x												
Transfer		x											
Aquaria (ICM)			x	x	x	x	x	x	x	x	x	x	x

2.3.1. On-board

After hauling in the net, the catch was deposited onboard on a nonslip black plastic surface, which followed the standard catch handling and sorting process of the vessel's crew. Immediately after this, *Nephrops* specimens that had been separated from the comercial fraction and were to be discarded were randomly sampled and transferred into one rectangular plastic holding tank containing surface sea water, which was renewed intermittently (Fig. 2). No more than 30 min after the catch was brought onboard, the initial CVA (T0) was conducted, and the animals were segregated according to their vitality status (Table 2) into one of four separate white plastic containers (50 L each). The initial sample size was limited to ~100 individuals to avoid overcrowding the specimens in any one of the four holding containers (for a maximum of 40 animals per container). The white plastic containers were supplied with running surface sea water and 5 Dajana oxygen-producing tablets per hour to prevent hypoxia. In addition, to controlling the water temperature in the containers during the warmer seasons (spring and summer), the containers were placed on ice packs during transit to the port and then transported to the laboratory in an air-conditioned vehicle (18 °C). It took a maximum of four hours to transfer the specimens back to the shore-based aquaria (i.e., a maximum of 3 h transit to the port, and 1 h from the port to the laboratory).

Fig. 2: *Nephrops norvegicus* in the catch (left) and white plastic containers filled with surface seawater into which the samples were transferred (right).

2.3.2. Transfer

The specimens, which were transported in the white plastic containers, were transferred as quickly as possible to the experimental aquarium facilities at the Institute of Marine Science (ICM) in Barcelona. The first hours of the assessment were the most critical period for survival, so to achieve better outcomes, an additional vitality assessment was performed when the animals were transferred into the aquaria (T1). At this moment, the CL of the largest individuals was measured to assess the maximum size of the discards. Here, individuals were again segregated into separate sections in the aquarium tanks according to their vitality status, and animals in state 4 (dead or moribund) were removed.

2.3.3. In the aquarium (ICM laboratory)

This phase was conducted in the experimental tanks at ICM. Eight further assessments (T2…T9) were conducted in the first 94 h of the observation period, with one assessment every 12 h. Later, three further assessments were made 1 week, 1.5 weeks and 2 weeks after the start of the experiment (T10, T11 and T12).

Each aquarium was partitioned into three sections, with one for each state of vitality (i.e., 1, 2 and 3). Each section had dimensions of 80 cm in length, 45 cm in width and 25 cm in depth and a maximum of 20 specimens per section to avoid overcrowding. When there was a change in the state of vitality in a specimen, it was isolated into another reserved aquarium with the same characteristics as previously described to avoid confusing it with other specimens.

To simulate natural conditions, each aquarium had an open-circuit seawater system; a water temperature between 13 and 14 °C; a photoperiod adapted to the natural light cycle; a black canvas to dim the light; periodic controls of salinity, nitrates, nitrites and silicates; and bricks and rocks to provide artificial shelter and free movements within all sections. The specimens were not fed during the assessment as in the other works (Mehault et al., 2016; Merillet et al., 2018) because *Nephrops* can naturally survive long periods without eating

2.4. Data analysis

The vitality at the initial time (T0) per season was explored by calculating the percentages of individuals in each vitality state.

2.4.1. Kaplan-Meier

Kaplan-Meier (KM) analysis (Kaplan and Meier, 1958) was used to describe survivorship over time (with a 95 % confidence interval) for the pooled season data, pooled CVA data, replicates and pseudo-controls. To study the possible significant differences among the seasons and vitality statuses over time, a log-rank test was conducted (see below). These analyses were conducted using the “survival” and “survminer” packages in R 3.3.1 (R Development Core Team, 2016).

2.4.2. Parametric survival modelling

The generalized parametric survival model proposed by Benoît et al. (2015) was fitted to each replicate experiment in each season to estimate the survival rate at the asymptote, as per WKMEDS guidelines (ICES CRR, 2020). The time to reach the asymptote can also be estimated from this model to determine whether the monitoring period was sufficient to allow all treatment-related mortality to be expressed.

This approach models the survivorship of *Nephrops* over time based on the mortality and censoring times observed in the experiment. The model was written as follows:

$$S(t) = \tau \cdot \left(\pi \exp[-(\alpha t)^{\gamma}] + (1 - \pi)\right)$$

where $S(t)$ is the survivorship at time t, α and γ are parameters of a Weibull survival distribution that describes the mortality of *Nephrops* as a result of the treatment (i.e., capture, transfer and captivity in aquaria), τ is the initial survival rate and π describes an asymptote in the discard mortality following the treatment (for a derivation, see Benoît et al., 2015). In this model, α, γ, π, and τ are all estimated parameters. From these parameters, one can separately estimate the initial capture and handling mortality rate, $1 - \tau$, and the post-transfer mortality rate (i.e., in captivity), $\tau\pi$; thus, from these metrics, the total treatment mortality rate is $1 - +\tau\ \tau\pi$. One can also estimate the time at which total treatment mortality has approximately reached its asymptote (i.e., within 99.9 %) as follows:

$$t_{asymptote} = \alpha^{-1} \log(1000)^{1/\gamma}$$

This variable was estimated for each replicate to confirm that all discard-related mortality had occurred by the end of the monitoring period.

The survival model was fitted to the data using maximum likelihood. The fit suitability was assessed by comparing the model predictions and non-parametric Kaplan-Meier estimates of survivorship.

2.4.3. Factors of survival

After confirming that mortality had reached an asymptote in all treatments by the end of the monitoring period (see Results), the relationship between the survival of *Nephrops*, season and vitality status (at T0) was investigated using a GLM with a binomial distribution and logit link function fitted to the overall survival data (i.e., after 14 days (336 h) of monitoring). To define survival after 14 days as the dependent variable, the *Nephrops* specimens with vitality 1, 2 and 3 were assigned codes of 1 (alive), while those in state 4 were assigned codes of 0 (dead). The best model was selected with a stepwise procedure based on the minimization of the Akaike information criterion (AIC), and the variance explained by the model was estimated using Nagelkerke's pseudo R2 (Nagelkerke, 1991).

3. Results

3.1. Survival and vitality analysis

The mean overall survival at day 14 was 0.43 (95 % confidence interval, CI: 0.40−0.46). There was substantial variability between replicates (Table 4), with the highest survival in trial "Win4" (Table 1) with 0.85 (CI: 0.78−0.91). The pseudo-controls (animals with vitality status: 1) typically had high survival rates between 0.67 and 1, except in the summer, which had survival rates of 0.5 or less.

Table 4: Results of the survival rates (S.rate) together with their respective pseudo-controls and 95% confidence intervals (C.I.) for each replicate.

Rep ID	S. rate	C.I.	n	Control S. rate	Control C.I.	Control n
Spr1	0.23	0.17-0.32	141	0.67	0.50-0.89	24
Spr2	0.51	0.41-0.62	87	0.91	0.79-1	21
Spr3	0.4	0.32-0.5	111	0.83	0.69-1	23
Sum1	0	-	112	0	-	4
Sum2	0.11	0.06-0.19	100	0.28	0.13-0.59	18
Sum3	0.07	0.03-0.14	101	0.5	0.23-1	6
Win1	0.73	0.65-0.81	109	0.91	0.81-1	32
Win2	0.69	0.6-0.78	108	0.85	0.74-0.98	34
Win3	0.7	0.62-0.79	115	0.94	0.87-1	36
Win4	0.85	0.78-0.91	116	1	-	35

The mean survival at day 14 showed significant seasonal variation: that in winter was 0.74 (CI: 0.67−0.78); in spring, 0.36 (CI: 0.31−0.41); and in summer, 0.06 (CI: 0.04−0.09) (Fig. 3). There were also significant differences in mean survival over time between vitality statuses (at T0), with the highest survival for *Nephrops* that were initially in excellent (1) condition, followed by those in good (2) and poor (3) conditions and ultimately those that were dying or dead (4) (Fig. 4). The highest mean survival (at day 14) was for those that were initially in the excellent (1) state, with 0.81 (CI: 0.76−0.86), while the lowest survival was observed in dead and dying *Nephrops* (vitality status 4), with 0.07 (CI: 0.04−0.11).

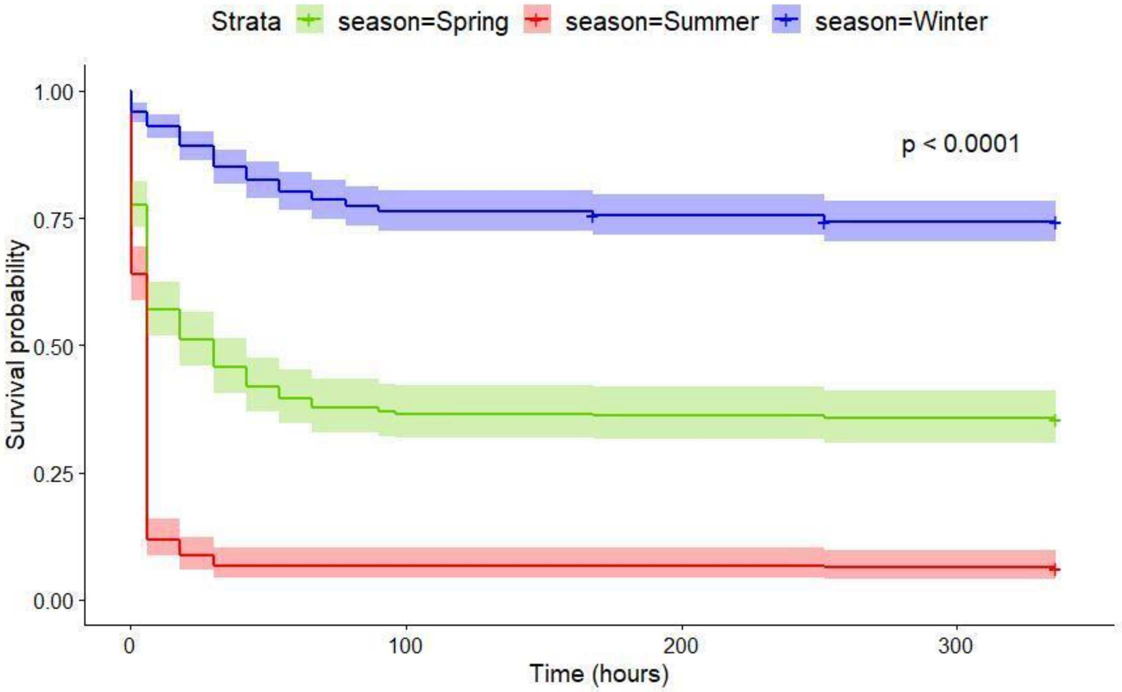

Fig. 3: Kaplan-Meier survival curves (with 95% confidence intervals) for *Nephrops norvegicus* caught in demersal trawls in different seasons: spring (green), summer (red) and winter (blue); data from different trials/replicates were pooled within seasons.

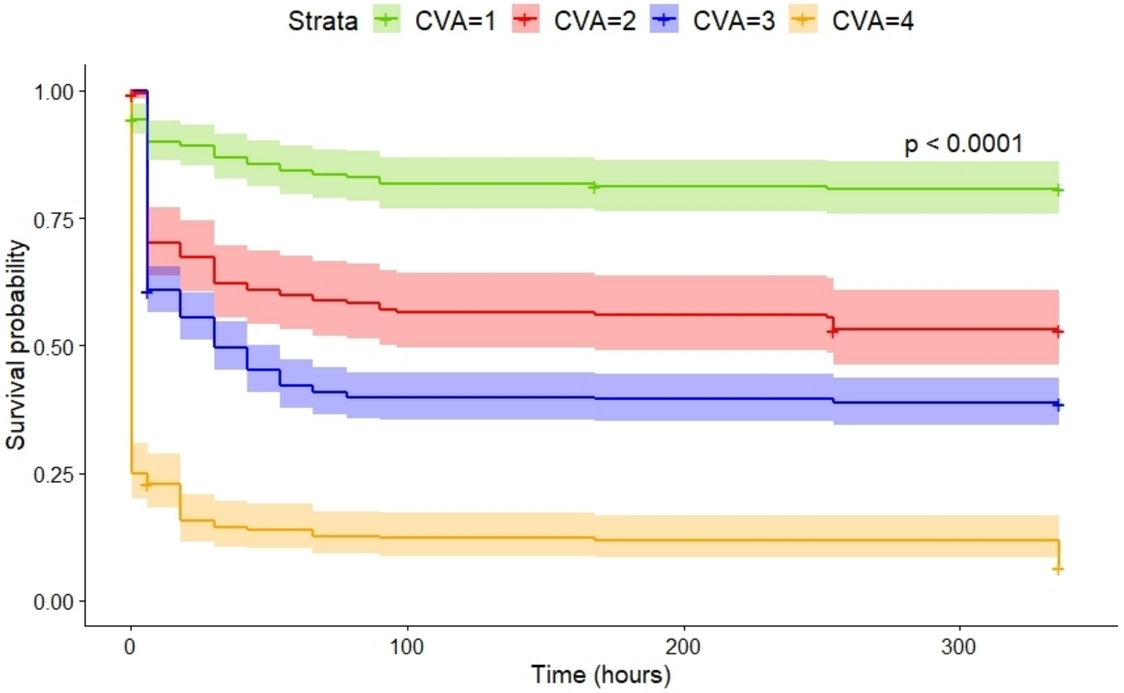

Fig. 4: Kaplan-Meier survival curves (with 95% confidence intervals) for *Nephrops norvegicus* with respect to vitality (CVA category at t_0): excellent (1) (green), good (2) (red), poor (3) (blue) and dead or dying (4) (yellow); data were pooled across replicates/trials and seasons.

The results of the vitality status analysis at T0 by season showed higher percentages of animals in excellent and good conditions in winter, which decreased in spring and was the lowest in summer (Table 5). In addition, the percentage of dead or dying individuals at the initial time (T0) was lower during winter and increased in spring until reaching the highest percentage in summer.

Table 5: Percentage of *Nephrops* individuals at each state of vitality per season at the beginning of the experiment (T0).

Season/CVA	1 (% excellent)	2 (% good)	3 (% poor)	4 (% dying or dead)
Spring	20.6	17.3	36.9	25.3
Summer	9.2	11.4	41.8	37.6
Winter	30.6	19.4	42.9	7.2

3.2. Parametric survival modelling

The predicted survivorship functions from the parametric survival model followed the Kaplan-Meier estimates very well (Fig. 5). Estimates of tasymptote from the model were within the range of those during the monitoring periods for all experimental trials (Table 6).

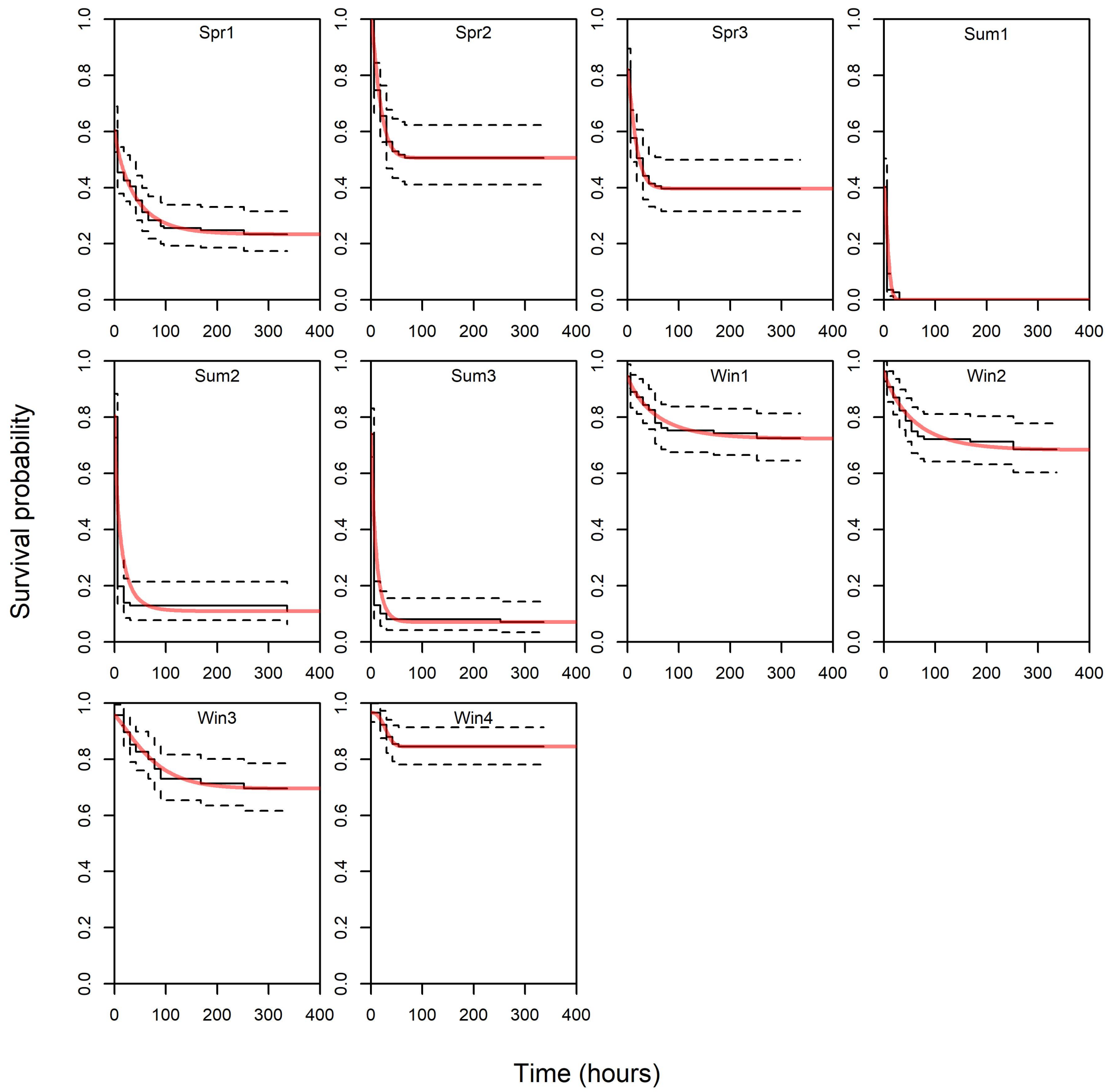

Fig. 5: Survivorship functions for *Nephrops norvegicus* sorted chronologically from left to right from spring to winter in the 10 experimental trials based on non-parametric Kaplan-Meier estimates (solid line; 95% confidence interval, dashed line) and predictions from the parametric survival model (red line).

Table 6: Estimates of $t_{asymptote}$ from the parametric model for each replica (Rep ID).

Season	Rep ID	$t_{asymptote}$
Spring	SPR1	227.4
	SPR2	64.1
	SPR3	60.9
Summer	SUM1	23.8
	SUM2	93.5
	SUM3	54.3
Winter	WIN1	307.1
	WIN2	314.0
	WIN3	261.6
	WIN4	58.4

The estimated initial mortality (0 h, 1 − τ) was the highest and most variable for the spring and summer experimental trials, ranging from 0.18 to 0.60, except for trial “Spr2″, which had no initial mortality (Fig. 6). In contrast, the estimated initial mortality was substantially lower in the winter, ranging between 0.04 and 0.06.

The estimated total discard mortality (at day 14) based on the parametric model was very similar to the Kaplan-Meier survival results (Table 4). The greatest mortality was in the summer experiment and ranged from 0.9 to 1.0 (Fig. 6). The uncertainty for the estimate for trial “Sum1″ was elevated because all individuals died, resulting in uncertainty in the survival asymptote parameter π. Estimates for π for spring were somewhat lower, ranging from 0.49 to 0.77, while estimates for winter were by far the lowest, ranging from 0.16 to 0.32. The confidence intervals for those estimates did not overlap those for the trials in other seasons.

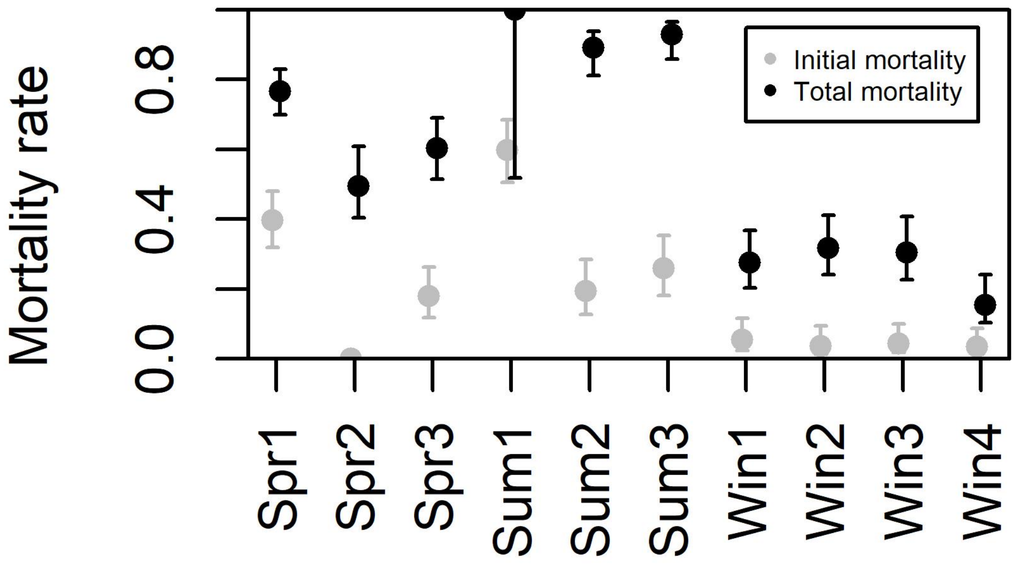

Fig. 6: Estimates (with 95% confidence intervals) of the initial (grey) and total (black) mortality for each trial. Each trial is numbered sequentially within each season: spring (Spr), summer (Sum) and winter (Win).

3.3. Factors of survival

The deviance explained in the GLM model of survival (at day 14) was 39.79 %. Survival differed significantly between seasons, as did the vitality status (Table 7). The parameter estimates (Table 7) confirmed that survival was higher in winter and lower in summer in comparison to in spring (baseline). Moreover, the survival for vitality statuses 2, 3 and 4 was consecutively lower than that for state 1 (baseline). A significant interaction between season and vitality resulted from the survival patterns for *Nephrops* with vitality status 3 in winter.

Table 7: GLM model of survival at day 14 (336 hours), with significance Pr (> | z |) where significant values are shown in bold font and standard error (Std.error), the value of the Z statistic and the estimate are shown for each parameter. The proportions of deviance explained by the increasingly more complex models were: 27.13% (season only, AIC:1100.1), 39.16% (season and vitality status, AIC: 935.58), 39.79% (season and vitality status with interaction AIC: 928.01).

Time (14 days)	Estimate	Std.error	Z value	Pr(>|z|)
Intercept	1.35	0.30	4.50	**6.76E-06**
Factor(Season)Summer	-2.65	0.55	-4.82	**1.43E-06**
Factor(Season)Winter	1.09	0.43	2.51	**0.01**
factor(vitality)2	-1.61	0.40	-3.97	**7.68E-05**
factor(vitality)3	-2.02	0.36	-5.65	**1.65E-08**
factor(vitality)4	-4.81	0.66	-7.30	**2.97E-13**
Factor(Season)Summer:factor(vitality)2	1.05	0.78	1.35	0.18
Factor(Season)Winter:factor(vitality)2	0.31	0.57	0.55	0.58
Factor(Season)Summer:factor(vitality)3	0.30	0.72	0.41	0.68
Factor(Season)Winter:factor(vitality)3	0.23	0.50	0.45	0.65
Factor(Season)Summer:factor(vitality)4	1.33	1.29	1.04	0.30
Factor(Season)Winter:factor(vitality)4	1.99	0.81	2.44	**0.01**

4. Discussion

This study has for the first time provided empirical evidence for the survival of discarded *Nephrops* removed from the catch of a commercial trawl in the Mediterranean Sea. The overall mean survival of 0.43 (CI: 0.40−0.46) was comparable to the mean survival rates of ~0.5 observed for *Nephrops* sampled from trawl catches in the Atlantic at comparable latitudes (e.g., Mehault et al., 2016; Merillet et al., 2018). All of these survival assessments, including that in the present study, used similar methods to determine post-capture and handling mortality, namely, captive observation (ICES CRR, 2020).

Captive observation assesses the effects of a treatment by monitoring specimens in a suitable holding facility for a sufficient period for any resultant mortality to be expressed. Containment facilities and conditions can influence the survival of marine animals in captive observation studies (ICES WKMEDS, 2014). In previous studies, separate boxes and/or cells have been used to house individual *Nephrops* due to their cannibalistic behaviour (Sarda and Valladares, 1990). While this avoids cannibalism, it can restrict freedom of movement, which could negatively impact welfare and therefore survival. Our approach provided freedom of movement and places to hide, which better replicated conditions experienced by *Nephrops* when they are returned to their natural habitat.

To demonstrate that captivity itself has not contributed to the observed mortality, it is recommended that suitable captivity control be employed (ICES CRR, 2020). In other survival assessments of *Nephrops* (e.g., Campos et al., 2015; Merillet et al., 2018), control animals were captured using traps or short duration trawl hauls to try to obtain *Nephrops* in excellent condition. However, traps tend to select larger *Nephrops* that are not representative of discards from the trawl fishery (García -de-Vinuesa et al., 2018), while short trawl haul control groups can have survival rates that are only marginally higher than those of the treatment groups (Merillet et al., 2018). In our study, we selected individuals identified by the CVA as having vitality status 1 (excellent) as the pseudo-controls (as suggested by ICES CRR, 2020). This provided us with a subset of *Nephrops* with an appropriate size range and no notable injuries. This method works on the premise that if the pseudo-control survival is close to 1.0, then the handling and captivity that the animals are subjected to after sampling has not been detrimental to them and so is less likely to have affected the mortality observed in other specimens (ICES CRR, 2020). Conversely, if the pseudo-control survival is substantially less than 1.0, this does not conclusively infer that there was a captivity effect, but it does reduce our confidence that the observed survival in the treatments was not biased (underestimated) (ICES CRR, 2020). In general, the pseudo-control survival was high, with a mean value of 0.81 (CI: 0.76−0.86). However, summer was an exception, with pseudo-control survival of between 0 and 0.5. In addition, during the summer, the number of control animals was the lowest in the study, which could lead to less precise estimation of its survival. As already demonstrated in other studies (e.g., Giomi et al., 2008), we theorized that the temperature changes between the normal, stable habitat of *Nephrops* on the seabed (∼13 °C) and the higher temperatures at the water surface (∼24 °C), in the air (∼25 °C) and on the catch-sorting mat (30−37 °C) induced thermal shock, which led to high mortality during the first hours of experimentation (T0-T1) on animals that previously seemed to be in an excellent state of vitality.

Refrigeration during the transfer of individuals may also not have been sufficient to achieve an appropriate water temperature (∼13 °C), and future studies should employ water cooling systems during the transfer, as has already been tested in another *Nephrops* survival study (Merillet et al., 2018). Water temperatures above 13 °C during transfer could have resulted in the underestimation of survival rates during warmer periods. However, this possible underestimation does not contradict the seasonal effects on *Nephrops* survival because the CVA carried out at the beginning of the experiment (T0) was not subject to this potential experimental bias. Moreover, the initial mortality (at T0) showed marked seasonality, with higher mortality in summer (0.26−0.6) than in winter (0.05−0.1) and high percentages of animals in excellent and good condition in winter, which decreased in spring and were the lowest in summer.

Seasonal variation in *Nephrops* survival has also been observed in the Atlantic (Castro et al., 2003; Lund et al., 2009; Merillet et al., 2018), although those specimens showed greater survival during the summer. In our study, the air temperature reached 25 °C. This temperature was higher than those in other studies carried out in the Atlantic Ocean, where the temperature in summer was approximately 19.4 °C (Merillet et al., 2018). In addition, in the Mediterranean during late spring and summer (i.e., between May and September) large *Nephrops* moult prior to reproduction (Sarda and Valladares, 1990) and ovary maturation and brooding occurs in female *Nephrops* (Orsi Relini et al., 1998). This could make them more vulnerable to injury during trawling, and the reduced metabolic capacity may reduce the animal's ability to cope with the stresses of capture and handling. Thus, it would be inappropriate to discard *Nephrops* during this period because it will result in low survival. The potential for sex-biased survival should be further investigated in *Nephrops* and other crustaceans.

Another challenge faced by all captive observation survival assessments was ensuring that the monitoring period had sufficient resolution and was long enough to observe all treatment-related mortality. In this study, most mortality was observed during the first 72 h post-treatment, while we monitored mortality every 12 h up to 96 h and then at a coarser interval until 14 days. Furthermore, the survival functions in all replicates were shown to have reached asymptote within the 14-day (336 h) monitoring period.

This study provided a systematic definition of vitality status for *Nephrops*, which is the ICES recommendation for survival assessments (ICES, 2014). The GLM analysis showed a significant relationship between vitality status and survival. In addition, a log-rank test showed consistent significant differences in survival over time between all vitality levels, where the highest survival was for the excellent state (1) and the lowest was for the dead or moribund state (4). This agreed with similar studies carried out in the Atlantic (Armstrong et al., 2016; Merillet et al., 2018) and suggested that vitality may be a useful mortality predictor for discarded *Nephrops* in the Mediterranean. However, there were some inconsistencies noted between the behavioural and injury criteria used to define the *Nephrops* vitality status. Moreover, the fact that the latter group, "dead or moribund" (vitality status 4), did not consistently have zero survival indicated that the criteria used to define this vitality status could benefit from some refinement. Future work should more thoroughly investigate the relationship between initial vitality status and survival of *Nephrops* with the aim of improving our mortality predictor for discarded *Nephrops* in the Mediterranean.

5. Conclusion

The seasonal variation in post-release mortality, which had very high values in summer, suggests that a survival exemption to allow post-release discarding in summer would be ineffective. Only seasonal fishery closure during the summer months would be appropriate to avoid producing fishing mortality on undersized *Nephrops*. Alternatively, technical improvements should be made onboard fishing boats to promote the survival of unwanted catches, such as protection from direct sunlight, refrigerated holding tanks, and white (or refrigerated) non-slip sorting tables.

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