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By-catch species susceptibilities and potential for survival in Algarve (South Portugal) deep-water crustacean trawl fishery

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By-catch species susceptibilities and potential for survival in Algarve (South Portugal) deep-water crustacean trawl fishery

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Declaração da autoria do trabalho

Declaro ser a autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

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Ana Catarina Adão

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ABSTRACT

Bottom trawling for crustaceans in Portuguese coastal waters constitutes a rather important fishery in revenue terms, despite its clear negative impacts on deep-sea communities and marine ecosystems. This poorly selective harvest strategy catches large amounts of unwanted species that are thrown overboard for various reasons. However, survival of discards is not yet properly assessed and constitutes an essential parameter for the upcoming landing obligation, with an exemption for species with "high survival". In this work, time-to-mortality and a vitality assessment were used to estimate immediate mortality and identify important biological characteristics on the susceptibility of a group of 14 by-catch species, most with commercial interest (*Conger conger*, *Galeus melastomus*, *Helicolenus dactylopterus*, *Lepidorhombus boscii*, *Lophius budegassa*, *Lophius piscatorius*, *Merluccius merluccius*, *Micromesistius poutassou*, *Mullus surmuletu*s, *Phycis blennoides*, *Scyliorhinus canicula*, *Trigla lyra*, *Trachurus trachurus* and *Trachurus picturatus*). Only *S. canicula* and *C. conger* were identified as species with potential to survive after the discarding process. Present results on time-to-mortality show significant differences when comparing individual sizes for some species, with smaller individuals dying faster than larger ones. Furthermore, species with scales, gas bladder and high metabolic rates are more vulnerable to die after being discarded. A short captive observation experiment with *C. conger* was conducted, with 84% survival after 65 hours of monitoring. However, this survival rate is likely to be overestimated due to two facts: 1) the mortality rate did not stabilize at the end of the experiment; and 2) the majority of individuals showed severe injuries (scratches, bruises and deep wounds). These outcomes can help to identify species that will likely survive the discarding process, factors influencing its survival and provide reliable estimates of unaccounted fishing mortality, essential for stock management and conservation.

Keywords: by-catch and discards; time-to-mortality; biological traits; survival; trawling; South Portugal

RESUMO

O arrasto de fundo é uma das artes de pesca mais comum em todo o mundo, apesar dos seus impactos destrutivos: desde modificações na morfologia dos fundos marinhos e ressuspensão de sedimentos a alterações nos ciclos de nutrientes e biodiversidade em águas profundas. Devido à sua natureza não selectiva, as redes de arrasto capturam grandes quantidades de espécies não desejadas que são posteriormente rejeitadas, no caso de não terem valor comercial, quota ou outras restrições legislativas (tamanhos mínimos legais, limites de percentagem de espécies não-alvo). De modo a evitar este problema, uma obrigação de desembarque tem vindo a ser implementada com a reforma da Política Comum de Pescas, que força os pescadores a manter a bordo e descarregar todas as espécies que possuem uma quota (ou total admissível de captura). No entanto, existem várias excepções a esta legislação. Uma delas é para espécies com 'elevada sobrevivência', que podem continuar a ser rejeitadas desde que haja evidências científicas que suportem esta decisão, daí a importância de averiguar a sobrevivência das rejeições.

A frota de arrastões de marisco em Portugal tem como espécies-alvo o lagostim (*Nephrops norvegicus*), a gamba-branca (*Parapenaeus longirostris*) e o camarão-vermelho (*Aristeus antennatus*), operando sobretudo ao largo dos canhões de Lagos, Portimão e Faro. Este segmento da frota representa menos de 4 % do total de licenças de pesca, mas os crustáceos capturados são o grupo mais caro, atingindo preços de 16 euros por kg em 2016. Contudo, a taxa de rejeição de pescado aproxima-se dos 70 %, com mais de 90 espécies de vertebrados e invertebrados. Vários projectos dedicados ao estudo das rejeições têm vindo a ser realizados, com foco na identificação de espécies, quantificação das taxas de rejeição e impactos ecológicos e económicos. Estratégias de mitigação como diminuição do comprimento do saco da rede e sistemas de grelhas selectivas foram testadas, mas apenas as grelhas demonstraram eficiência em reduzir a captura de peixe não desejado, sem afectar significativamente a captura de crustáceos. Um aumento do tamanho da malha no saco de 55 para 70 mm também produziu resultados significativos em termos de redução de pescado rejeitado. No entanto, nenhuma destas alterações técnicas foi adaptada pelos pescadores, devido a possíveis impactos económicos. Artes de pesca estáticas como armadilhas para lagostim e gamba têm vindo a ser investigadas como alternativa ao arrasto de fundo, pois produzem reduzidos impactos nos habitats marinhos e quase nenhum pescado é rejeitado.

Quanto à sobrevivência das rejeições, experiências com espécies não desejadas de peixe e caranguejos pescados com ganchorra mostraram taxas de sobrevivência entre 54% e 81% após 48 horas em cativeiro. No cerco, 20% a 80% da sardinha sobreviveu após a captura e durante um mês em tanques. No arrasto de fundo, apenas estudos com lagostim foram concretizados, com taxas de sobrevivência entre 13% e 60%, mas nenhum sobre espécies rejeitadas. Há três metodologias principais que podem ser usadas na investigação da sobrevivência: estudos de vitalidade; observação em cativeiro; e estudos de marcação-recaptura ou biotelemetria. Escalas de vitalidade e indicadores como tempo-para-mortalidade podem ser aplicados para avaliar a sobrevivência imediata. Apesar de não conseguirem prever mortalidade após rejeição, estes métodos são simples de aplicar e fornecem informação relevante para um elevado número de espécies. A observação em cativeiro consiste na captura de animais selvagens que, antes de serem rejeitados, são transferidos para tanques ou outras instalações e monitorizados durantes dias até semanas. Idealmente, a experiência de sobrevivência deve prolongar-se até a taxa de mortalidade estabilizar. Esta metodologia implica maiores custos e logística quando comparada com a anterior. No entanto, permite estimar de forma mais fiável a taxa de sobrevivência e fazer inferências acerca da sobrevivência a longo-prazo após rejeição.

Este trabalho está dividido em duas partes: 1) estudo das susceptibilidades das espécies rejeitadas; 2) experiência de sobrevivência a curto prazo com congro (*Conger conger*). Ambas foram realizadas a bordo de um arrastão em pesca comercial. No primeiro, foram usados indicadores de sobrevivência imediata para um grupo de 14 espécies: escalas de vitalidade com 4 categorias, desde excelente estado a moribundo; tempo-para-mortalidade, ou tempo até o animal não apresentar qualquer sinal de vida, quando exposto ao ar; e registo de vários tipos de lesões externas, como perda de escamas, arranhões ou feridas expostas. Estes indicadores permitem distinguir espécies com potencial de sobrevivência. Porém, a avaliação da vitalidade necessita de ser adaptada às particularidades de cada espécie, sobretudo a animais de profundidade que possuem estratégias de conservação de energia quando em situações de stress extremo. Foram também identificadas características biológicas que determinam a vulnerabilidade destas espécies: tamanho do animal, bexiga-natatória, escamas e taxa metabólica. Para algumas espécies, o tamanho dos indivíduos representou um factor relevante, pois animais mais pequenos são mais vulneráveis e morrem mais rapidamente do que

animais de maiores dimensões. Para além disso, espécies com escamas, bexiga-natatória e taxas metabólicas elevadas também são menos resistentes e têm reduzidas hipóteses de sobreviverem após rejeição. Apenas duas espécies surgiram como candidatas a estudos mais pormenorizados para averiguar o seu potencial de sobrevivência: pata-roxa (*Scyliorhinus canicula*) e congro ou safio (*Conger conger*). Assim, na segunda parte deste estudo, 84% dos congros sobreviveram após 65 horas de monitorização. Contudo, esta taxa de sobrevivência está sobrestimada devido a duas problemáticas: a mortalidade não estabilizou no final do período de observações, ou seja, se a experiência tivesse sido mais prolongada, ainda iria registar-se maior mortalidade; e cerca de 70% dos animais apresentaram lesões externas severas, o que coloca sérias questões acerca da sua recuperação e sobrevivência após rejeição. Para estudos futuros, recomenda-se experiências de sobrevivência mais prolongadas, em tanques em terra, de modo a evitar os problemas de espaço e logística que implicam trabalhar num navio. As metodologias escolhidas neste trabalho limitaram as conclusões obtidas, mas ao mesmo tempo implicaram uma estratégia de obter informação nova e importante, com baixo custo, que pode ser usada como ponto de partida para outros projectos. Estes resultados identificaram espécies que poderão sobreviver após a sua rejeição, factores que influenciam a sua sobrevivência e estimativas de mortalidade provocadas pela pesca que são essenciais à gestão e conservação das populações.

Palavras-chave: rejeições; tempo-para mortalidade e vitalidade; características biológicas; sobrevivência; arrasto de fundo; Algarve

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1. INTRODUCTION

Most marine fisheries are mixed fisheries directed towards only a few commercial target species but along with the target species, a variable and often high number of by-catch species is captured (Borges & O'Dor 2010). The Algarve deep-water trawl fishery targets three main crustacean species (*Parapenaeus longirostris*, *Nephrops norvegicus* and *Aristeus antennatus*) at fishing depths of between 200 and 700 meters. Sweeping the seabed with heavy ground gear imposes serious consequences for benthic ecosystems. Also, a number of unwanted species are caught and often rejected overboard. This study aimed at studying the vulnerability and survival potential of these discarded species, taking into account biological factors that determine their mortality.

1.1 The by-catch problem

Bottom trawling represents one of the most common fishing practices around the world despite its severe impacts on marine ecosystems, causing modifications in sea-bottom morphology and resuspension of sediments. As a result, alterations on the nutrient cycles and benthic biodiversity are evidences of its potential consequences (Pusceddu *et al*. 2014; Clark *et al*. 2015). Trawl nets as the ones used in crustacean demersal trawlers collect an extensive range of species and sizes, thus have high by-catch rates, meaning amounts of unwanted species that are hauled on-board. For a clear definition, by-catch is defined as any organism caught unintentionally, while discards are the portion of the catch which is not used and thrown away at sea (Borges & O'Dor 2010). In fact, by-catch can represent a large proportion of total catch and may be high in species diversity, especially in demersal mixed-species fisheries. In the Portuguese demersal trawl fishery, close to 80% of total catch weight is discarded, composed by more than 90 vertebrate and invertebrate species (Borges *et al.* 1997). Some of these species have economic value and are retained and sold, while others are discarded overboard due to no marketable value, quota or other legal restrictions (minimum landing sizes, catch composition regulations; Borges *et al*. 2001; Costa *et al*. 2008; Erzini *et al*. 2002). "The discard problem" raises a number of issues, from policy and ethical implications, fisheries management, to ecological, economic and technical concerns (Kelleher 2005).   Discards, when dead, may have serious consequences on the populations of target and non-target species, especially if the majority of the rejected specimens are undersized, which can lead to decreases in future yield and reproducing possibilities (Tingley *et al.* 2000). At the same time, fisheries discards and offal represent an important source of food mainly for benthic scavengers and invertebrates, and also for mid-water opportunistic species such as sharks, marine mammals and seabirds (Veiga *et al.* 2015; Votier *et al.* 2004; Olaso *et al.* 1998). The combined effect of trawling disturbance and discards may favour the rapid and short-term growth of small scavenger benthic species of amphipods and isopods for example, leading to important alterations of deep-water trophic webs and species interactions (Bellido *et al.* 2011; Kelleher 2005; Castro *et al.* 2005).

When comparing reported discard estimates from European Union (EU) logbooks with those obtained by scientific observers, the reported by-catch represented only 0.06% of the weight recorded by researchers (STECF 2013). Thus, by-catch and discards studies are essential not only to quantify the impacts of discarding practices on fish stocks but also to understand to which extent fishing activities affect and alter the marine environment.

1.2 The Landing Obligation

In terms of fisheries management of European Union fleets, the Common Fisheries Policy (CFP) aims to promote fisheries that should exploit marine resources in an environmentally, economically and socially sustainable way. To achieve this, an ecosystem-based approach shall be applied to minimise negative impacts of fishing activities on marine ecosystems. Moreover, the CFP targets the restoring or maintenance of fish stocks above biomass levels capable of producing maximum sustainable yield, which means the highest theoretical amount that can be constantly taken from a stock under regular environmental conditions without significantly disturbing the reproduction process (European Union 2013). Another management tool focusing on protection of marine biodiversity resources is the Marine Strategic Framework Directive (MSFD) with the main goal to achieve a good environmental status of EU's marine waters (European Commission 2016). All these goals were planned to be achieved no later than 2020, together with a gradual elimination of discards through the introduction of a discard ban. This key modification obligates fishermen to keep on board and land all regulated species; i.e., species under quotas (total allowable catches or TACs) or minimum conservation reference sizes (MCRS, in the Mediterranean). For this, species with TACs will suffer quota increments in order to represent real total catch, consequently increasing fishing mortality. For species with limited quota, these can become ‘choke species’, forcing the vessel to stop fishing once the quota is reached. However, there are several exceptions to this regulation in which the release of animals will still be permitted. This includes species with special conservation status as rays and sharks for which fishing is prohibited (European Union 2017).

1.2.1 ‘High survival’ exemption

An exemption for “species for which scientific evidence demonstrates high survival rates” is in place, but the term ‘high survival’ is not absolutely defined and might induce some ambiguity. To capture and keep on-board species that may survive the discarding process can also have negative impacts on spawning stock biomass, so this exemption should be applied in a case-by-case basis considering detailed characteristics of the species and fisheries (STECF 2013; paragraph 4, Article 15, European Union 2013).

1.2.2 ‘De minimis’ exemption

Moreover, *de minimis* exemptions were also created that allow for discarding of 5% of TACs for each stock, in the most challenging circumstances, namely when “improvements in selectivity are considered to be very difficult” and “disproportionate costs of handling unwanted catches do not represent more than a certain percentage”, concepts that also contain subjectivity in how to express ‘difficulties in improving selectivity’ and ‘disproportionate costs’. The problem of improving selectivity is that implies changing the current fishing practices that might decrease the revenues of the fleet. So, the real issue are the economic repercussions of increasing selectivity instead of a technical ‘difficulty’ (STECF 2013; paragraph 5, Article 15, European Union 2013).

1.2.3 Inter-species and inter-annual quota flexibilities

In order to avoid ‘choke species’, a mechanism of inter-species quota flexibility was also introduced to offer a method of exchanging quota from a target species (donor) to a non-target species (recipient), up to 9% of the quota of the target species. This can occur when a vessel has finished its quota for a specific stock and needs to stop fishing in areas where that species is caught, irrespective of how much quota it has for other stocks in the same area (STECF 2013; paragraph 8, Article 15, European Union 2013). However, the recipient non-target stock must be inside safe biological limits, otherwise this flexibility cannot be applied. An inter-annual quota flexibility of 10% of the TAC can similarly be used for species under the landing obligation (paragraph 9, Article 15, European Union 2013).

Theoretically, these exceptions shall only be applied after other technical methods to avoid capture of unwanted species have been tested, because the combined application of the exemptions might provide a way of continuing to discard species as formerly. This might discourage the objectives of improving exploitation patterns and minimising unwanted catches (STECF 2013, 2014).

1.2.4 Possible negatives outcomes

Following this landing obligation, logistic and economic complications might emerge due to increased sorting time and storage on board of former non-kept species that can lead to greater costs and/or lower incomes. Currently, there are no prepared installations or procedures on harbours to manage landed discards (Veiga *et al.* 2015). Besides, previously discarded species cannot be used for direct human consumption, so should be processed for other means as fish meal and oil, pet food, pharmaceuticals and cosmetics (European Union 2013). This additional burden may cause non-compliance with the no-discard policy if considered as unfeasible by fishermen (STECF 2014). Examining fishers' perceptions and behaviour can help to understand likely complications that might arise from the landing obligation (Villasante *et al.* 2016). As an example, even when applying exemptions, the results of modelling the impact of the landing obligation in UK demersal fisheries show that only 39% of the available quota could be landed in 2019, causing a drastic reduction of revenue, mainly due to the problematic of choke species (Russell *et al.* 2016).

The evaluation of economic and social impacts of the landing obligation at this initial period is still being developed (European Commission 2016b). In Norway, the 'discard ban package', implemented throughout the last 30 years, combines strategies such as a system of little compensation for fishermen with landing discards (only to pay the costs) and real-time closed areas when by-catch reached a certain percentage, which would obligate the vessel to travel to another fishing area. Together with other technical measures (e.g. grid sorting systems), this scheme promoted incentives to fish more selectively and allowed a quick recovery of the exploited stocks (Diamond & Beukers-Stewart 2011). However, current economic incentives in European fisheries seem to encourage the usual discarding routines and retain the maximum amount of commercial species instead of focusing in changing exploitation patterns and fisher's practices (STECF 2013; Zimmermann *et al.* 2015).

Accurate information about discard rates and survival of rejected specimens is essential to determine sources of unaccounted fishing mortality and thus calculate correctly fishing at maximum sustainable yield level and other biological reference points (STECF 2013). Moreover, with increases in quotas for regulated species in order to account for the former discards that will be landed, the stock assessments need to be as exact as possible to maintain the primary goal of sustainable use of fisheries resources.

1.3 The Portuguese trawl fishery

The trawling fleet in Portugal is composed mainly by vessels targeting fish and crustaceans, with distinct métiers and regulation measures, operating at a minimum distance of six nautical miles off the coast. The crustacean bottom trawlers have limited licenses, for two separate mesh size categories: 55-59 mm mesh size with which target species have to compose 30% of total catch and 70 mm net mesh size when targeting Norway lobster (Diário da República 2000). The fish trawling fleet targets mainly semi-pelagic species, such as horse mackerel (*Trachurus trachurus*) and Atlantic mackerel (*Scomber scombrus*); and uses nets with 65-69 mm mesh size, where 70% of total catch must be composed by these target species. These by-catch limitations have been one of the main reasons for discarding of commercial species (Campos 2003). Besides being managed by catches limits (total allowable catches or TACs) and technical measures (minimum conservation reference size or MCRS), control of the fishing effort can also be done for these fleets by implementing closed seasons, as for rose shrimp (*Parapenaeus longirostris*; Diário da República 2006). However, the implementation of a minimum landing size is not always in agreement with the biological characteristics of the species, often being below the size of first maturation (Campos 2003). For instance, minimum landing size for European hake is set at 27 cm, though the length of first maturity is nearly 33 cm for male and 45 cm for female (Piñeiro & Saínza 2003). The same occurs for other species: *Trachurus trachurus* has a MCRS of 15 cm, while the length of first maturation ranges from 21 to 30 cm; *Conger conger* reaches first maturation at minimum of 2 meters length, but the MCRS in Portuguese legislation is 57 cm. For one of the main target, Norway lobster, a minimum landing size of 20 mm was set despite reaching maturity at 23 mm of carapace length (Abelló & Sardá 1982; Froese & Pauly 2016; Diário da República 2001).

This deep-water crustacean fishery targets the rose shrimp *P. longirostris*, the red shrimp *Aristeus antennatus* and the Norway lobster *Nephrops norvegicus* and concentrates around the Canyons of Lagos, Portimão and Faro (Barlavento or occidental coast of the Algarve). The substrate of these fishing grounds is composed mainly by a mixture of silt and mud, the preferred habitat of the target species (Silva *et al.* 2014). The distributions of the three crustacean species overlap, but *P. longirostris* is commonly found between 200 and 400 m depths, while *A. antennatus* from 300 to 600 m depths; *N. norvegicus* has an uneven distribution between 200 and 700 m (Monteiro *et al*. 2001). During recent years, the landings of rose shrimp have been increasing in contrast with the declining catches of Norway lobster, possibly due to the restrictions in quota and fishing closures imposed for *N. norvegicus* to allow for the recovery of the stock, as well as an increased abundance of *rose shrimp* (Diário da República 2016; Silva *et al*. 2010). Despite the fact that trawlers only represent around 3.7% of the total number of Portuguese fishing licenses, they are a very important segment of the Portuguese fleet. For example, the mean commercial value of the crustacean species was more than 16 euros per kg and at the same time represented around 1% of total landings in 2016 (Figures 1.1 and 1.2; INE 2017).

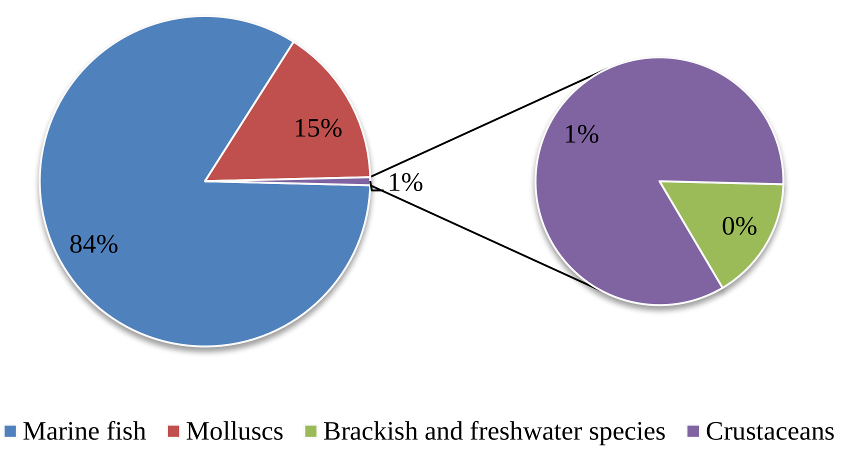

Figure 1.1 - Mean landings of landed fish on Portuguese ports by main species groups in 2016. Weight in tonnes was converted in weight as percentage (INE 2017).

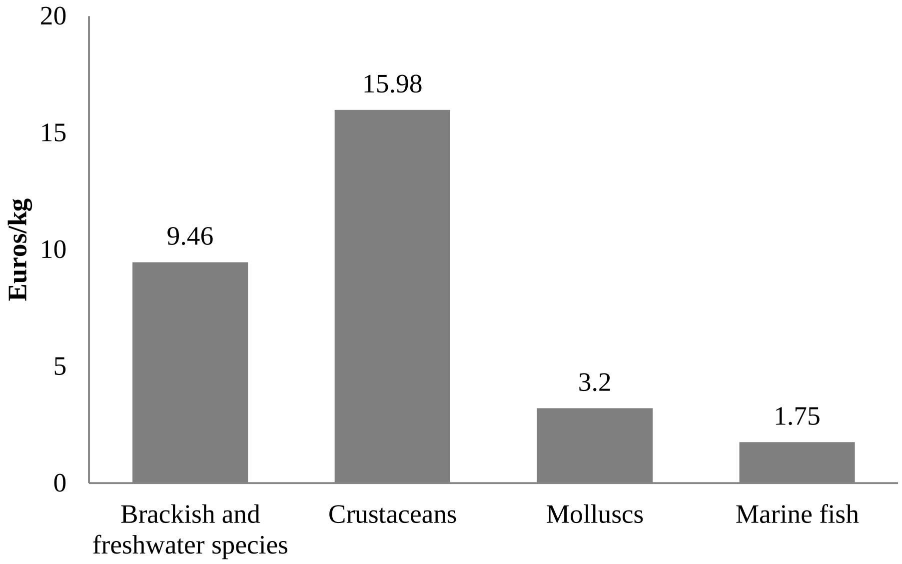

Figure 1.2 - Mean prices per kilogram of landed fish on Portuguese ports by main species groups in 2016 (INE 2017).

After 60 years of heavy trawling the seafloor in south and southwest Portuguese coasts, there is evidence of erosion and degradation of the sea bottom. This leads to alterations in habitat structure and biodiversity, with increased risk for particular ecosystems such as crinoid beds that host a diverse number of taxa (Morais *et al*. 2007; Fonseca *et al*. 2014). Another relevant effect of this fishing activity is the diversity and amounts of unwanted species discarded primarily due to low or no commercial value

(Borges *et al*. 1997, 2000, 2002). Previous work has shown discard rates of approximately 70% in crustacean trawlers (Monteiro *et al*. 2001; Costa *et al*. 2008), meaning weight of by-catch surpassing largely the weight of target species. The main discarded fish species are blue whiting (*Micromesistius poutassou*), European hake (*Merluccius merluccius*), Atlantic horse mackerel (*Trachurus Trachurus*) and blue jack mackerel (*Trachurus picturatus*), conger (*Conger conger*) and shark species (*Scyliorhinus canicula*, *Galeus melastomus*, *Etmopterus spp.*). Fish communities change according to depth range; in shallow waters until 120 meters deep, horse mackerel and axillary seabream compose most of the total biomass; while in deeper waters the biomass is dominated by blue whiting and hake, so by-catch composition will reflect these differences (Gomes *et al.* 2001). By-catch species under catch quotas (or TACs) in this fishery are *M. merluccius*, *Trachurus spp.*, *Lophius spp.*, *Micromesistius poutassou*, *Lepidorhombus spp.*, and more recently *Phycis blennoides* (European Union 2016, 2017). All are commercially valuable species, but discarded due to quota limitations and minimum landing sizes (or minimum conservation reference sizes).

Several exceptions to the landing obligation are already in place for this fishery. Blue whiting (*M. poutassou*), despite representing the most discarded species in terms of weight, is not included in the 70% maximum percentage of by-catch species (Diário da República 2000; Monteiro *et al.* 2001). A 'de minimis' exemption for hake (*M. merluccius*) is in place; up to 7% of the TAC can still be discarded based on the fact that 'viable increases in selectivity are very difficult to achieve', that could make the fishery possibly unprofitable (European Union 2015)[1]. Hake was considered a choke species in the North Sea Nephrops trawlers, obligating the fishery to stop working earlier due to quota exhaustion, even when applying quota uplifts of 20% (STECF 2014). In Portuguese waters, if an exemption was not in place, hake would also be a choke species because it is one of the most discarded species and at the same time only 3142 tonnes can be landed by all fleets in national waters (European Union 2017). A recovery plan for hake and Norway lobster was agreed in 2005 with the aim to rebuild the stocks to safe biological limits until 2015 (European Union 2005). Based on this recovery plan, the efforts should go to decreasing fishing mortality rates, which the recent exemption entirely contradicts since allows fishermen to continue discarding this species. These regulative measures do not encourage fisher's compliance or adoption of alternatives to reduce by-catches (Campos *et al.* 2014).

1.4 By-catch in Portuguese fisheries

The knowledge about by-catch and discards in the Algarve (South Portugal) and the resultant ecological and economic consequences has been enriched since mid-90, with three European-funded projects with the goal to quantify and manage discards ("DISCARDS", "DISCALG" and "BYDISCARD" projects; Borges *et al*. 1997, 2000, 2002). The first two projects (from 1996 to 2000) focused on identifying and quantifying discards and understanding the reasons for discarding of the most important fishing métiers of southern Portugal (trammel net, demersal purse seine, pelagic purse seine, fish trawl and crustacean trawl). The demersal purse seine and the trammel net in particular were shown to have low discard rates. The latter study ("BYDISCARD", 1998-2000) was dedicated only to the three other gears (pelagic purse seine, fish trawl and crustacean trawl) that represent most concern in terms of discard quantities and rates. Nevertheless, discard studies on pelagic purse seine and trammel nets continue to be done (Batista *et al*. 2009; Gonçalves *et al*. 2007; Marçalo *et al*. 2006, 2008). Reasons for discarding were generally economic such low market value, damaged or poor quality fish or species for which there is no readily available market or regulatory measures (excess quota, minimum size restrictions). Within the BYDISCARDS project, aspects of the biology of by-catch species were considered together with accurate identification and recording of discard rates; ecosystem's dynamics and economic outcomes of reduction of by-catch were also taken into account. A number of written-outcomes on by-catch and discarding practices from different fishing gears in the Algarve were published (Monteiro *et al*. 2001; Borges *et al.* 2001; Erzini *et al*. 2002; Costa *et al*. 2008). Two devices aiming to minimise by-catch rates were investigated on crustacean trawlers: changes in the sweep length of the trawl nets (18 and 90 m lengths) and a sorting-grid system. The modification in sweep length did not produced any relevant results in terms of by-catch amounts and composition, but the sorting-grid demonstrated significant efficiency in maintaining the catch of target species (95% of the crustaceans) while allowing for the escapement of the most discarded fish species (73% escapement of blue whiting; Borges *et al*. 2002). Other studies related to codend selection investigating different mesh configurations and sizes were also performed, with varying results (Campos *et al*. 2003; Fonseca *et al*. 2007; Campos & Fonseca 2004). For instance, an increase in cod end mesh size (55 to 70 mm) would be a reasonable and effective approach to avoid the catch of undersized shrimp and reduce by 40% the amount of by-catch (Campos 2003). The use of a modified Nordmore sorting grid was also tested for by-catch reduction with high levels of exclusion of non-commercial bycatch (48 to 74% exclusion rates), although simultaneously with short-term losses of target species, from 4 to 15% (Fonseca *et al*. 2005). None of the latter by-catch reduction devices was adopted into national law or used by fishermen. Present-day studies have proposed measures to transform bottom trawlers into more selective gears because the shift to low impact gears (e.g. traps) is a serious challenge. Until their effectiveness is proven, alternative gears will not be accepted, even if it means conserving fuel and consequently decreasing costs with almost no by-catch. Low impact, fuel efficient gears should be given more investment in order to explore their potential as future sustainable harvesting methods (Suuronen *et al.* 2011).

Since 2014, a large-scale European Union funded project is being conducted with the aim to "minimise unwanted catches by incentivising the adoption of fishing technologies and practices that reduce pre-harvest mortality and post-harvest discards, while avoiding damage to sensitive marine species and habitats" (MINOUW 2016). Solutions that incentivise fishermen first to avoid unwanted catches will be developed, and where this cannot be feasible, approaches on how to utilise the landed by-catch sustainably will be experimented but without profit to the producer. The Algarve demersal crustacean fishery is a case study in this project, since this harvest technology explores deep-water habitats with great biodiversity resulting in high by-catch rates and discards ratios. By-catch reduction devices are further investigated and the possibilities for alternative gears such as traps for Norway lobster and Deep-water rose shrimp are being tested in order to reduce unwanted catches and minimize overall impact.

[1] To note that the trawling fleet is highly dependent on government subsidies not only to support the fuel costs but also for maintenance and technological improvements of the vessels (Innes & Pascoe 2009; Jacket & Pauly 2008).

1.5 Discard survival

During the discarding process, a fish will be exposed to different influencing factors and injurious events that will affect its potential for survival and must be properly identified and described for the species and harvest strategy (ICES 2014).

Relevant works on estimating discard survival have been conducted in commercial fisheries and research vessels around the world (Davis 2002; Broadhurst *et al*. 2006; Revill 2012; Uhlmann & Broadhurst 2015). For the Portuguese fisheries, a number of studies of post-release survival of discarded fish and invertebrate species have been done recently. Survival of *N. norvegicus* caught with trawlers and placed into cages ranged between 12.5% and 60% (Castro *et al*. 2003). In contrast, for individuals initially caught by traps, average survival rate reached 86% (Campos *et al*. 2015). Survival experiments of by-catch fish (*Trachinus vipera*; *Dicologlossa cuneata*) and crab (*Polybius henslowii*) from bivalve dredges showed that 54 % to 81% of the individuals died after 48 hours captivity (Leitão *et al*. 2014). On purse seine fishing, Marçalo *et al*. (2008) identified the main factors that affect early survival and stress reactions of sardine (*Sardina pilchardus*) after capture and holding in tanks, with survival rates varying from 20% to 80% after a month of captivity. No studies have yet been done on the survival of by-catch fish species in demersal trawlers in Portuguese waters.

1.5.1 Methodologies to study discard survival

The ICES working group on Methods for Estimating Discard Survival (WKMEDS) has been regularly producing reports for guidance on how to estimate levels of discard survival in commercial fisheries (ICES 2014). Discard survival assessments can be conducted through three different methodologies: vitality assessment that gives estimates of immediate mortality; captive observation; and tagging/biotelemetry. There is no standard time frame to conduct a survival experiment, since it depends on the study species, the factors influencing survival and the logistical limitations of the investigation. Ideally, the experiment should continue until the mortality rates stabilizes, i.e. reaches an asymptote. Otherwise, mortality will be underestimated.

Vitality assessments can be applied as a first step of a survival study. Detailed and prolonged evaluations of discard mortality obtained by captive observation and tagging are costly, technically difficult to acquire, and only available for a limited number of species and fisheries (e.g. Huse & Vold 2010; Marçalo *et al*. 2008; Campos *et al*. 2015; Laptikhovsky 2004). So, semi-quantitative indicators that estimate the degree of injury given by a vitality assessment and a coarse mortality indicator that calculates time-to-mortality (TTM) are more cost effective preliminary assessments. Complementary studies are still needed to predict post-release survival of discards and to justify an exemption to the landing obligation. Nevertheless, these methods allow estimating immediate mortality, for a large number of species and over a wide range of conditions. Based on the outcomes, they can be used to distinguish species that demonstrate potential for survival after discarding and may need further investigation (Benoît *et al*. 2013; Benoît *et al*. 2010; Depestele *et al*. 2014; STECF 2014). Captive observation consists of transferring wild-caught animals (before being discarded) into tanks or other holding facilities (e.g. underwater cages) and monitoring them until the mortality rate stabilizes, which might take days to weeks or months. The survival estimate using this approach is representative of real fishing conditions and when combined with previous vitality assessments, can give a more exact survival rate (excluding predation) illustrative of the particular fishery and species in study (ICES 2014). This methodology has higher costs and logistics when compared with vitality assessments, but allows making inferences on long-term survival more realistically and supported with statistical modelling.

1.5.2 Factors influencing discard survival

Discard mortality results from the interactions of the individuals with the fishing gear and is affected by environmental, technical and biological factors. A combination of these should be considered when designing a discard survival study (ICES, 2014; Davis 2002; Broadhurst *et al.* 2006; Revill 2012; Uhlmann & Broadhurst 2015). There are a wide range of variables that can possibly be measured and taken into account. Biological traits, such as body size, presence and type of gas bladder, as well as other covariates i.e. water and air temperature, sea conditions, tow duration and speed, size of total catch and its composition, have been shown to affect discard mortality, both within and between species (Benoît *et al*. 2013; Davis 2002; Broadhurst *et al*. 2006). This work prioritized the biological characteristics of the different species: body size, deciduous scales, injuries, presence and type of gas bladder and metabolic rates. These appear to be the most influential factors and could be easily measured and analysed given the available resources. This information was compiled from general literature and field observations; when detailed information was not available, species were given the same trait category as other species within its family or order (Froese & Pauly 2016; WoRMS Editorial Board 2016; IUCN 2016; FAO 2016; ICES-FishMap 2014; Hill & Wassenberg 1990; Jacobsen *et al*. 2002; Revill *et al*. 2005; Encyclopaedia Britannica 2017).

Body length

Smaller individuals appear to be more vulnerable to discard mortality due to increased susceptibility to injury while crushing during the haul or greater exhaustion from swimming and trying to escape the trawl (Suuronen 2005). However, one effect that can be considered more relevant is that fish of smaller size are likely to be more susceptible to hypoxia due to their increased mass-specific metabolic rates and consequently higher energy expenditures in breathing activities (Benoît *et al*. 2013).

Gas bladder

Species that possess gas bladder, especially a closed swim bladder, where there is no connection between this organ and the gut (physoclistous condition), suffer significantly increased mortality due to depressurization when fish are brought to surface, such as extrusion of internal organs and the rupture of the gas bladder itself (Benoît *et al*. 2013). On the other hand, physostomous gas bladders (open bladders where there is a connection to the gut) allow for a regulation of the amount of gas via esophagus and therefore depressurization may not have such drastic effects. For individuals without a swim bladder, it is assumed that depressurization effects would not be so damaging when compared with organisms that hold a gas bladder (Broadhurst *et al*. 2006).

Deciduous scales

Loss of scales can contribute to higher mortality of fish in the medium to long term due to risk of infection. In the context of this work, deciduous or soft scales that easily fall off can represent a biological trait that increase susceptibility to injury and desiccation when the fish are stressed and compressed together inside the trawl net as well as when exposed to air after capture and handling (Benoît *et al*. 2013; Broadhurst et al 2006).

Metabolic rate

Metabolic rates of low activity level fish can be related with increased resistance to stress as a strategy to conserve energy of sedentary species (Helfman *et al.* 2009). So, metabolic rate was considered as a possible explanatory variable in species susceptibilities. This information was gathered from metabolism studies and when metabolic rate values were not found at the species level, major groups were used (Clarke & Johnston 1999; Yang *et al.* 1992; Cowles & Childress 1995; Carlson *et al.* 2004).

1.6 Objectives

This project had two main goals: first, to study bycatch species susceptibilities; and second, to assess the potential survival of *Conger conger* in a short experiment. Both were conducted on-board a commercial trawler. In the first part, vitality assessments (time-to-mortality or TTM and a categorical vitality assessment, CVA) were used to estimate immediate mortality of a group of 19 by-catch species, in order to prioritise which species might have the possibility to survive for further investigations. Also, the effects of biological traits (e.g. size, presence and type of gas bladder, scales, injuries and metabolic rate) on TTM were considered since these determine to a great degree the susceptibility of a species to die after being caught and discarded. For the second study, individuals of *C. conger* were caught and maintained in captivity to estimate the mortality rate. Types and number of injuries were also registered and related with the observed mortality. *C. conger* was chosen because it demonstrated high possibility of survivorship in the initial assessment and no previous survival studies were found.

2. MATERIALS AND METHODS

2.1 Study of by-catch species susceptibilities

2.1.1 Methodologies

Three assessment methods were applied: a categorical vitality assessment (CVA), time-to-mortality (TTM) and evaluation of external injuries.

Categorical vitality assessment (CVA)

CVA is based on four vitality classes (table 2.1) that consider at one extreme very lively and responsive fish (score 1) and at the other extreme, unresponsive and without any movement fish (score 4). This method provides a scored index of an individual's vitality may be used to infer potential discard survival (ICES 2014).

Time-to-mortality (TTM)

TTM is the time required to induce mortality during air exposure; previous works have indicated that air exposure is one of the greatest contributors to discard mortality rates, making this estimator a good discard mortality proxy (Benoit *et al*. 2012; Davis 2002; Broadhurst *et al*. 2006). Besides, provides a rough measure of the sensitivity of different species that can be used to rank them and identifying which ones have greater probabilities of survival (ICES 2014).

External injuries

External damages were also registered for each individual based on simple descriptions of 4 types of injuries – scale loss, bruises, superficial wounds and deep wounds (scored as present, 1, when clearly observed and as absent 0, when not present or when was not obvious its occurrence; table 2.2). This assessment was applied separately from CVA because different species shown specific characteristics. For example, European conger, monkfish and the sharks do not have scales. Presence of injuries has a direct relationship with trauma and possible infections, and consequently mortality (ICES 2014).

2.1.2 Sampling design

Sampling was conducted in December 2016 (during six days) and February 2017 (five days duration) in a commercial fishing vessel along the south coast of Portugal, at a minimum distance of 6 nautical miles off the coast (figure 2.2). Positional data (latitude and longitude), depth, fishing operation details and environmental information (air temperature, sea state and light level) were recorded for most of the forty hauls. Water temperature ranged between 17 °C and 18 °C and the sea was mostly at moderate state, with waves reaching 1.3 meters. The temperature of the air varied from 17 °C to 20 °C, with cloudy sky in half of the period. The trawl gear was towed for 4 hours (95% confidence interval, or CI, of 3.6 to 5.2 hours), between 123 and 841 meters depth. Average speed of towing was 2.9 to 3 knots (given by the skipper), and hauling took 20 minutes on average (95% CI from 16.4 to 23 minutes). The cod end was emptied into a container below deck and in most hauls the net was re-deployed prior to catch sorting, which lasted around seven minutes. Total catch was roughly estimated to be 181 kg (95% CI from 155.1 to 208.1 kg) and the sorting process took on average 18 minutes (95% CI 15.6 to 20 minutes). Time 0 was defined as when the catch was dropped into this container below deck. The crew sorted the catch by hand and samples were taken right after the sorting process started.

For practical reasons, random samples of 10 to 15 individuals were collected from each species, depending on the by-catch composition of the haul, for further mortality monitoring. Individuals were monitored for 2-3 seconds for vitality assessment (table 2.1) and injury evaluation (table 2.2), until there were no signs of life. Immobile individuals were manipulated and tested for reflexes responses in order to be sure of dead state (if at least one of the reflexes was present, the individual was considered still alive; table 2.3). At the end of the monitoring period, total body length was measured and individuals of some species frozen for later observations of the type of gas bladder.

Table 2.1 - Description of the codes used to score the vitality (Benoît *et al.* 2010)

Vitality state	Score	Description
Excellent	1	Vigorous body movement without stimuli
Good	2	Weak body movement, but responds to touching
Poor	3	No body movement, no obvious response to stimuli, but fish can move operculum/mouth/fins
Dead/moribund	4	No body or opercular movements, no response to touching or grabbing

Table 2.2 - Description of the codes used to score the injuries (Catchpole *et al.* 2015)

Fish injury	Description
Scale loss	Visible area of scale loss
Bruises	Red bruising visible on the body
Wounding	Visible shallow cuts on the body
Deep wounding	Visible deep cuts on the body

Table 1.3 - Description of the reflex responses tested (Catchpole *et al.* 2015)

Name	Stimulus action	Reflex response
Operculum closure	The operculum of the fish is gently opened with a blunt object	Ability to tightly close/clamp its operculum after being opened within 5 seconds
Mouth closure	The mouth of the fish is gently opened with a blunt object	Ability to tightly close/clamp its mouth after being opened within 5 seconds
Gag response	A blunt object is inserted in the mouth of the fish and touch the throat	Fish gagged/vomit

Concerning the biological traits, injuries and deciduous scales were scored as present (1) or absent (0). Gas bladder was divided in three categories: no gas bladder, open gas bladder or closed gas bladder. The metabolic rate was classified in three levels (low, medium and high metabolic rate), according to the range of values found for the group of taxa.

2.1.3 Data analysis

CVA and TTM

Immediate mortality for each species was calculated, given by the number of individuals that were classified as dying/dead state (state four of vitality assessment) at the first minute of visual evaluation. Survival analysis was conducted using statistical R programming language ('survival package', R Development Core Team 2008) to model survival probability as a function of time, as a measure of fish tolerance to stress and air exposure.

Simple non-parametric Kaplan-Meier models were applied to estimate time to 50% mortality (TTM), after data censoring (table 2.4). This median value and its confidence intervals (CI) were defined by drawing a horizontal line at 0.5 on the plot of the survival curve and its confidence bands. The intersection of this line with the lower CI band defined the lower limit for the median's interval, and similarly for the upper band (error bars represented in figure 3.3). Data censoring occurs when the exact time of an event, in this case time of mortality after capture and handling, is not known (Benoît *et al*. 2013). Fish that were dead (score four on vitality assessment) when first observed at time 0 (before monitoring period, time T) are treated as left-censored observations in that their actual time of mortality occurred before time T. Fish that were still alive when mortality monitoring ceases are considered right-censored observations ($t > T$); the remaining individuals (i.e. those that died during TTM monitoring) are considered as uncensored observations ($t \approx T$). In addition, interval censoring was applied when mortality was known to occur between two times ($a<T<b$) but the exact time of mortality was not registered. This type of censoring was used specifically to join the TTM data for 12 species.

After data censoring, from a group of 19 species, 7 were excluded from the further statistical analysis (table 2.4, species with *) due to small sample size (*Citharus linguatula*, *Lepidopus caudatus*, *Hoplostethus mediterraneus*, *Scomber spp.*) or because the individuals were taken just from one haul (*Capros aper*, *Nezumia sclerorhynchus*, *Setarches guentheri*).

Table 2.4 – Results of censoring and further statistical analysis for each species. * represents species with small sample size or data coming from only one haul. KM – Kaplan-Meier

Species	**Censoring**			**Total**	**Data analysis**
	None	Right	Left		
*Capros aper**	22	0	0	22	Excluded
Conger conger	13	27	0	40	KM & Weibull
*Citharus linguatula**	2	0	2	4	Excluded
Galeus melastomus	14	0	6	20	KM
Helicolenus dactylopterus	51	4	8	63	KM & Weibull
*Hoplostethus mediterraneus**	4	0	6	10	Excluded
Lepidorhombus boscii	13	0	7	20	KM
*Lepidopus caudatus**	0	0	3	3	Excluded
Lophius spp.	41	0	9	50	KM & Weibull
Merluciius merluccius	19	0	14	33	KM
Micromesistius poutassou	6	0	24	30	KM
Mullus surmuletus	17	0	14	31	KM
*Nezumia sclerorhynchus**	0	0	33	33	Excluded
Phycis blennoides	6	0	34	40	KM
Scyliorhinus canicula	16	24	0	40	KM & Weibull
*Setarches guentheri**	4	0	11	15	Excluded
*Scomber spp.**	5	0	3	8	Excluded
Trigla lyra	13	0	25	38	KM
Trachurus spp.	45	0	3	48	KM & Weibull

Parametric Weibull models were used to correlate survival (from time-to-mortality estimates) with vitality at first observation and individual size. This was applied only for species with at least 40 individuals whose observations were mainly uncensored or right censored. In addition, the Akaike information criterion (AIC) was calculated to give the goodness-of-fit of each model and likelihood tests were performed in order to check if there were significant differences in AIC when comparing distinct models.

Biological traits analysis

TTM interval censored data was collated for 12 species that have distinct combinations of biological traits (injuries, scales, gas bladder and metabolic rate; table 2.5) and Weibull models were applied to determine whether these factors have an influence in the TTM results. Gas bladder was divided in three categories – absence of gas bladder, open gas bladder and closed gas bladder. Deciduous scales were defined as present or absent as well as injuries of different types. Metabolic rate was separated in three classes – low metabolic rate (0 – < 3 mg O2/h per 50 g body mass), medium (3 – 6 mg O2/h per 50 g body mass) and high metabolic rate (> 6 mg O2/h per 50 g body mass). Information from different groups and publications was converted into the same unit of consumption of milligrams of oxygen per hour, per 50 grams of body mass, assuming oxygen solubility 7.9 mg/L or 5.9 mL/L at salinity 35 g/kg, pressure of 1 bar and temperature around 15 °C.

Table 2.5 – Summary of the biological traits for each species and major groups – gas bladder, deciduous scales, metabolic rate class and metabolic rate values.

Group	**Species/Variables**	**Gas bladder**	**Deciduous scales**	**Metabolic rate class**	**Metabolic rate (mg O2/h) per 50 g body mass**
Anguilliformes	*Conger conger*	Yes (open)	No (tough skin)	Low	2.75 (15°C)
Scorpaeniformes	*Helicolenus dactylopterus*	No	Yes (ctenoid)	Medium	6.61 (10°C)
Lophiiformes	*Lophius spp.*	No	No	Low	0.77 (5 °C)
Carcharhiniformes	*Scyliorhinus canicula*	No	No	Low	1.91 (15°C)
Perciformes	*Trachurus spp.*	Yes (closed)	Yes	Medium	6.18 (15°C)
Carcharhiniformes	*Galeus melastomus*	No	No	Low	1.91 (15°C)
Pleuronectiformes	*Lepidorhombus boscii*	No	Yes	Medium	5.54 (15°C)
Gadiformes	*Merluccius merluccius*	Yes (closed)	Yes	High	13.34 (15°C)
Gadiformes	*Micromesistius poutassou*	Yes (closed)	Yes	High	13.34 (15°C)
Perciformes	*Mullus surmuletus*	Yes (closed)	Yes	Medium	6.18 (15°C)
Gadiformes	*Phycis blennoides*	Yes (closed)	Yes	High	13.34 (15°C)
Scorpaeniformes	*Trigla lyra*	Yes (open)	Yes	Medium	6.61 (10°C)

2. Potential for survival of by-catch – an experimental trial

2.2.1 Sampling design and methodology

The experiment was conducted in July (23 to 27) on-board a commercial demersal trawler along the south coast of Portugal, at a minimum distance of 6 nautical miles off the coast (figure 2.2). Positional data (latitude and longitude) and fishing depth were recorded. Once the net was emptied to the container below deck and the catch started to be sorted, 20 to 30 individuals (*C. conger*) were collected from each haul (if there are enough individuals) and placed in the tanks (first haul – tank 1; second haul – tank 2; figure 2.1). The fish were maintained on the tanks for up to 65 hours and monitored every 4 to 6 hours to check for dead fish and remove them. Water temperature in the tanks ranged between 13 °C and 18 °C using the on-board cooling system. It was not possible to have a continuous water flow, but the water in the tanks was completely renewed every 3 hours. When first placed in water, the position and behaviour of the fish was noted: if it floats or sinks (FL/SK) and if it tries to swim or not (SW: 1/0), within the first 5 seconds on water. The behaviour in water was also noted during the monitoring period: immobile (IM) or swimming (SW) and on the bottom (BT) or in the water column (WC). Major injuries were assessed at the end of the experiment – scratches, deep cuts, wounds or bruises (scored as presence/absence, 1/0). In-water reflexes were observed to confirm the vitality/survival status and at the end of the experiment (scored as 1, present if a response is clearly present or 0, absent if the response was not present or of weak or questionable strength; table 2.6). Total length and a photographic record was taken for all individuals.

Table 2.6 – Description of the codes used to evaluate in-water reflexes (Catchpole *et al*. 2015)

Name	Stimulus action	Reflex response
Startle touch	Fish is underwater and hand approaches to touch fish	Actively moves away before or at first touch
Tail grab	Fish is grabbed gently by its tail	Actively struggles to escape within 5 seconds
Orientation/Righting	Fish is held on the palm of the hand on its back just below the water surface and released	Actively righting itself underwater within 5 seconds

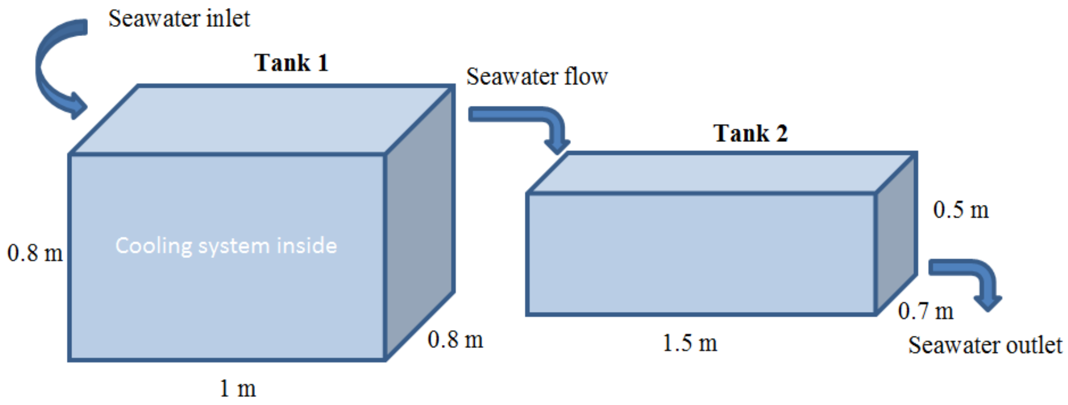

Figure 2.1 - Experimental design

2.2.2 Data analysis

Non-parametric Kaplan-Meier models were used to describe survival over time and plot survival curves to visually explore the obtained mortality rates. Log-rank tests compared and check for significant differences between the survival curves of the two tanks. Also, the percentage of individuals showing different types of injuries was calculated and number of injuries in each individual as added as a possible explanatory variable of the mortality results in a Weibull model. These analyses were performed using the ‘survival’ package in R software.

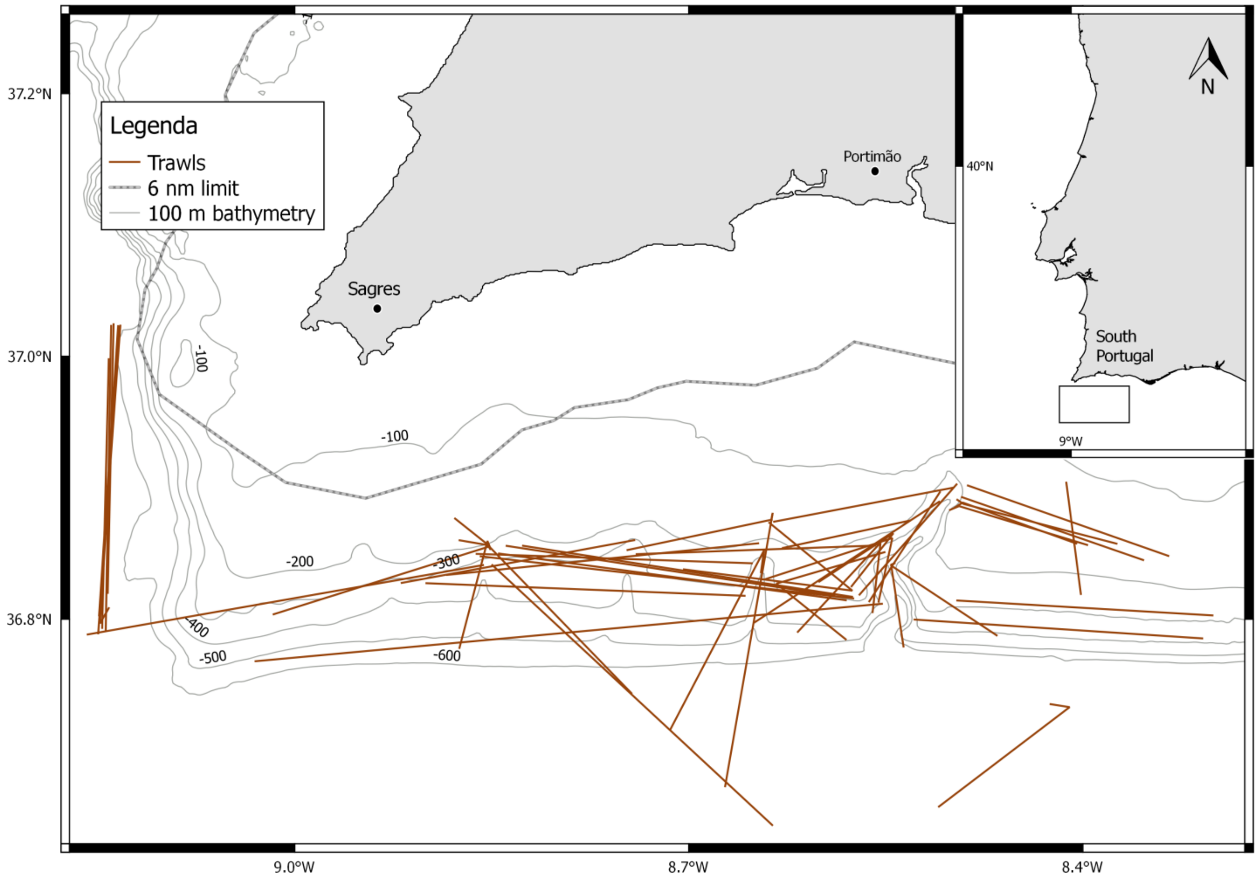

Figure 2.2 - Location of the study area off South Portugal (Algarve) where the trawls were performed (brown lines). The dashed line represents the 6 minimum nautical miles limit of fishing for trawlers. The grey lines represent the bathymetry every 100 m depth.

3. RESULTS

3.1 Study of by-catch species susceptibilities

Data on time-to-mortality was collected for a total of 502 individuals, belonging to 19 species, from 40 hauls. The number of individuals dead when first observed was computed for each species, expressed as immediate mortality in percentage (table 3.1).

Table 3.1 - Results of immediate mortality (%) calculated for each species.

Species	**Immediate mortality (%)**	**Species**	**Immediate mortality (%)**
		Merluciius merluccius	52
*Capros aper**	15	*Micromesistius poutassou*	80
Conger conger	3	*Mullus surmuletus*	45
*Citharus linguatula**	50	*Nezumia sclerorhynchus**	100
Galeus melastomus	30	*Phycis blennoides*	85
Helicolenus dactylopterus	13	*Scyliorhinus canicula*	8
*Hoplostethus mediterraneus**	60	*Setarches guentheri**	73
Lepidorhombus boscii	44	*Scomber spp.**	38
*Lepidopus caudatus**	100	*Trigla lyra*	66
Lophius spp.	18	*Trachurus spp.*	17

The species with * were excluded from further statistical analysis because were not representative of the population. The further analysis was conducted with the remaining group of 12 species (*M. poutassou*, *P. blennoides*, *L. boscii*, *T. lyra*, *M. merluccius*, *M. surmuletus*, *Trachurus spp.*, *H. dactylopterus*, *G. melastomus*, *Lophius spp.*, *S. canicula*, *C. conger).*

When looking at the injuries, more than 80% of the individuals from the group of 12 species shown some sort of injuries, except *Trachurus spp.*, *S. canicula* and *T. lyra* (figure 3.1).

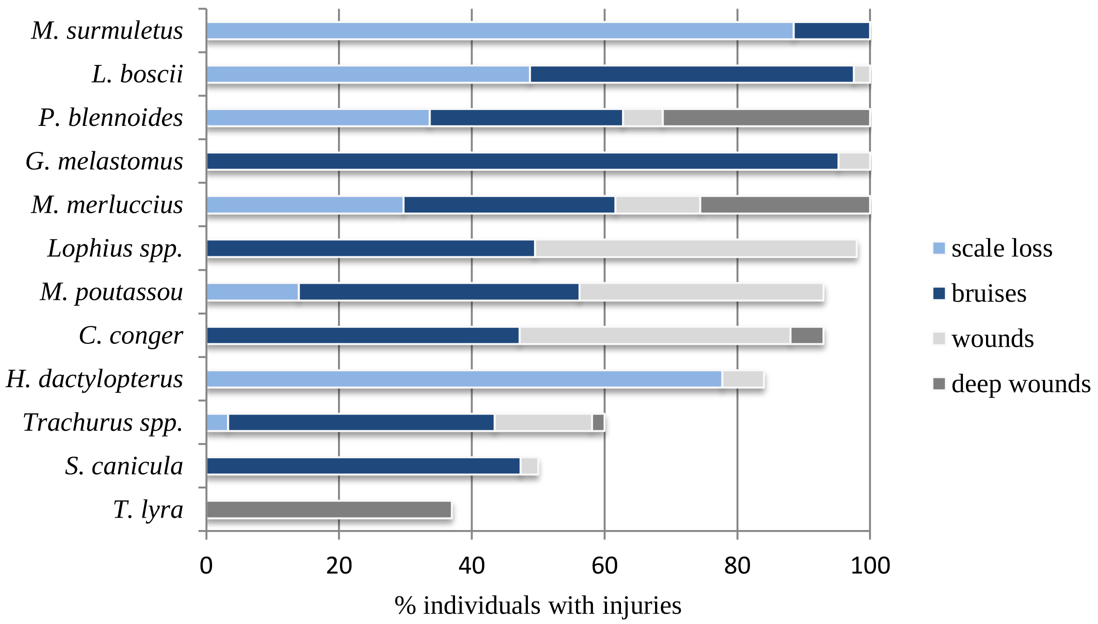

Figure 3.1 - Percentage (%) of individuals with 4 types of injuries (scale loss, bruises, wounds and deep wounds) for each species.

M. merluccius, *P. blennoides* and *T. lyra* often exhibited severe forms of damage, as inflated gas bladder and eversion of stomach (personal observations). The shark *G. melastomus* and *S. canicula* appeared mostly with bruises on the skin. Most fish with scales (*M. surmuletus*, *L. boscii*, *P. blennoides*, *M. merluccius*, *M. poutassou*, *H. dactylopterus*) suffered scale loss during the capturing and handling process; *Trachurus spp*. and *T. lyra* were the exceptions and conserved their scales. *Trachurus spp.* presented frequently bruises on the pectoral fins, as well as *C. conger*; this last one also showed scratched skin.

Kaplan-Meier models were applied individually for 12 species (*M. poutassou*, *P. blennoides*, *L. boscii*, *T. lyra*, *M. merluccius*, *M. surmuletus*, *Trachurus spp.*, *H. dactylopterus*, *G. melastomus*, *Lophius spp.*, *S. canicula*, *C. conger*), in order to calculate time to 50% mortality and visually explore the effect of vitality state on TTM (figures 3.2 and 3.3). Vitality states 1 and 2 were not observed for *G. melastomus*, *M. merluccius*, *T. lyra*, *M. poutassou* and *P. blennoides* (figure 3.2). For the species *G. melastomus* and *M. poutassou*, vitality 3 and 4 survival curves and confidence intervals overlapped throughout the graphics. On the contrary, for *M. merluccius*, *T. lyra* and *P. blennoides*, the two observed vitality states could be distinguished in the plots. Vitality

1, 2 and 4 was registered for *M. surmuletus*, but the KM model could not separate the survival curve for each vitality category. The opposite occurred for *L. boscii*, for which the vitality states 2, 3 and 4 had clearly separated survival curves in the plot.

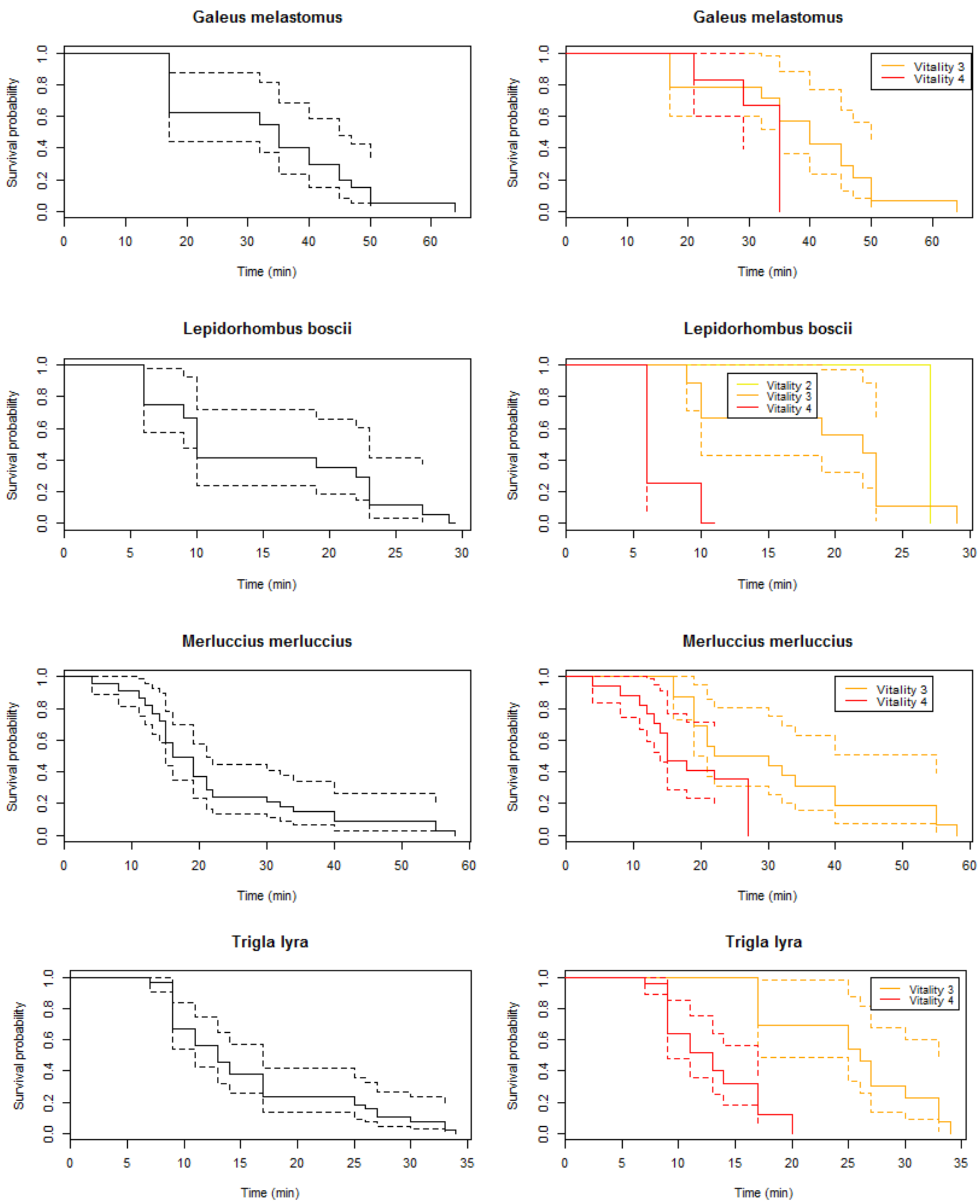

Figure 3.2 – Results of Kaplan-Meier for the species where only this model was applied (*Galeus melastomus*, *Lepidorhombus boscii*, *Merluccius merluccius*, *Trigla lyra*, *Micromesistius poutassou*, *Mullus surmuletus* and *Phycis blennoides*). In the left column are shown graphs with the base survival curve and in the right graphs with survival curves separately for each vitality category. Dashed lines represent 95% confidence intervals.

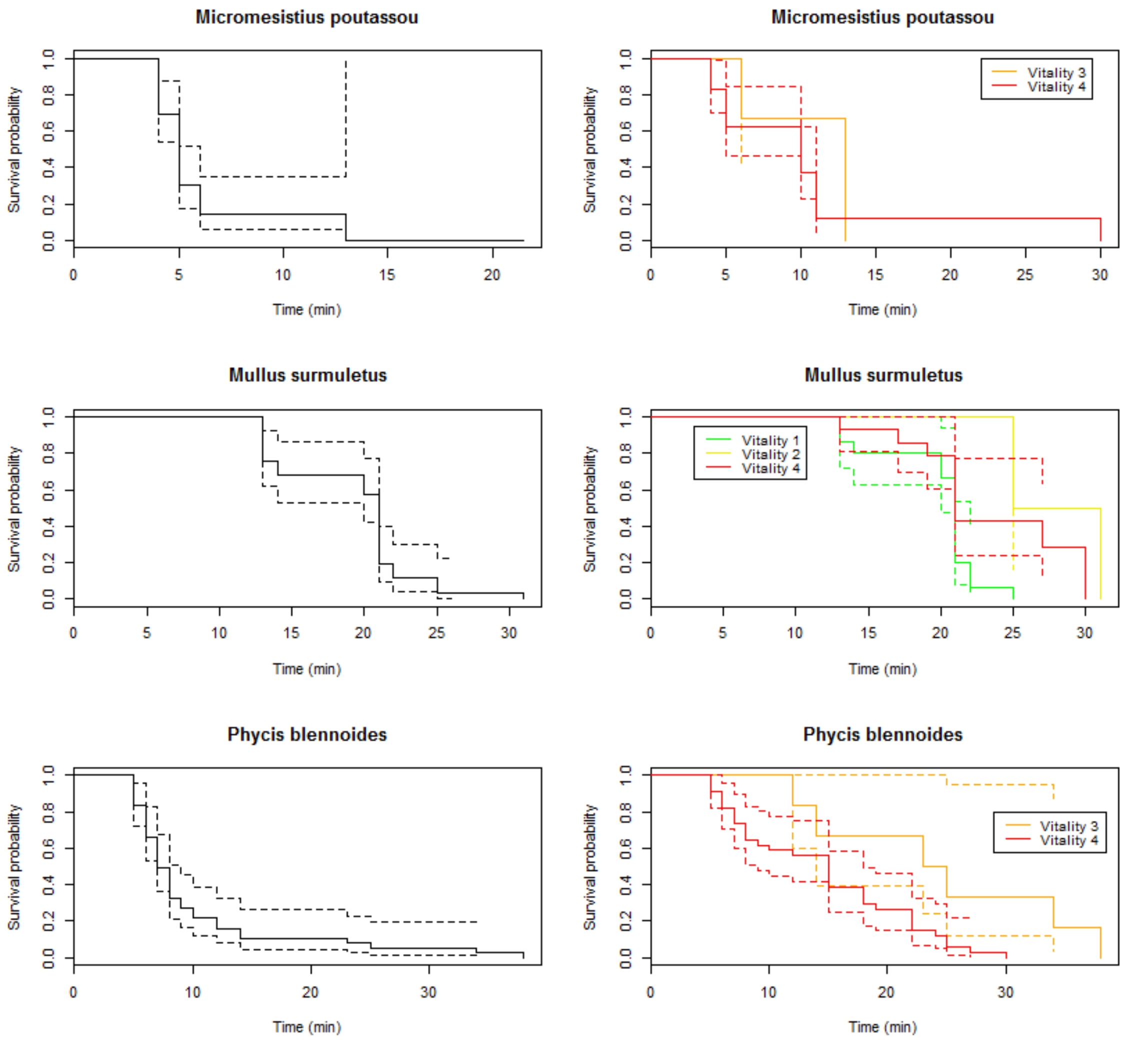

Figure 3.2 (cont.) – Results of Kaplan-Meier for the species where only this model was applied (*Galeus melastomus*, *Lepidorhombus boscii*, *Merluccius merluccius*, *Trigla lyra*, *Micromesistius poutassou*, *Mullus surmuletus* and *Phycis blennoides*). In the left column are shown graphs with the base survival curve and in the right graphs with survival curves separately for each vitality category. Dashed lines represent 95% confidence intervals.

In figure 3.3, there are two highly resistant species, *S. canicula* and *C. conger*, with mean times to 50% mortality of over 100 minutes. In fact, these two species together with *H. dactylopterus* were the only that had right censored observations, the remainder died during the monitoring period.

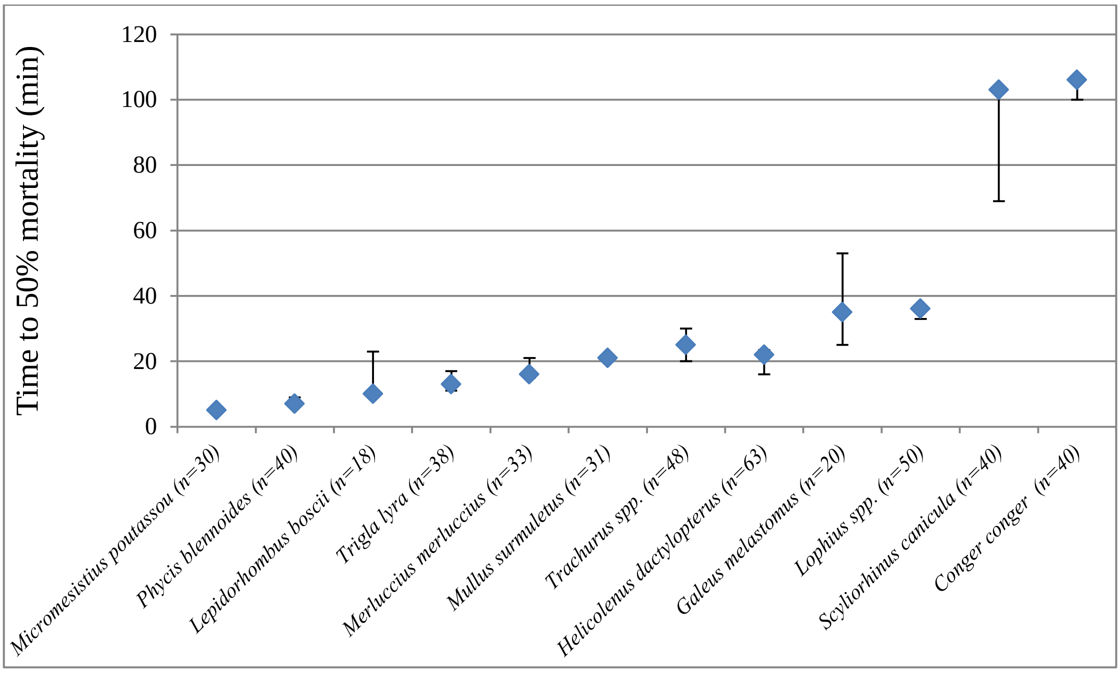

Figure 3.3 – Time to 50% mortality for the species included in the analysis. Error bars represent 95% confidence intervals (CI).

Weibull models were applied for *S. canicula*, *H. dactylopterus*, *C. conger*, *Lophius spp*. and *Trachurus spp.* in order to verify the effect of vitality state in TTM results (figures 3.4 – 3.8 and tables 3.2 – 3.6). Body size was also added as a possible explanatory variable, but only when size was registered for at least 40 individuals. This was not the case for *Lophius spp*. and *Trachurus spp*.

Scyliorhinus canicula

Vitality state 1 was not observed in *S. canicula*. Also, vitality states 2 and 3 overlap, with non-significant differences between them (vitality 2: n=16; vitality 3: n=21). Vitality state 4 individuals died significantly faster than vitality state 2 and 3 animals (vitality 4: n=3). Individual animal size was a significant explanatory variable, meaning that smaller individuals died at a faster rate than larger ones (figure 3.4 and table 3.2).

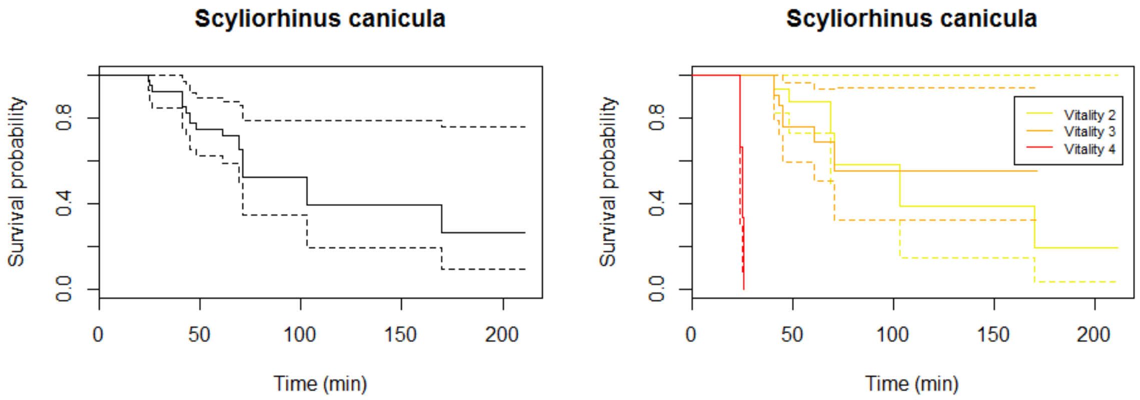

Figure 3.4 – Results of Kaplan-Meier for *Scyliorhinus canicula*, with base survival curve on left and survival curves for each vitality category shown on the right.

Table 3.2 – Results of Weibull models for *Scyliorhinus canicula* – without covariates and with vitality and size as possible explanatory variables.

Parameters/ Models for *S. canicula*	**Base model (without covariates)**			**+ Vitality**			**+ Size**		
	Value	Std. Error	p-value	Value	Std. Error	p-value	Value	Std. Error	p-value
Intercept	4.84	0.16	6.37e-198	5.00	0.188	1.05e-155	2.16	0.55	8.99e-5
Vitality state 3	-	-	-	-0.267	0.255	0.294	-	-	-
Vitality state 4	-	-	-	-1.780	0.324	3.79e-8	-	-	-
Size	-	-	-	-	-	-	0.073	0.018	3.06e-5
AIC		194.230			184.540			174.221	
p-value likelihood test		-			0.0011			2.7126e-6	

Helicolenus dactylopterus

According to the Weibull model, in particular the AIC value, vitality was significantly correlated with TTM (table 3.3). However, closer examination of the data in figure 3.5 (right) shows that only vitality states 1 and 4 have significantly different TTM curves. Due to the wide confidence interval around vitality state 4, it is indistinguishable from states 2 and 3 (vitality 1: n=2; vitality 2: n=28; vitality 3: n=25; vitality 4: n=8). Body size had no significant effect on TTM.

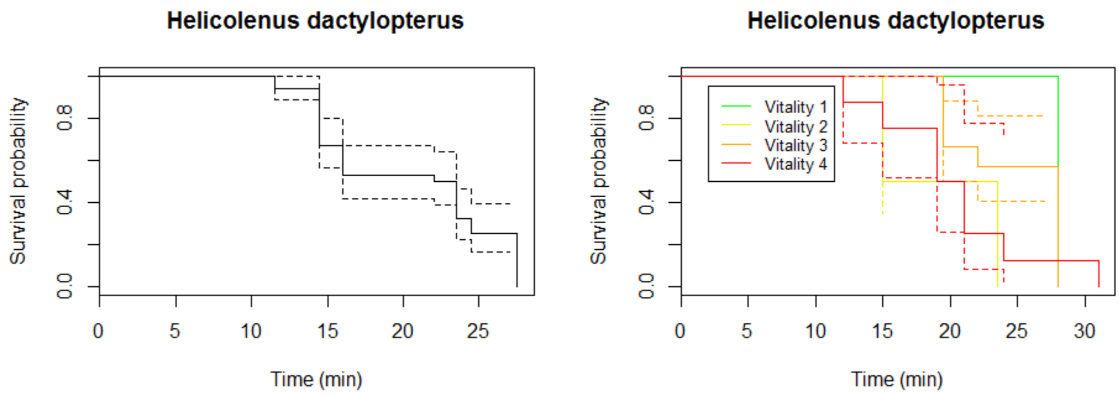

Figure 3.5 – Results of Kaplan-Meier for *Helicolenus dactylopterus*, with base survival curve on left and survival curves for each vitality category shown on the right.

Table 3.3 – Results of Weibull models for *Helicolenus dactylopterus* – without covariates and with vitality and size as possible explanatory variables.

Parameters/Models for *H. dactylopterus*	Base model (without covariates)			+ Vitality			+ Size		
	Value	Std. Error	p-value	Value	Std. Error	p-value	Value	Std. Error	p-value
Intercept	3.13	0.0556	0.00	3.450	0.351	8.91e-23	2.667	0.343	7.81e-15
Vitality state 2	-	-	-	-0.392	0.365	0.283	-	-	-
Vitality state 3	-	-	-	-0.165	0.360	0.646	-	-	-
Vitality state 4	-	-	-	-1.408	112.63	0.99	-	-	-
Size	-	-	-	-	-	-	0.023	0.017	0.172
AIC		53.651			33.803			53.962	
p-value likelihood test		-			1.027e-5			0.194	

Conger conger

Vitality has a significant effect on TTM in the Weibull model for *C. conger*. However, only vitality state 4 can be separately distinguished from the other three states, having a significantly more rapid TTM (figure 3.6; vitality 1: n=3; vitality 2: n=7; vitality 3: n=29; vitality 4: n=1). Size also had a small (0,009) but significant effect on TTM, with larger animals surviving longer (table 3.4).

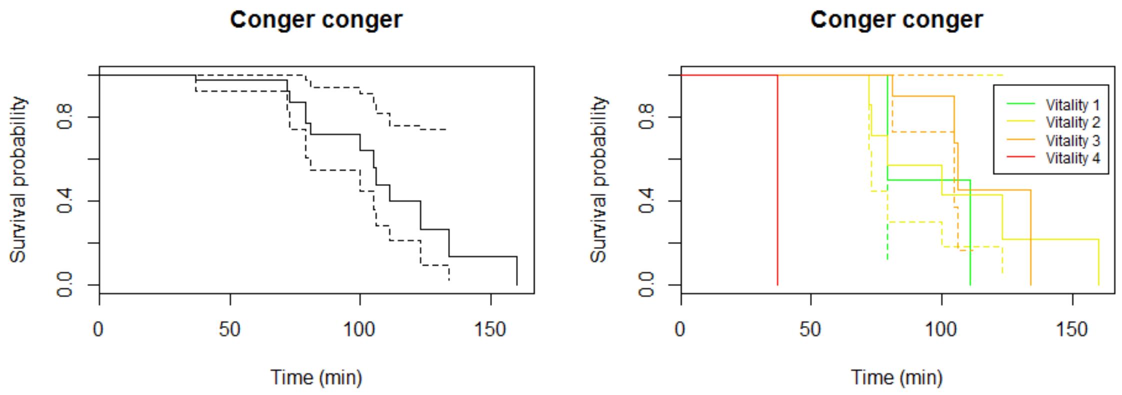

Figure 3.6 – Results of Kaplan-Meier for *Conger conger*, with base survival curve on left and survival curves for each vitality category shown on the right.

Table 3.4 – Results of Weibull models for *Conger conger* – without covariates and with vitality and size as possible explanatory variables.

Parameters/Models for *C. conger*	Base model (without covariates)			+ Vitality			+ Size		
	Value	Std. Error	p-value	Value	Std. Error	p-value	Value	Std. Error	p-value
Intercept	4.78	0.065	0.000	4.621	0.136	1.1e-253	4.285	0.204	1.33e-97
Vitality state 2	-	-	-	0.198	0.158	0.212	-	-	-
Vitality state 3	-	-	-	0.192	0.169	0.255	-	-	-
Vitality state 4	-	-	-	-1.01	0.235	1.76e-5	-	-	-
Size	-	-	-	-	-	-	0.009	0.004	0.025
AIC		138.516			134.377			134.951	
p-value likelihood test		-			0.017			0.018	

Lophius spp.

For *Lophius spp.*, only vitality categories 3 and 4 were observed due to its sedentary behaviour when on-board and exposed to air (figure 3.7 and table 3.5; vitality 3: n=41; vitality 4: n=9). Moreover, the estimated value for vitality state 4 did not produce significant differences when compared with vitality state 3 and has a rather large standard error. However, the fit of the model improved compared with the base model, which makes it difficult to correctly interpret these results.

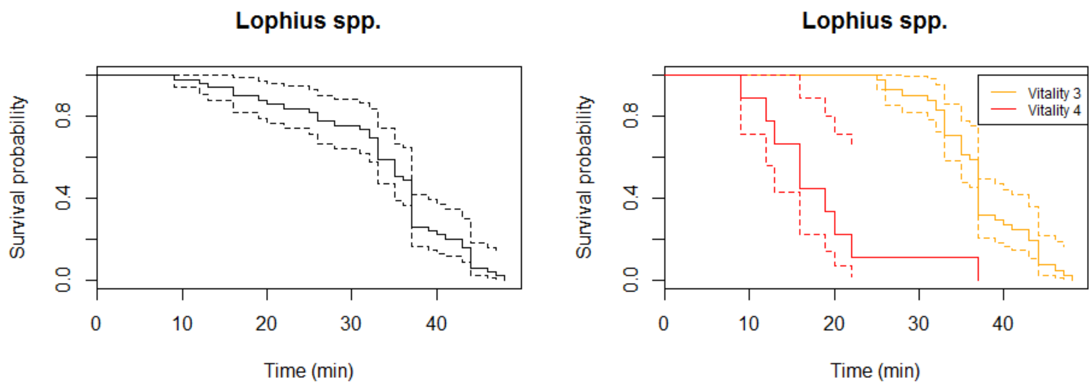

Figure 3.7 – Results of Kaplan-Meier for *Lophius spp*., with base survival curve on left and survival curves for each vitality category shown on the right.

Table 3.5 – Results of Weibull models for *Lophius spp.* – without covariates and with vitality as possible explanatory variable.

Parameters/Models for *Lophius spp.*	Base model (without covariates)			+ Vitality		
	Value	Std. Error	p-value	Value	Std. Error	p-value
Intercept	3.59	0.039	0.000	3.67	0.023	0.000
Vitality state 4	-	-	-	-1.88	87.558	0.983
AIC		340.136			264.941	
p-value likelihood test		-			1.548e-18	

__Trachurus spp.__

Although the Weibull model shows a highly significant effect of vitality (at first observation) on TTM (table 3.6), the relationship is counter-intuitive and not very informative. Figure 3.8 shows that the Kaplan-Meier (KM) curves for vitality states 1 and 4 are highly overlapped, while state 3 has the longest TTM (vitality 1: n=19; vitality 2: n=11; vitality 3: n=10; vitality 4: n=8). This contradicting relationship is most likely due to the highly active behaviour of healthy animals (vitality 1 and 2) when exposed to air, which thus rapidly exhaust themselves leading to a premature TTM.

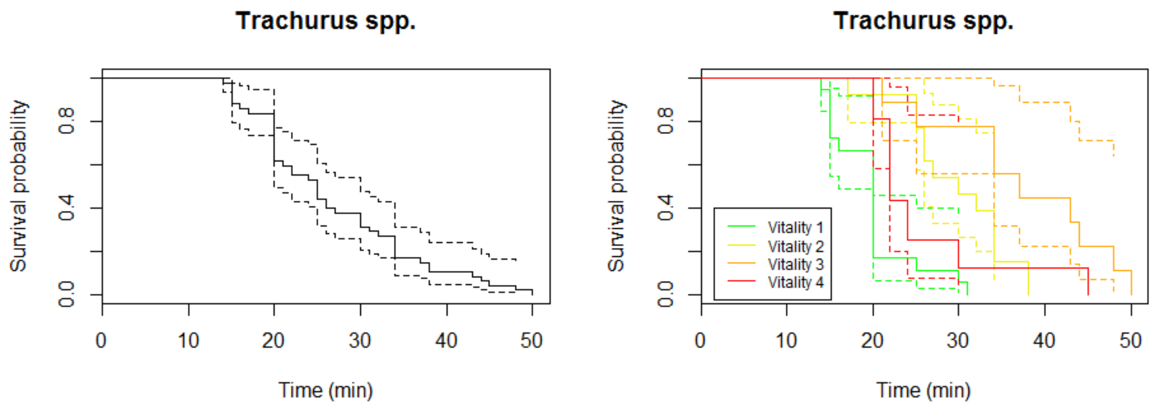

Figure 3.8 – Results of Kaplan-Meier for *Trachurus spp.*, with base survival curve on left and survival curves for each vitality category show on the right.

Table 3.6 – Results of Weibull models for *Trachurus spp.* – without covariates and with vitality as possible explanatory variable.

Parameters/Models for *Trachurus spp.*	Base model (without covariates)			+ Vitality		
	Value	Std. Error	p-value	Value	Std. Error	p-value
Intercept	3.39	0.052	0.000	3.076	0.0576	0.000
Vitality state 2	-	-	-	0.368	0.0868	2.2e-5
Vitality state 3	-	-	-	0.626	0.0971	1.15e-10
Vitality state 4	-	-	-	0.329	0.102	1.26e-3
AIC		337.937			312.407	
p-value likelihood test		-			6.572e-7	

Biological Traits Analysis

Information on four biological traits was compiled from general literature and field observations for 12 species (table 2.5).

Not all the biological traits had relevant effects – injuries and open gas bladder appeared to have no influence on TTM for these species, shown in table 3.7. On the contrary, deciduous scales, closed gas bladder and high metabolic rates have significantly reduced time-to-mortality results (p-value < 0.0001).

Table 3.7 – Results of Weibull models for 12 species – without covariates (base model) and models including each biological trait as possible explanatory variable.

Parameters	**Base model**			**AIC**	**p-value likelihood test**
	Value	Std	p-value	1648.13	————
Intercept	3.16	0.045	0.00		
+ Injury	0.23	0.153	0.141	1386.75	7.07e-32
+ Gas bladder (no bladder)					
Open gas bladder	0.22	0.102	0.0301	1527.90	1.06e-27
Closed gas bladder	-0.88	0.081	3e-27		
+ Scales	-1.16	0.066	4.82e-70	1419.09	3.55e-52
+ Metabolic rate (High)				1377.64	2.49e-60
Low	1.61	0.086	2.18e-78		
Medium	0.59	0.085	3.30e-12		

Kaplan-Meier graphs (figure 3.9) explore visually the fitted values from the Weibull models. A clear separation in the survival curves is seen when comparing closed and no gas bladder; presence as opposed to absence of deciduous scales and between the three classes of metabolic rates.

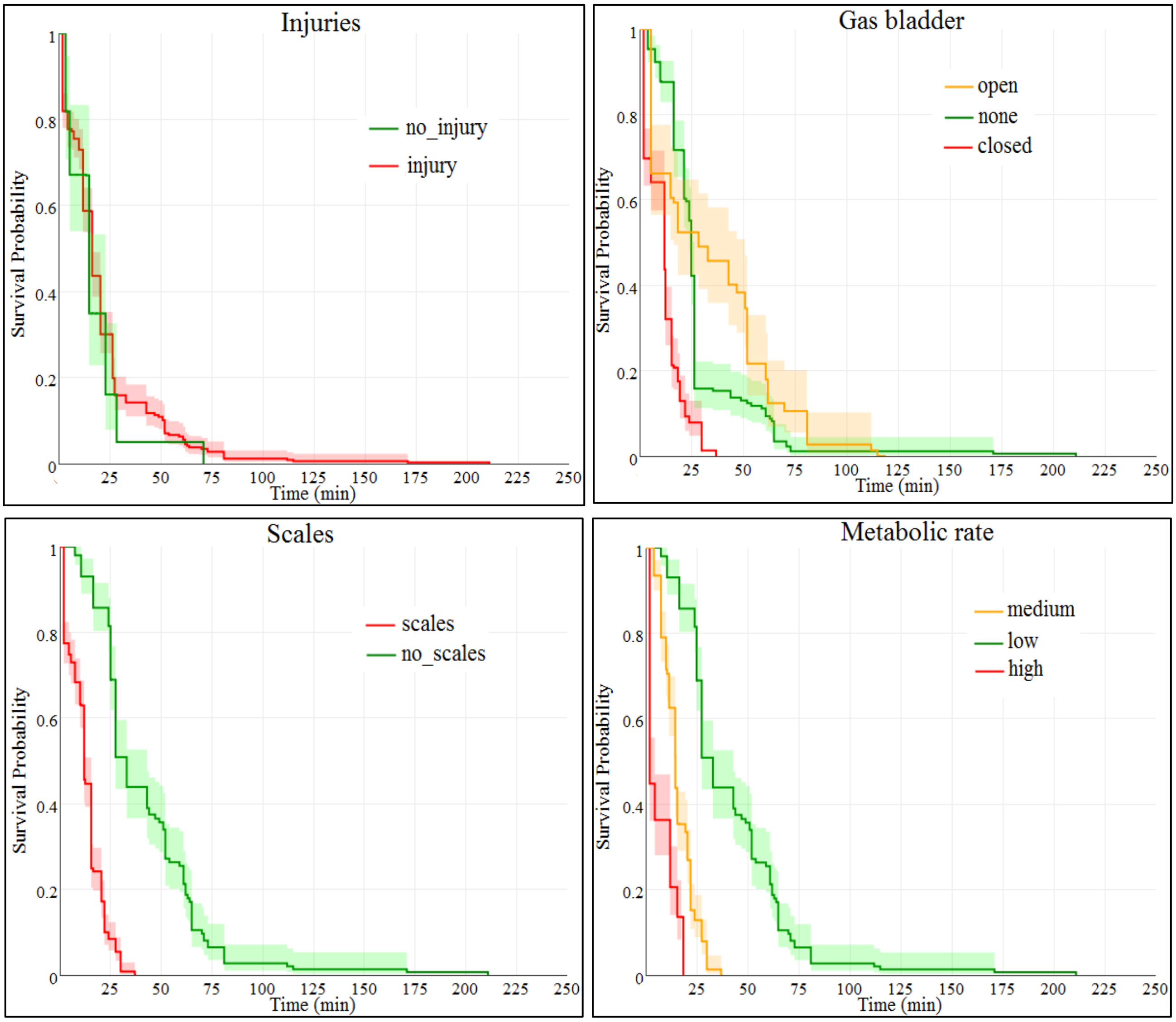

Figure 3.9 - Kaplan-Meier results for the effects of biological traits (injuries, gas bladder, scales and metabolic rate) on time-to-mortality for data pulled from 12 species (*M. poutassou*, *P. blennoides*, *L. boscii*, *T. lyra*, *M. merluccius*, *M. surmuletus*, *Trachurus spp*., *H. dactylopterus*, *G. melastomus*, *Lophius spp*., *S. canicula* and *C. conger*). Shaded areas represent 95% confidence intervals.

3.2 Potential for survival of by-catch – an experimental trial

Overall, 64 fish from 2 tows, maintained in different tanks, were used in the survival analysis. In tank 1, 4 out of 36 total fish died during the monitoring period, meaning a survival rate of 89% (95% CI: 78.6 to 99.2 %). In the second tank, 22 fish survived (total of 28 fish), which matches a survival proportion of 79% (95% CI: 63.4 to 93.8 %) at the end of the experiment (figure 3.10).

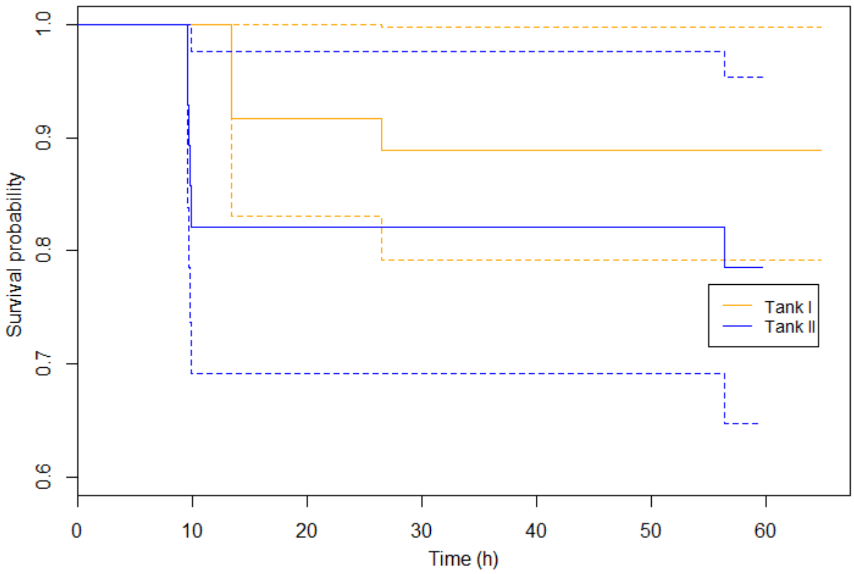

Figure 3.10 – Kaplan-Meier survival curves for *C. conger* separate by tanks. Dashed lines represent 95% confidence intervals.

Most mortality occurred in the first 14 hours: 8% in tank 1 (95% CI: 1 to 17.4%) and 18% in tank II (95% CI: 3.7 to 32%). However, the mortality rate did not reach an asymptote at the end of the observation period.

When comparing survival results between the two tanks, there were no significant differences (p=0.218), so the data was grouped into one survival curve, corresponding to an overall survival probability close to 84% (95% CI: 75.5 to 93.3%). All fish started to swim immediately after being placed in water and 98% responded positively to the in-water reflexes tested.

Every individual had some sort of injury, from scratches in the surface of the skin to more severe ones, e.g. deep wounds in tail and fins (Annex - images 7.1 to 7.6). The frequency of injuries is shown in figure 3.11, with almost 70% of *C. conger* exhibiting scratched skin, likely due to crowding inside the trawling net. Around 40% of the fish had bruises on the tail and pectoral fins; 5% shown affected eyes, maybe due to infection or result of abrasion; and 3% presented an inflated stomach, a probable sign of barotrauma. Nevertheless, most of the fish appeared to be swimming calmly during the experiment and were eating after 22h in water.

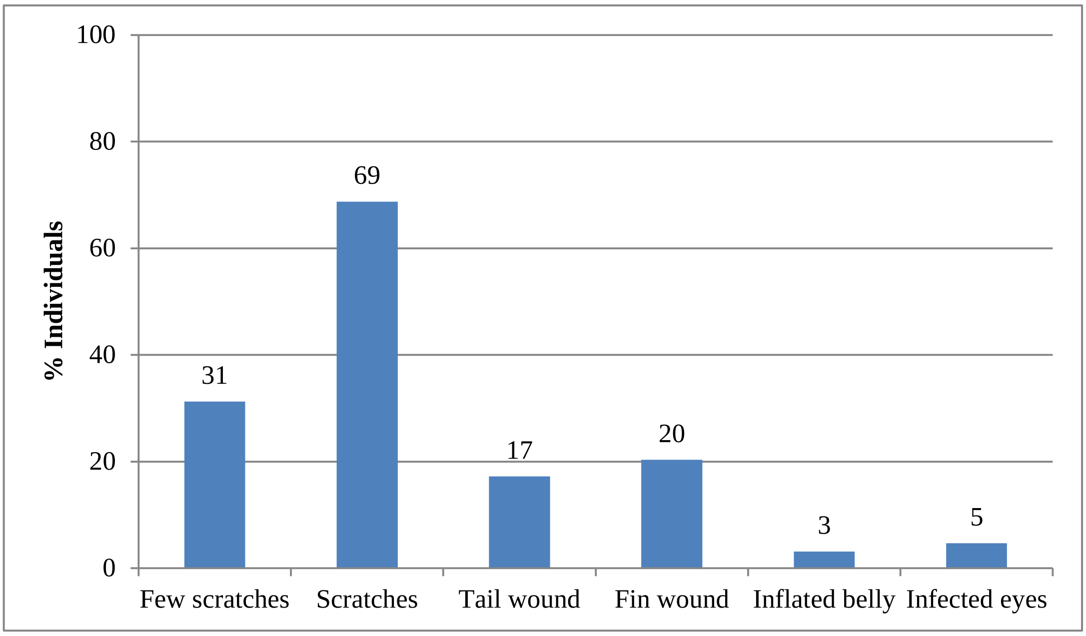

Figure 3.11 – Frequency of injuries shown as percentage (%) of individuals with each type of injury.

The number of injuries to each individual was included as a covariable in a Weibull model in order to see whether the presence of injuries influenced the observed mortality. Only individuals that presented 3 types of injuries had a significant and negative effect on the results (value = -0.32; p-value =0.01; table 3.8); meaning that fish with more injuries suffered higher mortality. As shown in figure 3.12, this can be confirmed because the survival curves of individuals that presented zero, one and two injuries (green, yellow and orange lines) are identical, but all the individuals that died during the course of the monitoring period presented 3 types of injuries (red line).

Table 3.8 – Results of Weibull model for survival *of C. conger* - without covariates (base model) and model including number of injuries as possible explanatory variable.

Parameters/Models	**Base model**			**p-value likelihood test**
	Value	Std	p-value	
Intercept	6.62	0.904	2.41e-13	________________
AIC (base model)	140.03			
+ Number of injuries (1)				0.038
0 injuries	1.38	1.44	0.34	
2 injuries	0.35	0.95	0.71	
3 injuries	-0.32	1.25	0.01	
AIC	137.62			

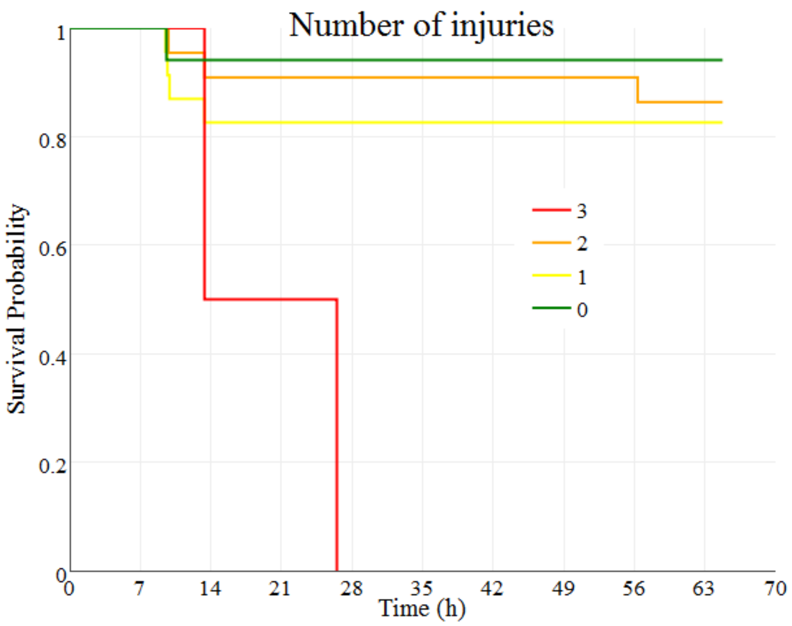

Figure 3.12 - Kaplan-Meier results for the effects of number of injuries (0, 1, 2, and 3 types of injuries) on survival of *C. conger*.

4. DISCUSSION

4.1 Study of by-catch species susceptibilities

As a preliminary survival assessment, this work provided estimates of immediate mortality and time-to-mortality (TTM) data for a wide range of species: *C. conger*, *G. melastomus*, *H. dactylopterus*, *L. boscii*, *Lophius spp.* (*L. budegassa* and *L. piscatorius*), *M. merluccius*, *M. poutassou*, *M. surmuletus*, *P. blennoides*, *S. canicula*, *Trigla lyra* and *Trachurus spp.* (*T. picturatus* and *T. trachurus*). This enabled a preliminary evaluation of the vulnerability of these animals to the stressors associated with capture and exposure to air, from which some inferences can be made on their potential to survive the discarding process.

Time to 50% mortality (50% TTM), immediate mortality (%) and the Kaplan-Meier survival probability curves identify *M. poutassou*, *P. blennoides*, *L. boscii*, *T. lyra*, *M. merluccius*, *M. surmuletus*, *Trachurus spp.* and *H. dactylopterus* as highly susceptible to air exposure; with 50% TTMs of less than 30 minutes, and in most cases less than 20 minutes. However, *S. canicula* and *C. conger* appear to be relatively resistant with 50% TTMs in excess of 100 minutes.

Almost all species were observed with some form of external injuries, mostly scale loss, bruises and shallow wounds. In particular, *M. merluccius*, *P. blennoides, T. lyra*, *N. sclerorhynchus* and *S. guentheri* often exhibited evidence of barotrauma, in the form of everted stomach, inflated abdomen or popped eyes (personal observations). For this species, survival after discarding is virtually impossible.

Based on the Kaplan-Meier and Weibull models, vitality at first observation is a very limited predictor of time-to-mortality. Species specific behavioural traits need to be considered when interpreting the results. Two major inconsistencies were identified: the absence of vitality states 1 and 2; and species with high vitality scores but low time-to-mortality. *Lophius spp.*, *C. conger*, *S. canicula* and *G. melastomus* are sedentary, benthic species with body musculature and skeletons adapted for energy saving mechanisms (Helfman *et al.* 2009). When subjected to extreme stress conditions such as air exposure, these species reduce activity to a minimum in order to conserve energy. Consequently, vitality states 1 and 2 were never observed. The same was the case of *L. boscii*, *M. merluccius*, *T. lyra*, *M. poutassou* and *P. blennoides*. These species suffer decompression effects, injuries or/and immense extenuation which leads to a state of most individuals dying or arriving dead on deck (vitality state 3 & 4). In contrast, *Trachurus spp.*, *M. surmuletus* and *Scomber spp.* display signs of intense activity and occasionally shivering movements when brought on deck; in accordance with the vitality scale, this response is “positive” and classified with high vitality scores for these species. But in reality, these signs seem to be an advanced stress-response. When placed in water, these individuals are disorientated, not able to hold position in the water column and/or unable to swim (personal observations). These observations are confirmed by the substantially reduced TTM. Behavioural disturbances such as loss of equilibrium have been related with different stressors, one being air exposure (Gingerich *et al.* 2007; Davis 2005).

Therefore, two separate vitality scales are proposed (tables 4.1 and 4.2) to distinguish the behaviour categories for benthic/sedentary species and bathypelagic, fast swimming fish. For sedentary species, a combined methodology of vitality together with testing reflex actions evaluates more adequately the overall state of the animal. In particular, the absence of a clear transition from alive to dead requires adaptions in the visual assessments. The solution is the use of innate or reflex responses to precisely stimulate, for example, the gag response or opening the operculum. Reflex actions are innate fixed movement patterns that are directly related to vitality, without being confounded by other factors. The vitality scale for bathypelagic and pelagic species stays identical but should be complemented if possible with testing reflex actions in-water, recorded as presence (1) or absence (0) of the reflex response.

Table 4.1 – Proposed vitality scale with description of the codes for bathypelagic and pelagic species (based on ICES 2014 and Catchpole *et al.* 2015)

Vitality state	**Score**	**Description**
Excellent	1	Vigorous body movement without stimuli
Good	2	Weak body movement, but responds to touching
Poor	3	No body movement, no obvious response to stimuli, but fish can move operculum/mouth/fins

Dead	4	No body or opercular movements, no response to touching or grabbing
Reflex actions in-water	**Stimulus action**	**Reflex response**
Startle touch	Fish is underwater and hand approaches to touch fish	Actively moves away before or at first touch
Tail grab	Fish is grabbed gently by its tail	Actively struggles to escape within 5 seconds
Orientation/Righting	Fish is held on the palm of the hand on its back just below the water surface and released	Actively righting itself underwater within 5 seconds

Table 4.2 – Proposed vitality scale with description of the codes for benthic/sedentary species (based on ICES 2014 and Catchpole *et al*. 2015)

Vitality state	**Score**	**Description**
Good	1	Some spontaneous body movements, responds to all reflex actions
Poor	2	No spontaneous body movements, responds to at least one reflex action
Moribund/Dead	3	No body movement, no response to any reflex action
Reflex actions	**Stimulus action/** **Description of response**	
Operculum closure	The operculum of the fish is gently opened with a blunt object/Ability to close or clamp its operculum after being opened within 5 seconds.	
Mouth closure	The mouth of the fish is gently opened with a blunt object/Ability to tightly close its mouth after being opened within 5 seconds.	
Gag response	A blunt object is inserted in the mouth of the fish and touch the throat/Fish gagged or vomit.	

Body size was a significant explanatory variable for *S. canicula* and *C. conger* which endure long periods of air exposure. Smaller fish have a higher rate of mortality, explained by their increased susceptibility to injuries in the net and higher respiration demands (Benoît *et al.* 2013). However, for species with high susceptibility to air exposure and consequent low TTM, such as *Trachurus spp.* and *H. dactylopterus*, body size seems to be less relevant.

Concerning the analysis of biological traits, there were particular traits that had a significant influence on TTM. Species with a closed gas bladder died at a faster rate compared to individuals without a gas bladder. Fish with a closed gas bladder cannot resist depressurization effects when brought to surface. Hauling occurs at such high speeds (~20 meters per minute) causing over-inflation or a rupture of the swim bladder, and consequently release of gas into the body cavity and eversion of stomach and gut (ICES 2014; Nichol & Chilton 2006; Rummer & Bennett 2005; Breen 2004).

There were no differences in TTM for species with open and no gas bladder, mainly due to *C. conger* possessing an open gas bladder but at the same time representing one of the most resistant species to air-exposure. For the group of 12 species (*M. poutassou*, *P. blennoides*, *L. boscii*, *T. lyra*, *M. merluccius*, *M. surmuletus*, *Trachurus spp.*, *H. dactylopterus*, *G. melastomus*, *Lophius spp.*, *S. canicula*, *C. conger)*, presence of scales and medium/high metabolic rates significantly decreased time-to-mortality. Fish with deciduous scales are more vulnerable to water losses due to scale loss and consequent collapse of the osmoregulatory functions of the skin, resulting in increased sensitivity to hypoxia (ICES 2014; Breen 2004). These outcomes are in line with Benoît *et al.* (2013), where mass-specific respiration demand, physoclistous and physostomous bladders and deciduous scales all had significant and negative effects on survival. They identified as well that sedentariness, defined as a species' average activity level that relates to its overall resistance to stress, was the most influential trait on TTM. In this work, metabolic-rate-class can be considered comparable to sedentariness, and indeed has a pronounced effect on how different fish species cope with the stressors related with hypoxia. Energy conserving behaviour of low metabolic rates, characteristic of species such as *Lophius* and *S. canicula*, is translated into greater time-to-mortality results when compared with species with fast metabolism and high susceptibility to air exposure (*M. surmuletus*, *M. poutassou*). When injuries are included in the analysis, contradicting results show up of a non-significant but positive result (value = 0.225; p-value > 0.01), meaning injured animals survive longer than healthy individuals. This bias may be due to anomalies in the unbalanced sample distribution of only 40 individuals without injuries against 367 with injuries.

It is important to identify species' characteristics that can explain the observed mortality rates. A biological traits analysis (BTA) of by-catch and target species was performed as an assessment of species vulnerability, also taking into account the diversity of life strategies (Demestre *et al.* 2017). Vulnerability was assessed into three components: catchability or susceptibility to be caught; resistance or potential survival on-board; and resilience of a population to fishing. This method provided a final score that reflected susceptibility at an individual, species and population level to the fishing operations. Most by-catch fish species were highly vulnerable, mainly attributable to high catchability together with low to moderate resistance. However, most of these species were at the same time considered highly resilient to fishing, explained by long/average life span, annual reproduction and age at maturity lower than 5 years. *Scyliorhinus canicula* was the only species considered less vulnerable when compared with the others (moderately vulnerable category).

The results of this BTA match the ones from the current work, that is the majority of by-catch fish species are fragile and do not resist the overload of stressors coming from the capture, handling and the discarding process. Only crustacean, bivalves, echinoderm and occasionally elasmobranch species are likely to survive but these also represent a low percentage of discarded weight (Hill & Wassenberg 2000; Depestele *et al.* 2014; Monteiro *et al.* 2001). The most likely fate is being eaten by marine birds or other predators in the water column or by bottom scavengers if the animals sink (Hill & Wassenberg 2000; Castro et al 2005). Complementary studies are still needed to accurately predict post-release survival of discards, since these methods are restricted to the specific stressors observed in hypoxia conditions and cannot be inferred for a range of stressors associated with the whole fishery (ICES 2014). Nevertheless, these indicators are certainly useful to calculate immediate mortality for a large number of species, in an easy and simple manner, providing valuable information of real fishing conditions with little costs. In addition, biological traits and other explanatory variables can be addressed in determining to which extent can influence mortality of these species.

4.2 Potential for survival of by-catch – an experimental trial

As a result of the first part of this work, *Conger conger* emerged as one of the species with a high likelihood of survival. A specific on-board experiment was conducted to investigate the survival potential of this species. 84 % (95% CI: 75.5 to 93.3%) of individuals survived the observation period of 65 hours. It is a rather high survival rate, taking into account that these fish had been exposed to extreme stressors associated with capture by a heavy chain gear, compression in the codend of the net and possible effects of vessel motion while on tanks. In fact, individuals started to feed on crustaceans after 22 hours on the system.

The experimental conditions between the two tanks were not exactly the same. The water temperature in tank II was slightly higher (2 to 4 °C higher) than in tank I; and the two tanks had different capacity and were made of different materials (tank 1 made of metal, tank 2 made of plastic). Nevertheless, these differences had no significant effect on the survival curves, so the two tanks were treated as replicates and the survival estimate grouped from both.

There are no reference studies on survival of *C. conger*. In the Northwest Mediterranean similar experiments were conducted with *C. conger* and *S. canicula* (Demestre *et al.* 1998). After three days in tanks, *C. conger* had 75% survival, but due to small sample size (3 individuals) no further conclusions could be reached. For *S. canicula*, all 11 individuals survived the experiment. However, mortality did not stabilize during the observation period and individuals kept dying. Also, dissections showed multiple internal traumas (personal observation from Demestre *et al.* 1998). On the other hand, recent studies show that *S. canicula* was able to fully recover after 48 hours, with a survival rate of around 98% (95% CI: 96 to 100%; Revill *et al.* 2005) and 78% (95% CI: 47.1 to 90.5%; Rodriguez-Cabello *et al.* 2005).

It is questionable that conger will show a similar survival success. All individuals in this work exhibited some form of injury, including severe wounds and scratches, bruises on skin and fins, inflated stomach and affected eyes (figures 7.1 – 7.6 in Annex). In fact, individuals that presented more types of injuries had higher mortality. Impaired fish have reduced ability to avoid predation and are more susceptible to physiological disturbances or disease, thus the survival rate calculated here is likely to be over-estimated (Davis 2002; Davis 2010; Davis & Ottmar 2006;

Breen 2004). As in the study by Demestre *et al.* (1998), the monitoring time of this experiment was not enough for the mortality rate to stabilize; meaning that if the study had been longer, more congers would probably die. Ideally, captive observation studies should terminate only once the mortality of held fish becomes constant, but this can take up to several weeks (Depestele *et al.* 2014), which in this case was not practical due to logistic limitations of working on board the vessel.

Another methodological concern is the absence of control fish. When survival is less than 100%, control individuals can help to distinguish whether it was the treatment (having gone through the capture and handling process) or the method (having been contained in tanks) which was associated to the observed mortality (ICES 2014). However, in this study it was not possible to have control individuals of *C. conger* caught with other fishing gears mainly due to financial constraints. There are always stressors associated with capturing fish that might induce mortality, even if static gears are used (e.g. traps), so proper controls are quite difficult to arrange, especially in such deep waters. Moreover, it is plausible to assume that in this experiment the effects of capturing and handling are much more relevant to the observed mortality than any stressor related with holding the fish in tanks.

5. CONCLUSIONS

Overall, the categorical vitality assessment used in this study does not seem to be a good predictor of time-to-mortality. Adjustments in this methodology to account for differences in behaviour between species should be considered in future work, as suggested previously.

It can be concluded that *M. poutassou*, *P. blennoides*, *L. boscii*, *T. lyra*, *M. merluccius*, *M. surmuletus*, *Trachurus spp*. (*T. trachurus* and *T. picturatus*) and *H. dactylopterus* are all vulnerable to the stressors associated with capture in this bottom trawl fishery, as well as exposure to air on deck. *G. melastomus* and *Lophius spp.* (*L. piscatorius* and *L. budegassa*) seem more resistant, but the only species that demonstrated resilience to these stressors were *S. canicula* and *C. conger*.

Smaller individuals in this study appear to be more susceptible than larger ones. Specific biological traits such as open gas bladder, presence of scales and high metabolic rates also increase a species' vulnerability. This allows inferences on probable mortality of not yet examined species; although, only for the same gear in identical fishing conditions. Since there is a large number of interacting variables of various types (environmental, technical, biological) that influence by-catch mortality, the implications of the results of this study are limited by the experimental factors taken into account (ICES 2014).

Regarding the survival experiment, *C. conger* had high survival (84%; 95% CI: 75.5 to 93.3%) but likely overestimated due to the fact that the mortality rate did not stabilize by the end of the observation period and also severe injuries were present in almost 70% of the individuals. For additional investigation of survival potential, inland tank trials are recommended in order to conduct a longer study, preferably using control fish. Analysis of cortisol, glucose and ions (Na^+, Cl^- and K^+) from blood sampling can provide a method to study stress levels of fish in captivity (Marçalo *et al*. 2008) . Also, mark-and-recapture studies using tags can be applied, considering that the proportion of tag returns is often minimal (ICES 2014). Every method has its advantages and drawbacks. The chosen methods for this work limited the conclusions that can be made, but also implied a cost-effective approach of having a large amount of new information that can be used as starting point for future projects.

Bottom trawling results in a large proportion of dead discards that are consumed immediately or sink. In this case study, 5% of total discards might survive, constituted by *S. canicula* and *C. conger* and without considering other phyla (e.g. molluscs or tunicates). The primary solution to reduce discard mortality is avoiding catching unwanted species in the first place; a difficult task considering that many discarded species are frequently retained due to their high market value (e.g. hake and monkfish). Both mesh configurations and sorting grids to improve trawl selectivity resulted in a slight decrease of crustacean catches and therefore never were implemented (Fonseca *et al.* 2004; Campos *et al*. 2003). More promising are minor changes to the procedures, such as reducing trawls duration and handling the catch with urgency and care, minimizing injuries and exposure to air (Breen *et al.* 2017). However, considering the extensive physical disruption of deep-sea habitats and the little post-discard survival in this particular fishery, alternative gears should be considered. Low-impact, no-discard gears can substitute crustacean demersal trawling (e.g. traps, creels), besides being a more economically viable fishery with reduced costs and fuel consumption (Leocádio *et al.* 2012).

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7. ANNEX

Figure 7.1 – *Conger conger* specimen exhibiting few scratches

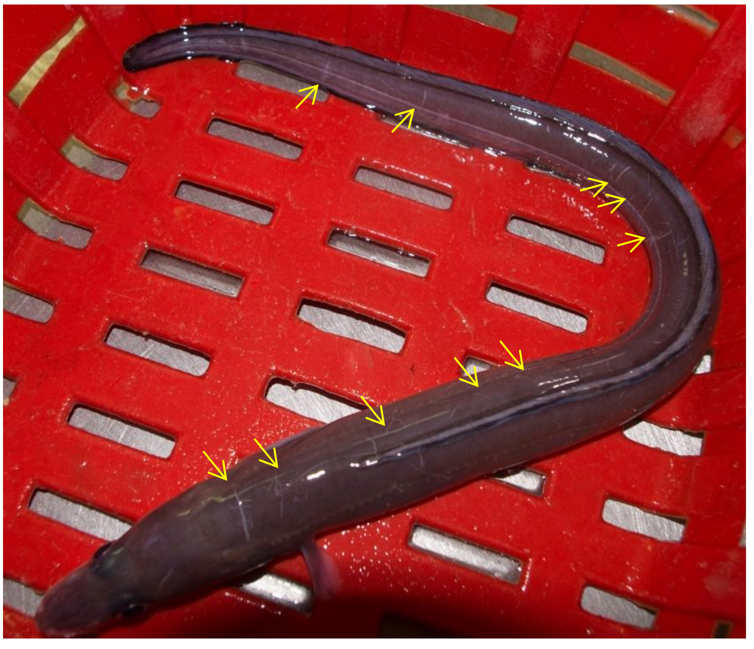

Figure 7.2 – *Conger conger* specimen exhibiting scratches

Figure 7.3 – *Conger conger* specimens exhibiting tail injuries

Figure 7.4 – *Conger conger* specimens exhibiting pectoral fin injuries

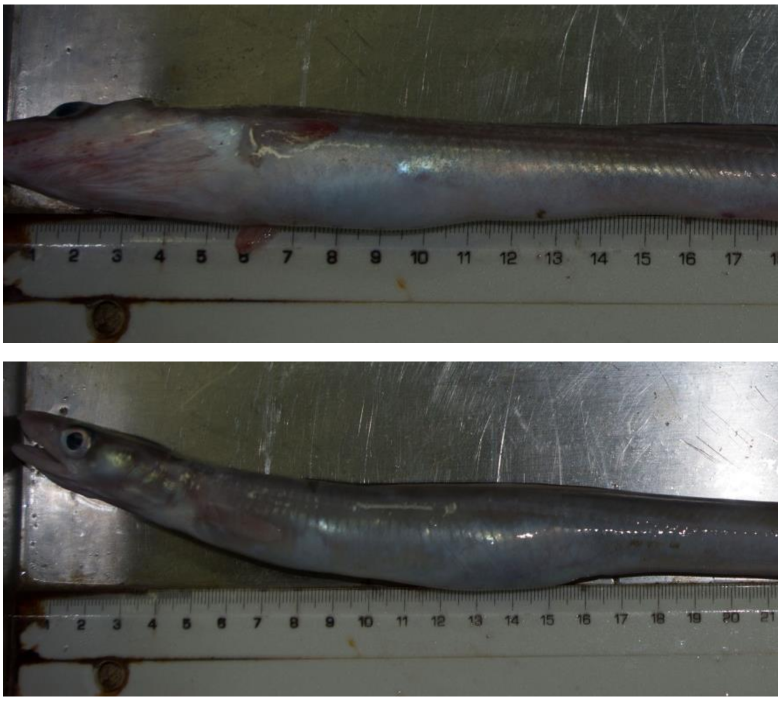

Figure 7.5 – *Conger conger* specimens exhibiting inflated stomach

Figure 7.6 – *Conger conger* specimens exhibiting affected eyes