"The Estimated Survival of species of skates after being captured by mixed fisheries using trammel nets in Peniche, Portuguese coast, and kept in captive."

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Abstract

By-catch fisheries are a large problem nowadays, affecting marine populations that are a non-target for world fisheries. The creation of the Landing Obligation (LO) was an important law introduced in the management and control of European fisheries, by aiming for more sustainable fisheries and promoting the bycatch reduction of species managed by quotas. Yet, a stock can be exempted from the LO if scientific studies support a high survivability to a given fishing gear. This means that with this exemption, based on high survivability, the bycatch of a stock if discarded can be delivered alive to the sea, promoting the recuperation of the stock. Currently, there is already a provisional exemption by high survival for most skate and ray species (Rajiformes) caught in European waters, yet the European Commission will only grant a definitive exemption of member states provide sufficient scientific evidence to support it. Therefore, the present study aims to gather scientific information on the survivability after capture of Rajiformes species caught in the polyvalent fleet operating with trammel nets off Peniche, central Portugal, which are the main fishing gears and area where Rajiformes are caught in mainland Portugal. For this study a holding tank with 1000 L was prepared for the individuals and weekly they were tested with CVA – Categorical Vitality Assessments, RAMP tests and lesion coverage. The results were utilized to evaluate discard survival for this two species, using a non-parametric Kaplan-Meier it was observed that R. brachyura species had 76% survival probability and R. montagui species had 54% survival probability, also it was observed that their vitality status influence the survival probability.

Key-words:

Landing Obligation; High survival exemption; Trammel nets; Survival; Rajiforms

Resumo

As capturas assessorias ou by-catch são hoje um grande problema, afetando as populações marinhas que não são o objetivo foco da pesca mundial. A criação da Obrigação de Desembarque (LO) foi uma lei importante introduzida na gestão e controlo das pescas europeias, visando uma pescaria mais sustentável e promovendo a redução da pesca das espécies geridas por quotas. No entanto, uma população pode ser isenta da LO se os estudos científicos apoiarem uma elevada sobrevivência a uma determinada arte de pesca. Isto significa que com esta isenção de elevada sobrevivência os stocks capturados por by-catch e que apresentem elevada sobrevivencia, possam ser devolvidos ao mar vivos, permitindo uma recuperação deste mesmo stock. Atualmente, existe já uma isenção provisória por elevada sobrevivência para a maioria das espécies de raias (Rajiformes) capturadas em águas europeias, mas a Comissão Europeia apenas concederá uma isenção definitiva aos Estados-Membros se estes suportarem com evidências cientificas que justifiquem a mesma. Por isso, o presente estudo visa recolher informações científicas sobre a sobrevivência após a captura de espécies rajiformes capturadas pela frota polivalente que opera com redes de tresmalho ao largo de Peniche, no centro de Portugal, tanto a arte de pesca como a área portuguesa são os dois fatores que influenciam uma maior captura de Rajiformes em Portugal continental. Para este estudo, um tanque com 1000 L foi preparado para manter os indivíduos e semanalmente foram testados com CVA – Avaliações Categóricas de Vitalidade, testes RAMP e cobertura de lesões. Os resultados foram utilizados para avaliar a sobrevivência para esta duas espécies, usando uma teste não-parametrico Kaplan-Meier, foi possivel observar que as espécies de *R. brachyura* tinham 76% de probabilidade de sobrevivência e os individuos *R. montagui* tinham 54% de probabilidade de sobrevivência, também se observou que o seu estado de vitalidade influencia a probabilidade de sobrevivência.

Palavras-chave:

Obrigação de desembarque; Elevada sobrevivência; Tresmalho; Sobrevivência; Rajiformes.

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Introduction

Marine ecosystems provide ecological processes that are fundamental to our world functionality (Costanza et al., 1997). The marine ecosystem helps in many different ways to sustain all the other ecosystems in our world, and yet it is one of the most exploited by humans (Adão et al., 2018). This is a very important topic, because as we exploit its resources for our own benefit, we need to acknowledge that these are limited, and in fact, they represent a big role in our markets, and because of that, sometimes the political choices that are being made, towards this, aren't fully measuring its importance in order to keep its wealth (Costanza et al., 1997).

One of the biggest intakes of protein for humans comes from fisheries, which is one of the ways that we extract resources from the marine ecosystems (Cheung et al., 2010). As our population grows, the fishing ratio will increase even more (Teixeira et al., 2014). Fisheries represent a big problem to the marine environment, as climate change does (Cury et al., 2008). The way in which humans overexploit marine habitats is leading to major consequences, like the decrease in abundance of some species and destruction of their habitat (Cury et al., 2008). All human practices that involve exploiting any kind of living form in our world, if not kept at controlled and sustainable levels, can damage its biodiversity (Ceballos et al., 2010) and consequently its functionality and ecological services start to fail (Costanza et al., 1997).

The United Nations has developed a scientific essay that revealed that in the past 10 years the biomass of living organisms in our oceans has reduced drastically mainly due to bad fisheries management , the lack of importance given to the wellbeing of this ecosystems has been the main issue (Lee M., 2009).

One of the problems in fisheries, is that most of the targeted fisheries (fishing vessels that target only certain species) end up catching a lot of non-targeted stocks, commonly defined as "bycatch" (Adão et al., 2018). Bycatch species generally have lower market value or have some type of restrictions that limits its selling (Ellis et al., 2010), and are being marginalized together with the ecosystem they incorporate (Adão et al., 2018). There is also another controversial practice which is the discards, the term used to describe organisms that are unintentionally fished are returned to the sea, for being unwanted specimens, based on their low economic value, size (some stocks are regulated with a minimum landing size, and some have also a maximum), or appearance (Adão et al., 2018).

Discards and by-catch can represent a variety of economic and ecological problems, as they interfere in the ecosystem functionality and therefore problems with the abundance of the target stocks start to appear (Adão et al., 2018). Therefore, some legislations and restrictions were developed to prevent overexploitation of specific stocks and to avoid discards, being the Landing Obligation (LO) under the Common Fisheries Policy (CFP) one of the examples. In summary, the LO demands that all fishing vessels should return to land all the captured resources regulated with quotas and recommend the adoption of more sustainable fishing practices to reduce bycatch, as other three exemptions that implicate on quota management (Rätz & Lloret, 2018).

All around the world there are several industrial fisheries with big fishing vessels, but in Portugal the fishing fleet is mainly composed of small scale fisheries (SSF; represents more than 70% of the Portuguese fleet), with traditional practices and small vessels that target a variety of important species due to its geography and seasonality (Teixeira et al., 2014). One of the most used fishing gear in Portugal are the trammel nets (Gaspar et al., 2014). There are also other southern European countries that practice this type of fisheries for centuries and it has become a tradition. Nowadays, SSF reveal a significant importance in the socioeconomic status quo, being very important socio-economically for local fisherman communities, since it has been their way of self-sustain for decades (Stergiou et al., 2006).

One of the fishing resources caught by SSF and other fisheries operating with trammel nets are elasmobranch, which are also usually caught by the Portuguese fleet, but mainly as bycatch (e.g.,.Coelho et al., 2005; Figueiredo et al., 2020; ICES, 2020). Rays and skates (Order: Rajiformes, Class: Chondrichthyes) is one of the group of species in Portuguese markets with higher importance (Farias et al., 2006; Baeta et al., 2010), representing in 2017, around 48% of the reported landings of elasmobranchs in Portuguese continental waters (Figueiredo et al., 2020). Skates and rays like many other elasmobranchs are one of the most vulnerable fishes that suffer overexploitation around the world, due to their particular life history traits, like higher longevity, slow growth, late maturity, low fecundity and use of specific habitats for reproduction and nursery (Stevenset et al., 2000). Additionally, their market values are lower compared with other target species that are landed, which leads to a lack of importance when it comes to managing the conservation of this group (Ball et al., 2016). Elasmobranchs are considered one of the most discarded groups of the world (Ball et al., 2016). In 1800 skates and rays had almost no market value, but since then, it has been growing the aim to catch this group, and because of that and given the increasing global concern on their conservation due to its biological vulnerability, the legislation around this group of species has been largely increasing (e.g., TACs and quotas, inclusion of some species as prohibited species, implementation of minimum landing size, seasonal closures)(Breen & Catchpole, 2021; Ellis et al., 2010; Figueiredo et al., 2020). Nevertheless, these resources are still discarded, and as demonstrated previously discards can lead to a destabilization of the ecosystem, but several studies indicate that elasmobranchs and rays and skates in particular, have potentially a high capacity of survival, being often alive when reaching the fishing vessel (Enever et al.,, 2010; Serra-pereira & Figueiredo, 2019). As Ellis et al., (2010) refers in their study, the survival can depend on many variables, like size or species, so there is a need to actually evaluate the survival of this animals to verify if the discard of non-wanted individuals is a better alternative to the obligatory landing.

State of the art

Skates and Ray species

Elasmobranchs perform a top predator role in our marine ecosystems, they serve as a controller of other species (prey) abundance in the food chain (Follesa et al., 2010). One of the most abundance families of elasmobranchs is the Rajidae, playing also a very important role as a marine resource caught by commercial fleets (Serra-pereira & Figueiredo, 2019). Around, 40% of the total weigh of elasmobranchs landed, in the NE Atlantic Ocean, correspond to skates and rays (Machado et al., 2004; Farias et al., 2006). Yet, due to misreporting, the total amount of landed weight per year may be an underestimation of what they represent in reality, and the total amount of discarded weight (i.e., catch not landed) is unknown (Enever, 2009).

The life history traits of this group of animals (batoids) is characterized by few offspring, slow growth, late maturity and low reproductive output, which translates is high vulnerability to overexploitation (Enever et al., 2009). Each skate and ray species represent different levels of resilience, as some appear to be more vulnerable to certain types of fisheries than others, which can be reflected in differences in abundance between species (Machado et al., 2004). For example, bigger specimens are more susceptible to fishing than smaller ones (Walker & Hislop, 1998).

There are at least eight species of skates and rays described to occur in Portugal mainland: the white skate *Rostroraja alba* (Lacepède, 1803), the blonde ray *Raja brachyura* Lafont, 1873; the small-eyed ray *Raja microocellata* Montagu, 1818; the thornback ray *Raja clavata* Linnaeus, 1758; the brown ray *Raja miraletus* Linnaeus, 1758; the spotted ray *Raja montagui* Fowler, 1910; the undulate ray *Raja undulata* Lacepède, 1802 and the cuckoo ray *Leucoraja naevus* (Müller and Henle, 1841) (Machado et al., 2004).

The species that entered this experiment were *R. brachyura* and *R. montagui*, *R. clavata* (only one individual) and *R. miraletus* (only one individual). Following is presented the main features by species:

Thornback ray (R. clavata)

Kingdom: Animalia;

Phylum: Chordata;

Class: Elasmobranchii (sharks and rays);

Order: Rajiformes (Skates and rays);

Family: Rajidae (Skates);

Genus: *Raja;*

Species: *Raja clavata* Linnaeus, 1758.

Raja clavata is one of the largest species of the Rajidae family, also one of the most exploited batoids in the NE Atlantic (Hunter et al., 2005), being considered by the IUCN red list as near threatened due to overexploitation (J. Ellis, 2016). Their capture and landings until the end of the XX century were declining (Hunter et al., 2005), but in the last 20 years the trends in biomass of the different European stocks are increasing indicative of a good stock status (Breen & Catchpole, 2021). This species are known for seasonal movements from offshore waters in winter to shallow coastal waters in spring/summer, where they lay their offspring (eggs) (Hunter et al., 2005). The juveniles hatch around 12 cm of length which makes them vulnerable to the non-selective fishing gears, like demersal trawling, and as a consequence, when caught, they end up being discarded (Stergiou et al., 2006). The youngsters' don´t do migrations and until they reach their first maturity they tend to stay near the coast (McEachran, 1998).

This species can reach the 1020 m depth but usually lives between 100-300 m (McEachran, 1998). It reaches its first maturity around 78.4 cm in the case of females and 67.6 cm in males (Serra-Pereira et al., 2011). It can grow till 105 cm, and has a live expectancy around 15 years old (McEachran, 1998). It is distinguishable from other species by its size and specific coloration pattern of the tail, with alternating dark and light bands (Stehmann, & Burkel, 1984).

In 2017, this species was the most landed species in Portugal mainland, corresponding to a total of 55% of the total landed weight of rays and skates; its landings were mainly from polyvalent vessels using trammel nets (80%) (Serra-pereira & Figueiredo, 2019).

The blond ray (*R. brachyuran)* & the spotted ray (*R. montagui)*

Kingdom: Animalia;

Phylum: Chordata;

Class: Elasmobranchii (sharks and rays);

Order: Rajiformes (Skates and rays);

Family: Rajidae (Skates);

Genus: *Raja;*

Species: *Raja montagui* Fowler, 1910.

Species: *Raja brachyura* Lafont, 1873.

Raja brachyura is a large skate specie that reaches up to 120 cm length, normally confounded with *R. montagui*, which is one of the smallest species that can only reach 84 cm of length (McEachran, 1998). Both are characterized by a coloration pattern with black dots all over their dorsal surface, but in *R. brachyura* the dots reach the limit of their fins and have bigger white spots surrounded by small black dots, while the *R. montagui* only have the black dots but they don't reach the limit of their fins, there is also a difference where the males from *R. brachyura* mature at higher sizes than *R. montagui* males (J. R. Ellis et al., 2009; J. Ellis et al., 2007). They are both benthonic species that occur until 300 m depth, yet *R. montagui* can be found deeper, at 500 m depth (McEachran, 1998). According to the IUCN red list, *R. brachyura* is considered near threatened and *R. montagui* is considered least concerned (J. R. Ellis et al., 2009; J. Ellis et al., 2007). The corresponding Iberian stocks for these two species, evaluated by ICES, have a stabilized biomass or even has a tendency to grow (Breen & Catchpole, 2021).

Raja brachyura is one of the batoid species that have more market value in spite of being captured as by-catch, their large sized body and weigh is considered good for the market (Dailianis et al., 2016). It is also, the second most landed species in Portugal, with a corresponding 235 tonnes in 2017, where 88% of the landings corresponds to polyvalent fisheries and it represents a total of 21% of the total landed rays and skates (Serra-pereira & Figueiredo, 2019). The landings of *R. montagui* only corresponded to 8% of the total landed rays and skates in Portugal, with a corresponding 76% of that landings being delivered by polyvalent fisheries (Serra-pereira & Figueiredo, 2019).

The brown ray (*R. miraletus)*

Kingdom: Animalia;

Phylum: Chordata;

Class: Elasmobranchii (sharks and rays);

Order: Rajiformes (Skates and rays);

Family: Rajidae (Skates);

Genus: *Raja;*

Species: *Raja miraletus* Linnaeus, 1758.

Raja miraletus is a small ray that grows until 63 cm, distinguished by two ocelli, coloured black, yellow and blue, one in the middle of each side of the disc. They can go up to 462 m of depth but usually lives between 50-150 m deep and are considered benthic animals. This species is considered least concerned by the IUCN red list their population it is considered growing (Dulvy, 2019). As other skates and rays, it is non-target for fisheries, but never the least sometimes ends up being captured in the nets (Dulvy, 2019; Press, 2016).

Peniche

In Portugal Skates are landed as a generic specie without any kind of differentiation (*Raja spp*.) and only represent aggregated landings. Since 1991 their market value has been growing, which influences the fisherman´s decisions (Machado et al., 2004).

In 2019, the TAC (total allowed catch) for the Iberian and Biscay ecoregion was 4759 tonnes with a corresponding 1463 tonnes of quota for Portugal (Serra-pereira & Figueiredo, 2019). Peniche has one of the most important fishing ports in Portugal and when it comes to abundance of landings, this port is mostly composed by trawl and artisanal fisheries, including mixed fisheries that also use trammel gear (Machado et al., 2004). Since 1990 to 2010, Portugal has landed around 1200 tonnes of skates and rays per year (Serra-pereira & Figueiredo, 2019).

Portugal had a corresponding 1024 polyvalent fishing vessels in 2017, and 83% of their landings corresponded to rays and skates, so it represents a big portion on their landings (Serra-pereira & Figueiredo, 2019).

There are two species of skates that appear the most in Peniche landings, which are the *R. clavata* and *R. brachyura,* but in contrast the *R. miraletus* is the least spotted, also the *R. brachyura* specie exhibits the smallest sizes of all landed species (Machado et al., 2004; Farias et al., 2006).

Portugal has developed specific laws for capturing rays and skates, from May to June there is a fishing closure that prevents the capture of this animals, only a 5% quota is allowed if it corresponds to by-catch (Serra-pereira & Figueiredo, 2019), also the CAPA (Cooperativa dos Armadores de Pesca Artesanal) has developed a minimum landing size (MLS), which dictates that rays and skates with 52 cm bellow can´t be captured (Serra-pereira & Figueiredo, 2019).

Trammel fishery

The small fisheries contribute for more than half of all landing in the European waters, small fishing vessel under 12 m usually represent heterogeneous fishing, this means that they catch different species with different gear at different seasons of the year (Stergiou et al., 2006). They can use a variety of gear like gill nets, trammel nets, longlines, traps, etc. (Stergiou et al., 2006). Trammel nets are composed by 3 parallel nets vertically hanged by posts, this type of fishery isn´t much selective to different animal sizes and it can catch fish by entangling and trapping large individuals in the inner net

(Karakulak et al., 2008). Trammel nets are a very common gear utilized in the southern Europe fleets, it´s already a tradition, it´s a type of gear that catches a variety of fish because of its length and therefore, these heterogeneous fishing nets compensates compared with other gears, like gill nets (Gonçalves et al., 2007). This kind of small fisheries are under studied compared to large industrial fisheries, but they are a lot less harmful for marine ecosystems and for that reason, the information about small polyvalent fisheries needs to be collected continuously in order to develop even more informed conservation and management plans (Stergiou et al., 2006).

Another problem with the LO that affects the mixed fisheries, is the monetary losses that the non-wanted fish incur, it´s a cost that fisherman's don´t want to take, they are obligated to bring to land all this fish and it inflicts a loss on their earnings (van Hoof et al., 2018). The only help the fisherman have is the exemptions which allows some discards for certain species (Adão, Breen, Eichert, & Borges, 2018).

By-catch and Discards

The By-catch meaning was described by FAO (The United Nations Food and Agricultural Organisation's) in 2018 which describe as *"the part of a catch of a fishing unit taken incidentally in addition to the target species towards which fishing effort is directed. Some or all of it may be returned to the sea as discards, usually dead or dying"* (van Hoof et al., 2018).

The by-catch not always refers to no market value species, some species represent other quotas that have market value, other don´t and for this reason, the discards practice appears in order to save space in the fishing vessel (van Hoof et al., 2018).

The described meaning of discards that FAO revealed in 2018 is *"the proportion of the total organic material of animal origin in the catch, which is thrown away or dumped at sea, for whatever reason. It does not include plant material and post-harvest waste such as offal"* (van Hoof et al., 2018).

The main reasons for the high ratio of discards is due to the practice itself, directly involved in the gear utilized, particularly the nets involved (the size of the mesh and the soaking time), the managing and legislation surrounding the practice also helps to raise the ratio of discards (Gonçalves et al., 2007). Most of the organisms captured by this nets (by-catch) are undersized compared to the exemplars desired, which means they can´t be captured, or if the individuals are too damaged and therefore have no market value (Gonçalves et al., 2007). It all sums up to four different aspects that lead to discard: when the fish is too small, when there is some type of restriction to capture a certain species, when the individual is too damaged or when the animal as low market value (van Hoof et al., 2018).

This problem has major consequences on the biodiversity of the marine ecosystem, once the dead organisms are thrown back to the ocean, lots of other problems can occur (Adão et al., 2018). It can damage entire populations or even destabilise an entire food chain, because it unbalances the food for certain species that play very important roles, for example, the scavenger species can grow too much, which creates an unbalance in the structure of the ecosystem (Gonçalves et al., 2007).

Some authors like Zeller & Pauly., (2005) refer to discards as a declining practice nowadays, this isn't necessarily good news, because as this happens also the number of total catches declines, if we see this in another perspective it means that the total abundance of stocks is declining also (Zeller & Pauly, 2005).

The number of discards around Europe are huge, estimating around 10 million tons of catches being discarded per year, there are locations designated as discard hot-spots due to the intensity of this practice (van Hoof et al., 2018). Sometimes discards can be perceived as a good practice, when the animals are considered an endangered species or if the animals returned to the sea are unharmed (van Hoof et al., 2018).

As reviewed in Enever et al., (2009) study, there are captured in the UK, by the Bristol Channel Fishery, around 3.8 million skates per year and 80% are discarded, but 20% of this 80% are discarded because of weigh.

Landing Obligation

The Landing Obligation (LO) comes in the Article 15 of the CFP reform of 2013, it stipulates that all fishing vessels are obligated to bring to land all catches of quota (regulated sized species) with the objective to reduce the ratio of discards in European fisheries (van Hoof et al., 2018).

The CFP- Common Fisheries Policy, created a reform in 2011 in order to move towards a better management of the system of the European fisheries (van Hoof et al., 2018), this reform was created because of the urge to protect both the marine ecosystems wealth and also the fisherman (Lee M., 2009). This efficient fishing ways leads to better results when it comes to the abundance of available stocks (van Hoof et al., 2018). Only after 2 years, in 2013, that the CFP could impose their policy that was divided in tow flagships: "Reach the objective of maximum sustainable yield (MSY) in 2020 and introducing the Landing Obligation (LO)" (van Hoof et al., 2018).

The LO is a wide legislation that affects a big variety of deferent fisheries, it doesn't take into account specific types of fisheries, and there are some fisheries like the mixed fisheries in Portugal, that may suffer the so called choke effect, because their way of fishing catches a lot of different species and they are exposed to the fact that most of the fishes they catch are unwanted compared with targeted fisheries, and this can lead to a socio-economic problem for this fisherman (van Hoof et al., 2018).

The discards are one of the main reasons that contribute to the fishing over the maximum sustainable yield (MSY) level, with most of the reported results being omitted, this may be the strongest reason to develop the second flagship (the Landing Obligation) (van Hoof et al., 2018).

In order to stop the choke effect there was 3 exemption that available discards, species that have fishing prohibitions can be discarded, the species under the Minimis exemption can also be discarded and finally species that appear to have high survival ratios after being captured could also be returned to the see (Salomon et al., 2014).

Choke effect and "De Minimis" exemption

The choke effect is directly related to the LO, the landing obligation goes against the previous capture quotas, because of all organisms captured needed to be landed, there will be some quotas that are underachieved, due to nowadays production levels of different populations biomass, when the quotas where developed there were different levels of population biomass and therefore other by-catch species can have more biomass nowadays, which are more captured than the targeted species (Mortensen et al., 2018). Also because there is a bigger percentage of by-catch than the actual target species, this can lead to a choke, in terms of disrupting this species populations instead of just capturing the targeted species, what can lead to the early closure of mixed fisheries, which is bad for fisherman´s (Mortensen et al., 2018).

In order to avoid the choke effect there are some exemptions created, “De Minimis” exemption which helps these fisheries by allowing for each quota a discard rate of 5% of their total catches, this gives the possibility at least to fish 5% more of certain target specie (Adão et al., 2018). Other exemptions are the high survival exemption and inter-species and inter-annual quota flexibilities, this exemptions relates to the exchanging of quotas while fishing, if the target specie (donor) quota is already been reached, it can be swapped for a non-target specie quota that hasn’t been reached, this can go up to 9% only, also a similar exchange of inter-annual quotas from different species can be made up to 10% (Adão et al., 2018).

High survival exemption (HSE)

The HSE is another exemption created in order to protect species that appear to have high survival probabilities after being captured by a certain type of fishery (Adão et al., 2018). The CFP describes this exemption as: *“species for which scientific evidence demonstrates high survival rates, taking into account the characteristics of the gear, of the fishing practices and of the ecosystem”* (van Hoof et al., 2018).

This way, it becomes a very important topic to evaluate and quantify, different species need to be studied in order to conclude if their survival ratio to different gears of fisheries is high enough to be considered a protected species by this exemption (van Hoof et al., 2018).

One of the main aspects of this study is about this exemption, HSE, and the evaluation of different ray species survival, to trammel fisheries.

Survival

The discards are not always alive, it depends on the gear utilized on the fishery, the duration of catching and also the injuries inflicted on the organisms can influence the vitality of the individuals (van Hoof et al., 2018).

Studies to estimate survival of fish after being captured are based on different factors that influence the mortality of the individuals, different factors like size of the individual, sex or even if the fish suffers grate changes of pressure during the hunt (Benoît et al., 2010). This studies can be made on a laboratory or while the investigators are on board (Benoît et al., 2010). For studies where the animals are kept in captivity, there are some limitations, like not being able to hold many individuals in the laboratory and this may lead to few results as a result of the low percentage of studied individuals (Benoît et al., 2010). There is also another difference in doing survival studies in laboratory compared with studies on board, because there will be limitations that incapacitates the investigator from accessing important factors that influence mortality ratios (Benoît et al., 2010).

In order to study the survival of discards there is a predictor called Reflex Action Mortality Predictor (RAMP) which is utilized to compare lots of different variables that may cause stress on the individuals and the response that the individual demonstrates after being exposed to this stress variables, however it must only be related to fisheries procedures and it can´t be influenced by the handling of the individuals (ICES, 2018).

In the ICES (2018) study, the animals were kept in cages at the bottom of the sea floor after which they collected data from the water temperature every month and compared with the survival of each individual, giving RAMP or vitality scores to each one. There was a problem with their study which was in winter and summer, the holding of the fish in water filled tanks for 1h before testing, this may have been the major influencer on the high rate mortality in summer, because temperatures rise on the tanks and may cause stress on the animals (ICES, 2018). The RAMP test evaluates if the animals respond to stimulus and it can be divided in 4 different tests: tail grab; body flex; startle touch and respiration for 30 s, this tests can be treated with a non-parametric Kaplan-Meier survival models (Serra-pereira & Figueiredo, 2019).

The Categorical vitality assessments (CVA) studies are usually used to access the vitality of rays and skates captured by polyvalent fisheries that use trammel nets, normally this tests give a score based on vitality parameters that goes from 1 to 3, in that 1 is excellent movement and 3 is poor/dead, which means that the individuals are almost dead (Serra-pereira & Figueiredo, 2019).

In the pilot study mentioned in Serra-pereira & Figueiredo ., (2019) they evaluated the soaking duration on each haul and also the size of the mesh, these are another variables that may influence the vitality score of the individuals, also in this study they had results that suggested that the size of the individuals didn´t interfered with the vitality scores for the retained individuals, but for the discarded animals the results suggested otherwise, the larger the individual the higher the vitality score. A GLM (Generalized Linear Model) was used to predict if there was a difference between discarded and retain individuals compared with sex and vitality scores (Serra-pereira & Figueiredo, 2019). There are also evaluations that can be made for lesion and internal bleeding correlated with the vitality scores and RAMP scores (Serra-pereira & Figueiredo, 2019).

In Enever et al., (2009) study, they considered that the individuals were dead when they had no muscular movement, when there was no spiracle closure and when the individual had a *rigor mortis* (the fins contract forming an upside-down "V" shape). In their study they achieved a 55% survival of the total number of individuals for big fishing vessels and 87% survival for small fishing vessels (Enever, Catchpole, Ellis, & Grant, 2009).

The Enever et al., (2009) study also categorized the individual's vitality by levels from 1 to 3, in that 1 is when the skates were dead or almost dead with no or almost no muscular movement or a slight closure of spiracles; 2 for animals that appear to have a moderate state of health characterized by moving but not vigorously and finally number 3 was when animals demonstrated vigorous movements and were extremely responsive to stimulus or had a faster spiracle closure ratio. The variable that had most significance was the health state when the individuals entered the tanks, corresponding to 1=79% died, 2=16% and 3=5%, the other variables like sex, species or size didn't had a significant p value that correlates with vitality in the long-term (Enever et al., 2009).

Objective and hypothesis

The following objectives and hypothesis were developed according to the ICES 2020 studies, IPMA guidance, with PPCENTRO project and also based on the literature previously demonstrated.

1. Estimate and characterize the survival after capture for two different skate species, *R. brachyura* and *R. montagui*, caught by trammel nets off Peniche (central Portugal, ICES division 27.9a), based on captivity studies. In order to estimate the following hypothesis (H1 and H2) were developed.

   H1: *Raja brachyura* has a survival rate, to trammel net fisheries, higher than 50%.

   H2: *Raja montagui* has a survival rate, to trammel net fisheries, higher than 50%.

2. Evaluate the relationship between the vitality scored (immediately) after capture and the estimated survival rate to trammel net fisheries in both species. In order to estimate the following hypothesis (H3 and H4) were developed.

   H3: The higher the vitality score, the lower the survival rate to trammel net fisheries for *R. brachyura*.

   H4: The higher the vitality score, the lower the survival rate is in *R. montagui* species.

All the experiments performed in the current study were made under the PPCENTRO project which is related to management and of the small fisheries, in Portugal, captures, it can be related to target species or not and it monetarizes the abundance and time distribution of the species.

Materials and Methods

On-board sampling and collection of samples for the study

This study was developed in IPMA´s laboratory in Peniche with association of the PPCENTRO project and fisherman help, there were three different fishing vessels involved with the study, all from polyvalent fisheries that used trammel nets, this type of fishing was the only one relevant for the study. The species that entered this experiment were *R. brachyura* and *R. montagui*, *R. clavata* (only one individual) and *R. miraletus* (only one individual) as mentioned previously.

Samples were either collected on-board the fishing vessels (with an observer on-board) or provided by fishing vessels collaborating with the PPCENTRO project. During the former, all specimens of skates and rays caught the following information was recorded: species, total length (cm), sex, Categorical Vitality Assessment (CVA), time of capture and if retained or discarded by the fishermen. The scale adopted to record the CVA was based on (Serra-Pereira & Figueiredo, 2019) and can be found in Table 1. From those to be discarded (mainly because of its size being below the minimum landing size of 52 cm), some were selected for the survivability experiments. Specimens selected for this purpose were placed on a holding tank (capacity of around 100 L) installed in the vessel, with constant sea water entry. Per trip, a maximum of 6 skates were selected to be used in the experiments.

Apart from the information mentioned above, collected for all skates and rays caught in the trip, the individuals that were kept in the holding tanks were observed for Reflex Action Mortality Predictors (RAMP), immediately after being placed in the tank. The RAMP was an aggregated test to fulfil the vitality data, which was utilized as a categorical reflex response to different stimulus. In the current study the assessed RAMP were:

Respiration – The individual's respiration (closure and opening of the operculum) was observed during 30 s;

Tail grab – The observer must apply a stimulus on the tip of the individual's tail, for example with the fingers and register if the animal responded or not (response=1/no response=0);

Body flex – The observer must grab the individual, in a non-harmful way, out of the water and register if the animal responds with body movement like body contraction or tail movement (response=1/no response=0);

Startle touch – The observer must apply a stimulus with his finger in the middle of the individual´s eyes and register if the animal closes its operculum's or if it contracts is eyes (response=1/no response=0).

Additionally, scars and internal bleeding coverage was also assessed using a categorical system described (Table 2). The forms used to register this data can be consulted in annex 1 and annex 2.

Numerical scoring	Categorical scoring	Description
1	**(excellent) -**	when the individual had vigorous body movement
2	**(good)**	Weak body movement but responded to touching/prodding
3	**(bad)**	almost no body movement but fish can still move operculum
4	**(dead)**	No body or opercular movements (no response to touching or prodding)

Table 1 - Categorical Vitality Assessment (CVA) adopted for skates and rays.

Level	Description
0	When the body part had no injurie coverage;
1	When the body part had below 10% injurie coverage;
2	When the body part had between 10-50% injurie coverage;
3	When the body part had more than 50% injurie coverage.

Table 2 - Categorical levels adopted to describe the degree of coverage of injuries (scars and/or internal bleeding) of studied specimens.

Given the COVID-19 pandemic, the access to on-board sampling was limited, and therefore a collaboration with fishermen from Peniche and operating with trammel nets (n=3 vessels) was established to get live animals for the study. Most of the samples used were provided under this collaboration. The specimens were kept alive on-board in the holding tank belonging to the vessel, which features varied between vessels (capacity ranged from 100-400 L), and with constant feed of sea water. When the vessel arrived to the landing port, the specimens were removed from the tank on-board and transferred to 50 l transport tanks, each with 1-3 fish. The distance from the landing port to the laboratory was around 2 min walking. Before entering the tank, an acclimatization was made to diminish drastic temperatures changes for the fish. This took around 2h while changes of water from the main holding tank (tank 1) to the transport tanks were made. After acclimatization the individuals were introduced in the holding tank 1.

Additionally, while on-board or after a fishing vessel collaborating with the project and bringing specimens reached the landing port, all data from the fishery, like soaking time, coordinates, net size, number of nets, per haul, were recorded. This data was registered in a table like the one in annex 3.

Laboratory captivity conditions

To evaluate discard survival vitality assessments are imperative, it can be used to estimate the vitality or survival of the individuals by evaluating indicators like injuries and reflexes they present, it can be performed while on the vessel or in captive and the main purpose is to attribute a vitality score that can be compared with survival likelihood.

The facilities in the laboratory, used to perform the experiments included a holding tank (tank 1) with a capacity for 1200 l (closed circuit), equipped with a biologic filtering system (with bioballs, dracalon wool and activated carbon), a sterilization system with UV light to control unwanted microorganisms, a cooler (set to 15ºC) and an air pump coupled with six diffuser stones and a protein skimmer (external to the tank). The cooler was kept at low temperature around 15ºC in order to diminish the fish metabolism, because each time the individuals were fed the ammonia levels started to rise drastically, and also because of climacteric conditions by keeping the temperature at low levels, sudden rises of the holding tank water temperature were prevented.

An additional tank of 600 l (tank 2) served as a container for mixing salt water used for the partial exchanges of water between tanks. Since the laboratory was not fed with natural sea water, and because logistics to transport sea water from other sources was too expensive, sea water was synthetized at a concentration of 35 ppm, using tap water and Premium REEF-Salt. The right proportions of water mixed with salt used were: 17.5 kg of salt for 500 l of tap water in order to achieve a concentration of 35 ppm (usual the salinity concentration of the NE Atlantic ocean). The cooler was kept at lower temperatures around 15ºC in order to diminish the fish metabolism, because each time the individuals were fed the ammonia levels started to rise drastically, and also because of climacteric conditions by keeping the temperature at low levels, sudden rises of the holding tank water temperature were prevented.

Once the holding system was prepared, after 2 months of stabilizing the water parameters, the experiment began. The water physico-chemical parameters were frequently measured using probes: an oxygen probe from Hanna Instruments model HI98198, a pH probe from Hanna Instruments model HI 98127, a salinity probe from Hanna Instruments model HI98319 and chemical tests to measure ammonia, nitrites and nitrates from Tropic Marin. The table where these parameters were registered can be consulted in annex 4.

Since, the system was virgin for this experiment, before starting the experiment, three skate specimens were introduced in tank 1 so that the biological filtering system was populated with nitrifying bacteria. The same three individuals also served as control to evaluate if the holding conditions weren´t the factor influencing the mortality of the specimens used in the experiments.

On board sampling

The procedure on board consisted in, when a desired individual discarded by the fisherman was captured, while they were hauling, it was putted on a holding tank with constant sea water circulating, with 100 L. Then some individuals were kept to bring to land alive, the criteria used was that the holding tank in the laboratory only was capable to receive 6 individuals at maximum, so the first 6 individuals were kept in different holding tanks (2 per tank).

The individuals that were kept in the holding tanks were observed instantly, they were evaluated in different categories: Species; sex; size; time of capture; vitality score (CVA); startle touch/ body flex/ tail grab/ respiration (RAMP); scar coverage and internal bleeding coverage. The table used to register this data is observable in annex 1 and annex 2.

When the individuals arrived on land they were transported to the laboratory, before entering the tank an acclimatization was made to diminish drastic temperatures changes for the fish, this take around 2h while changes of water from the principal holding tank (tank 1) to the other tanks was made. After acclimatization the individuals were introduced in the holding tank.

While on board it is important to register all data from the fishery, like soaking time, coordinates, haul number, net size, number of nets, etc., this data was registered in a table like the one observable in annex 3.

Laboratory captivity experiments

While the animals were in the holding tank 1, they were routinely submitted to a RAMP test (body flex, startle touch, tail grab and respiration) described in detail in the previous section, and the CVA assessed. This data was registered in a table like the one observable in annex 1. Also, a categorical degree (in percentage) of external (scars) and internal (internal bleeding- IB) injuries in segmented parts of the body, precisely head, body and tail injuries for each animal, were registered. Each specimen, if alive, was observed and kept in captivity for a minimum of 21 days.

Data analysis

In order to estimate the survival rate for both species a non-parametric Kaplan-Meier model was adopted. All the analysis was run in R language, using RStudio. The Kaplan-Meier helps to understand the survival rate over time of the sample, also it is observable when certain results were censured and when certain events occurred, and in this case the event is dying.

After analysing the survival rate for both species, considering all samples combined, another analysis was performed to evaluate if the vitality score influences the estimate of survival rate. The results were compared with the initial vitality score attributed on first observation of each individual. All the analysis was run in R language, using RStudio.

Results

The aim of this study in the beginning was to experiment with *R. clavata* individuals, but the majority of the individuals, that were sampled either on-board or obtained from the fishermen, were from *R. brachyura* and *R. montagui* species. The total amount of individuals (n) in this study was 42 individuals of those species, 28 identified as *R. brachyura* and 14 as *R. montagui*, plus one individual of *R. miraletus* and one *R. clavata*. From the 42 specimens, only 21 *R. brachyura* and 13 *R. montagui* were used in the captivity experiment (Table 3). The aforementioned specimens were obtained from a total of 11 fishing trips.

To note that that except for 10 specimens (individual 7 till individual 16) that were sampled on-board, for all the remaining, the CVA was assess inland, around 6 hours after capture, and thus cannot be considered as immediate vitality after capture. Given this fact, it is to notice that in both species the majority of individuals were classified with a CVA level 2 (moderate).

Table 3 – Number (n) of individuals by sex and vitality score and which ones were sorted to the captivity experience, by species (n=11 fishing trips sampled).

	Brachyura				Montagui			
	Vitality assessment		Survival		Vitality assessment		Survival	
Vitality score	F	M	F	M	F	M	F	M
1 - Good	0	0	0	0	1	0	1	0
2 - Moderate	17	2	11	2	6	5	6	5
3 - Bad	2	6	2	6	1	1	1	0
4 - Dead	1	0	0	0	0	0	0	0
Total	20	8	13	8	8	6	8	5

In figure 1 and figure 2 it is represented, for each species, the number of individuals by gender and size (CT in cm). The CT of females ranged from 35 to 57 cm, while males measured from 48 to 56 cm in *R. brachyura* species (figure 1), for *R. montagui* species the CT of females ranged from 42 to 57 cm, while males measured from 47 to 53 cm (figure 2). Not all individuals in this experiment were under the MLS that corresponds to 52 cm.

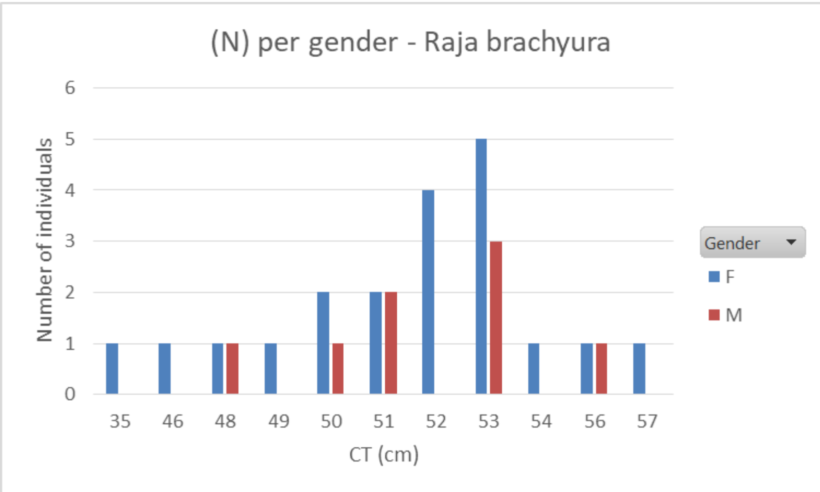

Figure 1 – Length frequency of *Raja brachyura* by gender, sampled (n=11 fishing trips sampled).

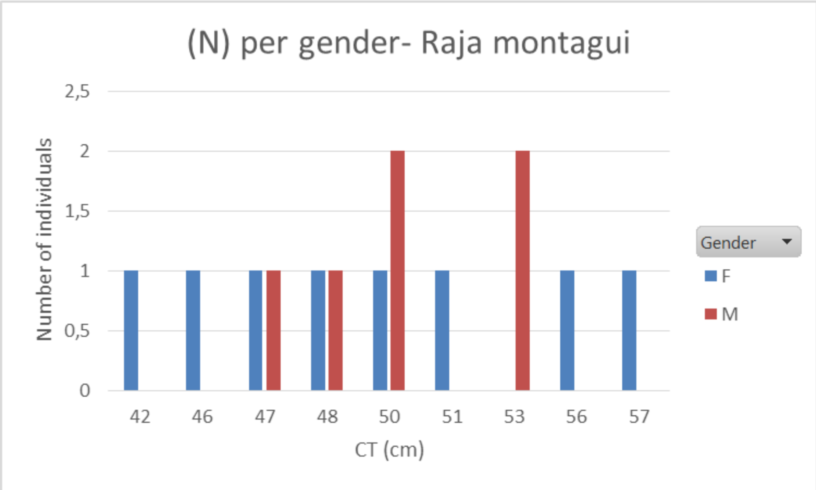

Figure 2 – Length frequency of *Raja montagui* by gender sampled (n=11 fishing trips sampled).

In figure 3 and figure 4 it is represented the size of the individuals (CT in cm) by vitality score. In figure 3 (*R. brachyura*) the smaller individuals represented better vitality scores, in figure 4 (*R. montagui*) is more homogenous; the only individual categorized as 1 (vitality score) was a 47 cm.

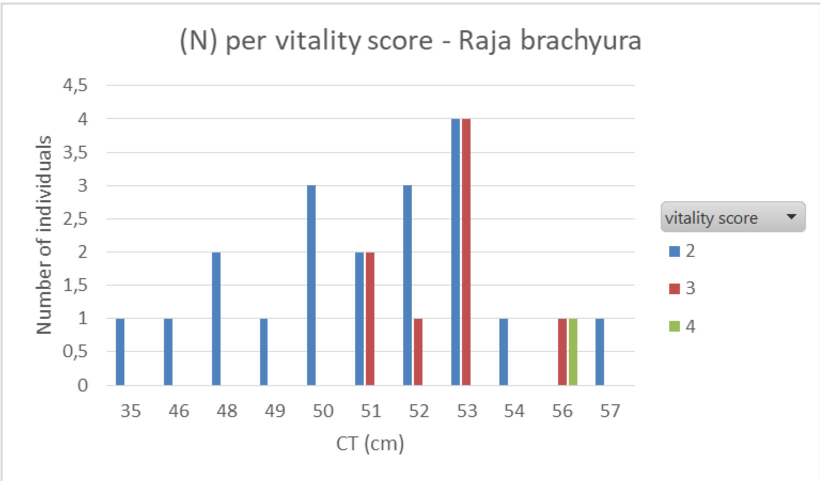

Figure 3 – Length frequency of *Raja brachyura* by vitality score (n=11 fishing trips sampled).

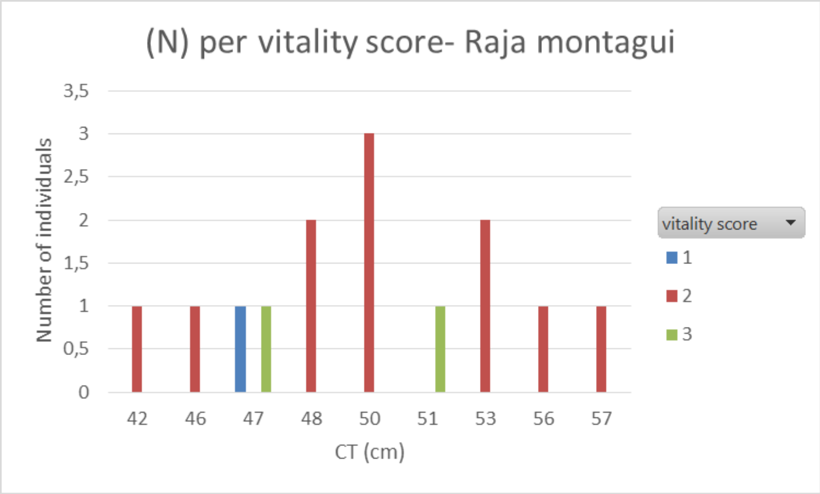

Figure 4 – Length frequency of *Raja montagui* by vitality score (n=11 fishing trips sampled).

It is important to characterize the specimens' vitality score for the different species by fishing parameters; table 4 and 5 were developed to compare the number of individuals by:

1. Mesh size (mm) – it could be 100 mm or 260 mm;
2. Number of nets – it could be 50, 100 or 125;
3. fishing time (h) – if the duration was under of 10 h or equal/ above 10 h;
4. Size class (cm) – If the individuals were under the MLS (52 cm), or equal/ above MLS;
5. Depth (m) – if the individuals were captured bellow 14 m depth or above;
6. Surface temperature (Cº) – If the temperature of the surface of the water was bellow or equal/ above 19 Cº ;
7. Wave height (m) – the size of the waves when the individuals were captured, if it was under 2 m or equal/above.

Table 4 – Number (N) of individuals by species and vitality score, according with the conditions that they were captured: by mesh size (100 m or 260 mm), number of nets and fishing time (h); and also the size class (if below or above the Minimum Landing size (MLS) of 52 cm CT.

Species	Vitality score	Net size (mm)		Number of nets			Fishing time (h)		MLS	
		100	260	50	100	125	<10	≥10	< 52	≥52
R. brachyura	1- Good	0	0	0	0	0	0	0	0	0
	2- Moderate	8	11	8	0	11	18	1	9	10
	3- Bad	0	8	0	0	8	8	0	1	7
	4- Dead	1	0	1	0	0	0	1	0	1
	N	9	19	9	0	19	26	2	10	18
R. montagui	1- Good	1	0	1	0	0	0	1	1	0
	2- Moderate	4	7	2	2	7	8	3	7	4
	3- Bad	1	1	0	1	1	1	1	2	0
	4- Dead	0	0	0	0	0	0	0	0	0
	N	6	8	3	3	8	9	5	10	4
R. miraletus	1- Good	1	0	0	1	0	0	1	0	1
	2- Moderate	0	0	0	0	0	0	0	0	0
	3- Bad	0	0	0	0	0	0	0	0	0
	4- Dead	0	0	0	0	0	0	0	0	0
	N	1	0	0	1	0	0	1	0	1
R. clavata	1- Good	0	0	0	0	0	0	0	0	0
	2- Moderate	0	0	0	0	0	0	0	0	0
	3- Bad	1	0	0	1	0	0	1	0	1
	4- Dead	0	0	0	0	0	0	0	0	0
	N	1	0	0	1	0	0	1	0	1

Most of the individuals sampled of *R. brachyura* had a CT equal or above the MLS; most were caught with nets with mesh size of 260 mm, from fishing vessels using around 125 nets, fishing for less than 10 h, at a surface temperature below 19 ºC. For the *R. montagui* species more individuals were under the MLS than other wise, in terms of number of nets and net size the results were similar to *R. brachyura*, but the fishing time for this species was the opposite, were when the fishing time was equal or above 10 h more individuals were captured (Table 4).

The depth (m) where specimens were collected and the size of the waves (m) observed in the sampling day were homogeneous for both species (Table 5).

Table 5 – Number (N) of individuals by species and vitality score, according with the conditions that they were captured: by depth (below or above 14 m depth) on, surface temperature (ºC), wave height (Undulation - m).

Species	Vitality score	Depth (m)		Temperature (Cº)		Ondulation (m)		MLS	
		< 14	> 14	< 19	≥ 19	< 2	≥ 2	< 52	≥52
R. brachyura	1- Good	0	0	0	0	0	0	0	0
	2- Moderate	13	6	15	4	12	7	9	10
	3- Bad	0	8	0	8	0	8	1	7
	4- Dead	1	0	1	0	1	0	0	1
	N	14	14	16	12	13	15	10	18
R. montagui	1- Good	1	0	1	0	1	0	1	0
	2- Moderate	5	6	7	4	5	6	7	4
	3- Bad	0	2	1	1	1	1	2	0
	4- Dead	0	0	0	0	0	0	0	0
	N	6	8	9	5	7	7	10	4
R. miraletus	1- Good	0	1	1	0	1	0	0	1
	2- Moderate	0	0	0	0	0	0	0	0
	3- Bad	0	0	0	0	0	0	0	0
	4- Dead	0	0	0	0	0	0	0	0
	N	0	1	1	0	1	0	0	1
R. clavata	1- Good	0	0	0	0	0	0	0	0
	2- Moderate	0	0	0	0	0	0	0	0
	3- Bad	0	1	1	0	1	0	0	1
	4- Dead	0	0	0	0	0	0	0	0
	N	0	1	1	0	1	0	0	1

For *R. brachyura* species the depth (m) captured the same amount of individuals in both categories, but there were more individuals with worst vitality score when captured above 14 m, when the temperature was below 19ºC more individuals were captured but the individuals appear to have better vitality scores, also when the waves were above 2 m more individuals were captured and had worst vitality scores. The *R. montagui* individuals had more individuals captured above 14 m and they were with worst vitality scores, the temperature below 19ºC reflected more captures and when the wave size was equal or above 2 m also more individuals were captured.

In figure 5 and 6 it is observable that over time the vitality score starts to get lower (vitality improved), this also reflects that the individuals recover over time.

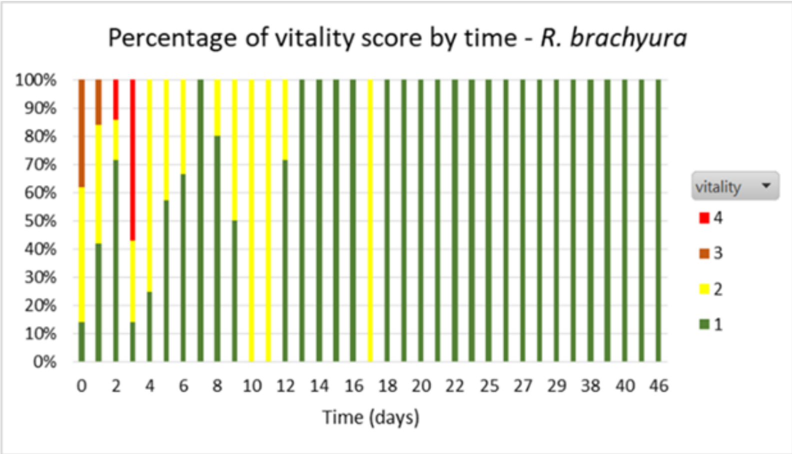

Figure 5 – Percentage of vitality scores over time for *R. brachyura* specimens.

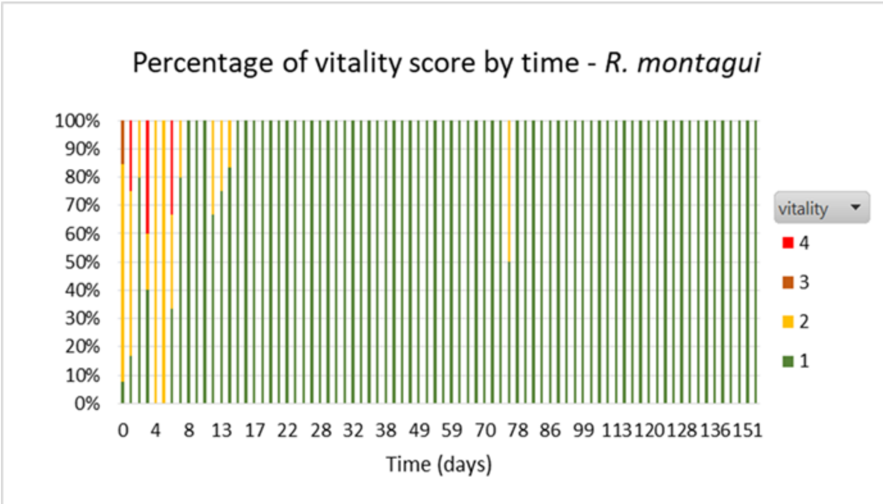

Figure 6 – Percentage of vitality scores over time for *R. montagui* specimens.

In figure 7 and 8 we can see for both species that each individual changes vitality score over time and in the great majority the individuals start at worst vitality scores and over time they get better vitality scores (level 1), except the individuals that die which corresponds to level 4.

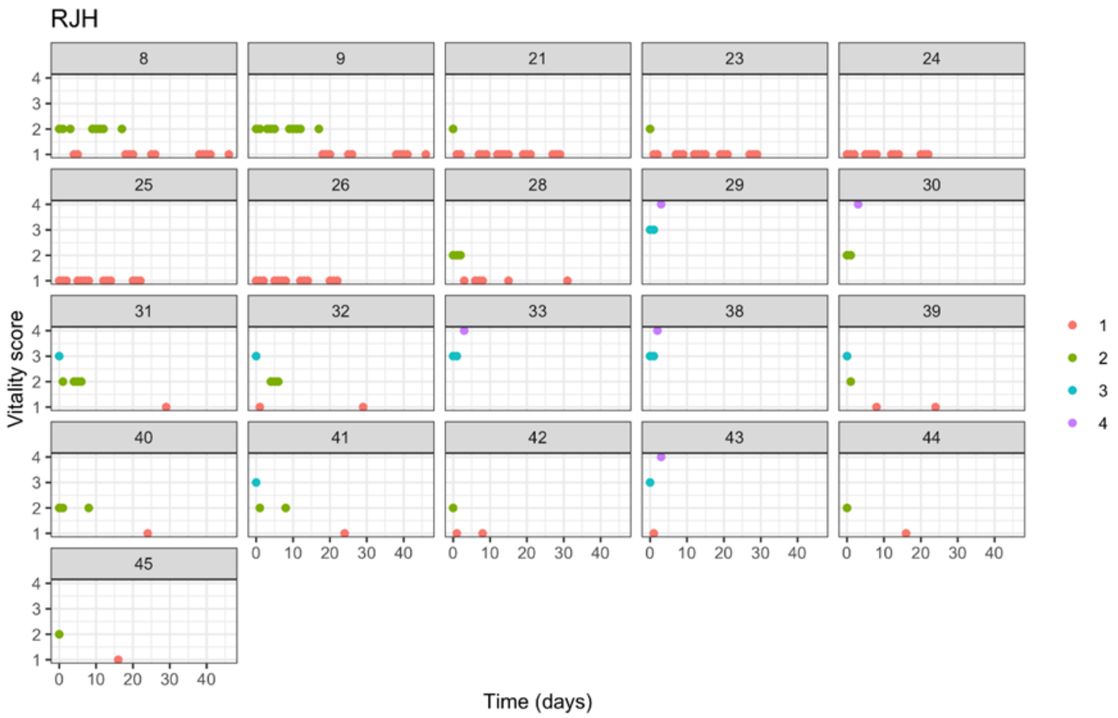

Figure 7 - *Raja brachyura* individual's vitality score over time.

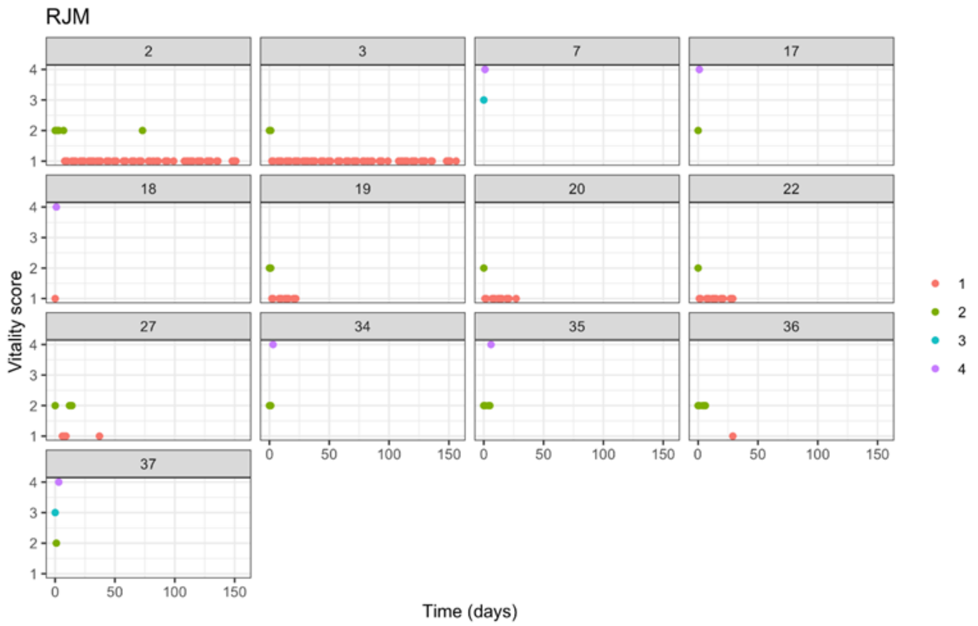

Figure 8 – *Raja montagui* individual's vitality score over time.

In figure 9 and figure 10 it is possible to observe the respiration results for each species along the time series by vitality score (1-4). The mean respiration results for *R. brachyura* was around 15 times per 30 seconds, individuals with better vitality score kept their respiration around this value, compared with individuals with worst vitality score that died or improved their vitality score, that´s why the values for individuals with worst vitality scores disappear over time. The *R. montagui* respiration results were similar to those from *R. brachyura*, and it is observable that their mean respiration in 30 seconds goes around 15 times also.

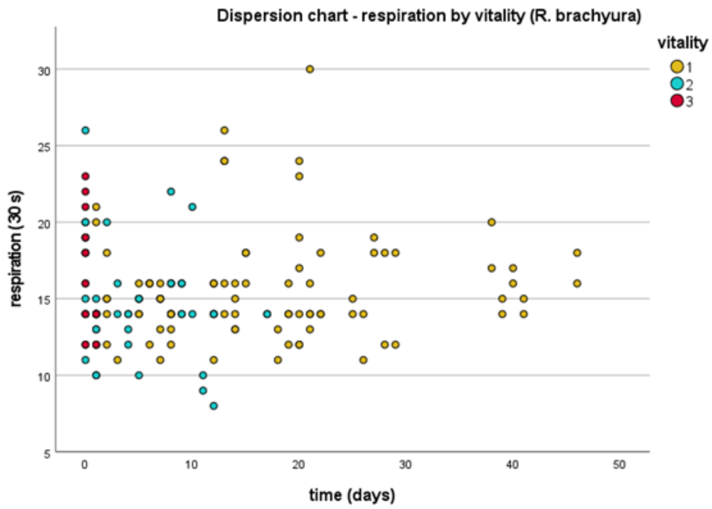

Figure 9 – The respiration results sorted by vitality by time for *R. brachyura* specimens. (n=21)

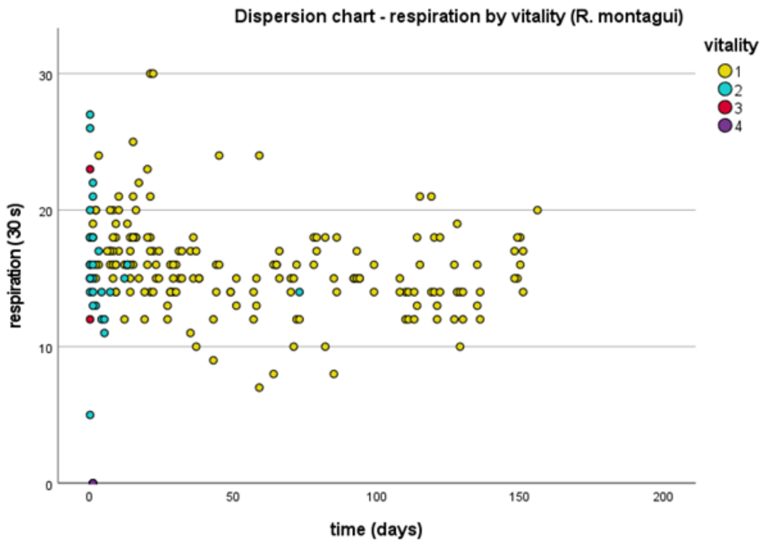

Figure 10 – The respiration results sorted by vitality by time for *R. montagui* specimens. (n=13)

The percentage of lesion coverage (internal bleeding – IB, and scars) by vitality score, for both species, can be observed in figure 11 and figure 12. The IB for the head and the scar coverage on the body are the ones who impact the most on the vitality score, as the vitality score gets worst the percentage of lesion coverage gets higher for both species, for the other lesion coverage categories is more aleatory, never the less in all categories, the worst vitality scores have more percentage of lesion coverage.

Figure 11 – Percentage of lesion coverage sorted by vitality score or *R. brachyura* specimens. (n=21)

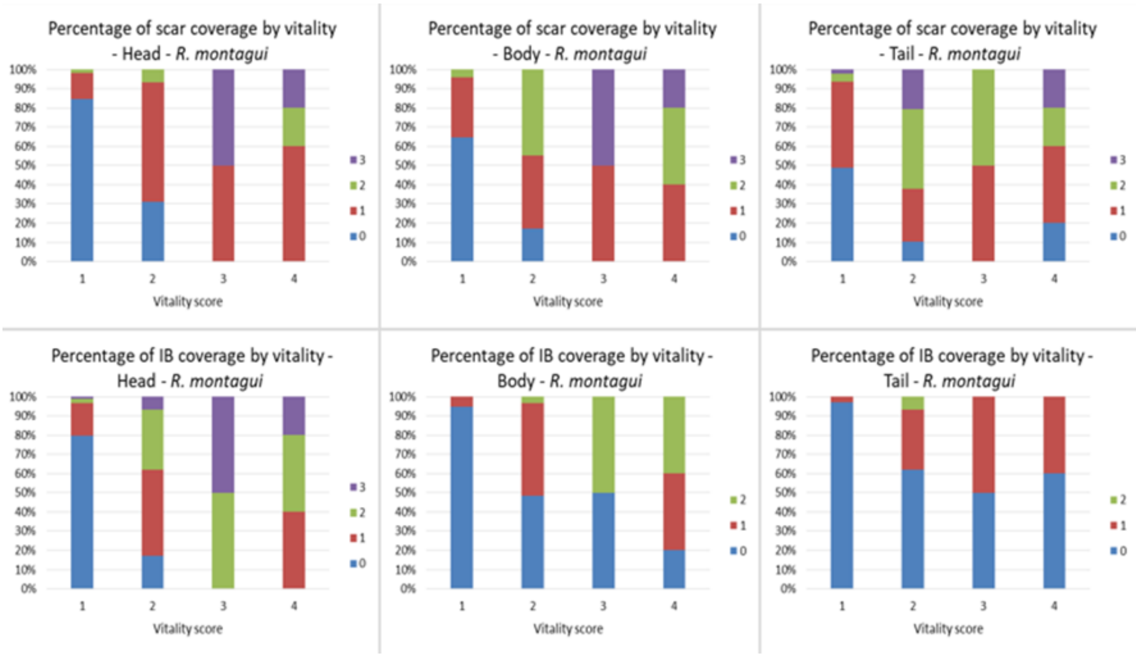

Figure 12 – Percentage of lesion coverage sorted by vitality score or *R. montagui* specimens. (n=13)

In figure 13 it is observable the percentage of RAMP reflexes by vitality score for each species, and in both species the higher the vitality score the less they respond to the RAMP tests positively. Individuals with vitality score 1 always responded positively to all RAMP tests.

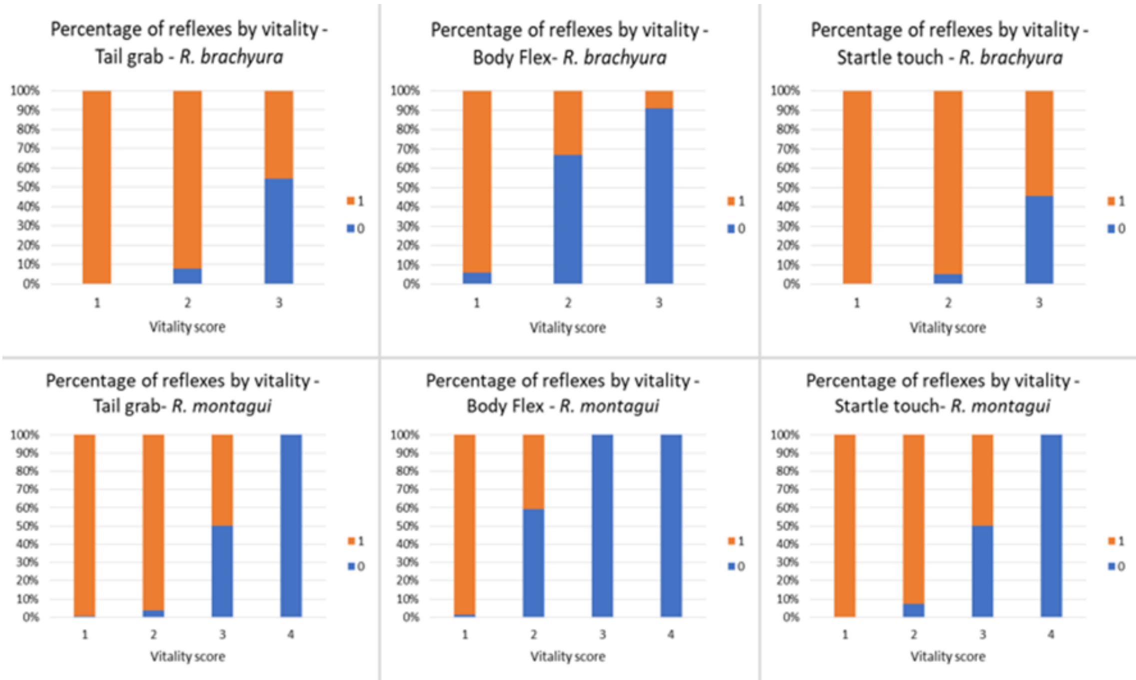

Figure 13 – Percentage of RAMP results sorted by vitality score.

In figure 14 it is observable a Kaplan-Meier model for the *R. montagui* species and with the help of table 6 it is noticed that entered 13 individuals on this experiment and occurred 3 events in the first day, and the survival rate was 77% after the first day in the experiment, after 3 days there were only 10 individuals that hold this long and occurred 2 events, leading to a survival rate of 62%, the last day which events occurred was at the day 6 mark where inly 1 event occurred where there were only 6 individuals at this point, which resulted in a 54% final survival rate. After this point the chart reaches an asymptote line which indicates that the individual's didn´t dyed, but the confidence intervals are wide because of the few individuals observed with more than 3 days.

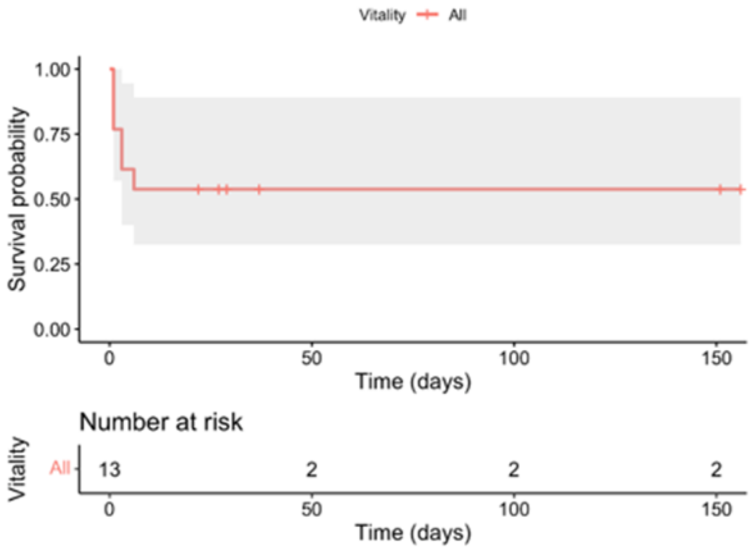

Figure 14 – Kaplan-Meier for *R. montagui* species

Table 6 – Kaplan-Meier results for *R. montagui* survival rate.

Time	n at risk	n of events	P(survival)	std.err	Lower 95% CI	Upper 95% CI
1	13	3	0.769	0.117	0.571	1.000
3	10	2	0.615	0.135	0.400	0.946
6	8	1	0.538	0.138	0.326	0.891

For *R. brachyura* (Figure 15 and table 7) 21 individuals entered on this experiment and occurred only 1 event in the second day, and the survival rate was 95%; after 3 days there were 20 individuals that survived and 4 events occurred, leading to a survival rate of 76%. After this point the chart reaches an asymptote line which indicates that the individual's didn´t dyed, this time the confidence intervals are more robust because there were more individuals to support these results.

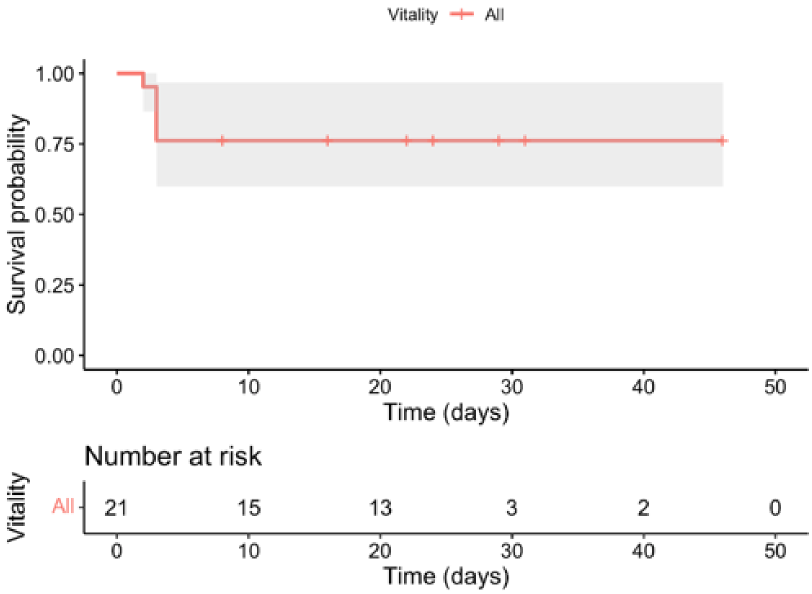

Figure 15 – Kaplan-Meier for *R. brachyura* species.

Table 7 - Kaplan-Meier results for *R. brachyura* survival rate.

Time	n at risk	n of events	P(survival)	std.err	Lower 95% CI	Upper 95% CI
2	21	1	0.952	0.0465	0.866	1.000
3	20	4	0.762	0.0929	0.600	0.968

In figure 16 there 3 vitality scores for the individuals, but the corresponding 1 and 3 vitality scores data died and this data isn´t reliable, for this figure 17 represents only individuals with vitality score 2 without the other scores.

With the help of table 8 we can observe that vitality score 1 and 3 only had 1 individual, which die at day 1 and day 3 respectively. For vitality score 2 it observable that at day 1 11 individuals were present in the experiment where 2 events occurred leading to a survival rate of 81%, at day 3 only 1 event occurred and only 9 individuals were present at the experiment and the survival rate was 73%, finally at day 6 the last individual dyed leading to a 64% survival rate reaching the asymptote level where no more individuals dyed.

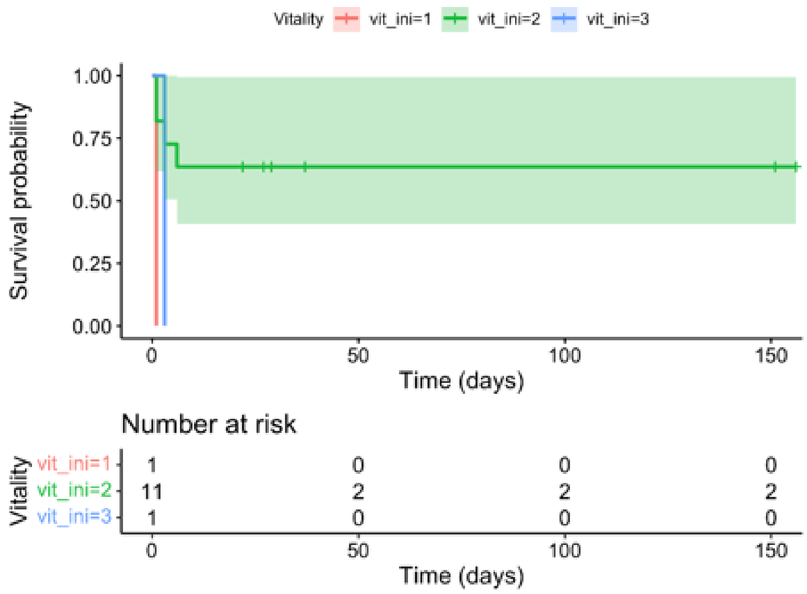

Figure 16 - Kaplan-Meier for *R. montagui* species by vitality score.

Table 8 - Kaplan-Meier results for *R. montagui* survival rate by vitality score.

Vitality score	Time	n at risk	n of events	P(survival)	std.err	Lower 95% CI	Upper 95% CI
1	1	1	1	0	NaN	NA	NA
2	1	11	2	0.818	0.116	0.619	1.000
	3	9	1	0.727	0.134	0.506	1.000
	6	8	1	0.636	0.145	0.407	0.995
3	3	1	1	0	NaN	NA	NA

The *R. brachyura* Kaplan-Meier by vitality score is demonstrated in figure 17, and with the help of table 9 it is observable that only two categories of vitality score were registered, level 2 and level 3, where for level 2 only occurred 1 event at day 3 where 13 individuals where present in the experiment, which lead to a survival rate of 92%, for level 3 individuals the first event occurred at day 2 where 8 individuals where present and the corresponding survival rate is 88%, at day 3 occurred the last 3 events which indicate a 50% survival rate.

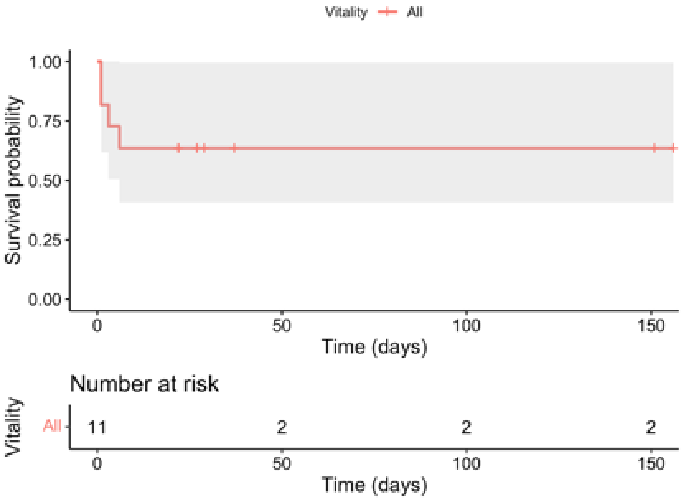

Figure 17 - Kaplan-Meier for *R. montagui* species by vitality score 2.

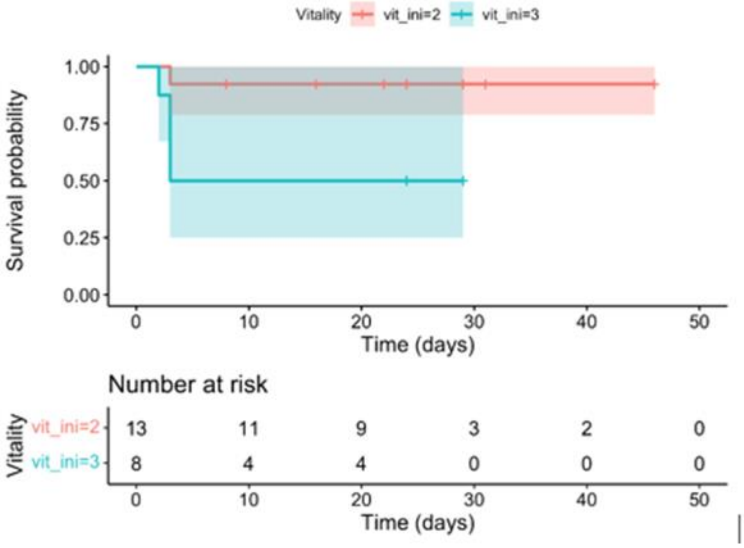

Figure 18 - Kaplan-Meier for R. brachyura species by vitality score.

Table 9 - Kaplan-Meier results for *R. brachyura* survival rate by vitality score.

Vitality score	Time	n at risk	n of events	P(survival)	std.err	Lower 95% CI	Upper 95% CI
2	3	13	1	0.923	0.074	0.789	1.000
3	2	8	1	0.875	0.117	0.673	1.000
	3	7	3	0.500	0.177	0.250	1.000

Discussion/ conclusion

Based on the results obtained in this study, which corroborate other studies (e.g., Eneever et al., 2010, Serra-Pereira & Figueredo, 2019) different species of skates and rays demonstrate different discard survival rates. In order to estimate the survival in captivity for two different species, *R. brachyura* and *R. montagui*, and based on the ICES guidelines for survival studies (ICES, 2021), a non-parametric Kaplan-Meier was fitted to the observations; the estimated survival rate for *R. brachyura* was 76% and for *R. montagui* was 54%, which validates the two first hypothesis (H1 and H2), this way is possible to assume that the discard survival rate of this two species along 3 weeks in captivity may be greater than 50%. Yet, to highlight that the majority of the individuals were only observed after 6 h of their capture when the observer wasn´t on-board, also because of this aspect the individuals weren´t controlled by the observer and were handled by the fisherman, so there is a non-controllable variable that may influenced my results, which one can assume it may be overestimated.

In Serra-pereira & Figueiredo, (2019) study it is revelled that skates and rays tend to be in better conditions when they are captured by polyvalent fisheries, like when trammel nets are used. In the results of this study, the Excellent vitality score that most of the individuals were categorized after being capture, compared with the individuals in my study, may be influenced due to the transportation and accommodation of the animals, most of the times the individuals were only observed after a few hours from the capture and were transported for a long time without ideal conditions, it may reflected lower vitality scores and also lesion coverage as the response to RAMP tests. If this study was conducted on board of a fishing vessel, maybe the results would be similar to Serra-pereira & Figueiredo, (2019) study, so one can assume that the results of vitality score would be around 1 level lower with a corresponding good status for each individual if the transportation conditions were adequate (Serra-pereira & Figueiredo, 2019).

In Serra-pereira & Figueiredo, (2019) study it is referred that the fisherman with influence of the CAPA (Cooperativa dos Armadores de Pesca Artesanal) understand the functionality of this exemption (HSE) and believe in the outcomes, that individuals can recover and reproduce after being captured and their population abundance may grow after some time, it was observed in the fishing trips that the individuals that were unwanted by the fisherman, were always returned back to the ocean, which corroborates the information provided in this study (Serra-pereira & Figueiredo, 2019).

In Serra-pereira & Figueiredo, (2019) study the survival rate of *R. clavata* to trawl corresponded to 64% for species. Besides being a different species and caught by a different gear, the estimates can be considered similar to the ones obtained in this study, with survival rates estimates above 50%. Yet, for *R. montagui* caught by trammel nets, the survival rate is lower (corresponding to 54%) than for *R. clavata* and for *R. brachyura* is higher (76%),

In Figueiredo et al., (2020) study the discard survival for *R. brachyura* is around 67% for beam trawl and 87% for otter trawl, in my study the results correspond for trammel fisheries was 76% survival rate, which is close to both results, also this study was conducted in the North Atlantic Sea, while my study was conducted in the costal line of Peniche, Portugal. In Enever et al. (2009) study revealed that for otter trawls the survival rate of *R. brachyura* was between 55-67% which is bellow my results, confirming the lower impact of trammel nets when compared to trawls.

The *R. montagui* results in Figueiredo et al., (2020) study were fewer and for that reason the results may be underestimated, but the corresponding survival for beam trawlers is around 27%, compared with the 54% survival rate from my study, which is way bellow my results, as their study may be underestimated, my study must be considered also overestimated.

In Catchpole et al., (2018) study, the experiment was conducted on board of a fishing vessel, with the conditions to minimize stress factors, the survival probability of Thornback rays corresponded to 92%. This information corroborates with the idea that the transportation of the individuals may influenced the final vitality results, if the individuals with higher vitality score have lower survival probabilities, then as the transportation lowers the individual vitality status also may influence in their survival probability (Catchpole, 2018).

The Kaplan-Meier models for each species reach to the asymptote level, which indicates that the individuals after a period of at least 21 days may survive in captivity, but this results may be overestimated, because the individuals weren´t returned to the ocean and because of that the predation variable is censured, which may have a big influence in the final results, for that, studies to estimate discard survival must continue in order to tag the individuals and register their survival after being returned to the ocean, only this way it can be assumed if the discards are a good practice or if it is a viable solution for unwanted catches (Figueiredo et al., 2020).

In order to evaluate if vitality score influences the survival rate in both species a Kaplan-Meier model was applied, separated by vitality score. In *R. montagui* species, only the vitality score correspondent to level 2 was considered, due to lack of data from other levels, so the survival rate for level 2 in *R. montagui* was 64% which is greater than the total survival rate of 54%, for the *R. brachyura* the survival rate for level 2 individuals was 92% and for level 3 individuals was 50%, clearly the vitality score in both species had impact, were individuals in levels 2 had higher survival rates than the total of the individual survival rate and level 3 individuals from *R. brachyura* species had lower survival rates that level 2 individuals. This way H3 and H4 are confirmed where in both species the vitality score influenced the survival rates.

It was also observed that the longer the individuals stayed in captivity, their vitality score lowers which means they recover over time, also the RAMP reflexes, respiration and lesion coverage are connected to vitality score, in the figures 11-13, it´s observable that each individual from both species and over time, their vitality status gets better if they don´t die. When the vitality score gets better the individuals in both species respond more to RAMP reflexes, which makes sense, because when an individual appears to be in good state of vitality, it has more chances to respond to stimulus and this can be applied to respiration also, if the individual appears to be in a good vitality status it would demonstrate a respiration close to the mean which is around 15 times per 30 s, like in my study. If an individual appears to have bigger lesion coverage we can assume that it isn´t in a good vitality status, this is observable in figures 11 and 12, that the individuals with higher vitality status had more scar coverage and also internal bleeding coverage, mainly in head internal bleedings and body scars.

All the individuals in this study were captured by polyvalent fisheries that used trammel nets, but there were different conditions in each fishing trip. The fishing condition that had most influence was when the fishing vessels had 125 nets with 260 mm of mesh size and with fishing durations were below 10 h, when this occurred more animals were captured and exhibited worst vitality scores, also most of the individuals on this study were above the MLS stipulated in Portugal.

With this study we can observe that for both this species (*R. montagui* and *R. brachyura*) their discard survival rate is above 50% and the vitality score associated influences this results, where individuals with better vitality scores have more chances to survive after being captured by trammel nets, so it can be assumed that the HSE (High survival exemption) is a viable solution at the moment, never the less it must be mentioned that the transportation of the individuals must be well addressed, and the conditions to minimize stress factors are an important aspect to control while conducting studies like this one. In my opinion the observer has great impact in the decision of the category attributed to each individual for vitality score, in order to minimize error, a good solution would be creating more categories instead of just 4 levels of categories, it could be 5 or 6, this specifies more each category, also if the lesion coverage and RAMP reflex are connected with the vitality of each individual, it would be an improvement if they were associated with the vitality score categories to help the observer to identify more easily the correct category.

Limitations

There were some limitations in this study, the fact we were in a world pandemic (Covid-19) made it more difficult to do this study, for instance this study was designed to be done inside a fishing vessel during the fishing time, at the beginning when nobody was vaccinated it was impossible because of safety measures imposed by the government, only the fisherman were allowed to continue. Another problem with this study was in May when occurred the closing of rays and skates fishing, this is a restriction to prevent overexploitation of the populations of this species and it was a whole month that delayed the assembly of data needed to finish the practical work. Another problem that occurred was the fact that I got seasickness which incapacitated me to do the work on board of the fishing vessel, this problem led to another, the fact I couldn’t be on board let me to only trust and rely on the fisherman to bring to land the studding individuals that I needed, but sometimes the accommodation of the animals was not the desired, sometimes they didn´t catch any of the specimens that I needed and also some of the individuals were not the specie that I requested, all of this adjacent problems had impact sometimes in the results. Because of weather conditions, sometimes the fisherman weren’t able to go to the see because of the danger, this also delayed the collection of data. The water from the research post also had problems that we couldn’t solve during the working time, the TAP water already had residues of nitrates and this may influenced the nitrates and nitrites of the studding tank, all along the working process there were some alterations to see if it would help to solve the problem, like adding a skimmer, adding more bio balls or even adding activated carbon to the filter, but the only significant changes was that the ammonia levels were more stabilized and controlled at the minimum. There was also some problems with the measuring equipment (probes), the oxygen probe had problems with the calibration at the beginning and the pH probe was damaged at the end which disabled the collection of the last day’s data.

For future experiments, it is needed to continue the final stage of discard survival experiments, the tagging and monitoring stage, with vitality assessments and captive observation we can´t measure the predation and survival post releasing the individuals, so it is important to evaluate if besides the individuals represent high survival ratios, if they survive to a release and for how long.

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Annexes

Annex 1 – Table to collect data from the RAMP tests, their lesion coverage and correspondent vitality score.

Data	Hora	Sexo	Espécie	Indivíduo	Tail grab	Respiração (30 s)	Body flex	Startle touch

Cicatrizes			Hemorragias				
Cabeça	Corpo	Cauda	Cabeça	Corpo	Cauda	Estado de vitalidade	Observações

Annex 2 – Table to register the data from the individual after being captured.

Indivíduo	Código_especie	Espécie	Sexo	CT (cm)	Hora de captura	Hora de observação	Duração de observação/transporte no barco (h)	Rejeição	Motivo de rejeição

Mantidos em cativeiro	TTM	Grau de vitalidade	Body Flex	Startle touche

lesão_cabeça	Lesão_disco	Lesão_cauda	Lesão_cloaca	Lesão_clasper	Lesões	Hemorragias	Tanque a bordo	Marca	Observações

Annex 3 – Table to collect information about the fishery that captured the individuals.

Viagem	Data	Observador a bordo	Embarcação	Arte	Rede	Lance	Largada_data	Largada_hora	Tempo de pesca (h) calculado	Numero de redes

Comprimento da caçada ou redes - INDICAR (m)	Hora alagem_inicio	Hora alagem_fim	Velocidade de alagem (milhas)	Tempo_alagem (calculado)	Velocidade_alagem (calculado)

Profundidade	prof_ini (m)	prof_fin (m)	latitude_ini	longitude_ini	latitude_fin	longitude_fin	Local de pesca	temperatura_sup_do_mar

ondulacao (Peniche: lat: 39.37, lon: -9.38)	Vento (Peniche: lat:39.37, lon: -9.38)	Vento_direcçao (Peniche: lat:39.37, lon: -9.38)

Annex 4 – Table used to collect information about the holding tanks.

Tanque	Data	Hora	Ph	Salinidade	Temperatura (ºC)	Cooler (ºC)	Nitritos (mg/L)	Nitratos (mg/L)	Amónia (mg/L)	Oxigénio (mg/L)

Oxigénio (% saturação)	Água adicionada (L)	Sal adicionado (kg)	Trocas de água do taque 2 para1 (%)	Aparelhos calibrados	Observações