HIGHLIGHTS

- This study evaluated the survival rates and physiological recovery after bottom-trawling of two crustaceans, *Nephrops norvegicus* and *Squilla mantis*, in the Gulf of Cadiz (Spain).
- Survival rates depended on the period of the year, being higher in spring than in autumn.
- *S. mantis* seemed to be more resilient than *N. norvegicus* under the circumstances of the study.

Physiological recovery after bottom trawling as a method to manage discards: the case study of *Nephrops norvegicus* and *Squilla mantis*

Abstract

The European Fisheries Policy aims at a progressive elimination of discards. An exception from this regulation includes the release of species with high survival rates after capture. In south-western Atlantic waters of Europe, Norway lobster (*Nephrops norvegicus*) and spottail mantis shrimp (*Squilla mantis*) are amongst the most important crustacean species captured by bottom trawling. We evaluated their short-term survival probability, survival rates and recovery capacities after being trawled by an oceanographic vessel. Seasonal differences were considered by sampling in spring and autumn. In order to characterise the full recovery after capture, physiological responses were also analysed along a time-course of 24 h. Our results confirm that bottom trawling is a stressful process to these crustacean species, as seen by changes in plasma and muscle metabolites, hemocyanin and immune system parameters. However, maintaining captured animals in onboard water tanks evidenced the full physiological recovery of survivors after 6 h and before 24 h. Survival in Norway lobster and spottail mantis shrimp varied according to the season, being higher in spring (68.4 ± 7.1 % and 87.0 ± 4.7 %, respectively) than in autumn (33.8 ± 7.8 % and 63.8 ± 9.3 %, respectively), probably due to the higher temperatures registered after summer months. The employment of the presented techniques for the evaluation of other crustaceans, fishing gears and geographical areas can be contemplated. Fisheries stakeholders might use this approach to better manage discards in Europe.

Physiological recovery after bottom trawling as a method to manage discards: the case study of *Nephrops norvegicus* and *Squilla mantis*

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1. Introduction

The Gulf of Cadiz belongs to the ICES Subdivision IXa-South according to the European Council Regulation (EEC no. 3094/86). It is situated in the south-western Atlantic waters of Europe between the Atlantic Ocean and the Mediterranean Sea. There is an important bottom trawling fleet in this area, with around 140 vessels characterised by a multi-species fishery [1]. Target species include fish like hake (*Merluccius merluccius*), molluscs like octopus (*Octopus vulgaris*) and crustaceans like pawn (*Parapenaeus longirostris)* Norway lobster (*Nephrops norvegicus)* and spottail mantis shrimp (*Squilla mantis*).

Norway lobster (*N. norvegicus*) and spottail mantis shrimp (*S. mantis*) are two of the most important crustacean species caught in this area. Although both species are captured by the same vessels and fishing gears, their bathymetric distribution and preferred seabed characteristics are different. *Nephrops norvegicus* inhabits sandy-muddy bottoms averaging 200–800 m depth [2, 3], while *S. mantis* in the Gulf of Cadiz can be found within 20–165 m depth in silty sands and sandy muds with strong influence of the estuaries [1, 4, 5]. In the last year, the most important local markets registered about 130 t of *N. norvegicus* [6] and 500 t of *S. mantis* (IEO Database). *Nephrops norvegicus* is managed under total allowable catches (TAC) and minimum conservation reference size (MCRS) in the Gulf of Cadiz in accordance to the International Council for the Exploration of the Sea (ICES), being its minimum size of fishing allowed around 20 mm of cephalothorax. *S. mantis* in the same area does not have special management restrictions, with 20–25 mm of cephalothorax as marketable size limit throughout the year.

According to the Article 15 of Regulation 1380/2013/UE, all catches subjected to MCRS or TAC should be landed by 2019. This Article, as part of the new Common Fisheries Policy (CFP), aims at a progressive reduction of fisheries discards. However, the possibility of giving exemptions from the landing obligation is described in Article 13, "Fishermen should be allowed to continue discarding species which, according to the best available scientific advice, have a high survival rate when released into the sea", while

Article 2 states that "The CFP shall implement the ecosystem-based approach to fisheries management so as to ensure that negative impacts of fishing activities on the marine ecosystem are minimised, and shall endeavour to ensure that fisheries activities avoid the degradation of the marine environment". As it is mentioned in this Regulation, scientific advices must be supported by studies conducted for each fishing gear, area and species. Due to the great fishing pressure in the Gulf of Cadiz, the Spanish Institute of Oceanography (IEO) has evaluated demersal stocks in the area since 1993 by means of bi-annual bottom trawling surveys [7]. In 2016, the IEO also implemented methodologies to evaluate survival of discarded species aboard fishing vessels.

The ICES Workshop on Methods for Estimating Discard Survival (WKMEDS) has described some guidelines for designing and conducting discard survival studies, such as vitality assessment, captive observation and tagging and biotelemetry. It is clear that there are many factors influencing survival rates including environmental temperature, fishing gear, geographic area, season of the year, age, size, etc [8, 9]. Previous studies on *N. norvegicus* include the evaluation of their vitality on-board commercial vessels [8] as has been advised by ICES WKMEDS, and maintaining captures in tanks for weeks at land facilities [10]. However, captivity may introduce some potential limitations with respect to survival estimates, because physiological recovery and further stress responses are barely considered in these studies [9, 11, 12]. Moreover, short-term (less than 24 h after the challenge) and long-term (more than 24 h) acclimation processes also induce differentiated physiological mechanisms in the animals that may be of great importance when evaluating their survival. Thus, a sharp description of the processes affecting captured animals, and the induced physiological stress responses should be mandatory for a proper evaluation of the survival rates after capture in fishing conditions.

Fishing is a stressful and dramatic process that may affect the survival of invertebrates and vertebrates [8, 13, 14, 15]. This stressful process in animals can be divided into different responses. The primary stress responses include the release of hormones into the blood-stream [16], including the hyperglycaemic hormone in crustaceans [17, 18]. These hormones induce secondary responses such as changes in the cardiorespiratory system and mobilisation of energy metabolites into the blood like glucose [19], which may lead

to an increased anaerobic metabolism, showing plasma lactate as a benchmark biomarker of this type of stress. Secondary stress responses also include hydric imbalances [14], and changes to the immune system like peroxidase and lysozyme activities [20, 21], and in the oxygen-transporter hemocyanin of some invertebrates [22, 23]. After an acute-stress situation, allostatic changes lead to basal homeostatic levels [24], allowing the animal to physiologically recover and maintain its internal balance for long periods [25]. However, if the stressful situation lengthens in time it can lead to metabolic exhaustion, depression of the immune system, and eventually death of the animal [26]. Thus, evaluating the physiological recovery capacity after bottom trawling should be mandatory to describe whether or not captured crustaceans may survive if released into the ocean.

The main goal of this study was to evaluate short-term survival probability of two commercially relevant crustacean species (*N. norvegicus* and *S. mantis*) in the Gulf of Cadiz (SW Europe) captured by bottom trawling on-board an oceanographic vessel. Furthermore, the effect of air exposure on their survival was also evaluated. As a secondary objective, this study aimed at evaluating the physiological recovery capacity after trawling, unravelling if surviving crustaceans are irreversibly damaged or if they fully recover from the catch-and-handling processes. Finally, the season of the year was also evaluated as a factor influencing survival and physiological recovery. The results obtained herein may assist in identifying survival methodologies that include physiological recovery of captures as a new approach to improve fisheries and ecosystem management.

2. Material and methods

2.1 Geographic location, vessel and tows characteristics

In order to carry out the experiment under controlled and bounded conditions, animals were captured during three different bottom trawling surveys off the Gulf of Cadiz (south-western waters of Europe, Spain) aboard the O/V "Miguel Oliver" (length: 70 m; engine power: 2 × 1000 kw) in March (spring) of 2017 and 2018 and in November (autumn) of 2017. The weather conditions were optimal for trawling in all hauls.

Figure 1 here.

Fishing procedures followed international standards [7]. A BAKA 44/60 type bottom trawl was used in each survey. The sampling gear was towed at three knots for one hour along a specific isobath for each haul by two warps. MARPOL sensors were placed on the otter boards to record the horizontal opening of the otter boards, in the footrope to record the horizontal opening of the fishing gear and on the headline rope to record the vertical opening of the fishing gear. This system was used to measure the contact and removal of the net at the bottom, as well as its horizontal and vertical openings (on average, 20.8 m and 1.9 m, respectively). The starting and ending position of each fishing manoeuvre was controlled through a global position system (GPS).

2.2 Experimental setup and animal maintenance

Animals from each haul were randomly selected for all the experiments, 'i.e.' animals were collected from different sections of the capture and pooled, removing putative effects due to time of capture within each trawl. The condition of the animals was only taken into account by the presence or absence of cheliped. Some animals were sampled after capture (time 0 h) while others were individually introduced into a portable system [27]. This system consisted of 30 independent aquariums, painted in black with an upper light entrance, of 5 L each with a flow- through system. The system also had a charcoal filter and a protein skimmer to remove possible contaminants and/or dissolved nitrogenous molecules. Seawater was collected from the sea surface during navigation, and the experimental animal were subjected to environmental changes of the sea. The number and volume of the aquariums, as well as the filtering system, may be modified to accommodate different aquatic organisms. It has been successfully tested for crustaceans, cephalopods, teleosts, small elasmobranches and algae. A temperature controlling device and LED-lights (light emitting diodes) can be included to better control environmental conditions in the tanks. Animals fasted during the experiment. Besides, the present study was conducted in the absence of control groups of animals that had not undergone the catch process. All survival study procedures were based in the recovery capacity on survivors within the first 24 hours. So that, animals that manage to survive and recover completely will be more likely to survive once they are released into the sea [27].

2.3 Short-term discard survival

The sampled individuals were introduced into the tanks after 60 minutes of air exposure. Following ICES WKMEDS advice [6], survival was evaluated every hour along a time-course of 24 h. Animals were observed inside their tanks (through a transparent window in the cover) to assess breathing, swimming and cheliped and pereiopod movements. Death was confirmed in those animals evidencing a lack of breathing and movement responses after stimulating them by a gentle touch with a stick inside the tanks.

2.4 Effect of air exposure and season on survival rates

In less than three minutes after the cod-end of the net was exposed to air, captures were transferred to a lower fishing deck, maintained in a low-irradiance and humid environment, and exposed to air for 15, 30, 60 or 100 min. These conditions were considered the most benign that these species can experience after trawling by a fishing vessel [28]. After air exposure, the crustaceans were introduced into individual recovery aquariums for 24 h. Survival rates were evaluated at the end of this period.

Considering the temperature effects observed in survival rates from previous studies in both species [25, 29], *N. norvegicus* and *S. mantis* were captured by bottom trawling and sampled in the Gulf of Cadiz. Thus, both species were collected in spring and autumn. After the triage process on the lower fishing deck (60 min air exposure [29]), crustaceans were transferred into the aquariums, and 24 h later the survival rates were evaluated.

2.5 Physiological time-course recovery

The physiological recovery capacity of *N. norvegicus* and *S. mantis* was evaluated after bottom trawling in spring 2017. The sampled animals were randomly introduced into the recovery aquariums. Samples were taken at times 0 h (60 min after air exposure and triage process, maintained outside water in the lower fishing deck, with high environmental humidity, no sun radiation, low room irradiance and temperatures below 23 ºC), 1, 3, 6 and 24 h in order to establish a time-course of their physiological recovery process. These times were selected as previous studies on other crustaceans and other invertebrates

indicated that physiological recovery after an acute stressor, such as being captured by bottom trawling, occurs within the first 6 hours [25, 27, 30].

2.6 Physiological recovery due to season

Two surveys were carried out to describe the seasonal effect (spring and autumn) on physiological recovery. The animals were maintained in the same on-board aquariums. Haemolymph and muscle samples were taken at times 0 h (just before introducing the animals into the aquariums) and 24 h later (samples taken only from survivors).

2.7 Physiological sampling procedures

To analyse the physiological recovery capacity of crustaceans, haemolymph and muscle were sampled for later analyses in the laboratory. Animals were collected by hand and their cephalothorax covered with a wet tissue in order to minimize their stress reactions. Haemolymph samples were taken with sterile syringes (circa 300 μL) in the ventral sinus of the thoracic somites, followed by cephalothorax severing with a sharp knife in order to confirm euthanasia. Samples were taken quickly to avoid an impact of handling stress on haemolymph variables [25, 31]. Plasma was obtained after centrifugation (10 000 *g*, 4 minutes) and frozen at -20 ºC. Muscle samples were collected from the abdomen and frozen at -20 ºC. All the procedures were conducted in less than 1 minute per animal. Because animals were maintained in individual aquariums, with darkened walls, sampling did not affect those animals still in the aquariums (no possible disturbances due to noise, visual contact or chemical distress).

2.8 Plasma and muscle variables

The reasons for selecting the parameters measured in this study to evaluate physiological recovery will be explained below. Plasma lactate increases due to long air exposure periods experimented in trawling, along with the high oxygen demand produced by intense physical exercise (trying to scape from the net) [12, 25]. In this sense, hemocyanin (Hc) is the oxygen transport protein, being a good stress indicator in these situations [27]. Besides, plasma glucose increases to meet the demand for extra energy required after fishing [12, 27]. Otherwise, the activation of the immune system was produced in a stress

situation such as trawling. Thereby, Hc is involved in the innate defence mechanism, peroxidase have antimicrobial activity and lysozyme activity seems to act non-specifically against a wide range of invaders [27]. Therefore, these parameters have been measured to assess the physiological recovery capacity of animal captured by trawling.

Plasma glucose (as the primary energy metabolite in these species) and lactate levels (as a representative secondary stress biomarker in crustaceans), were measured using commercial kits from Spinreact (St. Esteve de Bas, Girona, Spain) adapted for 96-well microplates. Plasma hemocyanin concentration (related to oxygen transport but also to the immune system) was measured spectrophotometry as previously described for *N. norvegicus* and other invertebrates [32, 33].

Peroxidase activity (another immune-related parameter) was measured as described [34], with some modifications: 15 µL plasma in duplicate were diluted in 135 µL of HBSS without Ca^{2+}/Mg^{2+} (H6648, Sigma-Aldrich) in a flat-bottomed 96-well plate, followed by the addition of 50 µL 10 mM TMB (T8768, Sigma-Aldrich) and 50 µL 5 mM H_2O_2. After 2 min the reaction was stopped with 50 µL 2 M H_2SO_4. Blank was done with 150 µL HBSS. Optical density was read at 450 nm. Peroxidase activity (U mL^{-1}) was determined defining 1 unit as that which produces an absorbance change of 1 OD. Plasma lysozyme activity (also related to the immune system) was measured as described [35]: 20 µL of sample and 180 µL of a solution of *Micrococcus lysodeikticus* (N3770, Sigma-Aldrich; 0.2 mg mL^{-1}, 0.04 M sodium phosphate buffer, pH 6.2) were added into a 96-well microplate. Blanks for each sample were done with 20 µL of the sample and 180 µL of sodium phosphate buffer. Reaction proceeds for 20 minutes at 37 ºC and afterwards absorbance was measured at 450 nm. A standard curve was done with lyophilized hen egg white lysozyme (L6876, Sigma-Aldrich) serially diluted in Na_2HPO_4 buffer.

Frozen muscle was finely minced on an ice-cooled Petri dish and homogenized and neutralised by ultrasonic disruption in 7.5 volumes ice-cold 0.6 N perchloric acid, neutralized using 1 M potassium bicarbonate, centrifuged (30 min, 3220 *g* and 4 ºC), and the supernatant used to determine tissue metabolites. Muscle lactate levels were determined spectrophotometrically with Spinreact kits Muscle glycogen concentration was assessed with aminoglucosidase as described [36]. Glucose obtained after glycogen

breakdown (after subtraction of free glucose levels) was determined with a commercial kit (Spinreact, see before). All assays were performed using a PowerWave™ 340 microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT, USA) and KCjunior™ data analysis software for Microsoft®. Muscle water content was analyzed by dehydrating pre-weighted muscle at 65 °C until achieving constant weight (around 48 hours). The percentage of water was calculated as the difference in weight between the fresh and the dry muscle divided by the fresh weight [37].

2.9 Data analysis

The survival probability across time (0, 1, 3, 6 and 24 hours) for each studied species was graphically assessed using Kaplan-Meier curves [38]. In order to evaluate the survival rates in spring and autumn, we considered the factor "Season" (fixed, with two levels. "spring" and "autumn"), while "Haul" was included as a random factor. In addition, to test for significant differences in the survival of the species studied in function of time of air exposure, we considered the factor "Time" (fixed, with four levels: "15", "30", "60" and "100" minutes) and "Hauls" as random factor. We used generalized linear mixed models (GLMM's) under the models explained above specifying a binomial distribution.

To explore the physiological recovery capacity of the studied species in spring 2017, we considered the different plasma and muscle variables as response variables and the factor "Time" (fixed, with five levels: "0", "1", "3", "6" and "24" hours). "Haul" was included in the design as a random factor. For the analysis of the differences in physiological variables after recovery between seasons, we considered the different plasma and muscle variables as response variables, and two fixed factors: "Season" (two levels: "spring" and "autumn") and "Time" (two levels: "0" and "24" hours). "Hauls" was included in the design as a random factor. Linear mixed effects (lme) models were fitted using the package 'nlme' (R package version 3.1-128) [40] until we selected the best model based on akaike information criterion (AIC) and variable significance. The normality and homogeneity of the residuals were checked with a Shapiro-Wilk and Bartlett's tests respectively. All data were analysed using the statistical program R version 3.3.3 [39].

3. Results

3.1 Samples characteristics

Three, five and seven hauls were carried out in spring 2017 and 2018 and autumn 2017, respectively in the case of *N. norvegicus*. *S. mantis* were captured in eight, seven and six hauls in spring 2017, spring 2018 and autumn 2017, respectively (Table 1). In the survey performed in spring 2017, Kaplan-Meier curves were carried out in order to study short-term survival probability in *N. norvegicus* and *S. mantis* (n = 36 and n = 95, respectively). In the same survey, 28 and 85 individuals of *N. norvegicus* and *S. mantis*, respectively were sampled for the evaluation of the physiological recovery time-course (Table 1). However, in spring 2018 and autumn 2017 surveys, air exposure challenge and the effect of the season on survival rates were carried out (n = 42 in spring and n = 144 in autumn) in *N. norvegicus* and (n = 37 in spring and n = 68 in autumn) in *S. mantis*. Recovery capacity experiments were performed for both species and seasons (n = 27 and n = 83 for *N. norvegicus* in spring and autumn, respectively; and n = 30 and n = 50 for *S. mantis* in spring and autumn, respectively). It is important to highlighter that 24 recover hours after trawling, 15 and 38 animal survivors were employees in the seasonal recovery study (against 15 and 62 who failed to survive) in spring and autumn, respectively in the case of *N. norvegicus*. Otherwise, in the *S. mantis*, 22 and 23 survivors were sampled (versus 7 and 16 animals that were unable to recover, causing the death of the individuals) in spring and autumn, respectively.

Moreover, Table 1 shows carapace length (mm), depth (m), bottom and tanks temperatures (°C) and bottom and tanks salinity. In this sense, *N. norvegicus* was capture in deeper waters than *S. mantis* (Table 1). Bottom water temperatures were higher in the autumn than spring for *S. mantis*, but without differences for *N. norvegicus*. However, tanks temperatures were higher than at the sea bottom in both species. By last, salinity was constant over the seasons and years, and similar between the sea bottom and on-board tanks.

Table 1 here.

3.2 Short-term survival

S. mantis presented higher survival probability that *N. norvegicus* under these conditions. The survival probability for *N. norvegicus* (n=36) ranged between 56.2 ± 0.1 % and 96.4 ± 0.0 % (mean ± SEM) and between 78.5 ± 0.1 % and 94.8 ± 0.0 % in the case of *S. mantis* (n=95) (Figure 2). Kaplan-Meier curves showed that an asymptote was obtained 6 hours after bottom trawling for both species.

Figure 2 here.

3.3 Effect of air exposure and seasons on survival rates

Nephrops norvegicus and *Squilla mantis* did not show different survival rates when exposed to air (fishing deck conditions, including no sun irradiance, no wind and a humid environment) for 15, 30, 60 or 100 minutes ($p > 0.05$, GLMM).

Further analyses were conducted by doing the average of all fishing sets within the same season of the year. Thus, survival rates in spring were 68.4. ± 7.1% and 87.0 ± 4.7% for *N. norvegicus* and *S. mantis*, respectively; and 33.8 ± 7.8% and 63.8 ± 9.3% for *N. norvegicus* and *S. mantis*, respectively in autumn (mean ± SEM). Both species had significantly higher survival in spring ($p < 0.05$, GLMM) (Table S1).

3.4 Physiological time-course recovery

Plasma lactate showed the highest concentrations in both species just after bottom trawling (Figure 3). These levels decreased gradually after 3 h recovery, reaching the lowest concentrations between 6 h and 24 h after being introduced into water recovery-tanks ($p < 0.001$, LMM) (Table S2 and Table S3). Final plasma lactate concentrations 24 h after recovery were 0.10 ± 0.05 mM and 0.20 ± 0.06 mM for *N. norvegicus* and *S. mantis*, respectively.

Figure 3 here.

No differences were found for plasma glucose in *N. norvegicus* for any of the recovery time tested (Table 2). However, these plasma glucose levels increased during the first hours after capture in *S. mantis* (Table 3). Plasma hemocyanin increased in both species 2–3 h after recovery, maintaining its levels without significant differences till the end of the experiment (24 h). Peroxidase activity increased its plasma levels 3 h after recovery in *N. norvegicus* and *S. mantis*, with no significant differences between the groups sampled at 3, 6 and 24 h. However, plasma lysozyme did not show any variation for neither species (Table 2 and Table 3).

Table 2 and Table 3 here.

Metabolites such as lactate in muscle increased significantly ($p < 0.001$) (Table S2 and Table S3) during the first hours after the stress, and reached the lowest concentrations 24 h after recovery in both species (Table 2 and Table 3). Muscle glucose concentration was low during the first 6 h after capture in *N. norvegicus* but increasing significantly ($p < 0.001$) at the end of the experiment (Table 2). *S. mantis* presented a muscle glucose concentration increase 1 hour after capture, to a level that remained stable' 24 hours later (Table 3). Glycogen, as an important storage of carbohydrates in crustaceans, did not show any differences between recovery times or species in this study (Table 2 and 3). The muscle water content was maintained around 78% in *N. norvegicus* along the experiment, with no significant differences between groups. However, muscle water content reached values circa 85% in *S. mantis* during the first 3 h post-stress, decreasing to circa 83% after 6 h recovery (Table 3).

According to the obtained results, where no differences in most of the analysed parameters were described between times 6 and 24 h, we considered that survivors of *N. norvegicus* and *S. mantis* captured by bottom trawling in the Gulf of Cadiz (Spain) were physiologically recovered 24 h after capture. Thus, further experiments were conducted including sampling just at times 0 and 24 h after capture.

3.5 Physiological recovery in spring and autumn

The highest plasma lactate concentrations were observed in all crustaceans sampled just before the recovery process in water tanks (time 0 h), with a 10-fold decrease 24 h later (Table 4 and5). Significant differences were found between spring and autumn, with lowest concentrations in spring for *S. mantis* (Table S5).

Plasma glucose presented significant differences between 0 and 24 h in *N. norvegicus* in both seasons, with highest concentrations at time 0 h. In *N. norvegicus*, samples showed significant differences between spring and autumn in plasma glucose, with highest levels in spring (Table 4). However, *S. mantis* did not present differences in plasma glucose between 0 and 24 h in any season, but significantly ($p < 0.001$) higher concentrations were found in animals sampled in spring (Table S5). Hemocyanin showed a similar trend for both species and seasons, with a significantly ($p < 0.001$) higher concentration 24 h after recovery than just after capture (0 h), and highest concentrations in spring (Table 4 and 5) Plasma peroxidase activity increased significantly ($p = 0.003$) 24 h after recovery in the case of *N. norvegicus* , with no significant differences due to season in any species (Table 4 and Table 5) . Plasma lysozyme showed significantly ($p < 0.001$) differences between season in *N. norvegicus*, with the highest level in autumn (Table 4). However, in *S. mantis* statistically differences were observed due to time, being lowest 24 h after recovery (Table 5).

Table 4 and Table 5 here.

Muscle lactate (Table S4 and Table S5) values were significantly ($p < 0.001$) higher just after bottom trawling in both species and seasons (Table 4 and 5). Differences between spring and autumn were observed, with higher muscle lactate in spring for both species. Muscle glucose levels only presented significant differences in *N. norvegicus*, with the highest values 24 h after recovery, and no differences were described between seasons. No differences were described in muscle glucose for *S. mantis* between any sampling

point or season (Table 4 and 5). Statistically differences were described in muscle glycogen concentration in *N. norvegicus* during the experimental time, being lower just after bottom trawling (0 h). However, no differences were described in the case of *S. mantis* (Table 5). Finally, no significant differences were described in muscle water content of *N. norvegicus* in any season, being 78.1 ± 0.5% at time 0 h and 77.5 ± 0.4% at time 24 h in spring, and 79.1 ± 2.4% and 78.6 ± 0.3% (at times 0 and 24 h, respectively) in autumn (mean ± SEM). However, *S. mantis* presented changes in muscle water content, being higher just after bottom trawling with an average of 82.6 ± 0.9% and 80.2 ± 0.3%, (0 and 24 h, respectively) in spring and 83.5 ± 0.6% and 81.2 ± 0.4% (0 and 24 h, respectively) in autumn. No differences between muscle water content were described in any species or seasons (Table 4 and 5).

4. Discussion

The present study describes some of the physiological responses experienced by *N. norvegicus* and *S. mantis* after bottom trawling in the Gulf of Cadiz (SW Spain) as a method to calculate survival rates and recovery capacity. Moreover, the main results highlighted that if maintained in proper environmental conditions, survivors are completely recovered in less than 24 h. Thus, survival rates after bottom trawling were evaluated in spring and autumn, being circa 68% and 33% for *N. norvegicus* (respectively) and 86% and 63% for *S. mantis* (respectively). Higher survival rates were found in spring, probably associated with lower water temperatures. These survival results could be considered as valid conclusions due to the high recovery capacity presented by all survivors. However, owing to the absence of controls, some initial experiments should be carried out in the future, in order to ascertain survival rates that are inclusive of method effects, such as water temperature. Thus, the results of the present study should be taken with caution since these survival rates are likely to decrease improving on-board tanks conditions.

4.1 Short-term survival

The Kaplan-Meier curves of crustaceans captured by bottom trawling in spring 2017 showed a horizontal survival asymptote was reached after 6 h recovery. This outcome

suggests that 24 h was time enough to evaluate short-term survival on-board the vessel as has been described before in other species [41, 42]. Studies with longer evaluation periods may lead to a long-term acclimation processes, including reassignment of the physiological machinery, thus compromising survival of the animals due to the processes not related to fishing capture alone.

4.2 Survival rates by season

The survival rates observed in the present study are in accordance with those obtained by other authors in other geographical areas for captured *N. norvegicus* [8, 10, 25, 30, 43, 44] and *S. mantis* [29, 45]. Under this framework, the methodologies employed in this study may be consider as valid, while the time required for the assessment of these results is clearly diminished to just 24 h (or even 6 h, according to the Kaplan-Meier curves obtained). Thus, this study may reduce the effort directed towards the evaluation of the survival rates of captured crustaceans.

With the experimental approach described in this study, a higher survival rate occurs in *N. norvegicus* and *S. mantis* during spring when compared to autumn. For *N. norvegicus* the sea bottom temperature did not change between seasons (probably because Norway lobster were captured in deeper waters around 440-494 m). However, high surface water temperatures were registered in autumn, 5 °C higher than in spring, due to water heating during the summer months [25, 46, 47]. Otherwise, the difference between the bottom and tank temperature in autumn 2017 was as much as 8°C. Thus, the tank conditions during recovery may have caused the decreased survival together with a rapid transition between temperatures [12]. Survival rates are 20% higher in *S. mantis* at each season compared to *N. norvegicus*, which may indicate an uppermost resilience of this species to environmental fluctuations [29, 45]. Nevertheless, for *S. mantis* both the bottom and tank temperatures increased between spring and autumn in addition to higher temperatures in the tanks compared with the bottom (Table 1). Thus, both season and tank conditions may have contributed to the increased survival in the autumn, being able to underestimate the survival capacity of this specie. By last, the aquarium system has been improved so that the tank temperature can be controlled and match the bottom temperature in future studies (patent ES2712348).

4.3 Physiological recovery capacity after bottom trawling

To the best of our knowledge, physiological recovery after capture has not been used before as a method to evaluate survival capacity in crustaceans within the first 24 recovery hours after trawling, maintained in individual recovery aquariums (ES2712348). To date, the time required to induce mortality (TTM) is a simple metric that can be used to study landing exemption candidates [48]. In this sense, animals recovering their homeostasis after a capture process indicate a proper physiological recovery and highlight a high survival capacity. However, the lack of a physiological recovery capacity in animals captured by fishing may show an overwhelming situation where the metabolic limits are exceeded, leading to a delayed mortality.

In this study, plasma metabolites, such as lactate and glucose, evidenced an acute stress response after bottom trawling. This stress is given not only by the obvious physical damage due to trawling (blows due to confinement and net trawling, leading to broken appendages or carapaces), but also by hypoxia due to air exposure and confinement [30]. Lactate increases have been previously associated to air exposure, indicating the occurrence of anaerobic processes leading to the inefficient combustion of carbohydrates [49, 50]. Our results confirmed an increase in plasma lactate during the first hours after capture/air exposure, as previously described in *N. norvegicus* [25] and *S. mantis* [29, 45]. Once introduced into recovery tanks, these crustaceans maintained high lactate concentrations during the first 3 h, followed by a sharp decrease in this metabolite, and reaching the lowest levels 6 to 24 h after recovery coinciding with previous studies [29, 30]. The lactate concentrations shown herein after 24 h corresponded to previously reported basal homeostatic levels of unstressed *N. norvegicus* [25] and *S. mantis* [29], confirming complete recovery of these crustaceans in less than 24 h after bottom trawling.

The plasma lactate recovery time-course profile was paralleled by the lactate levels in muscle. Muscle, as a highly energy demanding tissue in crustaceans, is fatigued after exercise [51], and lactic acidosis occurred due to anaerobic glycolysis. In this sense, glucose is the main energy metabolite mobilized as a secondary stress response in

different crustacean species [52, 53, 54]. In the present study, after a significant increase in plasma glucose, indicating the stressful situation induced by bottom trawling, *N. norvegicus* and *S. mantis* managed to return to basal unstressed levels in plasma and muscle after 24 h recovery. Another described consequence of stressful processes is muscle hydric imbalances [55]. In this sense, *S. mantis* showed muscle overhydration after bottom trawling, reaching lower and stable levels a few hours after bottom trawling. In summary, these results agree with those previously described for both species [25, 29, 30, 43], and suggested that these species managed to completely recover their physiological homeostasis within the first 24 h after capture.

Air exposure/hypoxia also affected plasma hemocyanin, the main oxygen transport protein in crustacean species [25]. The results obtained herein showed lower hemocyanin concentrations just after bottom trawling in both species and an increase after 3 h in *N. norvegicus* and 1 h in *S. mantis*, maintaining high and constant levels till the end of the experiment (24 h). These differences in the recovery time between the species could be related to the intensity of the stress experienced by *N. norvegicus* and *S. mantis* animals during bottom trawling. It has been described in laboratory experiments with *N. norvegicus* that the intensity of environmental hypoxia greatly affected the increase of hemocyanin after the challenge [22, 23]. Considering these studies, we postulate that *S. mantis* managed to recover earlier than *N. norvegicus* after bottom trawling in the Gulf of Cadiz. The reasons for the faster recovery time in *S. mantis* could be due to a less intense stress situation experienced by this species due to the less depth of capture, or by a higher resilience of it, recovering basal hemocyanin levels in less than 1 h after bottom trawling.

It was demonstrated that stress situations induced alterations of immune parameters in crustaceans [20, 21]. In this sense hemocyanin is also involved in the immune system, as some studies described its activity as a phenoloxidase enzyme after proteolysis activation [43, 56, 57, 58], as well as in defence mechanisms such as coagulation processes [59]. We thus postulate that the lowest hemocyanin concentrations described just after trawling could be due to the conversion of hemocyanin into phenoloxidase as part of the immune response. Alternately, peroxidase is produced in response to oxidative stress situations in crustaceans [60], being the peroxidase activity usually related to phagocytosis processes

[20, 57]. The present study showed an increase in its activity 3 h after a bottom trawling process, followed by species-specific differences 24 h after recovery. Thus, the capture processes induced oxidative stress damage that could be counteracted by peroxidase activity as it was observed in our study. However, plasma lysozyme activity which is related to bacterial recognition [61], did not show any variations in the present study. These suppositions point to the specificity of the damage caused by trawling and the activated immune responses, mainly aimed at coping with hypoxia process and eliminating free radicals produced by oxidative stress during the first hours after capture.

We aimed at describing whether survivors were able to be physiologically recovered, and thus reinforce the idea that released crustaceans after capture were not chronically affected by this process or have been metabolically exhausted, limiting their options to survive in the wild. In this study we have demonstrated that 24 h is time enough to recover the physiological homeostasis of unstressed crustaceans after bottom trawling as a tool to study survival rates. In all cases, survivors returned to basal homeostatic levels within 4 to 6 h, restoring this complete homeostasis in less than 24 h, as it has been described in fishes [62, 63, 64, 65, 66]. From 24 hours onwards, animals enter into a process of acclimatization to the new state, which implies the mobilization of energy resources and endocrine imbalance at the systemic level. This acclimation capacity of captivity, at least in fishes, requires no less than 7 days [67, 68, 69, 70]. This study present similar concentrations of metabolites such as lactate and glucose that those obtained in previous studies carried out with animals acclimated to captivity during several days. However, these concentrations showed differences due to captivity conditions [25, 29].

As described by the ICES WKMEDS, previous recovery studies have been conducted in onshore holding facilities maintaining captured crustaceans for weeks, and even months [8, 9, 25, 29, 30, 45]. This approach should be cautiously addressed as it may better describe the acclimation capacity to captivity of these species as has been described before in other animals such as fish [71] and crustaceans [72]. In this study we have demonstrated that 24 h is time enough to recover the physiological homeostasis of unstressed crustaceans after bottom trawling. Studies including longer periods may be useful to evaluate other putative physiological modifications, such as changes in

reproductive behaviour, as has been described in teleost fish [73], but inefficient to evaluate the physiological recovery produced by an acute short-term stress process.

4.4 Physiological recovery depending on season

Previous studies stated that differences in basal concentrations of some physiological parameters between seasons (as those described in the present study such as plasma glucose) did not influence the recovery ability of these species [25]. However, this study described differences between energy metabolites basal levels (after 24 h recovery) for both species, being higher in spring, coinciding with higher survival rates. Stored carbohydrates in crustaceans like *N. norvegicus*, could be higher in winter and spring than in summer-autumn [74]. We thus hypothesize that lower energy metabolites stores in autumn may lead to weaker crustaceans with limited energy resources to face highly demanding processes such as trawling. Otherwise, higher temperatures in autumn could lead to faster depletion of the metabolic stores. It has been described in other lobsters that animals without reserves could compromise their physiological condition [75]. It should be mentioned that carbohydrate stores (as seen by muscle glycogen) in *S. mantis* is 10-times higher than those in *N. norvegicus*, reinforcing our hypothesis. Altogether, *N. norvegicus* and *S. mantis* captured in the Gulf of Cadiz by bottom trawling in spring and autumn, if manage to survive for 24 h, are physiologically recovered from the stress process induced by fishing.

This study offers experimental results obtained under oceanographic vessel´s conditions, which may differ from those of commercial fisheries [7]. Thus, differentiated operational and environmental conditions between both fishing practices may affect the survival and recovery ability of these species. Some of these variables include (amongst others): i) haul duration and trawling speed are higher in commercial vessels [8, 10, 18, 25]; and ii) mesh size and geometry, catch size and time of the day for trawling [7, 25]. Due to the multitude of factors that can affect the survival of these species, it results additional work should be done on-board commercial vessels in order to assert the specific effect of each putative condition on crustacean survival and recovery capacity.

All these results lead to discuss, under the compulsory European Union landing regulation, if these species captured by bottom trawling in the Gulf of Cadiz can be discarded or not. Two conclusions are derived from the present work: i) the evaluation of the physiological recovery is a useful method to assert if surviving crustaceans are irreversibly damaged or are in full physiological conditions to cope with their release back into the wild; and ii) as already known, survival rates depend at least on the geographical area, fishing process and captured species. The methodologies described in the present study will be useful to evaluate survival rates in captured crustaceans, as well as other taxa, minimizing the times required according to what has been described so far.

5. Conclusions

Survival rates of *N. norvegicus* and *S. mantis* are higher in spring 68.3 ± 7.1% and 86.0 ± 4.7%, respectively) (mean ± SEM) than in autumn (33.8 ± 7.8% and 63.8 ± 9.3%, respectively). Survivors of these species managed to completely recover their physiological homeostasis within the first 24 h after capture. This conclusion allows us to shorten the current times of survival evaluation, which required the observation in captivity of captured animals for long periods, and are more focused on the ability to acclimate to captivity rather than evaluating their recovery after capture. However, more studies are necessary to ascribe the specific effect of all putative factors affecting survival and recovery of captured crustaceans in this area after bottom trawling. Furthermore, the implementation of these studies in the commercial fleet results mandatory to evaluate real survival rates under commercial vessel conditions.

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References

[1] Y. Vila, I. Sobrino, M.P. Jiménez, Fishery and life history of spot-tail mantis shrimp, *Squilla mantis* (Crustacea: Stomatopoda), in the Gulf of Cadiz (eastern central Atlantic), Sci Mar 77 (2013) 137–148.

[2] F. Maynou, F. Sardá, *Nephrops norvegicus* population and morphometrical characteristic in relation to substrate heterogeneity. Fish Res, 30 (1997) 139-149.

[3] Y. Vila, C. Burgos, J.L. Rueda, M. Soriano, M. Gallardo, C. Farias, I. Gonzalez-Herráiz, J. Gil, Estimación de la abundacia de cigala *Nephrops norvegicus* en el golfo de Cádiz a través de imágenes submarinas. Final Report of the Project supported by Biodiversity Foundation (Spanish Ministry of Agriculture, Food and Environment) and European Fisheries Funds (EFF) (2015).

[4] C. Froglia, Growth and behaviour of *Squilla mantis* (mantis shrimp) in the Adriatic Sea, EU Study DG XIV/MED/93/016, Final Report (1996).

[5] R.J.A. Atkinson, C. Froglia, E. Arneri, B. Antolini, Observation on the burrows and burrowing behaviour of *Squilla mantis* (L.) (Crustacea: Stomatopoda), Mar. Ecol 18(4) (1997) 337-359.

[6] ICES, Report of the Working Group for the Bay of Biscay and the Iberian waters Ecoregion (WGBIE). 3-10 May 2018, ICES HQ, Copenhagen, Denmark. ICES CM 2018/ACOM: 12 (2018) 642 pp.

[7] ICES, Manual of the IBTS North Eastern Atlantic Surveys. Series of ICES Survey Protocols SISP 15 (2017) 92 pp.

[8] S. Méhault, F. Morandeau, D. Kopp, Survival of discarded *Nephrops norvegicus* after trawling in the Bay of Biscay, Fish. Res 83 (2016) 396–400.

[9] ICES, Report of the Workshop on Methods for Estimating Discard Survival (WKMEDS), 17–21 February 2014, ICES HQ, Copenhagen, Denmark. ICES CM 2014/ACOM:51. (2014). 114 pp.

[10] L. Mérillet, S. Méhault, T. Rimaud, C. Pito, F. Morandeau, M. Morfin, D. Kopp, Survivability of discarded Norway lobster in the bottom trawl fishery of the Bay of Biscay. Fish Res 198 (2018) 24-30.

[11] R.L. Snyder. Behavioural Stress in Captive Animals. In Research in zoos and aquariums. A symposium held at the 49th Conf. Am. Ass. Zool. Parks aquariums, Houston, Texas. Oct. 6-11, 1973 (1976) pp41-76. Washington, Nat. Acad. Sci.

[12] D.E. Portz, C.M. Woodley, J.J. Cech. Stress-associated impacts of short-term holding on fish. Reviews in Fish Biology and Fisheries 16 (2006) 125-170.

[13] M.E. Conners, M. Levine, Characteristics and discard mortality of octopus bycatch in Alaska groundfish fisheries, Fisheries Research 185 (2013) 169-175.

[14] L.H. Frick, R.D. Reina, T.I. Walker, Stress related physiological changes and post-release survival of Port Jackson sharks (*Heterodontus portusjacksoni*) and gummy sharks (*Mustelus antarcticus*) following gill-net and longline capture in captivity, J. Exp. Mar Bio and Eco 385 (2010) 29-37.

[15] R.E. Olsen, F. Oppedal, M. Tenningen, A. Vold, Physiological response and mortality caused by scale loss in Atlantic herring. Fisheries Research 129 (2012) 21-27.

[16] S.G. Reid, N.J. Bernier, S.F. Perry, The adrenergic stress response in fish: control of catecholamine storage and release, Comparative Biochemistry and Physiology Part C Pharmacology, Toxicology and Endocrinology 120 (1998) 1-27.

[17] J.L. Kallen, S.L. Abrahamse, F. Vanherp, Circadian rhythmicity of the crustacean hyperglycemic hormone (Chh) in the hemolymph of the crayfish, Biol Bull-Us 179 (1990) 351-357.
[18] S. Lorenzon, P.G. Giulianini, E.A. Ferrero, Lipopolysaccharide-induced hyperglycemia is mediated by CHH release in crustaceans. Gen. Comp. Endocrinol. 108 (1997) 395-405.
[19] T.P. Mommsen, M.M. Vijayan, T.W. Moon, Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Reviews in Fish Biology and Fisheries 9 (1999) 211-268.
[20] G. Le Moullac, C. Soyez, D. Sauliner, D. Ansquer, J. Avarre, P. Levy, The effect of hypoxic stress on the immune response and resistance to vibriosis of the shrimp *P. stylirostris*, Fish Shellfish Immunol, 8 (1998) 621–629.
[21] L. Vazquez, J. Alpuche, G. Maldonado, C. Agundis, A. Pereyra-Morales, E. Zenteno, Review: Immunity mechanisms in crustaceans, Innate Immunity 15 (2009) 179-188.
[22] J.I Spicer, S.P. Baden, Environmental hypoxia and haemocyanin variability in Norway lobsters *Nephrops norvegicus* (L.), Mar Biol 139 (2001) 727–734.
[23] S.P. Baden, C.L.J. Håkansson, J.I. Spicer, Between-individual variation in haemocyanin concentrations in the Norway lobster *Nephrops norvegicus* following exposure to hypoxia and manganese, Mar. Biol. 143 (2003) 267–273.
[24] B.S. McEwen, J.C. Wingfield, The concept of allostasis in biology and biomedicine, Horm Behav 43 (2003) 2-15.
[25] H.S. Lund, T. Wang, E.S. Chang, L.F. Pedersen, E.W. Taylor, P.B. Pedersen, D.J. McKenzie, Recovery by the Norway lobster *Nephrops norvegicus* (L.) from the physiological stresses of trawling: influence of season and live-storage condition, J. Exp. Mar. Biol. Ecol 373 (2009) 124–132.
[26] S.E. Wendelaar Bonga, The stress response in fish, Physiological Reviews 77 (1997) 591-625.
[27] C. Barragán-Méndez, I. Sobrino, A. Marín-Rincón, S. Fernández-Boo, B. Costas, JM. Mancera and I. Ruiz-Jarabo. Acute-stress biomarkers in three octopodidae species after bottom trawling. Front. Physiol. 10 (2019) 784. Doi: 10.3389/fphys.2019.00784.
[28] M.W. Davis, Key principles for understanding bycatch discard mortality, Can. J. Fish. Aquat. Sci 59 (2002) 1834–1843.
[29] S. Lorenzon, M. Martinis, D. Borme, E.A. Ferrero, Hemolymph parameters as physiological biomarkers for monitoring the effects of fishing and commercial maintenance methods in *Squilla mantis* (Crustacea, Stomatopoda), Fish Res 137 (2013) 9–17.
[30] A. Albalat, S. Sinclair, J. Laurie, A. Taylor, D. Neil, Targeting the live market: Recovery of Norway lobsters *Nephrops norvegicus* from trawl-capture as assessed by stress-related parameters and nucleotide breakdown, J. Exp. Mar. Biol. Ecol. 395 (2010) 206–214.
[31] E.W. Taylor, M.G. Wheatly, The effect of long-term aerial exposure on heart rate, ventilation, respiratory gas exchange and acid-base status in the crayfish *Austropotamobius pallipes*, J. Exp. Biol. 92 (1981) 109–124.
[32] R.R. Harris, M.B. Andrews, Physiological changes in the Norway lobster *Nephrops norvegicus* (L.) escaping and discarded from commercial trawls on the West Coast of Scotland - II. Disturbances in haemolymph respiratory gases, tissue metabolites and swimming performance after capture and during recovery, J. Exp. Mar. Biol. Ecol. 320 (2005) 179–193.

[33] J. Aguila, G. Cuzon, C. Pascual, P.M. Domingues, G. Gaxiola, A. Sanchez, T. Maldonado, C. Rosas, The effects of fish hydrolysate (CPSP) level on *Octopus maya* (Voss and Solis) diet: Digestive enzyme activity, blood metabolites, and energy balance, Aquaculture 273 (2007) 641-655.

[34] M.J. Quade, and J.A. Roth. A rapid, direct assay to measure degranulation of bovine neutrophil primary granules. Vet. Immunol. Immunopathol. 58 (1997) 239–248. doi: 10.1016/s0165-2427(97)00048-2.

[35] P. Swain, S. Dash, P.K. Sahoo, P. Routray, S.K. Sahoo, S.D. Gupta, PK Meher. And Sarangi, N. Non-specific immune parameters of brood Indian major carp Labeo rohita and their seasonal variations. Fish Shellfish Immunol. 22 (2007) 38–43. doi: 10.1016/j.fsi.2006.03.010.

[36] D. Keppler, K. Decker, Glycogen determination with amyloglucosidase. Bergmeyer, H.U., ed. In Methods of Enzymatic Analysis, Vol 3, Academic Press, New York, (1974) 1127-1131.

[37] C. Foster, E.M. Amado, M.M. Souza, C.A. Freire, Do osmoregulators have lower capacity of muscle water regulation than osmoconformers? A study on decapods crustaceans, J. Exp. Zool 313 (2010) 80–94.

[38] E.L. Kaplan and P. Meier. Nonparametric estimation from incomplete observations. Journal of the American Statistical Association, 53 (1958) 457-481. http://dx.doi.org/10.1080.

[39] R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing. (2017). Vienna, Austria. URL http://www.R-project.org/.

[40] J. Pinheiro, D. Bates, S. DebRoy, D. Sarkar and R core Team. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1 (2017) 131.

[41] M. Morfin, D. Kopp, H.P. Benoît, S. Méhault, P. Randall, R. Foster and T. Catchpole. Survival of European plaice discarded from coastal otter trawl fisheries in the English Channel. Journal of Environmental Management, 204 (2017) 404–412. https://doi.org/10.1016/j.jenvman.2017.08.046

[42] M. Eskelund, C. Methling, P. Vilhelm Skov, N. Madsen. Survival of discarded plaice (*Pleuronectes platessa*) from Norway lobster (*Nephrops norvegicus*) ottertrawl fishery. J Appl Ichthyol. (2019). 00:1–10. https://doi.org/10.1111/jai.13888.

[43] I.D. Ridgway, A.C. Taylor, R.J.A. Atkinson, E.S. Chang, D.M. Neil, Impact of capture method and trawl duration on the health status of the Norway lobster, *Nephrops norvegicus*, J. Exp. Mar. Biol. Ecol 339 (2006) 135–147.

[44] A. Albalat, S. Sinclair, D. Neil. Validation of a vigour index for trawl-caught Norway lobsters (*Nephrops norvegicus*) destined for the live market: Underlying links to both physiological condition and survivability, Fis Res 191 (2017) 25-29.

[45] S. Raicevich, F. Minute, M.G. Finoia, F. Caranfa, P. Muri, L. Scapolan, M. Beltramini, Synergistic and antagonistic effects of thermal shock, air exposure, and fishing capture on the physiological stress of *Squilla mantis* (Stomatopoda), PLoS One 9 (2014) e105060.

[46] R. Gamito and H. Cabral. Mortality of brown-shrimp discards from the beam trawl fishery in the Tagus estuary, Portugal. Fisheries research. (2003). 423-427. doi:10.1016/S0165-7836(03)00108-5.

[47] P. Suuronen. Mortality of fish escaping trawl gears. FAO Fisheries Technical Paper. NO. 478. Rome, FAO. (2005). 72p.

[48] M. Morfin, S. Mehault, H.P. Benoît and D. Kopp. Narrowing down the number of species requiring detailed study as candidates for the EU Common Fisheries Policy

discard ban. Mar. Policy 77 (2017) 23e29. http://dx.doi.org/10.1016/j.marpol.2016.12.003.
[49] J.I Spicer, A.D. Hill, A.C. Taylor, R.H. Strang, Effect of aerial exposure on concentrations of selected metabolites in the blood of the Norwegian lobster *Nephrops norvegicus* (Crustacea: Nephropidae), Mar. Biol. 105 (1990) 129–135.
[50] A.S.C. Schmitt, R.F. Uglow, Haemolymph constituent levels and ammonia efflux rates of *Nephrops norvegicus* during emersion, Mar. Biol. 127 (1997) 403–410.
[51] L.B. Gladden, Lactate metabolism: a new paradigm for the third millennium, J. Physiol 558 (1) (2004) 5-30.
[52] M. Spindler-Barth, A bacterial infection in the common shore crab *Carcinus maenas* and the fiddler crab *Uca pugilator*, Mar. Biol. 36 (1976) 1-4.
[53] A.C. Taylor, J.I. Spicer, Metabolic responses of the prawns *Palaemon elegans* and *P. serratus* to acute hypoxia, Mar. Biol. (Berl.) 95 (1987) 521–530.
[54] E.A. Santos, R. Keller, Regulation of circulating levels of the crustacean hyperglycemic hormone: evidence for a dual feedback-control system. Comp Biochem Physiol B 163 (1993) 374–379.
[55] W. Dall, Indices of nutritional state in the western lobster, *Panulirus longipes* (Milne Edwards). I. Blood and tissue constituents and water content, J. Exp. Mar. Biol. Ecol 16 (1974) 167-180.
[56] K. Adachi, K. Wakamatsu, S. Ito, N. Miyamoto, T. Kokubo, T. Hirata, Anoxygen transporter hemocyanin can act on the late pathway of melanin synthesis, Pigment Cell Research, 19 (2005) 214–219.
[57] R.E.L. Lamela, R.S. Coffigny, Y.C. Quintana, M. Martínez, Phenoloxidase and peroxidase activity in the shrimp *Litopeneaus schmitti*, Pérez-Farfante and Kensley (1997) exposed to low salinity, Aquacult. Res 36 (2005) 1293–1297.
[58] C.J. Coates, J. Nairn Hemocyanin-derived phenoloxidase activity: a contributing factor to hyperpigmentation in *Nephrops norvegicus*, Food Chem 140 (2013) 361–369.
[59] T. Nagai, S. Kawabata, A link between blood coagulation and prophenol oxidase activation in arthropod host defense, J. Biol. Chem 275 (2000) 29264–29267.
[60] M. Muñoz, R. Cedeño, J. Rodriguez, W.P.W. Van der Knaap, E. Mialhe, E. Bachere, Measurement of reactive oxygen intermediate production in haemocytes of the penaeid shrimp, *Penaeus vannamei*, Aquaculture 191 (2000) 89–107.
[61] C. Gallo, F. Schiavon, L. Ballarin, Insight on cellular and humoral components of innate immunity in *Squilla mantis* (Crustacea, Stomatopoda), Fish Shellfish. Immunol, 31 (2011) 423-431.
[62] M.K. Broadhurst, R.B. Millar, C.P. Brad, and S.S. Uhlmann. Modified sorting technique to mitigate the collateral mortality of trawled school prawns (*Metapenaeus macleaya*). Mar Fresh w Res 53 (2002) 1189–1196. http://fishbull.noaa.gov/1073/broadhurst.pdf
[63] R. Laiz-Carrión, P.M. Guerreiro, J. Fuentes, A.V. Canario, M.P. Martín del Río, and J.M. Mancera, J.M. Branchial Osmoregulatory Response to Salinity in the Gilthead Sea Bream, *Sparus auratus*. J Exp Zool A Comp Exp Biol. 303 (2005) 563-76. Doi:10.1002/jez.a.183.
[64] B. Costas, L. Conceicao, C. Aragao, J.A. Martos, I. Ruiz-Jarabo, J.M. Mancera, et al. Physiological responses of senegalese sole (*Solea senegalensis* Kaup, 1858) after stress challenge: effects on non-specific immune parameters, plasma free amino acids and energy metabolism. Aquaculture 316 (2011) 68–76. doi: 10.1016/j.aquaculture.2011.03.011.

[65] J.C. Leland, P.A. Butcher, M.K. Broadhurst, B.D. Paterson, and D.G. Mayer, D.G. Relatie trap efficiency for recreationally caught eastern Australian blue swimmer crab (*Portunus pelagicus*) and associated injury and mortality of discards. Fish Res, 147 (2013) 304-311. https://doi.org/10.1016/j.fishres.2013.07.006

[66] A.K. Skrzynska, E. Maiorano, M. Bastaroli, F. Naderi, J.M. Miguez, G. Martinez-Rodriguez, et al. Impact of air exposure on vasotocinergic and isotocinergic systems in gilthead sea bream (*Sparus aurata*): new insights on fish stress response. Front. Physiol. (2018) 9:15. doi: 10.3389/fphys.2018.00096

[67] R. Laiz-Carrión, S. Sangiao-Alvarellos, J.M. Guzmán, M.P. Martín del Río, J.L. Soengas, and J.M. Mancera, Growth performance of gilthead sea bream *Sparus aurata* in different osmotic conditions: Implications for osmoregulation and energy metabolism. Aquaculture. 4 (2005) 849-861. doi.org/10.1016/j.aquaculture.2005.05.021.

[68] F.J. Arjona, L. Vargas-Chacoff, I. Ruiz-Jarabo, O. Gonçalves, I. Páscoa, M.P. Martín del Río, and J.M. Mancera. Tertiary stress responses in Senegalese sole (*Solea senegalensis* Kaup, 1858) to osmotic challenge: Implications for osmoregulation, energy metabolism and growth. Aquaculture. 287 (2009) 419-426. 10.1016/j.aquaculture.2008.10.047.

[69] R. Laiz-Carrión, I.R. Viana, J.R. Cejas, I. Ruiz-Jarabo, S. Jerez, J.A. Martos-Sitcha, E.B. Almansa, and J.M. Mancera. Influence of food deprivation and high stocking density on energetic metabolism and stress response in red porgy, *Pagrus pagrus* L. Aquaculture International. 20 (2011) 585-599. doi:10.1007/s10499-011-9488-y

[70] L. Hornstein, E.P. Espinosa, R.R. Cerrato, K.M.M. Lwiza, and B. Allam. The influence of temperature stress on the physiology of the Atlantic surfclam, Spisula solidissima. Comp Biochem Physiol A Mol Integr Physiol. 222 (2018) 66-73. doi: 10.1016/j.cbpa.2018.04.011

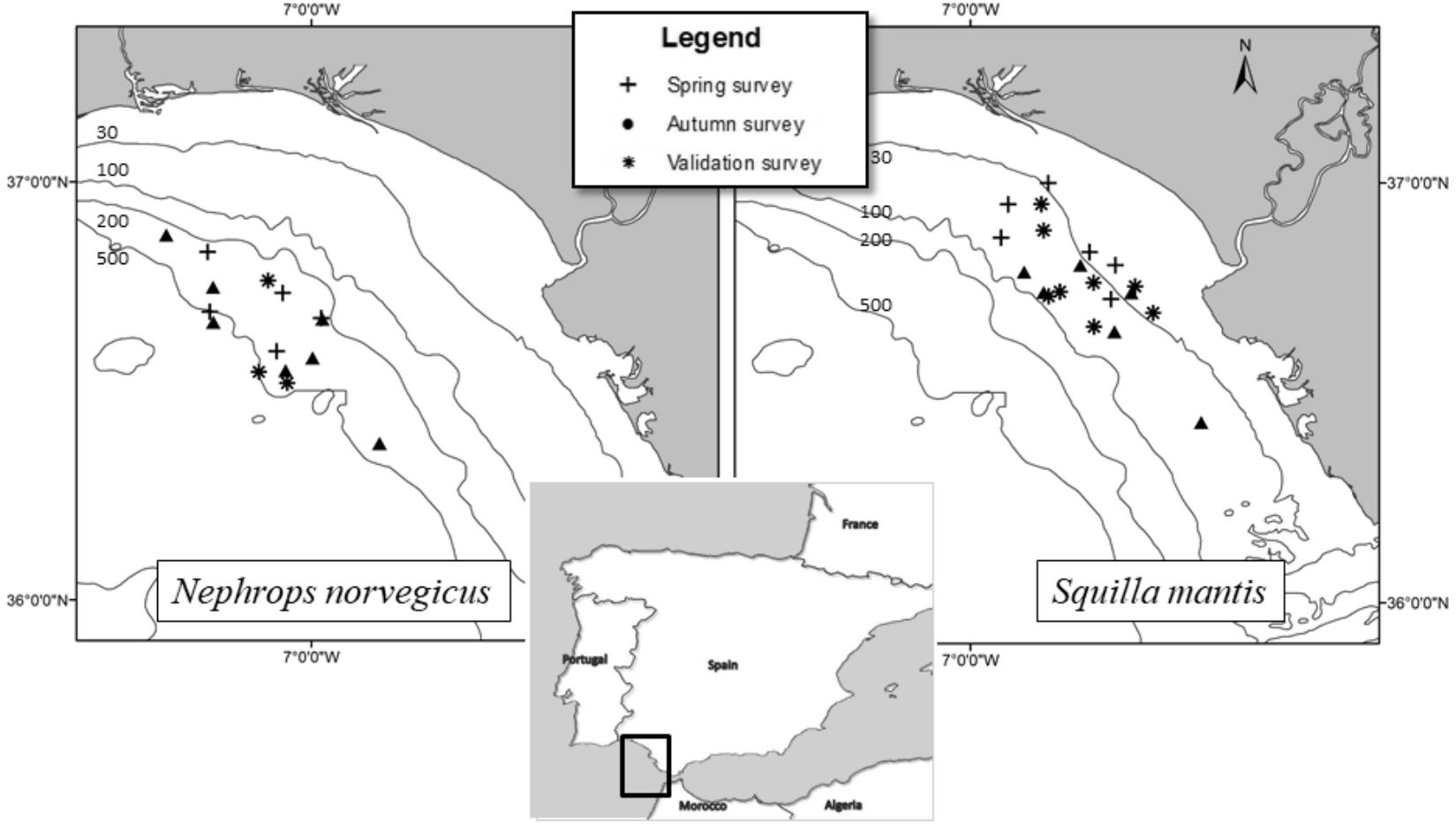
[71] A. Marçalo, P. Pousão-Ferreira, L. Mateus, J.H. Duarte Correia, Y. Stratoudakis, Sardine early survival, physical condition and stress after introduction to captivity. Journal of Fish Biology 72 (1) (2008) 103-120.

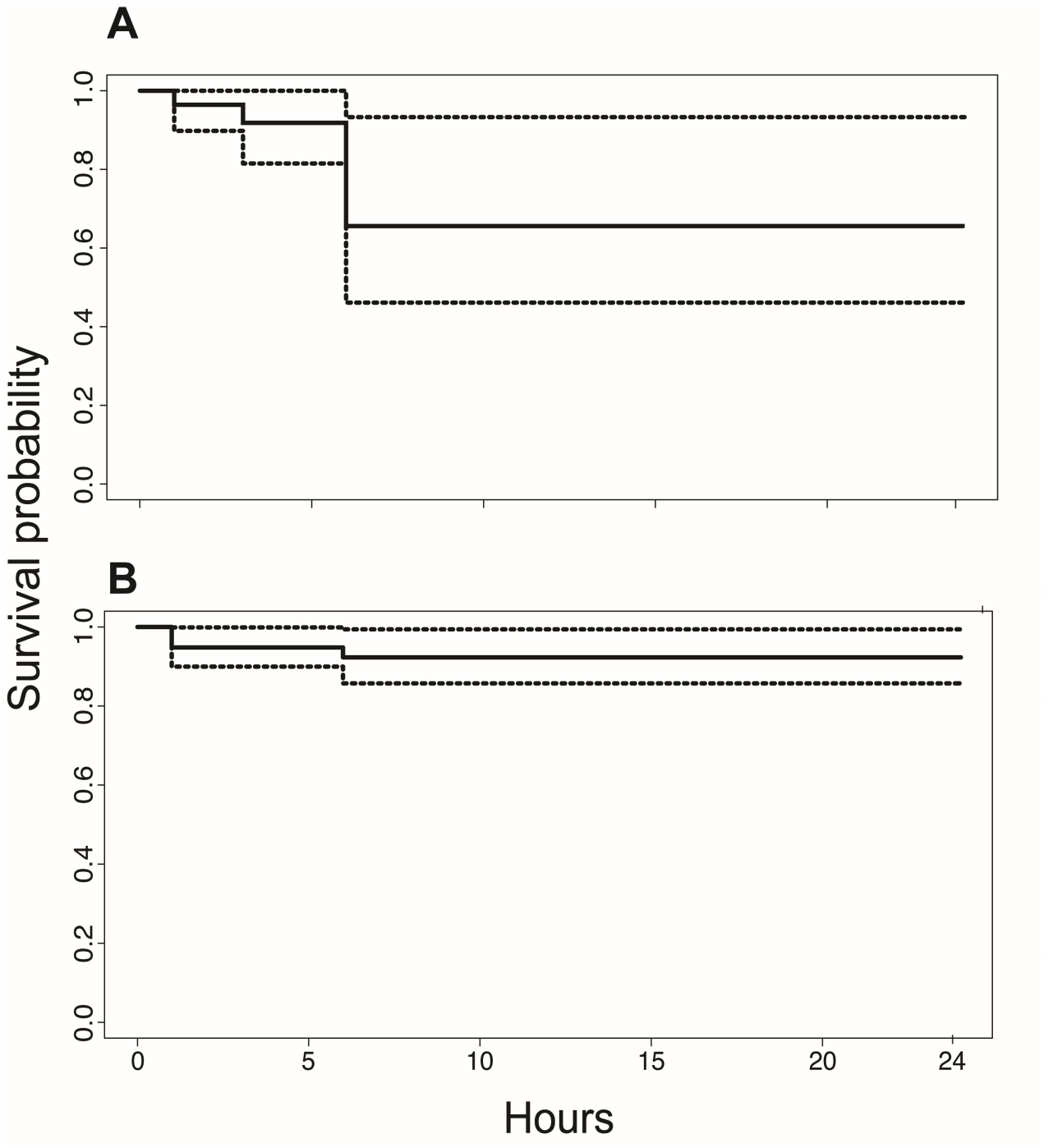
[72] A. Sánchez, C. Pascual, A. Sánchez, F. Vargas-Albores, G. Le Moullac, C. Rosas, Hemolymph metabolic variables and immune response in *Litopenaeus setiferus* adult males: the effect of acclimation. Aquaculture 198 (2001) 13–28.

[73] H.M.J. Van Overzee, A.D. Rijnsdorp, Effects of fishing during the spawning period: implications for sustainable management, Rev Fish Biol Fisheries 25 (2015) 65-83.

[74] G. Rotllant, J.B. Company, I. Alvarez-Fernández, J.A. García, J. Aguzzi, M. Durfort, The effects of seasonal variation on the nutritional condition of *Nephrops norvegicus* (Astacidea: Nephropidae) from wild populations in the western Mediterranean, J. Mar. Biol. Assoc. UK 94 (2014) 763–773.

[75] Albalat, A., Johnson, L., Coates, CJ., Dykes, GC., Hitte, F., Morro, B., Dick, J., Todd, K. and Neil, DM. The Effect of Temperature on the Physiological Condition and Immune-Capacity of European Lobsters (*Homarus gammarus*) During Long-Term Starvation. Front. Mar. Sci. (2019). 6:281. doi: 10.3389/fmars.2019.00281.


Legend
Spring survey
Autumn survey
Validation survey
7°0'0"W
37°0'0"N
36°0'0"N
30
100
200
500
N
Nephrops norvegicus
Squilla mantis
France
Portugal
Spain
Morocco
Algeria


A
B
Survival probability
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0.2
0.4
0.6
0.8
1.0
0
5
10
15
20
24
Hours

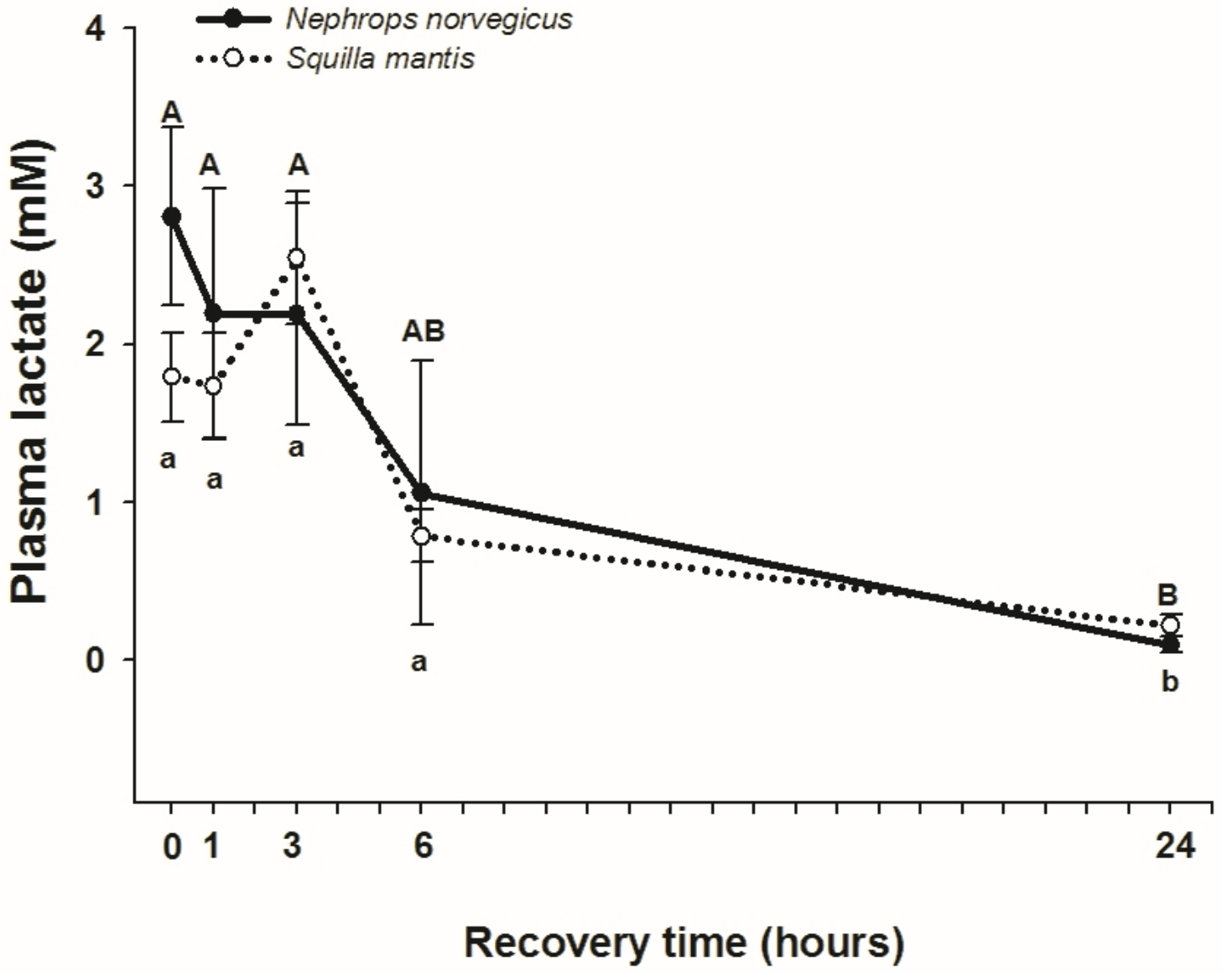
Nephrops norvegicus
Squilla mantis
Plasma lactate (mM)
4
3
2
1
0
A
A
A
AB
B
a
a
a
a
b
0
1
3
6
24
Recovery time (hours)

FIGURE LEGENDS

Figure 1. Sampled area off the Gulf of Cadiz (south-western Atlantic waters of Spain). Asterisks indicate validation surveys for both species (*Nephrops norvegicus* and *Squilla mantis*). Triangles indicate the spring survey and circles indicate the autumn survey. Bathimetric lines and their depths (in m) are indicated.

Figure 2. Kaplan-Meier survival curves for each species (A) *N. norvegicus* and (B) *S. mantis*, captured by bottom trawling. Animals were sampled just after bottom trawling (0 h), and after being introduced in onboard recovery tanks at times 1 h, 3 h, 6 h and 24 h. Dashed lines represent 95% confidence interval.

Figure 3. Time-course recovery of plasma lactate (mM) in bottom trawled *N. norvegicus* and *S. mantis* in the Gulf of Cadiz (Spain). Plasma samples were taken just after bottom trawling (0 h) and at different recovery times (1, 3, 6 and 24 hours). Data are expressed as average ± standard error (n = 6 and n = 17 per group in *N. norvegicus* and *S. mantis*, respectively). Different letters indicate significant differences between groups, with capital and lowercase letters employed to differentiate between *N. norvegicus* and *S. mantis*, respectively (LMM, $p < 0.05$).

Figure 1. Barragán-Méndez *et al*.

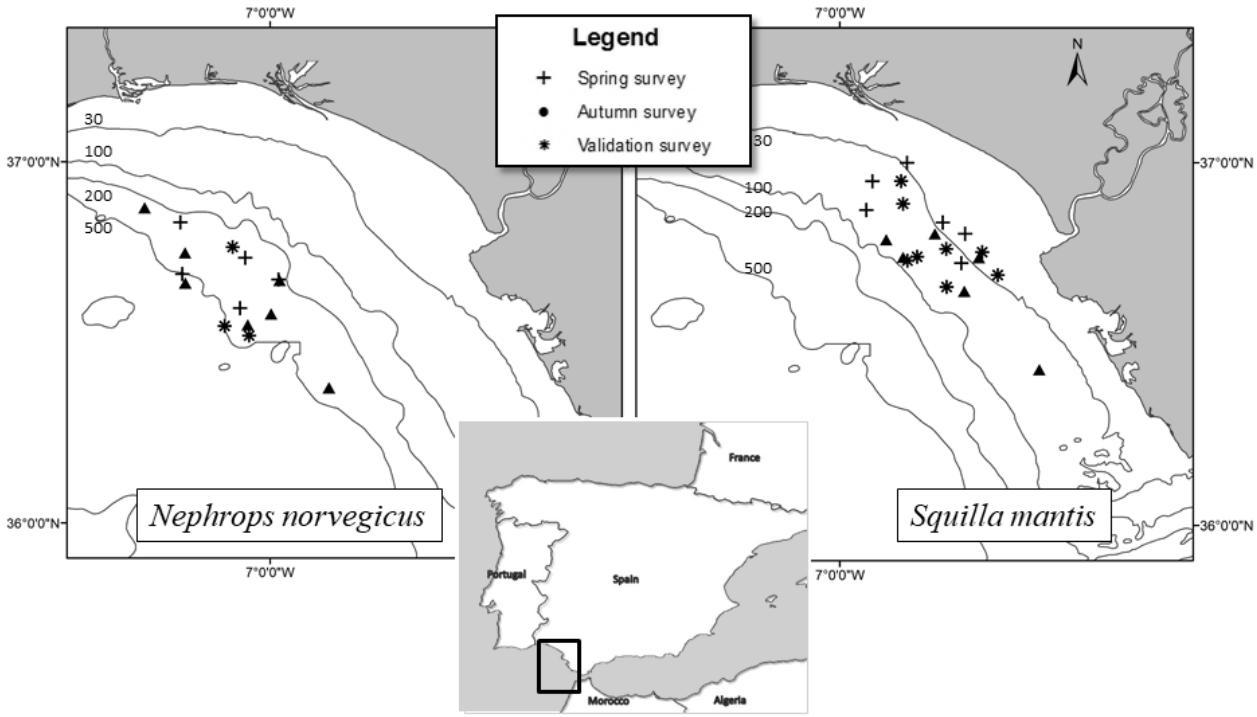

Figure 2. Barragán-Méndez *et al*.

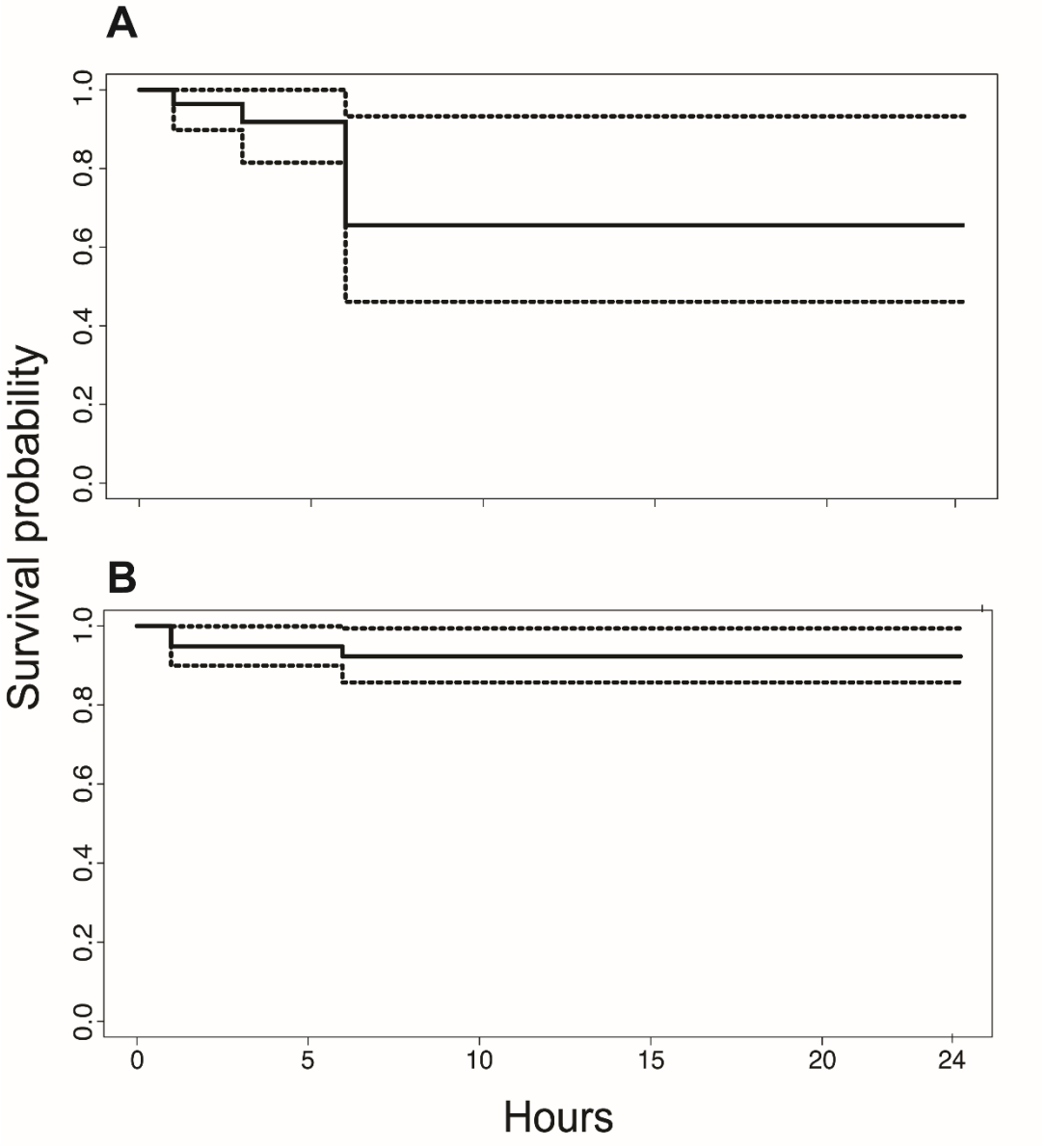

Figure 3. Barragán-Méndez, et al.

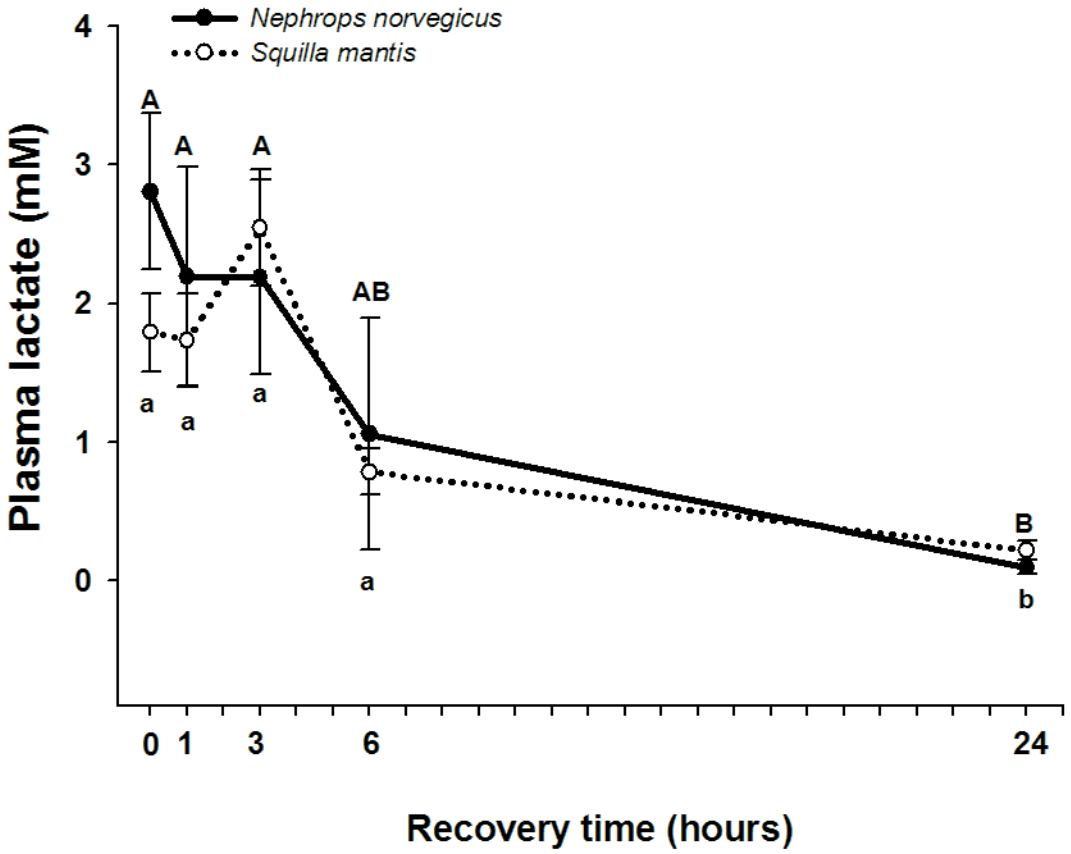

Table S1. Generalized Linear Mixed models of the differences in mortality between spring (S) and autumn (A). in the Gulf of Cádiz (Spain) of *N. norvegicus* and *S. mantis*. * = significant differences; SE = Standard Errors.

N. norvegicus	Estimate	SE	z value	Pr(>|z|)
Intercept	1.45	0.50	2.90	<0.001*
Season (A)	-1.14	0.53	-2.13	0.033*

S. mantis	Estimate	SE	z value	Pr(>|z|)
Intercept	0.95	0.27	3.51	<0.001*
Season (S)	1.11	0.39	2.87	<0.001*

Table S2. Linear mixed-effect models of the significant plasma and muscle physiological parameters in *N. norvegicus* after bottom trawling in spring 2017. * = significant differences; SE = Standard Errors. df = degrees of freedom; 0 = 0 h; 1 = after 1 h recovery in onboard tanks; 3 = after 3 h; 6 = after 6 h; 24 = after 24 h.

Plasma lactate	Estimate	SE	df	t-value	p-value
Intercept	2.83	0.58	20	4.91	<0.001*
1	-0.60	0.80	20	-0.75	0.464
3	-0.47	0.81	20	-0.58	0.568
6	-0.58	0.96	20	-0.61	0.551
24	-2.63	0.76	20	-3.48	0.002*

Plasma hemocyanin	Estimate	SE	df	t-value	p-value
Intercept	316.55	46.74	14	6.77	<0.001*
0	-200.54	65.59	14	-3.06	0.008*
1	-187.77	71.15	14	-2.64	0.019*
3	-97.89	58.68	14	-1.67	0.118
6	-158.37	71.15	14	-2.23	0.043

Plasma peroxidase	Estimate	SE	df	t-value	p-value
Intercept	10.55	3.57	19	2.96	<0.001*
1	-3.46	4.87	19	-0.71	0.486
3	10.95	3.83	19	2.86	0.010*
6	6.86	4.87	19	1.41	0.175
24	11.71	3.83	19	3.05	0.007*

Muscle lactate	Estimate	SE	df	t-value	p-value
Intercept	2.35	0.20	20	11.76	<0.001*
1	-0.17	0.25	20	-0.69	0.500
3	-0.65	0.25	20	-2.62	0.016*
6	-0.30	0.30	20	-0.98	0.338
24	-0.96	0.25	20	-3.88	0.001*

Muscle glucose	Estimate	SE	df	t-value	p-value
Intercept	263.27	27.64	20	9.52	<0.001*
0	-171.15	35.99	20	-4.76	<0.001*
1	-149.36	39.16	20	-3.81	0.001*
3	-197.99	37.32	20	-5.31	<0.001*
6	-173.04	45.79	20	-3.78	0.001*

Table S3. Linear mixed-effect models of the significant plasma and muscle physiological parameters in *S. mantis* after bottom trawling in spring 2017. * = significant differences; SE = Standard Errors. df = degrees of freedom; 0 = 0 h; 1 = after 1 h; 3 = after 3 h; 6 = after 6 h; 24 = after 24 h.

Plasma glucose	Estimate	SE	df	t-value	p-value
Intercept	5.10	0.71	73	7.13	<0.001*
1	-0.04	0.78	73	-0.05	0.964
3	-0.55	0.77	73	-2.01	0.048*
6	-1.64	0.80	73	-2.05	0.044*
24	-0.85	0.79	73	-1.07	0.288

Plasma lactate	Estimate	SE	df	t-value	p-value
Intercept	0.45	0.12	73	4	<0.001*
0	0.84	0.15	73	6	<0.001*
1	0.78	0.15	73	5	<0.001*
3	1.08	0.15	73	7	<0.001*
6	0.38	0.15	73	3	0.014*

Plasma peroxidase	Estimate	SE	df	t-value	p-value
Intercept	8.45	1.10	65	7.71	<0.001*
1	-0.42	1.43	65	-0.30	0.769
3	3.52	1.41	65	2.49	0.015*
6	1.82	1.45	65	1.26	0.214
24	0.71	1.49	65	0.47	0.637

Muscle lactate	Estimate	SE	df	t-value	p-value
Intercept	1.62	0.23	70	6.95	<0.001*
0	0.23	0.28	70	0.83	0.411
1	0.64	0.27	70	2.39	0.019
3	0.71	0.27	70	2.60	0.012*
6	0.16	0.27	70	0.60	0.550

Muscle glucose	Estimate	SE	df	t-value	p-value
Intercept	476.48	56.26	72	8.47	<0.001*
0	-175.52	67.08	72	-2.62	0.010*
24	-71.09	66.09	72	-1.08	0.286
3	-131.57	65.92	72	-2.00	0.04*
6	-122.81	66.04	72	-1.86	0.04*

Muscle water	Estimate	SE	df	t-value	p-value
Intercept	82.81	0.65	73	128.38	<0.001*
0	1.89	0.89	73	2.12	0.037*
1	2.19	0.91	73	2.42	0.017*

3	2.63	0.88	73	2.99	0.003*
6	0.76	0.89	73	0.85	0.396

Table S4. Linear mixed-effect models of the significant plasma and muscle physiological parameters in *N. norvegicus* after bottom trawling in the Gulf of Cadiz (0 h) and 24 h after recovery in water tanks in spring and autumn. * = significant differences; SE = Standard Errors. df = degrees of freedom; 24 = after 24 h; S = spring.

Plasma gucose	Estimate	SE	df	t-value	p-value
Intercept	2.17	0.11	93	20.09	<0.001*
Season (S)	0.46	0.17	13	2.64	0.020*
Hours (24)	-0.77	0.15	93	-5.31	<0.001*

Plasma lactate	Estimate	SE	df	t-value	p-value
Intercept	2.08	0.10	92	20.27	<0.001*
Season (S)	0.07	0.17	13	0.40	0.693
Hours (24)	-1.92	0.12	92	-16.11	<0.001*

Plasma hemocyanin	Estimate	SE	df	t-value	p-value
Intercept	147.57	12.90	84	11.44	<0.001*
Season (S)	198.87	28.39	13	7.01	<0.001*
Hours (24)	185.41	28.24	84	6.57	<0.001*

Plasma peroxidase	Estimate	SE	df	t-value	p-value
Intercept	109.07	6.98	71	15.62	<0.001*
Season (S)	-8.37	10.40	13	-0.80	0.435
Hours (24)	2.71	8.89	71	3.05	0.003*

Plasma lysozyme	Estimate	SE	df	t-value	p-value
Intercept	0.12	0.01	78	18.84	<0.001*
Season (S)	-0.07	0.01	13	-6.20	<0.001*
Hours (24)	-0.01	0.01	78	-0.76	0.450

Muscle lactate	Estimate	SE	df	t-value	p-value
Intercept	1.69	0.06	91	30.27	<0.001*
Season (S)	0.60	0.12	13	4.91	<0.001*
Hours (24)	-0.58	0.08	91	-7.26	<0.001*

Muscle glucose	Estimate	SE	df	t-value	p-value
Intercept	13.14	0.62	93	21.18	<0.001*
Season (S)	-2.20	1.03	13	-2.12	0.060
Hours (24)	2.94	0.71	93	4.15	<0.001*

Muscle glycogen	Estimate	SE	df	t-value	p-value
Intercept	3.96	0.30	80	13.14	<0.001*
Season (S)	-1.01	0.52	13	-1.96	0.072

Hours (24)	0.94	0.30	80	3.08	0.003*

Table S5. Linear mixed-effect models of the significant plasma and muscle physiological parameters in *S. mantis* after bottom trawling in the Gulf of Cadiz (0 h) and 24 h after. * = significant differences; SE = Standard Errors. df = degrees of freedom; 24 = after 24 h; S = spring.

Plasma gucose	Estimate	SE	df	t-value	p-value
Intercept	2.60	0.30	62	8.73	<0.001*
Season (S)	1.50	0.37	62	4.06	<0.001*
Hours (24)	-0.18	0.34	62	-0.53	0.601

Plasma lactate	Estimate	SE	df	t-value	p-value
Intercept	2.36	0.14	61	16.38	<0.001*
Season (S)	-0.39	0.17	61	-2.23	0.029*
Hours (24)	-1.90	0.14	61	-13.27	<0.001*

Plasma hemocyanin	Estimate	SE	df	t-value	p-value
Intercept	266	43	50	6	<0.001*
Season (S)	115	52	50	2	0.031*
Hours (24)	183	52	50	4	<0.001*

Plasma lysozyme	Estimate	SE	df	t-value	p-value
Intercept	0.431	0.082	55	5.28	<0.001*
Season (S)	-0.086	0.110	55	-0.78	0.438
Hours (24)	0.389	0.105	55	3.69	<0.001*

Muscle lactate	Estimate	SE	df	t-value	p-value
Intercept	1.26	0.12	60	10.82	<0.001*
Season (S)	0.42	0.14	60	2.93	0.005*
Hours (24)	-0.50	0.13	60	-3.73	<0.001*

Muscle water	Estimate	SE	df	t-value	p-value
Intercept	83.25	0.53	55	156.78	<0.001*
Season (S)	-1.24	0.65	55	-1.90	0.070
Hours (24)	-1.60	0.51	55	-3.14	0.002*

TABLES

Table 1

Summary of collected data for each species (*Nephrops norvegicus* and *Squilla mantis*) and sampling survey. Carapace length and environmental variables are given as average ± SEM.

	N. norvegicus			*S. mantis*		
Survey	**Spring 2017**	**Spring 2018**	**Autumn 2017**	**Spring 2017**	**Spring 2018**	**Autumn 2017**
Valid hauls	3	5	7	8	7	6
Mortality studies	Kaplan-Meier	Air exposure Seasonal mortality	Air exposure Seasonal mortality	Kaplan-Meier	Air exposure Seasonal mortality	Air exposure Seasonal mortality
Animals sampled	36	42	144	95	37	68
Recovery studies	Time course	Seasonal	Seasonal	Time course	Seasonal	Seasonal
Animals sampled	28	27	83	85	30	50
Carapace length (mm)	24.7 ± 0.8	25.9 ± 0.5	25.7 ± 0.3	29.5 ± 2.8	27.7 ± 0.9	24.8 ± 0.5
Depth (m)	454 ± 50	440 ± 28	494 ± 32	48 ± 8	52 ± 13	60 ± 11
Bottom T (°C)	14.1 ± 0.3	13.7 ± 0.0	13.5 ± 0.1	15.4 ± 0.0	13.6 ± 0.1	17.3 ± 0.1
Tanks T (°C)	14.7 ± 1.0	16.4 ± 0.2	21.3 ± 0.3	14.7 ± 1.0	16.4 ± 0.2	21.3 ± 0.3
Bottom salinity	36.5 ± 0.0	36.4 ± 0.2	36.4 ± 0.0	36.4 ± 0.0	36.6 ± 0.1	36.2 ± 0.1
Tanks salinity	36.4 ± 0.3	36.4 ± 0.1	36.3 ± 0.1	36.4 ± 0.3	36.4 ± 0.1	36.3 ± 0.1

Table 2

Time course recovery of plasma and muscle physiological parameters in *N. norvegicus* after bottom trawling in spring 2017. Samples were taken previously to the introduction into recovery tanks (0 h) and along a time course of 24 h (1, 3, 6 and 24 h). Data is shown as mean ± SEM (n = 6 per group). Significant differences between sampling times are indicated with different capital letters (Linear mixed-effect models, $p < 0.05$).

Parameter	Time-course recovery				
	0 h	**1 h**	**3 h**	**6 h**	**24 h**
Plasma glucose (mM)	1.59 ± 0.23	1.38 ± 0.27	1.05 ± 0.19	1.21 ± 0.44	1.09 ± 0.14
Plasma hemocyanin (μM)	114 ± 26 B	138 ± 39 B	223 ± 39 AB	168 ± 62 AB	318 ± 61 A
Plasma peroxidase (U/mL)	10.0 ± 2.0 B	4.7 ± 0.9 B	20.3 ± 4.0 A	15.0 ± 3.4 AB	21.0 ± 4.1 A
Plasma lysozyme (μg/mL)	0.10 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.10 ± 0.01
Muscle lactate (mg/g wet weight)	2.32 ± 0.16 A	2.15 ± 0.20 A	1.67 ± 0.13 B	1.99 ± 0.38 A	1.36 ± 0.18 B
Muscle glucose (mg glc/g wet weight)	96.0 ± 16.3 B	138.3 ± 29.0 B	66.6 ± 11.0 B	131.2 ± 34.0 B	264.0 ± 30.8 A
Muscle glycogen (mg glc/g wet weight)	27.5 ± 8.7	14.9 ± 6.9	15.0 ± 3.5	42.0 ± 14.0	35.3 ± 7.7
Muscle water (%)	78.9 ± 0.8	78.2 ± 0.9	78.9 ± 0.2	78.1 ± 1.5	78.7 ± 0.8

Table 3

Time course recovery of plasma and muscle physiological parameters in *S. mantis* after bottom trawling in spring 2017. Samples were taken previously to the introduction into recovery tanks (0 h) and along a time course of 24 h (1, 3, 6 and 24 h). Data is shown as mean ± SEM (n = 17 per group). Significant differences between sampling times are indicated with different capital letters (Linear mixed-effect models, $p < 0.05$).

Parameter	Time-course recovery				
	0 h	**1 h**	**3 h**	**6 h**	**24 h**
Plasma glucose (mM)	5.06 ± 0.86 A	5.16 ± 0.78 A	3.63 ± 0.36 B	3.50 ± 0.52 B	4.05 ± 0.43 A
Plasma hemocyanin (µM)	277 ± 33 B	384 ± 54 AB	386 ± 52 AB	364 ± 43 AB	420 ± 36 A
Plasma peroxidase (U/mL)	8.45 ± 0.90 B	8.02 ± 0.77 B	11.97 ± 1.11 A	10.27 ± 0.98 AB	9.15 ± 0.95 B
Plasma lysozyme (µg/mL)	0.28 ± 0.02	0.29 ± 0.02	0.29 ± 0.02	0.28 ± 0.03	0.34 ± 0.03
Muscle lactate (mg/g wet weight)	1.85 ± 0.15 B	2.28 ± 0.19 A	2.39 ± 0.23 A	1.82 ± 0.23 B	1.67 ± 0.23 B
Muscle glucose (mg glc/g wet weight)	68.8 ± 10.9 B	91.2 ± 9.9 A	68.4 ± 9.6 B	65.6 ± 9.5 B	75.9 ± 9.2 A
Muscle glycogen (mg glc/g wet weight)	111 ± 28	167 ± 41	128 ± 30	131 ± 33	87 ± 16
Muscle water (%)	84.7 ± 0.55 A	85.0 ± 0.7 A	85.4 ± 0.7 A	83.6 ± 0.5 B	82.8 ± 0.6 B

Table 4

Plasma and muscle physiological parameters in *N. norvegicus* after bottom trawling in the Gulf of Cadiz (0 h) and 24 h after recovery in water tanks in spring and autumn. Data is shown as mean ± SEM (n = 12 and 44 per group in spring and autumn, respectively). Asterisks (*) indicate significant differences between 0 h and 24 h for each season; hashtags (#) indicate significant differences between spring and autumn for the same sampling time (Linear mixed-effect models, $p < 0.05$).

Parameter	Spring		Autumn	
	0 h	24 h	0 h	24 h
Plasma lactate (mM)	1.95 ± 0.66	0.40 ± 0.63*	2.16 ± 0.80	0.12 ± 0.25*
Plasma glucose (mM)	2.54 ± 0.21	1.93 ± 0.20*	2.20 ± 0.96	1.38 ± 0.08*#
Plasma hemocyanin (µM)	341 ± 59	549 ± 49*	148 ± 69	328 ± 29*#
Plasma peroxidase (U/mL)	86 ± 20	136 ± 13*	113 ± 39	133 ± 6*
Plasma lysozyme (µg/mL)	0.05 ± 0.01	0.04 ± 0.01	0.11 ± 0.01	0.12 ± 0.01
Muscle lactate (mg/g wet weight)	2.32 ± 0.19	1.69 ± 0.15*	1.69 ± 0.42	1.12 ± 0.05*#
Muscle glucose (mg glc/g wet weight)	93 ± 18	184 ± 8*	159 ± 11	212 ± 14*
Muscle glycogen (mg glc/g wet weight)	10.0 ± 0.9	14.0 ± 1.7*	18.2 ± 2.2	23.1 ± 2.4*
Muscle water (%)	78.1 ± 0.5	77.5 ± 0.4	79.1 ± 2.4	78.6 ± 0.3

Table 5

Plasma and muscle physiological parameters in *S. mantis* after bottom trawling in the Gulf of Cadiz (0 h) and 24 h after recovery in water tanks in spring and autumn. Data is shown as mean ± SEM (n = 15 and 23 per group in spring and autumn, respectively). Asterisks (*) indicate significant differences between 0 h and 24 h for each season; hashtags (#) indicate significant differences between spring and autumn for the same sampling time (Linear mixed-effect models, $p < 0.05$).

Parameter	Spring		Autumn	
	0 h	24 h	0 h	24 h
Plasma lactate (mM)	1.50 ± 0.48	0.13 ± 0.07*	2.50 ± 1.02	0.33 ± 0.20*
Plasma glucose (mM)	4.03 ± 0.70	3.88 ± 0.34	2.52 ± 0.39	2.39 ± 0.17#
Plasma hemocyanin (µM)	313 ± 94	588 ± 67*	289 ± 70	432 ± 35*#
Plasma peroxidase (U/mL)	105 ± 39	177 ± 17*	158 ± 16	179 ± 16*
Plasma lysozyme (µg/mL)	0.37 ± 0.07	0.72 ± 0.09*	0.43 ± 0.10	0.83 ± 0.09*#
Muscle lactate (mg/g wet weight)	1.82 ± 0.21	1.11 ± 0.14*	1.20 ± 0.17	0.83 ± 0.12*
Muscle glucose (mg glc/g wet weight)	190 ± 67	274 ± 54	126 ± 23	164 ± 28
Muscle glycogen (mg glc/g wet weight)	151 ± 11	112 ± 35	114 ± 34	120 ± 34
Muscle water (%)	82.6 ± 0.9	80.2 ± 0.3*	83.5 ± 0.6	81.2 ± 0.4*

Physiological recovery after bottom trawling as a method to manage discards: the case study of *Nephrops norvegicus* and *Squilla mantis*

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Short Title: Physiological recovery of discarded crustaceans.

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