

Ecological importance of survival of unwanted invertebrates discarded in different NW Mediterranean trawl fisheries

Montserrat Demestre¹, Paolo Sartor², Alfredo Garcia-de-Vinuesa¹, Mario Sbrana²,
Francesc Maynou¹, Andrea Massaro³

¹ Institut de Ciències del Mar, CSIC, Pg. Marítim de la Barceloneta, 37-49, 08003 Barcelona, Spain.

(MD) (Corresponding author) E-mail: montse@icm.csic.es. ORCID iD: <https://orcid.org/0000-0003-2866-4821>

(AGV) E-mail: agvinuesa@icm.csic.es. ORCID iD: <https://orcid.org/0000-0002-6645-6217>

(FM) E-mail: maynouf@icm.csic.es. ORCID iD: <https://orcid.org/0000-0001-7200-6485>

² Consorzio per il Centro Interuniversitario di Biologia Marina ed Ecologia Applicata (CIBM). Viale Nazario Sauro 4, 57128 Livorno, Italy.

(PS) E-mail: psartor@cibm.it. ORCID iD: <https://orcid.org/0000-0001-7239-8255>

(MS) E-mail: msbrana@cibm.it. ORCID iD: <https://orcid.org/0000-0002-9562-8372>

³ Aplysia Soc. Coop. r.l. Via Menichetti, 35 – 57121 Livorno, Italy.

(AM) E-mail: andrea.massaro@aplysia.it. ORCID iD: <https://orcid.org/0000-0001-9224-3883>

Summary: There is currently very little information on the survival of discards of unwanted and unregulated catches of invertebrates after the stresses caused by capture. A great number of the unregulated invertebrate species form the basis of essential fish habitats for important fisheries resources such as hake, red mullet and cuttlefish. Thus, data on their survival after discarding may help to interpret the role of these species within the benthic ecosystems. Furthermore, descriptor 6 of the Marine Strategy Framework Directive (EU Directive 2008/56/E) foresees maintaining sea floor integrity at a level that ensures that the structure and functions of the ecosystems are safeguarded, and Article 7(d) of the Common Fisheries Policy (EU Reg. 1380/2013) foresees the implementation of management measures for fishing with low impact on the marine ecosystem and fishery resources. Survival measurements by direct recovery of tagged discarded species are not effective in bottom trawl fisheries, for which alternative studies such as semi-quantitative measures obtained on board prior to discarding can be considered as appropriate for mortality estimation. The present work assessed the survival of unwanted species using a semi-quantitative assessment on the deck of trawlers and at the laboratory for a period of 96 hours in two Mediterranean areas (the Catalan coast and the Ligurian and Northern Tyrrhenian seas). A high number of discarded invertebrates showed a high percentage of survival (>70%) in both assessments. The results can be used to provide information that can help to achieve higher survival levels of discarded specimens and enhance the productivity of fishing grounds by increasing the health of benthic ecosystems.

Keywords: discard survival; invertebrates; mortality estimation; vitality levels; trawl fishery; Mediterranean.

Importancia ecológica de la supervivencia de invertebrados no-deseados descartados en distintas pesquerías de arrastre del Mediterráneo nor-occidental

Resumen: Actualmente, hay muy poca información sobre la supervivencia del descarte de los invertebrados no-deseados y no-regulados después del stress de la captura. Un gran número de especies de estos invertebrados son básicas para los hábitats esenciales de importantes recursos pesqueros como la merluza, el salmónete o la sepia. Por lo tanto, información sobre su supervivencia al ser descartados pueden ayudar a interpretar el papel de estas especies dentro de los ecosistemas bentónicos. Además, el punto 6 de la Directiva Marco de Estrategia Marina (MSFD, EU Directive 2008/56/E) prevé mantener la integridad del fondo marino a un nivel que garantice la protección de la estructura y de las funciones de los ecosistemas, y el artículo 7 (d) de la Política Pesquera Común (PPC EU Reg. 1380/2013) prevé la implementación de medidas de gestión para la pesca, que tengan bajo impacto tanto en el ecosistema marino como en los recursos pesqueros. En el caso de las pescas de arrastre de fondo, no es efectiva la estimación de la supervivencia a partir del método directo de captura-recapture de las especies del descarte marcadas, por lo que los estudios alternativos, como las medidas semicuantitativas obtenidas a bordo antes del descarte, pueden considerarse una estimación de la mortalidad apropiada. El presente trabajo evaluó la supervivencia del descarte de especies no-deseadas utilizando una Evaluación Semicuantitativa (SQA) mediante dos estudios: uno sobre la cubierta de los arrastreros y el otro en laboratorio durante un período de 96 horas, en dos áreas del Mediterráneo, las costas Catalanas y en los mares de Liguria y del Tirreno Norte. Los resultados mostraron en ambos estudios, que un alto número de invertebrados descartados tenía un alto porcentaje de supervivencia (>70%). El propósito de estos resultados es proporcionar información que puede ayudar a alcanzar niveles de supervivencia más altos de los individuos descartados. Al mismo tiempo, se espera que con esta mejora se consiga potenciar la productividad de los caladeros al aumentar la salud de los ecosistemas bentónicos.

Palabras clave: supervivencia del descarte; invertebrados; estimación de mortalidad; estados de vitalidad; arrastre, Mediterráneo.

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INTRODUCTION

Mediterranean fisheries are characterized by a high rate of unwanted catches and a great number of marine organisms that are discarded at sea (Leonart 2015, Tsagarakis et al. 2014). One of the fishing methods that produces most discards is otter bottom trawling, which is also one of the least selective fishing gears. The discards include both species with non-commercial value and marketable species that are undersized or of low value. Technical regulations, such as the introduction of the 40-mm square mesh or the 50-mm diamond mesh in the cod-end (EC 1967/2006) can reduce discards to some extent, but cannot solve the impacts of bottom trawling on habitats and benthic communities.

The investigated areas were the Catalan coast, corresponding to FAO division 37.1.1, Geographical Sub-Area 6 (GSA06), and the Ligurian and northern Tyrrhenian seas, corresponding to FAO division 37.1.3, Geographical Sub-Area 9 (GSA09), both comprising chronically exploited fishing grounds. In the last ten years the demersal fisheries carried out mainly by bottom trawl fleets in the two areas accounted for about 40% of the total landings and 70% of the economic value (STECF 2016).

A large fraction of this discarded biomass (30%-50% of the total biomass caught) is composed of species of commercial interest (small-sized or damaged specimens), while the remaining fraction is composed of species with low or no economic value (Machias et al. 2001, Sánchez et al. 2004, 2007). Furthermore, trawl fleets operate in a great variety of soft habitats (e.g. muddy-sand, sandy-muddy, mud, sandy-gravel, sand), so discards are characterized by extremely high species diversity with a high percentage of non-commercial species, some of which are macroinvertebrates (echinoderms, crustaceans, poriferans, ascidians, cnidarians, bryozoans, bivalves and gastropods). In many cases the discarded species belong to sensitive benthic habitats, such as maërl or crinoid beds.

The impact of bottom trawling on benthic habitats and communities and demersal species is little known. The impact depends on the fishing activity (Martín et al. 2014) and can create changes in the ecological functioning of benthic components that have important repercussions on the exploited populations (de Juan et al. 2007, Frid 2011, Hewitt et al. 2008).

The European Marine Strategy Framework Directive (EU 2008/56/E) encourages member states to move towards an ecosystem-based fishery management in order to protect the goods and services that marine ecosystems provide. Therefore, it is important

to take into account the link between benthic communities and habitats and fisheries resources, because a great number of ecological interactions may be adversely impacted by fishing. The capture of benthic invertebrates and their discarding at sea will impact benthic habitats to a certain degree, depending on the post-release survival of each species. The survival of habitat-structuring invertebrates, such as crinoids and echinoderms, can help to maintain the good status of the essential fish habitats where the most important commercial resources, such as European hake, red mullet, spiny lobster and cuttlefish, use them as areas of nursery, recruitment or growth (Abella et al. 2008, Colloca et al. 2009).

There is currently very little information on the survival of unwanted and unregulated invertebrates after the stresses of being captured, handled and discarded. The specific biological characteristics make an organism more or less vulnerable to different stressors of the capture method and release process (de Juan and Demestre 2012). Other factors affecting the survival of released animals are related to the handling practices during the sorting and release processes and to the environmental conditions during capture, hauling on board and sorting, such as hypoxia and temperature (Bergmann et al. 2001, Giomi et al. 2008, Tsagarakis et al. 2017).

Some unwanted and unregulated invertebrates such as crinoids and ophiuroids form the basis of essential fish habitats for commercial species such as hake and red mullet. Robust information on discard survival after fishing and release to the seabed can improve the interpretation of the role of unregulated invertebrates on the benthos (Benoît et al. 2012).

The main objective of this paper was to estimate survival rates of invertebrates discarded from trawlers working in two northwestern Mediterranean areas, the Catalan coast and the Ligurian and northern Tyrrhenian seas. The study focused on unwanted invertebrates which belong to the unregulated species and are likely to continue to be released after capture. To estimate the vitality rates, a vitality assessment on the captured organisms was carried out under normal fishing activity of the trawl fleets in both selected areas. The approach was developed using a semi-quantitative assessment (SQA) (ICES 2014) according to Benoît et al. (2012). Two different procedures of survival estimation were developed, considering first the survival on the deck of trawlers and second the long-term survival at the laboratory with captive observations. The survival rate of the discarded fraction in the trawl catch can be used to propose man-

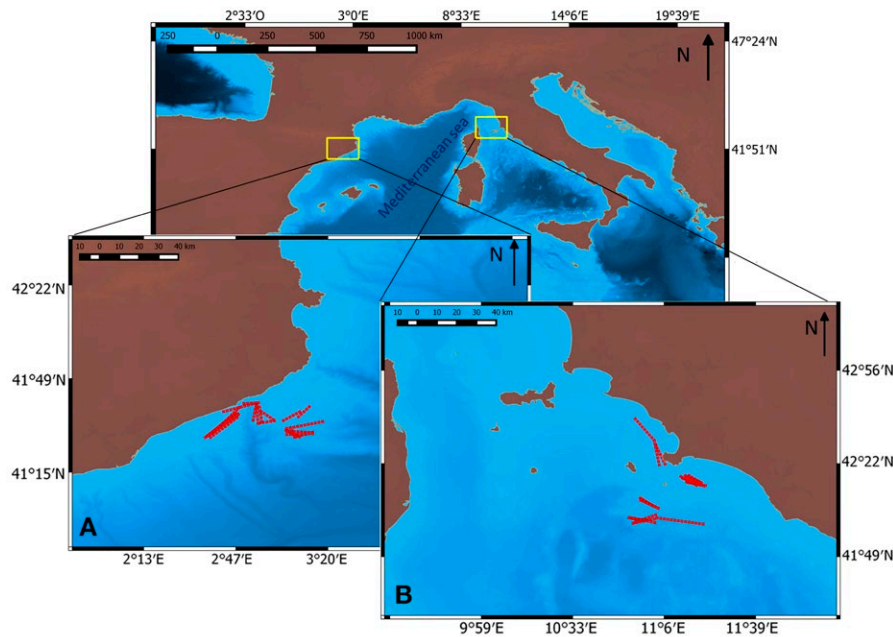


Fig. 1. – Study areas: A, Catalan coast; and B, Ligurian and northern Tyrrhenian seas. Red lines indicate hauls in the selected fishing grounds.

agement based on better control of discards or spatial restrictions to trawling that may accompany specific management plans based on conserving the functionality and health of benthic ecosystems.

MATERIALS AND METHODS

Study areas and sampling activities

The study was carried out in two NW Mediterranean trawl fishing areas, the Catalan coast (GSA 6), from March 2016 to February 2017, and the Ligurian and northern Tyrrhenian seas (GSA 9), from November 2016 to February 2017. Data were collected during fishing trips on board commercial vessels performed on a monthly basis.

Trawl sampling in the Catalan coast area was performed on board four different trawlers in five fishing grounds (Garotes, Las 40, Capets, Planassa and Malica) adjacent to the port of Blanes. The depth range was between 50 and 494 m, with a total of 23 hauls (Fig. 1A; Table 1). In the Ligurian and northern Tyrrhenian seas, 22 commercial hauls were carried out in a bathymetric range of 85–470 m in the Monte Argentario area (Fig. 1B; Table 1).

Table S1 of the Supplementary Material details the main characteristics and environmental data of each haul in the two selected areas. In both areas the average haul duration was about two hours.

At the end of each haul the trawl gear was retrieved on board and the cod-end was opened on the deck following the normal commercial fishing practices. After that, prior to sorting the catch into commercial and discard fractions, the net was shot for a new haul. Depending on the depth, this process took 10 to 25 minutes. During the fishing trips, there was no interference by the researchers on board with the habitual *modus operandi* of the fishermen in the daily fishing activity (position, duration, sorting, etc.) and the sorting processes of the catch, which lasted 20–30 minutes depending on the capture.

Survival experiments

The hauls considered for survival analysis were 4 on the Catalan coast and 19 in the Ligurian and northern Tyrrhenian seas (Supplementary material Table S1A). The average depth range was between 99 and 362 m on the Catalan coast and between 84 and 470 m in the Ligurian and northern Tyrrhenian seas. The

Table 1. – General information on trawl sampling in the study areas.

Study area	Fishing ground	No Hauls	Min. Depth (m)	Max. Depth (m)	Target species
Catalan coast (GSA06)	Las 40	3	85	120	Red mullet, monkfish, hake, octopus and sea cucumber
	Capets	2	70	113	Red mullet, monkfish, hake, octopus and sea cucumber
	Planassa	6	86	318	Red mullet, sea cucumber, hake and monkfish
	Garotes	2	55	107	Red mullet and pandora
	Malica	9	195	494	Norway lobster
Ligurian and northern Tyrrhenian seas (GSA09)	Argentario	22	85	470	Hake, red mullet, horned octopus and deep-water pink shrimp

Table 2. – Description of vitality levels of invertebrate organisms for a semi-quantitative assessment approach by groups of likely animals (From ICES 2014 and Demestre 2012).

Vitality levels	Code	Crustaceans	Echinoderms (Ophiuroidea and Asteroidea)	Echinoderms (Echinoidea)	Mollusca	Sessile (ascidians, corals, hydroids, etc.)
Excellent	1	Continued movement; no external injury.	Continued movement; no external injury.	Continued movement; no external injury.	Continued movement; no external injury.	Shape and size similar to natural state. No external injury.
Good	2	Weak movement; responds to contact; no external injury or superficial cuts on the exoskeleton or antennae.	Weak movement; responds to contact; no external injury or superficial cuts in limbs.	Weak movement; responds to contact; no external injury or few broken spines	Weak movement; responds to contact; scraping of shell or moderate loss of tegument.	Size and shape moderately different to natural state; moderate cuts or abrasions.
Poor	3	No apparent movement, but can move antennae or maxillipeds; loss of a member or deep cuts.	No apparent movement, but can move tube feet; deep cuts and loss of all or part of extremities.	No apparent movement, but can move tube feet; external injury and many broken spines.	No apparent movement, but can move feet, loss of parts of the shell or limbs.	Shape and size different to natural state; surface or serious cuts or abrasions; loss of body parts.
Dying or dead	4	No movement; does not respond to contact.	No movement; does not respond to contact; loss of central parts of body.	No movement; does not respond to contact; or broken shell.	No movement; does not respond to contact; or broken shell.	Loss of central parts of the body.

hauls were carried out on both the continental shelf and the upper slope, with a standard deviation of 117.66 and 152.89 m, respectively. The complexity of the experiment forced us to limit the number of hauls. While the catch was being sorted manually on deck by the fishermen, in both areas the vitality of the unregulated, non-commercial invertebrates was assessed just before the species were discarded. The SQA (ICES 2014) was performed by means of indicators of state of vitality according to mobility, injuries and lesions suffered by the organisms due to the fishing activity. To estimate the vitality of each individual, a categorical assessment scale of four vitality levels (VL) was applied: 1 (excellent), 2 (good), 3 (poor) and 4 (dying or dead) (Benoît et al. 2012, ICES 2017). A detailed explanation of each VL is presented in Table 2.

Two approaches to performing the survival experiments were developed: i) direct survival estimation on deck and ii) survival estimation at the laboratory for a period of 96 hours. In both study areas the studied invertebrates are only captured by trawling. Because of the challenges of obtaining viable control samples with other gears, the individuals who were classified in excellent state of vitality (Table 1) at the start of the captive experiment (time T₀, just as it was being released on deck) were taken as pseudo-controls for each haul (ICES 2014).

Survival estimation on deck

In both areas the immediate survival on deck was estimated following the same methodology. For the selected species, as many individuals as possible were taken and one of the four VLs was assigned to each one using the SQA (Table 2). The selection process was carried out only during the first 30 minutes after opening the net on the deck, starting before the gear was shot for a new haul. It was done as quickly as possible to decrease as far as possible the time of air exposure on deck.

Survival at the laboratory

The long-term survival rate was estimated for 96 h to achieve a deeper knowledge of the actual survival of the invertebrates discarded. It was only performed in the Catalan coast area. To analyse survival at the laboratory, the individuals of the selected species were sampled from the last daily haul, just when the catch was laid on deck and prior to sorting, but only during the first 30 minutes, as in the previous case. The VL was assessed according to the SQA (Table 2). The four possible VLs were observed for each specimen, which were individually introduced in four white containers (one for each VL), with running sea surface water to avoid hypoxia. The maximum number of selected animals was that which could be introduced in each container without causing stress. A total of 324 individuals from 22 species of invertebrates (10 echinoderms, 8 crustaceans, 2 cnidarians and 2 ascidians) were sampled but only the 6 most abundant species were selected for this survival assessment.

Survival at the laboratory was estimated for 96 hours in an aquarium at the ICM laboratory by applying an SQA. A total of ten time survival observations (T) were carried out during the experiment (from T₀ to T₉). The first observation, time T₀, just as animals were being released on deck, was executed on board. Individuals were selected for a maximum time of 30 minutes, and each one was introduced successively into the containers, as explained above. The second observation, T₁ (6 h), was performed just before each individual was transferred to the aquariums at the laboratory. The individuals that were in VL 4 (dead or moribund) were removed to avoid contamination in aquariums, but were accounted for. The following eight observation times, from T₂ (18 h) to T₉ (96 h), were made on the specimens placed in the aquariums, with a periodicity of 12 h until the study had lasted for 96 h. The aquariums were divided into three sections for each VL, 1, 2 and 3, and no more than ten

individuals were introduced per section. The time of the transport from the sea to the aquariums was a maximum of 2 hours and the animals were on the white plastic containers with oxygen pills during the whole transportation time.

The natural environment conditions were simulated in the aquariums through an open seawater circuit and water temperature was maintained between 13°C and 14°C, similar to the in situ temperature in the north-western Mediterranean fishing grounds. The photo-period was adapted to the natural luminosity with black canvas to dim the light. Controls of salinity, nitrates, nitrites and silicates were periodically performed. The whole process was carried out under food abstinence conditions.

Data analysis

The survival on deck was estimated by applying the survival index, which was calculated as the ratio between the number of specimens (VN) with a vitality level of 1 to 4 and the total number of discarded individuals (DN) and was expressed as a percentage.

A Wilcoxon test between the exploratory variables recorded (Supplementary material Table S1) and the

survival of invertebrates on deck for the first 30 min on board was carried out. The test analyses the relationship of each variable with survival, comparing the data related to live individuals (VLs 1, 2 and 3) with data of dead individuals (VL 4). Finally, if significant differences were observed between the variable and the survival, the group mean was calculated. The Wilcoxon test was implemented in R 3.4.3 (R Core Team 2017).

Because a seasonal sampling was not performed, variables related to seasonality such air and water temperature were not taken into account. We therefore conducted the analysis only with Depth and Haul Time.

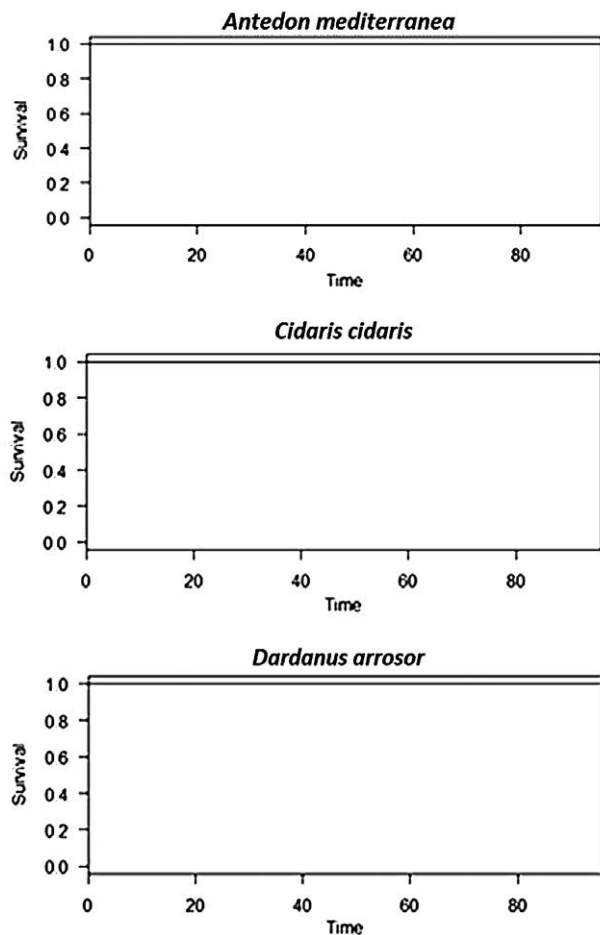
To calculate survival rate for each experiment over 96 h, the Kaplan-Meier analysis was used (Kaplan and Meier 1958). The Kaplan-Meier survival curve is a function of the data only, and in the absence of censored values it follows the proportion of individuals alive at each time interval during the holding phase of the experiment and seeks a point in time when survival stabilizes. Plots were made on six selected species for the three live VLs, showing their 95% confidence intervals. Analyses were conducted with the “survival” package in R 3.4.3 (R Core Team 2017).

Table 3. – Vitality levels (VL) and survival index of discarded invertebrates estimated on deck: A, Catalan coast; B, Ligurian and northern Tyrrhenian seas.

		Species	VL 1	VL 2	VL 3	VL 4	Total alive	Total assessed	Survival index		
A, Catalan coast											
Continental shelf	Crinoid bed	<i>Antedon mediterranea</i>	19	2	8	0	29	29	100		
		<i>Leptometra phalangium</i>	17	37	86	12	140	152	92.11		
		<i>Astropecten aranciacus</i>	0	2	4	0	6	6	100		
		<i>Echinus melo</i>	4	5	7	5	16	21	76.19		
		<i>Cidaris</i> sp.	2	3	7	0	12	12	100		
		<i>Spinolambrus macrochelos</i>	3	0	1	0	4	4	100		
	Muddy	<i>Echinaster sepositus</i>	9	3	4	0	16	16	100		
		<i>Anseropoda placenta</i>	0	0	6	0	6	6	100		
		<i>Astropecten irregularis</i>	1	1	0	0	2	2	100		
		<i>Diazona violacea</i>	4	0	0	0	4	4	100		
		<i>Liocarcinus depurator</i>	1	0	1	1	2	3	66.67		
		<i>Microcosmus sulcatus</i>	2	0	0	0	2	2	100		
		<i>Ophiura texturata</i>	0	0	38	0	38	38	100		
		<i>Macropipus tuberculatus</i>	4	0	2	0	6	6	100		
		Slope	Muddy	<i>Dardanus arrososor</i>	5	7	0	0	12	12	100
<i>Goneplax rhomboides</i>	2			0	0	0	2	2	100		
<i>Monodaeus couchii</i>	1			0	1	0	2	2	100		
<i>Munida intermedia</i>	0			0	4	2	4	6	66.67		
<i>Nephrops norvegicus</i>	72			143	222	354	437	791	55.25		
Total	147			204	387	375	738	1113	66.3		
B, Ligurian and northern Tyrrhenian seas											
Continental shelf		<i>Astropecten aranciacus</i>	0	0	3	0	3	3	100		
		Galeodea spp.	0	84	0	0	84	84	100		
		<i>Goneplax rhomboides</i>	7	0	0	236	7	243	2.88		
		<i>Macropodia</i> spp.	45	60	0	0	105	105	100		
		<i>Medorippe lanata</i>	21	20	6	955	47	1002	4.69		
		<i>Ophiura</i> spp.	0	3	0	0	3	3	100		
		<i>Parapenaeu slongirostris</i>	10	4	10	519	24	543	4.42		
		<i>Processa</i> spp.	0	0	2	0	2	2	100		
		<i>Solenocera membranacea</i>	88	134	187	8923	409	9332	4.38		
		<i>Squilla mantis</i>	45	103	114	1863	262	2125	12.33		
		Slope		<i>Dardanus arrososor</i>	4	10	0	28	14	42	33.33
				<i>Pagurus</i> spp.	17	17	6	0	40	40	100
				<i>Astropecten irregularis</i>	0	2	2	8	4	12	33.33
				<i>Macropipus tuberculatus</i>	22	31	32	187	85	272	31.25
				<i>Munida intermedia</i>	2	0	4	13	6	19	31.58
<i>Nephrops norvegicus</i>	2			5	25	173	32	205	15.61		
<i>Pagurus excavatus</i>	5			16	18	452	39	491	7.94		
<i>Paromola cuvieri</i>	0			0	2	0	2	2	100		
Total	268	489	410	13357	1167	14524	46.41				

Table 4. – Percentages of the four vitality levels in each study area for the two species of invertebrates subjected to minimum conservation reference sizes. CC, Catalan coast; LTS, Ligurian and northern Tyrrhenian seas.

	<i>N. norvegicus</i> (CC)	<i>N. norvegicus</i> (LTS)	<i>P. longirostris</i> (LTS)
Excellent (1)	9.10	0.98	1.84
Good (2)	18.08	2.44	0.74
Poor (3)	28.07	12.20	1.84
Dying or dead (4)	44.75	84.39	95.58

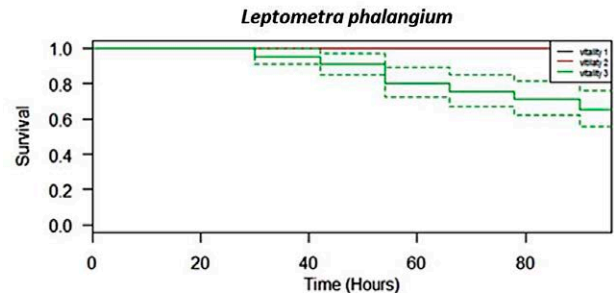
Fig. 2. – Analysis with the K-M model to assess the survival of *Antedon mediterranea*, *Cidaris cidaris* and *Dardanus arrosor*.

RESULTS

Survival on deck

The VLs of discarded invertebrates were identified for each individual of each selected species. Table 3 shows the results of each VL analysed in each study area, according to the continental shelf and slope and the corresponding habitat. From the discarded fraction, the species presented in Table 3 were scored with vitality levels in both study areas. Among the invertebrates captured, only *N. norvegicus* and *P. longirostris* were subject to minimum conservation reference sizes (MCRS) (Council Reg. EC 1967/2006), while the rest of the discarded invertebrates were non-regulated species.

A survival index of 100% was shown by 14 species in the Catalan coast area and 6 in the Ligurian

Fig. 3. – K-M model to assess the survival of *Leptometra phalangium* at vitality levels 1, 2 and 3.

and northern Tyrrhenian seas area, and *Astropecten aranciatus* and the genus *Ophiura* were common in both areas. The species *Munida intermedia*, *Goneplax rhomboids* and *Macropipus tuberculatus* showed a lower survival index in the Ligurian and northern Tyrrhenian seas area, where the processed number of individual was higher and the results were probably more reliable. On the other hand, *N. norvegicus* gave more robust results in the Catalan coast area, where more individuals were analysed.

The percentages of the four vitality levels (VL) of Norway lobster and deep water rose shrimp, the two commercial species subjected to MCRS, are presented in Table 4 for both areas. Values of each VL represent the percentage of the total number of individuals for each level from the total analysed hauls. The Catalan coast area showed the highest percentages of live VLs.

The Wilcoxon test was carried out only with the variables Haul Time and Depth. The results showed no significance between depth and survival. However, the test found significant differences in survival due to Haul time with a p-value <2.2e-16 and W=7898500. The mean duration of hauls with live animals was 217.39 min, while the mean duration with dead animals was 236.12 min (i.e. a 9% time increase).

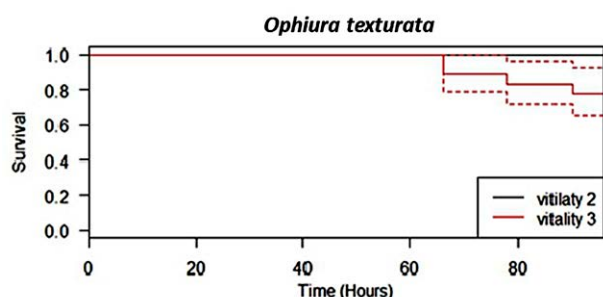
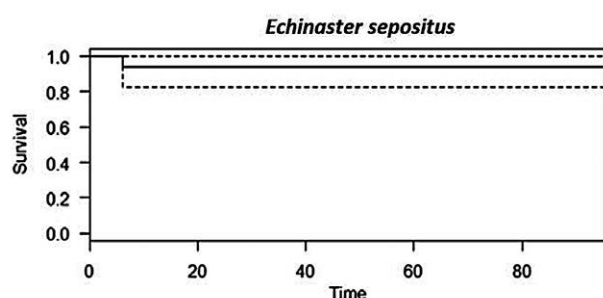
Survival at the laboratory

The survival estimation at the laboratory was carried out only for the specimens collected in the Catalan coast study area. The analyses were undertaken only with the invertebrate species with a higher number of individuals scored with VL on deck previous to the discard (Table 3A); a total of six species were analysed. Of the six invertebrates analysed, the three in Figure 2 showed 100% survival for 96 h in the aquarium experiment.

The species *Leptometra phalangium* showed more than 90% survival, and only after 30 h did the percent-

Table 5. Results of K-M model to analyse the survival of *Leptometra phalangium* (A and B), *Ophiura texturata* (C and D), *Echinaster sepositus* (E); n.risk, live animals; n.event, dead animals.

time	n.risk	n.event	survival	std.err	lower 95% CI	upper 95% CI
A. Longitudinal survival over 96 hours and three levels of vitality						
0	152	12	0.921	0.0219	0.879	0.965
30	140	4	0.895	0.0249	0.847	0.945
42	136	4	0.868	0.0274	0.816	0.924
54	132	9	0.809	0.0319	0.749	0.874
66	123	4	0.783	0.0334	0.720	0.851
78	119	4	0.757	0.0348	0.691	0.828
90	115	5	0.724	0.0363	0.656	0.798
B. Mortality events at the laboratory at vitality levels 2 and 3 at T0 (0.0H.=3)						
30	86	4	0.953	0.0227	0.910	0.999
42	82	4	0.907	0.0313	0.848	0.970
54	78	9	0.802	0.0429	0.722	0.891
66	69	4	0.756	0.0463	0.670	0.852
78	65	4	0.709	0.0490	0.620	0.812
90	61	5	0.651	0.0514	0.558	0.760
C. Longitudinal survival over 96 hours at the three vitality levels						
66	38	4	0.895	0.0498	0.802	0.998
78	34	2	0.842	0.0592	0.734	0.966
90	32	2	0.789	0.0661	0.670	0.930
D. Mortality events at the laboratory at vitality level 3 at T3 (T3.28.5H.=3)						
66	36	4	0.889	0.0524	0.792	0.998
78	32	2	0.833	0.0621	0.720	0.964
90	30	2	0.778	0.0693	0.653	0.926
E. Longitudinal survival over 96 hours at the three vitality levels						
6	16	1	0.938	0.0605	0.826	1

Fig. 4. – K-M model to assess the survival of *Ophiura texturata* at vitality levels 2 and 3.Fig. 5. – K-M model to assess the survival of *Echinaster sepositus* at vitality level 3.

age of mortality increase (Fig. 3; Table 5A, B) in the specimens with the VL 3. VLs 1 and 2 showed no evidence of mortality, but VL 3 showed an evident non-stability of survival.

Similar results were shown for the species *Ophiura texturata*. In this case the first evidence of mortality appeared after 66 h in VL 3, but the percentage of survival was still high (>79 %). VLs 1 and 2 showed no evidence of mortality (Fig. 4, Table 5C, D).

The last species studied in the aquarium was *Echinaster sepositus*, which showed mortality at 6 h of the experiment in VL 3, but maintained a steady survival rate >90% until the end of the experiment (Fig. 5 and Table 5E).

DISCUSSION

This study was carried out with those individuals that showed signs of vitality when arriving on board, which means they were still alive. In fact, there was a low number of specimens that could be assessed, and this may indicate the severe impact of trawling on the seabed and benthic communities (Kaiser and Spencer 1995, Jennings et al. 2001). The preservation of exploited resources is probably the main goal of fishery management, but the perturbation of chronic fishing activity on fishing grounds has negative ecological effects leading to high levels of mortality (DFO 2006, van Denderen et al. 2013).

Several studies have evidenced an improvement in the health of exploited resources when effort limitation and seasonal or temporal closures of trawl fishing activities are implemented (Demestre et al. 2008, Demestre et al. 2015, Pipitone et al. 2000), but the effects at the level of benthic communities remain less well known. The by-catch of invertebrates in bottom trawling yields a high amount of epifauna or infauna that have important functions for the sea floor ecology. For instance, echinoderms or gastropods are important bioturbators and comprise several feeding guilds, such as deposit or filter feeders, or predators (e.g. *Echinus melo*, *Spartangus purpureus*, *Echinaster sepositus*, *Ophiura texturata*, *Chlamys opercularis*, *Calliostoma granulatum* and *Aporrhais serresianus*). These organisms play an important role in ecosystem function by maintaining

or enhancing secondary marine production. They are very sensitive to disturbance and easily destroyed by fishing impact, and their decrease could have lasting consequences for benthos-pelagic processes (Lohrer et al. 2004, Demestre et al. 2017, de Juan et al. 2011), because the good status of the habitats in which the fisheries resources live depends to a large extent on these organisms.

In order to maintain the good status of the sea bottom, one of the priority actions to be taken is to determine the mortality levels of routinely discarded species. A study carried out near the Catalan coast area related the effects of trawl fishing and feeding of the red mullet *Mullus barbatus* (Muntadas et al. 2015), showing negative effects due to changes in benthic functional components in the fishing ground. In areas where there was no fishing (fishing closure areas) the macroinfauna which constitutes the food base for *M. barbatus* was significantly more abundant than in areas disturbed by the trawl. Changes in the habitat structure (homogenization) and functionality of benthic communities caused by fishing can alter the normal supply of food (e.g. polychaetes and crustaceans) for both adult and juvenile red mullet (Fiorentino et al. 2008, Muntadas et al. 2014). Furthermore, as a consequence of the habitat alteration, the characteristics of the seabed that serves as a nursery, spawning or growing habitat could be modified, with possible negative consequences on future recruitment of the species.

The rates of survival shown by invertebrates in both areas investigated in this study showed great variability between VLs of the same species once the individuals had been captured and deposited on the deck of the trawler. Mortality levels also vary from one species to another, depending mainly on the biological and functional traits of each species, such as fragility, emergent or surface position, filter feeding and sedentary motility (Costello et al. 2015, Muntadas et al. 2015). External protection is one of the most relevant traits for increasing survival, as evidenced by the monitoring of VLs on deck to analyse immediate mortality. In both areas the majority of crustaceans remained alive, even reaching percentages of 100%. Invertebrates with regeneration traits such as echinoderms also have a high level of survival.

We went one step further in estimating discarded invertebrate mortality by attempting to identify and separate the injuries of each individual on deck according to its VL. Individuals with VL 1 and 2 at time T0 (time of release on deck) survived on deck until they were released into the sea in a maximum time of 30 min, but those with VL 3 showed low survival on deck. The experiments at the laboratory to analyse survival at 96 h confirmed this behaviour for all analysed species. At the laboratory it was evident that when the survival was not 100% it was because the organism was at VL 3 when released on deck, and in fewer cases at VL 2.

The results of the Wilcoxon test indicated that Haul Time was an important factor for improving organisms' survival on deck. Injuries increased and VL decreased when invertebrates arrive on deck after long hauls, as was observed in the continental shell hauls,

which showed a higher survival of species of crinoids and crustaceans (Table 4 and Figs 3-5). Consequently, failure of individuals to survive for a long time in the laboratory experiment is due to their low VL when they were left on deck. It is therefore important to handle the organisms on deck quickly and safely to increase their survival when they are discarded back into the sea. During fishing operations on deck, it is recommendable to keep the organisms under a wet cover to avoid drying. Another easy method for improving the survival of discards could be a direct operating system such as a duct with water from the deck for throwing animals back into the sea.

It must be taken into account that, in addition to the unhealthy state of the invertebrates who died during the experiment, the mortality may also have been due to the captivity conditions, where no food was available and the environment was only similar to the most appropriate habitat. However, the possibility of discarded invertebrates escaping predators or obtaining food is low because of the injuries they suffer during capture (Ramsay et al. 1996, Bergmann and Moore 2001, Ingólfsson et al. 2007). Therefore, our experimental results can be assumed as a proxy to the level of survival of discards at sea.

According to the Common Fisheries Policy and the landing obligation to prevent discarding of regulated species (MCRS, Council Reg. EC 1967/2006, Art. 15.4b), a high level of post-capture survival can be adduced by member states to include an exemption from the landing obligation in their discard management plans. Our results for the survival of *N. norvegicus* can be considered a starting point with information focused on the aim of a possible species exemption but, obviously, more studies based on larger samplings need to be carried out before this exemption can be recommended. Conversely, a second crustacean species regulated by MCRS, *Parapenaeus longirostris*, showed low survival and would not be a good candidate for exemption.

The species selected for survival estimations were the most representative of different taxonomic levels and were of ecological importance in their habitats. In view of this, the 100% survival of the crinoids *A. mediterranea* and even *L. phalangium*, whose increase in post-release mortality started at VL 3, shows that the impact of trawling on crinoid beds may be less serious than assumed until now, as most crinoid individuals would survive the encounter with trawlers and post-catch release. Furthermore, in many cases crinoid beds are essential habitats for nursery and spawning areas of some commercial species (Colloca et al. 2004). The other two echinoderms that were assessed, *O. texturata* and *E. sepositum*, gave similar results, both starting mortality at VL 3. Therefore, the results may suggest again an optimistic possibility for maintaining a good environment status and a sustainable structure on the soft-sediment habitats that form the majority of trawl fishing grounds (Piet and Hintzen 2012). However, to maintain this optimistic perspective and a good environment status on the Mediterranean fishing grounds, it is mandatory to contain the current exploitation

levels, especially in other types of habitat that may be even more sensitive to trawling than crinoid beds, such as maërl and *Isidella* (Kamenos et al. 2004, Mastroianni et al. 2017). To achieve this, fishing activity and fishing effort must be reduced, temporal and spatial closures and even permanent closed areas must be implemented, and the measures regulating the reduction of discards must be implemented (FAO 2011).

The results of the present work offer some new knowledge on the survival of discarded invertebrates that may be useful for improving ecosystem health and productivity. Nevertheless, it should be regarded as a starting point, because mortality after discards at sea depends on many factors, such as susceptibility to predation and lower competitiveness for obtaining food (Bergmann and Moore 2001, Demestre et al. 2000, Kaiser et al. 2006). Knowing levels of survival of discarded invertebrates helps to obtain a more realistic image of the state of the benthic ecosystem, and consequently of the fishing grounds. The sustainability of the exploited populations depends on the conservation of these habitats, because a large part of their life cycle takes place in them.

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SUPPLEMENTARY MATERIAL

The following supplementary material is available through the on-line version of this article and at the following link:
<http://scimar.icm.csic.es/scimar/supplm/sm04784esm.pdf>

Table S1. – Information recorded during each haul in the two study areas.

Ecological importance of survival of unwanted invertebrates discarded in different NW Mediterranean trawl fisheries

Montserrat Demestre, Paolo Sartor, Alfredo Garcia-de-Vinuesa, Mario Sbrana,
Francesc Maynou, Andrea Massaro

Supplementary material

Table S1. – Information recorded during each haul in the two study areas. A, Catalan coast; B, Ligurian and northern Tyrrhenian seas. Hauls used for survival analysis in bold.

A	HaulNum	Date	Haulstart- ing time (h)	HaulDura- tion (min)	Avg Depth (m)	WindDir	Wind speed (km h ⁻¹)	Watersurfac- eTemp °C	Air Temp °C	Cloud- level (1-8)	SweptArea km ²	LATend	LATStart	LONGEnd	LONGStart	
Catalan Coast Continental shelf	1	21/03/2016	7:05	88	61.90	N-E	9	13.8	14	1	0.0962204	41.39.551	2.49.419	41.39.815	2.55.135	
	2	21/03/2016	9:09	196	92.90	N-E	12	13.8	17	1	0.2143091	41.38.281	2.55.039	41.32.096	2.55.989	
	3	22/03/2016	8:05	189	86.87	N	15	13.8	14.0	3	0.1082829	41.35.967	2.46.437	41.29.749	2.38.475	
	4	22/03/2016	11:10	270	85.77	N	14	13.8	19	2	0.1546898	41.28.988	2.38.230	41.38.390	2.48.060	
	5	21/04/2016	7:35	145	206.65	N	10	14	17	7	0.0830742	41.30.450	2.53.684	41.32.694	2.52.289	
	6	23/05/2016	7:35	206	126.55	N	7	15.9	16	1	0.1180226	41.33.425	3.03.749	41.36.895	3.10.201	
	7	15/07/2016	6:40	250	98.76	N	15	21.7	12	1	0.1432313	41.34.406	2.46.840	41.26.711	2.36.628	
	8	09/08/2016	7:30	125	117.04	N-E	3	22	24	1	0.1489606	41.32.783	2.58.6090	41.36.167	2.47.824	
	9	09/08/2016	12:15	165	97.84	N-E	4	22	30	1	0.0515633	41.32.760	2.58.820	41.32.600	3.00.030	
	10	10/08/2016	11:03	260	102.41	S-E	7	22	24	5	0.1432313	41.28.088	2.36.615	41.27.893	2.36.157	
	11	11/08/2016	6:54	75	83.21	N	9	22	24	6	0.0945327	41.35.528	2.46.138	41.35.420	2.54.570	
	12	30/11/2016	7:25	158	96.01	N	10	16	9	4	0.0905222	41.36.710	2.55.612	41.33.544	3.00.401	
	13	30/11/2016	10:30	144	138.07	N	10	16	16	2	0.0825012	41.33.348	3.00.333	41.32.418	2.54.525	
	14	30/11/2016	13:20	130	227.76	N	7	16	13	2	0.0744803	41.32.418	2.54.525	41.39.341	2.53.939	
Catalan Coast Slope	1	24/05/2016	8:30	350	315.47	W	2	15.9	20	1	0.4422270	41.33.016	3.17.870	41.30.396	3.04.074	
	2	13/06/2016	8:10	110	362.10	E	2	20.3	20	6	0.1389856	41.29.040	3.04.563	41.27.610	3.11.313	
	3	14/06/2016	12:30	130	363.93	E	7	20.3	23	2	0.1642557	41.27.645	3.11.175	41.30.292	3.04.659	
	4	30/06/2016	7:47	120	361.19	E	3	20.3	21	3	0.1516207	41.29.835	3.04.756	41.27.378	3.09.675	
	5	30/06/2016	10:38	118	355.70	E	12	20.3	28	1	0.1490937	41.27.472	3.08.312	41.30.970	3.06.147	
	6	30/06/2016	13:22	110	363.02	E	14	20.3	28	1	0.1389856	41.30.355	3.04.869	41.27.147	3.08.237	
	7	19/09/2016	8:20	145	358.44	N	7	22.5	24	1	0.1832083	41.29.520	3.04.975	41.28.964	3.15.186	
	8	20/09/2016	7:42	188	320.04	N	5	22.5	23	8	0.2375391	41.30.861	3.06.383	41.32.686	3.16.923	
	9	04/10/2016	8:57	358	342.90	N-E	7	22.6	20	4	0.4523351	41.34.274	3.17.754	41.29.628	3.04.433	
Thyrrhenian Continental Shelf	1	30/11/2016	13.45	90.00	112.00	NE	10.5	14.89	17.45	300.0	0.19265	42.14.353	42.15.843	11.20.701	11.14.209	
	2	30/11/2016	16.00	90.00	97.30	S	1.8	15.60	17.30	50.0	0.20658	42.18.533	42.15.531	11.14.321	11.19.519	
	3	01/12/2016	13.25	95.00	112.00	NE	6.0	14.81	17.40	100.0	0.19942	42.14.479	42.15.580	11.21.145	11.14.680	
	4	01/12/2016	15.40	70.00	101.00	E	5.5	14.93	17.40	25.0	0.15438	42.17.864	42.15.876	11.14.161	11.18.576	
	5	14/12/2016	3.30	95.00	112.00	E	7.0	14.85	17.43	8.0	0.20336	42.14.687	42.17.104	11.19.256	11.12.857	
	6	14/12/2016	15.15	105.00	109.00	S	6.5	14.71	16.95	13.0	0.0	0.23157	42.18.161	42.14.894	11.12.862	11.18.452
	7	15/12/2016	3.25	105.00	109.00	E	6.5	14.71	16.95	9.0	0.0	0.22476	42.14.455	42.17.771	11.18.051	11.12.094
	8	15/12/2016	15.20	100.00	105.00	N	3.6	14.72	17.00	14.0	20.0	0.23072	42.18.658	42.14.748	11.13.081	11.17.619
	9	26/01/2017	10.05	185.00	84.00	E	9.0	13.87	14.00	9.0	400.0	0.27143	42.21.729	42.29.289	11.06.602	11.02.910
	10	26/01/2017	13.44	204.00	92.00	S	5.0	13.84	14.10	14.0	300.0	0.31233	42.31.971	42.21.995	11.02.132	11.04.209
	11	26/01/2017	1.20	249.00	85.00	E	10.0	13.47	13.70	3.0	0.0	0.41849	42.39.602	42.77.830	10.55.204	11.02.800
	12	26/01/2017	6.09	201.00	84.00	E	11.0	13.40	13.95	1.5	0.0	0.32450	42.30.330	42.38.750	11.02.866	10.55.496
Thyrrhenian Slope	1	30/11/2016	7.30	95.00	230.00	NE	8.0	14.32	16.40	4.5	0.22745	42.10.349	42.07.173	10.56.611	11.01.718	
	2	30/11/2016	10.00	102.00	250.00	NE	18.0	14.27	16.47	8.0	0.22568	42.06.150	42.08.978	11.04.140	10.58.001	
	3	01/12/2016	7.03	97.00	238.00	E	10.0	14.28	16.40	1.0	0.0	0.22823	42.10.453	42.06.970	10.56.615	11.02.047
	4	01/12/2016	9.35	105.00	250.00	E	6.0	14.24	16.69	5.0	100.0	0.23469	42.06.198	42.09.113	11.03.814	10.57.476
	5	14/12/2016	7.55	85.00	425.00	E	4.5	14.11	16.20	7.5	30.0	0.18644	42.02.900	42.01.891	10.52.434	10.58.365
	6	14/12/2016	10.30	90.00	428.00	E	1.8	14.10	16.00	12.5	160.0	0.19160	42.02.390	42.00.804	11.02.701	10.54.763
	7	15/12/2016	10.40	120.00	418.00	NE	5.0	14.11	16.00	12.0	380.0	0.25547	42.03.887	42.01.249	11.03.190	10.55.510
	8	15/12/2016	8.00	90.00	433.00	E	7.2	14.11	16.00	9.0	90.0	0.19356	42.02.814	42.01.933	10.52.497	10.58.840
	9	16/02/2017	6.55	259.00	420.00	E	9.5	14.11	16.10	3.0	250.0	0.58481	42.02.718	42.00.486	11.03.459	11.20.189
	10	16/02/2017	12.20	180.00	470.00	SO	7.2	14.10	16.0	15.0	600.0	0.40643	42.03.032	42.01.024	10.52.760	11.02.563