



# Delayed response of hermit crabs carrying anemones to a benthic impact experiment at the deep-sea nodule fields of the Peru Basin?

Daphne Cuvelier<sup>a,\*</sup>, Mathilde Vigneron<sup>a,b</sup>, Ana Colaço<sup>a</sup>, Jens Greinert<sup>c</sup>

<sup>a</sup> Institute of Marine Sciences - Okeanos, University of the Azores, Rua Professor Doutor Frederico Machado 4, 9901-862, Horta, Portugal

<sup>b</sup> Sorbonne University, 4 place Jussieu, 75005, Paris, France

<sup>c</sup> GEOMAR –Deep-sea monitoring group, Helmholtz Centre for Ocean Research, Wischhofstrasse 1–3, 24148, Kiel, Germany

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## ABSTRACT

The deep Peru Basin is characterised by a unique abyssal scavenging community featuring large numbers of hermit crabs (*Probeebei mirabilis*, Decapoda, Crustacea). These are atypical hermit crabs, not carrying a shell, but on some occasions carrying an anemone (Actiniaria). The reason why some hermit crabs carry or not carry anemones is thought to be indicative of a changed environment, outweighing the cost/benefit of their relationship. Here we present the temporal variation of abundances of *P. mirabilis* with and without anemones, spanning more than two decades, following a benthic impact experiment. An overall decrease in hermit crab densities was observed, most noticeable and significant after 26 years and characterised by a loss of Actiniaria on the *Probeebei mirabilis*' pleon. Whether this is a delayed response to the benthic impact experiment carried out 26 years' prior or a natural variation in the population remains to be corroborated by an extension of the time-series. Attention is drawn to the limitations of our knowledge over time and space of the abyssal community dynamics and the urgent necessity to fill in these gaps prior to any type of deep-sea exploitation.

## 1. Introduction

*Probeebei mirabilis* Boone, 1926a (Decapoda, Crustacea) is amongst the deepest occurring benthic decapods of the Pleocyemata suborder and belongs to the hermit-crab superfamily Paguroidea (Riehl et al., 2020; Lemaitre and McLaughlin, 2023). Until recently, it was placed within the Parapaguridae family (Saint Laurent, 1972; Lemaitre 1998; Christodoulou et al., 2022) but Fraaije et al. (2022) reinstated the original Probeebeidae family (Boone, 1926b; Lemaitre and McLaughlin, 2023). It is an atypical hermit crab: they are freely roaming, not carrying a shell and their abdomen is armoured (carcinized) as that of a common or spiny lobster, unlike the soft abdomen regular shell-dwelling hermit crabs hide (Wolff, 1961; Lemaitre, 1998; Anker and Paulay, 2013). This hermit crab is abundantly present on the abyssal nodule fields of the deep Peru Basin (4150m depth, South-East Pacific Ocean) and dominates its scavenging community, which is unique for abyssal ecosystems (Bluhm, 1994, 2001; Drazen et al., 2019) *Probeebei mirabilis* are scarce in the more studied nodule fields of the Clarion-Clipperton Fracture Zone up north which are thus characterised by a different scavenger community (Drazen et al., 2021). In the Peru Basin, the *Probeebei mirabilis* hermit crabs are observed carrying anemones (Actiniaria, Cnidaria) on

their pleon (Drazen et al., 2019; Christodoulou et al., 2022) or, when without anemone, showing their abdomen bent underneath their bodies (Wolff, 1961). Upon the animal's first description and analysis of its systematics (Boone 1926a and b, Wolff, 1961 and reference therein, Lemaitre, 1998) this anemone carrying behaviour was not observed or described, but some of these organisms were collected by trawl and the anemones may have fell off during sampling. *P. mirabilis* are thought to actively grasp anemones for protection or housing, based on the presence of an appendage, a strong ventrodorsal spine on the propodus of the 4th pereopod, that is used by a member from the same family (*Tylaspis anomala* Henderson, 1885) for this purpose (Lemaitre, 1998). Cnidaria are among the most frequent epibionts of Crustacea and their relationship is a dynamic process between the benefits and disadvantages for the organisms involved as well as the environmental conditions (Fernandez-Leborans, 2013).

Five different cruises visited the deep Peru Basin between 1989 and 2015 where vast nodule fields were discovered in 1978 (Thijssen et al., 1981). The time-series studied here started off with the large-scale benthic impact experiment in 1989, called Disturbance and reCOLonisation experiment (DISCOL), in which the seafloor was ploughed through (Thiel and Schriever, 1990). The disturbance was monitored

\* Corresponding author.

E-mail address: [daphne.v.cuvelier@uac.pt](mailto:daphne.v.cuvelier@uac.pt) (D. Cuvelier).

with towed camera transects collecting imagery to assess the ecosystems' (non-)recovery. This disturbance experiment was carried out in a context of increased interest in deep-sea mining of polymetallic nodules, that started in the late 1970ies, featuring a number of small-scale commercial test mining or scientific disturbance studies (Jones et al., 2017). For several years the economic interest in these deep-sea ecosystems somewhat lessened in 1990s and 2000s but became very relevant again during the last decades. During the most recent cruise in 2015 to the DISCOL Experimental Area (DEA), 26 years after the disturbance, the impact was still very noticeable in faunal densities and seafloor disturbance (Simon-Lledó et al., 2019; Gausepohl et al., 2020).

Due to the abundance of the hermit crabs, and since they are easily recognisable on imagery, we hereby investigate their response to the disturbance over time and space. The hypothesis put forward is that the population has not recovered after 26 years. The peculiarity of the *P. mirabilis* individuals as to why some of them carry anemones, while others do not, is discussed in this context. By assessing their abundance over time, we can estimate impacts of the disturbance on a mobile scavenger and a sessile suspension feeder on a mobile platform, in addition to their interactions. Their possible delayed response to the impact as well as their possible role as an indicator for the Good Environmental Status (GES) is addressed.

## 2. Methodology

### 2.1. Study site

The DISCOL site received its name during the DISturbance and reCOLonisation experiment in 1989, one of the largest benthic impact experiments carried out on the abyssal deep-sea floor (Thiel and Schriever, 1990). During this experiment the nodule fields of the deep Peru Basin (at 4150m depth, Fig. 1a) were ploughed through with plough-harrow, disturbing ca. 11 km<sup>2</sup> of deep-sea floor. The main area for this disturbance experiment was called the DEA (DISCOL Experimental Area) where about 20% of the seafloor was ploughed through, thus burying the nodules and associated fauna as well as resuspending 10–20 cm of the upper sediment layer (Fig. 1c, Thiel and Schriever, 1990). The DISCOL site is located about 800 km of the Peruvian coast and features N–S striking graben and horst structures (Gausepohl et al., 2020). The central DEA area hosts gently sloping terrain with topographic range spanning 30m at most with the seafloor becoming more heterogeneous towards the north and the west with increased sloping and seamount features (Gausepohl et al., 2020). The water currents characterising the area are typically slow (<10 cm/s) and variable in direction (Klein, 1996; Baeye et al., 2022). Manganese nodules in the area have diameters of up to 15 cm and, prior to the DISCOL experiment, a nodule density estimated to be 5–10 kg/m<sup>2</sup> (Thiel and Schriever, 1990).

### 2.2. Imagery and data collection

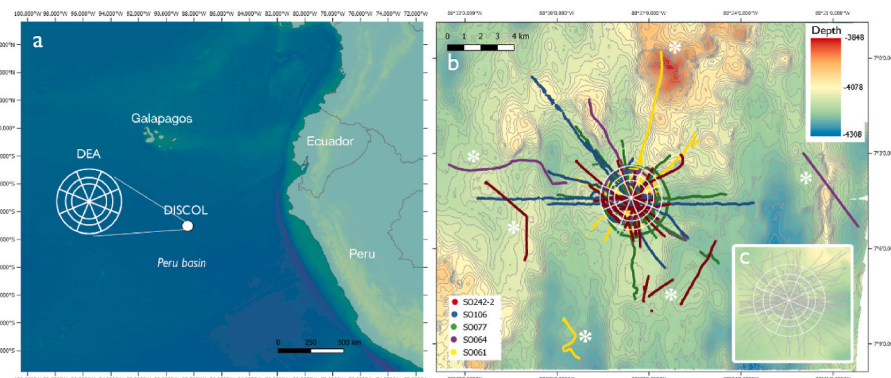
Images from five cruises (Table 1) were annotated using the Biigle online annotation software (Langenkämper et al., 2017). The first cruise was split in two legs; the first leg studied the baseline of the pre-impacted site (SO061\_Pre) and the second one imaged the seafloor immediately after the disturbance (SO061\_Post) (Table 1). Over time, images were collected with the Ocean Floor Observation System (OFOS) tow-cam, equipped with different cameras and different resolutions (Greinert, 2015; Boetius, 2015). A Benthos 372A camera was used during SO061 and SO064, a PhotoSea 5000 camera system (EXPLO-S/OFOS) was implemented during SO077 and SO106, followed by an iSITEC, CANON EOS 5D Mark III camera (AWI/OFOS) during SO242-2. However, since the organisms of interest for this study are fairly large (~5 cm), the image quality did not have an impact on organism detection and quantification. The camera system was towed at an altitude between 1.7 m and 3.5 m above the seafloor between 1989 and 1996 (Bluhm et al., 1995) and at 1.5m altitude during the most recent cruise (Boetius, 2015) (Fig. 1b). The DEA was the main area where the radial plough tracks cross and where the majority (n = 50) of the OFOS transects visualising the seafloor were carried out (Fig. 1b and c, Table 1). An additional nine transects were carried out in the so-called reference areas, situated outside of the DEA and not impacted by the plough harrow but might have been impacted by settling sediment.

A total of 41,335 images were analysed over time, available in Biigle. Organism of interest for this study was the hermit crab *Probeebei mirabilis* (Fig. 2) (Decapoda, Crustacea). This species occurs abundantly in the DISCOL area and can carry a, to date, unidentified anemone morpho-species (Actiniaria, Cnidaria) on its pleon (Fig. 2). Circle annotations

**Table 1**

Cruises to DISCOL, the year they took place and the time since disturbance (impact) as well as number of OFOS transects, number of images collected and area of seafloor visualised. Mo. = months, yrs = years.

Cruise	Year	Pre/post impact	# transects in DEA + REF	N images DEA + REF	Surface covered (m <sup>2</sup> ) DEA + REF
SO061_Pre	1989	Pre	7 + 1	3686 + 247	37272 + 2498
SO061_Post	1989	Post	9 + 1	4609 + 584	50937 + 6582
SO064	1989	6 mo post	5 + 2	3119 + 1318	35248 + 14895
SO077	1992	3 yrs post	7	5341	62526
SO106	1996	7 yrs post	7	5380	54705
SO242-2	2015	26 yrs post	15 + 5	14104 + 3919	79970 + 19595



**Fig. 1.** Location of the DISCOL site within the Peru Basin at 4150m depth and DISCOL Experimental Area (DEA, not to scale) (a), and location of OFOS images analysed across the DEA and in the reference areas (indicated with \*), outside the disturbed region (b). Inset shows the plough tracks across the DEA (c).



Fig. 2. *Probeebei mirabilis* with anemone on pleon (left) and without anemone (right) from SO242-2 (2015). Images from Purser et al. (2018).

were used to label the organisms with or without anemones, thus allowing pixel-based size estimations of the hermit crabs. The differences in sizes were addressed within each cruise year and relative differences compared between years. When hermit crab individuals were only partially visible on the image, e.g. only cephalothorax visible on the edge of an image, the individuals were not quantified in one of the categories discussed here. The seafloor surface covered ( $m^2$ ) was estimated based on image altitude and footprint.

Within the DEA various disturbance regimes were discerned, which were attributed as environmental condition labels besides the *P. mirabilis* and anemone identifications. Environmental attributes were thus *P. mirabilis* with/without anemones (i) on undisturbed surface, (ii) on track (plough track from plough harrow), (iii) next to track and (iv) on disturbed surface (sediment disturbance at surface) (Fig. 3). Undisturbed sediments were characterised by dark brown surface sediments which were fine-grained, semi-liquid silicate oozes. This surface material was nearly eliminated through the ploughing, bringing the sharp edge contoured light-grey clay from deeper layers to the surface (Borowski, 2001). Over the subsequent years, tracks were filled with soft material and their surface contours smoothed out (Borowski, 2001), but they were still very recognisable after 26 years (Simon-Lledó et al., 2019; Gausepohl et al., 2020) and the lighter coloured patches still indicated the presence of sediments originating from deeper layers (Borowski and Thiel, 1998) (Fig. 3).

### 2.3. Data analyses

All data analyses, graphs and statistical testing (correlations and t-tests) were carried out in R (R Core Team, 2020). Boxplots, stacked and unstacked barplots were created with ggplot2 (Wickham, 2016).

## 3. Results

### 3.1. Data collection

Data collection spanning more than two decades meant dealing with different (improving) camera systems, different image resolution and quality. Even within the same cruise or video transect, image quality differed substantially (Supp. Mat). Varying tow-cam altitudes, camera quality and lighting reflected by the seafloor sediments influenced image quality. Regardless of the differences in the overall colour palette of the image, brightness and other collection parameters, the size and the typical distinct shapes of both *Probeebei mirabilis* individuals and its occasional anemone companion allowed for a straightforward identification and quantification. While the imagery sampling between DEA and REF was unbalanced (more imagery transects, higher number of images and seafloor area covered in DEA compared to REF), no

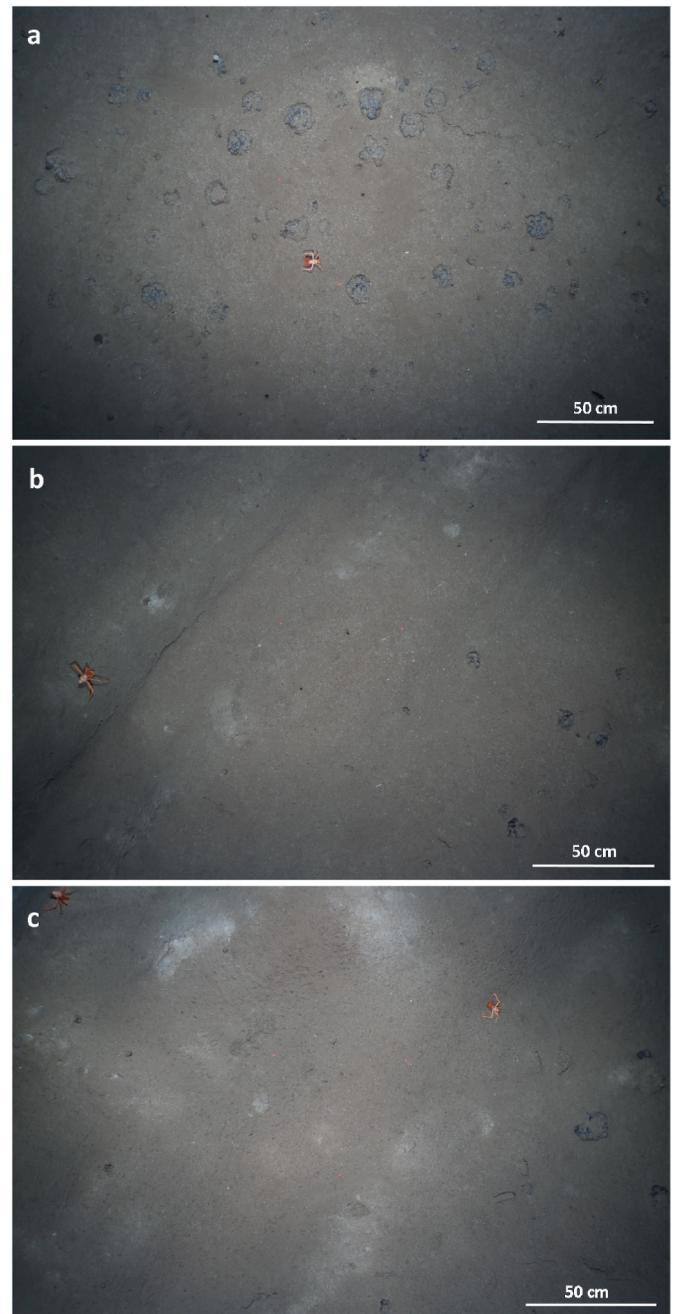
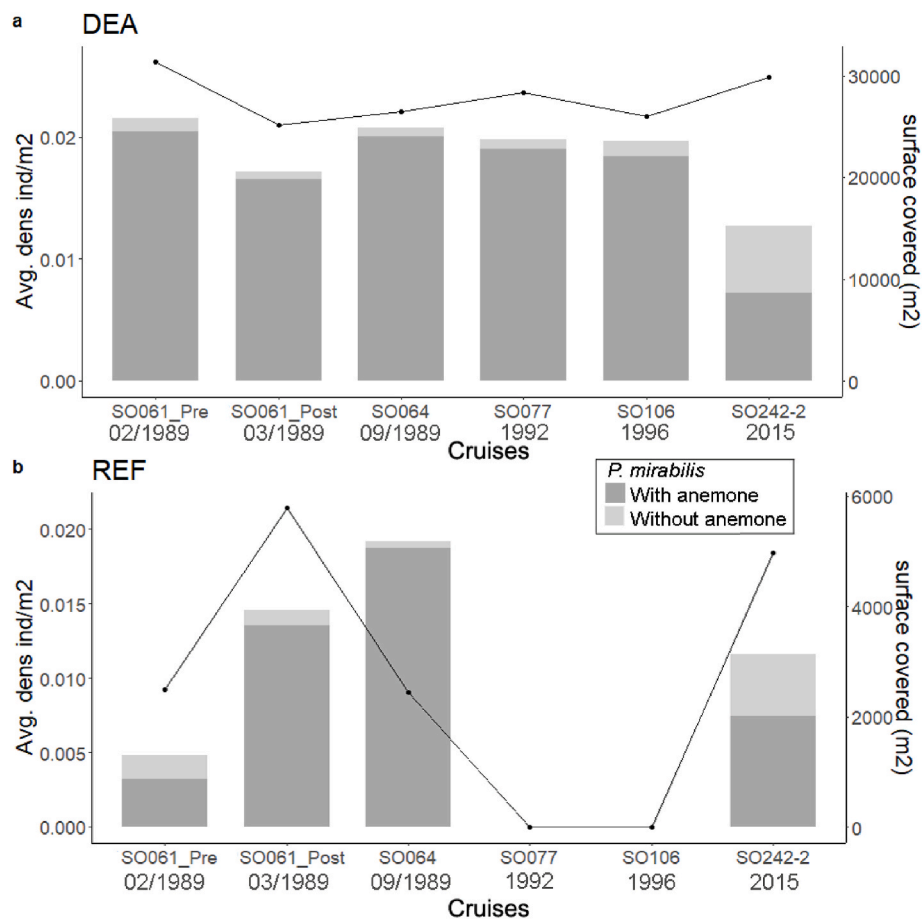


Fig. 3. Disturbance regimes annotated within the DEA and attributed as labels to the *Probeebei mirabilis* with/without anemones on undisturbed surface (a), on track and next to track (b) if the organism was positioned besides the plough track, and disturbed surface (c). Lighter coloured patches indicate the presence of sediments originating from deeper layers. Images from Purser et al. (2018). Laser dots are 50 cm apart.

significant correlations between organism densities and any of these metrics were revealed within these areas (Fig. 4,  $p > 0.1$ ). No significant trends were revealed in the number of organism observations with regard to the total number of images collected and annotated.

### 3.2. Densities between areas

On average, *P. mirabilis* with anemones were one third more abundant in the DEA than in the Reference areas (REF) (0.015 vs 0.010 ind/ $m^2$ ), while *P. mirabilis* without anemones were about 1/6th more abundant in the Reference areas compared to the DEA (0.0027 vs 0.0023



**Fig. 4.** Temporal variation of average *P. mirabilis* with and without anemones densities for the (a) central DISCOL experimental Area (DEA) and (b) reference areas (REF) presented in stacked bar plots, the black line represents the seafloor visualised in m<sup>2</sup> as represented by the second Y-axis.

ind./m<sup>2</sup>). Differences were not significant in *P. mirabilis* densities with anemones between DEA and REF, nor in *P. mirabilis* without anemones (two sample t-tests with unequal variance,  $p > 0.05$ , Fig. 4). In other words, variance in densities observed within the DEA and within the REF area was larger than between the areas.

### 3.3. Densities between years

When investigating the temporal component, different tendencies were observed. Within the DEA, there was a decrease in the densities of *Probeebei mirabilis* over time, which was due to the decrease of individuals with anemones (Fig. 4a). Highest densities characterised the pre-impact imagery from the DEA (SO061\_pre, average densities = 0.02 ind./m<sup>2</sup> ± 0.006) and lowest densities characterised the most recent visit, 26 years later (SO242-2, density = 0.007 ind./m<sup>2</sup> ± 0.003) (Fig. 4a). The reference areas (REF) (Fig. 4b), were not sampled during all cruises, but densities observed varied over time with lowest densities of *P. mirabilis* with anemones during the only pre-impact transect carried out in 1989 outside the DEA (0.003 ind./m<sup>2</sup>). Highest densities in the reference areas were reached 6 months after the disturbance (SO064, dens = 0.019 ind./m<sup>2</sup> ± 0.0002), but decreased again in 2015 (SO242-2, dens = 0.007 ind./m<sup>2</sup> ± 0.001) (Fig. 4). *P. mirabilis* without anemone densities were lower than those with anemones over time. Densities of individuals without anemones were rather low pre-impact (0.001 ind./m<sup>2</sup>), and decreased subsequently staying in similar ranges, immediately post-impact, after 6 months (SO064) and 3 years later (SO077, 0.00061–0.00075 ind./m<sup>2</sup> ± 0.0003). Contrastingly to *P. mirabilis* with anemones, average *P. mirabilis* without anemones densities increased in the DEA during SO106 in 1996 (7 years post disturbance), reaching

highest densities observed over time during SO242-2 (2015) (0.0055 ± 0.001 ind./m<sup>2</sup>), an almost tenfold increase when compared to SO064, 6 months post disturbance in 09/1989 (Fig. 4). The overall trend for *P. mirabilis* without anemones in reference areas was similar, decreasing post-impact and reaching highest densities in 2015, though lower than what was observed in the DEA.

There is a significant negative correlation between *P. mirabilis* with and without anemone densities in the DEA ( $r = -0.94$ ,  $p < 0.05$ , Fig. 5a and b), but not for the reference areas. Moreover, significant differences were observed (two sample t-test assuming unequal variance) between SO242-2 and all other cruises/years in *P. mirabilis* densities with and without anemones in the DEA ( $p < 0.05$ , Fig. 5a and b). Reference areas were not sampled sufficiently to allow for statistical testing, but SO242-2 appeared to stand out in the boxplots as well, especially for *P. mirabilis* without anemones (Fig. 5d).

### 3.4. Size

There were also differences in size of the hermit crabs with and without anemones per cruise. This was most noticeable during the most recent cruise (SO242-2, 2015) and the pre-impact cruise (SO061\_Pre, 02/1989) where *P. mirabilis* with anemones were respectively and on average 33% and 25% smaller in size (annotation circle sizes) than those without anemones. In the other post-impact data collecting years, differences were much smaller and ranged from 2.2% to 12% (with an average of 7%), but commonly *P. mirabilis* individuals with anemones were smaller than those without anemones. Growth estimation was not possible due to the peculiarities of the imagery collection over time. Size calculations were pixel-based and subject to improving image resolution

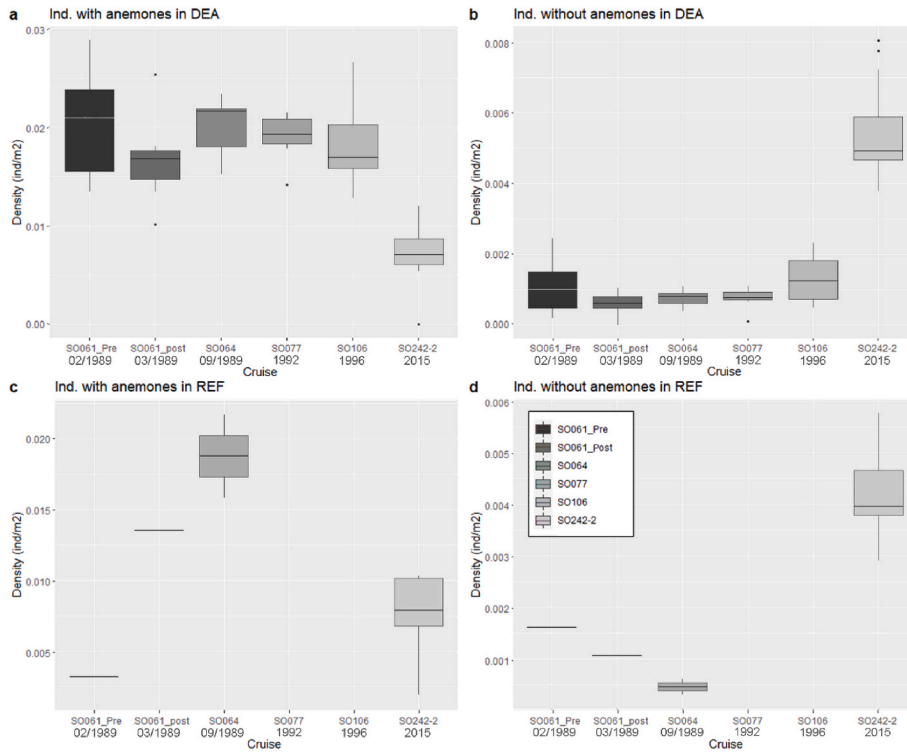


Fig. 5. Boxplots of densities (ind/m<sup>2</sup>) of *P. mirabilis* with and without anemones for the central DISCOL experimental Area (DEA) and reference areas (REF) for all cruises and years available.

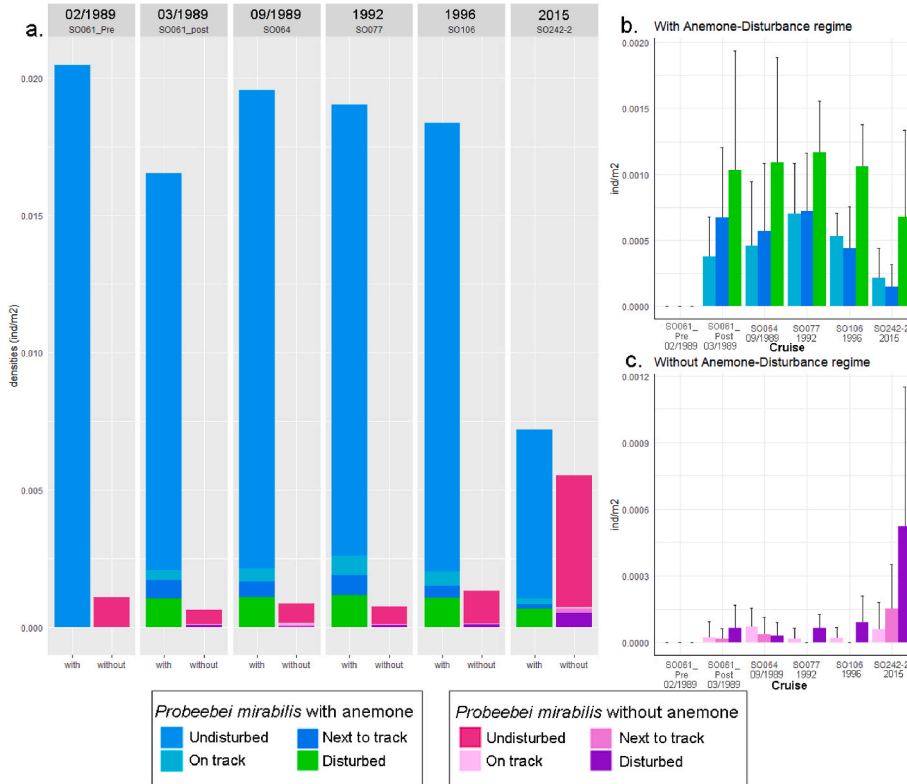


Fig. 6. (a). Stacked densities of *P. mirabilis* with and without anemones on the various undisturbed and disturbed surfaces as shown in Fig. 3. Right panel features an unstacked presentation of the left panel for the various disturbance regimes, omitting the densities of the undisturbed areas to facilitate interpretation. (b). Average densities and standard deviation per disturbance regime and per cruise for *P. mirabilis* with anemone and (c) without anemone.

over time. Results presented here represent the differences observed in relative average sizes between hermit crabs with and without organisms within the same collecting cruise.

### 3.5. Disturbance

Densities of *Probeebei mirabilis* with and without anemones were assessed per surface disturbance regime within the DEA. Overall, highest occurrences were observed on undisturbed surfaces (an average of 87% post disturbance). The remainder was distributed over disturbed surfaces (an average of 5.5–6.5%), on track (an average of 2.5%) and next to tracks (an average of 2%).

Highest densities of *P. mirabilis* with anemones on all disturbance regimes were encountered during SO77, 3 years after the disturbance, for all disturbance regimes (Fig. 6b), followed by a decrease. No linear increase over time was observed. For the *P. mirabilis* without anemones an increase in densities was noticeable during SO242-2 (2015, 26 years after disturbance) on all types of disturbed seafloor. These trends almost corresponded to or followed the trends in overall population densities over time (Fig. 4).

## 4. Discussion

### 4.1. Changes in population densities

Overall, hermit crab densities decreased post-impact but only significantly so in 2015, after 26 years. Despite the overall decrease, *P. mirabilis* with anemones were always more abundant than those without anemones. Smallest differences in densities between *P. mirabilis* with and without anemones were observed during the most recent cruise (SO242-2, 2015), revealing a higher abundance in hermit crabs without anemones than any of the previous years. For the individuals carrying anemones, there were some indications of initial recovery to pre-impact densities in the DEA during SO064 (6 months post-impact, 1989), but this increase did not follow through during the subsequent visits. The most prominent and statistically significant shift in densities and a shift in population structure was only noticeable after 26 years. Differences in faunal densities were not linked to increasing or decreasing sampling efforts per cruise and thus revealed an actual change in population structure.

The differences in densities between the impacted (DEA) and reference areas were not significantly different, but this was mostly due to the large variation occurring in *P. mirabilis* densities with and without anemones among the transects analysed within each area. The immediate decrease after disturbance in the DEA corresponded to an increase in densities in the reference areas for those individuals carrying anemones. This could be indicative of a fleeing behaviour of these hermit crabs away from the DEA. After 26 years, the densities from the reference areas from the most recent cruise (SO242-2, 2015) showed patterns similar to what was observed in the DEA in the same year. Natural fluctuations in faunal densities from other abyssal time series appeared to respond to changes in environmental variables and mainly food supply (Billett et al., 2001, 2010; Ruhl, 2007; Meyer et al., 2013; Sol-twedel et al., 2016). Nevertheless, it is important to bear in mind that the OFOS transects grouped as reference areas were spatially different; some transects were situated to the west, south and east of the DEA, sharing as most important characteristic that they were not ploughed or subject to a (visible) disturbance and sediment blanketing. This, alongside a smaller sampling effort (nine OFOS transects in REF areas compared to 50 OFOS transects across the DEA) might have contributed to the large variation observed. Differences in the physical environment between DEA and Reference areas cannot be ruled out either (Simon-Lledó et al., 2019) as for instance indicated by the visible differences in geomorphology and bathymetry. Alternatively, the impact of the benthic disturbance experiment over time could extend well beyond the DEA, affecting a larger part of the highly mobile Paguroidea population

frequenting the larger region, resulting in a significant decreasing *P. mirabilis* with anemones and a substantial increase in those not carrying anemones in areas more than 10 km outside the DEA.

Within the DEA, the highly mobile *Probeebei mirabilis* crabs with and without anemones tended to revisit the disturbed areas and plough tracks (also observed by Bluhm et al., 1995), with a higher density of individuals without anemones on disturbed surfaces during the most recent cruise. About 20% of the deep seafloor was disturbed vs. 80% undisturbed (Thiel and Schriever, 1990) and while the 10–15 cm deep tracks were smoothed out over time with soft material, the exposed sediment from deeper layers remained visible, as well as the actual tracks, even after 26 years (Borowski, 2001; Simon-Lledó et al., 2019; Gausepohl et al., 2020). No significant trends were revealed, though higher densities on the various disturbance regimes corresponded to or followed higher overall Paguroidea densities in observation years.

The observed decrease in Actiniaria on the hermit crab's pleon, 26 years after the disturbance, corresponded to the significant decrease in suspension feeders, a group dominated by Anthozoa (Cnidaria) at the DISCOL study site (Simon-Lledó et al., 2019). Most species of the Actiniaria are firmly attached to a substratum, with few species facultatively or obligatorily attached to motile species (Riemann-Zürneck, 1998). The presence of life forms on the surface of other organisms - epibiosis - is a common phenomenon in aquatic environments where hard substrata are limited (Wahl, 1989). As such, in an area that was ploughed through and in which the hard substrata were pushed into the underlying sediment making them no longer available for attachment, we would rather expect an increase of anemones on the hermit crab's pleon than a decrease. A changed environment could be the cause for the patterns observed.

### 4.2. Symbiotic interactions

Cnidarians are one of the most frequent epibionts on crustaceans (Fernandez-Leborans, 2013). The symbiotic relationships of anemones with their hosts is dynamic and can exhibit temporal change, going from commensalistic or mutualistic to parasitic depending on environmental and biological factors (Williams and McDermott, 2004; Fernandez-Leborans, 2013). The anemones on the hermit crab's pleon are sessile animals on a mobile platform which are essentially hitching a ride. In his case, *Probeebei mirabilis* are thought to actively grasp anemones (Lemaitre, 1998). As a consequence, the anemones gain mobility and increase their distribution, but do not choose the area frequented by the hermit crab, though advantages of their interaction are presumed for both (Williams and McDermott, 2004).

The closely related deep-sea hermit crab *Tylaspis anomala* from the same family, also no longer living in shells, was known to hold anemones for protection (Wolff, 1961; Lemaitre, 1998; Anker and Paulay, 2013). In this scenario, anemones would thus protect their mobile host from predators using their nematocysts for their defence or offering camouflage and in return benefit from leftover food or particles dropped by their hosts (Williams and McDermott, 2004). Contrastingly, the presence of an anemone epibiont could restrict growth and moulting of the organism providing attachment surface (the basibiont), in this case the hermit crab (Fernandez-Leborans, 2013).

Male and female *P. mirabilis* without epibionts only showed slight variations in adult size with females between 65 mm and 55 mm and males between 63 and 57 mm (Wolff, 1961) showing only a small effect of sexual dimorphism. Yet in our study, the difference in size added up to individuals without anemones being on average up to 1/4 (SO061\_Pre, 02/1989) and 1/3 (SO242-2, 2015) larger than individuals carrying anemones. The years visited in between showed similar discrepancies in size, but of a smaller order of magnitude. The decrease in size difference post-impact (average of 7%) could be attributed to the disappearance of the larger individuals fleeing the area post-impact. Though smaller, the size discrepancy remained with two possible explanations. One would be that the presence of an anemone restricted *P. mirabilis*' growth

(Fernandez-Leborans, 2013) and the energetic cost of carrying an anemone resulted in smaller individuals. Restrictions in size caused by the anemones could possibly keep the organism from moulting as well (Fernandez-Leborans, 2013). When moulting, the epibiont anemones would be shed with the rest of the exoskeleton, however, in Paguridae hermit crabs, it was shown that in 2/3 of the cases the pagurids moulted and recovered their anemones on their new exoskeleton immediately afterwards (Ross, 1975).

In case of the anemones offering protection against predators; if the predators (e.g. crabs, octopus, and fish) were absent, the host no longer needs protection and the anemones could become an energy burden for the host instead of offering the protective advantage (Williams and McDermott, 2004). The absence of anemones on the hermit crab's pleon could thus imply the absence of predators. Within this context, the population structure of *P. mirabilis* with and without anemones could be an indicator for environmental changes, the state of the environment and for the presence and abundance of larger predators. The decrease in megafaunal abundance from an average of ~1100 ind./ha pre-impact (Bluhm, 2001) to an average of ~700 ind./ha 26 years later (Simon-Lledó et al., 2019) is likely to have impacted both prey and predators and could very well explain the observations made here. Alternatively, the benefit for the anemone of being on the hermit crab's pleon and feeding on its leftovers may no longer outweigh the cost of being in a changed (potentially unfavourable) environment.

The absence of anemones on the crab's pleon could also be due to the decreased anemone abundance in the area (the significant decrease in suspension feeders reported by Simon-Lledó et al. (2019) 26 years after disturbance) and that there were less anemones "up for grasps". For all individuals carrying anemones that were annotated, only one to two different morphospecies of Actiniaria were observed on the hermit crabs, that appeared to demonstrate a preference for a certain actinian (as shown for two Paguridae species and their anemones by Ross (1975)). The absence of this specific Actiniaria morphospecies from anywhere else within DEA and reference areas, except the hermit crab's pleon, might indicate that this morphospecies is brought in from elsewhere by the highly mobile hermit crabs, but is dependent on the substrata offered and may not survive in the soft sediment. Nevertheless, it suffers the same consequences as the other sessile or sedentary actinian morphospecies associated to nodules in the Peru basin, namely a decrease in abundance over time post-impact, indicating a potentially changed and unfavourable environment, possibly extending beyond the DEA.

In all discussed scenarios, whether it is due to the changed equilibrium in the cost/benefit of the epibiont/basibiont relationship or due to the direct or indirect impacts of the disturbance on the ecosystem or a combination of thereof, the presence/absence of anemones on the pleon of the hermit crab appears to be an indication of a changed environment. If their relationship is indicative of the Good Environmental Status, the lower densities of *P. mirabilis* with anemones – or the loss of anemones on the pleon – could serve as an indicator in the Peru Basin for the decreasing health and diversity of the environment, characterised by a decline in both predators and abundance of food.

What is remarkable is that this significant pattern of decreasing anemone carrying behaviour only emerged after 26 years. Here, the intermediate years investigated (immediately after disturbance, after 6 months, 3 years, 7 years) showed no significant differences or shifts in the population between those with and without anemones. This finding highlights the importance of long time-series to monitor the impacts of a drastic benthic disturbance such as mining. Whether the population turnover and decrease in densities of *P. mirabilis* with anemones is in fact a delayed and lasting response to the disturbance impact can only be conclusively confirmed by additional data collection and a continuation of the time series.

## 5. Conclusion

The significant decrease of *Probeebei mirabilis* with anemones after 26 years is likely an indication of a changed environment and corresponds to the larger biological effects observed in the community from the Peru Basin nodule fields. Whether the loss of anemones on the hermit crab's pleon is in fact a delayed and lasting response to the benthic disturbance experiment needs to be corroborated with an extension of the time series. The observations presented here highlight the importance of long time-series and the need for long-term monitoring surveys post-disturbance, up to several decades in the abyss. This emphasizes the fact that we still know very little about the temporal scale and extent of anthropogenic impacts on the abyssal fauna. If the possible response of the Paguroidea community took up to 26 years before manifesting itself, caution is needed in assessing population's resilience and recovery over short(er) time-scales.

## Author contributions

DC: Conceptualization, investigation, data collection, Writing - original draft, review and editing, Visualisation.

MV: Investigation, data collection, Writing – review and editing.

AC: Writing – review and editing.

JG: Image data and metadata curation, Writing – review and editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.meres.2023.105899>.

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