ORIGINAL RESEARCH

Effects of artificial light at night on the mobility of the sea urchin *Paracentrotus lividus*

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ABSTRACT. Light pollution poses a significant global threat to biodiversity, driven by the increasing coastal urbanization and the resulting growth of artificial light at night (ALAN). However, to date, the scientific community has focused mainly on studying its ecological effects within the terrestrial environment. It is only recently that attention has turned to coastal marine systems which are crucial due to their essential contribution at the ecosystem level. These environments, characterized by their high productivity, also play a crucial role in protecting coasts against erosion. The aim of this case study was to investigate the possible effects of ALAN on the sea urchin species *Paracentrotus lividus* in four areas of an Italian rocky coast, selected according to a gradient of light intensity (0, 0.4, 3 and 25 lux), from April 2022 to February 2023. Effects of ALAN were examined by measuring the density and size of sea urchins and also their reactivity to a stress condition through an innovative technique of overturning sea urchins to study their physiological response in the presence or absence of artificial light. In addition, the permanence of sea urchins in the four areas was evaluated through an efficient tagging test. Results show how these organisms, typically nocturnal, suffer negative effects of ALAN in terms of minor density and mobility, expressed as the speed of response to an adverse event, compared to a dark area.

Key words: Italy, light pollution, mobility, sea urchin, tagging.

Efectos de la luz artificial nocturna sobre la movilidad del erizo de mar *Paracentrotus lividus*

RESUMEN. La contaminación lumínica plantea una importante amenaza global para la biodiversidad, impulsada por la creciente urbanización costera y el consiguiente crecimiento de la luz artificial nocturna (ALAN). Sin embargo, hasta la fecha, la comunidad científica se ha centrado principalmente en estudiar sus efectos ecológicos dentro del medio terrestre. Solo recientemente se ha prestado atención a los sistemas marinos costeros, que son cruciales debido a su contribución esencial a nivel de ecosistema. Estos ambientes, caracterizados por su alta productividad, también juegan un papel crucial en la protección de las costas contra la erosión. El objetivo de este estudio de caso fue investigar los posibles efectos de ALAN en el erizo de mar *Paracentrotus lividus* en cuatro áreas de una costa rocosa italiana, seleccionadas según un gradiente de intensidad de luz (0, 0,4, 3 y 25 lux), desde abril 2022 a febrero 2023. Se examinaron los efectos de ALAN midiendo la densidad y el tamaño de los erizos de mar y también su reactividad a una condición de estrés mediante una técnica innovadora de volcar los erizos de mar para estudiar su respuesta fisiológica en presencia o ausencia de luz artificial. Además, se evaluó la permanencia de los erizos de mar en las cuatro áreas mediante una prueba eficiente de marcaje. Los resultados muestran cómo estos organismos, típicamente nocturnos, sufren efectos negativos de la ALAN en términos de menor densidad y movilidad, expresada como velocidad de respuesta ante un evento adverso, en comparación con un área oscura.

Palabras clave: Italia, contaminación lumínica, movilidad, erizo de mar, marcado.

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INTRODUCTION

Numerous marine ecosystems are subjected to the influence of natural light regimes that modulate both the behavior of the species inhabiting them as well as their interactions across trophic levels (Davies et al. 2016). These light regimes determine critical aspects, such as the timing and success of predatory activity, consequently impacting the prey's ability to escape (Garratt et al. 2019). However, the use of artificial light sources by humans to carry out their activities is altering natural conditions even in the marine environment. These sources of artificial light at night (ALAN) range from temporary lights used for navigation and fishing, through intermittent ones produced by lighthouses, to permanent ones, such as those emitted by oil rings and cities on the coast (Davies et al. 2014), due to the constant illumination of residences, walks, piers and marinas (Garratt et al. 2019). This growing anthropic pressure is increasingly threatening key ecological processes shaping these ecosystems (Luijendijk 2018) and, in particular, intertidal and shallow subtidal habitats. They provide various ecosystem goods and services, such as tourism and recreation, raw material production, coastal protection and erosion control, nutrient cyclization, water purification and carbon sequestration (Barbier et al. 2011).

In particular, herbivorous sea urchins play a fundamental role in shallow subtidal marine environments, both as grazers by limiting algal biomass, and as prey of organisms belonging to different species, such as fish, crustaceans, starfish, otters and humans (Pearse 2006). Changes in their density can lead to sudden, persistent and large variations in the structure and functioning of the ecosystem in which they live (regime shifts), as in the case of the transformation of macroalgal forests into barrens or habitats dominated by algal felts (Benedetti-Cecchi et al. 1998). These habitats represent alternative states of the same system: the macroalgal forest is extremely productive because it accumulates a lot of biomass and maintains a high specific diversity within it; on the contrary, felts and barrens represent less diversified systems and accumulate less biomass (Stewart and Konar 2012; Filbee-Dexter and Scheibling 2014). Specifically, the sea urchin *Paracentrotus lividus* represents a model organism used for a long time in different fields of study of biology, such as immunology (Pinsino and Alijagic 2019), ecotoxicology (Macedo et al. 2017), development (Romancino et al. 2017), biochemistry (Karakostis et al. 2016) and ecology (Boarda et al. 2017). Thanks to these studies, many aspects of this species are known at different levels of biological organization and therefore, theoretically, it should be easier for researchers to develop hypotheses to test and carry out studies supported by previous knowledge acquired. In addition, *P. lividus* represents a species of commercial interest and potentially at risk due to overfishing by humans (Ceccherelli et al. 2011). In Italy its capture is regulated by the Ministerial Decree of 1995 which establishes the size, period and quantities permitted (MRAAF 1995).

Factors influencing the activity of sea urchins are varied, including hydrodynamics, food availability and the presence of predators (Benedetti-Cecchi et al. 1998), but also harvest by humans for food purposes (Farina et al. 2020). Herbivorous sea urchins carry out most of their activities at night (Dee et al. 2012) and it makes them theoretically more influenced by ALAN than other strictly diurnal organisms. In fact, during the day sea urchins remain safe from possible predators in shelters in the crevices among the rocks, while at night they come out to feed (Hereu 2005). Depending on the species of sea urchin, reactions to different daylight intensities include color change, ambulacral pedicel reactions, and hiding in shelters (Millott 1976). In addition, photoperiod variations have been shown to affect the reproduction of sea urchins, such as *P. lividus* (Shpigel et al. 2004) and *Arbacia lixula* (Wangesteen et al. 2013). In particular, it has been observed that in *P. lividus* prolonged periods of illumination reduce the rate of gametogenesis, while reduced periods of illumination increase it (Shpigel et al. 2004). However, there is no conclusive evidence of the presence of a particular structure capable of receiving light signals and it is assumed that these organisms rely solely on a photosensitive superficial epidermal nerve network (Millott 1976; Ullrich-Lüter et al. 2011).

Hypotheses tested were: 1) the density of the sea urchin *P. lividus* is greater at night than at day, outside their shelters among the rocks, in order to confirm the hypothesis that they are nocturnal organisms; 2) at night the density and size of *P. lividus* are greater in areas not subjected to ALAN and similar between areas subjected to ALAN and diurnal ones, such as possible stress conditions, variations in circadian rhythms and increased risk of predation; and 3) the time of the overturning test increases with a higher light intensity as a consequence of greater stress conditions.

MATERIALS AND METHODS

Study site

The study was carried out on a rocky coast along the promenade of Punta Righini (43° 40′ 08″ N, 10° 40′ 73″ E) in Castiglioncello in the province of Livorno (Tuscany, Italy) from April 2022 to February 2023 (Figure 1). The area is characterized by the presence of dark zones alternating with others with different types and intensity of night lighting, due to the presence of street lamps along the promenade and LED spotlights at the restaurant ‘La Baracchina’.

The habitat has an algal benthic population consisting mainly of algal turf and thin tubular sheet-like (TTS), to a lesser extent *Halimeda tuna*, *Laurencia obsuta*, *Ellisolandia elongata*, *Padina pavonica* and *Valonia macrophysa*. The most commonly present animal organisms are crabs, shrimps, hermit crabs, gastropods, actinias, mullets, sea stars, sea cucumbers and the two most common species of sea urchin along the Italian coasts in superficial subtidal habitat: *P. lividus* and *A. lixula*. The latter species is present in a minimum percentage (2%) and therefore it was considered appropriate to test hypotheses only on the sea urchin *P. lividus*, also to avoid including biological differences existing between the two species in the results.

In April 2022 four areas of equal extension and characterized by different ALAN levels were identified through light intensity measurements carried out using a lux meter (Digital Lux Meter LX1330B, range 0,1-200.000 lux) in the presence of a new moon (Figure 1). Proceeding from west to east, in the first area the light of street lamps of the walk does not reach the study habitat since it is located a few tens of meters from it remaining completely dark (Dark 1 area –0 lux). The second area was illuminated by the headlights of the restaurant ‘La Baracchina’, which produced a relatively strong light (Restaurant area –25 lux). Continuing south, we found the first street lamp of the promenade of Punta Righini, which however was not active and the area was almost completely dark (Dark 2 area –0.4 lux). The fourth area was below one of the working street lamps of the promenade. This LED lighting was very dim compared to that emitted by the restaurant’s spotlights, which allowed for an intermediate experimental condition between the two extremes of high illumination and total darkness (Promenade area –3 lux). Those four selected areas were similar in terms of rocky substrate and presence and abundance of main algae species.

Measurement and tagging methods

All measurements were replicated every two months for a total of 6 different sampling periods (April, June, August, October and December

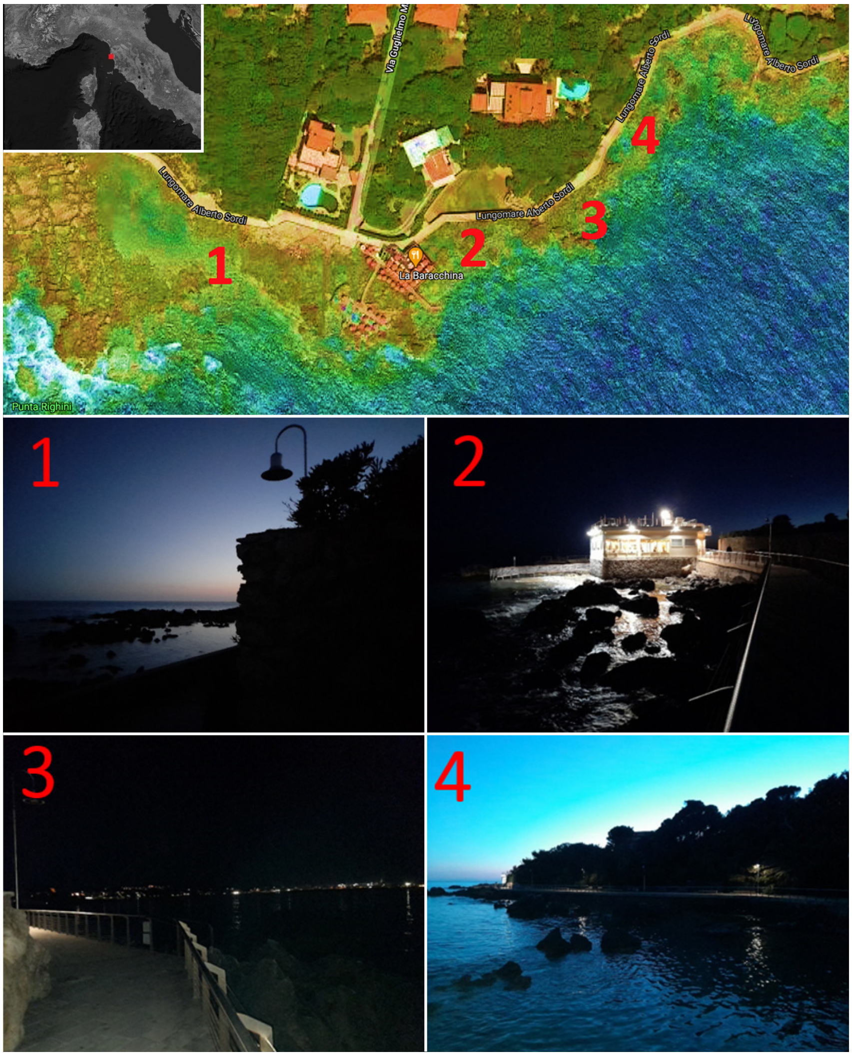

Figure 1. Study site indicating the four selected areas with different lighting levels. 1) Dark 1 area –0 lux. 2) Restaurant area –25 lux. 3) Dark 2 area –0.4 lux. 4) Promenade area –3 lux.

2022, and February 2023) to obtain results not attributable to the seasonal period but to an annual average. All measurements were carried out in subareas of similar extension for each of the four study areas. In each sampling period the subarea was changed but the same experimental condition of the area was maintained. Sea urchins were then relocated to an area not examined in this study and far from the sampling location to avoid possible pseudoreplication errors (Waller et al. 2013).

To determine whether sea urchins present in the four chosen sites were representative of them, it was considered appropriate to carry out a preliminary test by tagging them in each area and checking their presence a week later to determine if they were sedentary. To choose the most appropriate tagging method among those observed in the scientific literature (Duggan and Miller 2001; Boarda et al. 2015), a first test was carried out between the two most commonly used methods.

The first consisted of perforating the sea urchin with a hypodermic needle and passing a 0.25 mm monofilament fishing line through the hole created, into which a colored vinyl tube was inserted. This technique requires that gonads or other vital organs of the sea urchin not be pierced so that there are not high mortality rates (Ebert 1965). The second was the plastic beads method, which is based on the use of small 2 mm microplastics rings inserted and glued into the aboral part of sea urchins in a number equal to 5 per organism (Burnell 2015).

The test was performed in an area near the study site. Fifteen sea urchins of similar size (3-5 cm) were tagged for each of the two selected systems (Figure 2). After 7 days, sea urchins were recovered and viewed. The evaluation criteria included the number of sea urchins found, mortality, stress status and, for the second method tested, the number of beads left.

Once the most valid approach was established, 10 sea urchins were marked in each study area. After a week, characterized by calm seas, the areas were visited again to assess the number of urchins found and to give a qualitative estimate of the distance from the point of release.

Density and size

In order to evaluate possible differences in the average density of sea urchins in areas characterized by different night lighting, a non-destructive sampling was carried out in the four study areas by counting organisms outside their shelters among rocks. Since daytime sampling is generally carried out in the literature, it was considered interesting to compare densities in natural light conditions with those made at night in dark and artificially lit areas. In the same days, size measurements of sea urchins were also carried out within each zone using a caliper.

Overturning tests

Finally, an innovative overturning test was performed on sea urchins. It consisted of placing sea urchins in inverted position on a flat surface and measuring the time to return to the usual position with the oral surface facing the substrate and the aboral surface towards the water column (Figure 3). In fact, oral surface is less defended by spikes and potentially more vulnerable to attacks of possible predators. Therefore, sea urchins in good

Figure 2. Hypodermic needle (A) and beads (B) tagging methods used to test the mobility of sea urchins.

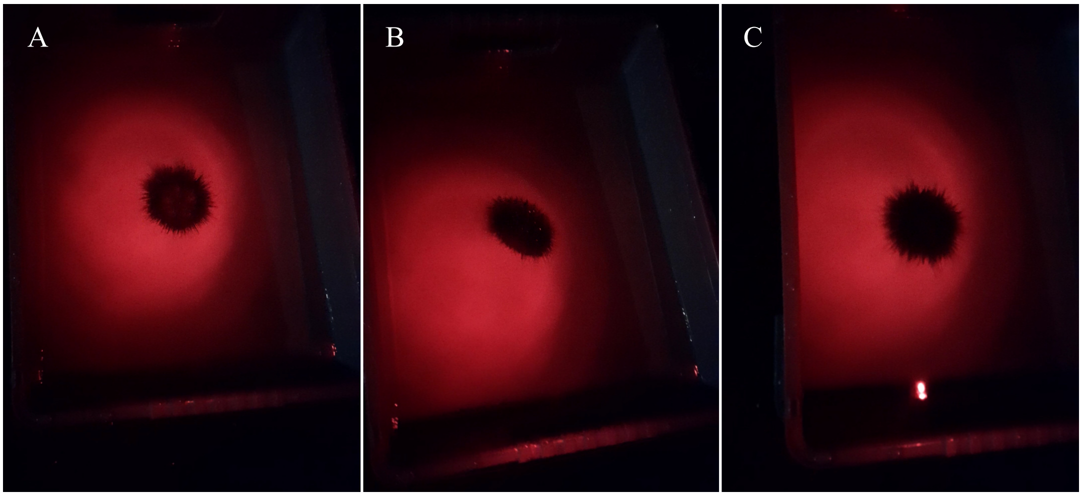

Figure 3. Three positions, overturned (A), intermediate (B) and natural (C), of a sea urchin during an overturning test.

health overturned very quickly, whereas if they were stressed, they took longer to return to the position that guarantees maximum defense (Bose et al. 2019). The hypothesis was that ALAN could generate stress conditions such as to compromise this anti-predatory behavior.

The test was conducted approximately one hour after dusk, placing organisms (n = 10 for each of 4 areas) of similar size (3-4 cm) in the center of a basin filled with water. After 30 sec in a natural position, so that they acclimatized to the new substrate, they were overturned. A red light was used to see during all measurements in the dark and not to distort the test. In fact, the light produced by this color was less dazzling than, for example, a white light (Figueiro et al. 2019).

Data analysis

The experimental design foresaw the comparison between annual averages of day and night densities of sea urchins in each of 4 zones; the comparison between annual averages of densities and nocturnal sizes of sea urchins in illuminated and unlit areas; and the comparison between annual averages of night overturning times of sea urchins in illuminated and dark areas. Independent samples were analyzed using the t-test to estimate the effect of ALAN on sea urchins. For the realization of analyses, the statistical software R was used considering $p < 0.05$.

RESULTS

Tagging methods

Hypodermic needle method

Fifteen sea urchins were randomly taken (11 *P. lividus* and 4 *A. lixula*) to which this first method was applied. After a week, sea urchins were all found, but it was noted that they had lost a large number of spikes, a symptom of a state of strong stress probably due to the treatment applied. Moreover, once placed in a poorly sheltered area and overturned, they were not very reactive in movements and 3 organisms were even dead (Figure 4).

Beads method

Fifteen sea urchins were randomly taken (11 *P. lividus* and 4 *A. lixula*) to which this second method was applied. After a week, sea urchins

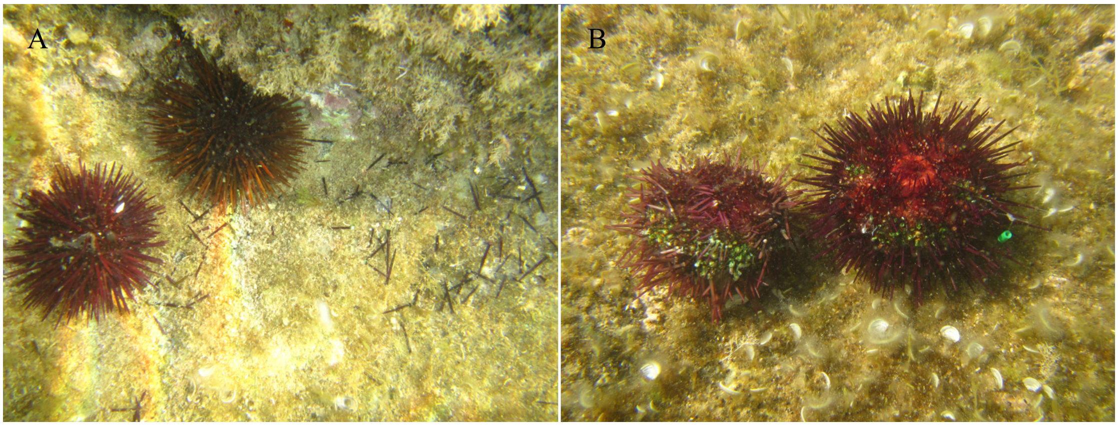

Figure 4. Effects of the hypodermic needle method: spikes lost by sea urchins (A) and dead sea urchins (B).

were all found. They did not lose spikes and the average of beads found per organism was 4.25 (Figure 5). Moreover, once placed in a poorly sheltered area and overturned, they were very reactive in their movements, and in a few minutes they moved to shelters between the rocks where they had been taken. Consequently, the bead method was considered appropriate to mark 10 sea urchins in each of 4 areas to assess their level of permanence. After a week, all the sea urchins had remained in the same area where they had been tagged, even within 2 m of the point of release following the application of the tagging.

Figure 5. Some of the sea urchins found after using the beads method.

Density and size

Analyses showed significant differences between day and night densities in the two areas not illuminated at night: Dark 1 $t_{0.05(1),10} = 7.92$ and Dark 2 $t_{0.05(1),10} = 8.29$. In the Dark 1 sampling area, the average number of sea urchins at night was statistically higher than during the day. Very similar results were also observed for the Dark 2 area. On the other side, there were no significant differences for the two illuminated areas. Particularly, nighttime values were almost the same as daytime values in the Restaurant area with greater illumination. In addition, results also showed significant differences between nocturnal densities in the two dark sites with the most illuminated density of the Restaurant area, e.g. Dark 1 $t_{0.05(1),10} = 6.51$ and Dark 2 $t_{0.05(1),10} = 5.48$, respectively. Night densities of sea urchins in dark areas were higher than in lit areas. Additionally, there was a drastic decrease in the number of sea urchins found outside their shelters in the most illuminated area (Figure 6). Instead, analyses showed no significant differences in the size of sea urchins under different experimental conditions of presence and absence of artificial lighting (Figure 7).

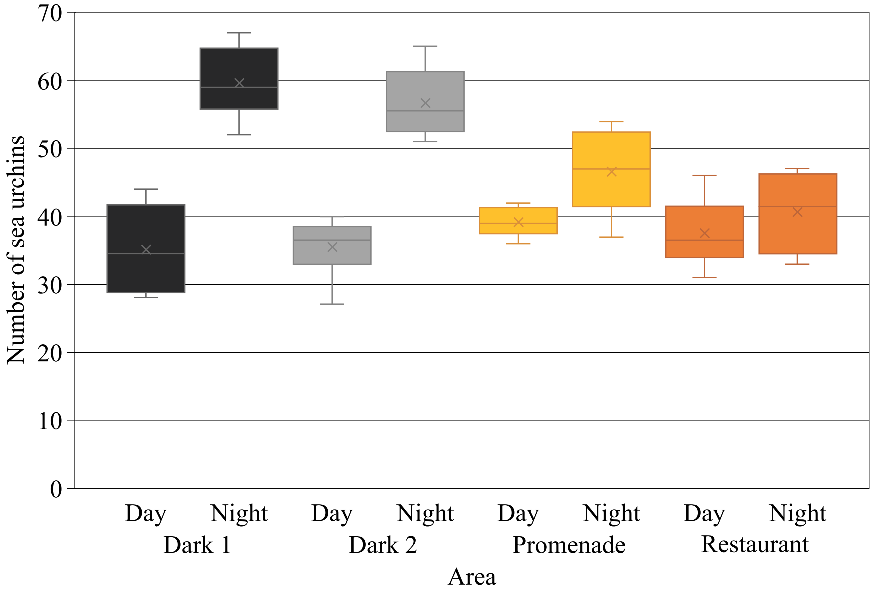

Figure 6. Box plots of the day and night densities of sea urchins in each of the four study areas. Mean (± standard error): Dark 1 (day) = 35.17 ± 2.61 sea urchins; Dark 1 (night) = 59.67 ± 2.17 sea urchins; Dark 2 (day) = 35.51 ± 1.88 sea urchins; Dark 2 (night) = 56.67 ± 2.08 sea urchins; Promenade (day) = 39.17 ± 0.87 sea urchins; Promenade (night) = 46.67 ± 2.56 sea urchins; Restaurant (day) = 37.52 ± 2.11 sea urchins; Restaurant (night) = 40.67 ± 2.35 sea urchins.

Overturning tests

Significant differences were observed between annual averages of Dark 1 and Dark 2 areas and that of the Restaurant area, $t_{0.05(1),118} = 13.47$ and $t_{0.05(1),118} = 13.41$, respectively; while there were no differences between the Promenade area with dim light and the others (Figure 8). Results also showed a high variability in times of the Restaurant area that was the most illuminated by light sources.

DISCUSSION

Results of this study confirm that sea urchins are nocturnal organisms that come out of their shelters mainly at night to carry out their needs. This study also supports the hypothesis that artificial light pollution at night has negative effects on the mobility of the sea urchin *P. lividus*. In fact, these nocturnal organisms have photoreceptors potentially alterable by ALAN (Ullrich-Lüter et al. 2011). In particular, this study showed that nocturnal densities of sea urchins were very sensitive to ALAN, especially higher in dark areas than in the restaurant, where there were LED spotlights at an intensity of 25 lux, while there appear to be no significant effects due to the light of streetlights of the promenade with a luminous intensity equal to 3 lux.

Regarding the size of sea urchins, no significant differences that support the hypothesis that sea urchins are larger in dark areas than in illuminated ones were observed. Rather, it seems to be an opposite trend, although not statistically proven, to what has been hypothesized. Satthong et al. (2019) postulated that night lighting may have a positive effect on the growth of algae sea urchins feed on that leading to increased growth of sea urchin and to counteracting visible effects of ALAN on them. However, results remain difficult to interpret due to some existing factors such

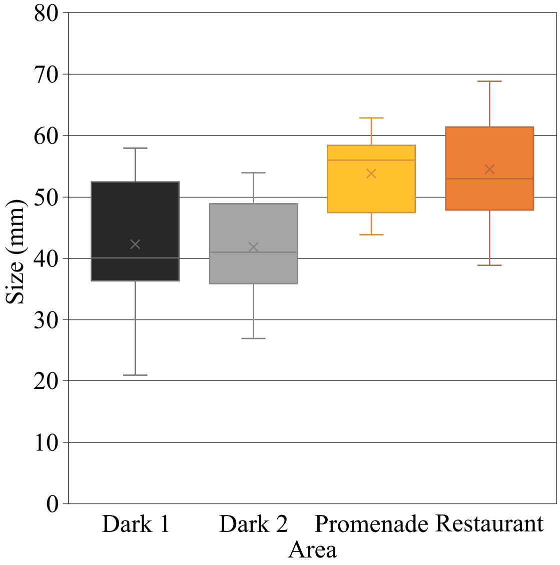

Figure 7. Box plots of the sizes of sea urchins at night in each of the four study areas. Mean (± standard error): Dark 1 = 42.46 ± 3.05 mm; Dark 2 = 41.85 ± 2.36 mm; Promenade = 53.92 ± 1.72 mm; Restaurant = 54.54 ± 2.51 mm.

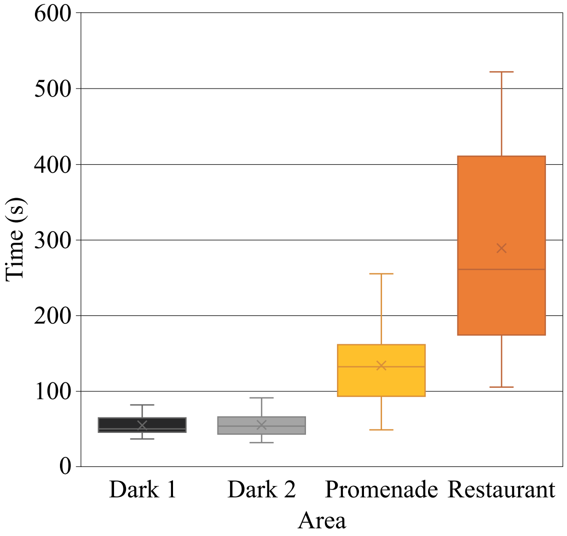

Figure 8. Box plots of the times to return to the natural position during the overturning tests on the sea urchins in each of the four study areas. Mean (± standard error): Dark 1 = 54.77 ± 2.09 s; Dark 2 = 55.47 ± 2.55 s; Promenade = 133.72 ± 8.72 s; Restaurant = 287.65 ± 21.03 s.

as different hydrodynamism and predation, which act on the size in different ways and intensities in the four areas.

Finally, regarding the reactivity of sea urchins evaluated with the overturning test, it was found as suggested, that exposure to high ALAN intensities determinates a decrease in their motility following an unfavorable event such as their accidental overturning. Probably, the anti-predatory behavior of rapid repositioning in the normal position was altered as a consequence of the increased stress condition of sea urchins due to an excessive alteration of their normal circadian rhythms. In fact, sea urchins are organisms highly susceptible to stress factors and even a simple handling of a few seconds can alter, at least in the short term, self-righting and predator escape speed (Bose et al. 2019). In addition, a high variability was observed in the times of the high illumination area compared to others, particularly dark ones, perhaps due to different effects of ALAN on individual organisms. These results support the hypothesis that ALAN affects the life of sea urchin with consequences on their activities, the extent of which is yet to be assessed.

Further studies could extend the research to other areas for a deeper understanding of the behavior of *P. lividus* and address the potential effects of ALAN on other nocturnal behaviors of sea urchin, partly already studied during the day, such as reproduction (Shpigel et al. 2004), predation (Sala and Zabala 1996) and different types of foraging (Bulleri et al. 1999). Moreover, it would be interesting to test the effects of an ALAN intensity gradient on sea urchin photoreceptors from a biochemical point of view to establish luminous intensity limits that determines its alteration.

In conclusion, it is unthinkable to be able to completely eliminate ALAN from our lives because it is now an integral part of modern society. Nevertheless, there are several practical and effective methods to try at least to limit this form of pollution such as keeping a proper distance between two adjacent light sources, reducing the intensity of the light source to that strictly necessary to carry out the activity for which it was appointed, turning off lights when not needed, replacing obsolete lighting types for others with good performance, as well as those with high consumption and cost for others with less environmental impact, and placing light sources with a correct direction of the light beam (Di Bari et al. 2023). Unfortunately, at European level, while widely recognizing ALAN as a serious threat to biodiversity, there are still no precise laws establishing common rules regarding this environmental problem. Thus, these decisions are left locally to politicians for whom in many cases it is difficult to introduce voluntary mitigation measures when they would conflict with economic gains and security concerns (Davis et al. 2014). In particular, due to increasing urban development, coastal marine environments are among the most affected areas of light pollution (Bird et al. 2004), often causing negative effects on organisms that inhabit them, as in the case of the mobility of *P. lividus*. However, the current knowledge of these impacts in marine ecosystems is not yet sufficient to determine the magnitude of the problem and its possible interactions with other anthropogenic pressures, so as to implement effective and realistic management strategies (Davies et al. 2014) that allow to find the best compromise between human requirements and the needs of other organisms (Gaston et al. 2012).

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