Influence of artificial lighting on feeding and movement of the purple sea urchin (Paracentrotus lividus)

Šiljeg, Anamarija

Master's thesis / Diplomski rad

2022

University of Zagreb
Faculty of Science
Department of Biology

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Prirodoslovno-matematički fakultet
Sveučilište u Zagrebu
Biološki odsjek

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Utjecaj umjetnog svjetla na hranjenje i kretanje hridinskog ježinca (*Paracentrotus lividus*)

Diplomski rad

Zagreb, 2022.

Ovaj rad izrađen je u Morskoj školi u Puli, pod vodstvom izv. prof. dr. sc. Petra Kružića. Predan je na ocjenu Biološkom odsjeku Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu radi stjecanja zvanja magistre ekologije i zaštite prirode.

Zahvaljujem mentoru izv. prof. dr. sc. Petru Kružiću na savjetima, susretljivosti i pomoći pri završnim koracima izrade diplomskog rada..

Zahvaljujem se cijeloj svojoj mnogobrojnoj obitelji na bezuvjetnoj ljubavi, podršci, strpljenju i razumijevanju i motivaciji.. Hvala bratu Antoniu za svaki zajednicki trenutak i osmijeh. Posebno se zahvaljujem mami Loreti i tati Miji, koji su mi uz sve iznad navedeno, svojom požrtvovnošću i mukotrpnim radom omogućili studiranje i dali mi mogućnost za bolji život.

Zahvaljujem se svom malom krugu velikih ljudi, svojim najboljim prijateljicama Ivani, Valentini, Žani I Ilenii koje su uvijek bile tu u svakoj situaciji i učinile mi sve ove godine studiranja lakšim i nezaboravnim. Zahvaljujem vam na prijateljstvu, radosti, podršci, ohrabrivanju, motivaciji.

Zahvaljujem se dečku Filipu! Hvala ti za enormno strpljenje, podršku, motivaciju, ohrabrivanje i utjehu svaki put kad je bilo potrebno.

Hvala vam što ste vjerovali u mene čak i kada ja nisam, svi ste vi dio moje diplome!

BASIC DOCUMENTATION CARD

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Master Thesis

Influence of artificial lighting on feeding and movement of the purple sea urchin (*Paracentrotus lividus*)

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Light pollution is an emerging anthropogenic stressor for marine ecosystems. About 20% of the world's coastlines are affected by artificial light at night - with exposure levels expected to increase as human populations grow. Despite the omnipresence of artificial light along urbanized coastlines, a comprehensive understanding of light pollution effects in marine environments is still lacking. The aim of this study was to investigate the influence of artificial light at night on the feeding and activity of benthic invertebrate *Paracentrotus lividus* (Lamarck 1816). The animals were exposed to a light intensity of 30 lux (first and second experiment) and 60 lux (third experiment). I conducted three temporally separate experiments which focused on feeding rates and activity patterns under different lengths of exposure to nighttime lighting. I found that exposure to artificial light at night does not influence the feeding rate in *P. lividus.* However, the light has an effect on the activity pattern. In the presence of artificial light at night, the sea urchin starts to hide more during the nighttime. Under the predicted increase of artificial light at night this knowledge is key to understand future changes in benthic communities.

(47 pages, 20 figures, 9 tables, 58 references, original in: English)

Thesis is deposited in Central Biological Library.

Keywords: light pollution, grazing, urbanization, Adriatic sea

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Thesis accepted: 10.02.2022

TEMELJNA DOKUMENTACIJSKA KARTICA

Sveučilište u Zagrebu
Prirodoslovno-matematički fakultet
Biološki odsjek

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Svjetlosno onečišćenje je novi antropogeni stresor za morske ekosustave. Oko 20 % svjetskih obala noću je pod utjecajem umjetnog svjetla, a očekuje se da će se razina izloženosti povećati kako ljudska populacija raste. Unatoč sveprisutnosti umjetne svjetlosti duž urbaniziranih obala, još uvijek nedostaje sveobuhvatno razumijevanje učinaka svjetlosnog onečišćenja u morskim okolišima. Cilj ovog istraživanja bio je istražiti utjecaj umjetnog svjetla noću na konzumaciju hrane i aktivnost bentoskog beskralježnjaka *Paracentrotus lividus* (Lamarck 1816). Jedinke su bile izložene intenzitetu svjetlosti od 30 luksa (prvi i drugi pokus) i 60 luksa (treći pokus). Provela sam tri vremenski odvojena pokusa koji su se fokusirali na konzumaciju hrane i aktivnosti pod različitim duljinama izlaganja noćnom svjetlu. Rezultati su pokazali da izlaganje umjetnom svjetlu noću *P. lividusa* ne utječe na količinu konzumirane hrane. Međutim, svjetlost ima utjecaj na aktivnosti, u prisutnosti umjetnog svjetla noću hidrinski ježinac se počinje više skrivati. Pod predviđanjima povećanjem umjetnog svjetla noću, ovo znanje je ključno za razumijevanje budućih promjena u bentoskim zajednicama.

(47 stranica, 20 slika, 9 tablica, 58 literaturnih navoda, jezik izvornika: engleski)

Rad je pohranjen u Središnjoj biološkoj knjižnici

Ključne riječi: svjetlosno onečišćenje, strugači, urbanizacija, Jadransko more

Voditelj: izv. prof. dr. sc. Petar Kružić

Ocjenitelji: izv. prof. dr. sc. Petar Kružić
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Rad prihvaćen: 10.02.2022

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1 Introduction

1.1 Urbanization and light pollution

Light pollution is an emerging anthropogenic stressor in marine ecosystems. In contrast to terrestrial ecosystems, marine ecosystems have received comparatively little attention concerning the effects of light pollution (Depledge et al. 2010). Environmental experts have identified urbanization as one of the key threats to global biodiversity (McKinney 2002). There has been a growing concern about the threat posed by urbanization to biodiversity (Becker 2013). With the expected growth of the human population in coastal areas, we can expect a proliferation of infrastructures such as jetties, wharves, and marinas in the coming decades. Often, such infrastructure is associated with artificial nightlights, yet we still don't know how these unnatural lighting regimes impact the fauna on coastal ecosystems. Population growth is particularly pronounced in coastal areas, where the density of humans is currently three times higher than the world average (Small & Nicholls 2003) and currently, 22 % of the world's coastlines are affected by artificial light at night (ALAN) (Davies et al. 2014).

Figure 1. Light pollution in Vigo, Spain

Over the past century, the proportion of the globe that is illuminated by artificial light at night has grown rapidly (Cinzano 2001). The widespread illumination of human settlements, roadways, and industrial infrastructure was facilitated by developing electric lighting and the growth of both grid-based and locally generated power. The unintended consequence of this process is the illumination of natural and semi-natural ecosystems, both directly and indirectly through scattered light in the atmosphere, or skyglow, which can extend the ecological effects of light pollution hundreds of kilometers beyond urban areas (Kyba 2001). The ramifications of artificial light at night are extensive, potentially spreading from the source for up to hundreds of kilometers, due to the expansion of metropolitan centers, illuminated infrastructure, and advances in lighting technology (Gaston et al. 2014). More than 95 % of global population growth is expected to occur in cities of economically developing countries over the next 50 years (Grimm et al., 2008), and levels of light pollution are closely linked to population density and economic activity (Gallaway, Olsen, & Mitchell 2010). The exposure of the marine environment to night lighting is expected to become more widespread as human coastal populations are predicted to double by 2060 (Neumann et al. 2015). Multiple stressors are present in the urban marine environment which, when combined can have serious consequences for the functioning of these systems (Johnston et al. 2015). Because of developments, lighting may impact animal behavior and impact the ecological systems of estuaries and coasts (Becker 2013). However, despite the omnipresence of artificial light in urbanized coastal areas, a comprehensive understanding of light pollution's effects on benthic ecosystems is still lacking.

1.2 Effect of light on animals

The vast majority of organisms have developed in conditions of moonlight, sunlight, and starlight that are natural and predictable. These regimes define a species' activity times (e.g., nocturnal, crepuscular, diurnal), serve as a navigational aid, aid in the regulation and coordination of maturation and reproductive events, and provide a relatively constant irradiance spectrum that can regulate physiology and inform visually guided behavior such as predation and communication (Gaston et al. 2013). The inclusion of artificial lighting is not only another unnatural feature, it has the potential to alter predator-prey interactions at multiple trophic levels by creating conditions that are favorable to predatory species at night and to disadvantage the prey (Becker 2013). By disrupting the natural rhythms of light and dark, artificial light obstructs the view of the night sky that animals rely on to navigate; it can alter diurnal and nocturnal animal behavior, disrupt movement and migration, and alter the timing of key events such as flowering, budburst, and reproduction (Bennie 2015). Light and dark cycles are among the most fundamental drivers of biological processes and interactions daily and yearly. Artificial lighting at night (ALAN) is disrupting these natural cycles, with far-reaching global implications. ALAN has an impact on species' behavior, distribution, and abundance at all scales, from the molecular to the entire ecosystem (Bolton 2017). As an example, light along the beachfront can cause hatchling turtles to become disoriented (Tuxbury & Salmon 2005), and lighting along urban streets can influence beach mice's foraging behavior (Bird et al. 2004). By changing the amount of time organisms spend hunting, hiding, and resting, ALAN can influence ecological processes such as predation, competition, and habitat usage (Bolton 2016). Many nocturnal predators and prey may see evolutionary changes when ALAN reduces the amount of time available for such activities (Minnaar et al. 2015). Many nocturnal animals rely on darkness for access to food and mates when competition and predation are low, but ALAN depletes this resource (Duffy et al. 2015). Furthermore, the loss of naturally dark space may result in the extinction of animals that rely on it for rest and recovery (Bolton 2016). Even low amounts of ALAN have been proven to have an impact on ecological processes (Cohen et al. 2010; Rotics et al. 2011), and several studies advocate that natural dark space be preserved as the best choice for management (Gaston et al. 2012).

1.3 Light-emitting diodes (LED)

ALAN's expanding influence is also being aided by advancements in lighting technology. As towns are under pressure to decrease their carbon footprints, more efficient lighting is being used. Although light-emitting diodes (LEDs) are less expensive than traditional lights, they have a bigger environmental impact (Bolton 2016). LEDs provide a broader spectrum (white) light with peaks in the blue and green wavelengths, which are dimmed at deeper depths (Elvidge et al. 2010). Urban lighting has been demonstrated to increase predator access to nocturnally foraging prey in the marine environment, as predators' capacity to detect prey is improved (Mazur & Beauchamp 2006). LED lighting is expected to become more popular because of its efficiency, low cost, and variety (Gaston et al. 2012). Technological advances in LEDs that would allow greater control over intensities and wavelengths emitted may provide greater mitigating the ecological impacts of ALAN (Gaston et al. 2012). LEDs have increased their share of the lighting market from 9% in 2011 to 45 percent in 2014, and are expected to reach 69 percent by 2020. (Zissis & Bertoldi 2014). LEDs are becoming increasingly popular due to the wide range of colors they can create, their improved energy efficiency over other electric light sources, and their ability to produce "white" light that is visually pleasant and improves visual performance (Pimputkar et al. 2009; Schubert & Kim 2005). While LEDs are frequently praised for their ability to cut global CO_2 emissions and the ability to modify their spectra to prevent negative environmental consequences, they also have several drawbacks (Davies 2017). LEDs' better energy efficiency may drive an increase in the amount of artificial light generated globally. This "rebound effect" may be seen in historical lighting trends. It helps explain why aesthetic and ornamental lighting installations are becoming more common in municipal centers, monuments, bridges, and waterfront developments (Davies 2017).

In England, 23 % of local authorities use permanent part-night lighting schemes, in which street lights are switched off between midnight and 04:00 to 05:00 a.m., while 39 % use permanent dimming schemes, in which lights are lowered for at least some of the night. Following the global financial crisis of 2008, increased budget limitations on local government budgets have spurred the adoption of various lighting strategies; nonetheless, the most common reasons claimed for their deployments are better energy savings and lower CO_2 emissions. Switching to LED and implementing central management systems that use wireless communication technologies to program individual street lights remotely improves dimming and part-night lighting. Dimming and part-night lighting have yet to be thoroughly explored in terms of their ecological benefits (Davies 2017). White light is increasingly illuminating nighttime surroundings as a result of the recent global boom in LED lighting. While these lights have the potential to save money and reduce CO_2 emissions, their broad spectral output, in comparison to traditional sodium-based technologies, covers a wider range of wavelengths to which a variety of light-guided behaviors, including larval recruitment, may be sensitive. By 2020, LEDs are expected to overtake incandescent bulbs as the primary light source in industrial, commercial, residential, and architectural lighting applications and they are becoming more common in shipping and oil and gas industries (Davies 2015).

1.4 *Paracentrotus lividus* (Lamarck 1816)

Paracentrotus lividus is a characteristic species of sea urchin in the Adriatic Sea. It inhabits areas of the North Atlantic and the Mediterranean, most often in a shallow area where it achieves high population density. The species belongs to the group of regular sea urchins, pentaradially symmetrical (Habdija et al. 2011). *P. lividus* can inhabit a variety of substrates from rocky substrates to seagrass settlements (Habdija et al. 2011). It moves through the ambulacral system as all echinoderm. The ambulacral legs at the top have a clamp that serves to attach them to the surface (Habdija et al. 2011). The color of the thorns is variable; they can be purple, dark red, dark green, brown. *P. lividus* is a species adaptable to different biological temperatures and food conditions. It feeds most frequently on algae and suspended organic particles (Bulleri et al. 1999). In the open sea, the sea urchin *P. lividus* occurs mainly on solid rocks and boulders, and in seagrass meadows such as *Posidonia oceanica* and *Zostera marina* (Lawrence 2013). Individuals in shallower seas are much more exposed to predators and the influence of waves. They resist the negative influence of wave energy by burying in the substrate (e.g. sandstone, limestone, granite), creating cup-shaped cavities in which they live temporarily or permanently. Such behavior also provides them with protection from predators. The dynamics of sea urchin populations may determine the form of benthic communities since the shift from erect algal communities to barrens is a consequence of sea urchin density (Hereu 2004). Young and small individuals, which are especially easy targets for predators, to protect themselves, live permanently in holes, cracks, under gravel and rocks, and sometimes under the dense cover of multicellular photosynthetic organisms. Larger individuals move more in search of food, after which they may or may not return to shelters. Populations in shallow habitats show the highest densities. They occur locally in very dense clusters, likely due to predator defense, feeding, and spawning. Populations can be stable in density for years, but rapid and long-lasting changes in the density of large individuals often occur. Short-term and long-term changes in population density are considered to be the result of a lot of different factors, such as uneven spawning, losses during the larval stage of life, migration, natural changes in predator numbers, predator overfishing, pollution, and disease. They feed mainly on multicellular photosynthetic organisms but can become opportunists and omnivores, especially in conditions of limited resources. This behavior and the ability to shift from preferred but limited resources to less preferred but more numerous resources significantly affect the structure of the benthic communities in which they live, and in particular the population density of multicellular photosynthetic organisms. They are recognized to play an essential part in community dynamics through their forage (grazing) behaviors, social aggregations, and predator-prey connections (Croo 2003).

Laboratory studies show that *P. lividus* movement may be important and that it is mediated by light, which can determine both direction and speed of movement (Domenici et al. 2003) They feed mostly at night. The sea urchin *P. lividus* often covers its upper body with various objects, such as leaves, empty shells, pebbles anandfragments of plastic (Lawrence 2013). It is believed that such behavior may be for protection from light, ultraviolet radiation, and predators. *P. lividus* is typically covered with non-living material to protect from light (Moore 1966) or to camouflage (Milligan 1915); however Crook et al. (1999) and Barnes & Crook (2001) suggest that covering may be a multifunctional behavior, and its benefits may vary based on the animal's size and habitat. *P. lividus* also had a clear circadian activity pattern, supporting previous evidence that it moves more at night (Kempf 1962; Millott 1976; Dance 1987). This nocturnal pattern has been suggested to be an escape behavior from diurnal predators (Dance 1987). During the daytime, Mediterranean *P. lividus* spends most of its time hiding in crevices to avoid predators (Sala&Zabala 1996). Light-stimulated locomotion in *P. lividus* is higher than spontaneous activity, which results from movement away from the light source (negative phototaxis). The explanation for this result is the sensitivity of *P. lividus* to light. It has been shown that most echinoderms possess photosensitive cells throughout the entire body surface and that the spines and podia have sensory functions as well (Reese 1966; Yoshida 1966). Additionally, many sea urchin species have negatively phototactic tube feet in bright light (Yoshida 1966). Dark-adapted animals tend to be negatively phototactic, while light-adapted animals may be positively phototactic. This is following the observed negative phototactic behavior of dark-adapted *P. lividus* (Domenico 2003).

2 Aim of the study

In this study, I aimed to investigate the effect of artificial light at night on the organism performance in the sea urchin *P. lividus*. Therefore, I measured the feeding rates and activity patterns of the animal.

This study aimed to research the following hypothesis:

1) **Artificial light at night will affect *P. lividus* feeding rates.**
   I assume that with the presence of artificial light at night, organisms will consume less food.
2) **Artificial light at night will decrease the duration of feeding activity in *P. lividus*.**
   Since they are feeding during the night, the presence of artificial light at night will be mistaken for daytime and they will not eat.
3) **Artificial light at night will increase the duration of hiding activity in *P. lividus.***
   The presence of artificial light at night will force them to hide more during the night since they are usually hiding during the day.
4) **Exposure to artificial light for half a night will have a smaller effect on the feeding rates and activity of *P. lividus*.**
   As organisms will be exposed to fewer hours of artificial light at night, the effect will be smaller than in full night light exposure.

3 Material and method

3.1 GAME – Global Approach by Modular Experiments

I conducted my master thesis in the framework of the international research and training programme GAME (Global Approach by Modular Experiments), founded by the GEOMAR Helmholtz Centre for Ocean Research, Kiel. This project enables students of environmental and biological sciences to conduct their master thesis in the field of marine ecology. The participants work together in up to eight binational teams, each consisting of a German and an international student. In general, ecological studies focus on a small number of species in specific habitats, while the GAME project aims to realise an international approach. The idea of GAME is to conduct the same experiment at numerous sites across the globe to get generalizable answers for up to date research questions. For the GAME 2021 project GEOMAR cooperated with researchers and marine institutes in Vigo, Indonesia, Madeira, Finland, Croatia and Japan. At the beginning of the program, all participants had together an online course and we designed an experimental set up, which we subsequently realised simultaneously in the following six months in the country of our team partner. Under the title "Effects of artificial light at night on benthic grazers " we aimed to investigate how artificial light affects food feeding rates in marine grazers around the world. To do so, we focussed on the most dominant and important grazer species in the respective ecosystems. Each student investigated one species and gained an independent data set, which could be used as the subject of a thesis. We applied standardized methods between sites to gain comparable results. After the practical part, all teams came back to Germany and we analyzed the data sets to develop a general global model.

3.2 Study site and field sampling

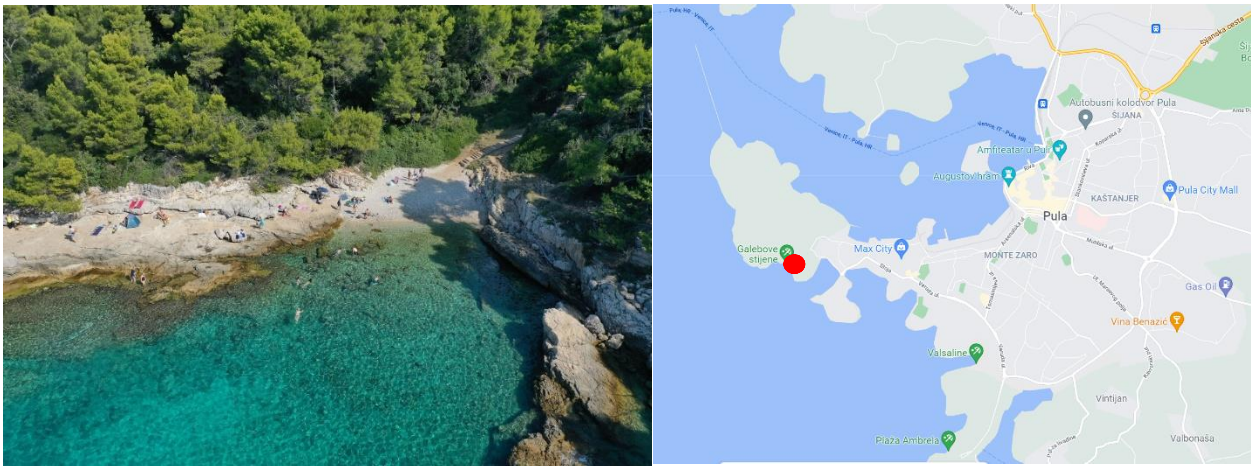

Figure 2. Location of the study site (right, adapted from Google maps, 27.12.2021) and collection site (left) at the cost of Pula (red circle)

I sampled my study organism from the subtidal in Muzil Bay (44°51’40.5 "N 13°48’24.5" E), which belongs to the city of Pula (see Figure 2). Muzil is located in the northern Adriatic, the shallowest (< 60 m), landlocked, and northernmost region of the Mediterranean Sea. In this region, stratification of the water column occurs from spring to mid-autumn, caused by the upwelling of fresh water and warming of the sea surface. In winter, cooling and cold northeast winds lead to intense mixing and the formation of dense water (Gianni 2012). I conducted sampling from June to September 2021. The ambient water temperature in the habitat was 25°C in June and 23 °C in September. *P. lividus* as my study organism is abundant in Muzil Bay and is an important local grazer, which made it well-suited for the experiment I planned. I collected medium-sized (shell plus the spine, 8-10cm; Figure 3), adult specimens for my experiment by free diving. The collection site was a pristine environment in which they were not under the direct influence of any artificial light sources. I did this to prevent them from showing any adaptive behavior during the experiments. Once the collection was done, I transferred them to the nearby facilities in 5 L plastic boxes (five individuals per box) within 15 minutes. After arriving in the lab, I transferred the sea urchin to 120-liter tanks, which had ~ 23 °C to minimize stress. The maximum number of individuals collected per experiment was 38. No individual died during transportation.

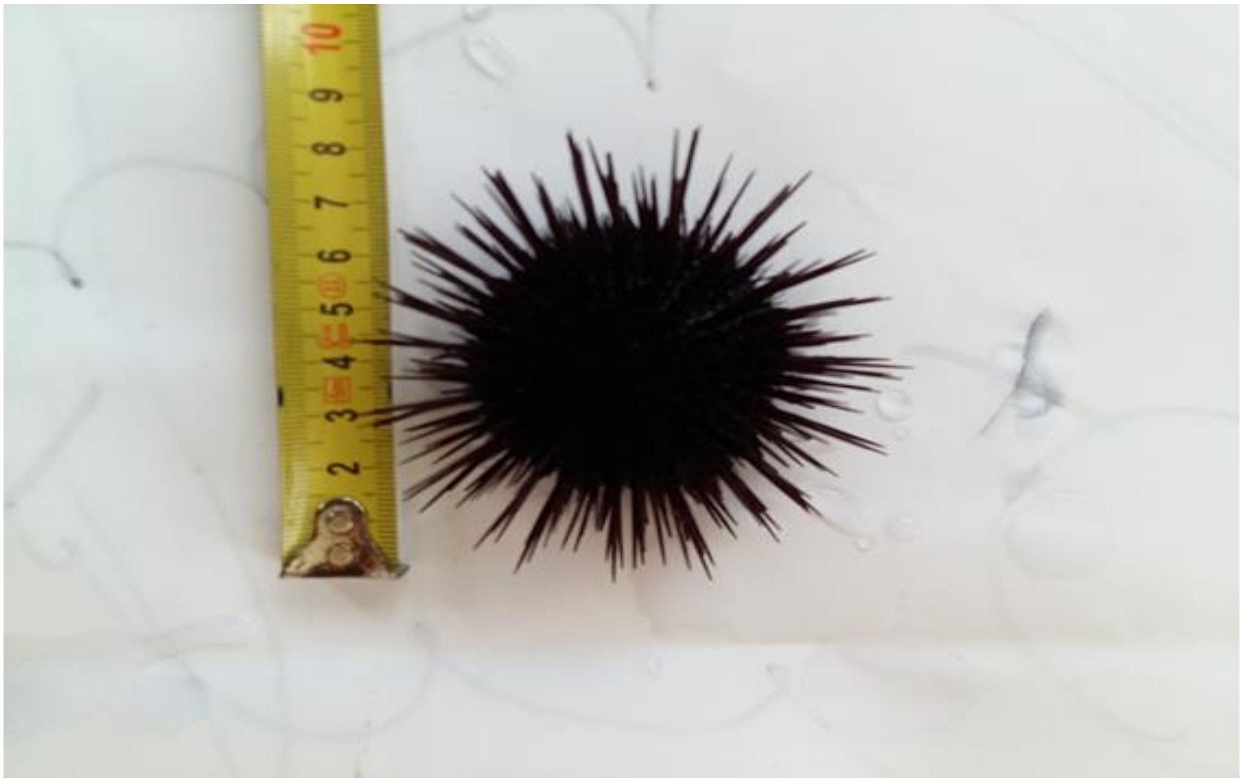

Figure 3. *Paracentrotus lividus* (Lamarck, 1816)

3.3 Experimental set up and time schedule

All laboratory experiments took place in the “Morska škola” facilities in Pula. In total, I performed three separate experiments: Experiment 1 with an acclimation of 12 days and a 30 lux exposure to artificial light at night; Experiment 2 without acclimation and a 30 lux exposure to artificial light at night; and Experiment 3 without acclimation and a 60 lux exposure to artificial light at night. In experiment 2 and experiment 3 I did not acclimate the animals to the light before the measurement phase because I wanted to see if there is an acute response to the light exposure. Before starting the main experiment, I conducted some pilot studies that determined the species sensitivity to red light and the possible autogenic changes in the food pellet weight during the measurement phase. These pilot studies also give me an idea about the test animal's general feeding activity (measured as mg*24 h-1*g individual-1). For this, I check daily if the provided algal material has been completely consumed within 24 h, adjusting the amount in a way that allows providing food *ad libitum.* This is essential to avoid starvation, which could influence the feeding behavior of the test animals. The latter could mask the possible effect of artificial light at night.

3.3.1 Light treatment

This thesis aims to investigate the effects of light pollution at night on marine benthic grazers *P. lividus*. For this purpose, I simulate the natural daylight period and additionally expose the test individuals to artificial light at night. I used three different light treatments (“None”,” Full-night”,” Half-night”) per experiment to investigate the possible effects of this artificial light-induced prolongation of the day on the test organisms. I also check for the possible acute response of the organism to artificial light at night. So in the first and second experiments, I observed the behavior of the test organisms at a light intensity of ~30 lux, and then in the third experiment, I exposed other individuals of the same species to a light intensity of ~60 lux. The behavior of each of the test individuals was observed for 24 hours. Before the start of the first experiment, I acclimated the individuals to laboratory conditions and the light regime of the respective length treatment for 12 days. During this period, the test individuals experienced the same light conditions as in the later measurement phase. In the second and third experiments, I did not acclimate the animals to the different light treatments before the measurement phase. Each experiment comprises three lengths of exposure to light. All of them have the same light intensity during the period from sunrise to sunset and during the daylight period. A white LED illuminated the experimental units with an intensity of ~3000 lux during the daylight period, which is equivalent to sunlight on a cloudy day (Gaston et al. 2013). The same white LED simulated artificial light at night, but the light intensity depended on the experiment. In the “None” = Darkness at night treatment, I exposed the test organisms to simulated daylight (~3000 lux) during the day and left them in complete darkness at night (0 lux) (Figure 4). In the "Full-night” light exposure", I dimmed the white LED to either ~30 lux (Experiment 1 and Experiment 2) or ~60 lux (Experiment 3). The dimmed LED then emits this lower light intensity throughout the night and is adjusted back to ~3000 lux at sunrise (Figure 6). In the "Half-night” light exposure the LED is dimmed to one of the two light intensities at night, but the LED does not stay on all night. It shines from sunset to midnight and from 5 am to sunrise. Between midnight and 5 am, I switched the LEDs off, and the test organisms were in complete darkness. Ayalon et al., 2019 (35–40 lux); Davies et al., 2012 (3 & 20 lux); Fobert et al., 2019 (26.5 lux); Maggi & Serôdio, 2020 (27 lux) have all used the same light intensities that I envision for the night period. In each experiment, all individuals of the test species have been exposed to one of the three light treatment levels (“None”, “Half-night” and “Full-night”).

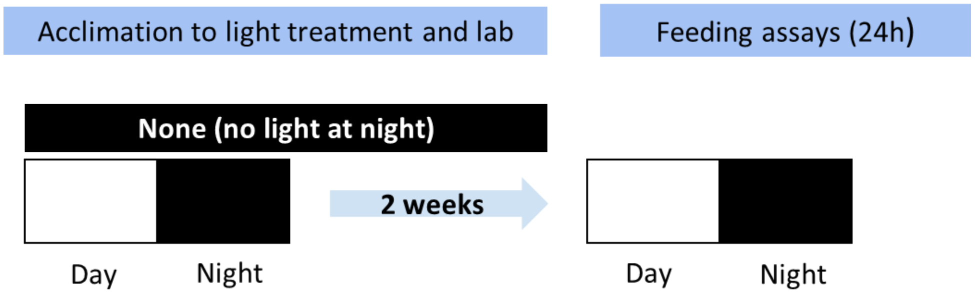

Figure 4. Time schedule of the “None” darkness at night light treatment for the first experiment with acclimation. During the day, the light intensity was ~3000 lux, while at night no light prevail.

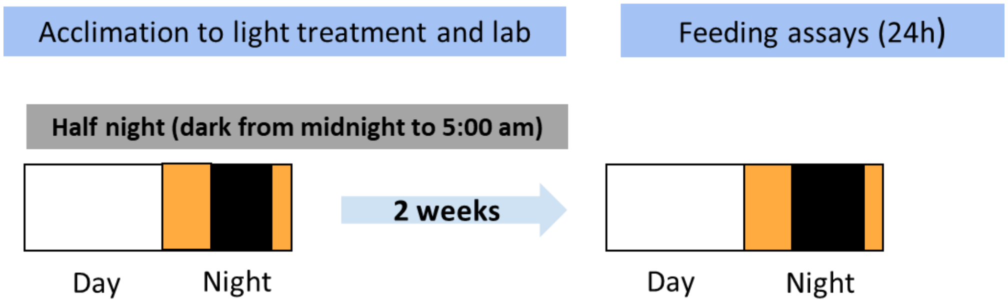

Figure 5. Time schedule of the "Half-night” light exposure treatment level. During the day, the light intensity was ~3000 lux, while at night, the intensity was ~30 lux (experiment 1 and experiment 2) or ~60 lux (experiment 3) for the time interval from sunset to midnight and from 5 am to sunrise. From 12 pm to 5 am, there was darkness.

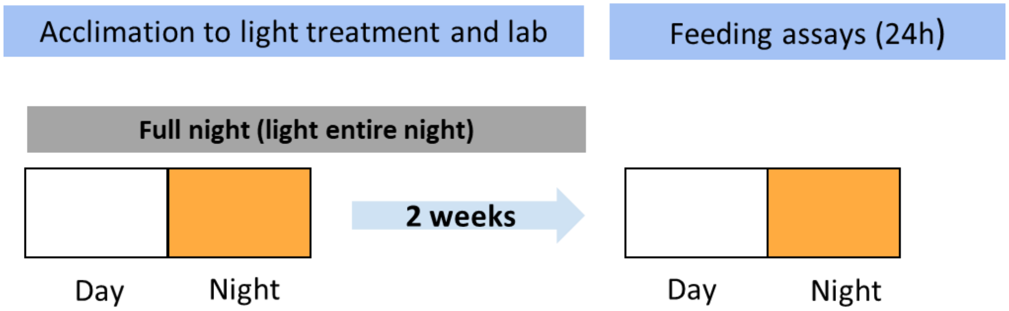

Figure 6. Time schedule for the "Full-night" light exposure treatment level. During the day, the light intensity was ~3000 lux, while at night there was a white LED with either ~30 lux (experiment 1 and experiment 2) or ~60 lux (experiment 3), from sunset to sunrise. The light regime was identical for the acclimation and the measurement phase.

3.3.2 Experimental units and LED cabling

I kept the animal individually in transparent tanks (i. e., experimental units) with manual water exchange, at which I replaced ¾ of the water volume per tank every second day. The ratio between the size of the sea urchin and the bottom area of the tanks was 1:10 (the bottom area is ten times the size of the individual) to ensure adequate habitat conditions and to allow unambiguous identification of the movement pattern during the measurement period. The water volume in the tanks was 5 liters. In every tank, there was a plastic shelter made of PVC tubes (Figure 7).

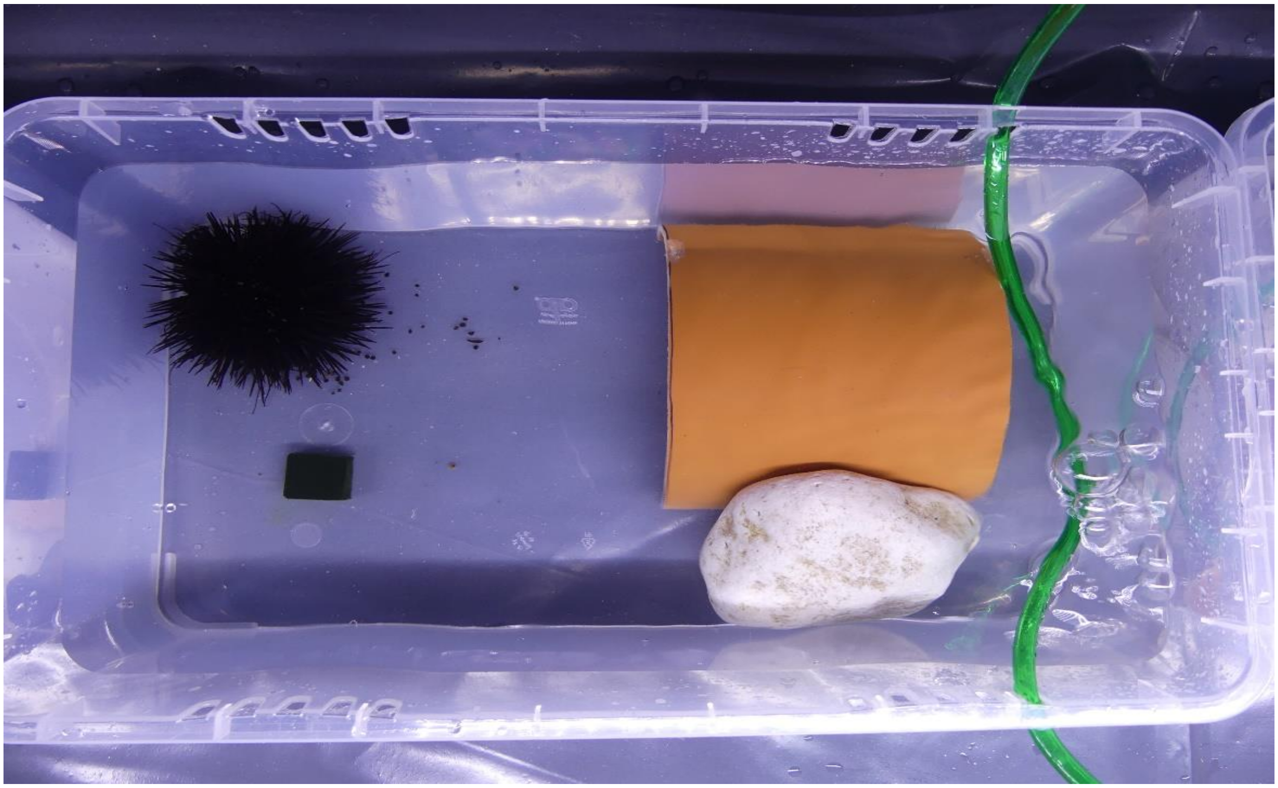

Figure 7. Experimental units with sea urchin *Paracentrotus lividus*, food pellet, and shelter

For the acclimation period, I used three different shelves. Each of the shelves represents one light treatment (Figure 8). I covered the shelves using black foil to mimic curtains to prevent light from neighboring treatment levels from falling in. To realize the different light treatment levels, I used a white 80 cm LED (eco+ LED strip SKY 6500 K) for the acclimation period, a 50 cm long white LED, and one 50 cm red LED (eco+ LED strip RED 625 nm) for the measurement period, during which I took the photos. I also use an automatic LED dimmer with four channels (Sunriser 4+), which can adjust the light regime for each of its four channels separately. Each channel was adjusting the light regimes to changes in the timing of sunrise and sunset. During acclimation, each of the 80 cm long white LEDs illuminated 16 experimental units with a minimum intensity of 3000 lux at the same time. During the measurement period, 50 cm long LEDs plus the red LED illuminated four or six experimental units for the "No light at night" light treatment. I placed the LED lights 30 cm above the water surface.

Figure 8. Shelf setup in which different light regimes are established on different shelf levels. The upper shelf contains the "None" treatment level, the middle shelf contains the "Full-night" treatment level, and the lower shelf contains the "Half-night" treatment level.

3.3.3 Sequence of experiments

The first experiment consists of two phases: (1) an acclimation phase of 12 days and (2) a measurement phase of 24 h. The second and third experiments consist of just the measurement phase of 24 h. Since I could process a maximum of n = 4 replicates per day, test animals enter the acclimation phase sequentially of n = 4 per day. In both cases, I starved the animals for five days before the measurement phase.

3.4 Measurement phase and response variable

I assessed two response variables: the feeding rates and the behavior of each grazer individual during the 24-hour measurement phase. I starved animals for 5 days before the measurement, i.e., the amount and quality of the food pellet were the same during the acclimation and the measurement phase.

3.4.1 Feeding rates

I used artificial food for the feeding essays because it is easy to standardize and I wanted to avoid the influence of light on live algae. I used pellets made with *Spirulina* powder and agar after the following recipe: I diluted 0.36 g of agar powder in 5 ml of seawater and 1 g *Ulva*-powder in 4 ml of seawater. Then I heated the Agar-water in a microwave at high energy till it seethed. I immediately added it to the *Spirulina*-water, stirred, and poured the mix onto the stamp. After letting the pellet-mix cooldown, I cut it with the small cubes (Figure 9).

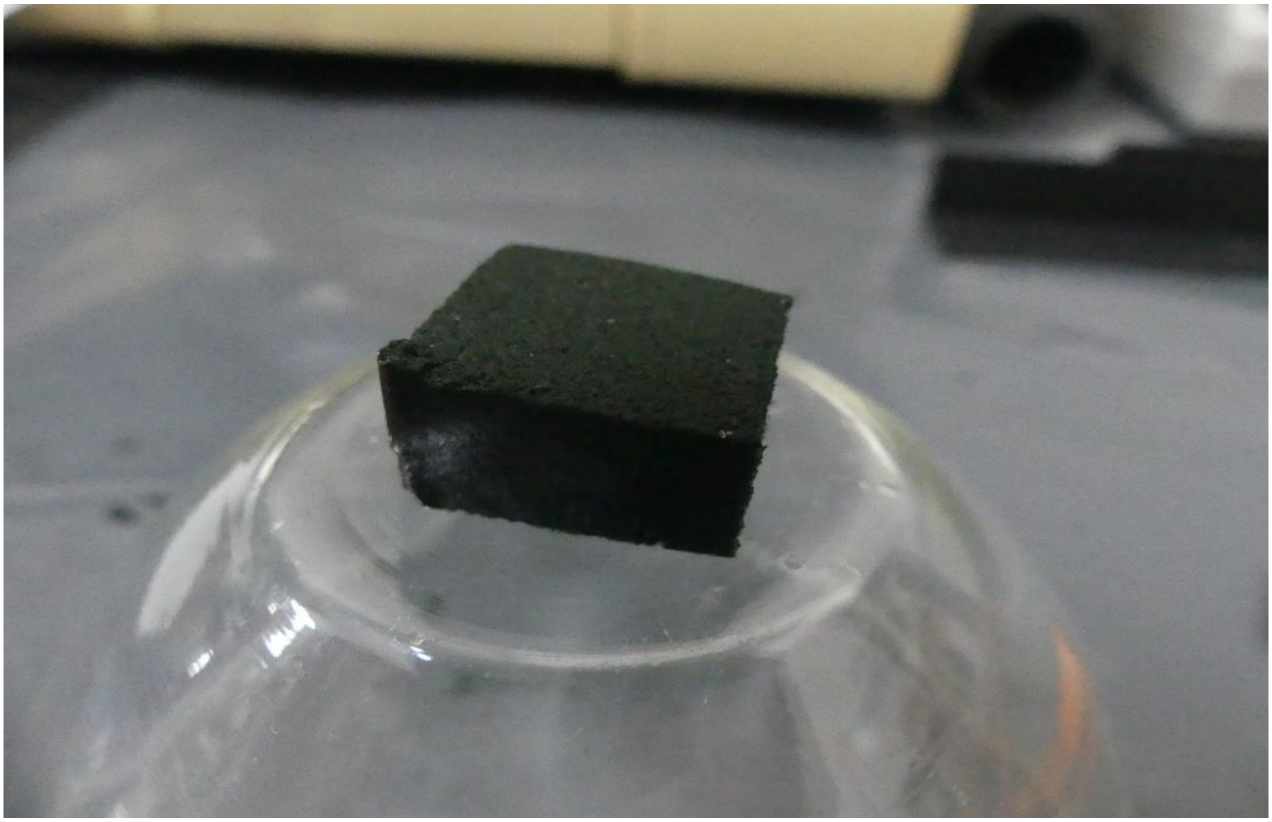

Figure 9. Finished *Spirulina*-pellet

At the beginning of the feeding essay, I put a pellet on the opposite side of the shelter. The measurement phase lasted 24 hours, and after that time, I collected the leftovers of the food pellets from the tanks. I completely remove water from the collected pellets by drying them in an oven at 60 °C for 48 hours. I calculated the food consumption rates rate by the difference between pellet dry weight before and after the measurement phase, divided by the animal dry weight. In a pilot study, I assessed the average dry weight of a food pellet.

$$\text{Consumption rate} = \frac{\Delta \text{ Pellet dry weight}}{\text{Animal dry weight} \times 24 \text{ h}}$$

In the pilot study I check for the autogenic change in the wet weight of the food pellets during the feeding assays, e.g. due to the soaking of water or the loss of material, is negligible and thus, does not bias the measurements that assess feeding rates.

3.4.2 Activity pattern

For the 24-hour measurement phase, during which photographs were taken at regular intervals, the camera set-up was placed outside the shelves where the replicates were located. This was necessary to avoid the influence of any external light source. When setting up the equipment for the measurements, I separated the food pellets and the shelter on the opposite sides of the tanks. The tanks were arranged so that four or six replicates could be photographed at a time so that one set of replicates was processed per day (Figure 10).

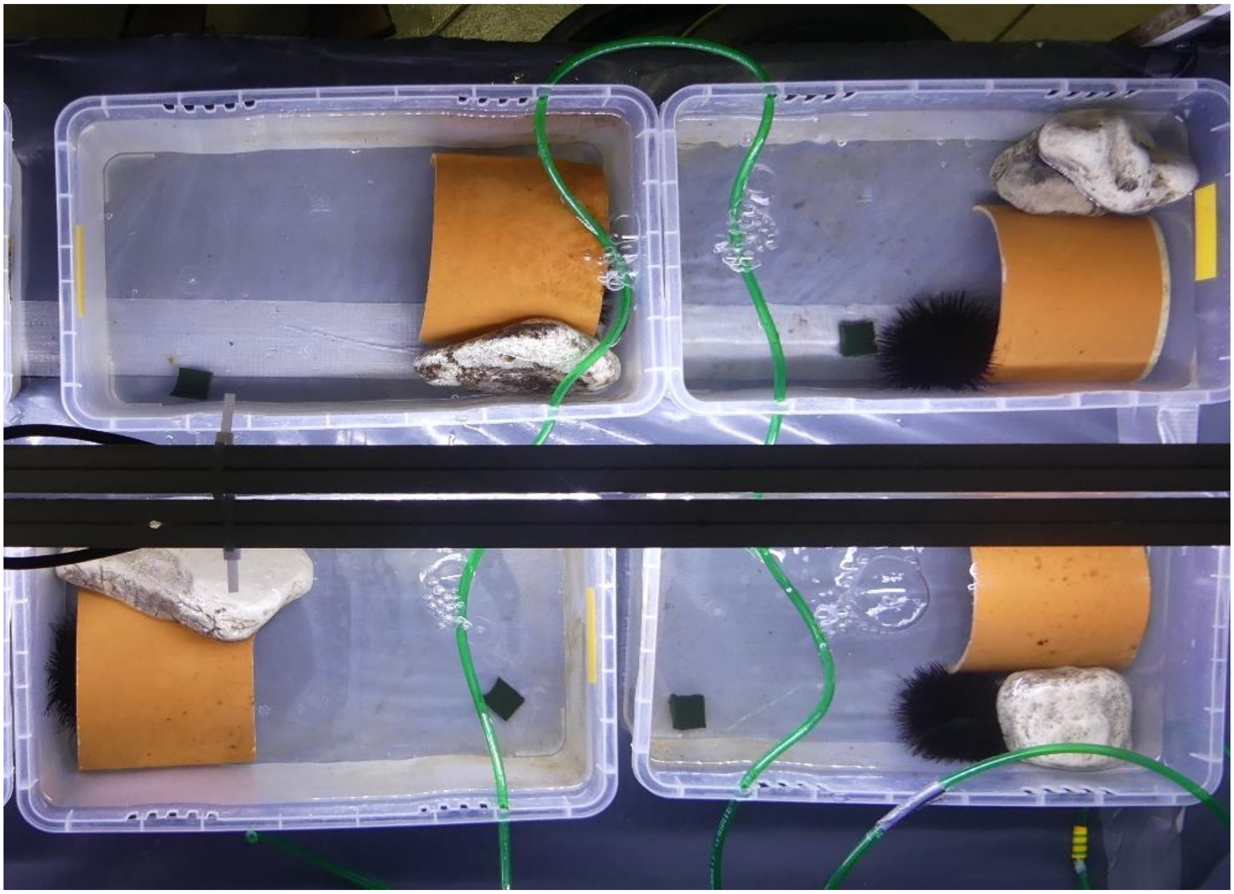

Figure 10. Tanks and set up of the measurement phase.

The behavior of the test individuals was documented by time-lapse photography (at 15-minute intervals) throughout the 24-hour measurement phase. Afterwards, I classified each photo as belonging to one of three categories: 1) “Pellet” is assigned if the grazer is in direct contact with the food pellet. 2) “Hiding” is assigned if at least 50 % of the grazer’s body is in the shelter. 3) “Elsewhere” if he is elsewhere in the tank (Figire11). Contact with the food pellet was interpreted as a feeding event while hiding in the shelter or presence elsewhere in the aquarium was regarded as two different behavioral responses. Following that, I entered all the data into the respective experiment’s excel sheet. I split the information into two separate binary data sets to analyze the frequency of “Pellet/Not pellet“ (1/0) and “Hiding/Not hiding” (1/0) under day and night conditions.

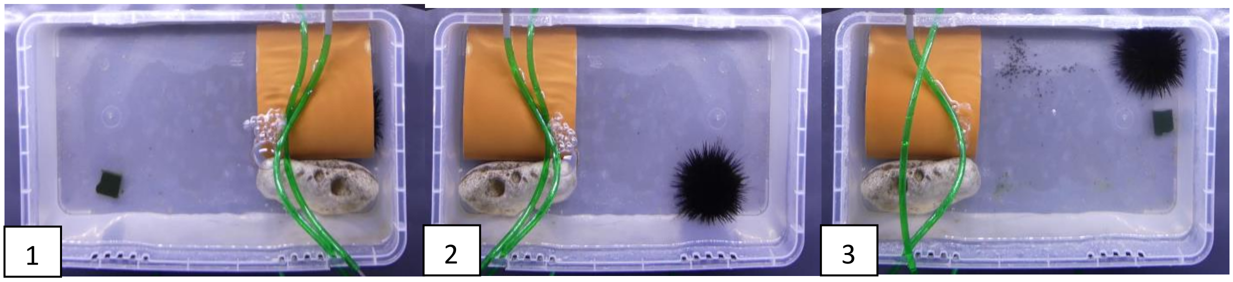

Figure 11. Possible position of the animal. 1) Hiding 2) Pellet 3) Elsewhere

In the main experiment, I assessed the activity of the grazer by taking photos. For this purpose, the camera I used needed a light source, for which I used dimmed red light. This is appropriate because previous studies claimed that numerous, especially marine invertebrate taxa, such as spiny lobsters or octopus, are insensitive to the light of wavelengths above 600 nm (Gaston et al. 2012, Peitsch et al. 1992, Hamasaki 1968, Hughes 1970, Meyer-Rochow & Tiang 1984, Weiss et al. 2006, Auster et al. 1990, Cronin 1986). A possible reason for the insensitivity of marine taxa to red light could be the strong attenuation of longer wavelengths within the water column (McFarland 1986). However, several other studies indicate a prevalent sensitivity to red light in fishes, crustaceans (Kohlberg et al. 2019, Widder et al. 2005), and sea urchins (Yang et al. 2020, Yang et al. 2021). For this reason, a pilot study to determine whether the sea urchin *P. lividus* is sensitive to red light was mandated. I ran feeding assays with non-light-acclimated individuals to test for red light sensitivity. Before the start of the pilot study, I checked for the minimum red light intensity that is required for photos of acceptable quality. In the end, it was 8 lux. The pilot study comprised two groups with 16 replicates each: a group called “Natural Darkness”, which experiences daylight and darkness at night (white LEDs, light intensity: day = 3000 lux, night = 0 lux). The second group, “Red light during the entire night” experiences daylight during the day (white LEDs, light intensity: day = 3000 lux) and red light during the night (red light LEDs, light intensity < 10 lux). During the pilot study, I only assessed the food consumption rates of the animals and no behavioral patterns. Feeding rates were measured for 24 h by examining the difference in the dry weight of the provided algal pellets before and after the feeding assays. After collecting the data, I did a t-test, which didn’t show a significant effect (n=0.311).

3.5 Statistics

I performed all the statistical analysis using the free computing software R. To evaluate the effect of light exposure on the feeding rates, I conducted a one-way analysis of variance (ANOVA). Using ANOVA, I tested for differences between the means of the treatment groups. I performed the Fligner–Killeen test to verify that variances were homogenous, and I used plots of Cooks’ distances to identify influential data points. To verify that the residuals were normally distributed, I visually controlled with histograms and statistically by applying the Shapiro-Wilk’s-Test. For the second and third experiments, I used the Kruskal-Wallis H test because the data were not normally distributed. For the activity pattern, I used generalized linear models (GLM). I check for the dispersion parameter and the model was highly overdispersed which can cause false positives. Because the model is highly overdispersed and the data were zero-inflated I use negative binomial glm.

4 Results

4.1 Feeding rates

4.1.1 First experiment

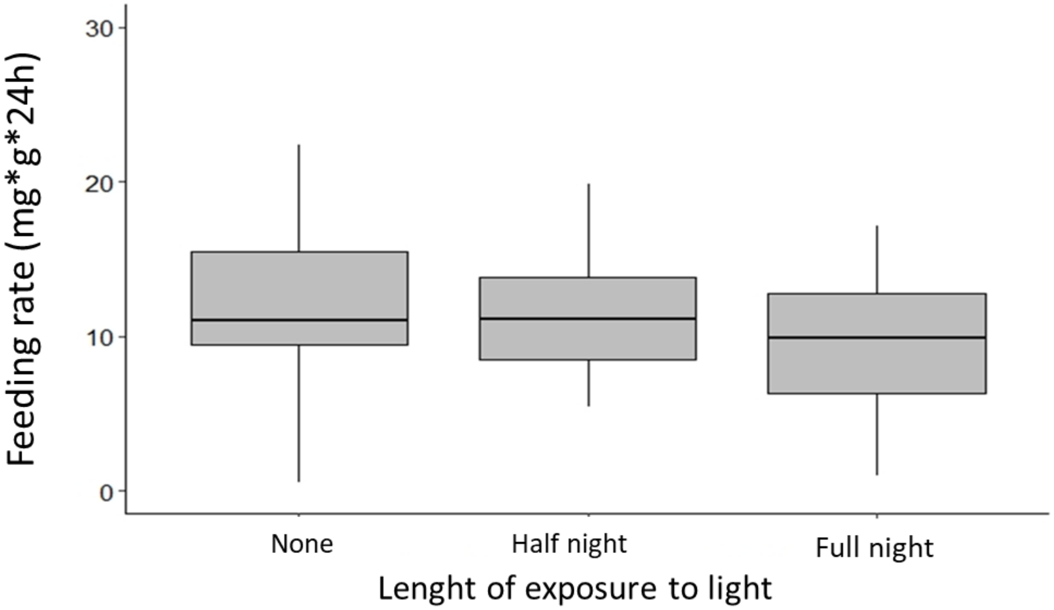

Figure 12. Feeding rates in *Paracentrotus lividus* individuals that were exposed to three different lengths of exposure to artificial light at night. "None" = Darkness (red light) at night. "Half-night" = ~30 lux artificial light at night from sunset to midnight and from 5 am to sunrise. "Full-night" = ~30 lux artificial light at night from sunset to sunrise. Feeding rates were assessed in 24 h feeding assays. Animals were acclimated to the light treatment for 12 days prior to feeding assays. Box plots show medians with interquartile range and non-outlier range The first box plot represents 24 replicates, the second 22 replicates, and the third 24 replicates.

In the first experiment, the animals were acclimated for 12 days to the light treatments and the feeding rates were assessed in the 24h feeding assays. Results show that feeding rates change insignificantly through the three different lengths of exposure to artificial light at night. The median feeding rates for the “None” (no light at night) light treatment is 12.17 mg*g*24h, for the “Half-night” (artificial light from sunset to midnight and from 5 am to sunrise) light treatment is 11.42 mg*g*24h and for the “Full-night” (artificial light at night from sunset to sunrise) light treatment is 9.91 mg*g*24h. The results show a trend in decreasing feeding rates with longer exposure to artificial light at night (“Half-night”,” Full-night”) (Figure 12). I applied a one-way analysis of variance (ANOVA) and can report a nonsignificant effect of artificial light at night ("None", "Half-night", "Full-night") on the feeding rates in *P. lividus* (Table 1).

Table 1. Effect of artificial light at night with three lengths of exposure ("None", "Half-night", "Full-night") on the feeding rates of the sea urchin *Paracentrotus lividus,* as assessed in 24 h feeding assays. Animals were acclimated to the light treatment for 12 days. Results from one-factorial ANOVA.

	df	**Sum sq**	**Mean sq**	**F-value**	**P-value**
Length of exposure to light	2	63.2	31.61	1.24	0.29

4.1.2 Second experiment

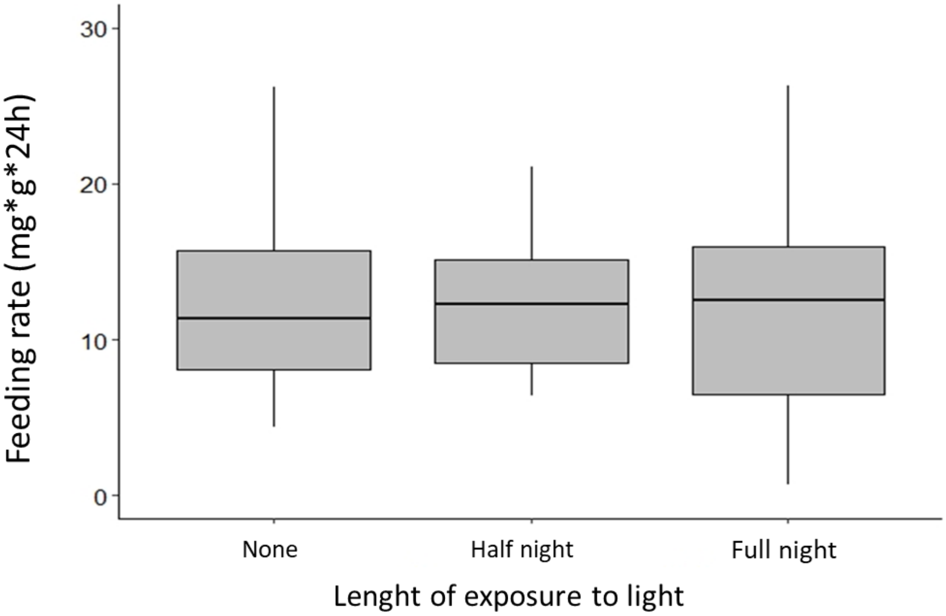

Figure 13. Feeding rates in *Paracentrotus lividus* individuals that were exposed to three different lengths of exposure to artificial light at night. "None" = Darkness (red light) at night. "Half-night" = ~30 lux artificial light at night from sunset to midnight and from 5 am to sunrise. "Full-night" = ~30 lux artificial light at night from sunset to sunrise. Feeding rates were assessed in 24 h feeding assays. Animals were not acclimated to the light treatment prior to feeding assays. Box plots show medians with interquartile range and non-outlier range. Every boxplot represents 18 replicates.

In the second experiment, where I checked for the acute response to the different light exposures to artificial light at night then animals were not acclimated prior to the feeding assay. The feeding rates didn’t change significantly between the different lengths of exposure to artificial light at night("None", "Half-night", "Full-night"). The median feeding rates for the “None” (no light at night) light treatment is 12.52 mg*g*24h, for the “Half-night” (artificial light from sunset to midnight and from 5 am to sunrise) light treatment is 13.55 mg*g*24h and for the “Full-night” (artificial light at night from sunset to sunrise) light treatment is 12.07 mg*g*24h. The results showed that acute exposure to the different lengths of artificial light at night had no significant effect on the feeding rates of the sea urchin *P. lividus* (Figure 13). Statistical analysis was not significant; I used Kruskal-Wallis because the data were not normally distributed (Table 2).

Table 2. Effect of artificial light at night with three lengths of exposure ("None", "Half-night", "Full-night") on the feeding rates of the sea urchin *Paracentrotus lividus*, as assessed in 24 h feeding assays. Animals were not acclimated to the light treatment. Results from Kruskal-Wallis.

	df	**chi-squared**	**p-value**
Length of exposure to light	2	0.30	0.86

4.1.3 Third experiment

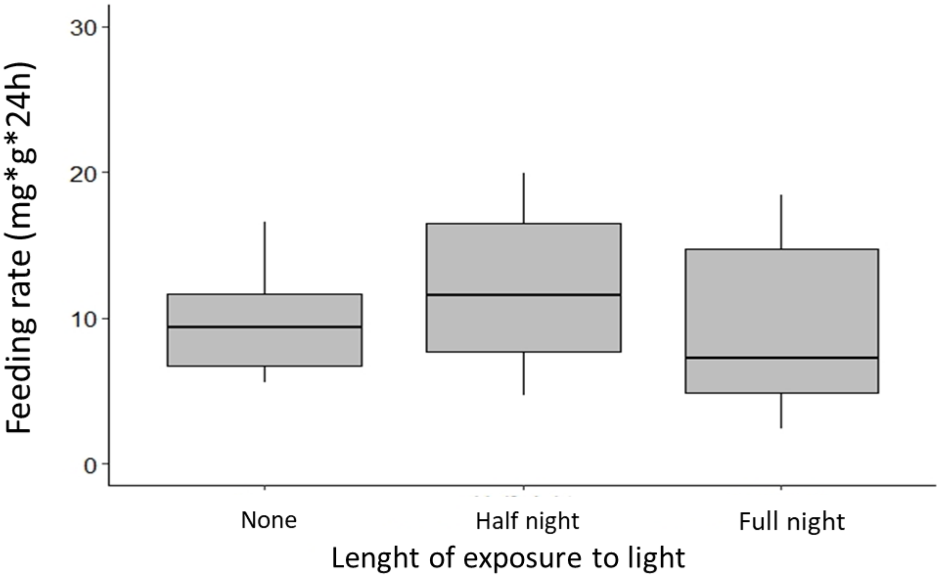

Figure 14. Feeding rates in *Paracentrotus lividus* individuals that were exposed to three different lengths of exposure to artificial light at night. "None" = Darkness (red light) at night. "Half-night" = ~60 lux artificial light at night from sunset to midnight and from 5 am to sunrise. "Full-night" = ~60 lux artificial light at night from sunset to sunrise. Feeding rates were assessed in 24 h feeding assays. Animals were not acclimated to the light treatment prior to feeding assays. Box plots show medians with interquartile range and non-outlier range. Every boxplot represents 18 replicates.

In the third experiment the animals were not acclimated to the different light exposure to artificial light at night before the feeding assay. The light intensity of the artificial light at night ("Half-night", "Full-night") was 60 lux. The result shows no significant change between the three different lengths of exposure to artificial light at night ("None", "Half-night", "Full-night"). The median feeding rates for the “None” (no light at night) light treatment is 9.95 mg*g*24h, for the “Half-night” (artificial light from sunset to midnight and from 5 am to sunrise) light treatment is 12.02 mg*g*24h and for the “Full-night” (artificial light at night from sunset to sunrise) light treatment is 9.10 mg*g*24h. The results showed that higher exposure to artificial light at night had no significant effect on the feeding rates of the sea urchin *P. lividus* (Figure 14). Statistical analysis was not significant; I used Kruskal-Wallis because the data were not normally distributed (Table 3).

Table 3. Effect of artificial light at night with three lengths of exposure ("None", "Half-night", "Full-night") on the feeding rates of the sea urchin *Paracentrotus lividus*, as assessed in 24 h feeding assays. Animals were not acclimated to the light treatment. Results from Kruskal-Wallis.

	df	chi-squared	p-value
Length of exposure to light	2	4.04	0.13

4.2 Activity pattern

4.2.1 First experiment

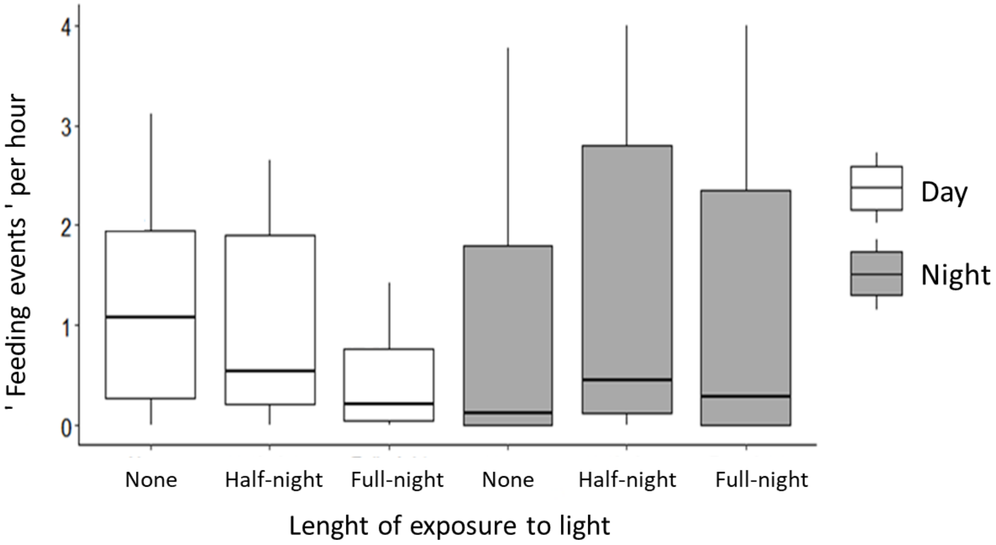

Figure 15. Day-night feeding activity of the sea urchin *Paracentrotus lividus* in response to three lengths of exposure to artificial light at night. “None” = Darkness (red light) at night.”Half-night” = ~30 lux artificial light at night from local sunset to midnight and from 5 am to sunrise. “Full-night” = ~30 lux artificial light at night from local sunset to sunrise. Animals were acclimated to the light treatment for 12 days prior to feeding assays. Feeding frequency is calculated from a presence/absence matrix (“Feeding”/”Not feeding”) derived from timelapse photography (23.5 h) at 15 min intervals. Box plots show medians with interquartile range and non-outlier range. Each replicate is plotted twice: once during day- and once during nighttime.

In the first experiment, after 12 days of acclimation to the different light treatments, the results show that there is no effect on the feeding activity of the animal under exposure to artificial light at night "Full-night" and "Half-night". Although there is a trend towards a decrease in feeding activity during the daytime when the animals experience exposure to artificial light at night ("Full-night" and "Half-night")(Figure 15). The result shows a statistically significant interaction between the time of the day and the length of exposure to light (Tabel 4). The result also shows that under normal conditions ("None" no light at night) the animals have more feeding activity during the daytime.

Tabel 4. Effects of exposure to artificial light at night on the day-night feeding activity (feeding events per hour) of the sea urchin *Paracentrotus lividus*. Data derived from timelapse photography (23.5 h) at 15 min intervals. Animals were acclimated to the light treatment for 12 days. Results from chi-square test based on two-factorial GLM negative binomial.

	Df	**Deviance**	**Res. Df**	**Res. Dev**	**Pr(>Chi)**
Time of the day	1	1.17	140	179.22	0.28
Lengh of exsposure to light	2	2.30	138	176.92	0.32
Time of the day/length of exposure	2	5.60	136	171.32	0.06

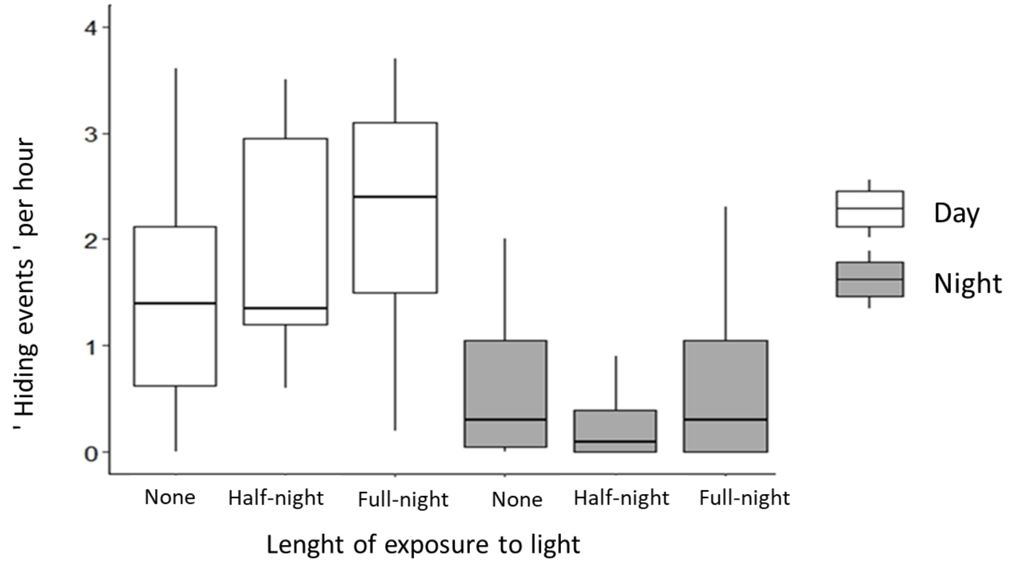

Figure 16. Day-night hiding activity of the sea urchin *Paracentrotus lividus* in response to three lengths of exposure to artificial light at night. "None" = Darkness (red light) at night."Half-night" = ~30 lux artificial light at night from local sunset to midnight and from 5 am to sunrise. "Full-night" = ~30 lux artificial light at night from local sunset to sunrise. Animals were acclimated to the light treatment for 12 days prior to feeding assays. Feeding frequency is calculated from a presence/absence matrix ("Hiding"/"Not hiding") derived from timelapse photography (23.5 h) at 15 min intervals. Box plots show medians with interquartile range and non-outlier range. Each replicate is plotted twice: once during day- and once during nighttime.

The results show a highly significant difference between daytime and nighttime hiding activity. The animal prefers to hide more during the daytime than during the nighttime. There is no effect on the hiding activity during the night while exposed to artificial light at night, neither the "Full-night" nor the "Half-night". Despite that, during the daytime, there is an increase in hiding activity for animals that experience "Full-night" and "Half-night" exposure to artificial light (Figure 16). When exposed to artificial light at night, hiding activity during the day and night follows opposing trends. The interaction between time of the day and length of exposure is statistically significant (Table 5). This significant result comes from more hiding activity during the daytime when exposed to artificial light at night, while during the night the hiding activity does not change in relation to the exposure to artificial light.

Tabel 5. Effects of exposure to artificial light at night on the day-night hiding activity (hiding events per hour) of the sea urchin *Paracentrotus lividus*. Data derived from timelapse photography (23.5 h) at 15 min intervals. Animals were acclimated to the light treatment for 12 days. Results from chi-square test based on two-factorial GLM negative binomial

	Df	**Deviance**	**Res. Df**	**Res. Dev**	**Pr(>Chi)**
Time of the day	1	32.83	136	171.22	0.08***
Lengh of exsposure to light	2	7.45	134	163.76	0.02*
Time of the day/length of exposure	2	9.06	133	154.70	0.01*

4.2.2 Second experiment

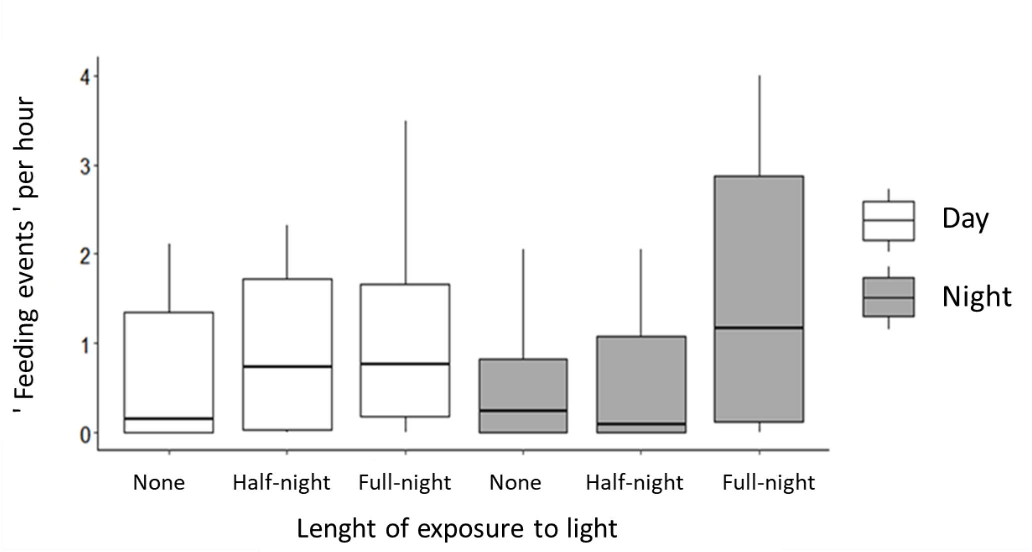

Figure 17. Day-night feeding activity of the sea urchin *Paracentrotus lividus* in response to three lengths of exposure to artificial light at night. “None” = Darkness (red light) at night.”Half-night” = ~30 lux artificial light at night from local sunset to midnight and from 5 am to sunrise. “Full-night” = ~30 lux artificial light at night from local sunset to sunrise. Animals were not acclimated to the light treatment. Feeding frequency is calculated from a presence/absence matrix (“Feeding”/”Not feeding”) derived from timelapse photography (24 h) at 15 min intervals. Box plots show medians with interquartile range and non-outlier range. Each replicate is plotted twice: once during day- and once during nighttime.

The results from the second experiment where the animals were not acclimated to the light treatment before the feeding assay showed no significant difference between daytime feeding and nighttime feeding. The animal eats equally during the 24 hours when exposed to artificial light at night (“Full-night and “Half-night”). Feeding activity during exposure to “Full-night” artificial light seems to increase if compared with the “None” and “Half-night” light exposure, but it is not significant (Figure 17). There is no statistically significant interaction between the time of the day and the length of exposure to light (Tabel 6). The immediate exposure to 30 lux of artificial light at night (“Full-night”,” Half-night”) does not have an effect on the feeding activity of the sea urchin *P. lividus.*

Tabel 6. Effects of exposure to artificial light at night on the day-night feeding activity (feeding events per hour) of the sea urchin *Paracentrotus lividus*. Data derived from timelapse photography (24h) at 15 min intervals. Animals were not acclimated to the light treatment. Results from chi-square test based on two-factorial GLM negative binomial.

	Df	**Deviance**	**Res. Df**	**Res. Dev**	**Pr(>Chi)**
Time of the day	1	0.54	106	131.95	0.47
Lengh of exsposure to light	2	3.22	104	128.73	0.20
Time of the day/length of exposure	2	1.28	102	127.44	0.53

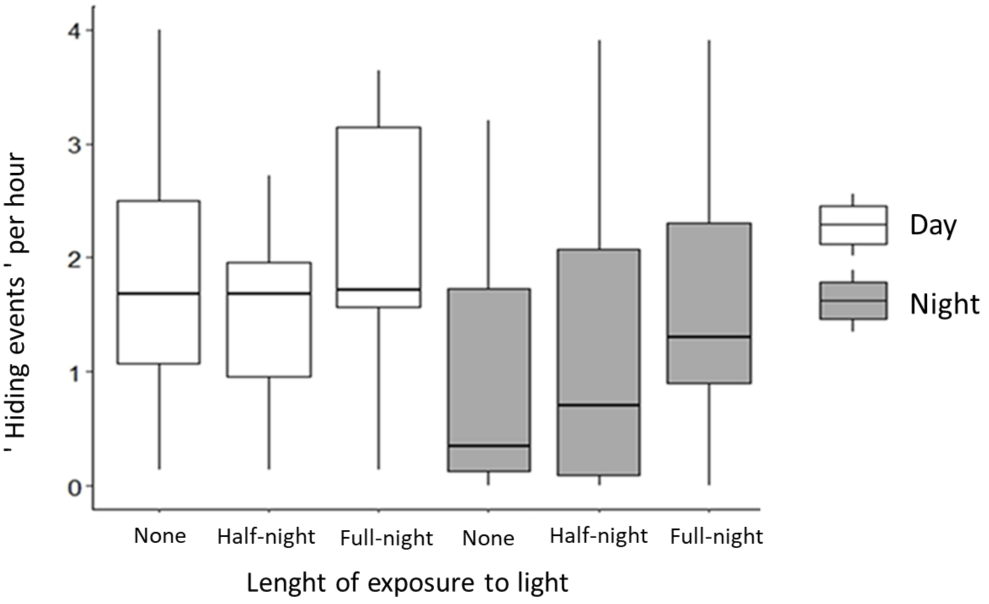

Figure 18. Day-night hiding activity of the sea urchin *Paracentrotus lividus* in response to three lengths of exposure to artificial light at night. “None” = Darkness (red light) at night.”Half-night” = ~30 lux artificial light at night from local sunset to midnight and from 5 am to sunrise. “Full-night” = ~30 lux artificial light at night from local sunset to sunrise. Animals were not acclimated to the light treatment prior to feeding assays. Feeding frequency is calculated from a presence/absence matrix (“Hiding”/”Not hiding”) derived from timelapse photography (24 h) at 15 min intervals. Box plots show medians with interquartile range and non-outlier range. Each replicate is plotted twice: once during day- and once during nighttime.

The time of the day has a significant effect on the hiding activity of the sea urchin. Animals hide more during the daytime than during the nighttime (Figure 18). However, exposure to artificial light at night ("Full-night" and "Half-night") does not have a significant effect on hiding activity. The result shows a trend during the nighttime exposure to artificial light, as the animals start to hide more during increasing exposure to artificial light ("Full-night" and "Half-night") (Tabel 7). The longer the exposure to artificial light at night, the more hiding activity is present. There is no significant interaction between the time of the day and the length of exposure to light.

Tabel 7. Effects of exposure to artificial light at night on the day-night hiding activity (hiding events per hour) of the sea urchin *Paracentrotus lividus*. Data derived from timelapse photography (24 h) at 15 min intervals. Animals were not acclimated to the light treatment. Results from chi-square test based on two-factorial GLM negative binomial

	Df	**Deviance**	**Res. Df**	**Res. Dev**	**Pr(>Chi)**
Time of the day	1	5.05	104	113.21	0.02*
Lengh of exsposure to light	2	3.74	102	109.41	0.15
Time of the day/length of exposure	2	2.14	100	107.27	0.34

4.2.3 Third experiment

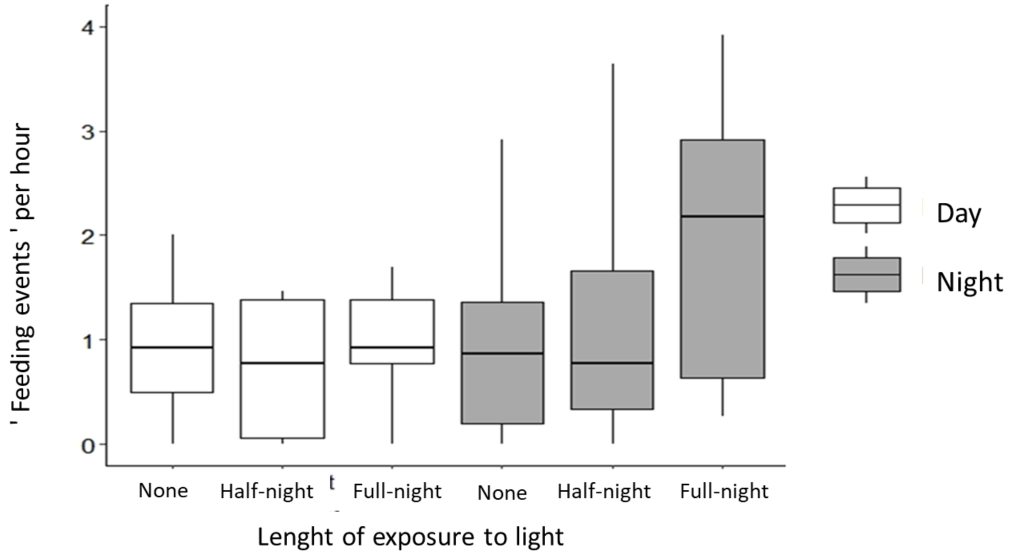

Figure 19. Day-night feeding activity of the sea urchin *Paracentrotus lividus* in response to three lengths of exposure to artificial light at night. “None” = Darkness (red light) at night.”Half-night” = ~60 lux artificial light at night from local sunset to midnight and from 5 am to sunrise. “Full-night” = ~60 lux artificial light at night from local sunset to sunrise. Animals were not acclimated to the light treatment. Feeding frequency is calculated from a presence/absence matrix (“Feeding”/”Not feeding”) derived from timelapse photography (24 h) at 15 min intervals. Box plots show medians with interquartile range and non-outlier range. Each replicate is plotted twice: once during day- and once during nighttime.

In the third experiment, where the animal experiences exposure to artificial light at night of 60 lux, the results show that there is no significant difference between daytime feeding and nighttime feeding (Figure 19). Nonacclimated animals have no preference when they feed. Different lengths of exposure to artificial light ("Full-night" and "Half-night") did not show a significant effect on the feeding activity of the sea urchin *P. lividus.* Besides that, the results also show an increase in feeding activity during the "Full-night" light exposure, which is not statistically significant (Tabel 8). There is no significant interaction between the time of the day and the length of exposure to light on the feeding activity. The feeding activity is uninfluenced by the light treatment.

Tabel 8. Effects of exposure to artificial light at night on the day-night feeding activity (feeding events per hour) of the sea urchin *Paracentrotus lividus*. Data derived from timelapse photography (24h) at 15 min intervals. Animals were not acclimated to the light treatment. Results from chi-square test based on two-factorial GLM negative binomial.

	Df	**Deviance**	**Res. Df**	**Res. Dev**	**Pr(>Chi)**
Time of the day	1	2.58	68	85.212	0.10
Lengh of exsposure to light	2	1.58	66	83.631	0.45
Time of the day/length of exposure	2	1.32	64	82.304	0.51

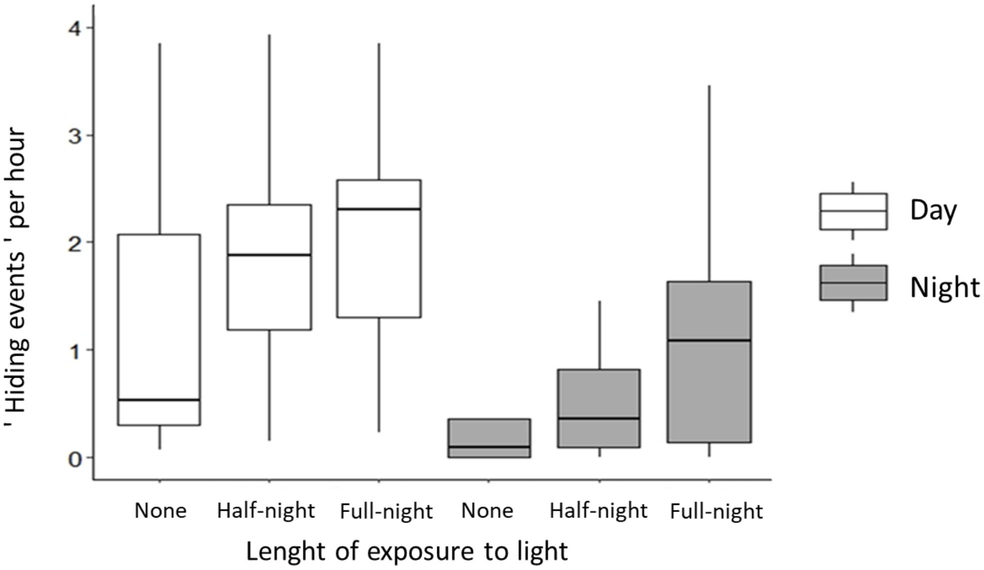

Figure 20. Day-night hiding activity of the sea urchin *Paracentrotus lividus* in response to three lengths of exposure to artificial light at night. “None” = Darkness (red light) at night.”Half-night” = ~60 lux artificial light at night from local sunset to midnight and from 5 am to sunrise. “Full-night” = ~60 lux artificial light at night from local sunset to sunrise. Animals were not acclimated to the light treatment prior to feeding assays. Feeding frequency is calculated from a presence/absence matrix (“Hiding”/”Not hiding”) derived from timelapse photography (24 h) at 15 min intervals. Box plots show medians with interquartile range and non-outlier range. Each replicate is plotted twice: once during day- and once during nighttime.

The result shows a significant effect of the time of day on hiding activity, as the animal hides more during the daytime (Figure 20). Exposure to artificial light at night ("Full-night" and "Half-night") has a significant effect on hiding activity. The animals have more hiding activity if exposed to artificial light at night. There is a clear trend during the nighttime which shows that animals start to hide more under increasing light exposure for the full night and half-night light exposure. The same trend is also present during the daytime, the animal hides more after being exposed to artificial light at night ("Full-night" and "Half-night"). There is no significant interaction between the time of day and the length of exposure to light on the hiding activity (Tabel 9). When exposed to 60 lux of artificial light during the night, the sea urchin will start to hide more during the nighttime and daytime.

Tabel 9. Effects of exposure to artificial light at night on the day-night hiding activity (hiding events per hour) of the sea urchin *Paracentrotus lividus*. Data derived from timelapse photography (24 h) at 15 min intervals. Animals were not acclimated to the light treatment. Results from chi-square test based on two-factorial GLM negative binomial

	Df	**Deviance**	**Res. Df**	**Res. Dev**	**Pr(>Chi)**
Time of the day	1	17.70	104	134.87	0.05***
Lengh of exsposure to light	2	6.19	102	128.67	0.04*
Time of the day/length of exposure	2	0.63	100	128.04	0.72

5 Discussion

To assess the potential consequences of light pollution on the benthic community, I studied the effect of artificial light at night on the food feeding rates and activity pattern for the herbivore grazer *Paracentrotus lividus*. In the first experiment, I used a light intensity of 30 lux and acclimated the animal for 12 days to this light regime. I have used a light intensity of 30 lux, based on the intensity measured on the water surface of Kiel's fjord, as well as the intensity found by Ayalon et al., 2019 (35-40 lux); Fobert et al., 2019 (26.5 lux); Maggi & Serôdio, 2020 (27 lux). Since these treatments did not show any effect on the feeding rates, I aim to exclude the acclimation period in the following trials. In the second experiment, the sea urchin did not experience acclimation before the measurement phase, but the light intensity was the same as in the first experiment, at 30 lux. The second experiment also did not show any effect on the feeding rates, so I increased the intensity for the third experiment. In experiment three, the sea urchins were confronted with a higher light intensity of 60 lux, which was higher than the average light pollution intensity in the field. Although the sea urchins were collected in different months (June, August, and September), I presume that the data from experiments 1, 2, and 3 are comparable. Through identical handling during collection and in the laboratory, and acclimation time to laboratory conditions, I aimed to compensate for seasonal differences between the groups of animals used in the three experiments.

5.1 Feeding rates

Because I participated in the GAME 2021 project, the center of my study was the feeding trial, which involved investing in the change in feeding rates with light pollution. In the first experiment, where the individuals acclimated to the light regime for 12 days, I did not find an effect of either exposure to light for the full night or half night. However, there is a trend of decreasing the feeding rates when exposed to full light at night. This can be explained by the fact that *P. lividus* is searching for food and feeding during the nighttime (Dance 1987) because of the necessity to avoid predators (Hereu 2005). Nevertheless, the sea urchin can interpret the presence of light at night as daylight and avoid feeding as a result. In the second experiment, I checked for the acute response of the animal when put under a light stressor. The result shows neither a decrease nor an increase in the feeding rates wheal exposed to 30 lux of artificial light at night. That could be because the animal did not have the time to perceive the effect of a change in artificial light like the one in the first experiment. Various studies showed that species are less sensitive to sporadic than to constant light exposure (Gehring 2009). In the third experiment, where I increased the light intensity to 60 lux, I found no effect either in the decrees or the feeding rates increase. Even though it is higher for 50 % than the one found in the field, the animal did not show an increase or decrease in consumption, probably again because of the short adaptation period.

5.2 Activity pattern

Laboratory studies show that *P. liviuds* movement is mediated by light, which can determine both the direction and speed of movement (Domenici et al. 2003). However, when exposed to artificial light the sea urchins did not show a change in their hiding activity during the night. Even though hiding is a common behavior during the day due to their tendency to avoid predators (Sala & Zabala 1996), artificial light at night increases hiding during the day. This can be caused by the stress that the animal experiences during the period of exposure to artificial light at night. Additionally, the size of the individuals can also contribute to these different responses, since different sizes influence light reception differently in sea urchins (Boudouresque & Verlaque 2001). However, the animals show an immediate effect of exposure to artificial light by increasing their tendency to hide. There is a trend towards increasing hiding in the presence of light at night when exposed to the lower intensity of light (30 lux). When the intensity is doubled (60 lux) the response is visible, and the animal hides during the night. This level of light intensity is not common in the field, but it may happen in the future, as Devis et. al (2012) suggest that artificial lighting is increasing at a rate of 6 percent per year globally. With this light intensity, I wanted to see if an increase in light intensity would affect the sea urchin’s food consumption and activity. Hiding also increases with longer exposure to light, the shorter exposure (half-night) has less impact. As a result of the shorter light exposure, the effect is also shorter. With a higher light intensity and longer exposure time, the hiding becomes more apparent. As *P. lividus* has a nocturnal activity pattern and usually moves in search of food at night (Dance 1987) the presence of light at night can be perceived as a prolongation of the day and they stay hidden waiting for the night. The reason can also be avoiding predators because in nature they hide in caves during the day in order to survive.

As a nocturnal species, the sea urchin *P. lividus* searches for food and feeds at night. In this study, they eat equally throughout the 24-hour period. This contradicts the findings of Dance (1987), which stated that *P. lividus* usually move when looking for food at night. When artificial light is introduced, the feeding during the daytime decreases and the feeding at night stays the same. The decrease during the daytime can be provoked by the fact that sea urchins are stressed by the presence of light at night. When looking for an acute response, the animals did not show any preference when consuming food. During the 24 hours of the measurement, feeding activity was equal. Besides that, I observed no other effect of light on the feeding activity. The same result is also found when they experience higher light intensity (60 lux). When exposed to higher light intensities at night, the animals did not show any effect on their feeding activity. If there is no effect of light on feeding activity, it could be because feeding behavior in sea urchins is not controlled by light. Even though the literature suggested that they move during the night in search of food. It may also be due to the absence of predators in the tanks. The presence of predators reduces the sea urchins' diurnal foraging pattern (Underwood 2017) and consequently, their grazing effect is reduced. Without the presence of predators, sea urchins can eat undisturbed the whole day.

The sea urchin, when acclimated to the presence of artificial light, did not show a change in either hiding or feeding. This suggests that *P. Lividus* can adapt to the stressful environment. After some time being exposed to artificial light, they will not perceive light as a stressor anymore. Nevertheless, an acute response is visible in hiding activity. They hide more under the immediate stress of artificial light during the night. Shorter time of exposure to artificial light has a smaller effect on the hiding of the sea urchin the reason can be the fact that spines and podia react to a sudden increase or decrease in illumination (Lawrence 2013). Rocky shore organisms including sea urchin will be increasingly exposed to a range of anthropogenic stressors throughout the 21st century (including ocean acidification, climate change, and noise pollution) that have affected their distribution, behavior, and morphology (Nevenhad et al. 2008; Kurihara 2013). Here, it is demonstrated that the potential effect of artificial light at night is a globally widespread, rapidly expanding and yet understudied source of anthropogenic change. It has acute effects on the behavior of sea urchins that are comparable with those observed in response to ocean acidification and climate change in similar laboratory studies (Detree et al. 2020).

5.3 *P. lividus* in the light of global GAME results

In the global study GAME 2021, which this thesis was part of, successfully found that benthic marine grazers can be affected by light pollution. The effect can go either way and lead to an increase or decrease in feeding rate. If species are affected, the effect can be substantial. We investigated eight species in six different locations with a latitudinal gradient of 54°. The artificial light at night did not affect the feeding rate in all species and differed in the direction of the effect. We found a significant effect in three experiments without acclimation and in two experiments with acclimation to light exposure. There was no clear pattern across latitudes which emerged.

Matching my study organism, I will focus on the sea urchin *P. lividus*. *P. lividus* was studied in two more locations other than Croatia: in Vigo (Spain) and on Madeira (Portugal). The results from Portugal showed a decrease in consumptio rate of sea urchins exposed to artificial light at night (with acclimation). These findings support the assumption that *P. lividus* is sensitive to light pollution. Unlike the results from the Atlantic populations, the sea urchins from the Mediterranean did not show significant differences in feeding rate in any conducted experiment.

Why do sea urchins of the same exhibit different responses to artificial light at night? All sampled populations are known to show nocturnal biorhythms (Boudouresque & Verlaque 2001; Hereu 2005). *P. lividus* in Spain and Portugal were sampled in the intertidal, a very exposed environment, while the sea urchins from Croatia were collected in the subtidal. *P. lividus* from Croatia is less exposed to the tidal rhythm. Furthermore, the habitats differ in several other environmental factors such as temperature, nutrient availability, and the influence of upwelling currents.

All over, these examples could play a role in explaining the various effects of artificial light at night exposure and indicate an intraspecific difference in light susceptibility of *P. lividus* from different populations. If we put all the findings into context of the current predictions, an increase of artificial light at night worldwide will not affect coastal communities hosting *P. lividus* equally.

More research is needed to fully understand the effects of light pollution on grazer-grazer and grazer-algae interactions across spatial distribution. However, GAME 2021 concluded that light pollution can have an effect on benthic grazers. Therefore, it adds a significant biological finding for coastal ecosystems to the study by Davies & Smyth (2017) supporting the conclusion that artificial light at night should be a focus for global change research in the future.

6 Conclusion

- The presence of artificial light at night does not affect the feeding rate in *P. lividus*. The feeding rate does not change when exposed to a lower light intensity of 30 lux or a higher light intensity of 60 lux

- When *P. lividus* is exposed to artificial light at night, it begins to hide more at night as an acute response. However, when exposed to the same light treatment over a longer period of time, it adapts and no longer perceives the light as a stressor.

- The effect of artificial light on activity patterns shows that individuals are sensitive to the presence of light in terms of hiding. The sea urchin hides more at night when it perceives artificial light.

- When individuals of *P. lividus* are exposed to artificial light for only half of the night from midnight to 5 a.m., feeding rates are not affected by the light, but there is an effect on the activity pattern that decreases the hiding compared to full exposure to light at night.

- This study provides evidence that the effects of light pollution as a stressor affect the activity pattern of *P. lividus.* Given the projected increase in artificial light at night, a more sophisticated understanding of light pollution is key to predicting future changes in urbanized coastal environments.

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Životopis

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