



Contents lists available at ScienceDirect

## Science of the Total Environment

journal homepage: [www.elsevier.com/locate/scitotenv](http://www.elsevier.com/locate/scitotenv)

# Artificial light pollution influences behavioral and physiological traits in a keystone predator species, *Concholepas concholepas*

Patricio H. Manríquez<sup>a,b,\*</sup>, María Elisa Jara<sup>a,b</sup>, María Isabel Díaz<sup>a,b</sup>, Pedro A. Quijón<sup>c</sup>, Stephen Widdicombe<sup>d</sup>, José Pulgar<sup>e</sup>, Karen Manríquez<sup>e</sup>, Diego Quintanilla-Ahumada<sup>e</sup>, Cristian Duarte<sup>e</sup>

<sup>a</sup> Centro de Estudios Avanzados en Zonas Áridas (CEAZA), Coquimbo, Chile

<sup>b</sup> Laboratorio de Ecología y Conducta de la Ontogenia Temprana (LECOT), Coquimbo, Chile

<sup>c</sup> Department of Biology, University of Prince Edward Island, Charlottetown, PE, Canada

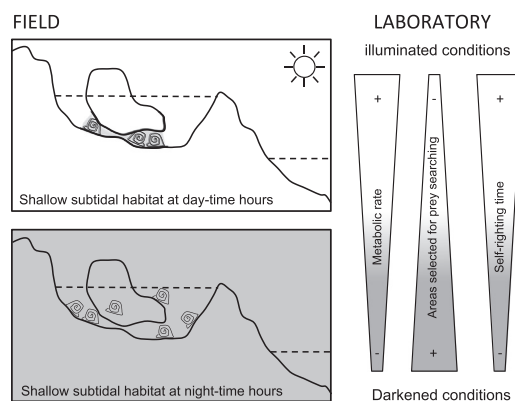
<sup>d</sup> Plymouth Marine Laboratory, Prospect Place, West Hoe, Plymouth PL1 3DH, UK

<sup>e</sup> Departamento de Ecología y Biodiversidad, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, Chile

## HIGHLIGHTS

- Juveniles of *C. concholepas* seek out and choose their prey in dark conditions.
- Light pollution increases righting times of juvenile *C. concholepas*.
- Light pollution increases metabolism of juvenile *C. concholepas*
- In nature small *C. concholepas* are more abundant in darkened habitats.
- Influence of light pollution at night may have implications on community structure.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

## Article history:

Received 12 November 2018

Received in revised form 7 January 2019

Accepted 13 January 2019

Available online 15 January 2019

Editor: Henner Hollert

## Keywords:

Intertidal

Light pollution

Ecology

Prey-searching behavior

## ABSTRACT

Artificial Light At Night (ALAN) is an increasing global problem that, despite being widely recognized in terrestrial systems, has been studied much less in marine habitats. In this study we investigated the effect of ALAN on behavioral and physiological traits of *Concholepas concholepas*, an important keystone species of the south-eastern Pacific coast. We used juveniles collected in intertidal habitats that had not previously been exposed to ALAN. In the laboratory we exposed them to two treatments: darkness and white LED (Lighting Emitting Diodes) to test for the impacts of ALAN on prey-searching behavior, self-righting time and metabolism. In the field, the distribution of juveniles was observed during daylight-hours to determine whether *C. concholepas* preferred shaded or illuminated microhabitats. Moreover, we compared the abundance of juveniles collected during day- and night-time hours. The laboratory experiments demonstrated that juveniles of *C. concholepas* seek out and choose their prey more efficiently in darkened areas. White LED illuminated conditions increased righting times and metabolism. Field surveys indicated that, during daylight hours, juveniles were more abundant in shaded micro-habitats than in illuminated ones. However, during darkness hours, individuals were not seen to aggregate in any particular microhabitats. We conclude that the exposure to ALAN might disrupt important

\* Corresponding author at: Centro de Estudios Avanzados en Zonas Áridas (CEAZA), Coquimbo, Chile.

E-mail address: [patriciohmanriquez@gmail.com](mailto:patriciohmanriquez@gmail.com) (P.H. Manríquez).

Self-righting  
Metabolism

behavioral and physiological traits of small juveniles in this species which, as a mechanism to avoid visual predators, are mainly active at night. It follows that ALAN in coastal areas might modify the entire community structure of intertidal habitats by altering the behavior of this keystone species.

© 2019 Published by Elsevier B.V.

## 1. Introduction

Marine environments are facing a growing number of stressors associated with global climate change, local human activities and the urbanization of coastal areas. In the face of this proliferation of human impacts, artificial light pollution has often been overlooked despite growing evidence that ALAN could pose a threat to the diversity and functioning of biological communities in terrestrial (Gaston et al., 2014; Davies et al., 2016; Davies et al., 2017; Davies and Smyth, 2017) and marine communities (Becker et al., 2013; Gaston et al., 2014; Davies et al., 2015; Bolton et al., 2017; Davies and Smyth, 2017). For example, exposure to ALAN increased the total abundance, and modified the community composition, of spiders and beetles in a grassland ecosystem (Davies et al., 2017). In marine ecosystems, Lorne and Salmon (2007) showed that sea turtle orientation was negatively affected by ALAN, impairing the ability of hatchlings to respond to natural orientation cues. Similarly, in nocturnally migrant birds ALAN altered multiple behaviors (Van Doren et al., 2017) and even human health traits such as sleep, circadian timing, next-morning alertness and increased risk of breast cancer has been shown to be affected by ALAN (e.g. Chang et al., 2015; Keshet-Sitton et al., 2015; Zielinska-Dabkowska, 2018).

Shifts in spectral signatures associated with ALAN might affect visually guided behaviors across a broad taxonomic group of animals (Davies et al., 2013). It has been estimated that ~19% of the global land area of the world it is now affected, to some extent, by ALAN (Cinzano et al., 2001; Kyba et al., 2017; Kyba, 2018). Moreover, it is estimated that the total area affected by this anthropogenic change in lighting technology is increasing by 6% per year (Hölker et al., 2010), which suggest that this stressor might have far reaching consequences. Light-emitting diodes (LEDs) are cheap, bright, highly efficient and reduce energy consumption. All of which means that LEDs are rapidly becoming one of the world's most important light sources (Zissis and Bertoldi, 2014) and are increasingly being used for lighting in both residential and commercial areas as well as the transport routes between them. In the marine environment this will specifically include beachfront developments, ports, marinas and shipping. Therefore, the potential impact of this change to LED illumination on marine communities needs to be considered (Gaston et al., 2015).

In the marine realm, many species have evolved behavioral and morphological responses to minimize visual predation (Troscianko et al., 2009; Manríquez et al., 2009). For instance, some intertidal species are most active during the night as a mechanism to avoid visual predators (Wells, 1980). In addition, being active at night minimizes thermal abiotic stress and desiccation at low tide. This is particularly advantageous for organisms performing energy-demanding activities (e.g. Kennedy et al., 2000). Recently, ALAN has also been shown to affect the locomotor activity, circadian rhythm and growth rate of intertidal amphipods (Luarte et al., 2016) as well as the small-scale diel vertical migrations of zooplankton species (Ludvigsen et al., 2018). Therefore, the modification of the natural light-dark regime by ALAN in coastal environments could have important consequences for the species inhabiting these areas.

The “Loco” or “Chilean abalone”, *Concholepas concholepas* (Bruguière, 1789), is a keystone species (i.e. its presence maintains the structure and integrity of the community) in rocky shores of the south-eastern Pacific Ocean coast (Castilla and Paine, 1987; Power et al., 1996; Castilla, 1999). This species is an economically and ecologically important component of the rocky intertidal and subtidal

communities along the Chilean coast (Castilla, 1999). According to observations conducted under laboratory conditions with intertidal individuals, *C. concholepas* prey mainly at night (Castilla et al., 1979; Castilla & Guisado, 1979; Castilla and Cancino, 1979; Guisado and Castilla, 1983). Meanwhile, studies conducted using subtidal individuals indicated that *C. concholepas* prey over the entire 24-h cycle (Stotz et al., 2003) suggesting that, in this species, intertidal and subtidal populations display different activity patterns. Competent larvae of *C. concholepas* show a marked circadian rhythm in their swimming behavior, displaying most of their activity at night (Manríquez & Castilla, 2001). However, it is not yet known if the behavior of benthic stages of this species is also timed over the lunar or tidal cycle. Among the most important prey items of *C. concholepas* are barnacles, mussels and ascidians (Stotz et al., 2003; Manríquez and Castilla, 2018), all of which are sessile or have limited mobility. Therefore, it is highly unlikely that preying at night in this species is a mechanism that evolved to avoid being perceived while approaching prey. Instead, it can be argued that preying at night might be a potential mechanism evolved by *C. concholepas* to avoid its own visual predators: the crab *Acanthocyclus hassleri* (Manríquez et al., 2013a, 2013b), the birds *Larus dominicanus* and *Haematopus ater* (Castilla and Cancino, 1979), the sea otter *Lontra felina* (Castilla and Bahamondes, 1979), and the fish *Pimelometopon maculatus* and *Syciasis sanguineus* (Viviani, 1975).

Similar to most mollusks, *C. concholepas* can use chemical and visual stimuli during sensory perception (Manríquez et al., 2014; Domenici et al., 2017). In this species, the detection of chemical cues associated with prey and predators play an important role in feeding and predation avoidance (Manríquez et al., 2013a; Manríquez et al., 2014). Moreover, as in other marine gastropods, chemoreception of odor cues emanating from food items, conspecifics or predators, involves the osphradium, an external sensory organ, which monitors the physiochemical properties of the surrounding seawater (Huaquín and Garrido, 2000). The structurally simple eyes of these gastropods are situated in each tentacle, and provide information on gross differences in light intensity (distinguishing light and dark), regulate daily and seasonal activities, egg laying behavior, mediate phototactic behavior and locomotion, and in some species, provide also visual detection of forms (Serb, 2008; Ter Maat et al., 2012). Tentacles withdraw in response to sudden decreases in light intensity, exhibiting a shadow response consisting of partial or total retraction of the body into the shell and downward movement of one or both tentacles (Stoll, 1972; 1976). In *C. concholepas* specifically, shadow response is observed under field and laboratory conditions once light intensity is suddenly interrupted near the cephalic region of the individuals with an opaque object (Manríquez PH. pers. obs). This suggests that the cephalic eyes, or other sensitive areas in the cephalic region, might play an important role in detecting habitats with appropriate light illumination. Hence, ALAN is likely to have a significant effect on the activity of this species.

Coastal urbanization and tourism development is followed by coastal land reclamation, creation of artificial beaches (Chee et al., 2017) and beachfront lighting (Hölker et al., 2010). This is particularly important in Antofagasta, northern Chile, where the urban fringe is narrow and urbanization takes place near the coast (Corsin, 2001). As previously mentioned, the intertidal habitat is subject to a wide range of stressors including ALAN (Underwood et al., 2017), so the rocky intertidal zone and the organisms inhabiting there are good models to investigate the eco-physiological consequences of ALAN. In this study, we conducted laboratory experiments using juveniles of *C. concholepas* to

investigate the potential effects of ALAN (using LED lighting) on prey searching, self-righting speed and metabolism. In this particular environment, prey searching and self-righting success are important traits in mediating both predator-prey interactions and the ability to return to a normal posture after dislodgement, respectively. Moreover, we conducted a field survey to determine whether the distribution of juveniles in shallow subtidal rocky habitats was influenced by the ambient light conditions. Our hypothesis was that exposure to ALAN has significant effects on behavioral and physiological traits of juveniles of this key-stone species. We expected that ALAN exposure would inhibit the activity of small juveniles of *C. concholepas* and prompt them to incur increased metabolic costs in searching for food in darkened areas. Given that overturned individuals are more vulnerable to visual predators, we also expected that ALAN would speed up self-righting.

## 2. Methods

### 2.1. Influence of natural lighting conditions on the abundances of loco in the field

This field sampling was conducted to explore the link between the response of small juveniles of *C. concholepas* to ALAN in the laboratory and their natural abundances during night hours. Daytime natural abundances on shaded or illuminated shallow subtidal microhabitats might give cues about where the small juvenile of this species prefer to be more active (e.g. searching for prey). Similarly, night-time abundances might help to know if this pattern changes in absence of light. The location and abundance of juvenile *Concholepas concholepas* (~1.5 cm in shell length) were determined from surveys conducted during the day at low tide conditions in rock boulders mainly encrusted with barnacle stands. The analyzed boulders were located in subtidal shallow-water habitats inside pools that remain as pockets of seawater when the tide ebbs. These surveys were conducted in Antofagasta, northern Chile, at El Lenguado (23°46'S; 70°28'W) and Trocadero (23°35'S; 70°23'W). In southern Chile, surveys were conducted at Calfuco (39°46'S; 73°23'W) during both day and night-time hours. All the surveys were conducted during austral summer months to match those months when high abundances of small juvenile *C. concholepas* are present at the chosen sampling sites (Manríquez et al., 2009; Manríquez et al., 2012). During the day the average ( $\pm$ SE; N; weather condition at sampling time) light intensities at seawater-level were 56.26 (0.67; 9; sunny), 46.64 (2.33; 9; sunny) and 3.16 (0.61; 9; partly cloudy) kilo-lux for El Lenguado, Trocadero and Calfuco, respectively. At night, during the sampling, light intensity measured in Calfuco was zero. Light intensities were measured using a Pro's Kit MT-4017 Light Intensity Meter. However, previous studies have shown that light intensity during full moon under clear conditions could be around 0.1–0.3 lx (Gaston et al., 2013, 2104).

The presence and abundance of juvenile *C. concholepas* was noted on both the upper and lower surface of rock boulders that were loose and small enough to be able to be turned by the observer ( $\leq$  20 cm in maximal length). In the pools, and only considering individuals below the water level, juveniles inhabiting the upper, illuminated surface of the boulders were counted before the boulder was turned over and those inhabiting the lower, shaded surface were then counted. After being assessed, all the boulders were returned to their original position. The number of juveniles in each of the 2 micro-habitats (i.e. upper or lower side of the boulders) was standardized by searching time. When the boulders being surveyed were in the shallow subtidal pools, water level was always ~10 cm above the upper surface of the boulders. Therefore, small juveniles of *C. concholepas* were exposed to contrasting natural lighting but not to desiccation. At each site, the same observer was used locate juvenile *Locos* for the entire duration of the survey, with a second researcher present to measure searching times and the size of the individuals. The traveling time between micro-habitats and the time needed to turn the loose boulders was not considered as part of

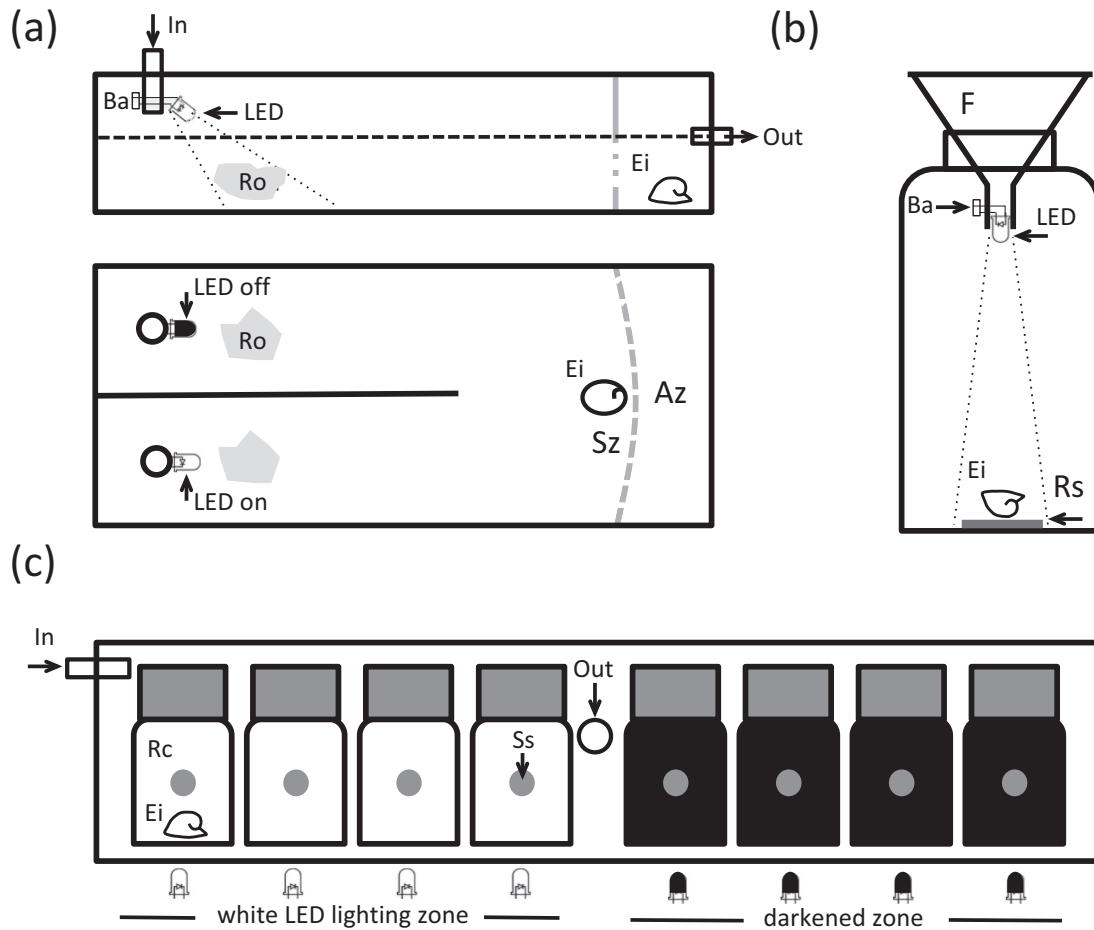
the search times. In southern Chile, the sampling schedule also included night-time samplings conducted in both micro-habitats during low tides occurring just a few hours after the daytime sampling. In those surveys, a headlamp equipped with a LED-generated dim red light was used to help observe the small juveniles.

### 2.2. Collection of individuals for experiments

During December 2017, early juvenile stages of *Locos* (<1 cm in shell length) were collected from rocky intertidal platforms located next to the remains of the old rail turntable at Caleta Coloso in northern Chile, Antofagasta (23°45'S; 70°27'W). In this study, we focused our observations and experiments on small juveniles of *C. concholepas*, because they are the more abundant stages in rocky intertidal and shallow subtidal habitats. Moreover, because of their small size these juveniles are the most susceptible to be attacked by visual predators, dislodged by wave action or disrupted by environmental stressors. At the study site, there is no urbanization or beachfront lighting in place and the only artificial light sources at night are from passing car headlights on the highway that runs parallel to the coast line, 50 m away and behind a 1 m high stone wall. Therefore, it can be assumed that sessile and low mobility organisms inhabiting this site have not been exposed to direct ALAN. The juveniles were collected from habitats mainly dominated by stands of the barnacle *Notochthamalus scabrosus*. Recently settled small individuals of the mussels *Perumytilus purpuratus* and *Semimytilus algosus* were also presents on the rocky intertidal platforms. Shell coloration in *C. concholepas* depends on the color of the more abundant prey available in the habitat (Manríquez et al., 2009). Therefore, all the individuals were cryptic with mixed shell color (dark and light colored) and therefore ingestively conditioned (Hall et al., 1982) to the prey used in the laboratory experiments (below). After collection, the individuals were moved to laboratory facilities at the Universidad Católica del Norte, Coquimbo, Chile (29°58'S; 71°21'W) where all the experiments were conducted. On arrival, individuals were maintained in Plexiglas® aquaria with running seawater, under a natural photoperiod for the austral summer months (12:12 h light:dark photoperiod without artificial control for gradual changes at dawn and dusk). During the entire rearing period, lighting in the laboratory was provided indirectly by fluorescent lamps and the light intensity at the level of the aquariums was on average ( $\pm$ SE; N) 38.6 lx ( $\pm$ 6.5; 6). During the first 2 weeks of rearing, individuals were provided with small rocks incrustated with stands of the barnacle *N. scabrosus* on which to feed and then the diet was switched to small individuals of the mussel *S. algosus*. At the end of each experiment (see below), individuals were weighed to the nearest 0.001 g and measured to the nearest 0.01 mm using an analytical balance (Adam AFA180 LC) and a digital vernier caliper (Mitutoyo 500-196-30), respectively.

### 2.3. Prey-searching under light-darkness conditions

This experiment was conducted on juvenile *C. concholepas* which had been acclimated to laboratory conditions for 1 week. During this acclimation period, individuals were fed ad libitum with barnacle stands collected from the same site used for *C. concholepas* collection. After acclimation, the directional responses of *C. concholepas* to artificial light were evaluated in a two-choice test using Plexiglas® Y-mazes. The left and right Y-maze arms were both 0.60 m long, 0.18 m wide and 0.10 m deep (Fig. 1). Three Y-mazes (black lateral walls, black lid and white floor) were run simultaneously with both arms of each maze receiving filtered (1  $\mu$ m) seawater at a constant rate of 1.0 L h<sup>-1</sup>. The seawater flowing into the arms of the Y-mazes dropped ~1 cm onto the surface of the water. As in a previous study (Manríquez et al., 2014), the displacement paths of the individual snails were verified at the end of the trials by observing the remains of fine sediment stuck to the mucous track generated by *C. concholepas* as the anterior part of their foot travels along the white Y-maze floor. The Y-maze experiments



**Fig. 1.** Schematic representation (not to scale) of the experimental setting used to evaluate the effect of white LED lighting on: (a) prey searching, (b) self-righting time and (c) metabolism of small juveniles of *C. concholepas*. In (a) a lateral view (top plot) and top view (bottom plot) of the Y-maze used to evaluate prey searching: Ba = battery; LED = white light-emitting diode; Ro = flat rock with barnacle stands; Ei = experimental individuals; Az = acclimation zone; Sz = starting zone. The dashed line depicts water level. In (b) a lateral view of the plastic chamber used to evaluate self-righting time: F = funnel; Rs = righting substrate conditioned with Safety Walk® 3 M®. In (c) an upper view of the black Plexiglas container used to hold the glass respirometry chambers (Rc) equipped with oxygen sensors (Ss) to measure metabolism.

were repeated on 8 consecutive days until a total of 24 replicate trails were obtained. After each run, the Y-mazes were cleaned with tap water to remove any chemical cue that could influence future measurements. At the beginning of each trial, a small flat rock with ~200 barnacles was placed near the seawater inlets in each of the Y-maze arms. The surface of the flat rock bearing the barnacle stands were always upwardly oriented. In each Y-maze, one arm was lit by attaching a white LED light to the seawater inlet while the other arm remained in darkness. All the LED lights used in this study (Chanzone®) were 5 mm round clear type, forward voltage of 3.2–3.6 V, 0.06 W, with a view angle of 120° and a correlated color temperature (CCT) of 8000 K. They also had the characteristic light source spectral power distribution (SPD) curve, displaying a first peak at 460 nm with a power of 0.028 W/nm (indicating blue content) followed by the bell shape of the second peak at 550 nm (indicating higher yellow content). The LED was powered by a button Lithium cell battery (3 V, CR1616) and oriented towards the upper surface of the rock in that arm (Fig. 1a). In the dark arm, a LED was also attached to the seawater inlet but was not switched on and therefore the surface of the rock was not illuminated. This was to ensure that the physical presence of the lighting equipment was the same in both arms and could not influence the results. During the experiments, the upper surface of the rocks was always immersed in water to depth of ~3–4 cm. The light intensities were measured in the air just above the water surface (~2 cm) and in the starting zone situated 50 cm away from the inlets using a L-100 PCE Lux Meter. Above the illuminated flat stones the light intensities were on average

( $\pm$ SE; N) 329.9 lx ( $\pm$ 22.4; 8). The light intensity used in this experiment (and in the others, see below) was within the natural range measured during daytime hours in the locality where the animals were collected (ca. 200 to 1000 lx). However, in the darkened arms and in the starting zone the light intensities were on average ( $\pm$ SE; N) 0.016 lx ( $\pm$ 0.001; 8) and 0.169 lx ( $\pm$ 0.011; 8), respectively. To standardize hunger levels, all trial specimens were starved for 24 h before the experiment. The test individuals were placed in the acclimatization zone for 1 h (~17:00) and then moved carefully to the starting zone. Each trial lasted 15 h, and the final position of the snails in the Y-mazes was recorded (~09:00). The final position of the juveniles in the Y-maze was categorized as lighting arm, darkened arm or in the starting zone (Fig. 1a). To maintain the temperature at 15 °C the Y-mazes were semi-immersed in a temperature-controlled water bath.

#### 2.4. Self-righting under light-darkness conditions

This experiment was designed to examine how light/darkness might influence self-righting success and speed of small juvenile individuals of *C. concholepas*. This experiment was conducted 1 week after the previous one on a different group of individuals that had been acclimated to laboratory conditions for 2 weeks. To account for the existence of an endogenous rhythm that might mask self-righting performance in response to light/darkness the experiment was run twice. The first experimental run was conducted during daytime hours (~10:00 to 19:00), while the second experimental run was conducted during the

night (~22:00 to 03:00). In each experimental run we used 24 different individuals. Each individual was placed in a plastic chamber (5 L), filled with 3.5 L of aerated seawater and immersed in a water bath to maintain the acclimation temperature (~15 °C). The entire chamber was covered with black plastic to prevent light from entering the system (Fig. 1b). The opening of the chamber was equipped with a modified plastic funnel used to close the chamber and to hold a white LED light (the same as the one described in the previous section) (Fig. 1b). The funnel spout was 1.0 cm in diameter and 3.0 cm in length and provided an efficient way to stop the room lighting from entering the chamber. When the white LED was on (during daytime or night hours), the light intensity in the bottom of the experimental chamber was on average ( $\pm$ SE; N) 485.5 lx ( $\pm$ 11.3; 8). However, when the LED was off (during daytime or night hours), the light intensity was 0.068 ( $\pm$ 0.001; 8). To improve adherence during righting, the bottom of the chamber was covered with a circular strip of a rough-textured rubber surface (3 M™ Safety-Walk™ tape). Each juvenile was placed in the chamber for 5 min of acclimatization and then was placed upside down in the middle of the chamber. In this way the plane of the shell aperture was parallel with respect to the horizontal plane, therefore with the same degree of difficulty to self-right (Manríquez et al., 2016, 2017). Self-righting time was defined as the total time taken by the individual to completely return to its normal upright position from the moment that the individual was placed upside down and was measured using a digital stopwatch. A maximum of 15 min to assess self-righting was allowed per individual. Inability to self-right within 15 min was considered as self-righting failure. Regardless of experimental run (diurnal or nocturnal), during darkness a dim red LED Petzl headlight (~15 lx) was used to intermittently illuminate the inside of the chamber to allow the juvenile to be observed. After each trial the chamber was cleaned and filled with fresh aerated seawater in preparation for the next trial.

### 2.5. Metabolic impact of ALAN

The effect of ALAN on oxygen consumption rate in small juvenile *C. concholepas* was measured on a randomly selected group of 32 individuals that had been acclimated to laboratory conditions for 3–4 weeks. Four experimental runs were conducted, each run consisting of 4 illuminated and 4 darkened 60 mL glass respirometry chambers. The illuminated chambers were lit with a white LED, the same as those used in the previous experiments, placed 3 cm above each chamber (Fig. 1c). The light levels in the artificially illuminated and darkened chambers, were on average ( $\pm$ SD; N) 480.8 lx ( $\pm$ 12.0; 4) and zero, respectively. The darkened chambers were identical except they were completely covered with sticky back vinyl tape leaving a small opening over the Oxygen Sensor Spot (SP-PSt3-NAU, PreSens). The chambers were placed horizontally and semi-immersed in a black Pexiglas® container connected to a refrigerated and heating water bath circulator (Lab Companion RW-2025G). In this container, all the chambers were left with the oxygen sensor spots (fixed at the inner side of the chambers) 0.5 cm above the water level. To avoid any additional stress during the oxygen measurements, the chambers were not manipulated when the fiber optical probe was applied to the spots. A fiber optic oxygen meter (Fibox 3, PreSens) was used for all oxygen consumption measurements. Calibration was performed using a Na<sub>2</sub>SO<sub>3</sub> solution (0% saturation) and using air bubbled seawater (100% saturation). Individuals were starved in tanks containing 1.0  $\mu$ m running filtered seawater for 24 h prior to the measurements. During this period individuals were maintained at light intensities that were on average ( $\pm$ SE; N) 38.6 lx ( $\pm$ 6.5; 6) and supplied by daylight fluorescent tubes. Metabolic measurements lasted for at least 6 h, with the first 5 min removed to avoid possible manipulation effects. Special care was taken to prevent oxygen levels from dropping below 70% of air saturation. Background respiration was determined by measuring oxygen consumption without a snail in the chamber, and that was subtracted from the experimental oxygen consumption rates. This experimental

setting did not allow us to test whether the return from illumination to darkness reduces oxygen consumption to maintain homeostasis. The temperature during the measurements was stabilized at 15 °C by connecting the container holding the respirometry chambers to a refrigerated and heating water bath circulator (Lab Companion RW-2025G). Wet weight of each individual was measured using an analytical balance (Adam AFA180 LC). To determine the presence of encrusting or shell-boring organisms in the small juveniles that might influence oxygen consumption each individual was immersed in a Petri dish filled with seawater and observed under a stereo-microscope (Olympus SZ 61).

### 2.6. Data analysis

Prey-searching and self-righting behavior can be affected by the size of snail performing that activity. Consequently, the sizes of juvenile *C. concholepas* used in the different lighting treatments were compared using a 1-way ANOVA, after the assumptions of normality and homogeneity of variance were tested. This was done for both the prey-searching and self-righting experiments. Oxygen consumption rates were analyzed with a 1-way ANOVA. Field abundances (square root-transformed data) of juvenile *C. concholepas* among microhabitats were analyzed by 2-way ANOVAs, considering in one case microhabitat type (above or underneath the boulders) and location as main factors and in the other case microhabitat type and hours of sampling (day or night-time hours) as main factors. Self-righting times were compared between treatments using a Kruskal-Wallis, as homogeneity of variance between treatments was violated. The final position of the trial individuals (percentage) in the prey-searching Y-maze experiment under contrasting lighting regimes and in the metabolism measurements were analyzed by a Fisher's exact-probability test. All statistical analyses were performed using SPSS v.18.0 (IBM Corp., Armonk, NY, USA). At the end of the experiments all the experimental individuals were returned to the same site where they were collected.

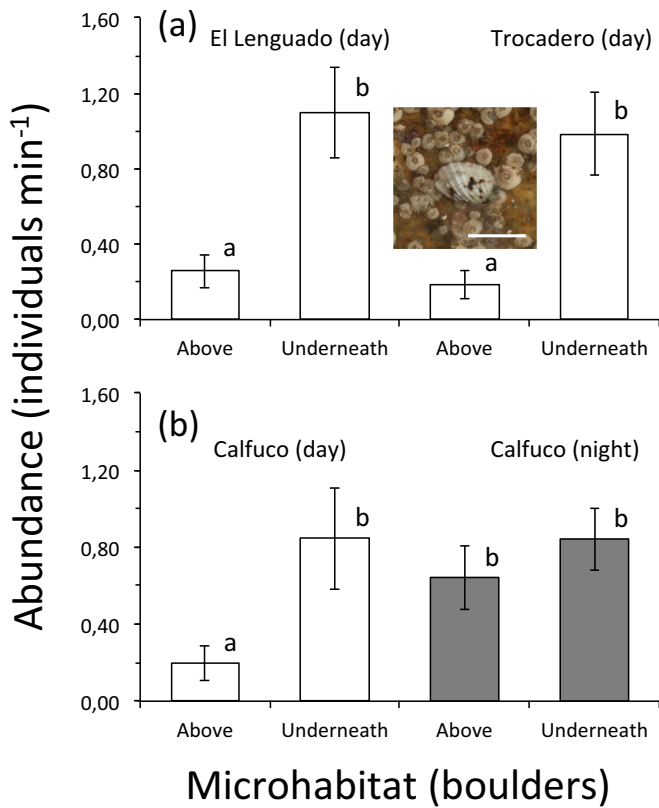
## 3. Results

### 3.1. Influence of natural lighting conditions on the abundances of loco in the field

The abundance of small juveniles of *C. concholepas* was significantly different between microhabitats ( $F_{1,28}$ : 0.9307;  $p < 0.00001$ ). Approximately 4 and 5 times more individuals were found in the shaded than in the illuminated microhabitats at El Lenguado and Trocadero, respectively (Fig. 2a). The same analysis found that sampling site ( $F_{1,28}$ : 0.05;  $p$  0.3429) and the interaction with microhabitats type ( $F_{1,28}$ : 0.05;  $p$  = 0.8261) were not significant. At Calfuco during day-time hours, almost 4 times more small juveniles were found underneath than above the boulders (Fig. 2b). However, during night-time hours the abundances were similar in both microhabitats (Fig. 2b). Overall, the abundance of juvenile *C. concholepas* was significantly higher underneath than above the boulders ( $F_{1,28}$ : 8.5653;  $p$  = 0.0067, Fig. 2a). Although more small juveniles were observed during night-time hours (Fig. 2b), the effect of sampling period was not significant ( $F_{1,28}$ : 3.4661;  $p$  = 0.0732). Similarly, the interaction between sampling period and microhabitat type was not significant ( $F_{1,28}$ : 0.05;  $p$  = 0.1702).

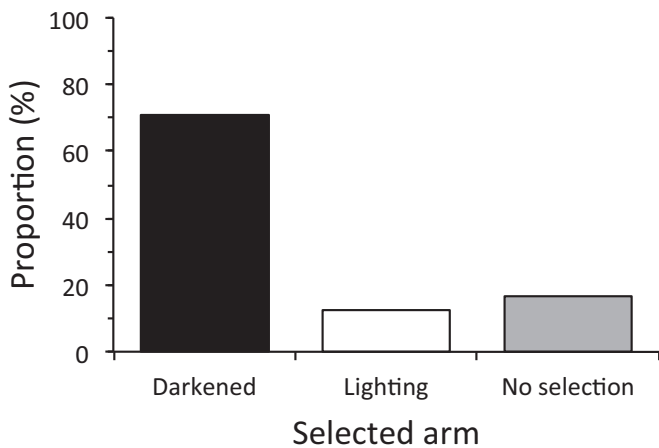
### 3.2. Prey-searching under light-darkness conditions

There were no significant differences (1-way ANOVA;  $F_{1,46}$  = 0.608;  $p$  = 0.440) in terms of size between the individuals used in the illuminated experiments (mean  $\pm$  SE = 7.75  $\pm$  0.16; N = 24) and those used in the dark experiments (mean  $\pm$  SE = 7.91  $\pm$  0.14; N = 24). At the end of the experiments, most of the trial individuals were found in the darkened arm (Fig. 3). The frequency of juvenile *C. concholepas* recorded in each of the 3 potential final positions (i.e. darkened arm,



**Fig. 2.** Average ( $\pm$ SE) abundances of small juveniles of *C. concholepas* recorded above and underneath rock boulders presents in shallow subtidal pools. In the top graphs are depicted the abundances in El Lenguado and Trocadero (a) during day-time hours at northern Chile. In the bottom graphs (b) the abundances are depicted for day (open bars) and night (filled bar) hours in Calfuco at southern Chile. Different letters indicate significant differences verified by ANOVA 2-way and Tukey's test ( $p < 0.05$ ). In the insert picture a small juvenile of *C. concholepas* preying on a barnacle stand during day-time hours in the underneath side of a rock boulder at Antofagasta, northern Chile. Scale bar 1 cm.

white LED illuminated arm or starting position) was significantly different from expected based on equal chance of being found on each positions ( $\chi^2 = 64.220$ ; DF = 2;  $p < 0.0001$ ; Fig. 3). When the juveniles were found in the darkened arms, the percentage of trial individuals found in each side of the rocks (above or below) or in other areas around the rocks was significantly different from expected (Table 1).



**Fig. 3.** The effect of white LED lighting on the selected Y-maze by the small juveniles of *C. concholepas*. White and dark bars are the proportion of individuals in the white LED illuminated and darkened arm of the Y-maze, respectively. The grey bar is the proportion of individuals that were found in the starting position of the Y-mazes.

**Table 1**  
*Concholepas concholepas*. Percentage of juvenile individuals recorded on or around the rocks placed in the selected darkened or white LED illuminated arm of the Y-maze.

Position of the trail snails	Darkened arm (%; n = 17)	White LED illuminated arm (%; n = 3)
On the rocks (Upper side-with barnacles)	70.58	0
On the rocks (Lower side-without barnacles)	23.53	100
Around the rocks	5.88	0

$\chi^2 = 66.911$ ; DF = 2;  $p < 0.0001$

Percentages of trial snails in each position at the end of the Y-maze experiments were compared using the Fisher's exact test with equal expected proportions in each position category. n, number of snail choosing the corresponding Y-maze arm.

In this condition the trial individuals were mainly found (~71%) in the upper surface of the rocks in which the barnacles were attached and no signs of mucous tracks were seen in the illuminated arm. When the trial individuals were found in the illuminated arm, no signs of mucous tracks were seen in the darkened arm and they were only found below the rocks (100%, Table 1). Finally, for the trial individuals found in the starting position, no signs of mucous tracks beyond that position were recorded.

3.3. Self-righting under light-darkness conditions

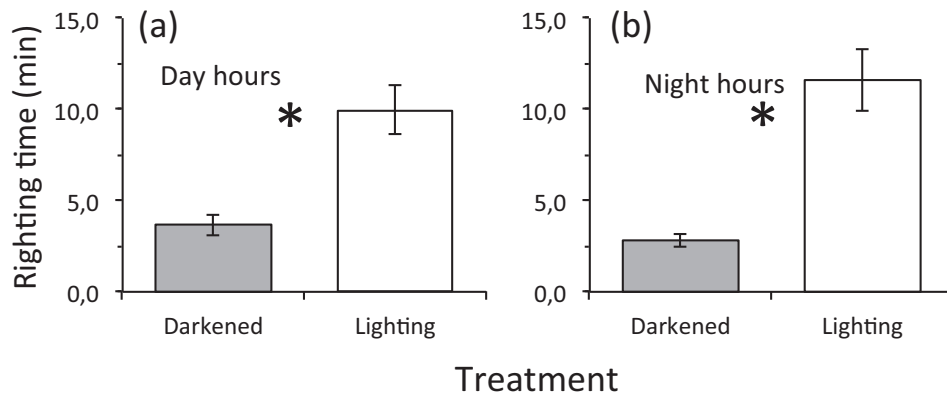
There were no significant differences (1-way ANOVA;  $F_{1,94} = 2.269$ ;  $p = 0.135$ ) in terms of size between the individuals used in the diurnal experiments (mean  $\pm$  SE =  $8.12 \pm 0.12$ ; N = 48) and those used in the nocturnal experiments (mean  $\pm$  SE =  $8.40 \pm 0.14$ ; N = 48). Moreover, no significant differences were found in size between individuals exposed to white LED illumination or maintained in darkened conditions during day time (1-way ANOVA;  $F_{1,46} = 0.472$ ;  $p = 0.495$ ) or night-time hours (1-way ANOVA;  $F_{1,46} = 0.364$ ;  $p = 0.549$ ) trials. Regardless of whether the observations were conducted during the day or at night, significantly shorter self-righting times were recorded for juvenile *C. concholepas* under dark conditions (Fig. 4a-b; Kruskal-Wallis,  $\chi^2 = 10.29$ ; DF = 1;  $p = 0.001$  and Kruskal-Wallis,  $\chi^2 = 10.29$ ; DF = 1;  $p = 0.001$  for day and night experiments, respectively). Righting success during the day was ~75% in the dark and 33% for white LED illuminated individuals. Similarly, righting success during the night was ~89% in the dark and ~28% for white LED illuminated individuals.

3.4. Metabolic impact of ALAN

There were no significant differences (1-way ANOVA;  $F_{1,34} = 0.4313$ ;  $p = 0.579$ ) in terms of size between the individuals used in the illuminated experiments (mean  $\pm$  SE =  $13.96 \pm 0.52$ ; N = 18) and those used in the dark experiments (mean  $\pm$  SE =  $13.68 \pm 0.41$ ; N = 18). Oxygen consumption rate was significantly higher (nearly twice as high) in juvenile *C. concholepas* that were exposed to white LED illumination than in those kept in darkness (1-way ANOVA;  $F_{1,34} = 9.637$ ;  $p = 0.004$ ; Fig. 5). Once the chambers were opened, the juveniles inside the white LED illuminated chambers were primarily found within the opaque blue screw caps away from the white LED lighting source (11 of 12 individuals;  $\chi^2 = 8.33$ ; DF = 1;  $p = 0.004$ ). Meanwhile, once the darkened chambers were opened this behavioral response was not generally observed (3 of 12 individuals;  $\chi^2 = 3.00$ ; DF = 1;  $p = 0.083$ ) and most trial individuals were found away from the screw caps.

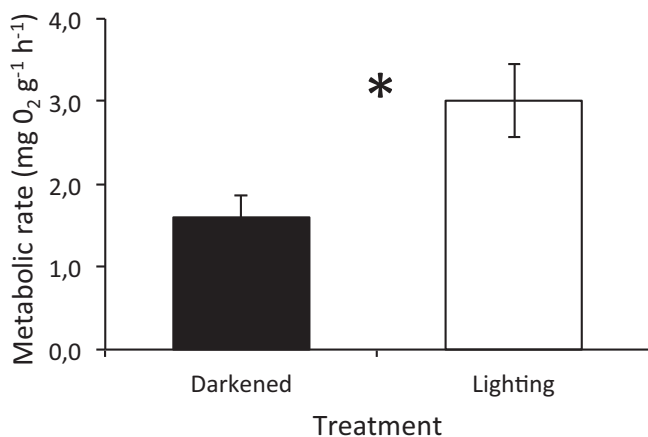
4. Discussion

Field surveys of rock boulders in shallow tide pools indicated that, during the day, juvenile *Concholepas concholepas* congregate on the underside of rock boulders. However at night, these juveniles were present



**Fig. 4.** The effect of white LED lighting on self-righting times of small juveniles of *C. concholepas* during day (a) and night hours (b). White and grey bars are average ( $\pm$ SE) values in illuminated and darkened conditions respectively. Significant differences ( $p < 0.05$ ) verified by 1-way ANOVA are indicated with an asterisk.

both on top of and on the underside of the boulders. Since these individuals were below the water level, they were not exposed to desiccation or thermal stress. Hence, those results suggest that they were actually avoiding light. Previous studies have indicated that large subtidal individuals of *C. concholepas* are active during both day and night (Stotz et al., 2003). Meanwhile, laboratory studies using intertidal adult individuals have indicated that they are mainly active at night (Castilla et al., 1979, Castilla and Cancino, 1979, Castilla et al., 1979, Guisado and Castilla, 1983). In rocky intertidal habitats, juveniles of this species remain motionless during daytime low tides, and are difficult to see due to their cryptic coloration (Manríquez et al., 2009). They are mainly found hiding in small cracks, crevices, on the undersides of boulders (Castilla and Cancino, 1979), inside shells of dead barnacles (Manríquez et al., 2004) and near/beneath the pedal disc of sea anemones (Moreno et al., 1993). Our study suggests the existence of a clear pattern in which juveniles of *C. concholepas* are more active at night than during the day, and select darkened micro-habitats during the day. In the rocky intertidal those sites might represent micro-habitats selected by small juveniles of *C. concholepas* during ebb tides to reduce desiccation and heating stress. However, our results suggest that similar microhabitats immersed within tide pools can also be used as refuges from stressful or inadequate lighting (i.e. scototactic behavior) and/or to avoid visual predators. If juveniles of *C. concholepas* are actually avoiding lit habitats, then ALAN could modify this species' distribution and prey-predation relationships, as it has been reported for other marine and terrestrial species (e.g. Bird et al., 2004; Becker et al., 2013; Luarte et al., 2016; Bolton et al., 2017; Pulgar et al., 2018).



**Fig. 5.** The effect of white LED lighting on metabolism of small juveniles of *C. concholepas*. White and grey bars are average ( $\pm$ SE) values in illuminated and darkened conditions respectively. Significant differences ( $p < 0.05$ ) verified by 1-way ANOVA are indicated with an asterisk.

The predation experiment showed that juvenile individuals tend to move to rocks with prey placed in the darkened arm of the Y-maze rather than the lit arm containing identical rocks and prey. This suggests that these organisms can distinguish between white LED illuminated and darkened areas and, therefore, in shallow subtidal habitats ALAN might affect the feeding activity, behavior and success of these snails at night. Given that the snails were able to reach the prey in darkness, these results suggest that prey finding in *C. concholepas* relies on chemical rather than on visual cues, which might be an important trait in a marine keystone predator species that move and prey during hours with low visibility. Absence of mucous tracks in both arms of the Y-mazes suggests that the final position of the experimental individuals represents their first choice. Such preference for darkened over lit areas seems natural and, we are confident, is not biased by the light levels used in the laboratory. The average light levels used to simulate ALAN treatments (330 lx) and the experiments discussed below (self-righting: 486 lx and metabolic impact: 481 lx) are relatively high but within the same order of magnitude of light intensities measured during night hours at the ground level of several field sites in the Chilean littoral (~100 to 150 lx; Pulgar et al., 2018, Duarte unpub. data). Moreover, light intensities used in our laboratory experiments are also within the order of magnitude of light intensities measured at night on the surface of rocky intertidal habitats next to promenades illuminated by lights equipped with white LEDs at Antofagasta (author's unpublished data).

Our findings also show that, unexpectedly, the time required for righting is negatively affected by exposure to ALAN. Fast self-righting is considered an adaptive trait that evolved to reduce lethal attacks by visual predators. Indeed, on wave-swept rocky shores, the ability of *C. concholepas* to self-right after dislodgement, and rapidly return to a normal up-right position, reduces the risk of predation (Manríquez et al., 2014). Therefore, increased righting times in intertidal habitats affected by ALAN, might increase an individual's susceptibility to a lethal attack by visual predators. Those predators are mainly active at night and include some species of crab (Silva et al., 2010), birds (Dugan, 1981) and small terrestrial mammals (Navarrete and Castilla, 1993). Although the negative effects of ALAN on the righting of overturned snails are clear, *C. concholepas* behavioral responses are complex and probably shaped by their interaction with other species. In our study, exposure to ALAN was done using individuals in isolation rather than as part of an interacting community. Therefore, further works addressing the impact of ALAN on multi-species systems are a logical follow up to this study.

We found that the metabolic rate of juveniles of *C. concholepas* was higher in the presence of ALAN than in a darkened environment. In our study, microbial respiration was minimised by using 0.45  $\mu$ m-filtered seawater and by ensuring that encrusting organisms, such as bryozoans, hydrozoans, ascidians and barnacles, were not present on the shell surface. Moreover, shell-boring phoronids and polychaetes were not present in any of the experimental individuals. Therefore,

differences in metabolic rates found in this study can only be attributed to the presence or absence of ALAN. A significant increase in the metabolic rate of juveniles exposed to ALAN suggests an increased demand for energy which otherwise would be allocated to other activities such as predator escape behavior or prey searching. This suggests that exposure to ALAN is a potentially powerful stressor during night-time that might have a selection effect on this species by impairing other energy-demanding activities, an issue that should be addressed in future studies. This also points towards the existence of a metabolic regulatory response in which light may lead to increased metabolic rates and hence ATP demand. Therefore, by minimizing day displacement and avoiding illuminated areas, *C. concholepas* may reduce metabolic rates. Our experimental setting did not allow us to test whether the return to darkness reduced oxygen consumption to maintain homeostasis. Future studies are required to investigate whether acute metabolic changes in response to ALAN induce a metabolic rebound once the individuals are back to not stressful conditions. If *C. concholepas* are conditioned to feed at night hours, exposure to ALAN would increase their normal energy requirements and, in order to meet this increased demand, they may start to undertake foraging activities with higher levels of boldness than normal, such as start foraging in the light. Such activities could make them more vulnerable to visual predators, reducing survival and impacting upon population success. The same applies to individuals that are dislodged when exposed to ALAN, where longer self-righting times will also make them more vulnerable to visual predators.

At the end of the metabolic measurements, the individuals maintained in the white LED illuminated chambers were usually found inside the screw cap away from the light source. In contrast, this behavior was rarely observed when the oxygen consumption was measured in individuals within darkened chambers. This is consistent with other observations showing that individuals of *C. concholepas* avoided lit areas and moved towards darkened areas in both the respirometry chambers and y-mazes. In nature, such microhabitats might be the underside of boulders submerged in an intertidal pool during low tides. This preferential movement to dark areas, or aversion to bright ones, suggests the existence of a scototactic behavior that has been previously reported in fish (Maximino et al., 2010, Blaser and Rosemberg, 2012, Thompson et al., 2016). Scototactic behavior has been suggested as a mechanism used by gastropods to return to coastal habitats after being displaced offshore (Chelazzi and Vannini, 1976). Studies conducted in mice have also found that short-term exposure to low levels of night-time fluorescent light increases their metabolic expenditure (Borniger et al., 2014). Consistently with that, in some species of fish, dark conditions decrease the individual's energetic requirements (Parker, 2002). Therefore, we suggest that the scototactic behavior in this species can be modulated by lighting and might have further consequences for other important traits, such as prey finding, predation susceptibility and metabolism.

## 5. Conclusions

Combined, our results indicate that when exposed to ALAN, small juveniles of *C. concholepas* showed significantly longer self-righting times, higher metabolic rates, and were less frequently found near the food items available. Moreover, in shallow subtidal habitats, small juveniles of this species preferred shaded areas during the day, but had no preference during night-time hours. Such evidence suggests that, due to ALAN, these juveniles become less efficient at finding food and more vulnerable to visual predators. A previous study found that juvenile *C. concholepas* that were stressed by a combination of elevated levels of  $p\text{CO}_2$  and the presence of a predatory crab showed significantly shorter self-righting times than those maintained under control conditions (Manríquez et al., 2013b). This was not the case with individuals exposed to ALAN. Therefore, we suggest ALAN can have far-reaching impacts on this and other species of rocky intertidal communities. We conclude that in the rocky intertidal, habitat complexity and natural or

artificial lighting can play an important role in micro-habitat selection by *C. concholepas* and other similar species. The reduction of darkness during night-time hours might alter the availability of appropriate habitat and affect those behavioral and physiological traits that are needed to make individuals less vulnerable to visual predators. Negative effects of night-time lighting, from the individual to the community levels, have been described in the literature (Davies et al., 2015; Luarte et al., 2016; Ludvigsen et al., 2018). However, our study is the first to report negative effects of ALAN on a keystone predator species, and thus might have negative implications for community dynamics. This far, available evidence on climate-driven effects on keystone species, and their cascading effects on coastal communities, has mainly focused on the effects of temperature (e.g. Sanford, 1999, Harley, 2011, Bonaviri et al., 2017), ocean acidification (Manríquez et al., 2013a, b, 2016) or a combination of both. In the light of the results presented here, the sustained growth of ALAN sources and their potential effects on keystone species and associated communities can no longer be ignored. In our study, artificial control of dawn and dusk was not feasible, so the potential crepuscular effect on the investigated behavioral responses cannot be disregarded.

In practical terms, our results imply that beachfronts equipped with white LED lighting at night might affect important behavioral (i.e. prey finding, self-righting) and physiological (i.e. metabolism) traits in *C. concholepas* or other similar species inhabiting intertidal habitats. This in turn can affect a whole range of species interactions and might have negative consequences for intertidal communities. Due to the fact that *C. concholepas* is a keystone species, changes in the balance of biological interactions caused by ALAN may, eventually, modify the whole community structure as has been described already in other intertidal invertebrates (Underwood et al., 2017) and ecosystems (Frank, 2006). The practical consequence of these results is the need for less biologically disruptive lighting (see Gaston et al., 2012). We argue that the development of beachfront lighting ordinance is required to help protect organisms inhabiting the coast-line. For instance, sea turtle friendly lighting has been designed to protect nocturnal nesting and hatching of sea turtle species (Salmon, 2003), and similar initiatives may be required to protect other organisms from ALAN. Further studies assessing the impacts of white LED lighting on feeding rate and survival of *C. concholepas* and other benthic species will allow additional predictions to be made about the effects of environmental changes on coastal habitats. Since LED lighting can be highly directional, one potential solution could be to direct light sources more precisely towards specific targets (i.e. coastal pedestrian paths, street lighting and routes), preventing or minimizing the illumination of coastal habitats in which sensitive species might inhabit.

## CRedit authorship contribution statement

**Patricio H. Manríquez:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Supervision, Visualization, Writing - original draft, Writing - review & editing. **María Elisa Jara:** Investigation, Methodology. **María Isabel Diaz:** Investigation, Formal analysis. **Pedro A. Quijón:** Conceptualization, Writing - review & editing. **Stephen Widdicombe:** Conceptualization, Writing - review & editing. **José Pulgar:** Funding acquisition, Conceptualization, Writing - review & editing. **Karen Manríquez:** Investigation, Methodology. **Diego Quintanilla-Ahumada:** Investigation, Methodology. **Cristian Duarte:** Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Writing - review & editing.

## Acknowledgments

The field part of this study was supported by the Fondo Nacional de Desarrollo Científico y Tecnológico, FONDECYT grant No 1050841 (2005–2007) to Patricio H. Manríquez (PHM). The experimental work of this study was supported by the FONDECYT grant No 1171056, to



Cristian Duarte (CD). During this study PHM was under the tenure of the grant "Climate driven Changes in the Habitat Suitability of Marine Organisms" (CLIMAR, ELAC2015/T01-0495) funded by the Network of the European Union, Latin America and the Caribbean Countries on Joint Innovation and Research Activities. During the writing of this article PHM and CD were under the tenure of the FONDECYT grants No 1181609 and 1171056. Pedro A. Quijón acknowledges the support from a NSERC-DG.

## References

- Becker, A., Whitfield, A.K., Cowley, P.D., Järnegren, J., Næsje, T.F., 2013. Potential effects of artificial light associated with anthropogenic infrastructure on the abundance and foraging behaviour of estuary-associated fishes. *J. Appl. Ecol.* 50, 43–50.
- Bird, B.L., Branch, L.C., Miller, D.L., 2004. Effects of coastal lighting on foraging behaviour of beach mice. *Conserv. Biol.* 18, 1435–1439.
- Blaser, R.E., Roseberg, D.B., 2012. Measures of anxiety in zebrafish (*Danio rerio*): dissociation of black/white preference and novel tank test. *Plos One* 7 (5), e36931.
- Bolton, D., Mayer-Pinto, M., Clark, G.F., Dafforn, K.A., Brassil, W.A., Becker, A., Johnston, E.L., 2017. Coastal urban lighting has ecological consequences for multiple trophic levels under the sea. *Sci. Total Environ.* 576, 1–9.
- Bonaviri, C., Graham, M., Gianguzza, P., Shears, N.T., 2017. Warmer temperatures reduce the influence of an important keystone predator. *J. Anim. Ecol.* 86, 490–500.
- Borniger, J.C., McHenry, Z.D., Abi Salloum, B.A., Nelson, R.J., 2014. Exposure to dim light at night during early development increases adult anxiety-like responses. *Physiol. Behav.* 133, 99–106.
- Castilla, J.C., 1999. Coastal marine communities: trends and perspectives from human-exclusion experiments. *Trends Ecol. Evol.* 14, 280–283.
- Castilla, J.C., Bahamondes, I., 1979. Observaciones conductuales y ecológicas sobre *Lutra felina* (Molina) 1782 (Carnívora: Mustelidae) en las zonas central y centro-norte de Chile. *Arch. Biol. Med. Exp.* 12, 119–132.
- Castilla, J.C., Cancino, J., 1979. Main predators of *Concholepes concholepes* (mollusca: gastropoda: muricidae) and preliminary observations about behavioural mechanisms of escape and defense. *Biol. Pesq. (Chile)* 12, 115–123.
- Castilla, J.C., Paine, R.T., 1987. Predation and community organization on Eastern Pacific temperate zone, rocky intertidal shores. *Rev. Chil. Hist. Nat.* 60, 131–151.
- Castilla, J.C., Guisado, Ch., Cancino, J., 1979. Aspectos ecológicos y conductuales relacionados con la alimentación de *Concholepes concholepes* (Mollusca: Gastropoda: Muricidae). *Biol. Pesq. (Chile)* 12, 91–97.
- Chang, A.M., Aeschbach, D., Duffy, J.F., Czeisler, C.A., 2015. Evening use of light-emitting eReaders negatively affects sleep, circadian timing, and next-morning alertness. *Proc. Natl. Acad. Sci. U. S. A.* 112, 1232–1237.
- Chee, S.Y., Othman, A.G., Sim, Y.K., Adam, A.N.M., Firth, L.B., 2017. Land reclamation and artificial islands: walking the tightrope between development and conservation. *Glob. Ecol. Conserv.* 12, 80–95.
- Chelazzi, G., Vannini, M., 1976. Researches on the coast of Somalia. The shore and the dune of Sar Uanle 9. Coastward orientation after displacement in *NERITA textilis*. Dillwyn (Gastropoda Prosobranchia). *Monitore Zoologico Italiano (NS Supplemento VIII)*. 4, pp. 161–178.
- Cinzano, P., Falchi, F., Elvidge, C.D., 2001. The first world atlas of the artificial night sky brightness. *Mon. Not. R. Astron. Soc.* 328, 689–707.
- Corsin, A., 2001. The Becoming of Space: A Geography of Liminal Practices of the City of Antofagasta, Chile. University of Oxford, D. Phil. thesis.
- Davies, T.W., Smyth, T., 2017. Why artificial light at night should be a focus for global change research in the 21st century. *Glob. Chang. Biol.* 24, 872–882.
- Davies, T.W., Bennie, J., Inger, R., Hempel de Ibarra, N., Gaston, K.J., 2013. Artificial light pollution: are shifting spectral signatures changing the balance of species interactions? *Glob. Chang. Biol.* 19, 1417–12166.
- Davies, T.W., Coleman, M., Griffith, K., Jenkins, S.R., 2015. Night-time lighting alters the composition of marine epifaunal communities. *Biol. Lett.* 11, 20150080.
- Davies, T.W., Duffy, J.P., Bennie, J., Gaston, K.J., 2016. Stemming the tide of light pollution encroaching into marine protected areas. *Conserv. Lett.* 9, 164–171.
- Davies, T.W., Bennie, J., Cruse, D., Blumgart, D., Inger, R., Gaston, K.J., 2017. Multiple nighttime light-emitting diode lighting strategies impact grassland invertebrate assemblages. *Glob. Chang. Biol.* 23, 2641–2648.
- Domenici, P., Torres, R., Manríquez, P.H., 2017. Effects of elevated carbon dioxide and increased temperature on locomotion and the repeatability of lateralization of a keystone marine mollusk. *J. Exp. Biol.* 220, 667–676.
- Dugan, P.J., 1981. The importance of nocturnal foraging in shorebirds: a consequence of increased prey activity. In: Jones, N.V., Wolff, W.J. (Eds.), *Feeding and Survival Strategies of Estuarine Organisms*: 251–260. Plenum Press, New York.
- Frank, K.D., 2006. Effects of artificial night lighting on moths. In: Rich, C., Longcore, T. (Eds.), *Ecological Consequences of Artificial Night Lighting*. Island Press, Washington, District of Columbia, pp. 305–344.
- Gaston, K.J., Davies, T.W., Bennie, J., Hopkins, J., 2012. Reducing the ecological consequences of night-time light pollution: options and developments. *J. Appl. Ecol.* 49, 1256–1266.
- Gaston, K.J., Bennie, J., Davies, T.W., Hopkins, J., 2013. The ecological impacts of nighttime light pollution: a mechanistic appraisal. *Biol. Rev.* 88, 912–927.
- Gaston, K.J., Duffy, J.P., Gaston, S., Bennie, J., Davies, T.W., 2014. Human alteration of natural light cycles: causes and ecological consequences. *Oecologia* 176, 917–931.
- Gaston, K.J., Visser, M.E., Hölker, F., 2015. The biological impacts of artificial light at night: the research challenge. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 370, 20140133.
- Castilla, J.C., Guisado, Ch., 1979. Conducta de alimentación nocturna de *Concholepes concholepes* (Mollusca: Gastropoda: Muricidae). *Biol. Pesq. (Chile)* 12, 125–130.
- Guisado, Ch., Castilla, J.C., 1983. Aspects of the ecology and growth of an intertidal juvenile population of *Concholepes concholepes* (Mollusca: Gastropoda: Muricidae) at Las Cruces, Chile. *Mar. Biol.* 73, 99–103.
- Hall, S.J., Todd, C.D., Gordon, A.D., 1982. The influence of ingestive conditioning on the prey species selection in *Aeolidia papillosa* (Mollusca: Nudibranchia). *J. Anim. Ecol.* 51, 907–921.
- Harley, 2011. Climate Change, Keystone Predation, and Biodiversity Loss. *Science* 334, 1124–1127.
- Hölker, F., Moss, T., Griefahn, B., Kloas, W., Voigt, C.C., Henckel, D., Hänel, A., Kappeler, P.M., Völker, S., Schwöpe, A., Franke, S., Uhrlandt, D., Fischer, J., Klenke, R., Wolter, C., Tockner, K., 2010. The dark side of light: a transdisciplinary research agenda for light pollution policy. *Ecol. Soc.* 15 (4), 13.
- Huaquín, L.G., Garrido, J., 2000. Morphology and discussion of the possible role of the osphradium in *Concholepes concholepes* (Brugüiere, 1789) (Neogastropoda: Muricidae). *J. Med. Appl. Malacol.* 10, 145–155.
- Kennedy, F., Naylor, E., Jaramillo, E., 2000. Ontogenetic differences in the circadian locomotor activity rhythm of the talitrid amphipod crustacean *Orchestoidea tuberculata*. *Mar. Biol.* 137, 511–517.
- Keshet-Sitton, A., Or-Chen, K., Yitzhak, S., Tzabary, I., Haim, A., 2015. Can avoiding light at night reduce the risk of breast cancer? *Integr. Cancer Ther.* 15, 145–152.
- Kyba, C.C., 2018. Is light pollution getting better or worse? *Nat. Astron.* 2 (4), 267.
- Kyba, C.C., Kuester, T., de Miguel, A.S., Baugh, K., Jechow, A., Hölker, F., Bennie, J., Elvidge, C.D., Gaston, K.J., Guanter, L., 2017. Artificially lit surface of earth at night increasing in radiance and extent. *Sci. Adv.* 3 (11), e1701528.
- Lorne, J.K., Salmon, L.M., 2007. Effects of exposure to artificial lighting on orientation of hatchling sea turtles on the beach and in the ocean. *Endanger. Species Res.* 3, 23–30.
- Luarde, T., Bonta, C.C., Silva-Rodríguez, E.A., Quijón, P.A., Miranda, C., Farias, A.A., Duarte, C., 2016. Light pollution reduces activity, food consumption and growth rates in a sandy beach invertebrate. *Environ. Pollut.* 216, 1147–1153.
- Ludvigsen, M., Berge, J., Geoffroy, M., Cohen, J.H., De La Torre, P.R., Normes, S.M., Singh, H., Sørensen, A.J., Daase, M., Johnsen, G., 2018. Use of an autonomous surface vehicle reveals small-scale diel vertical migrations of zooplankton and susceptibility to light pollution under low solar irradiance. *Sci. Adv.* 4, eaap9887.
- Manríquez, P.H., Castilla, J.C., 2001. Significance of marine protected areas in central Chile as seeding grounds for the gastropod *Concholepes concholepes*. *Mar. Ecol. Prog. Ser.* 215, 201–211.
- Manríquez, P.H., Castilla, J.C., 2018. Life history, knowledge, bottlenecks, and challenges for the aquaculture of *Concholepes concholepes* (Gastropoda: Muricidae) in Chile. *J. Shellfish Res.* 37, 1079–1092.
- Manríquez, P.H., Navarrete, S., Rosson, A., Castilla, J.C., 2004. Settlement of the gastropod *Concholepes concholepes* on shells of conspecific adults. *J. Mar. Biol. Assoc. U. K.* 84, 651–658.
- Manríquez, P.H., Lagos, N.A., Jara, M.E., Castilla, J., 2009. Adaptive shell color plasticity during the early ontogeny of an intertidal keystone predator. *Proc. Natl. Acad. Sci. U. S. A.* 106, 16298–16303.
- Manríquez, P.H., Galaz, S.P., Optiz, T., Hamilton, S., Paradis, G., Warmer, R.R., Castilla, J.C., Labra, F.A., Lagos, N.A., 2012. Geographic variation in trace-element signatures in the statoliths of near-hatch larvae and recruits of *Concholepes concholepes* (loco). *Mar. Ecol. Prog. Ser.* 448, 105–118.
- Manríquez, P.H., Jara, M.E., Optiz, T., Castilla, J.C., Lagos, N.A., 2013a. Behavioural and morphological traits in the early ontogeny of *Concholepes concholepes* in response to predation risk. *Mar. Ecol. Prog. Ser.* 472, 169–183.
- Manríquez, P.H., Jara, M.E., Mardones, M.L., Navarro, J.M., Torres, R., Lardies, M.A., Vargas, C.A., Duarte, C., Widdicombe, S., Salisbury, S., Lagos, N.A., 2013b. Ocean acidification disrupts prey responses to predator cues but not net prey shell growth in *Concholepes concholepes* (loco). *Plos One* 8 (7), e68643.
- Manríquez, P.H., Jara, M.E., Mardones, M.L., Torres, R., Navarro, J.M., Lardies, M.A., Vargas, C.A., Duarte, C., Lagos, N.A., 2014. Ocean acidification affects predator avoidance behaviour but not prey detection in the early ontogeny of a keystone species. *Mar. Ecol. Prog. Ser.* 502, 157–167.
- Manríquez, P.H., Jara, M.E., Seguel, M.E., Torres, R., Alarcon, E., Lee, M.R., 2016. Ocean acidification and increased temperature have both positive and negative effects on early ontogenetic traits of a rocky shore keystone predator species. *Plos One* 11 (3), e0151920.
- Manríquez, P.H., Torres, R., Matson, P.G., Lee, M.R., Jara, M.E., Seguel, M.E., Sepúlveda, F., Pereira, L., 2017. Effects of ocean warming and acidification on the early benthic ontogeny of an ecologically and economically important echinoderm. *Mar. Ecol. Prog. Ser.* 563, 169–184.
- Maximino, C., de Brito, T.M., de Mattos Dias, C.A.G., Gouveia, A., Morato, S., 2010. Scototaxis as anxiety-like behavior in fish. *Nat. Protoc.* 5, 221–228.
- Moreno, C.A., Ascencio, G., Ibañez, S., 1993. Patrones de asentamiento de *Concholepes concholepes* (Brugüiere) (Mollusca: Muricidae) en la zona intermareal rocosa de Valdivia, Chile. *Rev. Chil. Hist. Nat.* 66, 93–101.
- Navarrete, S.A., Castilla, J.C., 1993. Predation by Norway rats in the intertidal zone of Central Chile. *Mar. Ecol. Prog. Ser.* 92, 187–199.
- Parker, R., 2002. *Aquaculture Science*. Delmar, a Division of Thomson Learning, Inc. (621 pp.).
- Power, M.E., Tilman, D., Estes, J.A., Menge, B.A., Bond, W.J., Mills, L.S., Daily, G., Castilla, J.C., Lubchenco, J., Paine, R.T., 1996. Challenges in the Quest for Keystone Species. *BioScience* 46, 609–620.
- Pulgar, J., Zeballos, D., Vargas, J., Aldana, M., Manríquez, P.H., Manríquez, K., Quijón, P.A., Widdicombe, S., Anguita, C., Quintanilla, D., Duarte, C., 2018. Endogenous cycles, activity patterns and energy expenditure of an intertidal fish is modified by artificial light pollution at night (ALAN). *Environ. Pollut.* 244, 361–366.

- Salmon, M., 2003. Artificial night lighting and sea turtles. *Biologist* 50, 163–168.
- Sanford, E., 1999. Regulation of keystone predation by small changes in ocean temperature. *Science* 283, 2095–2097.
- Serb, J.M., 2008. Towards developing models to study the disease, ecology, and evolution of the eye in Mollusca. *Am. Malacol. Bull.* 26, 3–28.
- Silva, A.C.F., Hawkins, S.J., Boaventura, D.M., Brewster, E., Thompson, R.C., 2010. Use of the intertidal zone by mobile predators: influence of wave exposure, tidal phase and elevation on abundance and diet. *Mar. Ecol. Prog. Ser.* 406, 197–210.
- Stoll, C.J., 1972. Sensory systems involved in the shadow response of *Lymnaea stagnalis*. *Proceedings van de Koninklijke Nederlandse Akademie van Wetenschappen Section C, Biological and Medical Sciences.* 75, pp. 342–351.
- Stoll, C.J., 1976. Extraocular photoreception in *Lymnaea stagnalis* (L.). In: Sálanki, J. (Ed.), *Neurobiology of Invertebrates: Gastropoda Brain, Tihany 1975.* Akadémiai Kiadó Budapest, pp. 487–495.
- Stotz, W.B., González, S.A., Caillaux, L., Aburto, J., 2003. Quantitative evaluation of the diet and feeding behavior of the carnivorous gastropod, *Concholepas concholepas* (Bruguere, 1789) (Muricidae) in subtidal habitats in the southeastern Pacific upwelling system. *J. Shellfish Res.* 22, 147–164.
- Ter Maat, A., Pieneman, A.W., Koene, J.M., 2012. The effect of light on induced egg laying in the simultaneous hermaphrodite *Lymnaea stagnalis*. *J. Molluscan Stud.* 78, 262–267.
- Thompson, R.R.J., Paul, E.S., Radford, A.N., Purser, J., Mendl, M., 2016. Routine handling methods affect behaviour of three-spined sticklebacks in a novel test of anxiety. *Behav. Brain Res.* 306, 26–35.
- Troscianko, T., Benton, C.P., Lovell, P.G., Tolhurst, D.J., Pizlo, Z., 2009. Camouflage and visual perception. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 364, 449–461.
- Underwood, C.N., Davies, T.W., Queirós, A.M., 2017. Artificial light at night alters trophic interactions of intertidal invertebrates. *J. Anim. Ecol.* 86, 781–789.
- Van Doren, B.M., Horton, K.G., Dokter, A.M., Klink, H., Elbin, S.B., Farnsworth, A., 2017. High-intensity urban light installation dramatically alters nocturnal bird migration. *Proc. Natl. Acad. Sci. U. S. A.* 42, 11175–11180.
- Viviani, C., 1975. *Las comunidades marinas litorales en el norte grande de Chile.* Publicación Ocasional del Laboratorio de Ecología Marina. Universidad del Norte, Iquique, Chile (196 pp.).
- Wells, R., 1980. Activity pattern as a mechanism of predator avoidance in two species of acmaeid limpet. *J. Exp. Mar. Biol. Ecol.* 48, 151–158.
- Zielinska-Dabkowska, K.M., 2018. Make lighting healthier. *Nature* 553, 274–276.
- Zissis, G., Bertoldi, P., 2014. Update on the status of the LED market. *European Commission Report EU 27000 EN.*