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

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Abstract. Artificial Light At Night (ALAN) is an increasing global problem that, despite being widely recognized in terrestrial systems, has~~ve~~ 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 behavioral and physiological traits of small juveniles in~~of~~ 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.

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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 & Smyth 2017) and marine communities (Becker et al. 2013, Gaston et al. 2014, Davies et al. 2015, Bolton et al. 2017, Davies & 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. 2917, 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

91   and reduce energy consumption. All of which means that LEDs are rapidly becoming one

92   of the world's most important light sources (Zissis & Bertoldi 2014) and are increasingly

93   being used for lighting in both residential and commercial areas as well as the transport

94   routes between them. In the marine environment this will specifically include beachfront

95   developments, ports, marinas and shipping. Therefore, the potential impact of this change

96   to LED illumination on marine communities needs to be considered (Gaston et al. 2015).

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98   In the marine realm, many species have evolved behavioral and morphological

99   responses to minimize visual predation (Troscianko et al. 2009, Manríquez et al. 2009). For

100   instance, some intertidal species are most active during the night as a mechanism to avoid

101   visual predators (Wells 1980). In addition, being active at night minimizes thermal abiotic

102   stress and desiccation at low tide. This is particularly advantageous for organisms

103   performing energy-demanding activities (e.g. Kennedy et al. 2000). Recently, ALAN has

104   also been shown to affect the locomotor activity, circadian rhythm and growth rate of

105   intertidal amphipods (Luarte et al. 2016) as well as the small-scale diel vertical migrations

106   of zooplankton species (Ludvigsen et al. 2018). Therefore, the modification of the natural

107   light-dark regime by ALAN in coastal environments could have important consequences

108   for the species inhabiting these areas.

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110   The "Loco" or "Chilean abalone", *Concholepas concholepas* (Bruguière, 1789), is a

111   keystone species (i.e. its presence maintains the structure and integrity of the community)

112   in rocky shores of the south-eastern Pacific Ocean coast (Castilla & Paine 1987, Power et

113   al. 1996, Castilla 1999). This species is an economically and ecologically important

114   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 & Cancino 1979, Guisado & 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 areis also timed over the lunar or tidal cycle is unknown. Among the most important prey items of *C. concholepas* are barnacles, mussels and ascidians (Stotz et al. 2003, Manríquez & 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 & Cancino 1979), the sea otter *Lontra felina* (Castilla & Bahamondes 1979), and the fish *Pimelometopon maculatus* and *Syciasis sanguineous* (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 & 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 phototaxic 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 (Corsín 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

163 investigate the potential effects of ALAN (using LED lighting) on prey searching, self-

164 righting speed and metabolism. In this particular environment, prey searching and self-

165 righting success are important traits in mediating both predator-prey interactions and the

166 ability to return to a normal posture after dislodgement, respectively. Moreover, we

167 conducted a field survey to determine whether the distribution of juveniles in shallow

168 subtidal rocky habitats was influenced by the ambient light conditions. Our hypothesis was

169 that exposure to ALAN has significant effects on behavioral and physiological traits of

170 juveniles of this keystone species. We expected that ALAN exposure would inhibit the

171 activity of small juveniles of *C. concholepas* and prompt them to incur increased metabolic

172 costs in searching for food inand darkened areas. Given that overturned individuals are

173 more vulnerable to visual predators, we also expected that ALAN would speed up self-

174 righting.

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176 **2. Methods**

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178 **2.1 Influence of natural lighting conditions on the abundances of Loco in the field.**

179 This field sampling was conducted to explore the link between the response of small

180 juveniles of *C. concholepas* to ALAN in the laboratory and their natural abundances during

181 night hours. Daytime natural abundances on shaded or illuminated shallow subtidal

182 microhabitats might give cues about where the small juvenile of this species prefer to be

183 more active (e.g. searching for prey). Similarly, night-time abundances might help to know

184 if this pattern changes in absence of light. The location and abundance of juvenile

185 *Concholepas concholepas* (~1.5 cm in shell length) were determined from surveys

186 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 (±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'sKit 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 lux (Gaston et al. 2013, 2104).

The presence and abundance of juvenile *C. concholepas* was noted on both the upper and lower surface of rocks boulders that were loose and small enough to be able to be turned by the observer (≤ 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~~levels~~ 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 ~~visualizing~~ 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*

235 *purpuratus* and *Semimytilus algosus* were also presents on the rocky intertidal platforms.

236 Shell coloration in *C. concholepas* depends on the color of the more abundant prey

237 available in the habitat (Manríquez et al. 2009). Therefore, all the individuals were cryptic

238 with mixed shell color (dark and light colored) and therefore ingestively conditioned (Hall

239 et al. 1982) to the prey used in the laboratory experiments (below). After collection, the

240 individuals were moved to laboratory facilities at the Universidad Católica del Norte,

241 Coquimbo, Chile (29°58'S; 71°21'W) where all the experiments were conducted. On

242 arrival, individuals were maintained in Pexiglas® aquaria with running seawater, under a

243 natural photoperiod for the austral summer months (12:12 h light:dark photoperiod without

244 artificial control for gradual changes at dawn and dusk). During the entire rearing period,

245 lighting in the laboratory was provided indirectly by fluorescent lamps and the light

246 intensity at the level of the aquariums was on average (±SE; N) 38.6 lux (±6.5; 6). During

247 the first 2 weeks of rearing, individuals were provided with small rocks incrusted with

248 stands of the barnacle *N. scabrosus* on which to feed and then the diet was switched to

249 small individuals of the mussel *S. algosus*. At the end of each experiment (see below),

250 individuals were weighed to the nearest 0.001 g and measured to the nearest 0.01 mm using

251 an analytical balance (Adam AFA180 LC) and a digital vernier caliper (Mitutoyo 500-196-

252 30), respectively.

253

254 **2.3 Prey-searching under light-darkness conditions.** This experiment was conducted on

255 juvenile *C. concholepas* which had been acclimated to laboratory conditions for 1 week.

256 During this acclimation period, individuals were fed *ad libitum* with barnacle stands

257 collected from the same site used for *C. concholepas* collection. After acclimation, the

258 directional responses of *C. concholepas* to artificial light were evaluated in a two-choice

259 test using Plexiglas® Y-mazes. The left and right Y-maze arms were both 0.60 m long,

260 0.18 m wide and 0.10 m deep (Fig. 1). Three Y-mazes (black lateral walls, black lid and

261 white floor) were run simultaneously with both arms of each maze receiving filtered (1 μm)

262 seawater at a constant rate of 1.0 L h^{-1}. The seawater flowing into the arms of the Y-mazes

263 dropped ~1 cm onto the surface of the water. As in a previous study (Manríquez et al.

264 2014), the displacement paths of the individual snails were verified at the end of the trials

265 by observing the remains of fine sediment stuck to the mucous track generated by *C.*

266 *concholepas* as the anterior part of their foot travels along the white Y-maze floor. The Y-

267 maze experiments were repeated on 8 consecutive days until a total of 24 replicate trails

268 were obtained. After each run, the Y-mazes were cleaned with tap water to remove any

269 chemical cue that could influence future measurements. At the beginning of each trial, a

270 small flat rock with ~200 barnacles was placed near the seawater inlets in each of the Y-

271 maze arms. The surface of the flat rock bearing the barnacle stands were always upwardly

272 oriented. In each Y-maze, one arm was lit by attaching a white LED light to the seawater

273 inlet while the other arm remained in darkness. All the LED lights used in this study

274 (Chanzon ®) were 5 mm round clear type, forward voltage of 3.2-3.6 V, 0.06 W, with a

275 view angle of 120º and a correlated color temperature (CCT) of 8,000 K. They also had the

276 characteristic light source spectral power distribution (SPD) curve, displaying a first peak at

277 460 nm with a power of 0.028 W/nm (indicating blue content) followed by the bell shape of

278 the second peak at 550 nm (indicating higher yellow content). The LED was powered by a

279 button Lithium cell battery (3V, CR1616) and oriented toward the upper surface of the rock

280 in that arm (Fig. 1a). In the dark arm, a LED was also attached to the seawater inlet but was

281 not switched on and therefore the surface of the rock was not illuminated. This was to

282 ensure that the physical presence of the lighting equipment was the same in both arms and

283   could not influence the results. During the experiments, the upper surface of the rocks was

284   always immersed in water to depth of ~ 3-4 cm. The light intensities were measured in the

285   air just above the water surface (~2 cm) and in the starting zone situated 50 cm away from

286   the inlets using a L-100 PCE Lux Meter. Above the illuminated flat stones the light

287   intensities were on average (±SE; N) 329.9 lux (±22.4; 8). The light intensity used in this

288   experiment (and in the others, see below) was within the natural range measured during

289   daytime hours in the locality where the animals were collected (ca. 200 to 1000 lux).

290   However, in the darkened arms and in the starting zone the light intensities were on average

291   (±SE; N) 0.016 lux (±0.001; 8) and 0.169 lux (±0.011; 8), respectively. To standardize

292   hunger levels, all trial specimens were starved for 24 h before the experiment. The test

293   individuals were placed in the acclimatization zone for 1 h (~17:00) and then moved

294   carefully to the starting zone. Each trial lasted 15 h, and the final position of the snails in

295   the Y-mazes was recorded (~09:00). The final position of the juveniles in the Y-maze was

296   categorized as lighting arm, darkened arm or in the starting zone (Fig. 1a). To maintain the

297   temperature at 15 °C the Y-mazes were semi-immersed in a temperature-controlled water

298   bath.

299

300   **2.4 Self-righting under light-darkness conditions.** This experiment was designed to

301   examine how light/darkness might influence self-righting success and speed of small

302   juvenile individuals of *C. concholepas*. This experiment was conducted one week after the

303   previous one on a different group of individuals that had been acclimated to laboratory

304   conditions for 2 weeks. To account for the existence of an endogenous rhythm that might

305   mask self-righting performance in response to light/darkness the experiment was run twice.

306   The first experimental run was conducted during daytime hours (~10:00 to 19:00), while

307 the second experimental run was conducted during the night (~22:00 to 03:00). In each

308 experimental run we used 24 different individuals. Each individual was placed in a plastic

309 chamber (5 L), filled with 3.5 L of aerated seawater and immersed in a water bath to

310 maintain the acclimation temperature (~15 ºC). The entire chamber was covered with black

311 plastic to prevent light from entering the system (Fig. 1b). The opening of the chamber was

312 equipped with a modified plastic funnel used to close the chamber and to hold a white LED

313 light (the same as the one described in the previous section) (Fig. 1b). The funnel spout was

314 1.0 cm in diameter and 3.0 cm in length and provided an efficient way to stop the room

315 lighting from entering the chamber. When the white LED was on (during daytime or night

316 hours), the light intensity in the bottom of the experimental chamber was on average (±SE;

317 N) 485.5 lux (±11.3; 8). However, when the LED was off (during daytime or night hours),

318 the light intensity was 0.068 (±0.001; 8). To improve adherence during righting, the bottom

319 of the chamber was covered with a circular strip of a rough-textured rubber surface (3M™

320 Safety-Walk™ tape). Each juvenile was placed in the chamber for 5 min of acclimatization

321 and then was placed upside down in the middle of the chamber. In this way the plane of the

322 shell aperture was parallel with respect to the horizontal plane, therefore with the same

323 degree of difficulty to self-right (Manríquez et al. 2016; 2017). Self-righting time was

324 defined as the total time taken by the individual to completely return to its normal upright

325 position from the moment that the individual was placed upside down and was measured

326 using a digital stopwatch. A maximum of 15 min to assess self-righting was allowed per

327 individual. Inability to self-right within 15 min was considered as self-righting failure.

328 Regardless of experimental run (diurnal or nocturnal), during darkness a dim red LED Petzl

329 headlight (~15 lux) was used to intermittently illuminate the inside of the chamber to allow

330 the juvenile to be observed. After each trial the chamber was cleaned and filled with fresh

331 aerated seawater in preparation for the next trial.

332 **2.5 Metabolic impact of ALAN.** The effect of ALAN on oxygen consumption in small

333 juvenile *C. concholepas* was measured on a randomly selected group of 32 individuals that

334 had been acclimated to laboratory conditions for 3-4 weeks. Four experimental runs were

335 conducted, each run consisting of 4 illuminated and 4 darkened 60 mL glass respirometry

336 chambers. The illuminated chambers were lit with a white LED, the same as those used in

337 the previous experiments, placed 3 cm above each chamber (Fig. 1c). The light levels in the

338 artificially illuminated and darkened chambers, were on average (±SD; N) 480.8 lux

339 (±12.0; 4) and zero, respectively. The darkened chambers were identical except they were

340 completely covered with sticky back vinyl tape leaving a small opening over the Oxygen

341 Sensor Spot (SP-PSt3-NAU, PreSens). The chambers were placed horizontally and semi-

342 immersed in a black Pexiglas® container connected to a refrigerated and heating water bath

343 circulator (Lab Companion RW-2025G). In this container, all the chambers were left with

344 the oxygen sensor spots (fixed at the inner side of the chambers) 0.5 cm above the water

345 level. To avoid any additional stress during the oxygen measurements, the chambers were

346 not manipulated when the fiber optical probe was applied~~approached~~ to the spots. A fiber

347 optic oxygen meter (Fibox 3, PreSens) was used for all oxygen consumption

348 measurements. Calibration was performed using a Na_2SO_3 solution (0% saturation) and

349 using air bubbled seawater (100% saturation). Individuals were starved in tanks containing

350 1.0 µm running filtered seawater for 24 h prior to the measurements. During this period

351 individuals were maintained at light intensities that were on average (±SE; N) 38.6 lux

352 (±6.5; 6) and supplied by daylight fluorescent tubes. Metabolic measurements lasted for at

353 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 ºC 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. 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

378   were analysed by a Fisher's exact-probability test. All statistical analyses were performed

379   using SPSS v.18.0 (IBM Corp., Armonk, NY, USA). At the end of the experiments all the

380   experimental individuals were returned to the same site where they were collected.

381

382   **3. Results**

383   **3.1 Influence of natural lighting conditions on the abundances of Loco in the field.** The

384   abundance of small juveniles of *C. concholepas* was significantly different between

385   microhabitats ($F_{1,28}$: 0.9307; $p < 0.00001$). Approximately 4 and 5 times more individuals

386   were found in the shaded than in the illuminated microhabitats at El Lenguado and

387   Trocadero, respectively (Fig. 2a). The same analysis found that sampling site ($F_{1,28}$: 0.05; p

388   0.3429) and the interaction with microhabitats type ($F_{1,28}$: 0.05; $p = 0.8261$) were not

389   significant. At Calfuco, and during day-time hours, almost 4 times more small juveniles

390   were found underneath than above the boulders (Fig. 2b). However, during night-time

391   hours the abundances were similar in both microhabitats (Fig. 2b). Overall, the abundance

392   of juvenile *C. concholepas* was significantly higher underneath than above the boulders

393   ($F_{1,28}$: 8.5653; $p = 0.0067$, Fig. 2a). Although more small juveniles were observed during

394   nigh-time hours (Fig. 2b), the effect of sampling period was notn significant ($F_{1,28}$: 3.4661;

395    $p = 0.0732$). Similarly, the interaction between sampling period and microhabitat type was

396   not significant ($F_{1,28}$: 0.05; $p = 0.1702$).

397

398   **3.2 Prey-searching under light-darkness conditions.** There were no significant

399   differences (1-way ANOVA; $F_{1,46} = 0.608$; $p = 0.440$) in terms of size between the

400   individuals used in the illuminated experiments (mean±SE = 7.75±0.16; N=24) and those

401   used in the dark experiments (mean±SE = 7.91±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, 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). 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~~in~~ 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±SE = 8.12±0.12; N=48) and those used in the nocturnal experiments (mean±SE = 8.40±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

426   day and night experiments, respectively). Righting success during the day was ~75% in the

427   dark and 33% for white LED illuminated individuals. Similarly, righting success during the

428   night was ~89% in the dark and ~28% for white LED illuminated individuals.

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430   **3.4 Metabolic impact of ALAN.** There were no significant differences (1-way ANOVA;

431    $F_{1,34} = 0.4313$; p = 0.579) in terms of size between the individuals used in the illuminated

432   experiments (mean±SE = 13.96±0.52; N=18) and those used in the dark experiments

433   (mean±SE = 13.68±0.41; N=18). Oxygen consumption was significantly higher (nearly

434   twice as high) in juvenile *C. concholepas* that were exposed to white LED illumination than

435   in those kept in darkness (1-way ANOVA; $F_{1,34} = 9.637$; p = 0.004; Fig. 5). Once the

436   chambers were opened, the juveniles inside the white LED illuminated chambers were

437   primarily found within the opaque blue screw caps away from the white LED lighting

438   source (11 of 12 individuals; $\chi^2 = 8.33$; DF = 1; p = 0.004). Meanwhile, once the darkened

439   chambers were opened this behavioral response was not generally observed~~found~~ (3 of 12

440   individuals; $\chi^2 = 3.00$; DF = 1; p = 0.083) and most trial individuals were found away from

441   the screw caps.

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443   **4. Discussion**

444   Field surveys of rock boulders in shallow tide pools indicated that, during the day,

445   juvenile *Concholepas concholepas* congregate on the underside of rock boulders. However

446   at night, these juveniles were present both on top of and on the underside of the boulders.

447   Since these individuals were below the water level, they were not exposed to desiccation or

448   thermal stress. Hence, those results suggest that they were actually avoiding light. Previous

449   studies have indicated that large subtidal individuals of *C. concholepas* are active during

450   both day and night (Stotz et al. 2003). Meanwhile, laboratory studies using intertidal adult

451   individuals have indicated that they are mainly active at night (Castilla & Guisado 1979,

452   Castilla & Cancino 1979, Castilla et al. 1979, Guisado & Castilla 1983). In rocky intertidal

453   habitats, juveniles of this species remain motionless during daytime low tides, and are

454   difficult to see due to their cryptic coloration (Manríquez et al. 2009). They are mainly

455   found hiding in small cracks, crevices, on the undersides of boulders (Castilla & Cancino

456   1979), inside shells of dead barnacles (Manríquez et al. 2004) and near/beneath the pedal

457   disc of sea anemones (Moreno et al. 1993). Our study suggests the existence of a clear

458   pattern in which juveniles of *C. concholepas* are more active at night than during theat day

459   hours, and select darkened micro-habitats during the day hours. In the rocky intertidal those

460   sites might represent micro-habitats selected by small juveniles of *C. concholepas* during

461   ebb tides to reduce desiccation and heating stress. However, our results suggest that similar

462   microhabitats immersed within tide pools can also be used as refuges from stressful or

463   inadequate lighting (i.e. scototactic behavior) and/or to avoid visual predators. If juveniles

464   of *C. concholepas* are actually avoiding lit habitats, then ALAN could modify this species'

465   distribution and prey-predation relationships, as it has been reported for other marine and

466   terrestrial species (e.g. Bird et al. 2004, Becker et al. 2013, Luarte et al 2016, Bolton et al.

467   2017, Pulgar et al. 2018).

468

469   The predation experiment showed that juvenile individuals tend to move to rocks

470   with prey placed in the darkened arm of the Y-maze rather than the lit arm containing

471   identical rocks and prey. This suggests that these organisms can distinguish between white

472   LED illuminated and darkened areas and, therefore, in shallow subtidal habitats ALAN

473   might affect the feeding activity, behavior and success of these snails at night. Given that

474 the snails were able to reach the prey in darkness, these results suggest that prey finding in

475 *C. concholepas* relies on chemical rather than on visual cues, which might be an important

476 trait in a marine keystone predator species that move and prey during hours with low

477 visibility. Absence of mucous tracks in both arms of the Y-mazes suggests that the final

478 position of the experimental individuals represents their first choice. Such preference for

479 darkened over lit areas seems natural and, we are confident, is not biased by the light levels

480 used in the laboratory. The average light levels used to simulate ALAN treatments (330

481 lux) and the experiments discussed below (self-righting: 486 lux and metabolic impact: 481

482 lux) are within the same order of magnitude of light intensities measured during night hours

483 at the ground level of several field sites in the Chilean littoral (Pulgar et al. 2018, Duarte

484 unp. data). Moreover, light intensities used in our laboratory experiments are also within

485 the order of magnitude of light intensities measured at night on the surface of rocky

486 intertidal habitats next to promenades illuminated by lights~~luminaires~~ equipped with white

487 LEDs at Antofagasta (author's unpublished data).

488

489   Our findings also show that, unexpectedly, the time required for righting is negatively

490 affected by exposure to ALAN. Fast self-righting is considered an adaptive trait that

491 evolved to reduce lethal attacks by visual predators. Indeed, on wave-swept rocky shores,

492 the ability of *C. concholepas* to self-right after dislodgement, and rapidly return to a normal

493 up-right position, reduces the risk of predation (Manríquez et al. 2014). Therefore,

494 increased righting times in intertidal habitats affected by ALAN, might increase an

495 individual's susceptibility to a lethal attack by visual predators. Those predators are mainly

496 active at night and include some species of crab (Silva et al. 2010), birds (Dugan et al.

497 1981) and small terrestrial mammals (Navarrete & 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

minimisedcontrolled by using 0.45 μm-filtered seawater and by ensuring that, while

encrusting organisms, such as bryozoans, hydrozoans, ascidians and barnacles, were not

present oin 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

522   conditioned to feed at night hours, exposure to ALAN would increase their normal energy

523   requirements and, in order to meet this increased demand, they may start to undertake

524   foraging activities with higher levels of boldness than normal, such as start foraging in the

525   light. Such activities could make them more vulnerable to visual predators, reducing

526   survival and impacting upon population success. The same applies to individuals that are

527   dislodged when exposed to ALAN, where longer self-righting times will also make them

528   more vulnerable to visual predators.

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530   At the end of the metabolic measurements, the individuals maintained in the white

531   LED illuminated chambers were usually found inside the screw cap away from the light

532   source. In contrast, this behavior was rarely observed when the metabolism was measured

533   in individuals within darkened chambers. This is consistent with other observations

534   showing that individuals of *C. concholepas* avoided lit areas and moved towards darkened

535   areas in both the respirometry chambers and y-mazes. In nature, such microhabitats might

536   be the underside of boulders submerged in an intertidal pool during low tides. This

537   preferential movement to dark areas, or aversion to bright ones, suggests the existence of a

538   scototactic behavior that has been previously reported in fish (Maximino et al. 2010, Blazer

539   & Rosemberg 2012, Thompson et al. 2016). Scototactic behavior has been suggested as a

540   mechanism used by gastropods to return to coastal habitats after being displaced offshore

541   (Chelazzi & Vannini 1976). Studies conducted in mice have also found that short-term

542   exposure to low levels of night-time fluorescent light increases their metabolic expenditure

543   (Borniger et al. 2014). Consistently with that, in some species of fish, dark conditions

544   decrease the individual's energetic requirements (Parker 2002). Therefore, we suggest that

545   the scototactic behavior in this species can be modulated by lighting and might have further

546 consequences for~~in~~ other important traits, such as prey finding, predation susceptibility and

547 metabolism.

5. Conclusions

549 Combined, our results indicate that when exposed to ALAN, small juveniles of *C.*

550 *concholepas* showed significantly longer self-righting times, higher metabolic rates, and

551 were less frequently found near the food items available. Moreover, in shallow subtidal

552 habitats, small juveniles of this species preferred shaded areas during the day, but had no

553 preference during~~at~~ night-time hours. Such evidence suggests that, due to ALAN, these

554 juveniles become less efficient at finding food and more vulnerable to visual predators. A

555 previous study found that juvenile *C. concholepas* that were stressed by a combination of

556 elevated levels of $p\mathrm{CO_2}$ and the presence of a predatory crab showed significantly shorter

557 self-righting times than those maintained under control conditions (Manríquez et al.

558 2013b). This was not the case with individuals exposed to ALAN. Therefore, we suggest

559 ALAN can have far-reaching impacts on this and other species of rocky intertidal

560 communities. We conclude that in the rocky intertidal, habitat complexity and natural or

561 artificial lighting can play an important role in micro-habitat selection by *C. concholepas*

562 and other similar species. The reduction of darkness during~~at~~ night-time hours might alter

563 the availability of appropriate habitat and affect those behavioral and physiological traits

564 that are needed to make individuals ~~them~~ less vulnerable to visual predators. Negative

565 effects of night-time lighting, from the individual to the community levels, have been

566 described in the literature (Davies et al. 2015, Luarte et al. 2016, Ludvigsen et al. 2018).

567 However, our study is the first to report negative effects of ALAN on a keystone predator

568 species, and thus might have negative implications for community dynamics. This far,

569 available evidence on climate-driven effects on keystone species, and their cascading

570   effects on coastal communities, has mainly focused on the effects of temperature (e.g.

571   Sanford 1999, Harley 2012, Bonaviri et al. 2017), ocean acidification (Manríquez et al.

572   2013, 2016) or a combination of both. In the light of the results presented here, the

573   sustained growth of ALAN sources and their potential effects on keystone species and

574   associated communities can no longer be ignored. In our study, artificial control of dawn

575   and dusk was not feasible, so the potential crepuscular effect on the investigated behavioral

576   responses cannot be disregarded.

577

578   In practical terms, our results imply that beachfronts equipped with white LED

579   lighting at night might affect important behavioral (i.e. prey finding, self-righting) and

580   physiological (i.e. metabolism) traits in *C. concholepas* or other similar species inhabiting

581   intertidal habitats. This in turn can affect a whole range of species interactions and might

582   have negative consequences for intertidal communities. Due to the fact that~~Because~~ *C.*

583   *concholepas* is a keystone species, changes in the balance of biological interactions caused

584   by ALAN may, eventually, modify the whole community structure as has been described

585   already in other intertidal invertebrates (Underwood et al. 2017) and ecosystems (Frank

586   2006). The practical consequence of these results is the need for ~~the design of~~ less

587   biologically disruptive lighting (see Gaston et al. 2012). We argue that the development of

588   beachfront lighting ordinance is required to help protect organisms inhabiting the coast-

589   line. For instance, sea turtle friendly lighting has been designed to protect nocturnal nesting

590   and hatching of sea turtle species (Salmon 2003), and similar initiatives may be required to

591   protect other organisms from ALAN. Further studies assessing the impacts of white LED

592   lighting on feeding rate and survival of *C. concholepas* and other benthic species will allow

593   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.

Acknowledgments

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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.

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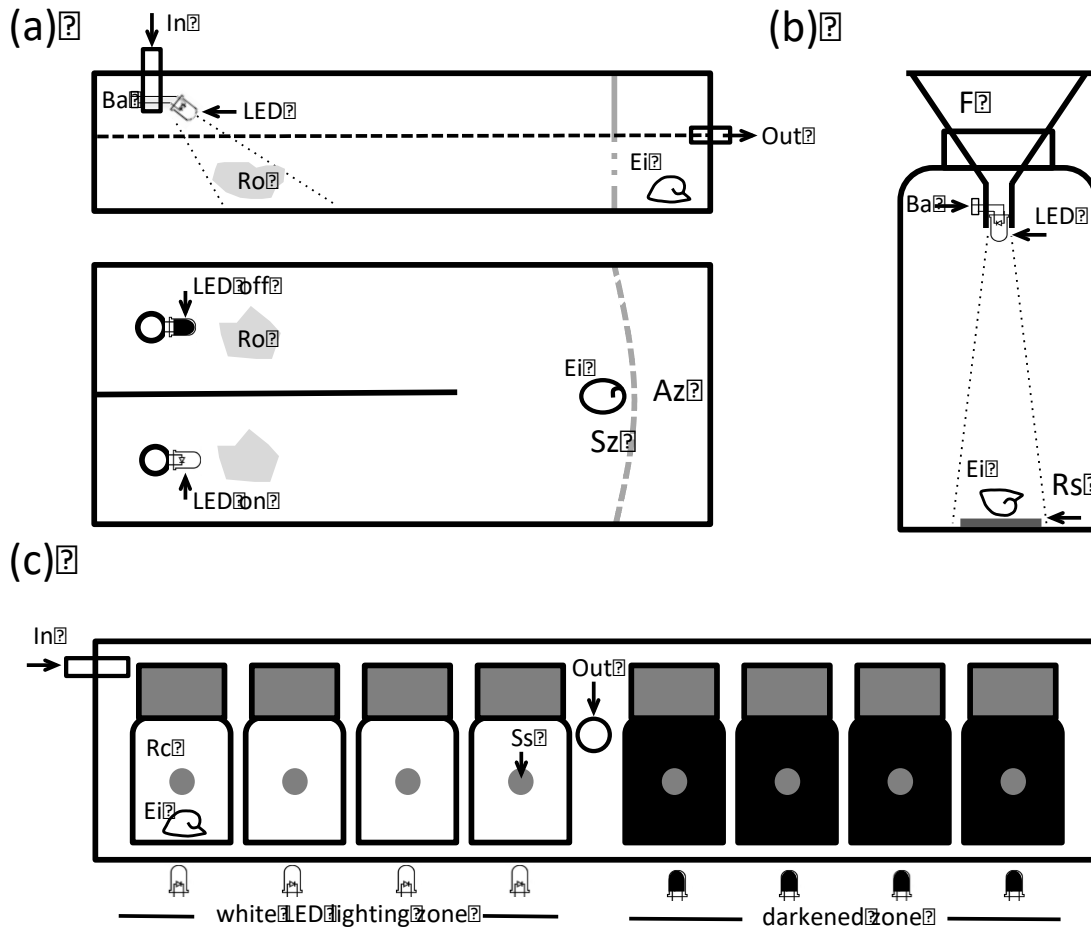
Figure 1. Schematic representation (not to scale) of the experimental setting used to evaluate the effect of white LED lighting on: (a) prey searching, (b) self-righting time and (c) metabolism of small juveniles of *C. concholepas*. In (a) a lateral view (top plot) and top view (bottom plot) of the 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 ® 3M ®. In (c) an upper view of the

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703   black Plexiglas container used to hold the glass respirometry chambers (Rc) equipped with
704   oxygen sensors spots (Ss) to measure metabolism.
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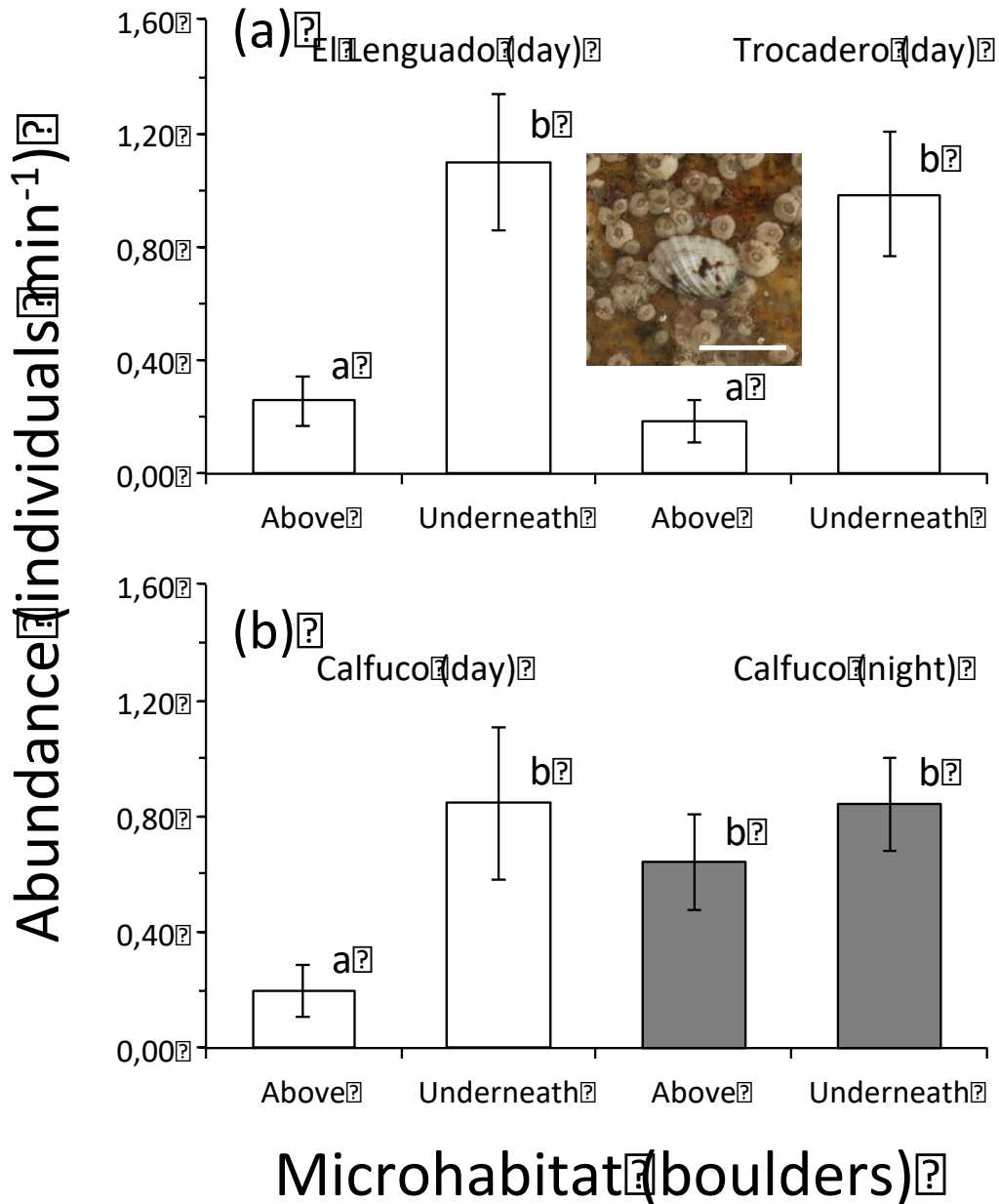
Figure 2. Average (± SE) abundances of small juveniles of *C. concholepas* recorded above and underneath rock boulders presents in shallow subtidal pools. In the top graphs are depicted the abundances in El Lenguado and Trocadero (a) during day-time hours at northern Chile. In the bottom graphs (b) the 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.
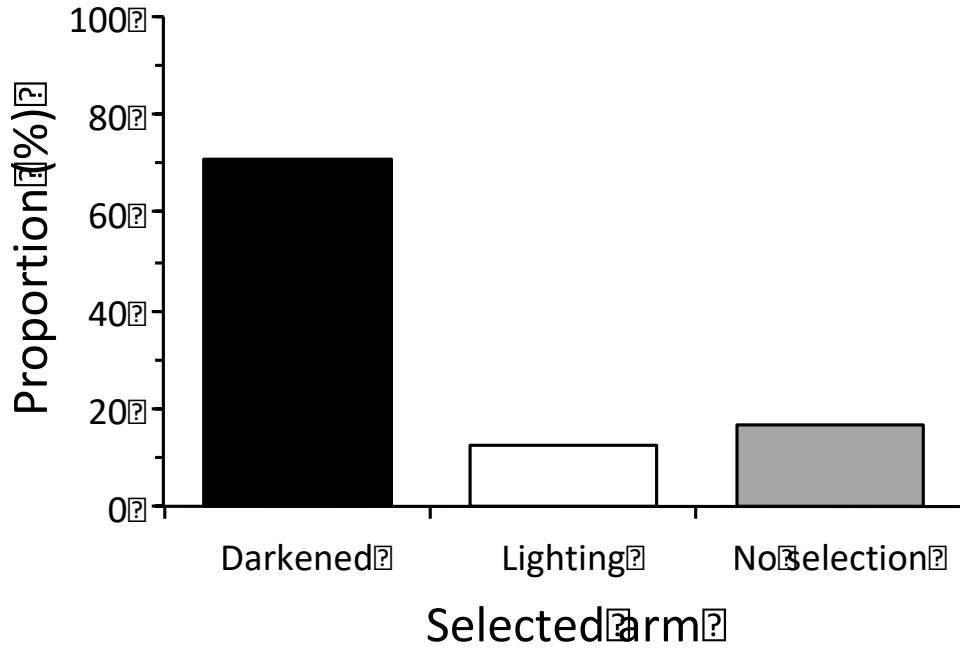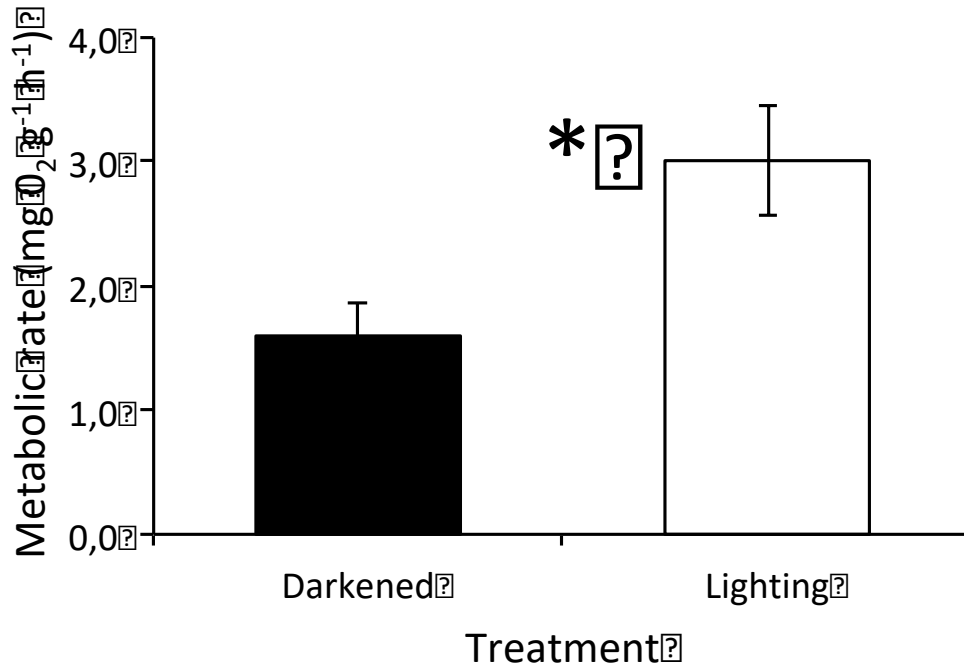
Figure 3. The effect of white LED lighting on the selected Y-maze by the small juveniles of *C. concholepas*. White and dark bars are the proportion of individuals in the white LED illuminated and darkened arm of the Y-maze, respectively. The grey bar is the proportion of individuals that were found in the starting position of the Y-mazes.

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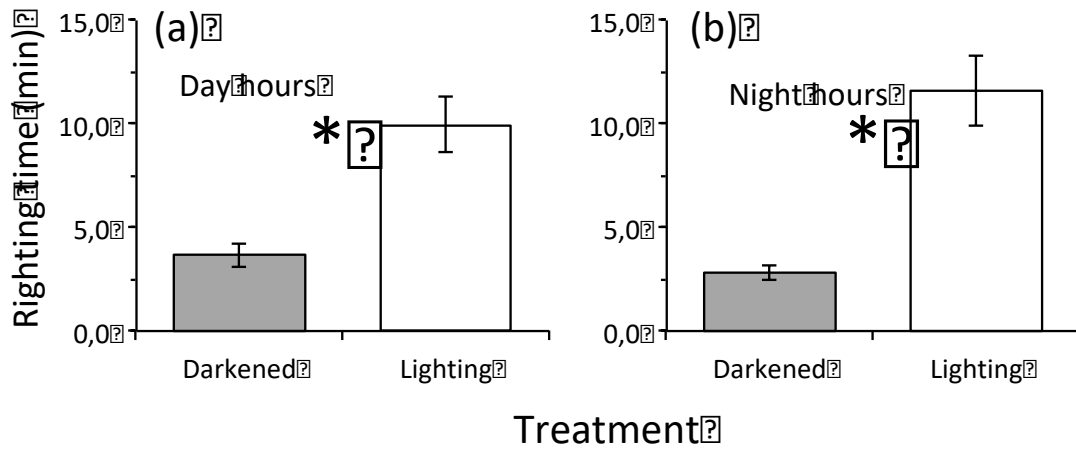
Figure 4. The effect of white LED lighting on self-righting times of small juveniles of *C. concholepas* during day (a) and night hours (b). White and grey bars are average (± SE) values in illuminated and darkened conditions respectively. Significant differences (p < 0.05) verified by 1-way ANOVA are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

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

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