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4 **ENDOGENOUS CYCLES, ACTIVITY PATTERNS**

5 **AND ENERGY EXPENDITURE OF AN**

6 **INTERTIDAL FISH IS MODIFIED BY ARTIFICIAL**

7 **LIGHT POLLUTION AT NIGHT (ALAN)**

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36 **ABSTRACT**

37 The increase of global light emissions in recent years has highlighted the need for
38 urgent evaluation of their impacts on the behaviour, ecology and physiology of
39 organisms. Numerous species exhibit daily cycles or strong scototaxic behaviours that
40 could potentially be influenced if natural lighting conditions or cycles are disrupted.
41 Artificial Light Pollution at Night (ALAN) stands for situations where artificial light
42 alters natural light-dark cycles, as well as light intensities and wavelengths. ALAN is
43 increasingly recognized as a potential threat to biodiversity, mainly because a growing
44 number of studies are demonstrating its influence on animal behavior, migration,
45 reproduction and biological interactions. Most of these studies have focused on
46 terrestrial organisms and ecosystems with studies on the effects of ALAN on marine
47 ecosystems being more occasional. However, with the increasing human use and
48 development of the coastal zone, organisms that inhabit shallow coastal or intertidal
49 systems could be at increasing risk from ALAN. In this study we measured the levels of
50 artificial light intensity in the field and used these levels to conduct experimental trials
51 to determine the impact of ALAN on an intertidal fish. Specifically, we measured
52 ALAN effects on physiological performance (oxygen consumption) and behaviour
53 (activity patterns) of “Baunco” the rockfish *Girella laevifrons*, one of the most abundant
54 and ecologically important intertidal fish in the Southeastern Pacific littoral. Our results
55 indicated that individuals exposed to ALAN exhibited increased oxygen consumption
56 and activity when compared with control animals. Moreover, those fish exposed to
57 ALAN stopped displaying the natural (circatidal and circadian) activity cycles that were
58 observed in control fish throughout the experiment. These changes in physiological
59 function and behaviour could have serious implications for the long-term sustainability
60 of fish populations and indirect impacts on intertidal communities in areas affected by
61 ALAN.

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63 Key words: Intertidal fish, Artificial Light Pollution, ALAN, Endogenous activity

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76 **INTRODUCTION**

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78 As human populations grow so does the footprint of human activities needed to
79 support this growth. One consequence of this is that global light emission levels are
80 currently increasing at a rate of around 6% per year (Hölker et al., 2010). It is well
81 established that environmental light levels, and particularly natural cycles of light and
82 dark, can exert a strong controlling influence over the behaviour and performance of
83 many organisms. It is reasonable therefore to expect that the alteration of natural light
84 conditions could have a significant impact on organisms, biodiversity and ecosystem
85 function.

86 Artificial Light Pollution At Night (ALAN) is a term that is gaining increased
87 recognition from researchers and that describes a disruption in which artificial light
88 alters the natural cycles of light and dark in ecosystems (Longcore and Rich, 2004). It is
89 proposed that ALAN has the potential to threaten biodiversity, through the effects of
90 changing light conditions on animal behavior, migration, reproduction, and biological
91 interactions (Longcore and Rich, 2004; Hölker et al., 2010). To date, most of the studies
92 evaluating ALAN effects have been carried out on terrestrial organisms (Bennie et al.,
93 2015). However, the rapid development of coastal areas and the increasing human use
94 of the coastal zone to support residential demand, food supply, recreation and the
95 transport of people and commodities, warrant the study of ALAN's impacts on these
96 habitats. It is estimated that currently 22% of the World's coastal areas are exposed to
97 artificial light at night (Davies et al., 2014). This suggests that coastal marine
98 ecosystems could be facing a significant and increasing risk from the impacts of ALAN.

99 The extent to which ALAN impacts marine organisms is not well known
100 (Depledge et al., 2010) although studies carried out with sea turtles and birds are
101 notable exceptions (Berger et al., 2009; Montevecchi, 2006; Mazor et al., 2013; Merkel
102 and Johansen, 2011). In fish, periods of light and dark have been shown to be important
103 for reproduction, growth rate, ontogenetic development, migration, locomotor activity,
104 food intake and diel vertical migration (Downing and Litvak, 2002; Mehner, 2012). For
105 example, the experimental alteration of the photoperiod can improve the growth rate in
106 a number of fish species (Ginés et al., 2003; Trippel and Neil, 2003). The continuous
107 exposure to light has also been shown to increase the growth of the Atlantic salmon
108 (*Salmo salar*) (Saunders et al., 1985) and the Atlantic cod (*Gadus morhua*) (Folkvord
109 and Ottera, 1993). However, Hallaraker et al. (1995) working with the fish
110 *Hippoglossus hippoglossus*, found no significant differences between the growth rate of
111 individuals maintained under a natural photoperiod compared with those maintained
112 under a continuous light regime. Recently the response of animals to light has been
113 referred as scototaxis behavior, which is the preferential movement of an organism to a
114 dark (safe) zone with aversion to a bright one. The active selection of dark zones by fish
115 has also been demonstrated in preference assays with individuals making fewer visits to,
116 and spending less time in illuminated (unsafe) aquarium zones (Maximino et al., 2010,
117 Blazer & Rosemberg 2012, Thompson et al., 2016). It is not just vertebrates that can be
118 affected by exposure to ALAN. A recent study by Underwood et al., (2017)
119 demonstrated that the foraging activity of dog-whelks (*Nucella lapillus*), a predatory
120 mollusk that structures biodiversity in temperate rocky shores, was altered by exposure
121 to ALAN.

122 The intertidal Baunco fish, *Girella laevisfrons*, is one the most abundant fish in
123 intertidal zones of the Chilean coast and plays an important ecological role as a predator

124 (Pulgar et al., 2015). As a juvenile this species inhabits high intertidal rocky pools, and
125 has been described as a diurnal fish (Helfman et al., 2009). After spending 2 years in
126 these intertidal rock pools individuals reach sufficient reproductive body size and
127 migrate out of the intertidal and into subtidal areas (García-Huidobro et al., 2017;
128 Pulgar et al., 2017). During their 2 years in the intertidal zone as juveniles, individuals
129 of *G. laeivfrons* are likely exposed to ALAN, with unknown energetic or behavioral
130 consequences. Hence, the aim of this study was to determine if exposure to ALAN had
131 an impact on activity levels, circadian and circatidal rhythm, weight and oxygen
132 consumption of *G. laeivfrons*.

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134 **MATERIAL and METHODS**

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136 Juveniles of *G. laeivfrons* (N = 30, standard length [L_s] = 4.98 (+/-0.32) s.d. cm,
137 and weight= 1.75(+/-0.36) s.d. [g]) were captured from high intertidal pools located at
138 Punta Choros, Chile (29°14S, 71°27W) during November 2017. All animals were
139 obtained applying a BZ-20 anesthetic (15 mL/100 L of seawater). Collected fish were
140 immediately placed in a cooler (25 l) containing fresh seawater with constant aeration,
141 and transported live to the laboratory for experiments. Once in the laboratory and prior
142 to the start of any experiments, all specimens were acclimatized and fed *ad-libitum* for
143 10 days with commercial marine flakes (Tetra GmbH, Herrenteich, Germany) in a
144 system with daily renewal of sea water at controlled temperature (17-18 °C) and salinity
145 (35‰), and with a 12h:12h photoperiod. During the acclimation time, fish were exposed
146 to 7222 lux intensity during the 12 h of daylight (laboratory light conditions).
147 Additionally, light intensity was measured in the intertidal pools from where the
148 experimental fish were collected. Light intensity was measured during day (n=5) and
149 night conditions (n=5) using a Luxometer (PCE-L 100, España). These measurements

150 indicated that during daylight hours the intertidal pools were exposed to 7222 (1029.98
151 s.d.) lux. Meanwhile, during the night hours these sites were exposed to 78.28 (5.4 s.d.)
152 of lux, i.e., the field sites and the fish used in the experimental trials were indeed
153 exposed to ALAN.

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155 **ALAN effects on fish activity**

156 To determine if ALAN modified the scototaxis behavior of juvenile *G. laevis*, fish
157 were maintained for 10 days under two contrasting light regimes: i) a fish group with a
158 natural 12:12 photoperiod (Control, n= 5, Body size [L_s] =4.85 (0.32 s.d.) cm, weight =
159 1.64 (0.32 s.d.) [g]) and ii) an ALAN group (Treatment, n= 5, Body size [L_s] = 4.92
160 (0.34 s.d.) cm, weight = 1.86 (0.41 s.d.) [g]). During daylight hours, both fish groups
161 were exposed to 7222 lux (laboratory light conditions). Fish exposed to ALAN were
162 exposed to 70 lux from dusk to dawn (the level of light exposure currently measured in
163 the area where the fish were collected, knowingly exposed to ALAN), whereas control
164 fish were exposed to natural (dark) conditions experienced in areas located away from
165 artificial sources of light. This light intensity was controlled using a Luxometer (PCE-L
166 100, España). During the experimental time, fish were fed daily with commercial
167 marine flakes (Tetra GmbH, Herrenteich, Germany), maintained with constant aeration
168 and the seawater was changed every day. To evaluate the impact of ALAN on fish
169 activity, individuals from each group (see above) were placed in a 25 l tank (50x30x20
170 cm) that included a rocky refuge (14 x 14 x 14 cm, equal to 10% of aquarium volume;
171 see Vargas et al., 2018). Although potential interactions among fish within a particular
172 group were possible (and we expect this to occur in nature), our main goal was to
173 describe the response of each group to the presence or absence of ALAN. Activity was
174 measured as the number of times a fish passed between a dark (refuge) and the zone

175 exposed to light, either natural daylight (both treatments) or artificial light (ALAN
176 treatment). The refuge entrance was a 5 cm gap from the bottom of the tank, that
177 allowed the free fish movement, and was equipped with actographs, an infrared
178 recording system (see Jaramillo et al., 2003), that recorded each occasion a fish passed
179 through the entrance. The capability of actographs to detect fish movements in seawater
180 between the two areas of the aquarium (refuge and light) was previously evaluated
181 using similar fish and experimental set ups.

182

183 **Oxygen consumption in fish exposed to ALAN**

184 Ten additional fish (different from those used in the activity experiments) were
185 placed individually into large flasks (1000 mL); five fish were exposed to ALAN (Body
186 size [L_s] =4.95 (0.24) cm, weight = 1.68 (0.16) [g] and five were maintained in control
187 conditions (Body size [L_s] =5.13 (0.21) cm, weight = 1.90 (0.23) [g] for 10 days. Fish
188 associated to different treatments had no visual contact between them. At the end of this
189 exposure period, oxygen consumption rates were determined in the morning hours for
190 each fish using a metabolic chamber following the methodology of Chapelle and Peck
191 (1995). To avoid digestive energetic costs, fish were starved for 24 hours prior to all the
192 measurements (Horn et al., 1999; Chabot et al., 2016; Benítez et al., 2017). Briefly,
193 1000 mL flasks were filled with filtered seawater and fully saturated with oxygen
194 through constant bubbling. Once seawater saturation was reached, the dissolved oxygen
195 concentration ($\text{mg O}_2 \text{ L}^{-1}$) was measured with an oxygen-meter (OXI-Check, HI9147-
196 04, Hanna Instruments). Each individual was placed for 120 min in the chamber without
197 bubbles. After this period, each metabolic chamber was carefully opened, and the
198 dissolved oxygen concentration was measured (Peck and Veal, 2001). For all the
199 experimental fish, rates of oxygen consumption were measured during similar day

200 hours. Then, oxygen consumption, expressed as $\text{mg O}_2 \text{ g}^{-1} \text{ min}^{-1}$ (VO_2), was calculated
201 as the difference between the final and initial concentrations of dissolved oxygen in
202 each test. All individuals were subjected to standard measurements of body length (cm)
203 and weight (g) before and after the ALAN exposure, and the variation in weight (Δw)
204 between these time-points was estimated.

205

206 **Statistical Analysis**

207 Data from the activity experiments were analyzed in R software (R Core Team,
208 2017) using generalized Additive Models for Location, Scale and Shape (GAMLSS,
209 Rigby and Stasinopoulos, 2005). The GAMLSS model is a general regression model
210 which assumes that the response variable has any parametric distribution (beyond
211 exponential family distribution), including highly skew and/or kurtotic continuous and
212 discrete distributions. In addition, within this framework, all the parameters of the
213 distribution of the response variable (i.e. μ , σ , τ , ν) can be modeled as
214 linear/non-linear or smooth functions of the explanatory variables. To determine if
215 ALAN modified fish activity, the daily record of fish passes through the refuge entrance
216 over the 10 d period were grouped in ten minutes intervals ($n=1438$ intervals, for both
217 the control and the ALAN). We modeled; (1) the probability of activity (at least one
218 detected movement per 10 minutes) and (2) the frequency of activity (total number of
219 detected movements per 10 minutes) in response to the treatment, the hour of the day
220 (0-23hrs) and the day of the experiment (1-10). The hour of the day was fitted non-
221 linearly (in order to adjust the circadian rhythm) using cubic smoothing splines function
222 available in the GAMLSS package. We included the interactive effects between the
223 treatment (as a factor) and the hour of the day (non-linearly) and the day of the
224 experiment (linearly). For the probability model we used a binomial error distribution

225 and a logit link function and for the frequency model we used a negative binomial error
226 distribution and a log link. The negative binomial distribution is a two parameter
227 distribution (*mu*, *sigma*), suited to dealing with count data, which usually present over-
228 dispersion (Zuur et al., 2009, O'Hara and Kotze, 2010). For both models an "anova-
229 like" likelihood ratio test was applied. Models selection and diagnostics were based on
230 the AIC, BIC and residuals plots available in the GAMLSS package (Rigby and
231 Stasinopoulos, 2005). Figures were produced using the ggplot2 package (Wickham,
232 2009) in R.

233 As oxygen consumption is affected by standard body length, this physiological
234 variable was compared using an ANCOVA (Variable factor: Photoperiod [Treatment=
235 ALAN, Control= 12:12], which controlled for the effects of standard body length ([L_s],
236 Co-variate) (Zar, 1996).

237

238

239 **RESULTS**

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241 For the activity experiment, we found increased activity ($P < 0.0001$, Table 1) associated
242 to ALAN, the hour of the day (fitted with cubic smoothing splines) and their interaction:
243 we recorded significant changes on (1) the probability (at least one pass across the
244 refuge per 10 minutes) and (2) the frequency of activity of fish (number of pass across
245 refuge entrance per 10 minutes). Under control conditions fish had a clear circadian
246 rhythm (Figure 1) with a mean of 71% probability and 6 pass across refuge throughout
247 the day. The highest probability and frequency of activity were observed between
248 12:00-14:00 h (mean of 83% probability and 23 pass across the refuge). Meanwhile,
249 fish exposed to ALAN showed a significant increase of activity, passing constantly
250 across the refuge entrance with a mean of 83% probability and 15 pass throughout the

251 whole day cycle (up to 93% of probability and 18 pass of activity at 13:00 h, Figure 1).
252 In addition, we found a significant interaction between the days (linearly) and the
253 treatment (i.e. different slopes) for the probability and frequency of activity of fish
254 ($P < 0.0001$, Table 1, Figure 1). At control conditions fish showed an increase in the
255 probability of activity (from ~55% to 84%), but with constantly lower frequency
256 throughout the ten days (i.e. fish did pass across the refuge but fewer times).
257 Conversely, fish exposed to ALAN presented a significant increase in both, the
258 probability and frequency of activity throughout the ten days of the experiment (Figure
259 1).

260 The oxygen consumption was significantly higher in fish exposed to ALAN
261 compared to control fish (ANCOVA $F_{(1,7)} = 5.86$; $P = 0.04$, Fig. 2). Regarding to weight
262 variation, no significant variations between fish exposed to ALAN and control fish were
263 detected (Final weight ALAN 1.56 (0.15 s.d.) [g], Control 1.82 (0.26 s.d.) [g], $P > 0.05$).

264

265 **DISCUSSION**

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267 This study demonstrates that exposure to ALAN increased activity levels and
268 oxygen consumption in the intertidal fish *G. laevifrons*, when compared to animals kept
269 under a more natural 12h:12h light-dark cycle. In addition, exposure to ALAN altered
270 the natural circadian and circatidal rhythm of this important intertidal species.

271 All organisms that regularly visit or inhabit the intertidal zone have tidally
272 organized behavioral rhythms that are driven by an endogenous clock system (Chabot
273 and Watson 2010). This endogenous cycle has been described in a variety of taxonomic
274 groups including fish (Helfman et al., 2009), sandy beach invertebrates (Jaramillo et al.,
275 2003; Luarte et al., 2016), the amphipod *Corophium volutator* (Harris and Morgan

276 1984) and the crabs *Liocarcinus holsatus* and *Uca crenulata* (Honegger 1973). In the
277 case of fish it has been shown that intertidal fish often rest during low tide periods and
278 swim actively during high tides (Helfman et al., 2009). The circadian and circatidal
279 clock are principally influenced by physical factors such as water depth (Gibson 1982,
280 1992), light levels, (Helfman et al., 2009) and biological interactions such as predation
281 (McFarland et al., 1999). The current study demonstrates that exposure to ALAN can
282 modify both the circadian and circatidal rhythm of an intertidal fish species. While,
283 control animals displayed one clear activity peak (13-14 h), which seemed directly
284 related to tide change (high to low tide and low to high tide), this activity peak was
285 altered or lost in fish exposed to ALAN. In fact the activity of ALAN-exposed animals
286 increased significantly across the whole daily cycle and throughout the entire duration
287 of the experiment. This altered activity contrasted the activity level observed in control
288 fish, which was characterized by a constant and low number of passes through the
289 refuge entrance. The probability and frequency of activity observed in control fish
290 during the experiment (Fig. 1) is intriguing and offer a venue for further research. This
291 pattern might be related to a loss in the internal circatidal rhythm over time, potentially
292 linked to the lack of tidal conditions (not replicated in our laboratory conditions).
293 Testing such hypothesis was beyond the scope of this study which represents the first
294 description of locomotor activity for this species. It is also the first study to assess the
295 influence of ALAN on endogenous cycles in a temperate intertidal transitory fish.

296 The exposure of *G. laevisfrons* to ALAN indicated that this stressor modified
297 locomotor activity levels and the fish's use of dark and light aquarium zones (Fig. 1). In
298 natural (wild) conditions, fish actively use dark zones and display an aversion to bright
299 environments (i.e. they display scototaxis). Such active selection of areas not exposed to
300 light has been already observed in experiments using light gradients, and suggest that *G.*

301 *laevifrons* are able to modify their activity patterns in response to light (Pulgar et al.,
302 2015). This makes sense as illuminated areas are deemed more dangerous due to an
303 increased risk of detection by visual predators (Thompson et al., 2016). Our results
304 indicate that fish exposed to ALAN showed an increased movement between the refuge
305 (dark) and the light zones of the experimental set up. This suggests that ALAN has the
306 potential to modify the normal scototaxis behaviour, and therefore poses a risk to these
307 individuals. This evidence is also consistent with previous studies that have shown that
308 exposure to ALAN can affect fish movement, habitat selection and can increase
309 foraging in marine fish (Oppedal et al., 2011, Becker et al., 2013).

310 Our study also shows that exposure to ALAN increased oxygen consumption in
311 *G. laevifrons*. During its first two years of life this species inhabits higher intertidal
312 pools and consequently experiences huge environmental variability in terms of
313 temperature, UV radiation and pH. All of these environmental factors have been shown
314 to have an impact on fish oxygen consumption (Pulgar et al., 2005, 2015 Benitez et al.,
315 2017, García-Huidobro et al., 2017, Vargas et al., 2018). Adding ALAN to these
316 existing environmental stressors could place even greater physiological demands on
317 these fish. The higher oxygen consumption observed in *G. laevifrons* when exposed to
318 ALAN (Fig. 2) was likely a consequence of the increase in fish activity seen under
319 ALAN conditions and could represent a change in the overall energetic balance in *G.*
320 *laevifrons*, increasing the metabolic cost of living in this species (Bridges 1993).
321 Although there were no differences in fish weight between control fish and those fish
322 exposed to ALAN at the end of the current study, we suspect that the significant
323 increase in activity of fish exposed to ALAN could lead to differences in fish weight
324 had the exposure period been longer.

325 Finally, it should be noted that the experimental ALAN levels used in the current
326 study (nearest to 70 lux) represent the observed light levels already being experienced in
327 the intertidal zone at night. Consequently, the influence of ALAN on fish activity,
328 endogenous cycles and energetic change may already be occurring in intertidal habitats
329 exposed to this stressor. This emphasizes the importance of future studies to further
330 document the impact of ALAN on intertidal species, such as *G. laevisfrons*, and the
331 implications for communities and coastal ecosystems as a whole. We specifically call
332 for further studies assessing the relationship between *G. laevisfrons* activity level and its
333 consumption of prey in rocky pools, and likewise, on studies assessing mortality risk
334 due to increased exposure to its own predators. Tradeoffs between consumption of prey
335 and risk of mortality due to visual predators are likely to be modified by their exposure
336 to ALAN, and warrant further research.

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530 Tables

531 Table1. Probability and frequency of activity of fish in response to the treatment
 532 (control and ALAN), the hour of the day and the days of the experiment. It is shown a
 533 likelihood-ratio test of dropping each term of the saturated models (i.e. models
 534 containing all terms).

A) Probability of activity				
Term	Df	AIC	LRT	Pr(Chi)
<i>None</i>		2932.4		
Treatment	1	3010.3	59.14	< 0.001
cs(Hour)	4.00	3027.1	81.92	< 0.001
Day	1	3007	55.80	< 0.001
Treatment x cs(Hour)	11.17	3014.2	104.17	< 0.001
Treatment x Day	1	2945.6	15.30	0.001
B) Frequency of activity				
Term	Df	AIC	LRT	Pr(Chi)
<i>None</i>		17114		
Treatment	1	17889	416.87	< 0.001

cs(Hour)	4	17784	318.4	<0.001
Day	1	17472	0.47	0.4911
Treatment x cs(Hour)	16.78	17718	636.83	<0.001
Treatment x Day	1	17162	51.96	<0.001

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536 AIC; Akaike's Information Criterio, LRT; likelihood ratio test, cs; cubic smoothing
537 splines, x; indicates interaction between predictive variables. The AIC values shows,
538 how much worsens the model became when each term is dropped (i.e. greater AIC
539 values).

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545 Figure Index

546 Figure 1. Fish activity registered during ten days in control (dark:light photoperiod
547 12:12) and fish exposed to ALAN. Data includes the probability (at least one pass
548 across the refuge per 10 minutes) and the frequency of activity (number of pass across
549 refuge per 10 minutes) in response to the hour of the day (top panels) and the days of
550 the experiment (lower panels). The hour of the day was fitted non-linearly using cubic
551 smoothing splines. Lines and shaded areas shows the mean \pm standard error,
552 respectively. Tide change is showed in top panels in the x axe: dotted line represent
553 hours of low tide and segmented line represent hours of high tide registered for sampled
554 zone.

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556 Figure 2. Oxygen consumption in control (dark:light photoperiod 12:12) and fish
557 exposed to ALAN. Bars indicate +/- 1 standard deviation

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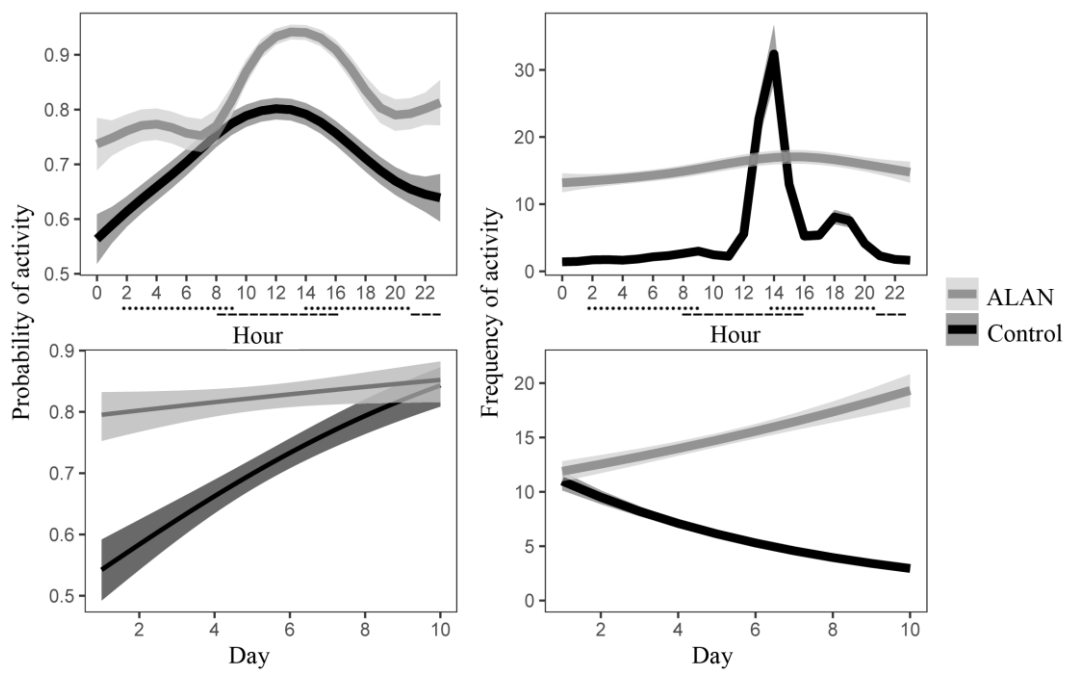
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575 Figure 1



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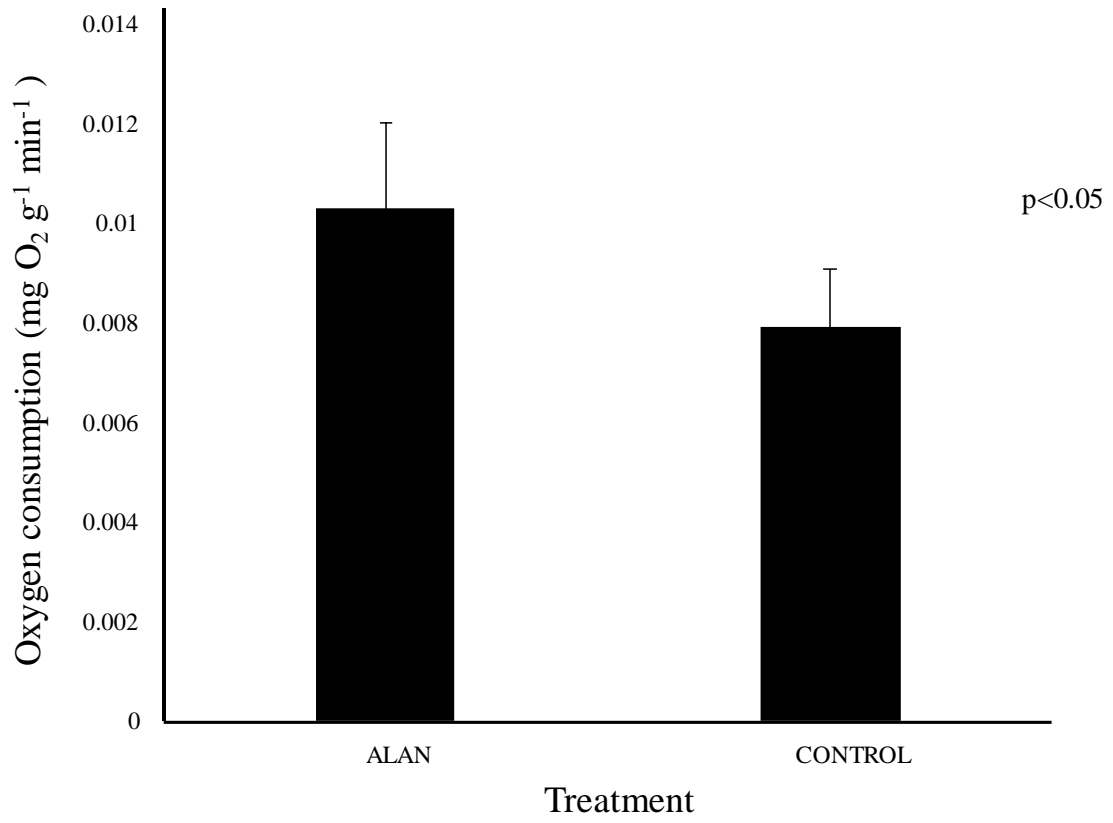
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592 Figure 2

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