ENDOGENOUS CYCLES, ACTIVITY PATTERNS AND ENERGY EXPENDITURE OF AN INTERTIDAL FISH IS MODIFIED BY ARTIFICIAL LIGHT POLLUTION AT NIGHT (ALAN)

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ABSTRACT

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

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

INTRODUCTION

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

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

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

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

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

MATERIAL and METHODS

Juveniles of *G. laevifrons* (N = 30, standard length [L_s] = 4.98 (+/-0.32) s.d. cm, and weight= 1.75(+/-0.36) s.d. [g]) were captured from high intertidal pools located at Punta Choros, Chile (29º14S, 71º27W) during November 2017. All animals were obtained applying a BZ-20 anesthetic (15 mL/100 L of seawater). Collected fish were immediately placed in a cooler (25 l) containing fresh seawater with constant aeration, and transported live to the laboratory for experiments. Once in the laboratory and prior to the start of any experiments, all specimens were acclimatized and fed *ad-libitum* for 10 days with commercial marine flakes (Tetra GmbH, Herrenteich, Germany) in a system with daily renewal of sea water at controlled temperature (17-18 °C) and salinity (35‰), and with a 12h:12h photoperiod. During the acclimation time, fish were exposed to 7222 lux intensity during the 12 h of daylight (laboratory light conditions). Additionally, light intensity was measured in the intertidal pools from where the experimental fish were collected. Light intensity was measured during day (n=5) and night conditions (n=5) using a Luxometer (PCE-L 100, España). These measurements indicated that during daylight hours the intertidal pools were exposed to 7222 (1029.98 s.d.) lux. Meanwhile, during the night hours these sites were exposed to 78.28 (5.4 s.d.) of lux, i.e., the field sites and the fish used in the experimental trials were indeed exposed to ALAN.

ALAN effects on fish activity

To determine if ALAN modified the scototaxis behavior of juvenile *G. laevifrons*, fish were maintained for 10 days under two contrasting light regimes: i) a fish group with a natural 12:12 photoperiod (Control, n= 5, Body size [L_s] =4.85 (0.32 s.d.) cm, weight = 1.64 (0.32 s.d.) [g]) and ii) an ALAN group (Treatment, n= 5, Body size [L_s] = 4.92 (0.34 s.d.) cm, weight = 1.86 (0.41 s.d.) [g]). During daylight hours, both fish groups were exposed to 7222 lux (laboratory light conditions). Fish exposed to ALAN were exposed to 70 lux from dusk to dawn (the level of light exposure currently measured in the area were the fish were collected, knowingly exposed to ALAN), whereas control fish were exposed to natural (dark) conditions experienced in areas located away from artificial sources of light. This light intensity was controlled using a Luxometer (PCE-L 100, España). During the experimental time, fish were fed daily with commercial marine flakes (Tetra GmbH, Herrenteich, Germany), maintained with constant aeration and the seawater was changed every day. To evaluate the impact of ALAN on fish activity, individuals from each group (see above) were placed in a 25 l tank (50x30x20 cm) that included a rocky refuge (14 x 14 x 14 cm, equal to 10% of aquarium volume; see Vargas et al., 2018). Although potential interactions among fish within a particular group were possible (and we expect this to occur in nature), our main goal was to describe the response of each group to the presence or absence of ALAN. Activity was measured as the number of times a fish passed between a dark (refuge) and the zone exposed to light, either natural daylight (both treatments) or artificial light (ALAN treatment). The refuge entrance was a 5 cm gap from the bottom of the tank, that allowed the free fish movement, and was equipped with actographs, an infrared recording system (see Jaramillo et al., 2003), that recorded each occasion a fish passed through the entrance. The capability of actographs to detect fish movements in seawater between the two areas of the aquarium (refuge and light) was previously evaluated using similar fish and experimental set ups.

Oxygen consumption in fish exposed to ALAN

Ten additional fish (different from those used in the activity experiments) were placed individually into large flasks (1000 mL); five fish were exposed to ALAN (Body size [L_s] =4.95 (0.24) cm, weight = 1.68 (0.16) [g] and five were maintained in control conditions (Body size [L_s] =5.13 (0.21) cm, weight = 1.90 (0.23) [g] for 10 days. Fish associated to different treatments had no visual contact between them. At the end of this exposure period, oxygen consumption rates were determined in the morning hours for each fish using a metabolic chamber following the methodology of Chapelle and Peck (1995). To avoid digestive energetic costs, fish were starved for 24 hours prior to all the measurements (Horn et al., 1999; Chabot et al., 2016; Benítez et al., 2017). Briefly, 1000 mL flasks were filled with filtered seawater and fully saturated with oxygen through constant bubbling. Once seawater saturation was reached, the dissolved oxygen concentration (mg O_2 L^{-1}) was measured with an oxygen-meter (OXI-Check, HI9147-04, Hanna Instruments). Each individual was placed for 120 min in the chamber without bubbles. After this period, each metabolic chamber was carefully opened, and the dissolved oxygen concentration was measured (Peck and Veal, 2001). For all the experimental fish, rates of oxygen consumption were measured during similar day hours. Then, oxygen consumption, expressed as mg O_2 g^{-1} min^{-1} (VO_2), was calculated as the difference between the final and initial concentrations of dissolved oxygen in each test. All individuals were subjected to standard measurements of body length (cm) and weight (g) before and after the ALAN exposure, and the variation in weight (Δw) between these time-points was estimated.

Statistical Analysis

Data from the activity experiments were analyzed in R software (R Core Team, 2017) using generalized Additive Models for Location, Scale and Shape (GAMLSS, Rigby and Stasinopoulos, 2005). The GAMLSS model is a general regression model which assumes that the response variable has any parametric distribution (beyond exponential family distribution), including highly skew and/or kurtotic continuous and discrete distributions. In addition, within this framework, all the parameters of the distribution of the response variable (i.e. mu, sigma, tau, nu) can be modeled as linear/non-linear or smooth functions of the explanatory variables. To determine if ALAN modified fish activity, the daily record of fish passes through the refuge entrance over the 10 d period were grouped in ten minutes intervals (n=1438 intervals, for both the control and the ALAN). We modeled; (1) the probability of activity (at least one detected movement per 10 minutes) and (2) the frequency of activity (total number of detected movements per 10 minutes) in response to the treatment, the hour of the day (0-23hrs) and the day of the experiment (1-10). The hour of the day was fitted non-linearly (in order to adjust the circadian rhythm) using cubic smoothing splines function available in the GAMLSS package. We included the interactive effects between the treatment (as a factor) and the hour of the day (non-linearly) and the day of the experiment (linearly). For the probability model we used a binomial error distribution and a logit link function and for the frequency model we used a negative binomial error distribution and a log link. The negative binomial distribution is a two parameter distribution (*mu*, *sigma*), suited to dealing with count data, which usually present over-dispersion (Zuur et al., 2009, O'Hara and Kotze, 2010). For both models an "anova-like" likelihood ratio test was applied. Models selection and diagnostics were based on the AIC, BIC and residuals plots available in the GAMLSS package (Rigby and Stasinopoulos, 2005). Figures were produced using the ggplot2 package (Wickham, 2009) in R.

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

RESULTS

For the activity experiment, we found increased activity (P<0.0001, Table 1) associated to ALAN, the hour of the day (fitted with cubic smoothing splines) and their interaction: we recorded significant changes on (1) the probability (at least one pass across the refuge per 10 minutes) and (2) the frequency of activity of fish (number of pass across refuge entrance per 10 minutes). Under control conditions fish had a clear circadian rhythm (Figure 1) with a mean of 71% probability and 6 pass across refuge throughout the day. The highest probability and frequency of activity were observed between 12:00-14:00 h (mean of 83% probability and 23 pass across the refuge). Meanwhile, fish exposed to ALAN showed a significant increase of activity, passing constantly across the refuge entrance with a mean of 83% probability and 15 pass throughout the whole day cycle (up to 93% of probability and 18 pass of activity at 13:00 h, Figure 1). In addition, we found a significant interaction between the days (linearly) and the treatment (i.e. different slopes) for the probability and frequency of activity of fish ($P<0.0001$, Table 1, Figure 1). At control conditions fish showed an increase in the probability of activity (from ~55% to 84%), but with constantly lower frequency throughout the ten days (i.e. fish did pass across the refuge but fewer times). Conversely, fish exposed to ALAN presented a significant increase in both, the probability and frequency of activity throughout the ten days of the experiment (Figure 1).

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

DISCUSSION

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

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

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

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

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

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

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

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Tables

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

A) Probability of activity				
Term	Df	AIC	LRT	Pr(Chi)
None		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)
None		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**

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

Figure Index

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

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

Figure 1

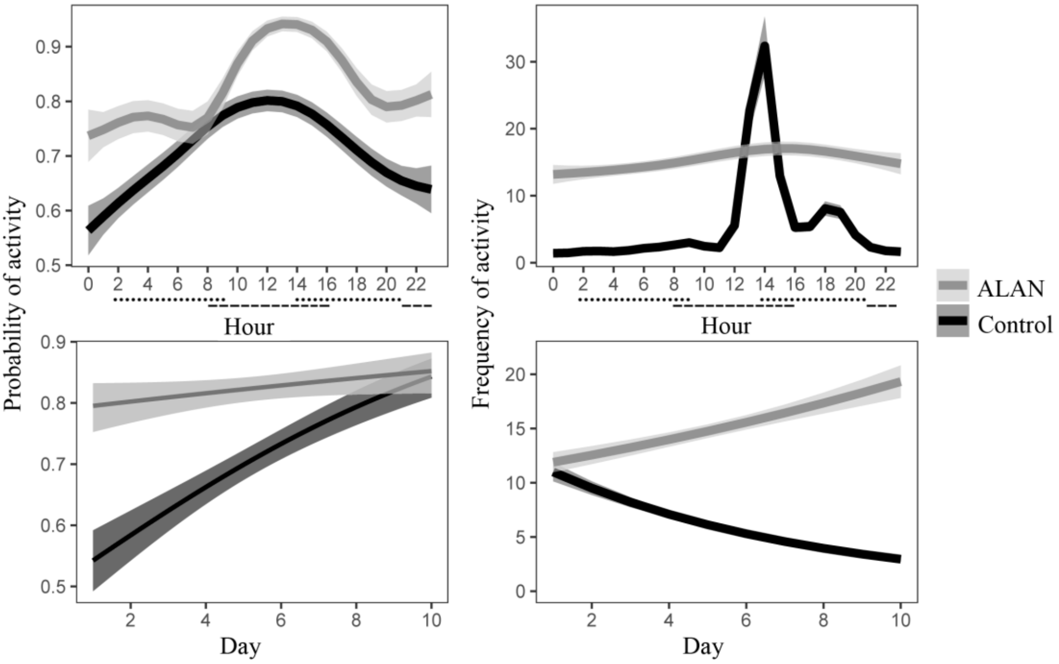
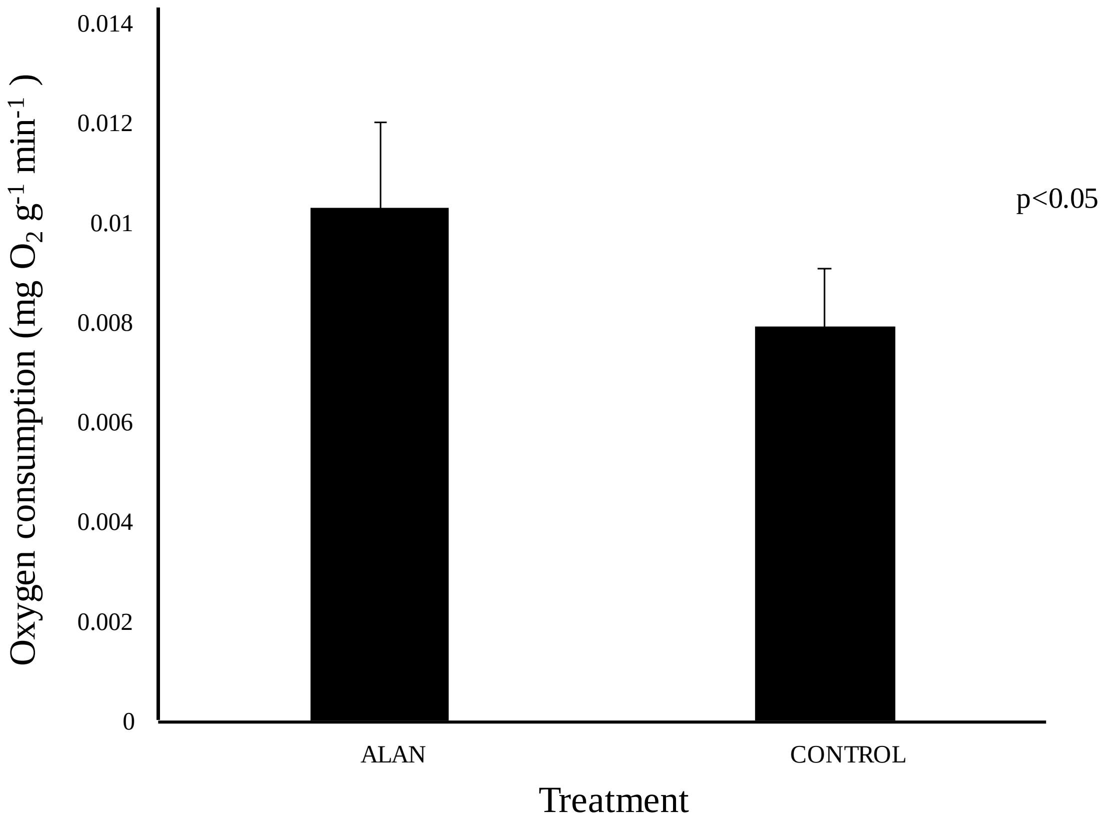

Probability of activity
0.5
0.6
0.7
0.8
0.9
0
2
4
6
8
10
12
14
16
18
20
22
Hour
Frequency of activity
0
10
20
30
ALAN
Control
Day
2
4
6
8
10
0
5
10
15
20

Figure 2


Oxygen consumption (mg O_2 g^{-1} min^{-1})
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
p<0.05
ALAN
CONTROL
Treatment