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Research Article

The effects of artificial light on the behavior of Eastern Mosquitofish (*Gambusia holbrooki)* in Iran

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Abstract

Around 83 percent of the world's population live in light-polluted areas, of which 40 percent live in places that are continuously lit by “ALAN (Artificial Light at Night)”. The light pollution caused by “ALAN” is growing day by day. Thus, the risk of environmental damage is growing in aquatic areas by increasing the “ALAN”. In this way, this research investigates the artificial light at night’s a “ALAN” effect on Gambusia Holbrooke “mosquitofish” in Iran. To obtain the ALAN data, we relied on the information from the operational linear satellite scanning data related to the Metrological Defense Satellite Program (DMSP/OLS). the data provided by the DMSP satellite belonged to 1992 and 2013 as images. The images were clipped by the geographical information systems (GIS), and the images were classified into five classes based on the light pollution intensity. Afterwards, the changes in light pollution by area were calculated and reported. The aim of this research was to examine the effect of ALAN on the *Gambusia holbrooki*. Two types of treatments were provided for *G. holbrooki*: control and ALAN treatments. In both treatments, fish were kept for 60 days, and after that period, their shoaling and hiding behaviors were examined and recorded by a web camera. The results indicated that by increasing the ALAN, especially near the coastlines, the risk of harmful effects on *G. holbrooki* could be increased. The findings suggested that the ALAN might influence *G. holbrooki* behavior. The shoaling behavior of the ALAN treatments samples revealed that the time spent shoaling individually at night and during the day was decreased. As a result, in the hiding behavior experiment, *G. holbrooki* moved less at night after ALAN exposure but did not hide, indicating that the fish are unlikely to swim away or hide when exposed to light at night. As a result, they may be more vulnerable to predation.

Keywords: DMSP, GIS, ALAN, Iran, Mosquitofish

Introduction

The advancement of electrical lighting technologies has changed human communities, extending the time available for both work and leisure (Gaston et al. 2015). Large regions of the Earth are now experiencing an illumination differing from regular patterns in terms of spacing, intensity, and spectrum. In line with human settlements, transport networks, and manufacturing, the natural night-time climate has also changed deeply (Gaston et al., 2013) (Gaston et al., 2015). The increased unplanned artificial lightning is generally considered a type of light pollution (Lo 2002). Some studies illustrate that light pollution can be considered in the presence of heavy artificial light during the nighttime, adversely affecting the environment and humans (Raap et al., 2015). Light disturbance affects the physiological activity of many insects, birds, and mammals. Even an insignificant amount of artificial light can disrupt their natural behaviors (Pun et al., 2012). Anthropogenic disturbances and intensified human use of natural resources lead to biodiversity destruction and modification and climate change (Dudgeon et al., 2006) (Ellis 2011). A class of anthropogenic emissions that alters the natural light and dark cycle of the environment is “artificial light at night” (ALAN) (Swaddle et al., 2015). The “ALAN” has caused an anthropogenic pressure on natural biological systems, because firstly such systems are most commonly organized by light, specifically by daily and seasonal light-dark cycles (Bradshaw and Holzapfel 2010). Secondly, given the seasonal periods of light and darkness, there are no natural analogues to the shape, extent, distribution, timing, or pace of the spread of artificial illumination on any timeline (Gaston et al., 2015).

As global urbanization is dramatically increased, “ALAN” is becoming so prevalent that 83 percent of the world's population lives in light-polluted areas, of which 40 percent live in places that are continuously lit by “ALAN” (Swaddle et al., 2015) (Falchi et al., 2016). In Iran, the light pollution caused by “ALAN” is growing day by day because the rate of migration to large cities is increasing due to the better social services and more job opportunities that these cities provide in comparison with small cities. Thus, it causes an unbalanced distribution of the population and results in the extreme growth of electricity consumption (Brahmandzadeh and Rezaei Ghahroudy 2014). Every major city in Iran has been exposed to the threat of light pollution due to a lack of legal provisions on light pollution (Salehipour 2020). Thus, the risk of environmental damage is growing in aquatic areas by increasing the “ALAN” in Iran. Furthermore, the shipments, have been increasing in Iran in the last decades; thus, they can affect aquatic species through their “ALAN” pollution.

Aquatic species are affected by “ALAN” because they are affected by photoperiod during life history, including reproduction, growth, development, and behavior (Downing and Litvak 2002). Among the restricted studies of “ALAN” on aquatic ecosystems and aquatic species reproduction, there is a knowledge gap regarding the impact of “ALAN” on some fish species in terms of increasing their activity, changing their shoaling behavior, and spending more time in open (riskier) areas, physiology, and ecology (Miner et al., 2021). Mosquitofish, *Gambusia. holbrooki (G. holbrooki)* is one of the species frequently found in freshwater all around the world, in Iran an exotic species introduced to all basins (Fig. 1). Males are translucent grey to light olive with a blue, sheen on the sides and percale. Iris with a purple sheen. Females with a large, triangular, black blotch on the lower flank, behind the pectoral fin (gravid spot). Peritoneum black. Males recognized by anal fin rays modified into an elongate gonopodium for intromittent fertilization. Inhabits clear and weedy streams and ditches, margins of rivers and lakes and brackish lagoons, over mud or sand bottoms. Maximum age 4 years and total length 63 mm in females and 45 mm in males. Survive in salinities up to 10‰, temperatures of 0.5-42°C, pH=4.46-10.2 and DO levels as low as 0.2 mg/l. Maturity in 2-3 months at 16 mm total length. About 17 days after fertilization female gives birth to as many as 428 live fish over a period of about 1 month. Eggs up to 1.8 mm in diameter and embryos about 6-8 mm at birth. Sperm is transferred in a spermatophore by several males and can be stored for up to 10 months. Omnivores, food includes aquatic and terrestrial insects, and also filamentous algae and worms. Females cannibalize larvae (Keivany and Nasri 1395). Mosquitofish has an important effect on the environment. It acts as an ecological and biological agent for eliminating *Anopheles spp* and controlling the mosquito population (Pyke 2008) (Pyke 2005).

Figure 1. Male and female *G. holbrooki* and the map of distribution in Iran (Keivany and Nasri 1395).

Studying light pollution based on field methods is costly and it is problematic to compare them with timely data. In contrast with other methods such as (ground observation technologies using remote sensing), night light remote sensing technology enables quick access to the night light image on a local and global scale (Cinzano et al., 2000), The database Operational Linear Scanning Satellite Data for the Meteorological Defense Satellite Program (DMSP / OLS) contains a wide range of data, from daily data to Stable Light data throughout time. DMSP / OLS satellite night light images have been widely utilized all over the world to save energy and electricity (He et al., 2014), monitor human settlements (Division et al., 1997), Socio-economic activities (Sutton and Costanza 2002), Estimation of urban population (Dobson et al., 2019) and population density (Sutton et al., 1997).

The aim of this study was to find out the “ALAN” growth between 1992, and 2013; also, the possible catastrophic effects of “ALAN” on “*G. holbrooki*” behavior. These investigations were approved by monitoring the “ALAN” of Iran and analyzing the *G. holbrooki* shoaling and hiding behavior under “ALAN” and normal conditions at the laboratory. There are no studies about the effect of “ALAN” on Iran’s aquatic environment. Thus, this problem needs more attention due to the possible environmental damage.

Material and methods

Monitoring the Light at Night

Iran is located between 25°03´ to 39° 47´ north latitude and 44° 05° to 63° 18 minutes’ east longitudes, with an area of about 1,648,000 km. The light at night “ALAN” of the year 1992, and 2013 was investigated by the data obtained from the DMSP/OLSP satellite with a resolution of

850 meters, which was provided by two satellites (F10, and F18). The GIS software was used to clip images according to the national borders; For better analysis of images, we classified the night brightness of images into five classes. The brightness in DMSP/OLS images is extracted from digital numbers (DN), and all pixels of the images are in the range of zero to 63. Classes with "DN" of 0-3.4 are divided as areas with very low luminosity, 3.4-12.3 areas with low luminosity, 12.3-26.1 areas with medium luminosity, 26.1-45.4 areas with high luminosity, 45.4-63 areas with very high luminosity. to illustrate the changes in light pollution in 1992 and 2013, the percentage of the area was calculated in all classes based on "DN". The high and very high luminosity classes were set on as light pollution indicators. This data would help us to indicate the possible effect of light pollution on "*G. holbrooki*" by modeling the highly polluted areas.

Collection and Sustaining Mosquitofish (*Gambusia. holbrooki*) and the ALAN design

In this research, *G. holbrooki* collected from the Caspian Sea coastlines in Gilan Province were collected from the low water levels shores during the breeding season (April-October). The collected fish (N=100 female) are placed in two tankers separately. Afterwards, they were placed in two laboratory aquariums (volume of 50 L) filled with dechlorinated water and gravel at the bottom (Fig. 2). We fed them ad libitum with ISO flake food daily and frozen bloodworms and brine shrimps three times a week. We prepared four aquariums, each of which contains N=50 females. Two of them with N=50 females were used in the control treatment of 14:10 hours of light-dark cycles, and the two other aquariums were used in (the ALAN) experimental treatment of 14:10 hours of light-dark cycles (i.e., 10 hours of ALAN) for a total of 10 replicates in each treatment for 50-60 days (Miner et al. 2021).

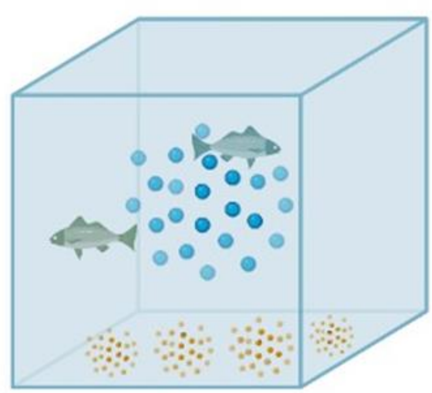

Figure 2. The laboratory aquarium (Created with BioRender.com)

The light levels at night on Caspian Sea shores had a great difference, we used a lux meter (Extech LT 45) to measure the light levels at our fish selection location; the night level was almost 250 lux near the crowded places, ports and harbors. In direct sunlight, the full daylight levels can reach up to 25,000 lux, with illuminances up to 100,000 lux. To provide the ALAN environment conditions in the laboratory, white LED lamps were used at 250 lux, and the daylight was stimulated by white LED lights at 850 lux, both were bought from (keyhan.co). Because lights from bridges, highways, boardwalks, and residences near water sources are ubiquitous, our measurements show the amount of light reaching the water where organisms can be located. The LED lamps are hung 50 cm above the aquariums, and all the aquariums are monitored every day (Fig. 3).

Figure 3. ALAN condition

Hiding behavior

In this part of the experiment, a 30 L aquarium was filled with deionized water (5 L) and water temperature was maintained between 23°C and 25°C. Then, a PVC pipe (45 cm long and opaque) was used as a hiding place, and another smaller pipe (30 cm long) was used for hiding out of view. Afterwards, N=25 female animals from both treatments were selected and placed into the aquarium after day 50 for the hiding test. After day 50, we took the fish from the ALAN treatment and placed them individually in the testing tank under an ALAN illumination, where they acclimated for 10-12 minutes under a transparent, plastic 1 L container. We used black paper to cover the sides of the tanks in case the fish were still distracted by their surroundings at night. We removed the transparent container after acclimating and began recording for 10-12 minutes.

Shoaling behavior

To investigate the shoaling behavior in this study, N= 25 female animals from both treatments are selected after day 60, and placed into five containers filled with deionized water (2 L) in groups of five to observe the shoaling behavior. In this study, the shoal size was used based on Tobler and Schlupp's previous study of Poeciliidae (Tobler and Schlupp, 2008). The groups of fish stay within the containers for 45 min, and their behaviors are recorded for analyzing shoaling (keeping a 2 cm distance from each other). We then recorded the shoaling behavior for almost 24h for logistical purposes, the average distance moved (cm), resting time, and shoaling (during day 14 h and night 10 h) were analyzed.

Results

Monitoring the ALAN

In this research, the area and the percentage of light pollution therefore the changes of the luminosity classes were measured to monitor the light pollution and find the trend of light pollution changes. Increasing light pollution has a possible effect on *G. holbrooki's* life.

In 1992, just 3.87% of the area in Iran has been classified as low to high brightness areas. Table 1 shows that classification, according to calculations made on night light images of this class (table. 1.), 1362469 (96.1%) belong to a region with very low luminosity, 32528 (2.29%) to the area with low luminosity, 12517 (0.88%) to the area with moderate luminosity, 5516 (0.38%) to the area with high luminosity and 4676 (0.32%) very high luminosity area. The results show that in 2013, the area of the region with very low luminosity was 1308182 (92.27 %), the region with low luminosity was 71711 (5.05%), the region with average luminosity was 19395 (1.36%), an area with a high luminosity was 8670 (0.61%) and an area with a high luminosity was 9743 (0.68%).

The dramatic growth in light pollution in Iran may be seen in the increase in the size and percentage of regions with very illumination (also known as areas with a very high degree of artificial light supply), (Fig. 4). According to data extracted from DMSP / OLS night sky satellite data, night light increased over the past years in Iran and the luminosity has increased in all classes (very low, low, moderate, high and very high). However, an increasing trend in night light between 1992 and 2013 has not been the same in all provinces of the country, but that is not the focus of this research to calculate and compare the night light changes in the scale of the province. The point of discovering night light is to describe the increasing process of night light in Iran to better understanding of possible night light effect on aquatic species. Thus, by relying on the changes in night light between 1992 and 2013 all around Iran the eastern mosquitofish *G. holbrooki* is under the effect of "ALAN".

Table 1. Changes in the amount of night light in Iran between 1992 and 2013

Class range	Area 1992 km^2	%Area 1992 km^2	Area 2013 km^2	%Area 2013 km^2	Changes area (km^2) between 1992-2013
0-3.4	1362469	96.1	1308182	92.27	54287
3.4-12.3	32528	2.29	71711	5.05	39183
12.3-26.1	12517	0.88	19395	1.36	6878
26.1-45.4	5516	0.38	8670	0.61	3154
45.4-63	4676	0.32	9743	0.68	5067

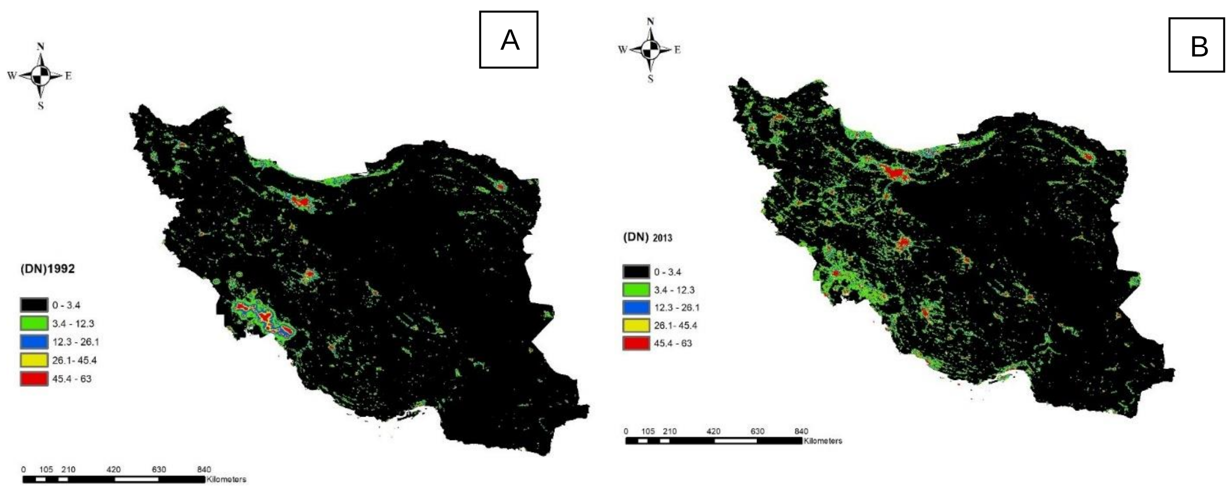

Figure 4. Classified night light distribution in Iran A:1992, B:2013.

Hiding behavior

After the hiding behavior test, the obtained data showed that the female *G. holbrooki* from the ALAN treatment spent more time resting compared to the fish in control treatments (LM: treatment: $F_{1,25} = 7.025$, $P = 0.018$, Table 2, and Fig. 5).

Table 2. Results of the general linear model testing of the ALAN effect on female G. holbrooki

		Estimate ± SE	*t-value*	*df*	*P-value*	*Lower 95% Cl*	*Upper 95% Cl*
Resting	Intercept	216.2 ± 152.3	1.58	25	0.19	-100	586.23
	SL (mm)	0.03 ± 4.56	0.0125	25	0.96	-91	10.08
	control	-49.8 ± 18.35	-3.012	25	0.02	-12	-12.56
	SL*control	-3.98 ± 4.21	-0.89	25	0.36	-18.5	4.89
Hiding	Intercept	-48.36 ±138.13	-0.35	25	0.69	-365.2	256.21
	SL (mm)	2.97 ± 3.98	0.65	25	0.52	-6.032	12.89
	control	3.12 ± 13.58	0.19	25	0.88	-30.65	38.21
	SL*control	-0.45 ± 4.15	-0.16	25	0.96	-9.12	6.51

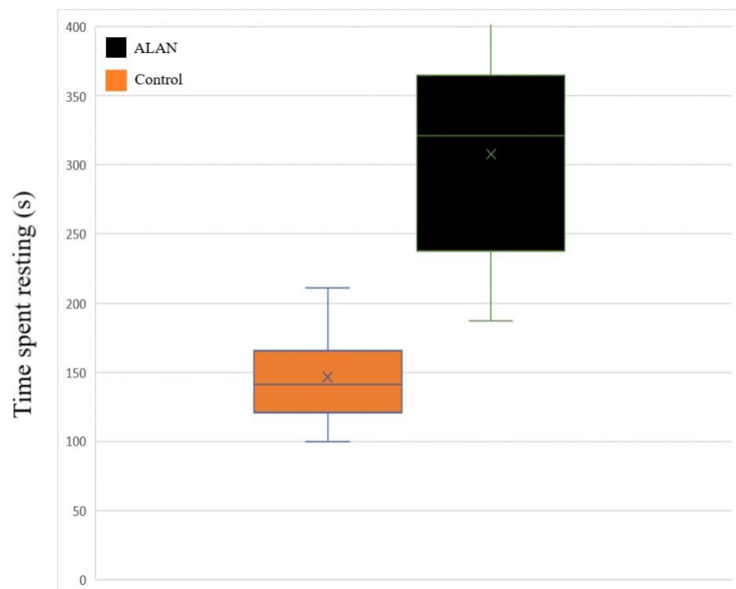

Figure 5. The mean time spent on resting by female *G. holbrooki* in control and ALAN treatments at the laboratory conditions.

Shoaling behavior

The results of the shoaling behavior test indicated that in the ALAN conditions, female *G. holbrooki* showed a significant increase in shoaling time during the day compared to the control treatment (Repeated measure ANOVA: $F_{1,25} = 6.81$, $P = 0.038$). Furthermore, the samples from both conditions showed significant movements by keeping a greater distance (in cm) during the daytime compared to the nighttime ($F_{1,25} = 9.02$, $P = 0.023$) (Fig. 6).

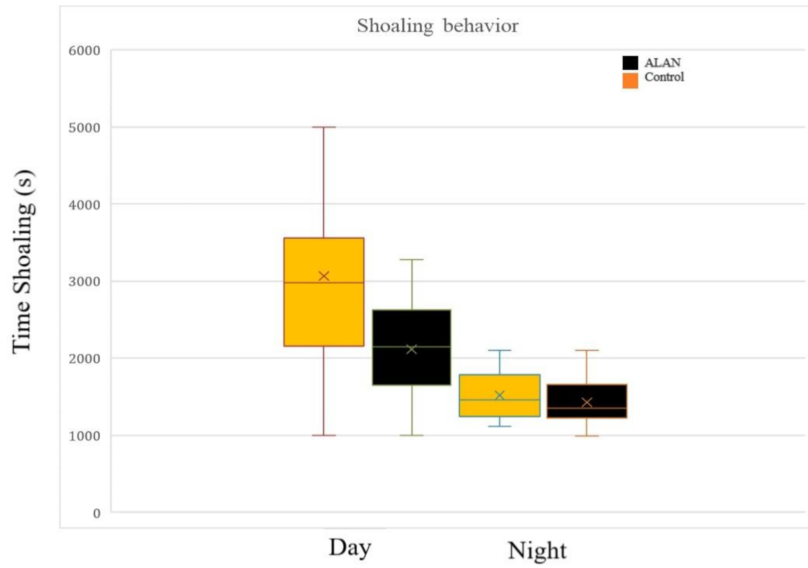

Figure 6. Time spent shoaling (s) in control and ALAN by *G. holbrooki*

Discussion

After 60 days of exposure to "ALAN" in the lab, *G. holbrooki* movement was generally reduced at night. After being exposed to "ALAN," *G. holbrooki* activity was reduced individually at night and in shoaling throughout the day. From an important point of view, *G. holbrooki* may be more vulnerable to predation since they did not spend more time hiding at night. This might lead to a better understanding of the mechanism underlying the previous discovery that fish exposed to ALAN reported greater predation rates (O'Connor et al., 2019). Recent findings imply that the effects of ALAN differ depending on the species. There are several studies show the effect of "ALAN" on various species, some of them describe that the "ALAN" could increase fish activity (Foster et al., 2016), (Kurvers et al., 2018), anoles (Dananay and Benard 2018) and zebra finches (Batra et al., 2019) after exposure to "ALAN". Shoaling is an essential advantage because it protects fish from predation. As a result, foraging becomes more efficient. Unfortunately, female *G. holbrooki* spent less time shoaling throughout the day following the same lab exposure to

"ALAN." As a result of the absence of shoaling, they are more vulnerable to predators and have a harder time finding food (Pitcher 1986) (LALAND and WILLIAMS 1997). There was a definite demonstration of diel shoaling activity in the control group, with fish being more active and swimming in closer proximity during the day than at night; this activity disappeared after exposure to "ALAN". At least, *G. holbrooki* in the "ALAN" situation showed us less shoaling during the day and decreasing movement at night, this behavior could cause a risk of being more susceptible to predation.

G. holbrooki is highly distributed and can be found in almost developed urban settings in Iran (Fig.1), in this research we used the maximum levels of light that we found in the coastline of the Caspian Sea (250 lux). This species lives in harsh light pollution conditions, and of course tends to be found in low-depth, slow-moving water (Belk and Lydeard, 1994). *G. holbrooki* moved less at night following ALAN exposure and did not hide, showing that the fish is unlikely to swim away from or hide when exposed to light at night.

Conclusion

In this research, the light pollution-light at night "ALAN"- in Iran and the harmful effects of ALAN on nature (in this case, *G. holbrooki*) were investigated. The growing population has led to extensive urbanization, and thereby, day-by-day increasing ALAN in the last decades. The ALAN can affect human life and also mammals, birds, and aquatic species. The subject of our study is *G. holbrooki*, which is one of the ecologically important aquatic species frequently found in freshwaters all around the world. Mosquitofish has an important effect on the environment. It acts as an ecological and biological agent for eliminating *Anopheles spp* and controlling the mosquito's population. The ALAN intensity all over Iran was investigated by using the DMSP/OLS satellite images, the obtained images were clipped by the GIS program, which were then classified into five categories based on the ALAN intensity. Furthermore, in these experiments, the ALAN effects on shoaling and hiding behavior of *G. holbrooki* were investigated based on two "ALAN" and control treatments. The findings suggested that the ALAN might influence the behavior of *G. holbrooki*. The shoaling behavior of the samples from ALAN treatments revealed that the time spent shoaling individually at night and during the day was decreased. As a result, in the hiding behavior experiment, *G. holbrooki* moved less at night after ALAN exposure and did not hide, indicating that the fish are unlikely to swim away from or hide when exposed to light at night. As a result, they might be more vulnerable to predation. The future risk would be the investigation of the ALAN effect on the *G. holbrooki* breeding mechanism; it depends on several aspects such as increasing the population and transportation, ALAN etc.

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