



## Does coastal light pollution alter the nocturnal behavior and blood physiology of juvenile bonefish (*Albula vulpes*)?

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**ABSTRACT.**—Light pollution is a prevalent, but often overlooked, ecological concern in a variety of ecosystems. Marine environments are subjected to artificial lighting from coastal development, in addition to offshore sources, such as fishing vessels, oil platforms and cruise ships. Fish species that rely on nearshore habitats are most significantly impacted by coastal light pollution, as they are often limited to nearshore habitats due to predation risk in deeper offshore waters, particularly as juveniles. Juvenile bonefish [*Albula vulpes* (Linnaeus, 1758)] inhabit the nearshore environment, and are therefore exposed to coastal lighting and other watershed development impacts. Here, we assessed juvenile bonefish behavior and physiology in the presence of two common light sources: constant street lighting (high pressure sodium) and intermittent car headlights (H4 halogen). The behavioral responses were compared with a night and day control, whereas physiology was compared only with a night control. Each behavioral trial had two time periods: light and recovery (2 hrs each). Physiology (blood glucose and whole body cortisol) was assessed after an overnight 8-hr exposure. The results suggest that there is no effect of light pollution on the swimming behavior or whole body cortisol of juvenile bonefish, but that both forms of light pollution resulted in elevated blood glucose concentrations (a simple stress indicator) relative to controls, with constant light glucose levels being significantly higher. Further research is needed to understand the ecological consequences of light pollution on bonefish and other coastal marine fish using additional endpoints, assessing fish over longer time periods, and ideally combining data from the laboratory and the field.

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In many respects, artificial lighting has been beneficial to humanity, particularly with regards to improving security and facilitating modern urban lifestyles (Jakle 2001, Doll et al. 2006). However, these benefits have come with a number of detrimental impacts to the natural world (Vitousek et al. 1997, Crutzen and Stoermer 2000). Most, if not all, metazoan species occurring outside of the abyssal zones of the oceans have evolved in the presence of natural diel variation, with the availability of light in the form of solar radiation (Heldmaier et al. 1989, Menaker et al. 1997, Panda et al. 2002, Laura Idda et al. 2012). This variation forms the basis of neuroendocrine-based circadian rhythms (see Kopperud and Grace 2017) that influence multi-scale phenologies, including metabolism, growth, and behavior (Dunlap 1999, Dodd et al. 2005, Ko and Takahashi 2006, Bass and Takahashi 2010). While more insidious pollutants, such as those arising from the combustion of various materials (i.e., coal, wood, or oil), have been on the scientific radar since the 1840s (Macfarlane et al. 1841), artificial lighting has only recently received the attention of the scientific community (Vinogradova et al. 2009). Ecological light pollution (described in Longcore and Rich 2004) arises from the excessive and inefficient use of artificial lighting, a phenomenon that is increasing at a rate of approximately 6% annually on a global scale (Hölker et al. 2010). Consequences of ecological light pollution on animals have been measured across taxa and scales ranging from individuals to entire ecosystems, with disruptions in spatial orientation, foraging patterns, migration, reproduction, predation, and communication (all reviewed in Longcore and Rich 2004). Despite this growing body of evidence, light remains an often-overlooked form of pollution (Rudd and Lawton 2013).

While some research efforts have focused on terrestrial ecosystems, freshwater and marine environments have not received the same level of attention (Depledge et al. 2010, Davies et al. 2014). Nearshore freshwater and marine species are exposed to artificial lighting due to coastal development, while some offshore species may be exposed to lighting on bridges, oil platforms, fishing vessels, and cruise ships (Hölker et al. 2010, Davies et al. 2014). Species relying on nearshore habitats get very little respite from lighting caused by coastal development, particularly in places like the southeastern coast of Florida, where essentially all available shoreline has been developed (Finkl and Charlier 2003). In addition, tropical and subtropical species may be more susceptible to alterations in ambient light, as these latitudes experience relatively little variation in diel light cycles throughout the year (Gliwicz 1999). Thus, light pollution along coastlines may leave the juvenile life stage of fishes vulnerable, as they cannot readily relocate and may experience elevated predation risk if they attempt to leave their nearshore nurseries (Werner et al. 1983, Laegdsgaard and Johnson 2001). Juvenile bonefish [*Albula vulpes* (Linnaeus, 1758)] are one such species that rely on nearshore habitats, and may be impacted by coastal lighting and development. In some regions of their range, adult bonefish populations are in decline, and some populations (e.g., Florida) have been designated as “near-threatened” under International Union for Conservation of Nature criteria (Adams et al. 2014). In addition, exhaustive efforts to locate juvenile bonefish along developed coastlines have been met with little success over the past two decades, especially in developed areas of Florida (Adams et al. 2008, Snodgrass et al. 2008), although they are readily found along less-developed shorelines in some regions of The Bahamas. Bonefish are a relatively long-lived species, with some adults having reached approximately 20 to 25 yrs of age (Larkin 2011; CR Haak, unpubl data); therefore, if there is a threat to or

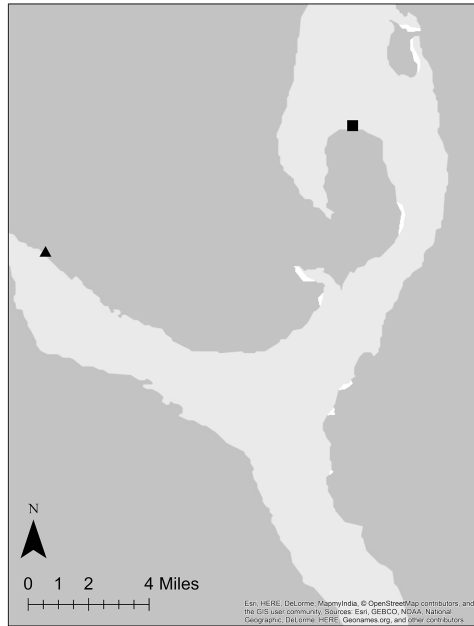


Figure 1. A map of southern Eleuthera, The Bahamas (developed using Google Earth Pro). The square denotes the capture and collection site of juvenile bonefish in Rock Sound, and the triangle denotes the location of the Cape Eleuthera Institute wet laboratory.

a decline in the juvenile population, it may not be evident in the adult population for several decades.

The objective of our study was to determine the acute effects of ecologically relevant light pollution on juvenile bonefish behavior and physiology. We examined juvenile bonefish behavior when exposed to light pollution, and measured blood glucose levels (a simple stress indicator; Barton 2002) post-exposure, and evaluated whole body cortisol. Wild-caught juvenile bonefish were exposed to two ecologically relevant light treatments: constant simulated street lighting (high-pressure sodium) and intermittent car headlights (H4 halogen), with responses compared to fish exposed to control conditions. Our work provides insights into the potential effects of light pollution on the behavior and physiology of juvenile bonefish, and may act as a model for other fish species utilizing similar habitat.

## METHODS

**CAPTURE AND TRANSPORT.**—The present study was conducted during April and August of 2015 in southern Eleuthera, The Bahamas ( $24^{\circ}50'05''\text{N}$ ,  $76^{\circ}20'32''\text{W}$ ), at the Cape Eleuthera Institute (CEI) (Fig. 1). A shallow nearshore area ( $<1$  m depth) was spot-seined (15.25 m length  $\times$  1.22 m height, 0.6 cm mesh size) for juvenile bonefish in Rock Sound (Fig. 1). Upon capture, juvenile bonefish were transferred from the seine to a flow-through net pen (1.50 m length  $\times$  0.7 m width  $\times$  1.20 m height) to keep fish in well-oxygenated and ambient water while seining continued for more individuals. Fish were then transferred to large coolers (90 cm length  $\times$  35 cm width  $\times$  20 cm height; 63 L) on the boat for transportation, with a maximum density of 10 fish per cooler. Approximately  $1/3$  volume water changes occurred every 10 min over

the course of the 30-min boat transport. Upon arrival at the CEI wet lab facility, fish were transferred to circular tanks (155 cm diameter  $\times$  25 cm height; 472 L), which were aerated and continuously supplied with fresh seawater (10 L min<sup>-1</sup>) at ambient water temperatures [27.8 (SD 2.1) °C]; densities never exceeded 20 fish per tank. Fish were collected using bonefish handling techniques (Murchie et al. 2009), but adapted for this more sensitive life stage (Laegdsgaard and Johnson 2001). To this end, when a fish was transferred, it was gathered from the water in a container (15 cm diameter  $\times$  20 cm height; 3.5 L) and gently poured into the new area of containment (e.g., from net to cooler). This technique ensured the fish were never exposed to air (Cook et al. 2015) and minimized abrasion caused by nets and handling (Murchie et al. 2009). In April, 20 juvenile bonefish [mean = 109 (SD 6 mm) fork length; 96–116 mm] were collected from Rock Sound for the behavior portion of the study, and 30 juvenile bonefish [mean = 71 (SD 5 mm) fork length; 60–78 mm] were collected from the same location in August for the physiology aspect of the study. Fish were given a minimum of 48 hrs to acclimate to laboratory conditions before experimentation.

**BEHAVIOR TRIALS.**—Each trial consisted of four treatments conducted over 3 d; the four treatments were: (1) control ambient day lighting; (2) control ambient night lighting; (3) constant lighting from a high-pressure sodium bulb (Globe Electric, Montreal, Quebec, Canada; 150 watt bulb), which simulated nearshore street lighting; and (4) intermittent lighting from a H4 halogen car light (Sylvania, Mississauga, Ontario, Canada; H4 bulb), which was meant to simulate passing cars in nearshore environments (light was on 1 min every 10 min using a timer). In this case, “ambient” refers to natural illumination (i.e., night sky or daylight), through the semi-transparent roof of the wet lab. Each juvenile bonefish ( $n = 20$ ) was used in each of the four treatment groups and experienced the treatments in a unique order, with four fish per trial.

Fish were moved from the circular holding tanks to the experimental raceways on the morning of the first day of trials at 08:00 hrs, 14 hrs before experimentation. The experimental set-up included two raceways that were situated in parallel and each raceway was split into two sections yielding four arenas in total (150 cm length  $\times$  62 cm width  $\times$  22 cm height; 205 L; Fig. 2). Sections were separated by black partitions that allowed for water flow-through, but prevented fish seeing other individuals. Each section of the experimental arena had lines on the bottom of the tank every 15 cm to measure the number of lines crossed as a proxy for horizontal distance traveled. The water leading into the raceways was aerated and pumped into a segmented end of the raceway [6 L min<sup>-1</sup>; 26.9 (SD 1.6) °C] to ensure aeration did not disturb the water in the experimental arenas. The high-pressure sodium and H4 halogen lights were mounted 3.5 m above the bottom of the raceways, directly above where the raceways were segmented to ensure no compartment had shadows during light exposure (Fig. 2). The illuminance (lux) was recorded using a lux meter (Dr. Meter®, Hisgadget Inc., Union City, California, United States of America) for both high pressure sodium and H4 halogen light at the surface of the water (48 and 80 lux, respectively) and in a nearshore area that had street and car lighting spill-over (25 and 68 lux, respectively). Ambient night conditions ranged from 0.02 to 1.6 lux. Raceways were located in an open-air wetlab, with a semi-transparent roof, which may have allowed for a small amount of moonlight. During the experiment, moon phase was between the last quarter and the first quarter (<50% full), although many of the nights were overcast.

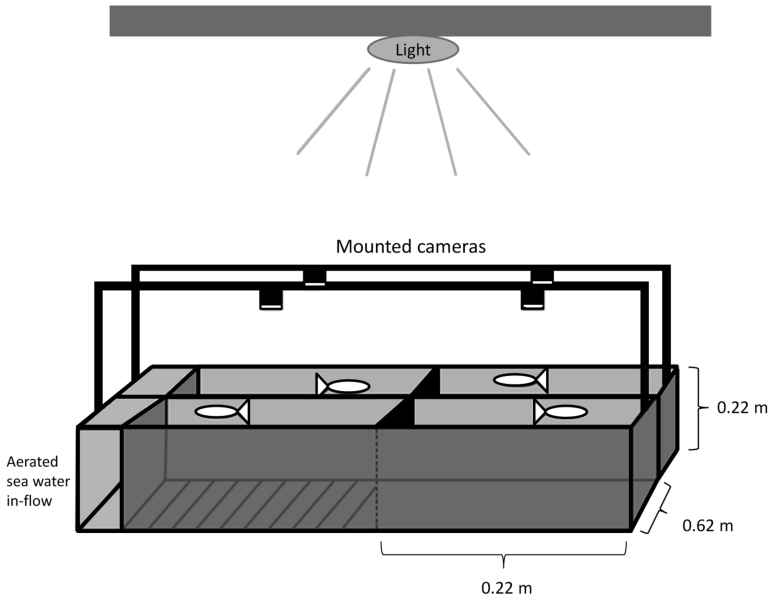


Figure 2. Diagram of the experimental set up to assess the behavioral response of juvenile bonefish to experimental lighting conditions (i.e., constant and intermittent lighting). Cameras were mounted above each individual arena, and each arena was further subdivided on the bottom of the tank with lines to measure horizontal distance traveled.

The light treatments (constant and intermittent) included two phases: first, fish were exposed to artificial light between 22:00 and 00:00 hrs, and then a recovery period ran from 00:00 to 02:00 hrs, where the fish were left in ambient darkness. The night and day controls were also assessed based on two time periods, without the influence of any artificial lighting. Fish were filmed (DVR9-4200 9 Channel 960H Digital Video Recorder and PRO-642 Cameras; Swann Communications U.S.A Inc.; Santa Fe Springs, California, United States of America) from 22:00 to 02:00 hrs for the night controls, constant lighting and intermittent lighting treatments, and 10:00–14:00 hrs for the day controls. While not being filmed, fish were left in a natural photoperiod with ambient night or day sky, as the semi-transparent roof would allow. Fish were fed small shrimp fragments *ad libitum* at 08:00 hrs each morning. After the four fish from the first trial were subjected to each of the four treatments, they were removed and put in a new holding tank, and another four fish were distributed as described above.

**VIDEO ANALYSIS.**—Following the experiment, the videos for each fish were analyzed using JWatcher (Blumstein and Daniel 2007). Videos were analyzed in 10 min segments for each hour of filming. Distance traveled was conservatively estimated using lines crossed as a proxy, and an additional three behaviors were considered: number of approximately 90° turns, number of freeze events (defined as the fish being entirely stationary for >1 s), and number of burst events (sudden, rapid, undirected swimming). These parameters were assessed to determine whether there was evidence for unusual or erratic swimming behavior during either of the light treatments when compared with control or recovery periods.

**PHYSIOLOGY.**—Juvenile bonefish ( $n = 10$  per treatment) were moved from their holding tanks (1.55 m diameter  $\times$  0.25 m height; 472 L) into segmented net pens in the experimental tank (35 cm length  $\times$  30 cm width  $\times$  20 cm height; 21 L; 0.3 cm fine mesh) at 16:00 hrs and left to rest; each treatment occurred on a different day. At 20:00 hrs, 10 fish were subjected to one of three treatments: (1) control ambient night conditions, (2) constant high-pressure sodium lighting, or (3) intermittent H4 halogen lighting (1 min on, 10 min off). Fish were kept in small groups (e.g., 3–4) in the net pens to ensure that all fish were not alerted as they were dip netted for lethal sampling the following morning. The fish were left in their treatment until 04:00 hrs, at which point they were netted out and euthanized using cerebral percussion and immediately ( $<3$  min; Clark et al. 2011) had blood taken from their caudal vasculature to assess blood glucose concentrations ( $\text{mmol L}^{-1}$ ) using a previously validated point-of-care device (ACCU-CHEK glucose meter; Roche Diagnostics; Basel, Switzerland; Stoot et al. 2014).

To measure total body cortisol ( $\text{ng g}^{-1}$ ), whole body samples were frozen at  $-20$  °C during transportation before being relocated to  $-80$  °C for short term storage. Whole body samples were individually ground over liquid nitrogen with a chilled mortar and pestle. Ground samples were weighed and homogenized with 400  $\mu\text{L}$  of homogenizing buffer (80 mM  $\text{Na}_2\text{PO}_4$ , 20 mM  $\text{NaH}_2\text{PO}_4$ , 100 mM NaCl, and 1 mM EDTA) using a hand-held homogenizer (Pellet Pestle Motor, Kimble® Kontes, Vineland, New Jersey, United States of America). The samples were then treated with 600  $\mu\text{L}$  of methanol, vortexed, and incubated at 4 °C in the dark for 60 min. They were then centrifuged for 5 min at 4 °C (3000 g) and flash frozen for 10 min at  $-80$  °C. The supernatant was pipetted into new tubes, and dried with an air stream until only a pellet remained. This process was repeated twice more. The samples were reconstituted in 900  $\mu\text{L}$  of extraction buffer and assayed using a cortisol ELISA kit (#402710, Neogen Corporation, Lansing, Michigan) to yield whole body cortisol.

**STATISTICAL ANALYSIS.**—Each of the four behavioral measures failed to meet the assumptions of normality (Shapiro-Wilk test: all  $P < 0.05$ ), thus the data were rank-transformed and examined with analysis of variance (ANOVA) (Scheirer et al. 1976, Conover and Iman 1981). Each behavioral measure was examined independently in a series of two-way repeated measures ANOVAs with time period (light, recovery) and treatment (four levels, described above) as main factors with an interaction term and individual fish as the repeated measure in ANOVA models. Any behavioral measure found to vary significantly with a main factor had the repeated measure term removed from the model for post hoc analysis using Holm-corrected Tukey's HSD tests. Variance component analysis was conducted to determine how much of the response was due to individual variation and how much was due to treatment effects. All analyses and figures were generated using R version 3.2.3 (R Core Team 2015) and the packages nlme (Pinheiro et al. 2015), ape (Paradis et al. 2004), varComp (Qu et al. 2013), and gplots (Warnes et al. 2015). Statistical analysis was conducted on 16 of the 20 individuals in the experiment.

Measurements of blood glucose concentrations also did not meet the assumptions of normality (Shapiro-Wilk test:  $P < 0.05$ ), in addition to having a small sample size per treatment. Therefore, a non-parametric Kruskal-Wallis test was used to examine the data, with a Nemenyi post hoc test. Although whole body cortisol met the

Table 1. Repeated measures two-way analysis of variance in four behavioral responses in juvenile bonefish (*Abuia vulpes*) during or after (Time) exposure to constant, intermittent light, and a dark control at night and a daytime ambient light control (Treatment). Bold values indicate statistical significance ( $P < 0.05$ ). # is number of events.

Behaviors	Main effects														
	Time			Treatment			Time*Treatment			Repeated measure (Fish ID)					
	F	P	df	F	P	df	F	P	df	F	P	df			
Distance (cm)	1.88	0.174	1, 103	1.40	0.246	3, 103	1.20	0.314	3, 103	0.58	0.458	1, 13	0.50	1, 13	0.492
Turns (#)	0.07	0.799	1, 13	1.13	0.342	3, 103	0.88	0.454	3, 103	0.47	0.506	1, 13	1.85	1, 13	0.197
Freeze (#)	1.62	<b>0.010</b>	1, 103	1.89	0.136	3, 103	1.62	0.188	3, 103	2.79	0.119	1, 13	0.18	1, 13	0.675
Burst swim (#)	12.49	<b>&lt;0.001</b>	1, 13	1.17	0.323	3, 103	1.57	0.201	3, 103	14.34	<b>0.002</b>	1, 13	1.45	1, 13	0.250

Table 2. Variance component analysis expressing the relative contributions (percent) of individual variability and treatment effects on observed behavioral responses of juvenile bonefish (*Albula vulpes*) exposed to constant, intermittent, and no light at night and a daytime ambient light control. # is number of events.

Behaviors	Variance components	
	Individual	Treatments
Distance (cm)	53.9%	46.1%
Turns (#)	46.4%	52.6%
Freeze (#)	37.8%	62.2%
Burst swim (#)	12.1%	87.9%

assumptions of normality, a Kruskal-Wallis test was used to account for the small sample size.

## RESULTS

**BEHAVIOR.**—Juvenile bonefish swimming was a mix of non-directional, random swimming, as well as swimming the perimeter of the arenas; however, neither behavior occurred substantially more than the other. Though not significant, juvenile bonefish appeared to demonstrate slightly lower levels of activity during recovery periods compared to light treatment periods, although this was not the case in the number of approximately 90° turns. There were no significant effects of treatment, time period or individual as a repeated measure on either the distance traveled or number of 90° turns in the repeated measures ANOVAs (all  $P > 0.05$ ; Table 1). Variance component analysis indicated that for distance traveled and number of 90° turns, individual variability was an approximately equal contributor to treatment effects (53.9% and 46.4%, respectively; Table 2).

For the number of freeze events, both the day and night controls froze more frequently than fish in either the constant or intermittent treatments. In all four treatments, fish froze more during the light than recovery periods. The two-way repeated measures ANOVA showed that there was no significant treatment effect; however, there was a significant effect of time period (light vs recovery,  $P < 0.05$ ; Table 1). The freeze responses of individuals did not vary significantly between treatment or time periods (Table 1). Variance component analysis revealed that individual variability accounted for 37.8% of the variation in freeze responses and the treatment effect accounted for 62.2%, suggesting the treatments had slightly more influence on the behavior (Table 2).

The analysis for the burst events revealed similar results to the freeze events. The number of burst events decreased for all treatments from the light to the recovery periods. Treatment did not have a significant effect on burst events, whereas time period did ( $P < 0.001$ , Table 1). In addition, there was a significant effect of time period within individuals ( $P < 0.05$ , Table 1). Individual variability was responsible for 12.1% of the variation in burst responses, while treatment contributed 87.9% (Table 2), indicating that treatment had a much greater effect on this behavioral measure.

**PHYSIOLOGY.**—Blood glucose concentrations ( $\text{mmol L}^{-1}$ ) varied significantly between treatments (Kruskal-Wallis test:  $\chi_{(2)}^2 = 7.5$ ,  $P = 0.02$ ). Mean glucose concentrations increased with increasing levels of artificial light exposure [control = 3.83 (SD



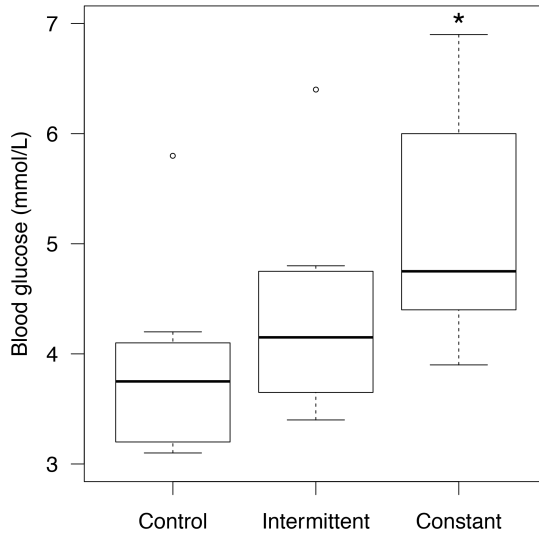


Figure 3. Boxplots illustrating median, 25<sup>th</sup> and 75<sup>th</sup> quartiles, and 95% CI blood glucose concentrations ( $\text{mmol L}^{-1}$ ) in wild-caught, captive juvenile bonefish (*Albula vulpes*) immediately following exposure to constant, intermittent (1 min of constant light every 10 min), or no artificial light (control) treatments between 20:00 and 04:00 hrs. Asterisk (\*) denotes significant pairwise differences between treatments from the Kruskal-Wallis test with Nemenyi test post hoc ( $P < 0.05$ ). Open circles illustrate outliers.

1.12)  $\text{mmol L}^{-1}$ ; intermittent = 4.36 (SD 0.96)  $\text{mmol L}^{-1}$ ; constant = 5.12 (SD 0.80)  $\text{mmol L}^{-1}$ ; Fig. 3]. Based on the Nemenyi post hoc test, glucose concentrations of fish in constant lighting differed significantly from those in the control group ( $P = 0.03$ ), but not when compared with intermittent light ( $P = 0.8$ ). Fish under intermittent lighting did not differ significantly from those under control conditions ( $P = 0.08$ ). Whole body cortisol ( $\text{ng g}^{-1}$ ) did not vary significantly between treatments [ $\chi_{(2)} = 3.6$ ,  $P = 0.16$ ; control = 0.86 (SE 0.03)  $\text{ng g}^{-1}$ ; intermittent = 0.87 (SE 0.05)  $\text{ng g}^{-1}$ ; constant = 0.80 (SE 0.04)  $\text{ng g}^{-1}$ ].

## DISCUSSION

Our study assessed the effect of artificial lighting on juvenile bonefish swimming behavior and physiology. Contrary to our predictions, there was no significant effect of light pollution on juvenile bonefish swimming behavior. Blood glucose, a common indicator of stress in fish (Barton 2002), was measured following an overnight 8-hr exposure to the two light treatments and compared with a night control. Following light exposure, blood glucose levels became elevated as light levels increased, with the constant treatment having significantly higher blood glucose concentrations than intermittent lighting, or the control group which was not exposed to any artificial lighting. Despite glucose being elevated, cortisol levels post light exposure were not significantly different from the controls, a common result from photoperiod manipulation studies on fish (Biswas et al. 2006, 2008, Brüning et al. 2015). To our knowledge, the present study is the first to specifically address the concern of near-shore artificial lighting on juvenile marine fish, and will add to the growing body of literature on the effects of artificial lighting on the natural world.

Physiological stress is an adaptive mechanism by which fish are able to compensate or prepare for perceived or realized external stressors (Barton 2002). As such, an increase in blood glucose concentration is considered an adaptive secondary stress response (see Barton and Iwama 1991). Following the overnight exposure to simulated constant street lighting and intermittent car headlights, juvenile bonefish experienced elevated blood glucose concentrations, with constant street lighting eliciting a significant difference from the intermittent and control groups. Furthermore, the extent to which they experienced elevated blood glucose appeared to be related to the duration of light exposure, with fish under constant lighting experiencing higher levels than those under intermittent lighting. Although ours is the first study to quantify juvenile bonefish blood glucose, the results can be compared with adult bonefish blood glucose responses to various stressors. Light pollution is considered a perceived stressor, whereas catch-and-release angling acts as a physical stressor, and a cold shock event could behave as a chemical stressor (see Barton 2002 for definitions). Brownscombe et al. (2015) evaluated the physiological stress associated with catch-and-release angling of adult bonefish, and considered blood glucose concentrations as an indicator of angling stress. They found that 1 hr after an angling event ranging in duration from 70 to 245 s, adult bonefish blood glucose concentration was measured at 5.2 mmol L<sup>-1</sup>; this value closely resembles the concentrations of the present study following an 8-hr exposure to constant street lighting (5.12 mmol L<sup>-1</sup>). In another study conducted by Szekeres et al. (2014), adult bonefish were exposed to a 2-hr cold shock event with water temperatures 7 and 14 °C below ambient conditions (approximately 25 °C) and noted glucose values of approximately 4.2 mmol L<sup>-1</sup> post treatment, similar to the intermittent results from the present study (4.36 mmol L<sup>-1</sup>); however, these values were not significantly different from controls in either study. In general, the elevation in glucose associated with constant light pollution for juvenile bonefish appears to be similar to that arising from catch-and-release angling of adults. Although glucose levels have been shown to be highest 1–2 hr post stressor, glucose levels often remain higher than controls even up to 24 hrs post stressor (Rotllant and Tort 1997, Vijayan et al. 1997). We were unable to study longer-term glucose dynamics in our study.

Cortisol concentrations had no significant difference among groups, yet there was an increase in glucose concentrations following light treatments; this suggests that there was a cortisol response, but it was transient. Cortisol can become elevated rapidly after a stressor (i.e., minutes), and even after an intense acute stressor, such as exercise, levels often returned to baseline within 4 hrs (Barton 2002), while glucose remains elevated for longer periods (Mommensen et al. 1999, Barton 2002). Given that cortisol mobilizes glucose reserves (Mommensen et al. 1999), it is reasonable to assume that a cortisol response occurred in the two light treatments, but was not statistically discernible some 8 hrs after the initial light exposure (Pickering et al. 1982). It is also worth noting that due to low levels of cortisol during this experiment, juvenile bonefish may have experienced very little stress during holding or experimentation. Future studies should consider sampling fish at other intervals (e.g., 30 min, 1 hr, 2 hrs) post treatment; we were unable to do so given limitations with the number of fish available (juvenile bonefish are difficult to capture in the wild) and the fact that only lethal sampling was possible given their small size.

Although artificial lighting has been an overlooked form of pollution for the last century, the evidence of negative consequences has been accumulating rapidly, with

impacts to human health and natural ecosystems (Navara and Nelson 2007). The overnight artificial light treatments of our study resulted in a secondary physiological stress response in the form of elevated glucose (Barton and Iwama 1991). The behavioral parameters assessed did not yield significant results, which may be due to several reasons. First, for a conservative estimate of distance travelled, only horizontal distance travelled was considered; including vertical distance would have given a more representative indication of total distance traveled. Second (and most notably), there may not have been a long enough light exposure period to elicit a behavioral response. As nearshore light pollution is not a “one-off” occasion and occurs every night, longer periods of light exposure over several nights may have been needed to trigger a behavioral response. Third, we suspect there may have been a dilution effect with the intermittent treatment, as the light was on only for 1 min of constant light every 10 min. Due to the frequency and nature of passing cars, this is an important factor to consider; if fish are acutely startled by the light emitted by a passing car, there is the possibility of alerting nearby predators as a result of the erratic swimming behavior. We concede that having a more frequent intermittent light may have been more indicative of passing cars in nearshore roadways; however, due to equipment and time restrictions we were required to use the current experimental design. Primary and secondary stress responses in fish often result in a tertiary response over time (Barton 2002). Thus, it may be speculated that if an overnight exposure to artificial light triggered a secondary response in the form of elevated blood glucose, that over many nights this effect may elicit a behavioral one as well. Indeed, there have been other studies where changes in physiological parameters do not always yield changes in fish behavior (Cull et al. 2015, Pleizier et al. 2015). Disturbances to the natural light:dark cycle have provoked several tertiary responses across various taxa, and may result in changes to growth, reproductive timing, metabolism, habitat usage, feeding and foraging, and predation or predator avoidance (Dunlap 1999; also reviewed in Longcore and Rich 2004 and Navara and Nelson 2007).

It is important to note that juvenile bonefish are difficult to capture at this life stage; thus, it is possible our results would change with a larger sample size. However, our study is still valuable as it represents the first attempt to study juvenile bonefish, and in a broader context, yields insight into the possible effects of artificial lighting on nearshore juvenile fish. There was no statistical support for behavioral consequences of light pollution with the behavioral metrics tested. As juvenile bonefish and other nearshore fishes are subject to a variety of natural lighting phenomena (i.e., storms, lunar cycles, etc.), it is a possible explanation for why the acute light experiments did not elicit dramatic changes to behavior. However, the results did determine that blood glucose levels became elevated with increasing exposure to artificial lighting. As chronic light exposure is more indicative of what nearshore juvenile bonefish experience, a study looking at long-term effects could address whether the secondary responses would eventually result in tertiary responses (e.g., in behavior). Future research should examine whether juvenile bonefish are attracted to or repulsed by artificial lighting, and if their foraging behavior is altered, which may have implications for growth, as well as predation risk. Additionally, a study examining the response of phototactic bonefish leptocephali to artificial lighting, with regards to habitat choice and predation, could inform on disturbances to leptocephali recruitment. As an extension, future research should also consider predation with respect to juvenile or prey fish, by determining how their predators utilize and possibly

optimize artificial lighting to hunt. The results from our study provide a framework for future research to pursue the consequences of light pollution on nearshore fish. Further research on the effects of light pollution on juvenile bonefish might consider using an ecosystem approach and determining predation risk, which might be mediated by light pollution.

#### ACKNOWLEDGMENTS

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#### LITERATURE CITED

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