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Impacts of a local music festival on fish stress hormone levels and the adjacent underwater soundscape☆

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ABSTRACT

An understudied consequence of coastal urbanization on marine environments is sound pollution. While underwater anthropogenic sounds are recognized as a threat to aquatic organisms, little is known about the effects of above-surface coastal sound pollution on adjacent underwater soundscapes and the organisms inhabiting them. Here, the impact of noise from the 2019 Ultra Music Festival® in Miami, FL, USA was assessed at the University of Miami Experimental Hatchery (UMEH) located directly adjacent to the music festival and on underwater sound levels in Bear Cut, a nearby water channel. In addition, stress hormone levels in fish held at UMEH were measured before and during the festival. Air sound levels recorded at UMEH during the Ultra Music Festival did not exceed 72 dBA and 98 dBC. The subsurface sound intensity levels in the low frequency band increased by 2–3 dB re 1 μPa in the adjacent waterway, Bear Cut, and by 7–9 dB re 1 μPa in the fish tanks at UMEH. Gulf toadfish (*Opsanus beta)* housed in the UMEH tanks experienced a 4–5 fold increase in plasma cortisol, their main stress hormone, during the first night of the Ultra Music Festival compared to two baseline samples taken 3 weeks and 4 days before Ultra. While this study offers preliminary insights into this type of sound pollution, more research is needed to conclude if Ultra caused a stress response in wild organisms and to fully understand the implications of this type of sound pollution.

1. Introduction

The world's population is rapidly urbanizing, with most people already living and moving to cities by the sea (Creel, 2003). Coastal cities are of significant interest to ecologists due to their interactions with and consequences for the marine environment (Todd et al., 2019). Underwater anthropogenic sounds associated with urbanization are recognized as a threat to aquatic organisms. For example, it is well-documented that underwater sound pollution activates the endocrine stress response (Wysocki et al., 2006; Nichols et al., 2015; Celi et al., 2015) and various physiological and behavioral disruptions such as effects on communication (Vasconcelos et al., 2007), hearing loss (Smith et al., 2004), auditory threshold shifts (Smith et al., 2006; Wysocki and Ladich, 2005; Codarin et al., 2009), cardiac output (Graham and Cooke, 2008), metabolism (Wale et al., 2013), spawning behavior and reproduction (de Jong et al., 2018), development (Nedelec et al., 2014), and predator-prey interactions (reviewed by Kunc et al., 2016). Since many aquatic animals use sound to communicate, sound pollution can have a serious impact either by harming auditory systems directly or masking the sounds of important communication (reviewed in Slabbekoorn et al., 2018; Popper and Hawkins, 2019; and Kight and Swaddle, 2011).

To date, most research on anthropogenic underwater sounds in the aquatic environment has focused on the effects of vessel noise and other industrial activities on marine mammals and other aquatic organisms (Popper and Hawkins, 2019). In contrast, less research has been done to investigate the effect of sound pollution from the air into water (Peng and Zhang, 2016). However, a potential consequence of coastal urbanization is for city noise to penetrate the water and increase subsurface sound levels. For example, little is known about the effect of the many large music

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concerts and festivals that occur along city coastlines or near facilities that hold aquatic organisms around the world. These events have the potential to impact the adjacent underwater soundscape and affect the inhabiting aquatic species. In addition to an increase in underwater sound levels, the random and intermittent nature of music may contribute to a stress response as these types of sounds can elicit a greater cortisol response than continuous or repetitive sounds in giant kelpfish (*Heterostichus rostratus*; Nichols et al., 2015).

One recent music festival that had potential to impact the adjacent underwater soundscape was the 2019 Ultra Music Festival®, which occurred three days in a row (March 29th – March 31st) and was the longest and largest music festival held on Virginia Key, FL, USA to date. Music stages and festival facilities associated with Ultra 2019 were distributed over large areas from the Miami Marine Stadium to the Historic Virginia Key Beach Park (Fig. 1). The stages and facilities were adjacent to the coastal marine environment surrounding Virginia Key that is home to many different species of marine fish, birds, and marine mammals such as manatees and bottlenose dolphins. In addition, the festival bordered on the University of Miami Experimental Hatchery (UMEH), which houses various species of commercially valuable fish such as mahi-mahi (*Coryphaena hippurus*), cobia (*Rachycentron canadum*), Japanese flounder (*Paralichthys olivaceus*), and red snapper (*Lutjanus campechanus*) in tanks outside the facility that are used for both aquaculture and research (Benetti et al., 2010; Steiglitz et al., 2017; Steiglitz et al., 2016).

The proximity of the 2019 Ultra Music Festival to the coastal environment and University of Miami gave us the opportunity to monitor the effects of sound pollution associated with music festivals. Thus, our first objective was to determine the magnitude of underwater sound pollution by recording sound before and during Ultra Music Festival along the coast. The coastal body of water adjacent to the festival was Bear Cut, which is a connecting tidal channel that is an important habitat for local coastal organisms and organisms that travel between the Atlantic Ocean and Biscayne Bay (Fig. 1). The second objective was to examine whether increases in sound levels from the festival were associated with plasma cortisol levels in fish held in tanks at UMEH in close proximity to the music festival. To address this, sound levels in air near the tanks and underwater sound levels within the tanks were recorded. These measurements were paired with measurements of stress hormone levels of Gulf toadfish (*Opsanus beta*) at UMEH prior to and during the music festival. Based on previous studies of fish responses to anthropogenic sound pollution (Celi et al., 2015; Nichols et al., 2015; Wysocki et al., 2006; Smith et al., 2004), we hypothesized that toadfish would experience an increase in plasma levels of the stress hormone, cortisol, during Ultra compared to baseline samples.

Fig. 1. Ultra Music Festival stages were on Virginia Key, FL, USA located A) near the Miami Marine Stadium and B) at the Historic Virginia Key Beach Park. The sample sites were: 1. University of Miami Experimental Hatchery (UMEH) where air and underwater recordings were collected and toadfish blood sampling occurred, 2. Bear Cut where underwater recordings occurred. Inset panel shows Bear Cut is the connecting channel between Atlantic Ocean and Biscayne Bay.

2. Methods

2.1. Air sound level recordings at UMEH

UMEH is home to commercially valuable fish in tanks outside the facility that are used for aquaculture and research. Ultra stages were located directly next to UMEH and in efforts to reduce sound travel, festival organizers installed a sound barrier along the fence between UMEH and Historic Virginia Key Beach Park (Fig. 1). Sound levels in air at UMEH were measured before, during, and after Ultra Music Festival using a REED R8050 Sound Level Meter. Over an approximately 30–60 s period, the maximum and minimum dB were recorded next to the toadfish tanks at UMEH pre-Ultra (3/26/19 at 00:16), during sound check (3/28/19 at 17:10), night 1 during Ultra (3/29/19 at 23:34), night 2 during Ultra (3/30/19 at 20:10), night 3 during Ultra (3/31/19 at 20:00), and post-Ultra (4/1/19 at 15:31). These measurements were collected for both dBA and dBC, where dBA puts more weight on higher frequencies (>1000 Hz) and dBC is weighted for lower frequencies (<1000 Hz).

2.2. Underwater sound level recordings at UMEH and Bear Cut

2.2.1. Toadfish tanks at UMEH

A HTI-96 hydrophone (High-Tech Inc, Long Beach, MS, USA) connected to a Zoom H1 recorder (Zoom, Hauppauge, NY, USA) was placed in the toadfish tanks at UMEH and continuously recorded underwater sounds on the same days that toadfish blood sampling occurred: 3 weeks before Ultra (3/7/19), 4 days before Ultra (3/25/19), and during the first night of Ultra (3/29/19). The HTI-96 hydrophone had a sensitivity of −164 dBV/μPa and the sampling frequency was 48 kHz.

2.2.2. Bear Cut

Ultra stages in the Historic Virginia Key Beach Park were also located directly next to Bear Cut (Fig. 1). This water channel is home to Gulf toadfish, *Opsanus beta*, and other species that rely on sound for communication (Sogard et al., 1987; Serafy et al., 1997a; Roessler, 1965). A SNAP hydrophone (Loggerhead Instruments, Sarasota, FL, USA) was deployed at a depth of 3 m in Bear Cut (25.732454°N, 80.160094°W) on 3/11/19 and continuously recorded 10 s every consecutive minute (i.e. recording for 10 s, not recording for 50 s) for as long as it was deployed. Underwater sound levels were analyzed at: 2.5 weeks before Ultra (3/12/19), 4 days before Ultra (3/25/19), and during the first night of Ultra (3/29/19). The SNAP hydrophone sensitivity was −169 dB re 1 V/μPa, sample rate was 32 kHz, and gain was adjustable from −4 dB to +18 dB.

2.2.3. Underwater recording analysis

Recording schedules were first standardized and subsampled for 10 s per minute. Secondly, two analyses were run: one for all sound frequencies (0-20,000 Hz) and one for the low band frequency of 100-1000 Hz associated with the hearing range of most marine fish (Yan et al., 2000; Ladich and Fay, 2013). In Bear Cut, the highest frequencies (>1000–20,000 Hz) are dominated by snapping shrimp, tidal currents, and wind. For each analyzed 10s of recording, the sound pressure level (SPL) was computed following Staaterman et al. (2014). The data was converted to dB by applying $20^{*}\log_{10}X$ transformation and then adding a scalar corresponding to the hydrophone sensitivity. The resulting values in dB referenced at 1 μPa (dB re 1 μPa) are the sound pressure level (SPL). For tidal signal removal, the MATLAB® (MathWorks Inc, USA) tidal fitting toolbox was used (Grinsted, 2008). SPL differences between before and during Ultra were computed on the mean SPL values of the tide-free signal. Moving average was then computed using a window of five 10s samples using MATLAB. We examined the ΔSPL defined as the difference between the sound pressure levels recorded underwater during and before Ultra, such that ΔSPL = SPL during Ultra − SPL before Ultra.

2.3. Toadfish stress hormone levels at UMEH

2.3.1. Gulf toadfish as a model species

Rather than using fish species held at UMEH, about which little is known regarding their endocrine stress response, Gulf toadfish (*Opsanus beta*) were used as the study species for three primary reasons. First, toadfish have well-characterized responses to various experimental stressors (McDonald et al., 2009; Medeiros and McDonald, 2012; Medeiros et al., 2014; Cartolano et al., 2019), making them a good model to study acoustic stress. Second, toadfish play an important ecological role in the local marine environment as they are common prey items for species such as bottlenose dolphins (Gannon et al., 2005; Remage-Healey et al., 2006). And third, toadfish produce vocalizations that contribute to the local marine soundscape and they rely heavily on sound and hearing for reproduction, social interactions, maintaining territories, and predatory avoidance (Gray and Winn, 1961; Fish and Offutt, 1972; Remage-Healey et al., 2006). Toadfish produce low frequency boatwhistles (mating call) and grunts and have a hearing range below 1000 Hz, which is similar to many other fish species (Fish and Offutt, 1972; Yan et al., 2000; Ladich and Fay, 2013). Additionally, studies on Gulf toadfish and other fish species have demonstrated reduced vocalizations in response to sound pollution (Vasconcelos et al., 2007; de Jong et al., 2018).

2.3.2. Experimental set-up and sampling

Gulf toadfish were collected from Biscayne Bay between August 2018 and February 2019 by local commercial shrimpers using trawl nets (Florida Fish and Wildlife Conservation Commission Special Activity License SAL-16-0729-SR). Upon arrival in the laboratory and every 2.5 weeks thereafter, toadfish were treated with freshwater, formalin, and malachite green to preemptively treat for the parasite *Cryptocaryon irritans.* Toadfish were then transferred on 3/2/19 to UMEH and placed in 2.4 m diameter outdoor inflatable pools (Intex®, Long Beach, CA, USA) that were approximately 0.5 m deep. Pools had continuous flow through UV-treated, 1 μm-filtered seawater (2 L min^{-1}) and aeration as well as PVC tubes and sand-covered bottoms to provide shelters and substrate for toadfish. There were six pools that contained 20 toadfish each. Each pool was covered by UV-protected shade cloth as well as shade tents. Fish were fed weekly with shrimp or squid and water chemistry (temperature, salinity, dissolved oxygen, and pH) was monitored throughout the experiment (Table 1). Toadfish were allowed to acclimate to these pools for at least 4–5 days prior to the first sampling date. During sampling, notes were taken on any abnormalities in body condition. Based on these written observations, four researchers were blinded to the identity of the fish and rated the health of individual fish using a health index (HI) on a 5-point scale (Table 2). The scores were averaged and fish with 1 point were deemed healthy and fish that received 2 points up to a maximum of 5 points based on increasing severity of *Crytopcaryon irritans* symptoms were deemed unhealthy (Table 2). Only healthy fish (HI = 1) were used for analysis.

Ultra Music Festival occurred from approximately 14:00 to 02:00 on 3/29/19, 3/30/19, and 3/31/19 with preceding sound checks occurring at various times from 3/26/19-3/28/19 and around 12:00 each day of the festival. The first baseline sample was taken 3 weeks before Ultra Music Festival on 3/7/19 at 23:45, before any set-up and construction associated with Ultra Music Festival began. Unfortunately, on 3/18/19, 11 days after that first blood sample, *Cryptocaryon irritans* infection was detected in toadfish found in

Table 1
Mean water chemistry parameters measured at UMEH in toadfish tanks on the three dates sampled before and during Ultra. Means include measurements on the day of sampling and the 4 days prior to sampling.

Date	pH	Dissolved Oxygen ($mg \cdot L^{-1}$)	Salinity (ppt)	Temperature (°C)
3/7/19	8.04 ± 0.01	6.85 ± 0.02	37.0 ± 0.1	26.6 ± 0.0
3/25/19	8.03 ± 0.01	7.25 ± 0.04	36.0 ± 0.2	24.2 ± 0.2
3/29/19	8.03 ± 0.01	7.05 ± 0.03	37.0 ± 0.0	25.0 ± 0.3

Data presented as mean ± standard error of mean.

Table 2
Health index (HI) scale.

HI	Description
1	No external signs of *Cryptocaryon* evident
2	Cloudy eyes after placed in MS-222
3	Mild cloudy eyes before placed in MS-222
4	Beginning of *Cryptocaryon* symptoms: cloudy eyes and skin abnormalities
5	Full *Cryptocaryon* symptoms

four of the six pools (Pools A-D). All the fish were removed, and the four pools were soaked in bleach and freshwater, scrubbed and rinsed thoroughly. On 3/19/19, the two pools (Pools E, F) still containing healthy fish were prophylactically treated with freshwater and formalin, as described above. On 3/21/19, the four clean pools (Pools A-D) were each re-stocked with healthy fish and the new fish were then allowed to acclimate for 4 days before the next sampling date.

On 3/25/19, the second baseline sample was taken at 22:00 after set up and construction started but before any sound checks began. Fish were sampled from each of the six pools so that half of the fish were from the original batch of fish (Pools E and F, 3 fish from each pool; $n = 6$) and half were from the new batch (Pools A-D, 6 fish randomly sampled from these four pools; $n = 6$) for a total of $n = 12$ fish. Notes on abnormalities in body condition were taken. On 3/28/19, the day before Ultra, *Cryptocaryon irritans* infection was again detected in one of the six pools (Pool A). On the morning of 3/29/19, the first day of Ultra, *Cryptocaryon irritans* was detected in three more pools (Pools B,C,D). These were the same four pools that had *Cryptocaryon irritans* infection originally. No fish from any of these pools were used for the remainder of the experiment.

The third sample was taken during Night 1 Ultra on 3/29/19 at 21:30, about 7 h after music began (in addition to preceding sound checks). Six fish were sampled from each of the two remaining pools (Pools E and F; $n = 12$). These two pools of fish had been carried through the entire experiment. Notes on body condition were taken. The experiment was concluded at this point, which was earlier than intended. All blood samples were collected at night for consistency. On the above sampling dates, toadfish were removed from the pools and a blood sample was immediately taken via caudal puncture. The blood was promptly centrifuged and the plasma was flash frozen in liquid nitrogen and stored at −80 °C for later analysis of cortisol using an ^{25}I-cortisol radioimmunoassay kit (MP Biomedicals, Santa Ana, CA, USA). Only fish caught fast enough (typically < 10 min, any difficulties in capture were noted at the time of sampling) to avoid elevation of cortisol from handling stress were included in analysis, which led to one fish being removed from the 3/6/19 data set. After blood collection, fish were euthanized in MS-222 buffered with NaHCO3 (3 g L^{-1}). All procedures involving toadfish were conducted according to an approved Institutional Animal Care and Use Committee (IACUC) protocol (19-032, Assurance #A-3224-01).

2.3.3. *Post-experiment assessment of health status on cortisol concentrations*

After the conclusion of the experiment, at noon on 3/30/19 (10 h after the Night 1 Ultra music ended), a health assessment on the remaining fish in the two pools (Pools E and F) sampled on Night 1 Ultra was completed and blood was sampled. Six fish from each of the two pools were sampled for a total of $n = 12$ and notes on abnormalities in body condition were taken. For these fish sampled, 8 out of the 12 fish had varying degrees of *Cryptocaryon* symptoms (unhealthy; HI > 1) and 4 fish had no symptoms evident (healthy; HI = 1). Blood samples for these 12 fish were analyzed for cortisol.

2.3.4. *Statistics*

Statistical tests were performed using GraphPad Prism software (ver. 7.0a, La Jolla, CA, USA). Data were log-transformed and a one-way analysis of variance (ANOVA) and Tukey's multiple comparisons test were conducted to compare cortisol concentrations between the three sampling dates. For the post-experiment assessment, a non-parametric Mann-Whitney *U* test was used to compare HI and plasma cortisol in healthy and unhealthy fish. Values are presented as mean cortisol concentrations ± standard error of the mean (SEM).

3. Results

3.1. *Air sound level recordings at UMEH*

Air sound levels in decibels (dBA and dBC) at UMEH increased during Ultra Music Festival compared to before and after this event and they did not exceed 72 dBA and 98 dBC at UMEH during Ultra Music Festival (Fig. 2).

3.2. *Underwater sound level recordings*

3.2.1. *Toadfish tanks at UMEH*

The Ultra Music Festival music was clearly heard from the UMEH underwater recordings (Supplementary File 1). Sound pressure levels (SPL) in the toadfish tanks increased by 7–9 dB re 1 μPa across all and low frequencies during the Ultra Music Festival (Table 3, Figs. 3 and 4). The end of the first Ultra festival day (3/30/19 2:00 a.m.) was evident in the low frequencies band (100–1000 Hz) with an abrupt decease in SPL (Figs. 3 and 4; Supplementary File 2).

3.2.2. *Bear Cut*

The sound pressure levels in Bear Cut increased by *ca.* 2 dB re 1 μPa in the low frequency range during the Ultra Music Festival (Table 3; Supplementary File 3) compared to two baseline dates before Ultra (Figs. 5 and 6). There was no detectable increase when taking the full range of frequencies into account (Table 3; Supplementary File 5).

Overall SPL were an order of magnitude higher before removal of tidal signals (Fig. 5a). After removing the tidal signal, an average increase of 1.8 dB re 1 μPa in amplitude was detected during the festival (start: 3/29/2019 2–4:00 p.m.; end: 4/1/2019 2:00 a.m.;

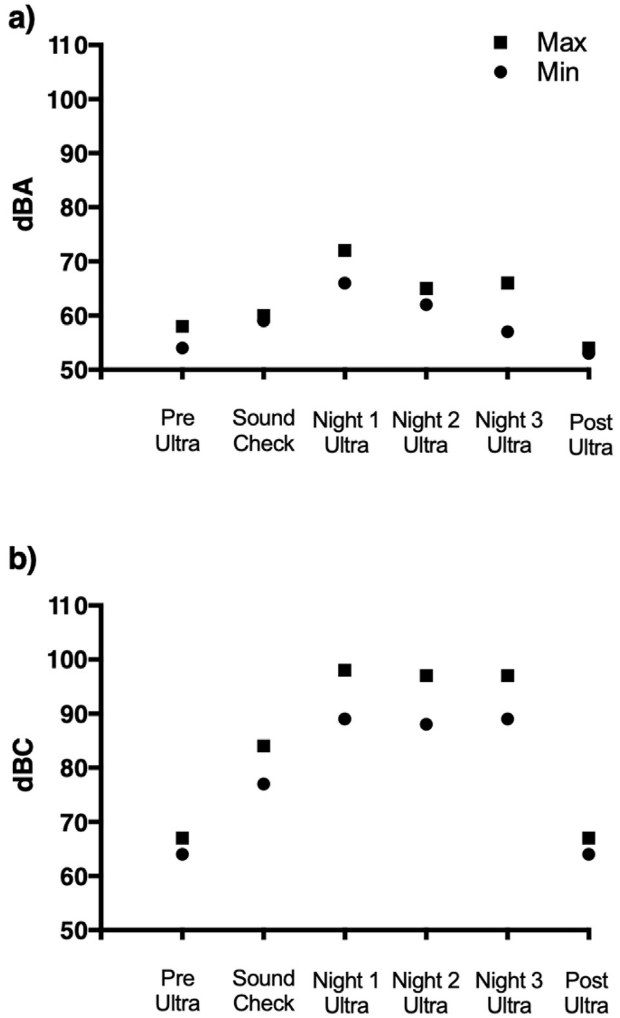

Fig. 2. Minimum and maximum sound levels measured in air at UMEH pre-Ultra (3/26/19 at 00:16), during sound check (3/28/19 at 17:10), night 1 Ultra (3/29/19 at 23:34), night 2 Ultra (3/30/19 at 20:10), night 3 Ultra (3/31/19 at 20:00), and post-Ultra (4/1/19 at 15:31) for a) dBA (weighted for higher frequencies) and b) dBC (weighted for low frequencies).

Fig. 5b). Clear music melodies could not be detected from the Bear Cut sound recordings during and before Ultra (Supplementary File 3, 4). Toadfish vocalizations were detected in Bear Cut (Supplementary Files 3, 5).

3.3. Toadfish stress hormone levels at UMEH

Water chemistry (pH, dissolved oxygen, temperature, and salinity) was consistent throughout the sample dates (Table 1). Toadfish plasma cortisol concentrations were 30.9 ± 14.8 ng mL^{-1} ($n = 11$) 3 weeks before Ultra (3/7/19). All the fish included in this first baseline sample had a health index (HI) of 1 (Table 2). Toadfish plasma cortisol concentrations were 24.7 ± 11.7 ng mL^{-1} ($n = 12$) 4 days before Ultra (3/25/19) and all fish included in this second baseline sample had an HI of 1 (Fig. 7; Table 2). Cortisol concentrations for these two baseline samples were not significantly different from each other ($p = 0.85$). During night 1 of Ultra (3/29/19), toadfish had 4 to 5-fold elevated plasma cortisol concentrations compared to baseline samples (120.5 ± 43.4 ng mL^{-1}; $n = 7$, (n = 2 from pool E and n = 5 from pool F)) (Fig. 7). All fish included in this sample had an HI of 1 (Table 2). At this time point n = 5 fish additional fish were sampled but had an HI > 1 and were thus not included in this analysis. Cortisol concentrations during the first night of Ultra were significantly higher than 3 weeks before Ultra ($p = 0.008$) and 4 days before Ultra ($p = 0.002$). Results from the one-way ANOVA and Tukey's multiple comparisons test on log-transformed cortisol values are reported in Table 4.

3.4. Post-experiment assessment of health status on cortisol concentrations

Of the 12 fish sampled after the conclusion of the experiment, the unhealthy fish had 4.2-fold higher HI ($p = 0.002$) and 23-fold higher plasma cortisol concentrations ($p = 0.0283$) than the healthy fish (Fig. 8).

4. Discussion

The underwater sound levels increased in the UMEH tanks due to music from Ultra, as music melodies were clearly heard in the underwater signal, with a ΔSPL of 7–9 dB re 1 μPa. This increase in sound levels occurred despite Ultra Music Festival adhering to the imposed air sound limit of 110 dB at UMEH. While underwater sound was elevated, we measured a significant elevation of toadfish plasma cortisol. The exact increase in air sound levels from average baseline sounds cannot be concluded due to short sound samples collected and should be taken into account in further studies. It is also unknown if the sound barrier put in place by festival organizers reduced sound levels and this should also be investigated in future work. In contrast, a change in the ΔSPL in the more complex coastal marine environment was measured but was not as apparent as in the tanks. First, the ambient noise in the coastal marine environment was variable with respect to time, location, and frequency. Second, the sound propagation depended on the currents and the topography of the sea floor. Natural sound sources such as wind, fish, and benthic invertebrates (as heard in Supplementary File 5) may have significant variability, making the identification of the various sound sources more difficult to determine due to the increase in the noise component of the ΔSPL. In addition, the transmission of sound from air to water is attenuated, or reduced, by the refraction at the water surface, which is affected by the roughness of the ocean surface (Peng and Zhang, 2016). Bear Cut is a narrow channel with strong tidal currents and heavy boat traffic that contributed to a relatively loud soundscape approximately 25 dB louder than the quieter open ocean's ambient noise (74–100 dB, Urick, 1986) and *ca.* 15 dB louder that the UMEH toadfish tanks. Despite the high ambient noise and attenuation in Bear Cut, we still detected an increase of 2–3 dB re 1 μPa in the low frequency band (100-1,000Hz) and a *ca,* 2 db re 1 μPa increase when tides were removed, indicating that the level of noise power of Ultra was detectable.

While underwater sound levels were elevated in UMEH tanks, toadfish experienced a significant increase in plasma concentrations of the stress hormone cortisol during the Ultra Music Festival. While we believe the increased stress hormone levels are due to noise associated with the festival, we must acknowledge the limitations in our study. Ideally, in addition to control baseline samples, we would also have had a control group in another location away from the festival. However, this was not possible as the music could be heard from miles away. Additionally, there was an unexpected infection of *Cryptocaryon irritans* that resulted lower sample sizes, a less than optimal sampling design, and the experiment ending sooner than planned. However, we are confident that our on-site health assessment was rigorous and excluded toadfish that many have experienced an elevation in plasma cortisol due to infection. Our post-experiment health assessment 10 h after Night 1 Ultra

Table 3
Average sound pressure level (SPL) underwater before and during Ultra and the resulting difference in SPL (ΔSPL; ΔSPL = during Ultra − before Ultra). The recordings 3 weeks before Ultra were taken on 03/07/19 and 03/12/19 at UMEH and Bear Cut, respectively and the recordings 4 days before Ultra were on 3/25/19. High frequency sounds represented 16% and 1% of the total frequencies (0-24,000 Hz) analyzed for Bear Cut and UMEH, respectively.

Location/Date	3 weeks Before Ultra SPL (dB re 1 μPa)	4 days Before Ultra SPL (dB re 1 μPa)	Night 1 During Ultra SPL (dB re 1 μPa)	ΔSPL (dB re 1 μPa)
UMEH All Frequencies	109	111	118	**7–9**
UMEH Low Frequencies	100	102	109	**7–9**
Bear Cut All Frequencies	126	126	126	**0**
Bear Cut Low Frequencies	106	105	108	**2–3**

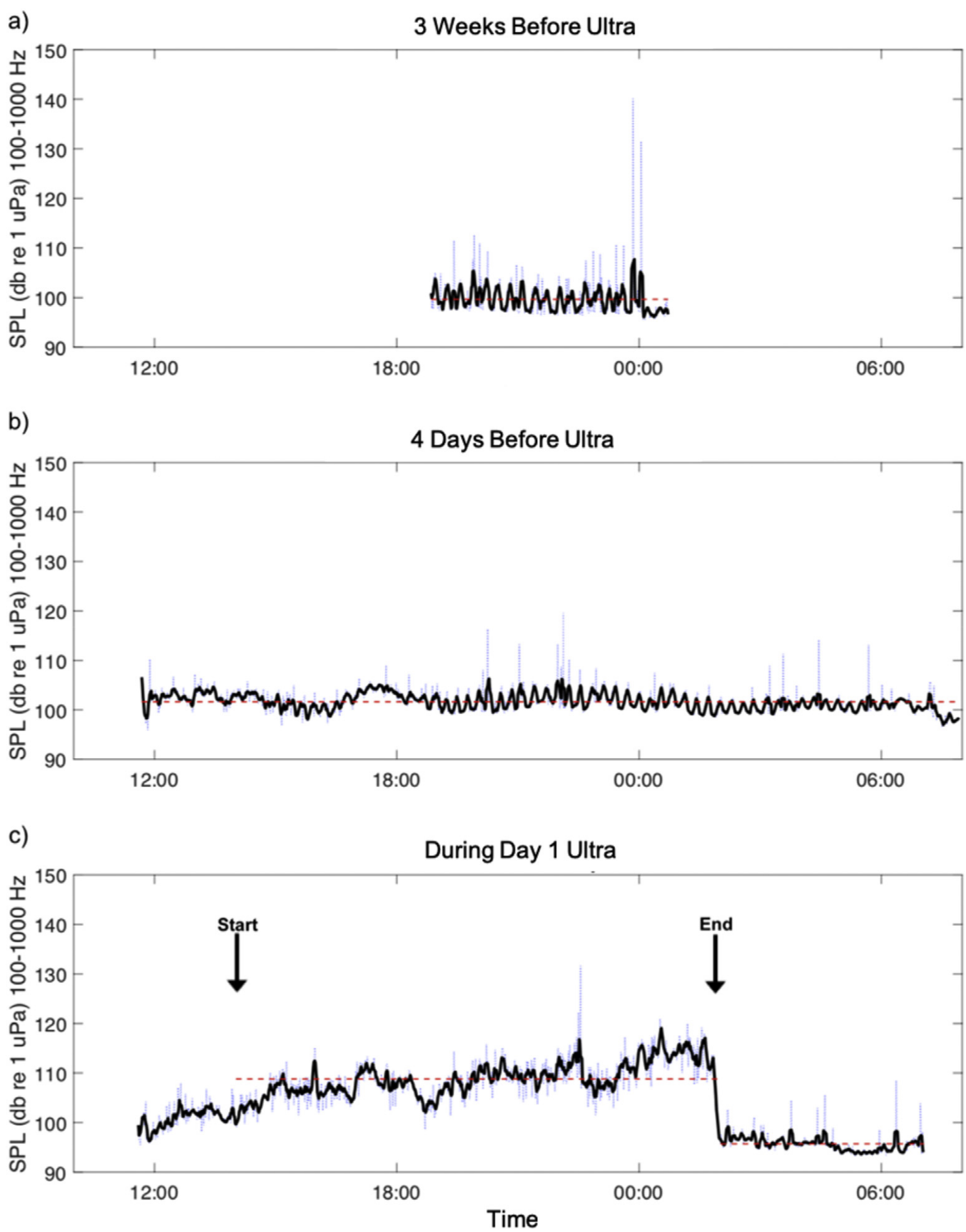

Fig. 3. Sound pressure levels (SPL) over time in the low frequency range (100-1000 Hz) in toadfish tanks at UMEH a) 3 weeks before Ultra (3/7/19), b) 4 days before Ultra (3/25/19), and c) during day 1 of Ultra (3/29/19) where the black arrows indicate the approximate start (14:00) and end (02:00) of Ultra. Raw data and 5 points moving average (black) are shown. Average SPL (dotted red lines) are taken during the sound control period before Ultra and during Ultra. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

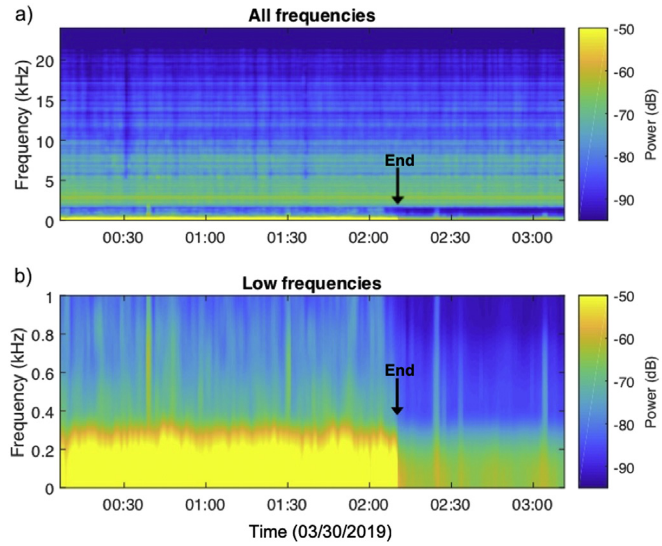

Fig. 4. Spectrogram for sounds recorded in toadfish tanks at UMEH for a) all frequencies (0-20,000 Hz) and b) low frequencies (100-1000 Hz). The sound detected in the toadfish tanks is loudest in the low frequency range as shown by the bright yellow in b), which abruptly reduces when the concert ends a little after 02:00 indicated by black arrows. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

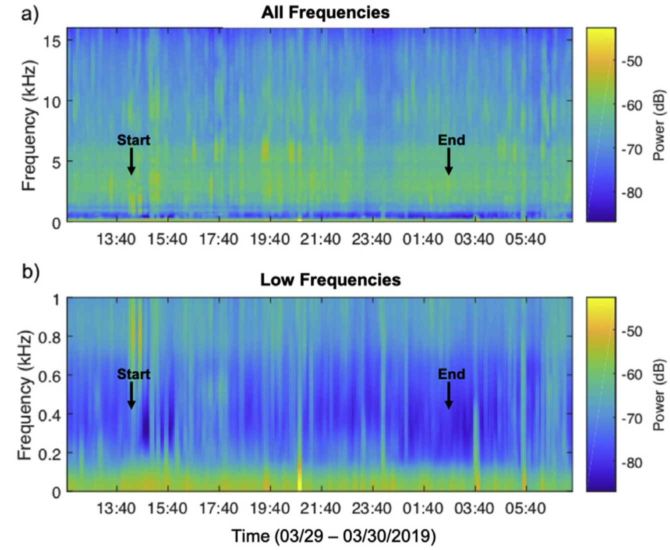

Fig. 6. Spectrogram for sounds recorded in Bear Cut for a) all frequencies (0-20,000 Hz) and b) low frequencies (100-1000 Hz).

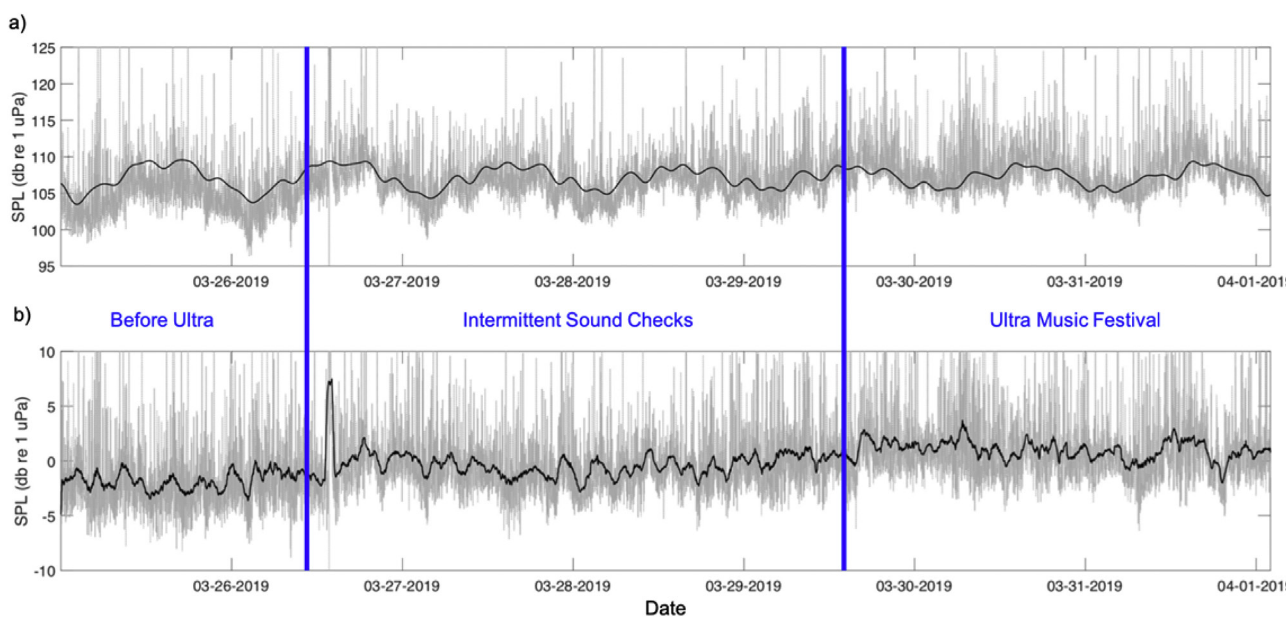

Fig. 5. Sound pressure levels (SPL) over time before and during Ultra in the low frequency range (100-1000 Hz) in Bear Cut (a) before and (b) after the removal of the tidal signal from 03/24/2019 to 04/01/2019 where intermittent soundchecks began on 03/26/19 and Ultra Music Festival lasted from 03/29/2019 to 04/01/2019 from approximately 14:00 to 02:00.

that showed low cortisol levels in healthy fish with HI = 1 (compared to high cortisol in unhealthy fish with HI > 1). Furthermore, our post-experiment health assessment data show that healthy fish do not have elevated cortisol when held in the same pool as fish that do have symptoms. Thus, the stress response observed in healthy fish during Night 1 of Ultra compared to healthy fish before Ultra was not due to presence of *Cryptocaryon irritans* in the pools. Despite these limitations and inability to repeat the experiment, we believe our study provides valuable preliminary insights into increases in endocrine stress responses that may be associated with elevated sound levels during music festivals. Future studies should be conducted to establish a more direct causal link.

The relative elevation in cortisol (a 4-5-fold increase) that toadfish experienced during Ultra was comparable to what toadfish experience in response to another acoustic stressor, the playback of dolphin foraging vocalizations (4-fold increase; Remage-Healey et al., 2006), which is one of their main predators. However, the relative elevation in cortisol during the Ultra Music Festival was less than the 10-fold increase in cortisol that toadfish experience when exposed to laboratory crowding, an experimental condition that

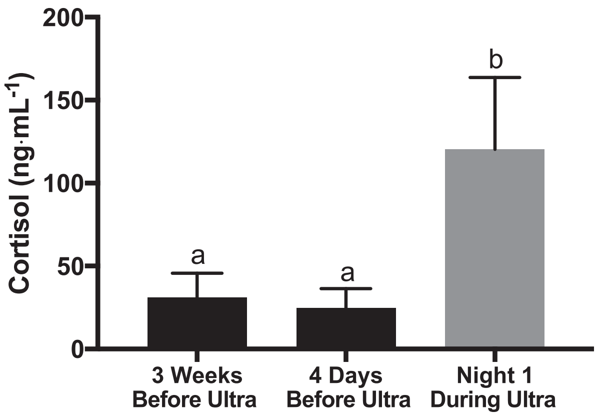

Fig. 7. Toadfish stress hormone levels before and during Ultra Music Festival determined by plasma cortisol concentrations (ng·mL-1). Cortisol was measured 3 weeks before Ultra (3/7/19 at 23:45; n = 11), 4 days before Ultra (3/25/19 at 22:00; n = 12), and during Night 1 of Ultra (3/29/19 at 21:30; n = 7). Values are mean ± SEM and different letters denote significant differences (p < 0.01).

toadfish are subjected to in the lab to investigate toadfish social behavior and the unique pulsatile urea excretion mechanism they use for chemical communication (McDonald et al., 2009; Cartolano et al., 2019). Indeed, being held in crowded conditions is a major social stressor for toadfish because they are aggressive, live individually, and defend large territories in the wild (Sloman et al., 2005; McDonald et al., 2011; Sogard et al., 1987). Measuring a statistically significant elevation in cortisol in a group of toadfish during sound pollution is noteworthy as the stress response is complex and can be highly variable. This complexity arises because there are several points of regulation along the stress axis, called the hypothalamic-pituitary-interrenal (HPI) axis, that need to be affected for a significant increase in blood cortisol to occur (reviewed by Wendelaar Bonga, 1997; Mommsen et al., 1999) and this coordination can result in a high level of interindividual variation in response to certain stressors. In general, short-term elevations in cortisol may reduce physiological processes that are not necessary for acute survival, such as digestion, social behaviors, and communication. On a long-term basis, an elevation in cortisol can have detrimental effects: it can cause reduced reproduction and growth, muscle wasting, and immune system impairment which might lead to long-term decreases in condition factor and, ultimately, decreases in population size (reviewed by Wendelaar Bonga, 1997; Mommsen et al., 1999). The data of the present study suggest toadfish experienced a significant endocrine stress response during the first day of Ultra Music Festival (a short-term exposure) and it is possible that this level of anthropogenic acoustic stress could have led to disruptions in behaviors such as

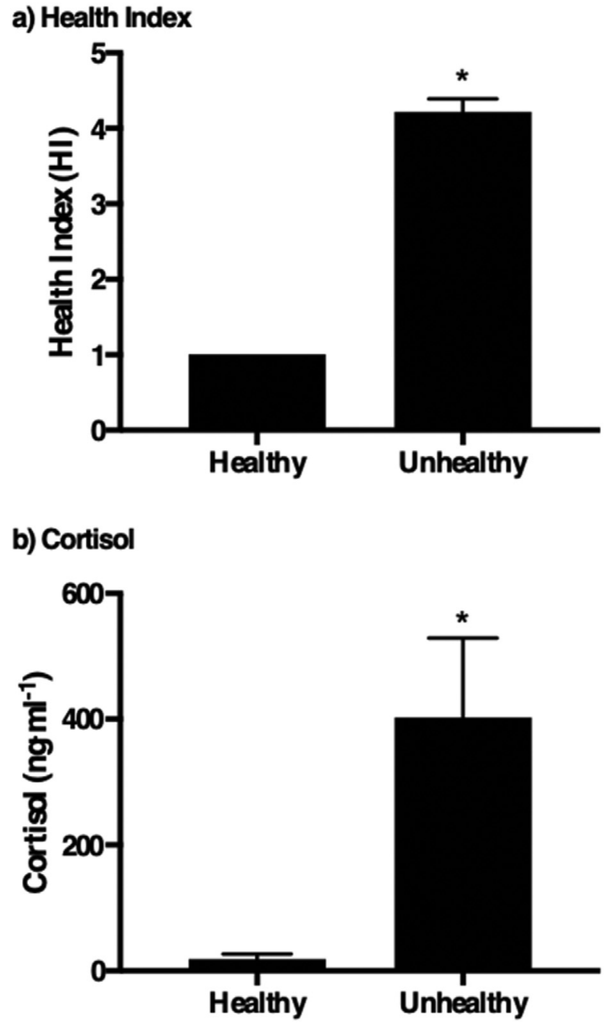

Fig. 8. For the post-experiment assessment of health status on cortisol concentrations completed on (3/30/19 at 12:00), fish health index (HI) and blood plasma cortisol concentrations were quantified in healthy (n = 4) and unhealthy (n = 9) fish. Values are means ± standard error of the mean and an asterisk denotes significant differences (p < 0.05).

altered social interactions and disruptions in communication on a short-term basis (Vasconcelos et al., 2007; de Jong et al., 2018). Our post-experiment health assessment suggests that cortisol concentrations of healthy toadfish returned to pre-Ultra levels 10 h after the end of Ultra music exposure. However, we cannot determine whether repeated exposure over the course of three days would

Table 4
Results from the one-way ANOVA and Tukey's multiple comparisons test on log-transformed cortisol values.

ANOVA Table	Sum of Squares (SS)	Degree of Freedom (DF)	Mean Square (MS)	F value	P value
Treatment (between columns)	3.86	2	1.93	7.85	0.0021
Residual (within columns)	6.64	27	0.25		
Total	10.49	29			

Tukey's Multiple Comparisons Test	Mean Difference	95% Confidence Interval of Difference	q	DF	P Value
3 Weeks Before Ultra vs. 4 Days Before Ultra	0.11	−0.40 to 0.63	0.78	27	0.8456
3 Weeks Before Ultra vs. Night 1 During Ultra	−0.78	−1.37 to −0.19	4.60	27	0.0083
4 Days Before Ultra vs. Night 1 During Ultra	−0.89	−1.48 to −0.31	5.36	27	0.0021

translate to long-term elevations in cortisol and the related long-term effects described above such as hearing sensitivity or changes in vocalizations.

Sound has been pointed out as a general concern for the health and growth of organisms in research and aquaculture facilities such as those found at UMEH. While some studies have shown that fish raised in noisier conditions can recover their hearing sensitivity (Smith et al., 2004) or found no overall effect on growth and survival (Wysocki et al., 2007), other studies have found that conditions that are louder than natural habitats can affect growth and reproduction rates, increase mortality, and increase metabolic rates in shrimp (Lagardè;re, 1982; Regnault and Lagardè;re, 1983) and reduce growth and egg viability in fish (Banner and Hyatt, 1973). More research is needed to determine the sound pollution effects on important aquaculture species held in facilities in proximity to events like Ultra since each species has different hearing sensitivities and levels of resilience to environmental stressors.

5. Conclusions

In summary, during Ultra we measured elevated sound pressure levels as well as a significant increase in plasma cortisol levels in toadfish held in tanks directly adjacent to the festival at UMEH. We also found a signal of sound pollution in the adjacent Bear Cut channel, which may have affected wild organisms. To our knowledge, this is the first study to assess the impacts of sound pollution due to a coastal music festival. Our study should be interpreted as preliminary insights into understanding the effects of sound pollution from air into water and demonstrate the need for further studies to make a causal relationship between sound from music festivals and the endocrine stress response in fish. We cannot conclude whether the short-term exposure to the festival would have translated to a long-term elevation in plasma cortisol levels and other long-term detrimental effects. Our results suggest that festivals that reach these sound volumes, especially in the low frequency range, have the potential to affect the marine environment. Thus, more studies are needed to assess the impact on the endocrine stress response and on vocal communication essential for reproduction in wild organisms (Popper and Hawkins, 2019; Vasconcelos et al., 2007; Codarin et al., 2009; Slabbekoorn et al., 2010; Kunc et al., 2016; de Jong et al., 2018) as well as determine appropriate levels of sounds and/or locations for these types of events.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.114925.

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