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ORIGINAL ARTICLE

Noise during mouthbrooding impairs maternal care behaviors and juvenile development and alters brain transcriptomes in the African cichlid fish *Astatotilapia burtoni*

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Abstract

Anthropogenic noise has increased underwater ambient sound levels in the range in which most fishes detect and produce acoustic signals. Although the impacts of increased background noise on fish development have been studied in a variety of species, there is a paucity of information on how noise affects parental care. Mouthbrooding is an energetically costly form of parental care in which the brooding fish carries developing larvae in the buccal cavity for the duration of development. In the African cichlid *Astatotilapia burtoni*, females carry their brood for ~2 weeks during which time they do not eat. To test the hypothesis that increased background noise impacts maternal care behaviors and brood development, we exposed brooding females to a 3-h period of excess noise (~140 dB) played through an underwater speaker. Over half of noise-exposed brooding females cannibalized or pre-maturely released their brood, but 90% of control females exhibited normal brooding behaviors. RNA-seq analysis revealed that transcripts related to feeding and parental care were differentially expressed in the brains of noise-exposed females. Juveniles that were exposed to noise during their brood period within the mother's mouth had lower body condition factors, higher mortality and altered head transcriptomes compared with control broods. Furthermore, onset of adult-typical coloration and behaviors was delayed compared with control fish. Together, these data indicate that noise has severe impacts on reproductive fitness in mouthbrooding females. Our results, combined with past studies, indicate that parental care stages are extremely susceptible to noise-induced perturbations with detrimental effects on species persistence.

KEYWORDS

anthropogenic noise, development, mouthbrooding, RNAseq, shoaling, teleost

1 | INTRODUCTION

Anthropogenic noise is pervasive to almost all terrestrial and aquatic environments,[1] and was designated as a pollutant of global concern by the World Health Organization in 2011. Underwater anthropogenic noise has risen rapidly in the past century due to increases in pile driving, sonar use and shipping travel, which has intensified ambient underwater noise levels in the frequency range in which most fishes produce and detect acoustic stimuli.[2-8] Fishes depend on their auditory system for anti-predator behaviors, detecting prey, orientation and social communication.[9] Aquatic anthropogenic noise is linked to changes in feeding and foraging behaviors,[4,10,11] decreased growth rates[12,13] and damage to the sensory hair cells in the inner ear.[14] Elevated and persistent noise can also induce stress,[15,16] further

interfering with an animal's ability to feed, reproduce, care for young, evade predators and navigate their environment. Despite well-documented impacts on behavior and physiology, no study has examined how anthropogenic noise impacts the brain in fishes.

Parental care life history stages are particularly sensitive to perturbations,[17] and fishes engaging in parental care behaviors are often at greater risk than fish species with other reproductive strategies.[18] Parental care (post-fertilization behaviors intended to promote offspring survival) occurs in approximately 22% of teleost fishes,[19,20] and can vary from nest defense, to egg fanning, to feeding and cleaning, to mouthbrooding. Most fish species that provide parental care live in shallow, nearshore areas[19] that are subjected to high amounts of anthropogenic disturbances from recreational and commercial boating and other activities. As such, anthropogenic noise may be particularly detrimental to fishes engaged in parental care behaviors and to early-life developing individuals, and especially to species that are site-attached and unlikely to leave a noisy environment. The impairment of parental care behaviors may have direct negative impacts on the developing offspring and the parents, and ultimately result in decreased reproductive fitness.

Mouthbrooding is an extreme form of parental care in which one fish carries the developing larvae for the full or partial duration of development inside their buccal cavity.[21] Mouthbrooding often results in the brooding fish undergoing forced starvation for an extended amount of time.[21] While brooding increases the likelihood of larvae hatching and success, it is costly and stressful for the brooding parent fish due to the physiological and energetic demands. To date, no study has examined the impact of noise on mouthbrooding fishes. Mouthbrooding fishes exposed to noise are not only themselves susceptible to noise-induced changes in behavior and physiology, but their brood can also be directly affected by the noise, extending the effects to future generations. The developing larvae may also suffer indirect consequences due to effects on the brooding parent. For example, developing larvae often feed on the mucus inside the buccal cavity, which indicates some form of maternal-embryo nutrient transfer[22] and potential transfer of immunity.[23] Investigating the impact of noise on mouthbrooding fishes is of extreme importance because any disruption to mouthbrooding, from the parent or offspring perspective, can have devastating effects on species persistence and biodiversity.

Although the impact of anthropogenic noise on fish behavior and physiology has become a prevalent research topic in recent years, relatively few studies have examined how larval fishes may cope with anthropogenic sounds, and no study has examined how noise exposure during the mouthbrooding period influences the young after they are released. Past studies found mixed results on the effects of noise on growth and development in fishes. For example, hatching success and growth was not affected by noise-playback in the substrate spawning African cichlid *Neolamprologus pulcher*.[10] Rainbow trout (*Oncorhynchus mykiss*) raised in noisy and silent conditions had similar growth and survival rates after 2 months, but fish raised in noisy conditions had slower growth rates for the first month.[24,25] However, seismic air guns caused up to 100% mortality in lake trout (*Salvelinus namaycush*) larvae,[26] and in the Atlantic cod (*Gadus morhua*), noise exposure to developing larvae impacted growth, use of yolk sac, condition factor and ability to avoid predators.[27] The reason for these varied results could be due to differences in exposure protocol, species variability due to hearing or other physiological differences, or timing of the exposure; however, the majority of these studies found some detrimental impacts of noise during early-life stages.

In the mouthbrooding cichlid fish *Astatotilapia burtoni*, females incubate developing larvae in their buccal cavity for ~14 days. In larval *A. burtoni*, the otolithic auditory endorgans develop ~5 days post fertilization (dpf) and larvae in the buccal cavity may be able to detect acoustic stimuli after this time, suggesting that acoustic overstimulation could directly impact developing young. Here, we examined how exposure to a single period of excess noise at a critical developmental point impacts mouthbrooding fish and their developing young. We examined noise-induced effects at multiple levels of biological organization, including brain transcriptome profiles, hormones and behavior. Together, our results provide a comprehensive picture of how noise exposure impacts maternal care behaviors and juvenile condition and suggest that a single exposure at a critical developmental timepoint can have detrimental consequences for species persistence through direct and indirect impacts on juvenile survival.

2 | MATERIALS AND METHODS

2.1 | Experimental animals

Astatotilapia burtoni were bred under laboratory conditions from a wild-caught stock. Community aquaria contained 10–20 adults and were maintained at conditions mimicking their Lake Tanganyika natural environment (pH = 7.6–8.0; 28–30°C; 12L:12D diurnal cycle). Community fish were monitored daily for the presence of mouthbrooding females, which were identified by the presence of a distended jaw (due to fertilized eggs in the mouth). All experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at Louisiana State University, Baton Rouge, LA, and were in accordance with the guidelines set forth by the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, 2011.

2.2 | Noise exposure protocol

To examine the impact of anthropogenic noise on maternal care behaviors and juvenile development, mouthbrooding females (with developing young in buccal cavity) were randomly assigned to either control or noise sound treatments (Figure 1). We used the same experimental setup and noise exposure protocol as previously published for *A. burtoni*[28] (Figure S1). Briefly, mouthbrooding females were transferred to the 38-L experimental tank on day 7 of mouthbrooding (i.e., halfway through the brood period). After a short acclimation (15 min), fish were exposed to their assigned sound condition for 3 h and allowed to recover in silence for 30 min before being placed in

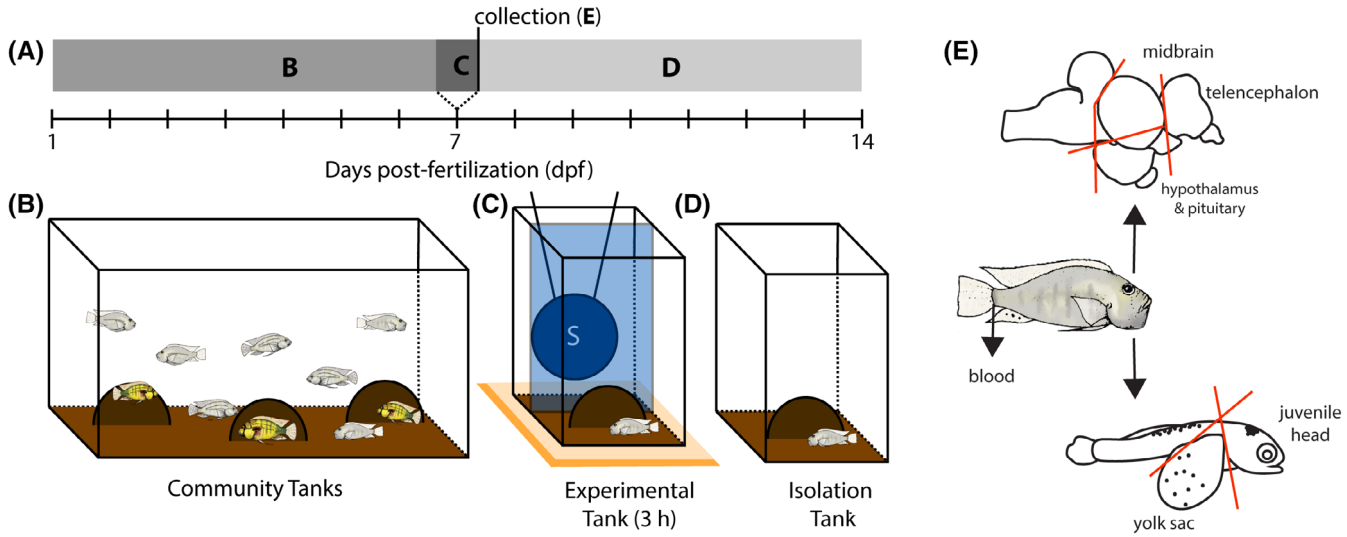

FIGURE 1 Experimental setup for noise exposure. Mouthbrooding females were monitored in community tanks (A, B). In the morning of the seventh day of mouthbrooding, females were placed in the experimental tank (C) with an underwater speaker (S) and exposed to either noise or silence for 3 h. They were then transferred to an isolation tank (D) and monitored daily. A second group of fish was collected immediately after noise exposure (E). Approximate cuts for female brain and juvenile dissections are depicted by red lines in (E)

isolation in a 38-L recovery tank. The noise sound condition was generated by playing an audacity-generated (Version 2.1.1 from https://audacityteam.org/) sound file comprised of pure tones ranging from 100 to 2000 Hz and lasted ~5 min in length but was played on a loop for a total of 3 h. *Tone* order and duration (0.5–4.0 s) was randomized. The computer-generated sound file was amplified (CA-160; TOA, Hyogo, Japan) before being played through the underwater speaker (UW-30; Electrovoice, NY). A hydrophone (HTI-94, High Tech, Inc., Gulfport, MS; sensitivity −163.7 dB re: 1 V/mPa; frequency response 2 Hz to 30 kHz) was placed at various locations in the front compartment to record sound pressure levels (SPLs) of sound playback. The amplifier was adjusted until the average SPL was ~140 dB re: 1 μPa just above the territory shelter. Estimated source levels in dBrms re: 1μPa were determined from the calibrated hydrophone recording system.[28] A second group of control fish were placed in the experimental setup but without a sound file selected (ambient sound levels just above the spawning shelter: ~90 dB re: 1 μPa). While *A. burtoni* likely responds primarily to the particle acceleration component of sound, short anterior projections of the swim bladder suggest that they may also respond to sound pressure but this requires further experimental study.[29] We used a total of 32 females: 10 per group in behavior experiments, and 6 per group for brain and hormone data.

We chose to use pure tones encompassing the hearing range of *A. burtoni* (<1500 Hz[30]) instead of boat noise recordings. The sound file described above was used in separate studies[28] in which we fully characterized the sound conditions, analyzed frequency-dependent impacts of noise on adult social behaviors, and provide a detailed discussion on the limitations of our experimental paradigm. Briefly, we hypothesized that the above sound file would be easier to characterize and reproduce in small aquaria. Sound playback in small aquaria, even under ideal conditions, cannot adequately mimic natural sound conditions.[31] Because of this, and in an effort to limit resonant frequencies and stay within the output range of the speaker, we used pure tones, which produced a largely broadband sound within the hearing range of *A. burtoni*.[30]

2.3 | Experiment 1: Behavioral responses to noise exposure

Mouthbrooding females transferred to isolation tanks were observed daily for the presence of prematurely released fry or potential filial cannibalism. Females had to be moved into isolation tanks instead of community tanks because other adults immediately cannibalize her brood upon release. While this isolation is not naturally occurring, all fish had similar handling and isolation stress. Once fry were released, the female was "threatened" by an observer quickly approaching the tank to examine if she would provide parental care by taking them back into her buccal cavity, a normal maternal response that typically lasts for ~1–3 days following fry release under these conditions. Following this test, the brooding female was quickly removed from the tank and measured for standard length (SL) and body mass (BM). The day and time of release, presence or absence of parental care behaviors, and number of juveniles were recorded (average of 22 fish per brood). Three to four fry per brood were sampled on days 0, 7, 14, 21 and 28 post release to measure their BM and SL prior to feeding. Fulton's condition factor was calculated as $[BM/(SL^3)]*100$ for each fish. Fish were fed 5% of their BM in crushed cichlid flakes daily for the first 2 weeks followed by a reduction to 3% of their BM in crushed cichlid flakes daily. Juveniles were monitored daily for mortality and onset of adult-typical behaviors and coloration. Each morning (8–10 am when fish were most active), an observer watched the fish for 10 min and recorded if any fish displayed coloration (e.g., eyebars, yellow coloration, etc.) or territorial behaviors, such as chasing or biting other juveniles.

To examine how noise exposure during development might impact freezing behaviors in *A. burtoni* juveniles, we examined the time spent stationary after an acoustic startle at 14 and 28 days post-release (dpr). Across taxa, freezing behaviors, or time spent motionless within an arena or aquarium, is used as a measure of stress, with increased stationary time correlated with higher stress.[32] Alternatively, freezing after a fear-inducing stimulus is a common anti-predator behavior in fishes.[33] Ten fry per brood were placed in a 38-L aquarium with no shelter. After a 10 min acclimation period, a padded hammer was gently tapped against the outside of the tank producing a ~ 130 dB re: 1 μPa acoustic stimulus. A video camera (Canon HFR400; Melville, NY) recording at 60 frames per second was placed immediately above the aquarium and recorded for 5 min after the acoustic startle. Videos were later analyzed by an observer blind to brood treatment identity. The first frame of the acoustic stimulus marked the "start time." The video was then slowly scanned to determine when at least 5 fish (50%) resumed swimming to quantify the latency to return to normal swimming (i.e., freezing time).

Juvenile *A. burtoni*, like many juvenile organisms, will shoal when placed in a large, open space. We measured shoaling behavior in control and noise-exposed broods at 14 and 28 dpr. Ten fry per brood per placed into a 38-L tank with a grid (2 cm × 2 cm, black lines on white background) placed underneath. The aquarium was filled to a depth of 10 cm to ensure fish spread out horizontally and not vertically in the water column. In one noise exposure group, less than 10 juveniles were present by the 28 dpr trial, so this brood was excluded from this time point. After a 15 min acclimation time, behaviors were video recorded from above the tank for 10 min. The video was later analyzed by an observer blind to brood treatment identity. We calculated average distance to nearest neighbor and average distance between fish in ImageJ (imagej.nih.gov/ij/) as the distance between points placed on the junction between the head and trunk of the fish (origin of the dorsal fin; Figure S2). Each measurement was taken for five randomly selected frames within the 10 min video and averaged together for each individual. Additional sampling (every 10 s for the duration of the trial) did not significantly affect the data because fish were generally stationary and moved very little after the initial acclimation period.

For all juvenile measurements, it is possible that the same fish were sampled at multiple timepoints. Because noise-exposed broods had higher mortality, this increases the likelihood that noise-exposed animals were repeatedly sampled compared with control juveniles. As such, it is possible that any observed behavior changes could be influenced by this repeated handling and sampling.

2.4 | Experiment 2: Neural and physiological responses to noise

2.4.1 | Tissue collection

Using the same noise exposure protocol described above, a second group of fish was collected for RNAseq analysis to examine the effects of noise exposure on brain transcriptomes. Immediately following the 3-h exposure, mouthbrooding females were measured for SL and BM, and blood was collected from the caudal vein, centrifuged at 8000 RPM for 10 min, and the serum stored at −80°C until processing for cortisol measurements. Fish were quickly sacrificed by rapid cervical transection, and the brain quickly removed from the head. The brain was macrodissected into five parts (Figure 1): (1) telencephalon and olfactory bulbs, (2) hypothalamus with pituitary, (3) tectum and thalamus, (4) cerebellum and (5) hindbrain. Cuts were made carefully to ensure that the preoptic area remained with the hypothalamic sample. Three offspring were also collected per brood. Their yolk sacs were dissected and collected for cortisol measurements, and likely included parts of the developing spinal column and overlying muscle and skin. Because of the small size of the fry, the entire heads were collected for RNAseq. Three juveniles were pooled to ensure enough RNA sample for processing. All tissue collected for RNAseq was immediately flash frozen in liquid nitrogen to preserve RNA integrity and stored at −80°C until extraction.

RNA was extracted using RNeasy Plus Mini kits (Qiagen, Germantown, MD) following the manufacturer's protocols. Briefly, the samples were homogenized using a tissue ruptor and vortexing, centrifuged and the supernatant was collected. Samples were then run through gDNA eliminator columns, processed per kit instructions and eluted with 32 μL of RNase free water. Samples for RNAseq were shipped on dry ice overnight to Novogene (Sacramento, CA) for further processing. RNA yields for cerebellum and hindbrain samples were too low for library prep and were excluded from sequencing. Samples with low RNA quantity, RNA quality and library prep efficiencies were excluded from downstream processing, resulting in a total of 4–5 biological replicates per region per sound condition.

2.4.2 | RNA-sequencing and analysis

RNA sequencing was done by Novogene using recommended protocols. Libraries were run on an Illumnia NovaSeq 6000 with a paired-end 150 read length and an average of ~50 million reads per sample. Paired-end clean reads were aligned with the *Astatotilapia burtoni* genome (NCBI/USCS/Ensembl; Burton's Mouthbrooder) using STAR v2.5. HTSeq was used to count the number of reads mapped of each gene and calculate FPKM (fragment reads per kilobase per million mapped reads). Differential expression of gene read counts was analyzed using DESeq2 v1.26.0 in R[34] using a negative binomial GLM model, lfcshrink set to apeglm, and *p*-values adjusted using the Benjamini and Hochber's approach. Each brain region was run independently with the main effect of noise being examined. Gene ontology (GO) enrichment analyses and KEGG analyses were done using the clusterProfiler v 3.12.0 R package.[35]

2.4.3 | Cortisol assay

To examine how noise exposure impacted cortisol levels, we used an enzyme linked immunosorbent assay to measure cortisol from serum

of control and noise-exposed brooding females and from yolk sacs of their broods (Cayman Chemical, Ann Arbor, MI). Steroids were extracted from the yolk using modified ethyl acetate protocols.[36,37] Briefly, yolks were thawed to room temperature, homogenized in ethyl acetate using a tissue ruptor for 15 s, centrifuged at full speed for 5 min, and the supernatant was reextracted with ethyl acetate. Samples were then left to evaporate overnight and reconstituted with 105 μL of kit ELISA buffer. Serum samples were diluted 1:35, and kit protocols were strictly followed. All samples were run in duplicate and fit on a single plate, which was read in triplicate at 405 nm. Concentrations were determined based on a standard curve with an intra-assay CV of 9.65%. Cortisol assay kits were previously validated for this species.[38]

2.5 | Statistics

RNAseq data were analyzed as described above in Section 2.4.2. All other statistics were performed in SigmaPlot 12.3 (San Jose, CA). Student's *t*-tests were used to compare data between the two sound conditions when not measured across time. Since data points were collected from the same brood of fish weekly (growth and mortality) and biweekly (shoaling and freezing time), a 2-way repeated measures ANOVA was used. Treatment (control vs. noise) and age (0, 7, 14, 21, 28 dpr) were fixed factors with brood identity as a random effect. ANOVAs were followed by Tukey's post-hoc testing. All data were checked for outliers using Grubbs outlier test, but none were detected, and normality and equal variance were met with all data sets. Mean ± SD is represented by closed circles and error bars in each figure.

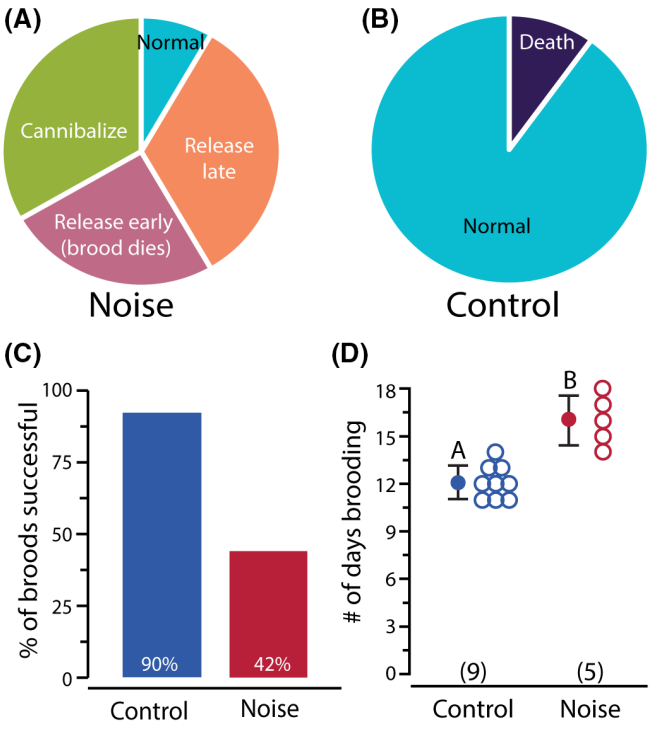

FIGURE 2 Exposure to noise during mouthbrooding impairs maternal care. Noise-exposed brooding females (A) were more likely to cannibalize (green, 33%) and prematurely release (pink, 25%) their brood compared with control females (B), which resulted in reduced brooding success (C). Noise exposed females also held onto their brood for significantly longer than control females (D). Release early: <10 dpf; Normal: 10–14 dpf; Release late: >14 dpf. *N* = 10 control and 12 noise brooding females, but only nine control and five noise females released broods for measurements in D. In (D), data points are plotted as unfilled circles with mean ± SD plotted to the side of each group. Different letters indicate statistical significance at $p < 0.05$

3 | RESULTS

3.1 | Noise impacts brooding success and maternal care

Mouthbrooding females typically carry developing broods for 10–14 days. Nine control females released their fry within this window and one female died (Figure 2B). In contrast, only one of the 12 noise-exposed brooding females released her brood within the normal time frame (Figure 2A). Of the remaining noise-exposed females, four released between 14 and 18 days post fertilization, three pre-maturely released underdeveloped fry (with un-resorbed yolk sacs) shortly after noise exposure, and four cannibalized their brood (verified by dissection). As such, control females had 90% successful broods, but noise-exposed females had only 41.67% success (Figure 2C). Of the successful broods, control females held their broods for significantly less time than noise-exposed brooding females (Figure 2D; $t(12) = -5.557$, $p = <0.001$). All nine control females performed post-release maternal care behaviors (i.e., taking released fry back into their mouths for protection) but only 60% (3 of 5) of noise-exposed brooding females performed this same maternal care behavior; however, this is not statistically different (Fisher's exact test; n = 14 total, $p = 0.110$). There was no difference in the size of the broods at release (Figure S3; $t(12) = 0.009$, $p = 0.993$) with an average of 22 fish in each brood.

3.2 | Noise exposure increases female cortisol and alters the brain transcriptome

Noise-exposed brooding females had higher circulating cortisol levels immediately after noise exposure compared with control females (*t*-test; $t(10) = 3.256$; $p = 0.008$; Figure 3A). There were no differentially expressed genes in the telencephalon between control and noise-exposed females, and only 15 (4 down, 11 up; Figure 3B) differentially expressed genes in the midbrain of noise-exposed females. However, there were almost 1200 differentially expressed genes in the hypothalamus of noise-exposed females compared with control females (Figure 3C). In the hypothalamus, neuroactive ligand-receptor pairs were differentially expressed, including many related to metabolism,

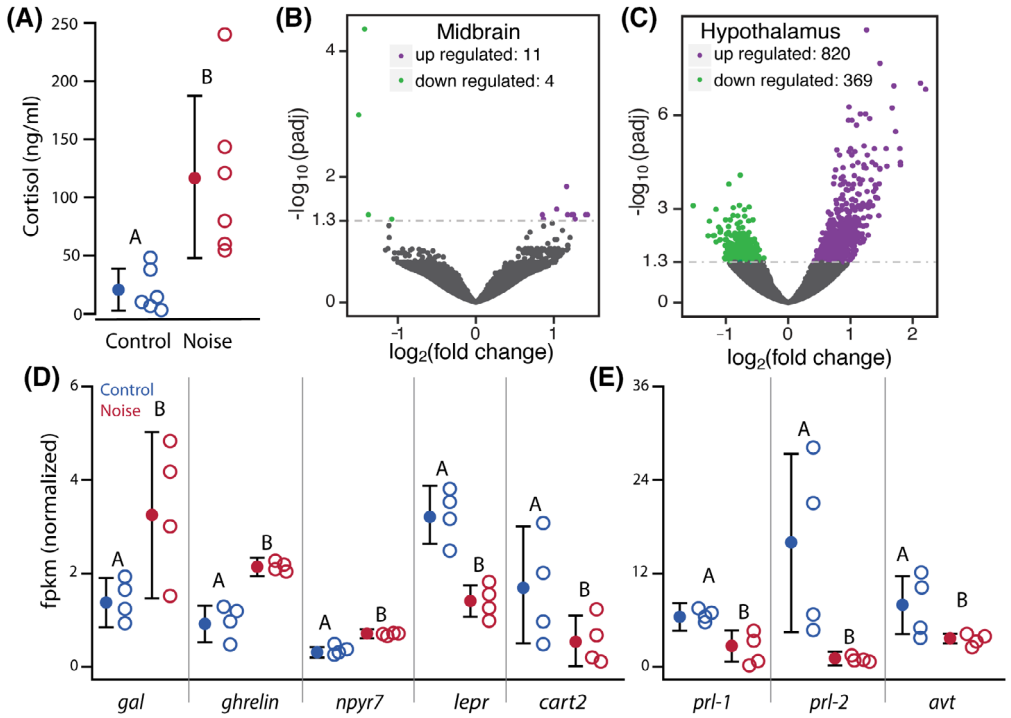

FIGURE 3 Noise exposure increases circulating cortisol and alters hypothalamic transcriptomes in mouthbrooding females. Noise-exposed brooding females have higher cortisol immediately after the exposure (A). There are 15 differentially expressed genes in the midbrain (B) and almost 1200 differentially expressed in the hypothalamus (C) of noise-exposed females. Feeding-related (D) and maternal care-related (E) genes are differentially expressed in the hypothalamus between control and noise-exposed females. Data points are plotted as unfilled circles with mean ± SD plotted to the side of each group. Different letters indicate statistical significance at $p < 0.05$ within a gene. Fpkm: fragments per kilobase of transcript per million mapped reads, normalized to fit on same scale. Gene abbreviations are as follows: *avt*: arginine vasotocin; *cart2*: cocaine and amphetamine related transcript 2; *gal*: galanin; *lepr*: leptin receptor; *npyr7*: neuropeptide Y receptor 7; *prl*: prolactin

reproduction and social behaviors. Appetitive or food-intake stimulating neuropeptides, such as galanin and ghrelin, and neuropeptide Y (NPY) receptors, were up-regulated in noise-exposed animals while leptin receptor and CART transcripts, which inhibit food intake, were down-regulated (Figure 3D). Prolactin and vasotocin, both of which are linked to maternal care behaviors, were down-regulated in the hypothalamus of noise-exposed compared with control animals (Figure 3E). A complete list of differentially expressed genes can be found in Table S1.

3.3 | Noise decreases fry condition and increases mortality

Released fry were assessed on the day of release (0 dpr) and 7, 14, 21 and 28 dpr (Figures 4 and S4; see Table 1 for detailed statistics). Since noise-exposed brooding females held their broods for longer, noise-exposed juveniles were inside the female's mouth for ~2 days longer than control fish, so their age is ~2 days older at each corresponding dpr timepoint. During the first 28 days post-release, juveniles exposed to noise during brooding differed in SL compared with control juveniles in an age-dependent manner (treatment*dpr: $p = 0.014$). SL did not differ at release ($p = 0.075$), but noise-exposed fish were significantly longer than controls at 7, 14 and 21 dpr ($p < 0.001$), possibly due to being slightly older. However, by 28 dpr, juveniles had a similar SL between treatments ($p = 0.381$). BM also differed with noise exposure and age. Overall, noise-exposed juveniles weighed less than control juveniles ($p = 0.010$), and all fish were significantly larger each successive week ($p < 0.001$). By calculating condition factor, which takes into account both BM and SL (see methods for formula), we found that control juveniles had higher condition factors than noise-exposed fish at most timepoints (Figure 4A; treatment*dpr: $p = 0.013$). Control juveniles had a higher condition factor at release ($p = 0.011$), and at 7 ($p < 0.001$), 14 ($p = 0.001$) and 21 dpr ($p < 0.001$), but by 28 dpr, fish had similar condition factors ($p = 0.550$). In addition, noise-exposed broods had higher mortality rates after release compared with control broods. Within control animals, there was no change in mortality among the different weeks, however, in noise-exposed broods, animals had significantly higher mortality during the first week compared with weeks 2–4 ($p < 0.05$). Up to 60% mortality during the first month was observed in noise-exposed broods, while control broods had at maximum 25% mortality during the first month (*t*-test; $t(12) = -4.407$, $p < 0.001$).

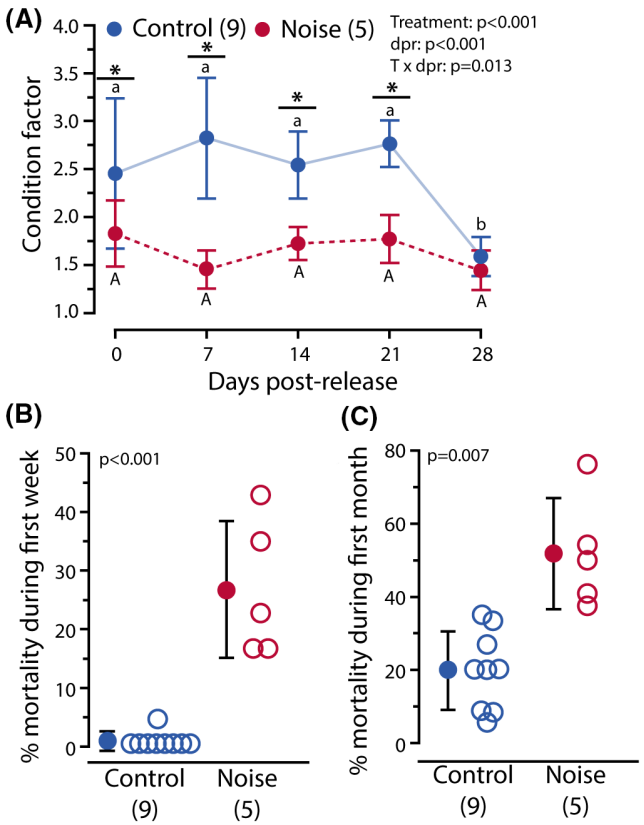

FIGURE 4 Juveniles exposed to noise during development have slower growth rates and higher mortality after release. Noise-exposed fish have lower condition factors than control fish for the first several weeks of development (A). More noise-exposed fish per brood died during the first week (B) and month (C) post-release compared with control broods. Filled circle with error bars represents mean ± SD and individual data points are plotted as unfilled circles with mean ± SD next to each group. Different capital letters represent differences within the noise group across time while lowercase letters represent differences within the control group across time. * indicates differences between groups (i.e., between noise and control treatments). Different letters indicate statistical significance at $p < 0.05$. Detailed growth and mortality data can be found in Figure S4

3.4 | Noise alters juvenile behaviors

When presented with an acoustic startle stimulus, all juvenile fish exhibited a startle response that resulted in freezing behaviors. We measured the time delay between the stimulus onset and when fish returned to normal swimming behaviors at 14 (Figure 5A) and 28 dpr (Figure S5). Juveniles exposed to noise during development took significantly longer to return to normal swimming compared with control fish at both time points ($p < 0.001$). Shoaling behavior, or how close together fish swam in an open aquarium, was dependent on both sound condition (Figure 5B) during development as well as the time tested (14 vs. 28 dpr). Overall, noise-exposed juveniles swam closer together than control juveniles ($p = 0.001$), and fish swam closer together at 14 dpr than at 28 dpr ($p = 0.027$).

To assess if appearance of adult-typical behaviors/coloration was affected in juveniles after they experienced noise exposure during the brooding period, we identified that the first day a fish from each brood was observed with adult-typical coloration or displaying an adult-typical aggressive behavior (Figure 5C,D). Control fish first displayed coloration ~30 dpr (29.78 ± 2.77 days) while noise-exposed broods did not develop colors until ~36 dpr (36.40 ± 3.36 days). Typically, only a single (often the largest) fish was observed with yellow coloration and a faint eyebar at this stage. More complex coloration (vertical banding, fin spots, egg dummies) were not present during the first 60 dpr in either group. A similar pattern was observed for the day of first aggressive behavior. A single fish was observed chasing other fish from the terracotta pot (i.e., territory), but no other adult-like aggressive behaviors were observed during the first 60 dpr. This chasing behavior was first observed at ~31 dpr (30.67 ± 2.06 days) in control broods, but not until ~38 dpr (37.60 ± 4.39 days) in noise-exposed broods. As such, both onset of adult-typical coloration ($p = 0.002$) and behaviors ($p = 0.002$) were delayed in noise-exposed broods. Control and noise-exposed fish had similar adult-typical social behaviors and coloration later in life at ~4 months of age.

TABLE 1 Detailed statistical outputs for analysis of juvenile growth and behaviors

		Noise		Age		Noise * Age	
	Test	*F/t*	*p*	*F*	*p*	*F*	*p*
Standard length	2-way RM ANOVA	32.179	**<0.001**	668.015	**<0.001**	3.509	**0.014**
Body mass	2-way RM ANOVA	9.276	**0.010**	287.885	**<0.001**	0.550	0.700
Condition factor	2-way RM ANOVA	61.544	**<0.001**	6.084	**0.011**	3.547	**0.013**
% Mortality	2-way RM ANOVA	17.921	**0.001**	19.393	**<0.001**	6.909	**<0.001**
Freezing time	2-way RM ANOVA	44.133	**<0.001**	0.002	0.964	5.240	**0.043**
Shoaling density	2-way RM ANOVA	20.788	**<0.001**	6.732	**0.027**	1.161	0.307
Coloration	*t*-test	3.981	**0.002**				
Aggression	*t*-test	4.083	**0.002**				

Note: Bold indicates $p < 0.05$.

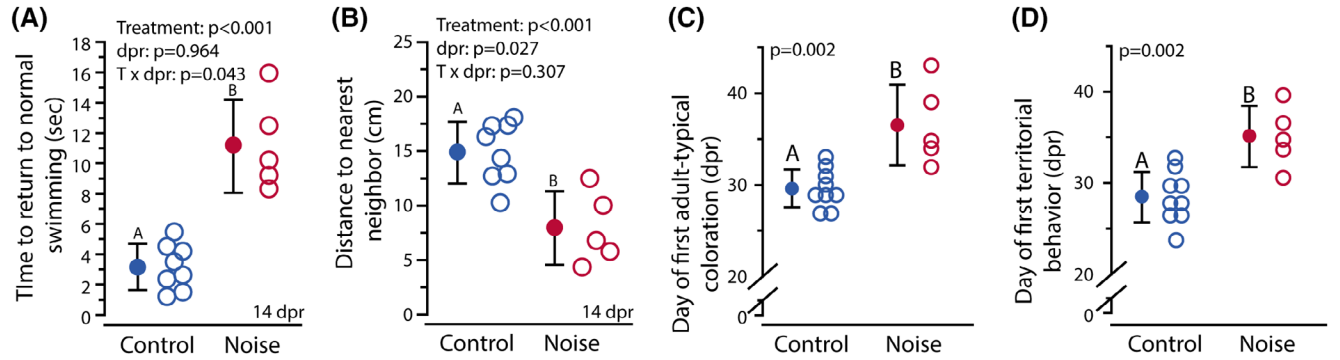

FIGURE 5 Noise exposure during the mouthbrooding development period impacts post-release juvenile behaviors. Fish exposed to noise during development take longer to return to normal swimming after an acoustic startle (A). Noise-exposed fish shoal closer together compared with control fish (B). Noise-exposed juveniles first display adult-typical color (i.e., eyebar, yellow/blue body colors, fin spots) (C) and behaviors (D) at a later age than control juveniles. Individual data points are plotted as unfilled circles with mean ± SD plotted next to each group. Dpr, days post-release. Different letters indicate statistical significance at $p < 0.05$. Only data for 14 dpr are plotted in (A) and (B), but data for 28 dpr are in Figure S5. Despite the treatment*dpr interaction, the general pattern in (A) is the same at 28 dpr

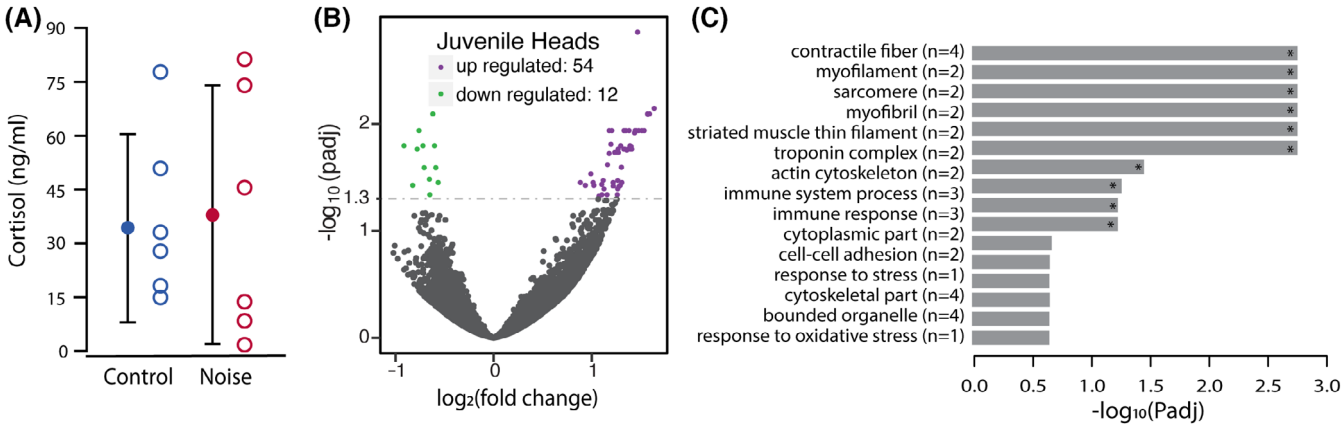

FIGURE 6 Noise exposure alters head transcriptome profile but not yolk cortisol levels in juveniles. Cortisol levels from extracted yolk sacs is not different between control and noise-exposed larvae (A). Noise exposure results in up- and down-regulated transcripts (B) related to growth, development and immune responses (C). Only the 15 GO classes with the highest adjusted *p*-values are plotted in (C). * in (C) represents GO analysis classes that are significantly differentially expressed between the two groups. *n*-values represent the number of transcripts differentially expressed in each GO class

3.5 | Noise exposure to developing larvae alters head transcriptome

Cortisol levels in yolk from control and noise-exposed developing juveniles were not different (*t*-test; $t(10) = 0.235$; $p = 0.820$; Figure 6A). Heads from noise-exposed larvae had 66 differentially expressed genes (12 down, 54 up). The gene for isotocin receptor was up-regulated in noise-exposed juveniles compared with controls (Figure 6B), an interesting find since nonapeptides are linked to altered sociality in some childhood neurodevelopmental disorders.[39,40] Of the other up-regulated genes, eight were related to immune function or inflammatory responses. Sixteen genes involved in growth and development of muscles, connective tissues and bone were up-regulated, while three down-regulated genes are involved in proper development of the nervous system (e.g., scribble and frizzled). GO analysis revealed that gene classes related to muscles and immune processes were differentially expressed in response to noise (Figure 6C). A complete list of differentially expressed genes can be found in Table S1.

4 | DISCUSSION

Anthropogenic noise is now pervasive to almost all aquatic and terrestrial environments. For fishes, the largest and most diverse group of vertebrates, underwater noise can have devastating effects on their growth, reproduction and communication, with impacts observed both at individual and population levels.[2] Although previous work found that anthropogenic noise can impact growth and development to varying degrees,[10,24-27] particularly in early life stages, no study has examined the impact of noise in mouthbrooding fishes. Mouthbrooding provides a unique situation because effects observed

in juveniles could be direct (i.e., on developing juveniles themselves) or indirect (i.e., impacts on the brooding parents that influence developmental conditions). We found that exposure to noise during mouthbrooding affected both the mouthbrooding females themselves and the developing juveniles, potentially in interconnected ways. For the first time, we also provide evidence that anthropogenic noise alters brain transcriptomic profiles, with notable changes observed in metabolic, reproductive and parental care related pathways.

Anthropogenic noise affects parental care across taxa, with animals being less attentive during periods of noise.[41-46] For example, tree swallow parents visit their nest less frequently during periods of noise-playback compared with silent periods.[45] Male smallmouth bass (*Micropterus dolomieu*) guarding nests with egg-sac fry-stage offspring had decreased parental care behavior during noise,[44] but effects were dependent on the stage of the offspring. Similarly, both cooperatively breeding cichlid *Neolamprologus pulcher* and spiny chromis damselfish (*Acanthochromis polyacanthus*) parents spent more time performing territorial, defensive behaviors and less time attending to their nest.[42,46] This change in time allocation means that nests/offspring are more prone to predation. In *A. burtoni*, a species completely reliant on mouthbrooding for reproductive success, exposure to noise during brooding resulted in dramatic changes to maternal care behaviors. Noise-exposed females were more likely to cannibalize or prematurely release underdeveloped larvae. In addition, females that did successfully carry and release a developed brood held onto their brood for significantly longer than control fish. Only one of the 12 noise-exposed fish fit the characteristics of a "typical" brooding period. Together, this resulted in >90% of noise-exposed females with altered maternal care behaviors and only a 42% successful brooding rate. Although noise-exposed females held onto their brood longer, this could be considered advantageous since it allowed for further growth and protection to the developing juveniles. However, upon release, females were less likely to perform protective parental behaviors. Only 60% of noise-exposed brooders retrieved their brood when presented with a threat, compared with 100% of control females performing this common maternal care behavior; although, with so few noise-exposed broods, this difference was not statistically significant. Noise exposure shifted parental care behaviors from short brooding time with high post-release retrieval behaviors to a longer brooding time with diminished retrieval behaviors. Changes to parental care behaviors can have devastating effects on reproductive fitness of animals with potential consequences on species survival.

Similar to that observed in several other fishes,[15,16] *A. burtoni* mouthbrooding females exposed to noise have higher circulating cortisol compared with control animals. This noise-induced cortisol rise could explain the changes in maternal care that happen shortly after noise exposure (i.e., spitting out underdeveloped fry, or cannibalism). Elevated cortisol negatively affects parental care behaviors and results in decreased nest success in smallmouth bass.[41] In tree swallows, glucocorticoids may be important for parents to strategically respond to offspring's needs within different social and environmental contexts.[47] While elevated circulating cortisol after noise exposure in female *A. burtoni* may be related to aspects of parental care, stress response, or shifts in allocation of energy resources, it is also important to recognize that glucocorticoid signaling is complex and involved in many other homeostatic processes that would require further study.

Parental care involves trade-offs between offspring-promoting and self-promoting behaviors. This is especially true for provisioning of energetic resources, with feeding and parental care strategies often being linked. In mouthbrooding species, they undergo a period of forced starvation for the duration of brood development. While the neural control of this is still not completely understood, it likely involves the integration of feeding-related and social behavior-related neural circuitry.[48-50] Our analyses reveal that many feeding-related neuropeptides are differentially expressed after noise-exposure. Galanin, which has emerged as key candidate molecule for regulating parental care and infanticide across taxa,[51,52] is up-regulated in noise-exposed animals. In general, orexigenic (appetite-stimulating) neuropeptides were upregulated while anorexigenic (appetite-inhibiting) neuropeptides were down-regulated. Prolactin, the hormone responsible for mammalian lactation[53,54] and closely tied to the display of parental care behaviors,[55-57] was also down-regulated in noise-exposed individuals. We found no differentially expressed genes in the telencephalon of noise-exposed females and only minimal differences in the midbrain/tectum samples. In contrast, the high number of differentially expressed genes in the hypothalamus likely reflects the role of this region in homeostatic functions, such as feeding, metabolism and stress. Together, changes in feeding-related and parental care-related neuropeptides could signal a switch from offspring-promoting behaviors (starvation) to self-promoting behaviors that could explain the increased cannibalism and decreased parental care observed in our noise-exposed group.

Since developing juveniles are contained in the buccal cavity of brooding females, changes in the mother's physiology and behavior could have direct consequences on juvenile development and/or noise could directly impact developing young themselves. We found that juveniles exposed to noise during development had lower condition factors. While these noise-exposed fish had similar body lengths, indicating similar growth rates, they had a lower BM and appeared to have very little fat or muscle mass. Despite identical feeding regimes, noise-exposed fish had a harder time putting on weight. Interestingly, Simpson et al.[58] found that exposure to anthropogenic noise increased the metabolic rate of developing Ambon damselfish (*Pomacentrus amboinensis*). While developing young did not have higher cortisol immediately after noise exposure, changes in stress physiology at later developmental stages could cause higher metabolic rates and decreased muscle mass,[59] possibly explaining lower BM in noise-exposed fry after their release. In addition to changes in growth, noise-exposed juveniles had higher mortality during the first month, most commonly within the first week after release. While <1% of control juveniles died during the first week after release, up to 50% of noise-exposed juveniles died during this same time. Mortality did stabilize slightly over the month, but total mortality of noise-exposed juveniles was 51.35% compared with 20.50% in control animals. This higher mortality during early life could be due to a lower condition and general poorer health. Nedelec et al.[46] also found that juvenile

A. polyacanthus exposed to noise during development had decreased survival likelihood but attributed this to higher predation. They did, however, observe that parental care-providing males performed less "glancing" behaviors, which transfers mucus to their offspring. This mucus contains proteins, hormones, immunoglobulins, ions and microorganisms, which are important for offspring development and growth.[46] Female *A. burtoni* are thought to provide mucus to their developing brood, which contains important components related to immunity, growth and health.[60] If the offspring receive less mucus from the mother, or if the mucus composition changes as a result of anthropogenic noise, this could decrease health of the offspring. Overall, decreased juvenile growth and increased mortality ultimately results in decreased reproductive fitness and can affect species persistence.

In addition to changes in physiology (i.e., growth), juveniles exposed to noise while still inside the mother's mouth also had altered behaviors after they were released. Shoaling is a natural behavior observed in approximately half of all fishes when individual fish stay in close proximity to each other for social reasons,[61] enhanced protection from predators, and increased foraging efficiency.[62] We found that noise-exposed fish formed tighter shoals (i.e., swam closer together) than control fish. Fish swimming closer together could reflect a higher perceived threat[63,64] or a greater need for information transfer between the fish.[65-67] In addition to shoaling, we used freezing time in response to an acoustic startle as a measure of stress (for review see[32].) or anti-predator behavior.[33,68] While all fish appeared to have a similar startle response to the acoustic stimulus (although this needs to be verified with high-speed video), the time it took for fish to return to normal behaviors after the stimulus differed with noise condition. In accordance with the changes observed with shoaling, noise-exposed juveniles took longer to return to normal swimming behaviors compared with the control juveniles. Together, these changes in behavior suggest that exposure to noise at a critical developmental timepoint can have lasting effects on social and stress-related behaviors.

We also found that noise-exposed larvae had altered head transcriptome profiles. Despite having lower BM at later developmental timepoints, noise-exposed larvae had higher levels of transcripts involved in muscle and bone formation. In contrast, several transcripts related to the proper development of the nervous system were down-regulated. Scribble and frizzle, which are both down-regulated in noise-exposed larvae, are important for cell polarity and general cellular health.[69-71] Their deficits are often linked to higher incidences of cancer, disrupted retinal, cochlear and vestibular function, and deficient neural development in mammals.[72-75] Many immune-related genes were up-regulated in noise-exposed individuals, suggesting a possible immune or inflammatory response that could have lasting effects on growth and development. Interestingly, isotocin receptors were up-regulated in noise-exposed juveniles, suggesting a possible increase in nonapeptide signaling. Nonapeptides (e.g., isotocin, homologous to oxytocin, and vasotocin, homologous to vasopressin) have emerged as candidate therapeutic agents for some childhood psychopathologies because they mediate social recognition and play behaviors.[76-78] There is robust clinical evidence that treatment with nonapeptides can ameliorate social functioning in neuro-atypical adults.[40,79] Although not examined in detail here, these changes in gene expression could indicate that exposure to noise during development can change developmental trajectories related to physiology and sociality, possibly influencing future social behaviors as adults.

Taken together, our data indicate that noise exposure during development affects early-life (<1 month) behaviors and physiology. However, by ~1 month post release, noise-exposed fish are not different from juveniles that were not exposed to noise during development but had ~50% mortality. By adulthood, these two groups of fish are indistinguishable based on condition factors and behavioral observations. Although more research is needed to test the exact physiological mechanisms leading to these changes, we propose that the observed early life effects are due to differences in stress physiology. While the noise may not directly impact the developing larvae, it is a stressor for the brooding female that resulted in higher levels of circulating glucocorticoids. Developing larvae feed on the mucus and secretions from inside the brooding female's mouth. We hypothesize that the noise-induced cortisol rise in brooding females is passed on to her brood through her mucus, which could affect their stress physiology and resilience.[80-82] A more reactive stress system, coupled with changes in immune and other growth-related transcripts, could lead to transient changes in physiology and behavior during the first few weeks following the noise.

Anthropogenic noise and increasing background sound levels are a prevalent problem in today's world and are only projected to worsen in coming years. Territorial and site-attached animals living in noise-polluted areas are unlikely to leave, even in unfavorable conditions. We show here that even a single exposure to noise during mouthbrooding has dramatic effects on maternal care behaviors, stress hormones and gene expression. Over half of noise-exposed females failed to complete mouthbrooding successfully. Of those that did, their offspring were initially smaller and had higher mortality despite a longer brooding time. Together, this results in significantly diminished reproductive fitness for the females. Since there is high diversity in parental care strategies among fishes and a broad range of acoustic communication and auditory capabilities, it is important to investigate noise-induced impacts on parental care and reproduction in a variety of species. Only after this has been done will we fully understand the detrimental impacts of human activities on fishes and be able to inform policy makers on empirically based ways to effectively alleviate this pervasive and worldwide problem.

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CONFLICT OF INTEREST

The authors declare no competing or financial interests.

DATA AVAILABILITY STATEMENT

All datasets will be made available upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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