The Journal of Experimental Biology 209, 4444-4451
Published by The Company of Biologists 2006
doi:10.1242/jeb.02525

Dolphin foraging sounds suppress calling and elevate stress hormone levels in a prey species, the Gulf toadfish

Luke Remage-Healey[1,*], Douglas P. Nowacek[2] and Andrew H. Bass[1]

[1]*Department of Neurobiology and Behavior, Cornell University, Ithaca, NY 14850, USA and* [2]*Department of Oceanography, Florida State University, Tallahassee, FL 32306, USA*

*Author for correspondence (e-mail: lrr4@cornell.edu)

Accepted 5 September 2006

Summary

The passive listening hypothesis proposes that dolphins and whales detect acoustic signals emitted by prey, including sound-producing (soniferous) fishes. Previous work showed that bottlenose dolphins (*Tursiops truncatus*) behaviorally orient toward the sounds of prey, including the advertisement calls of male Gulf toadfish (*Opsanus beta*). In addition, soniferous fishes constitute over 80% of *Tursiops* diet, and toadfishes alone account for approximately 13% of the stomach contents of adult bottlenose dolphins. Here, we used both behavioral (vocalizations) and physiological (plasma cortisol levels) parameters to determine if male Gulf toadfish can, in turn, detect the acoustic signals of bottlenose dolphins. Using underwater playbacks to toadfish in their natural environment, we found that low-frequency dolphin sounds ('pops') within the toadfish's range of hearing dramatically reduce toadfish calling rates by 50%. High-frequency dolphin sounds (whistles) and low-frequency snapping shrimp pops (ambient control sounds) each had no effect on toadfish calling rates. Predator sound playbacks also had consequences for circulating stress hormones, as cortisol levels were significantly elevated in male toadfish exposed to dolphin pops compared with snapping shrimp pops. These findings lend strong support to the hypothesis that individuals of a prey species modulate communication behavior in the presence of a predator, and also suggest that short-term glucocorticoid elevation is associated with anti-predator behavior.

Key words: acoustic startle, predation, eavesdropping, natural selection, stress, communication, corticosterone.

Introduction

Communication signals can be conspicuous to both intended receivers (e.g. conspecifics) and unintended receivers (e.g. predators or parasites). The conspicuousness of animal signals can be modified to maximize the benefits of advertisement while minimizing the costs of potential predation and/or parasitism (Bradbury and Vehrencamp, 1998; Zuk and Kolluru, 1998). Most examples of this 'conspicuousness tradeoff' occur in species that rely on visual communication signals (e.g. Endler, 1983; Endler, 1987; Endler, 1992; Forsgren and Magnhagen, 1993; Koga et al., 1998; Evans et al., 2002).

Acoustic advertisement signals should also be susceptible to eavesdropping [or 'interception' (see Myrberg, 1981)] by predators. Empirical evidence supports the hypothesis that acoustic signals become more cryptic in the presence of predators. For example, nesting petrels reduce advertisement calling in response to playback sounds of a predator, the brown skua (Mougeot and Bretagnolle, 2000). Similarly, silver perch that are exposed to playback whistles of the bottlenose dolphin [a primary predator (Barros, 1993)] show a transient reduction in population call amplitude (Luczkovich et al., 2000). Bat echolocation signals have also been shown to suppress or eliminate advertisement calling in both male túngara frogs (Ryan, 1985) and katydids (Faure and Hoy, 2000). Therefore, both predators and prey species monitor and respond to 'eavesdropped' acoustic information, although the proximate mechanisms for anti-predator behavior by vocalizing prey species are not well understood.

Acoustic signaling is the primary mode of communication during the breeding season in toadfishes, when males emit 'boatwhistles' to attract females to their nests and interact with rival males (Fig. 1) (Gray and Winn, 1961). Toadfishes constitute approximately 13% of the diet of adult bottlenose dolphins (Barros, 1993), and Gannon et al. (Gannon et al., 2005) recently showed that bottlenose dolphins exhibit positive phonotaxis toward acoustic playbacks of the vocalizations of Gulf toadfish. Thus, dolphin prey, such as Gulf toadfish, could be under selection to detect dolphin acoustic signals and use this information to adjust mate advertisement calling, though this possibility remains untested.

The mechanisms of behavioral adjustment during predation events are unclear, but may include brief changes in hormone

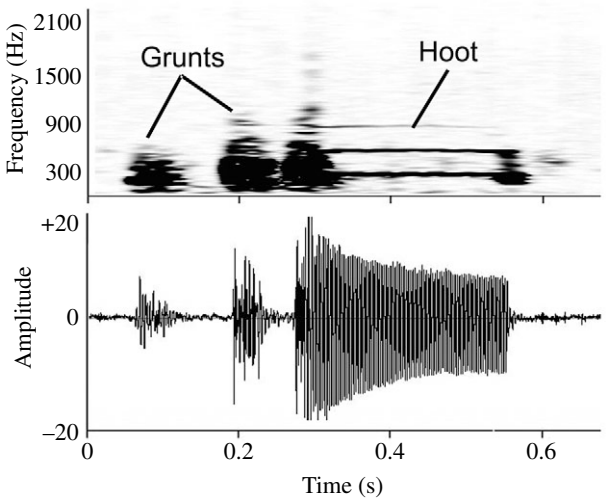

Fig. 1. The advertisement call of male Gulf toadfish is termed a boatwhistle. Shown here is a spectrogram (top) and oscillogram (bottom) of a representative boatwhistle recorded in May, 2002 at 28.6°C. A single call includes a series of non-harmonic 'grunts' that are followed by a multi-harmonic, tonal 'hoot'. Adapted with permission from Remage-Healey and Bass (Remage-Healey and Bass, 2005).

levels. Across vertebrates, including teleosts, exposure to predator cues in a wide-variety of contexts elicits robust increases in circulating stress hormones (Blanchard et al., 1998; Kagawa and Mugiya, 2000; Kavaliers et al., 2001; Cockrem and Silverin, 2002; Clinchy et al., 2004). Although elevated circulating stress hormones are linked to anti-predator behaviors such as defensive posture (Blanchard et al., 1998), it is unknown whether reductions in acoustic behavior and conspicuousness are also linked to changes in glucocorticoids [some evidence indicates that glucocorticoid levels vary in other systems in response to context-dependent acoustic playbacks (e.g. Rukstalis and French, 2005)]. Furthermore, glucocorticoids are known to cause rapid increases in the output of a hindbrain–spinal vocal pattern generator that establishes the temporal properties of natural calls in toadfishes (Remage-Healey and Bass, 2004; Remage-Healey and Bass, 2006), but the relationship of this observation to natural behavior is largely unexplored.

In this study, we test whether exposure to playback of vocalizations of bottlenose dolphins elicits rapid changes in the vocal behavior and/or stress hormone levels in a primary prey species, the Gulf toadfish. Bottlenose dolphins employ a variety of vocalizations during social communication and foraging. High-frequency whistles (5–20 kHz) are used during social communication with conspecifics (Fig. 2A) (Tyack and Clark, 2000; Janik et al., 2006) and echolocation clicks (20–100 kHz) are emitted during navigation and foraging (Au, 1993; Johnson et al., 2004). A third vocalization category, the low-frequency 'pop', has been recently documented during foraging bouts over habitats that may be less amenable to high-frequency echolocation clicks, such as seagrass beds (Fig. 2B) (Nowacek, 2005). However, dolphin prey species such as toadfish may be able to best detect low-frequency pops emitted by foraging dolphins, since toadfish auditory frequency encoding is most robust below 1 kHz (Fish and Offutt, 1972; Yan et al., 2000; Fay and Edds-Walton, 2000; Bass et al., 2001). As shown here, using behavioral (vocalization) and physiological (circulating cortisol levels) measures, Gulf toadfish can apparently recognize the foraging pops of predatory dolphins.

Materials and methods

Male Gulf toadfish (*Opsanus beta* Linnaeus, Günther) were collected from nests in the Turkey Point Basin, adjacent to the Florida State University Marine Laboratory (FSUML) in St Teresa, FL, USA (29°54.9′N: 84°30′W). Collection permit 03SR-688 was obtained from Florida Division of Marine Fisheries. Prior to experimentation, fish were kept in running seawater tanks at FSUML and offered live shrimp and squid as food. Fish were in captivity no longer than 48 h. All experimental procedures were approved by the Cornell University Institutional Animal Care and Use Committee.

Playback, recording and blood sampling procedures were similar to those presented previously (Remage-Healey and Bass, 2005). Playback experiments were performed using enclosures (70×70×10 cm) in the bay at FSUML, which has a water depth of 1–1.5 m. Enclosures were placed on the sea bottom (depth=1.5 m) within a natural group of calling male toadfish with active nests. A section of PVC pipe (8 cm diameter, 25 cm length) was inserted in the enclosure to provide nesting and hiding space. A single male *O. beta* was placed inside each enclosure and each male began emitting boatwhistles (Fig. 1) within 48 h. A hydrophone was placed in the center of the calling population (approximately 3–4 m from any one enclosure) and suspended 4 cm from the sea bottom by a hydrophone stake.

Acoustic stimuli were used with permission from William Tavolga (marine sounds atlas), and were brief clips of bottlenose dolphin and snapping shrimp sounds, as presented in Fig. 2. Stimuli were presented in a looped-mode playback (1 min loop comprising 45 s of stimulus followed by 15 s of silence) for a 5-min playback period (see below). Each fish received only one stimulus per experiment, and only one experimental playback occurred during a 24 h period (within the hours 12:00 to 16:00). Each fish was only used in one experiment, to control for possible habituation effects. Stimuli were broadcast from a portable compact disc player (Memorex) connected to a 12 V powered amplifier (Mofset XAF340 340 W 2/1 channel power amplifier, Namsung, Kent WA, USA) connected to an underwater playback speaker (Aquasonic 229, Clark Synthesis, Littleton, CO, USA). The speaker was suspended from the side of the research boat so that it was 1 m from the sea floor, directly above the calling population of toadfish. All stimuli were presented at approximately 136 dB (see Table 1) to minimize distortion due to over-amplification. The range of instantaneous source levels for dolphin vocalizations is 150–200 dB (Janik, 2000; Tyack and Clark, 2000) and for snapping shrimp 'pops'

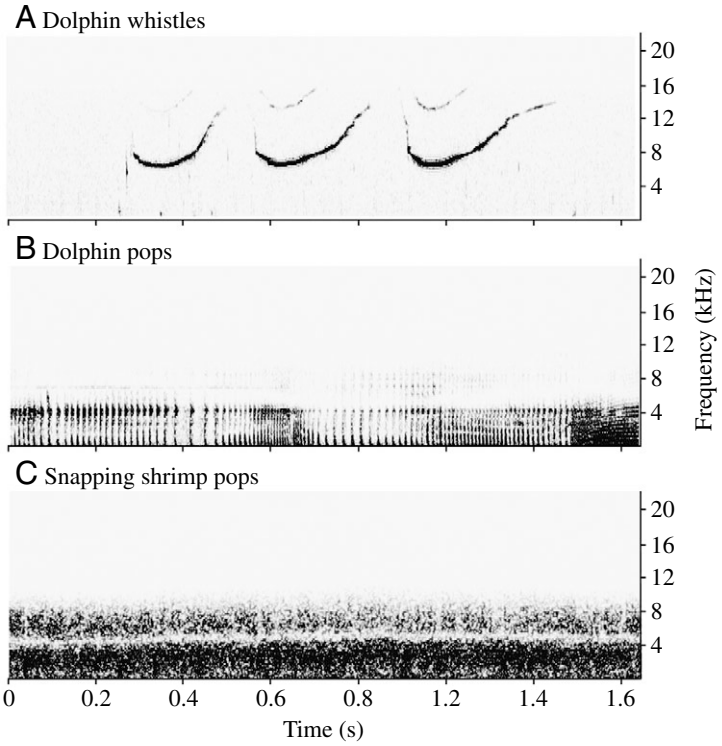

Fig. 2. Examples of playback stimuli sonograms recorded *via* hydrophones placed near vocalizing male Gulf toadfish. Stimuli were high-frequency dolphin whistles (A), low-frequency dolphin pops (B), and low-frequency snapping shrimp pops (C) that served as a control stimulus. Acoustic parameters for each stimulus type are described in Table 1.

is 180–210 dB (Au and Banks, 1998; Versluis et al., 2000). Recorded dolphin pops used in this study were repetitive trains of brief, broadband signals that had significant energy <1 kHz (see Fig. 1 and Table 1), and are similar to the low-frequency 'pops' previously reported to be involved in foraging behavior in bottlenose dolphins (Connor and Smolker, 1996; Nowacek, 2005).

The playback treatment for all groups was divided into three periods: (1) vocal activity was recorded for 5 min prior to playback ('5 Pre'); (2) one of the four stimuli (see Table 1) was then presented for 5 min, in a looped-mode playback ('5 Stim'). (3) 5 min of post-playback activity was then recorded with no playback sound presented ('5 Post'). The number of individuals for each group was: whistles alone (N=5), dolphin pops alone (N=4), dolphin whistles and pops (N=9), snapping shrimp pops (N=7).

Toadfish vocal responses were recorded onto a Sony laptop computer (digitized using Syrinx software, designed by John Burt, Cornell Lab of Ornithology, Ithaca, NY, USA). Levels of stimuli were monitored in the center of the calling population (see above) and are presented as sonograms in Fig. 2. All recording data were analyzed with CoolEdit Pro 1.2a software (Syntrillium, Phoenix, AZ, USA). Recorded individuals were identified unambiguously based on call duration, amplitude, and fundamental frequency (see Edds-Walton et al., 2002; Thorson and Fine, 2002a; Remage-Healey and Bass, 2005; Remage-Healey and Bass, 2006). Two acoustic measurements were quantified for each individual: 'call rate', which is the number of boatwhistles per 5 min playback period, and 'call duration', which is the duration of the hoot portion of each boatwhistle [Fig. 1, defined as the period of the constant-frequency portion of the boatwhistle, after Tavolga (Tavolga, 1958), Thorson and Fine (Thorson and Fine, 2002a; Thorson and Fine, 2002b) and Remage-Healey and Bass (Remage-Healey and Bass, 2005)].

Plasma sampling and analysis

Blood samples were taken from male toadfish following three playback treatments: dolphin whistles and pops, dolphin pops alone and snapping shrimp pops. Field conditions allowed sampling of plasma cortisol from animals in these three groups only. The two groups that were exposed to stimuli containing dolphin pops (dolphin whistles and pops, and dolphin pops alone) were grouped together as 'dolphin pops' for statistical purposes (see below). Following the last playback period (5 Post), individual fish were immediately brought to the surface and briefly anesthetized (<1 min; 0.025% benzocaine) for blood sampling (see Remage-Healey and Bass, 2005; Remage-Healey and Bass, 2006). The operculum was drawn back and gill exposed, and excess water from the gill slits was drawn into a transfer pipette to prevent dilution of the blood sample. A 0.5 ml blood sample was then drawn from the gill arch using a 1.0 c.c. heparinized syringe (26 gauge needle tip). No more than four fish were sampled following any one playback stimulus, and the length of time between the end of playback stimuli and collection of blood sample for all fish ranged from 4.22 min to 18.12 min (mean interval=10.99 min). Only one fish was brought to the surface at a time after playback stoppage, reducing disturbance effects on plasma cortisol levels. No significant difference in plasma cortisol levels was

Table 1. *Acoustic parameters of stimuli used in playback experiments*

Stimulus	Frequency range	Duration	Peak intensity	Repetition rate
Dolphin whistles	6–12.4 kHz	151–240 ms	137 dB (6.5 kHz)	Intermittent
Dolphin pops	410–4500 Hz	3–5 ms	137 dB (900 Hz)	62–117 Hz
Snapping shrimp	520–7400 Hz	4–5 ms	134 dB (2.1 kHz)	194 Hz

Source levels are in dB relative to 1 μPa at 1 m from the speaker.

found between early (4–6 min) and late (12–18 min) samples (*U*-test; $P>0.05$), therefore samples were pooled by group (dolphin pops *versus* shrimp pops) for analysis. Average sampling times did not differ between the two groups (dolphin pops *vs* shrimp pops; *t*-test; $P>0.05$). Whole blood was centrifuged at 400 ***g*** and plasma was stored frozen until later analysis. Plasma was analyzed for cortisol using radioimmunoassay (RIA) at the Diagnostic Laboratory, New York State College of Veterinary Medicine at Cornell University.

Analysis

Behavioral data were analyzed using Statview, version 4.57 and SAS V8 (Abacus, Berkeley, CA, USA) using repeated measures ANOVA followed by Tukey's *post-hoc* tests for within-subject differences over time (before, during and after playback). Data for both call duration and call rate were standardized to baseline levels (100%) to approximate normality. Plasma cortisol levels, as determined by RIA, were not normally distributed (Shapiro–Wilk goodness-of-fit test $P<0.006$), and so these data were analyzed using JMP 5.0.1a using Wilcoxon rank sum tests.

Results

Vocal responses

Call duration (mean call length), did not change significantly across treatment groups and time periods (data not shown; repeated measures ANOVA; $P>0.05$). For call rate (calls per unit time), repeated measures ANOVA revealed significant overall effects of stimulus type ($F=5.35$; d.f.=3,56; $P<0.002$), playback period ($F=3.03$; d.f.=2,45; $P<0.05$) and a period × stimulus interaction ($F=3.10$; d.f.=6,45; $P<0.01$). For the dolphin whistles + pops treatment Tukey's *post-hoc* test showed significant ($P<0.001$) within-group decreases in call rate between the 5 Pre and 5 Stim playback periods (Fig. 3; non-normalized call rate decreased from 9.33 to 4.85 calls/5 min), as well as decreases in call rate between the 5 Pre and 5 Post playback periods (Fig. 3; non-normalized call rate decreased from 9.33 to 5.22 calls/5 min). For the dolphin pops alone treatment, Tukey's *post-hoc* test showed significant ($P<0.03$) within-group decreases in call rate between the 5 Pre and 5 Stim playback periods (Fig. 3; non-normalized call rate decreased from 8.75 to 4.25 calls/5 min), as well as decreases in call rate between the 5 Pre and 5 Post playback periods (Fig. 3; non-normalized call rate decreased from 8.75 to 4.75 calls/5 min). All other within-group *post-hoc* comparisons were not significant ($P>0.05$). Thus, male toadfish groups that were exposed to dolphin pops showed significant reductions in call rate, both during (5 Stim) and following (5 Post) the playback of these stimuli (Fig. 3).

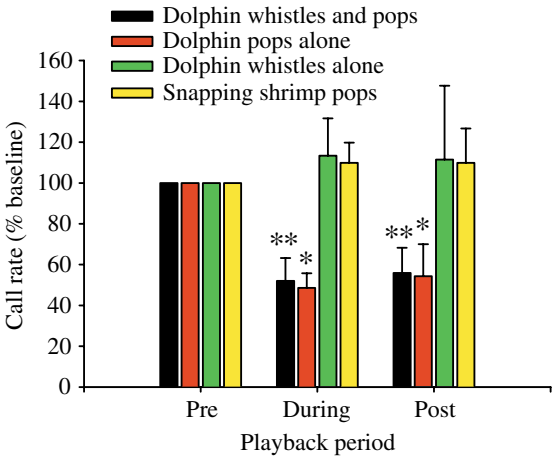

Fig. 3. Low-frequency acoustic signals of a predator, the bottlenose dolphin, rapidly suppress advertisement calling in a prey species, male Gulf toadfish. Relative to pre-treatment calling behavior (Pre), acoustic playback of dolphin whistles and pops ($N=9$) or dolphin pops alone ($N=4$) caused significant suppression of toadfish calling behavior both during (During) and immediately after (Post) 5 min of loop-mode playback. Similar treatment with dolphin whistles alone ($N=5$) or snapping shrimp pops ($N=7$) produced no significant changes in toadfish calling. Bars indicate mean ± s.e.m.; *$P<0.05$, **$P<0.001$.

Hormonal responses

Cortisol levels in the fish exposed to dolphin pops were significantly elevated compared to levels in fish exposed to snapping shrimp pops (Fig. 4; Wilcoxon rank sum test; $\chi^2=5.16$; d.f.=1; $P<0.02$). The dolphin pops group includes all animals exposed to dolphin pops alone ($N=4$) and a subset of animals exposed to dolphin whistles and pops ($N=4$); there were no significant differences between the two groups in cortisol responsiveness (*U*-test; $P>0.05$) and these groups were combined to increase power (overall $N=8$). Field logistics circumvented obtaining blood samples from all animals in the dolphin whistles and pops group.

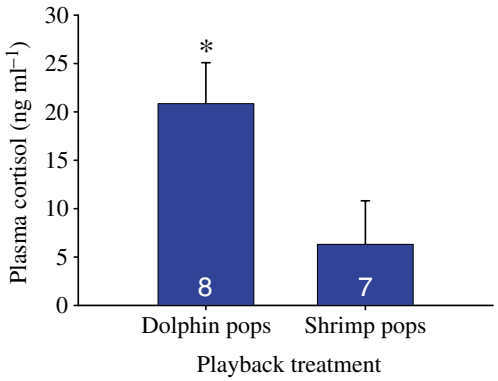

Fig. 4. Predator vocalizations (dolphin pops) induce a significant increase in baseline plasma cortisol levels, relative to control stimuli (snapping shrimp pops). Plasma was immediately sampled following the end of the playback periods. Sample sizes are indicated at the bottom of each bar. The dolphin pops group represents both animals receiving playbacks of dolphin pops alone ($N=4$) and a subset of the animals that received dolphin whistles and pops ($N=4$; also see Fig. 3). Bars indicate mean ± s.e.m. *$P=0.03$.

Discussion

Eavesdropping, or the perception of cues by unintended receivers, has been documented in a variety of species, and is known to influence intraspecific communication (e.g. Earley and Dugatkin, 2002). Interspecific eavesdropping, e.g. between predators and prey, could also exert strong selection on the evolution of acoustic communication when both predators and prey vocalize. This study now shows that the intermittent predation risk posed by dolphin predators has both behavioral and physiological consequences for one of their prey, male Gulf toadfish. Our experiments show that toadfish have adopted behavioral strategies to reduce the risk of predation during the protracted breeding season (May–September) when dolphin predators forage on near-shore grass beds. Reduction in call rate by male toadfish during dolphin foraging bouts probably reflects an evolutionary tradeoff between the fitness benefits of mate-advertisement and the high risk of predation. Recent experimental data demonstrate that bottlenose dolphins orient toward toadfish vocalizations (Gannon et al., 2005), and emit pops during foraging bouts (see below). Taken together, these results suggest that a dynamic, co-evolutionary relationship exists between acoustic communication systems of both members of a predator–prey interaction. Acoustic eavesdropping between predators and prey is very likely to be a widespread phenomenon among vertebrates and invertebrates (e.g. Tuttle and Ryan, 1981; Ryan, 1985; Belwood and Morris, 1987), and this report demonstrates such an interaction between a teleost species and a mammal (see also Luczkovich et al., 2000).

Detection of dolphin sounds

Bottlenose dolphins, as well as other toothed whales, use sound pulses during prey searching, and echolocation clicks are emitted during foraging dives (Johnson et al., 2004; Miller et al., 2004). In particular, bottlenose dolphins use echolocation clicks and low-frequency pops when prey are in seagrass beds, at seagrass edges and over open sand (Tyack and Clark, 2000; Nowacek, 2005), and these are the primary habitats for breeding (vocalizing) Gulf toadfish (Sogard et al., 1989; McMichael, 2002). The current results indicate that male Gulf toadfish can detect low-frequency pops employed by foraging dolphins and not high-frequency vocalizations, such as whistles. Some teleost prey species can detect ultrasonic frequencies produced by whales and dolphins (Mann et al., 1997; Mann et al., 1998; Mann et al., 2001; Higgs et al., 2004). However, the auditory frequency encoding of toadfishes is most robust for $\leq$1 kHz (see Introduction), which is consistent with the utilization of low-frequency sounds in intra-specific communication in toadfish. Our data indicate that toadfish detect the lowest-frequency components of the pops produced by bottlenose dolphins and that they can use this information to adjust advertisement calling.

Perception of dolphin sounds

Categorical perception has been defined as the "*behavioral segmentation of a stimulus that varies continuously along some physical parameter*" (Hoy, 1989). The current results suggest that Gulf toadfish employ categorical perception of auditory cues, in which the valence of sounds is encoded by a combination of frequency, repetition rate and pulse duration. Field playback experiments with toadfish together demonstrate that acoustic cues are divided into at least two categories: (1) stimulatory, such as the advertisement boatwhistles of conspecific males (Fish, 1972; Remage-Healey and Bass, 2005), and (2) suppressive, such as the foraging pops of bottlenose dolphins (current study). This study also used a third playback stimulus, snapping shrimp pops, which did not produce changes in toadfish vocal advertisement calls, despite the fact that snapping shrimp pops and dolphin pops share a common frequency range, peak intensity and pulse duration (Table 1). It is important to note that male toadfish emit individual grunts (30–75 ms duration) during vocal advertisement bouts, and that grunts can be used to interrupt the calling of neighbors in the closely-related *O. tau* (Winn, 1972). The possibility exists, therefore, that dolphin pops were perceived as acoustic signals of competing male toadfish, which then resulted in reduced calling of focal individuals in the current study. However, the temporal acoustic parameters of grunts (30–75 ms duration, <1 Hz repetition rate) differ widely from dolphin pops (Table 1). Moreover, acoustic playbacks of synthesized grunt-like calls at a natural repetition rate (1 grunt/3 s) to this same population of male toadfish did not cause reductions in call rate or changes in plasma cortisol levels (Remage-Healey and Bass, 2005). In addition, Winn (Winn, 1972) observed that calling was not significantly inhibited by grunt stimuli presented at supra-normal rates (>1 Hz) in a field population of the closely-related *Opsanus tau*. The importance of both pulse duration and inter-pulse gaps for signal recognition is shown by playback studies with the closely-related midshipman fish, *Porichthys notatus* (McKibben and Bass, 1998; McKibben and Bass, 2001). We suggest, therefore, that Gulf toadfish are able to differentiate conspecific grunts from dolphin foraging pops by temporal acoustic cues, including duration and repetition rate.

Since repetition rate also differs between snapping shrimp pops and dolphin pops in this study (Table 1), repetition rate may be a critical parameter employed by toadfish to distinguish harmless background noise from predator sounds. A critical test of the above 'categorical perception' hypothesis would be to present advertising toadfish with synthesized dolphin pops at the elevated repetition rate (~200 Hz) observed here for snapping shrimp sounds. Alternatively, the critical parameter that distinguishes dolphin sounds from snapping shrimp pops may be variability in repetition rate, as dolphin vocalizations occur at highly variable rates during foraging bouts (Cranford, 2000). The repetition rate for snapping shrimp pops emitted by individuals is not well understood (see Au and Banks, 1998), however, it is evident that the rate of pops emitted by shrimp populations constitute a uniform background rate with little fluctuation on a minute-by-minute time scale (see Fig. 2)

Stress hormones and anti-predatory behavior

In principle, acute elevation in stress steroid hormones (glucocorticoids) could be a proximate mechanism leading to the adjustment of advertisement signals during predator encounters in male Gulf toadfish [for similar results in roughskin newts see Orchinik et al. (Orchinik et al., 2002)]. Both stress hormones (cortisol) and androgens exert rapid and dramatic effects on vocal patterning *via* actions on the central nervous system in toadfishes (Remage-Healey and Bass, 2004; Remage-Healey and Bass, 2006). In addition, prior field work with Gulf toadfish has shown that rapid (within minutes) elevations in male advertisement calling during territorial challenges are due to similarly rapid actions of androgens on the central nervous system (Remage-Healey and Bass, 2005; Remage-Healey and Bass, 2006). Glucocorticoids and androgens may function differently to mediate the cost/benefit tradeoff between predation risk and mate advertisement, respectively, in Gulf toadfish.

The current results do not indicate whether acute elevations in plasma cortisol are directly responsible for reductions in calling behavior in male toadfish. Indeed, non-invasive treatment with cortisol does not produce significant changes in calling behavior in nesting males in the field (Remage-Healey and Bass, 2006). However, the transition from a non-calling to a calling state is accompanied by a several-fold increase in baseline cortisol levels (Remage-Healey and Bass, 2005), although these levels do not reach the stress-induced levels observed here. We previously hypothesized that this behavioral state-dependent elevation in baseline cortisol levels would aid the mobilization of energy reserves to support the physiological demands of high calling rates (but see Amorim et al., 2002), consistent with the stimulatory effects of cortisol on fictive calls in the laboratory (Remage-Healey and Bass, 2005; Remage-Healey and Bass, 2006). The results of the current study now suggest an inverted U-shaped function between cortisol levels and calling behavior in toadfish, whereby only mid-range, rather than sub-threshold and elevated levels of cortisol, can facilitate calling [for comparable glucocorticoid effects in other systems see Sapolsky et al. (Sapolsky et al., 2000), Breuner and Wingfield (Breuner and Wingfield, 2000) and Clement et al. (Clement et al., 2005)]. In addition, the current data emphasize the importance of the auditory environment, together with hormone levels, in determining changes in calling behavior. As observed with androgens and male-male aggressive calling in this same species (Remage-Healey and Bass, 2006), cortisol may not be sufficient for the suppression of calling behavior in the absence of auditory stimuli, in this case predator vocalizations.

As shown here for toadfish, experimental presentation of predators and predator stimuli causes significant glucocorticoid elevation in other systems (Blanchard et al., 1998; Kagawa and Mugiya, 2000; Plata-Salaman et al., 2000; Kavaliers et al., 2001; Canoine et al., 2002; Cockrem and Silverin, 2002). Predator exposure also causes behavioral responses that are similar to behavioral changes observed during stress in lizards (Van Damme and Quick, 2001) and manted howler monkeys (Gil-da-Costa et al., 2003). Furthermore, risk-taking behavior in mice is shifted in the presence of predator odors, and this was accompanied by increases in circulating corticosterone and decreases in circulating testosterone (Kavaliers et al., 2001).

The well-documented acoustic startle response in flying insects is mediated by auditory detection of predator cues by prey (Hoy, 1989). Similarly, the low-frequency, high-energy 'pops' documented recently in foraging bottlenose dolphins may be used to startle or flush fish prey species from nests or hiding refuges (Nowacek, 2005). However, the current data suggest that dolphin foraging pops also have consequences for detection by at least one prey species, male Gulf toadfish. Low-frequency dolphin foraging pops may be particularly effective for use in habitats that scatter high-frequency echolocation clicks such as seagrass and seagrass/sand edges (Nowacek, 2005), but this benefit is balanced against the potential costs of increased detection by low-frequency 'specialists' such as toadfish.

Startle behaviors generally occur in response to specific, noxious stimuli and achieve fast reduction in conspicuousness, and the rapidity of these responses depends on how fast prey must react to predators (Bullock, 1984). The stereotyped reduction in advertisement calling has been documented in katydids as part of the acoustic startle response to bat echolocation signals (Faure and Hoy, 2000). Moreover, acute stress and the accompanying increases in plasma glucocorticoids are associated with the acoustic startle response in rats (Pryce et al., 2001). Therefore, the current data are consistent with the hypothesis that some forms of anti-predator behavior have adapted existing neural mechanisms that link startle responses with fast activation of 'fight-or-flight' mechanisms, including elevation in plasma glucocorticoids.

The authors thank William Tavolga for access to playback stimuli; Barbara Shoplock and Andrew Tuccillo for assistance with field experiments; William Hernkind, Robert Henderson and the staff at the Florida State University Marine Laboratory for logistical support; David Mann for speaker and amplifier use and advice, and Ron Hoy for valuable discussion. Christiane Meyer and two anonymous reviewers provided helpful comments on an earlier version of the manuscript. This work was supported by a NSF predoctoral fellowship, NIMH training fellowship, NSF DDIG IBN-0407802 (to L.R.H.), and NSF IBN-9987341 and IBN-0516748 (to A.H.B.).

References

Amorim, M. C. P., McCracken, M. L. and Fine, M. L. (2002). Metabolic costs of sound production in the oyster toadfish, *Opsanus tau*. *Can. J. Zool. – Rev. Can. Zool.* **80**, 830-838.

Au, W. W. L. (1993). *The Sonar of Dolphins.* New York, NY: Springer-Verlag.

Au, W. W. L. and Banks, K. (1998). The acoustics of the snapping shrimp Synalpheus parneomeris in Kaneohe Bay. *J. Acoust. Soc. Am.* **103**, 41-47.

Barros, N. B. (1993). *Feeding Ecology and Foraging Strategies of Bottlenose Dolphins on the Central East Coast of Florida.* PhD Thesis, University of Miami, Miami, USA.

Bass, A. H., Bodnar, D. A. and Marchaterre, M. A. (2001). Acoustic nuclei

in the medulla and midbrain of the vocalizing gulf toadfish (*Opsanus beta*). *Brain Behav. Evol.* **57**, 63-79.

Belwood, J. J. and Morris, G. K. (1987). Bat predation and its influence on calling behavior in neotropical katydids. *Science* **238**, 64-67.

Blanchard, R. J., Nikulina, J. N., Sakai, R. R., McKittrick, C., McEwen, B. and Blanchard, D. C. (1998). Behavioral and endocrine change following chronic predatory stress. *Physiol. Behav.* **63**, 561-569.

Bradbury, J. W. and Vehrencamp, S. L. (1998). *Principles of Animal Communication*. Sunderland, MA: Sinauer Associates.

Breuner, C. W. and Wingfield, J. C. (2000). Rapid behavioral response to corticosterone varies with photoperiod and dose. *Horm. Behav.* **37**, 23-30.

Bullock, T. H. (1984). Comparative neuroethology of startle, rapid escape, and giant-fiber mediated responses. In *Neural Mechanisms of Startle Behavior* (ed. R. C. Eaton), pp. 1-11. New York: Plenum.

Canoine, V., Hayden, T. J., Rowe, K. and Goymann, W. (2002). The stress response of European stonechats depends on the type of stressor. *Behaviour* **139**, 1303-1311.

Clement, T. S., Parikh, V., Schrumpf, M. and Fernald, R. D. (2005). Behavioral coping strategies in a cichlid fish: the role of social status and acute stress response in direct and displaced aggression. *Horm. Behav.* **47**, 336-342.

Clinchy, M., Zanette, L., Boonstra, R., Wingfield, J. C. and Smith, J. N. M. (2004). Balancing food and predator pressure induces chronic stress in songbirds. *Proc. R. Soc. Lond. B Biol. Sci.* **271**, 2473-2479.

Cockrem, J. F. and Silverin, B. (2002). Sight of a predator can stimulate a corticosterone response in the great tit (*Parus major*). *Gen. Comp. Endocrinol.* **125**, 248-255.

Connor, R. C. and Smolker, R. A. (1996). 'Pop' goes the dolphin: a vocalization male bottlenose dolphins produce during consortships. *Behaviour* **133**, 643-662.

Cranford, T. W. (2000). In search of impulse sound sources in odontocetes. In *Hearing by Whales and Dolphins* (ed. W. W. L. Au), pp. 109-155. New York: Springer.

Earley, R. L. and Dugatkin, L. A. (2002). Eavesdropping on visual cues in green swordtail (*Xiphophorus helleri*) fights: a case for networking. *Proc. R. Soc. Lond. B Biol. Sci.* **269**, 943-952.

Edds-Walton, P., Mangiamele, L. A. and Rome, L. C. (2002). Variations of pulse repetition rate in boatwhistle sounds from oyster toadfish *Opsanus tau* around Waquoit bay, Massachusetts. *Bioacoustics* **13**, 153-173.

Endler, J. A. (1983). Natural and sexual selection on color patterns in Poeciliid fishes. *Environ. Biol. Fishes* **9**, 173-190.

Endler, J. A. (1987). Predation, light-intensity and courtship behavior in *Poecilia reticulata* (Pisces, Poeciliidae). *Anim. Behav.* **35**, 1376-1385.

Endler, J. A. (1992). Signals, signal conditions, and the direction of evolution. *Am. Nat.* **139**, S125-S153.

Evans, J. P., Kelley, J. L., Ramnarine, I. W. and Pilastro, A. (2002). Female behaviour mediates male courtship under predation risk in the guppy (Poecilia reticulata). *Behav. Ecol. Sociobiol.* **52**, 496-502.

Faure, P. A. and Hoy, R. R. (2000). The sounds of silence: cessation of singing and song pausing are ultrasound-induced acoustic startle behaviors in the katydid *Neoconocephalus ensiger* (Orthoptera; Tettigoniidae). *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* **186**, 129-142.

Fay, R. R. and Edds-Walton, P. L. (2000). Frequency response of auditory brainstem units in toadfish (*Opsanus tau*). *Biol. Bull.* **199**, 173-174.

Fish, J. F. (1972). The effect of sound playback on the toadfish. In *Behavior of Marine Animals*. Vol. 2 (ed. H. E. Winn), pp. 386-434. New York: Plenum.

Fish, J. F. and Offutt, G. C. (1972). Hearing thresholds from toadfish, *Opsanus tau*, measured in laboratory and field. *J. Acoust. Soc. Am.* **51**, 1318-1321.

Forsgren, E. and Magnhagen, C. (1993). Conflicting demands in sand gobies – predators influence reproductive behavior. *Behaviour* **126**, 125-135.

Gannon, D. P., Barros, N. B., Nowacek, D. P., Read, A. J., Waples, D. M. and Wells, R. S. (2005). Prey detection by bottlenose dolphins, *Tursiops truncatus*: an experimental test of the passive listening hypothesis. *Anim. Behav.* **69**, 709-720.

Gil-da-Costa, R., Palleroni, A., Hauser, M. D., Touchton, J. and Kelley, J. P. (2003). Rapid acquisition of an alarm response by a neotropical primate to a newly introduced avian predator. *Proc. R. Soc. Lond. B Biol. Sci.* **270**, 605-610.

Gray, G. A. and Winn, H. E. (1961). Reproductive ecology and sound production of toadfish, *Opsanus tau*. *Ecology* **42**, 274-282.

Higgs, D. M., Plachta, D. T. T., Rollo, A. K., Singheiser, M., Hastings, M. C. and Popper, A. N. (2004). Development of ultrasound detection in American shad (*Alosa sapidissima*). *J. Exp. Biol.* **207**, 155-163.

Hoy, R. R. (1989). Startle, categorical response, and attention in acoustic behavior of insects. *Annu. Rev. Neurosci.* **12**, 355-375.

Janik, V. M. (2000). Source levels and the estimated active space of bottlenose dolphin (*Tursiops truncatus*) whistles in the Moray Firth, Scotland. *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* **186**, 673-680.

Janik, V. M., Sayigh, L. S. and Wells, R. S. (2006). Signature whistle shape conveys identity information to bottlenose dolphins. *Proc. Natl. Acad. Sci. USA* **103**, 8293-8297.

Johnson, M., Madsen, P. T., Zimmer, W. M. X., de Soto, N. A. and Tyack, P. L. (2004). Beaked whales echolocate on prey. *Proc. R. Soc. Lond. B Biol. Sci.* **271**, S383-S386.

Kagawa, N. and Mugiya, Y. (2000). Exposure of goldfish (*Carassius auratus*) to bluegills (*Lepomis macrochirus*) enhances expression of stress protein 70 mRNA in the brains and increases plasma cortisol levels. *Zool. Sci.* **17**, 1061-1066.

Kavaliers, M., Choleris, E. and Colwell, D. D. (2001). Brief exposure to female odors 'emboldens' male mice by reducing predator-induced behavioral and hormonal responses. *Horm. Behav.* **40**, 497-509.

Koga, T., Backwell, P. R. Y., Jennions, M. D. and Christy, J. H. (1998). Elevated predation risk changes mating behaviour and courtship in a fiddler crab. *Proc. R. Soc. Lond. B Biol. Sci.* **265**, 1385-1390.

Luczkovich, J. J., Daniel, H. J., Hutchinson, M., Jenkins, T., Johnson, S. E., Pullinger, R. C. and Sprague, M. W. (2000). Sounds of sex and death in the sea: bottlenose dolphin whistles suppress mating choruses of silver perch. *Bioacoustics* **10**, 323-334.

Mann, D. A., Lu, Z. M. and Popper, A. N. (1997). A clupeid fish can detect ultrasound. *Nature* **389**, 341.

Mann, D. A., Lu, Z. M., Hastings, M. C. and Popper, A. N. (1998). Detection of ultrasonic tones and simulated dolphin echolocation clicks by a teleost fish, the American shad (*Alosa sapidissima*). *J. Acoust. Soc. Am.* **104**, 562-568.

Mann, D. A., Higgs, D. M., Tavolga, W. N., Souza, M. J. and Popper, A. N. (2001). Ultrasound detection by clupeiform fishes. *J. Acoust. Soc. Am.* **109**, 3048-3054.

McKibben, J. R. and Bass, A. H. (1998). Behavioral assessment of acoustic parameters relevant to signal recognition and preference in a vocal fish. *J. Acoust. Soc. Am.* **104**, 3520-3533.

McKibben, J. R. and Bass, A. H. (2001). Effects of temporal envelope modulation on acoustic signal recognition in a vocal fish, the plainfin midshipman. *J. Acoust. Soc. Am.* **109**, 2934-2943.

McMichael, R. (2002). *Fisheries-independent Monitoring Program 2002 Annual Data Summary Report*, pp. A14-A30. St Petersburg, FL: Florida Marine Research Institute.

Miller, P. J. O., Johnson, M. P. and Tyack, P. L. (2004). Sperm whale behaviour indicates the use of echolocation click buzzes 'creaks' in prey capture. *Proc. R. Soc. Lond. B Biol. Sci.* **271**, 2239-2247.

Mougeot, F. and Bretagnolle, V. (2000). Predation as a cost of sexual communication in nocturnal seabirds: an experimental approach using acoustic signals. *Anim. Behav.* **60**, 647-656.

Myrberg, A. A. (1981). Sound communication and interception in fishes. In *Hearing and Sound Communication in Fishes* (ed. W. N. Tavolga, A. N. Popper and R. R. Fay), pp. 395-426. New York: Springer-Verlag.

Nowacek, D. P. (2005). Acoustic ecology of foraging bottlenose dolphins (*Tursiops truncatus*), habitat-specific use of three sound types. *Mar. Mamm. Sci.* **21**, 587-602.

Orchinik, M., Gasser, P. and Breuner, C. (2002). Rapid corticosteroid actions on behavior: cellular mechansims and organismal consequences. In *Hormones, Brain and Behavior*. Vol. 3 (ed. D. W. Pfaff), pp. 567-600. San Diego: Elsevier.

Plata-Salaman, C. R., Ilyin, S. E., Turrin, N. P., Gayle, D., Flynn, M. C., Bedard, T., Merali, Z. and Anisman, H. (2000). Neither acute nor chronic exposure to a naturalistic (predator) stressor influences the interleukin-1 beta system, tumor necrosis factor-alpha, transforming growth factor-beta 1, and neuropeptide mRNAs in specific brain regions. *Brain Res. Bull.* **51**, 187-193.

Pryce, C. R., Bettschen, D., Bahr, N. I. and Feldon, J. (2001). Comparison of the effects of infant handling, isolation, and nonhandling on acoustic startle, prepulse inhibition, locomotion, and HPA activity in the adult rat. *Behav. Neurosci.* **115**, 71-83.

Remage-Healey, L. and Bass, A. H. (2004). Rapid, hierarchical modulation of vocal patterning by steroid hormones. *J. Neurosci.* **24**, 5892-5900.

Remage-Healey, L. and Bass, A. H. (2005). Rapid elevations in both steroid

hormones and vocal signaling during playback challenge: a field experiment in Gulf toadfish. *Horm. Behav.* **47**, 297-305.

Remage-Healey, L. and Bass, A. H. (2006). From social behavior to neural circuitry: steroid hormones rapidly modulate advertisement calling via a vocal pattern generator. *Horm. Behav.* **50**, 432-441.

Rukstalis, M. and French, J. A. (2005). Vocal buffering of the stress response: exposure to conspecific vocalizations moderates, urinary cortisol excretion in isolated marmosets. *Horm. Behav.* **47**, 1-7.

Ryan, M. J. (1985). *The Tungara Frog. A Study in Sexual Selection and Communication*. Chicago, IL: University of Chicago Press.

Sapolsky, R. M., Romero, L. M. and Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr. Rev.* **21**, 55-89.

Sogard, S. M., Powell, G. V. N. and Holmquist, J. G. (1989). Utilization by fishes of shallow, seagrass-covered banks in Florida Bay. 1. Species composition and spatial heterogeneity. *Environ. Biol. Fishes* **24**, 53-65.

Tavolga, W. N. (1958). Underwater sounds produced by two species of toadfish, *Opsanus tau* and *Opsanus beta*. *Bull. Mar. Sci. Gulf Carrib.* **8**, 278-284.

Thorson, R. F. and Fine, M. L. (2002a). Acoustic competition in the gulf toadfish *Opsanus beta*: acoustic tagging. *J. Acoust. Soc. Am.* **111**, 2302-2307.

Thorson, R. F. and Fine, M. L. (2002b). Crepuscular changes in emission rate and parameters of the boatwhistle advertisement call of the gulf toadfish, *Opsanus beta*. *Environ. Biol. Fishes* **63**, 321-331.

Tuttle, M. D. and Ryan, M. J. (1981). Bat predation and the evolution of frog vocalizations in the neotropics. *Science* **214**, 677-678.

Tyack, P. L. and Clark, C. W. (2000). Communication and acoustic behavior of dolphins and whales. In *Hearing by Whales and Dolphins* (ed. W. W. L. Au), pp. 156-224. New York: Springer.

Van Damme, R. and Quick, K. (2001). Use of predator chemical cues by three species of lacertid lizards (*Lacerta bedriagae*, *Podarcis tiliguerta*, and *Podarcis sicula*). *J. Herpetol.* **35**, 27-36.

Versluis, M., Schmitz, B., von der Heydt, A. and Lohse, D. (2000). How snapping shrimp snap: through cavitating bubbles. *Science* **289**, 2114-2117.

Winn, H. E. (1972). Acoustic discrimination by the toadfish with comments on signal systems. In *Behavior of Marine Animals; Current Perspectives in Research* (ed. H. E. Winn and B. L. Olla), pp. 361-385. New York: Plenum Press.

Yan, H. Y., Fine, M. L., Horn, N. S. and Colon, W. E. (2000). Variability in the role of the gasbladder in fish audition. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* **186**, 435-445.

Zuk, M. and Kolluru, G. R. (1998). Exploitation of sexual signals by predators and parasitoids. *Q. Rev. Biol.* **73**, 415-438.