Crocodile egg sounds signal hatching time

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Crocodilians are known to vocalize within the egg shortly before hatching [1,2]. Although a possible function of these calls — inducing hatching in siblings and stimulating the adult female to open the nest — has already been suggested, it has never been experimentally tested [1–5]. Here, we present the first experimental evidence that pre-hatching calls of Nile crocodile (*Crocodylus niloticus*) juveniles are informative acoustic signals which indeed target both siblings and mother.

As a first step, we studied behavioral responses of embryos using playback experiments. Eggs (*n* = 17) coming from six different clutches that were due to hatch within ten days were split into 'pre-hatch', 'noise' and 'silence' groups. 'Pre-hatch' group eggs (*n* = 7) were tested individually twice a day with playback of recorded pre-hatching calls (duration one minute, one to three calls per second, a different recording used for each egg tested; Supplemental Data). 'Noise' group eggs (*n* = 5) were challenged with the same playback except that pre-hatching calls had been replaced by noise sequences (Supplemental Data). 'Silence' group eggs (*n* = 5) were individually manipulated as in the other experimental groups; however, instead of sound treatment, these eggs were observed twice a day during one minute in total silence. For each egg, observations were repeated until hatching success or prenatal death (Supplemental Data). Results show that the pre-hatching call playback elicited a higher behavioral response than the two other stimuli (Figure 1A). 'Pre-hatch' group eggs answered vocally during 80 ± 31% (mean ± sd) of the tests, whereas eggs of the 'noise' and of the 'silence' groups emitted sounds in 22 ± 33% (significant difference with 'pre-hatch': Mann-Whitney test, *P* = 0.023, Bonferroni corrected, U = 2, Z = 2.52) and 7 ± 15% of the tests (significant difference with 'pre-hatch': *P* = 0.014, Bonferroni corrected,

U = 1, Z = 2.68), respectively. No significant difference was detected between 'noise' and 'silence' groups (*P* = 0.70, Bonferroni corrected, U = 8, Z = 0.94; Figure 1A). Moreover, 'pre-hatch' eggs moved during 55 ± 33% of the tests versus 10 ± 13 % for the eggs of the 'noise' group (Mann-Whitney test, *P* = 0.024, Bonferroni corrected, U = 2, Z = 2.52). We never observed a movement by a 'silence group' egg (significant difference with 'pre-hatch group': *P* = 0.0088, Bonferroni corrected, U = 0, Z = 2.84; no significant difference with 'noise group': *P* = 0.60, Bonferroni corrected, U = 7.5, Z = 1.04). Finally, all individuals that successfully hatched in the 'pre-hatch' group (*n* = 4) did so during the playback or during the following ten minutes (Supplemental Data). The probability of hatching during this time period by chance was 0.0153 (binomial test: z = 16.05, *P* < 0.0000001). This event happened only once in the 'noise' group (binomial test: z = 4.49, *P* = 0.0445). The remaining hatchings (*n* = 2 in the 'noise' group, *n* = 3 in the 'silence group') occurred at least five hours after the last test ('silence' group binomial test: z = –0.216, *P* = 0.956).

The second step of the study was to assess mothers' behavioral responses to these pre-hatching signals. Nile crocodile females lay their eggs in

the sand and guard their 'nest' until juveniles hatch [5]. In the zoo where we did the experiments, eggs are removed within a few days after laying. In spite of this, females continue to guard the nest. We conducted our experiments at the end of the incubation period (i.e., about three months after the laying date). Each female tested (*n* = 10) was first driven away in order to place the loudspeaker for playbacks 50 cm underground. Females returned to their nest within five minutes (3 ± 2.15 minutes, except one individual (female 'F5' in Figure 1B) which needed 25 minutes). After 30 minutes of motionless rest, females were challenged with a series of pre-hatching calls and a series of noise sequences (duration of each recording: 10 minutes, 1 to 3 calls or noise sequences/second, 38 ± 8 dB, 30 minutes of rest between pre-hatch and noise series). The presentation order of the series was counterbalanced between females. Results show that all females responded — at least by head or body movement — to the playback of pre-hatching calls while only four out of ten reacted during noise sequences (Figure 1B). Reaction latencies were significantly shorter with pre-hatching calls (Wilcoxon paired test, *n* = 10, *P* = 0.009, Z = 2.60). Above all, the
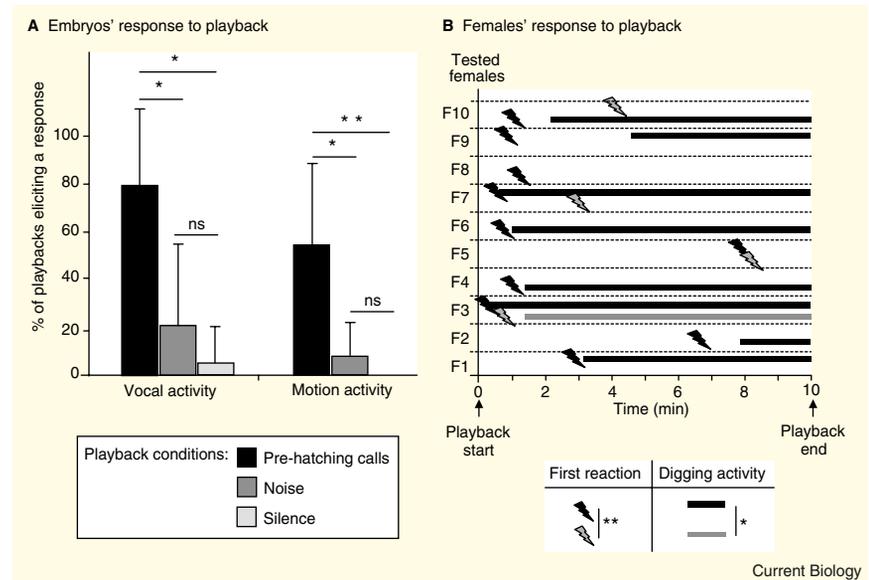

Figure 1. Behavioral responses of Nile crocodile embryos and adult females to sound playback.
(A) Embryos show higher behavioral activity — in terms of emitted vocalizations and movements — during playback of pre-hatching calls than during playback of noise or silence period. * (*P* < 0.05); ** (*P* < 0.01). (B) Crocodile mothers react strongly to playback of pre-hatching calls, most of them by digging the sand. * (*P* < 0.05); ** (*P* < 0.01) (ns: not significant) See also Supplemental Movie.

playback of pre-hatching calls elicited digging behaviour in eight of the females (Figure 1B and Supplemental Data), while this response happened only one time during the playback of noise sequences (Wilcoxon paired test, $n = 10$, $P = 0.012$, $Z = 2.52$). In summary, our experiments show that pre-hatching calls of the Nile crocodile carry relevant information for both embryos and mother. While still inside the egg, juveniles are responsive to nearby calls; egg vocalizations may act to fine-tune hatching synchrony as in some species of birds [4,6]. The other key effect of egg vocalizations is to stimulate the adult female to open the nest. With crocodile juveniles being highly susceptible to predation [1,3], both hatching synchrony and maternal assistance certainly increase the fitness of newborns.

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Rapid increase in fish numbers follows creation of world's largest marine reserve network

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No-take marine reserves (NTMRs) are much advocated as a solution to managing marine ecosystems, protecting exploited species and restoring natural states of biodiversity [1,2]. Increasingly, it is becoming clear that effective marine conservation and management at ecosystem and regional scales requires extensive networks of NTMRs [1,2]. The world's largest network of such reserves was established on Australia's Great Barrier Reef (GBR) in 2004. Closing such a large area to all fishing has been socially and politically controversial, making it imperative that the effectiveness of this new reserve network be assessed. Here we report evidence, first, that the densities of the major target species of the GBR reef line fisheries were significantly higher in the new NTMRs, compared with fished sites, in just two years; and second, that the positive differences were consistent for multiple marine reserves over an unprecedented spatial scale (>1,000 km).

Australia's Great Barrier Reef Marine Park (GBRMP) has an area of 344,400 km^2 and is an international icon that generates AU$5.8 billion annually from tourism and fisheries [3]. In mid-2004, the Australian Government rezoned the GBRMP. After extensive planning involving identification of bioregions and stakeholder consultation, ≥20% of each of 70 bioregions within the park was placed into the world's largest network of NTMRs [4] covering 115,395 km^2 (33.4% of the GBRMP) and spanning 14° of latitude. Because of the intense community interest, and as livelihoods were

affected [4], monitoring the effects of the new reserve network was imperative. Thus, an integrated and spatially extensive program was established to assess its value in protecting both biodiversity and exploited species.

A team from James Cook University used underwater visual census to survey reef biota at 18 sites in new coral reef NTMRs and in an equal number of control areas that remained open to fishing in three inshore island groups (10–30 km off the coast and spanning 4.5° of latitude; see Figure S1 in the Supplemental data available on-line with this issue) before and again 1.5–2 years after implementation of the NTMRs (see Supplemental data). Concurrently, sites on 28 pairs of no-take and open offshore reefs (30–200 km from the coast) in five GBRMP regions (spanning 7.5° of latitude, see Figure S1) were surveyed 1.5–2 years after implementation by the Australian Institute of Marine Science. All offshore survey reefs were initially open to fishing but one reef per pair was declared a NTMR in mid-2004, while the other remained open to fishing. Inshore and offshore surveys used similar methods (see Supplemental data).

After 1.5–2 years of protection, the density of the primary target of reef line fisheries, coral trout (*Plectropomus* spp.), increased significantly in inshore NTMRs in the Palm ($p < 0.05$) and Whitsunday ($p < 0.001$) Islands (+68% and +65%; Figure 1A). Changes were small and non-significant (+2% and –6%) where reefs remained open to fishing. Reefs in the other inshore region, the Keppel Islands, suffered extreme coral bleaching during March 2006 and coral trout density declined on both open reefs (–23%) and NTMRs (–19%). However, coral trout density in NTMRs increased relative to open reefs in all three inshore regions, significantly so in the Whitsunday Islands and marginally so in the Palm Islands (Palms +65%, $p < 0.10$; Whitsundays +75%, $p < 0.01$; Keppels +4%, $p > 0.10$). Offshore, average coral trout density was also higher in NTMRs than on open reefs in all five regions (Figure 1B), significantly so in four and marginally so in the fifth