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No. 12, Thiruvannamalai Nagar, Saravanampatti - Kalapatti Road, Saravanampatti,
Coimbatore, Tamil Nadu 641035, India
Ph: +91 9385339863 | www.threatenedtaxa.org
Email: sanjay@threatenedtaxa.org

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Cover: Dorsal view of Mantis Shrimp *Cloridina ichneumon* (Fabricius, 1798) & *Gonodactylellus demanii* (Henderson, 1893). © Fisheries Research Station, Junagadh Agricultural University, Sikka.

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COMMUNICATION

Post-release growth of captive-reared Gharial *Gavialis gangeticus* (Gmelin, 1789) (Reptilia: Crocodilia: Gavialidae) in Chitwan National Park, Nepal

Bed Bahadur Khadka [1], Ashish Bashyal [2] & Phoebe Griffith [3]

[1] Gharial Conservation and Breeding Center, Chitwan National Park, Kasara, Chitwan, Nepal.
[2] Biodiversity Conservancy Nepal, House 594, Manigram, Rupandehi-32903, Nepal.
[3] Institute of Zoology, Zoological Society of London, London, NW8 7LS, UK.
[3] Department of Zoology, University of Oxford, Oxford, OX1 3SZ, UK.
[1] bed.khadka@gmail.com, [2] a.bashyal@bioconnepal.org, [3] phoebe.griffith@zoo.ox.ac.uk (corresponding author)

Abstract: Supplementation of wild populations of the Critically Endangered Gharial *Gavailis gangeticus* with individuals reared in captivity is a widely used conservation management tool in Nepal and India, although its efficacy is uncertain. Measuring post-release growth in Gharial can provide valuable information on acclimation of captive-reared Gharial to the wild and provide growth rates to inform population recovery models. We studied post-release growth of Gharial reared in the Gharial Conservation Breeding Centre, Nepal, following their release into the Chitwan National Park. We used recapture data from known individuals to determine growth and change of mass for 26 Gharial recaptured 0.5–10 years after release. We found that Gharial recaptured two or more years post-release had increased in mass and length despite being over six years old at release, however there was a triangular relationship between time since release and growth: some Gharial had grown very slowly, whilst others had grown much faster. All Gharial recaptured less than two years since release had lost mass and had negligible growth in total length. This data show that there is considerable variation in post-release growth rates, which will lead to some individuals being very old before they reach a potentially mature size class, with unknown implications for reproduction. This variation is important for predicting or modelling recovery in populations where the release of Gharial from captivity is a management tool. Our results also suggest the two years after release are an acclimation phase—when Gharial lose mass and do not grow—which should be considered by release strategies in order to give Gharial the best chance of survival after release.

Keywords: Conservation, Crocodylia, growth rate, head starting, rear and release.

Abbreviations: CNP—Chitwan National Park | GCBC—Gharial Conservation and Breeding Centre | OLS—Ordinary least squares regression | SVL—Snout-vent length | TL—Total length.

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Competing interests: The authors declare no competing interests.

Author details: Bed Bahadur Khadka has wide experience in wetland and freshwater ecology and has been studying Gharials for the last 18 years. Presently, he works as consultant to the Gharial Conservation Breeding Centre. Ashish Bashyal is a co-founder of Biodiversity Conservancy Nepal—a non-profit dedicated to wildlife conservation in Nepal. He has been studying genetics and ecology of crocodilians including Gharials since 2009. Phoebe Griffith is a conservation scientist, specializing in crocodilians. She is currently a DPhil candidate at the Zoological Society London and University of Oxford, and research fellow at Himalayan Nature, studying the ecology and conservation of gharial crocodilians in Nepal.

Author contributions: BK conceptualized and designed the study. BK and PG collected data, and AB and PG performed data analysis. All authors interpreted the results and prepared the manuscript.

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INTRODUCTION

The Critically Endangered Gharial *Gavialis gangeticus* crocodile was once common in the Narayani River of southern Nepal, as well as its tributaries including the Kali Gandaki and Rapti, with hundreds of Gharial found in the lower Narayani prior to the construction of the dam on the Indo-Nepal border (Maskey 1989). However, by the 1970s the population had crashed, and in response the Government of Nepal instigated the Gharial Conservation Breeding Centre (GCBC), based at Kasara, Chitwan National Park (CNP) in 1978 (Maskey 1989). At the GCBC, Gharial eggs laid in captivity or collected from the wild are reared in captivity until they reach a size of 1.5–2 m, usually at an age of 5–7 years, when they are released into rivers within the species' range in Nepal (Khadka 2012). The goal of the GCBC is to reinforce Nepal's Gharial populations, with a major focus on Chitwan (Khadka 2010; Acharya et al. 2017).

Before 2004, the vast majority of Gharial were released in the Narayani River (375 from 1981–2003). However, the population of Gharial did not recover (Maskey & Percival 1998; Ballouard et al. 2010), and the release programme was shifted predominantly to the Rapti in 2004, with all Gharial released in the Rapti since 2008. To date (April 2022), 972 Gharial have been released in the Rapti, with 788 of these released from 2006 onwards (Bed Bahadur Khadka, pers. comm. April 2022). It was estimated that captive-released Gharial had survival rates of only 7% in the Narayani (Maskey & Percival 1998), however the rear-and-release programme has seen greater success since the shift to releasing the Gharial individuals in Rapti. The overall Gharial population in Chitwan is estimated to have increased from 39 in 2005, to a minimum count of over 200 in 2022 (Acharya et al. 2017; Khadka 2022). However, even if the entirety of this increase is attributed to the head-start programme, this still accounts for only about 30% of released the Gharials, which suggests that mortality and/or loss from the system remains high. Ballouard et al. (2010) suggested the first two years after release was a time of particularly low survival (~20% survival) for released Gharials.

There is currently less data on growth rates of Gharial reared in captivity followed by release into the wild. Work on captive and released crocodilians suggests that released animals may not thrive, especially immediately after release (Blake & Loveridge 1975; Singh 1978). Growth rate data for Gharial at different stages post-release into a natural system such as the Rapti River will be very informative to understand acclimation of captive-reared Gharial to wild conditions, provide growth rates to inform population recovery models, and also indicate at which stage there is likely to be high mortality as limited growth and negative or limited mass changes may indicate difficulties in adapting to conditions post-release.

The goal of this study was to investigate post-release growth rates in recaptured Gharials, in order to:

1. inform GCBC release strategy, by providing a better understanding of the post-release response of Gharial to their new environment in terms of change in mass and growth,
2. inform predictions of population recovery, by providing a better understanding of the variation in Gharial growth rates and the time taken for Gharial to reach a potentially reproductive size class.

MATERIALS AND METHODS

This study evaluated growth rates in length and mass following release of Gharials raised in captivity into the wild. We used recapture data from known individuals to determine growth and change of mass for 26 Gharials released from the GCBC, 0.5–10 years after release.

This study took place on the Rapti River and its tributaries, the Dhugre Khola and the Budhi Rapti, in and around Chitwan National Park (CNP), southern Nepal (Image 1). The Rapti is a tributary of the larger Narayani River, and Gharials freely move between the two rivers. The Rapti River forms the northern boundary of the CNP, whilst the Dhugre Khola and Budhi Rapti fall within community forest outside the northern park boundary. Our team was made up of staff from the GCBC and catchers from the indigenous fisherfolk communities. We captured the Gharials in daytime using either a throw net deployed from a dugout canoe, or via gill nets drifted along basking sand banks, with one end attached to a float and the other held by a person upstream. Once basking Gharial was located, long gill nets were set up under the water adjacent to the bank. The Gharials were captured by flushing them into water, after which they became entangled when they dived into the net, or were captured by traditional throw nets cast from the canoes offshore. In clear, shallow water Gharials were located underwater, and captured using throw nets. Following entanglement, we used hessian sacks to blindfold the Gharial whilst still in the water, then removed captures to the nearest shore, where the Gharials were restrained on ladders to minimise risks during measurements.

For all captured individuals, total length (TL; distance

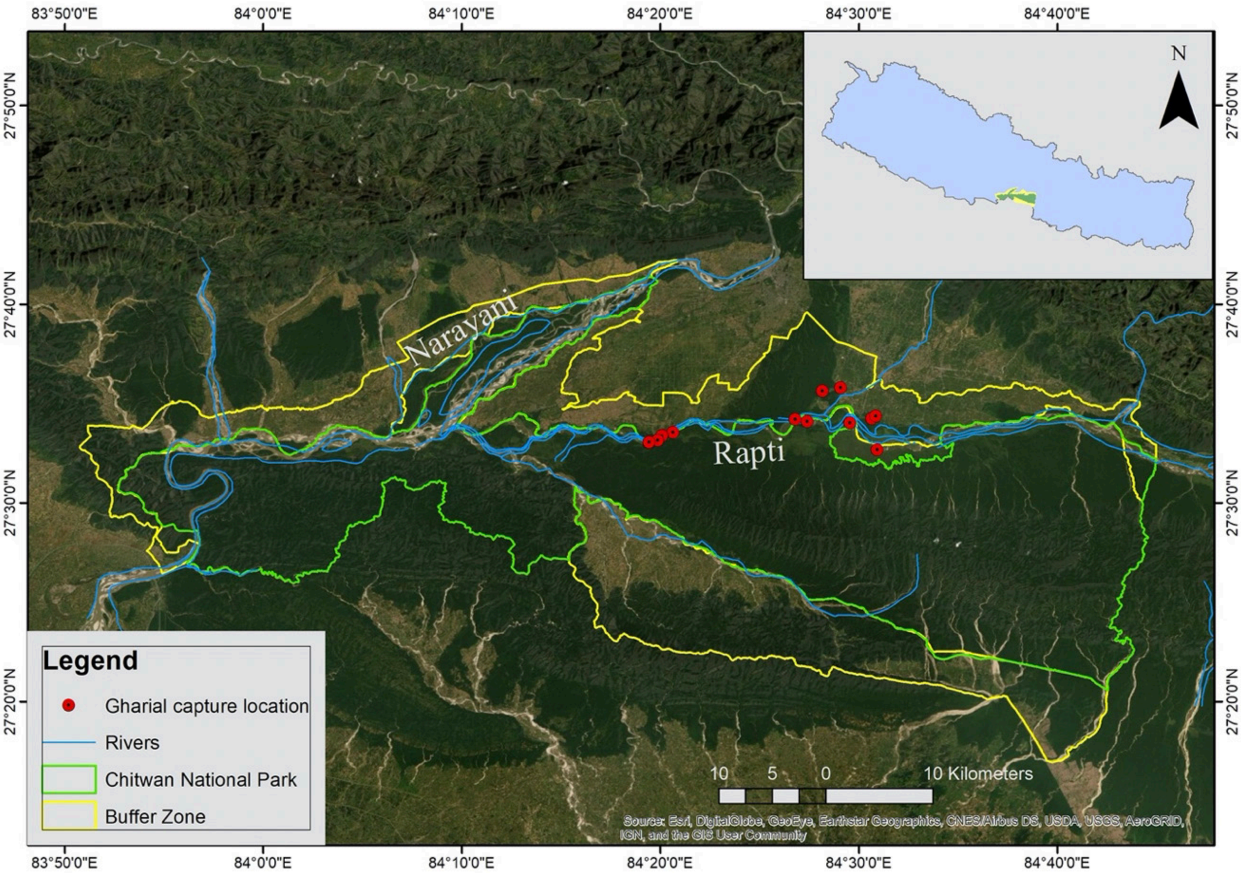

Image 1. Map of the Study Site in Chitwan National Park. The major rivers (Rapti and Narayani) are labelled, and locations of Gharial capture are indicated. Inset shows position of Chitwan National Park in Nepal.

from anterior tip of the snout to the posterior tip of the tail) was measured to the nearest 0.5 cm, and mass was measured to the nearest 0.5 kg. If captured Gharial had clipped tail scutes, we matched position of clipped scutes to the catalogue of previously marked Gharial maintained at the GCBC. Sex was determined at recapture by physical examination of the outer genitals and were designated male, female or indeterminate. Indeterminate individuals had intermediate sized genital organs that could not confidently be designated as either a clitoris or penis. After morphometric and sex measurements were made, Gharial were released back into the river at their capture location. The total process from capture to release took less than one hour. Gharial were recaptured from 2005–2019 as part of the GCBC programme for detangling Gharial from fishing nets (n = 7), and from 2018–2019 as part of an ongoing telemetry study (n = 19). Size classes were designated as adult (>300 cm TL), sub-adult (200–299 cm TL) and juvenile (100–199 cm TL).

To determine growth rates, morphometric values (TL, mass) were compared to the same values recorded at the time of release, (measured using the same protocol described above for recaptured Gharial) which are kept on record in the GCBC database. Morphometric measurements of all Gharial released from the GCBC are taken and recorded on the day of release as part of standard practice at the centre.

A linear regression was carried out to establish whether time since release significantly predicted growth (in TL and mass). A Breusch-Pagan test showed there was heterogenous variance in both the mass and TL linear regression models. Therefore, a quantile regression was used to model empirical relationships between time since release and mass or TL. To identify which quantile regression predictions fell outside of the confidence intervals of the ordinary least squares regression, we used a stepwise approach identify quantile regressions at 5% intervals from the 5–95 % quantile.

Total length was used for all analyses rather than snout-vent length (SVL), as TL and SVL in this study were highly correlated both at time of release ($r = 0.99$, $p < 0.001$, $n = 26$) and for recaptured Gharials ($r = 0.99$, p

<0.001, n = 18). We corrected for missing values (TL or mass) for some individuals by using a scaling relationship that predicted the missing values based on the data we had for recaptured Gharial for which complete measurements were available. The relationship was: mass = $1.3602*TL^{3.7175}$ (R^2 = 0.9824, n = 18). A scaling relationship of this form has been shown to be appropriate for crocodilians (Grigg & Kirshner 2015). These computed values are designated with asterisks in Table 2. We only used this method for Gharials in good condition for which we had a minimum mass estimate (>80 kg, the maximum of our equipment). For four Gharials we did not have a mass measure or minimum mass estimate, and these individuals had poor body condition. Therefore, the mass of these Gharials was excluded from the analysis.

Growth rates for Gharials released for two years or less (mass n = 8; TL n = 10) were calculated by taking the mean change in mass or TL and dividing it by time since release (in years) to give as estimated per-year change. We used a paired t-test to determine whether per-year change in mass and TL for these Gharials differed from the change in mass and TL predicted by the ordinary least squares regression. One Gharial was excluded from all mass analyses as it was recaptured for welfare reasons due to extreme emaciation following a long-term entanglement in a gill-net. Analysis was conducted in R (R Core Team 2013), with the package 'quantreg' (Koenker 2020) used for quantile regressions. Figures were produced using the package ggplot2 (Wickham 2016).

RESULTS

The 26 Gharials included in this study were recaptured 0.5–10 years after release. Gharial recaptured less than two years post-release had generally lost mass and grown negligibly. Gharial released over two years earlier had all increased in length and mass, but the relationship was triangular, with some Gharial growing very slowly, and others much faster. We collected morphometric measurement from a total of 28 Gharials, however two were excluded from this study as they had not been previously scute clipped.

Time since release significantly predicted post-release change in mass (B = 10.18±2.80, t = 7.86, p <0.01) and accounted for 74% (adjusted R^2) of variability in mass change, according to the ordinary least squares linear regression (OLS). However, the relationship was triangular in shape: all Gharials released within less than two years had lost or maintained mass, whereas Gharials released for after more than two years split into individuals that had grown considerably, and those that had grown very little (Figure 1). The quantile regressions showed that at the lowest and highest quantiles, the quantile coefficients fall outside the confidence intervals of the OLS coefficient. At the 5%, 10% and 15% quantiles the coefficient was lower than that of the OLS (slow growth), and at the 90% and 95% quantiles the coefficient was higher than that of the OLS (fast growth; Table 1, Figure 1a).

A very similar pattern was seen in post-release TL growth. Time since release (predictor variable) significantly predicted the response variable of growth in total length (B = 17.94±3.93, t = 9.42, p <0.01) and accounted for 78% (adjusted R^2) of variability in total length growth. This relationship was also triangular, with the quantile regression (see Figure 1b and Table 1) showing that at the 15% and 20% quantiles the coefficient was lower than that of the OLS (slow growth), and at the 90% and 95% quantiles the coefficient was higher than that of the OLS (fast growth).

This variation in post-release growth of both mass and TL can be seen clearly in the data (Table 2), for example two Gharials released 5.67 and 5.75 years before capture showed a difference in mass change of 30.5 kg and TL change of 40 cm, when at their release in the same year their difference in mass was just 2.5 kg and in TL was just 3 cm. As a consequence, Gharials from the GCBC will reach a size of 300 cm (thought to be adult size) at very different ages: one slow-growing Gharial is only 247 cm at 15.5 years old, whilst another is 306 cm at 9.92 years old.

There was no correlation between age and either TL or mass at release: older Gharials in our sample were

Table 1. Value of regression coefficient (estimated change in growth (TL or mass) per year post-release) for differing quantiles that fall outside of the confidence intervals of the ordinary least squares (OLS) model, with OLS regression as reference.

	Value of Regression Coefficient	
Quantile	Mass	Total Length
5%	5.93	
10%	5.93	
15%	5.46	13.57
20%		12.91
90%	13.01	22.01
95%	13.01	24.27
OLS Estimate	10.18±2.80	17.94±3.93

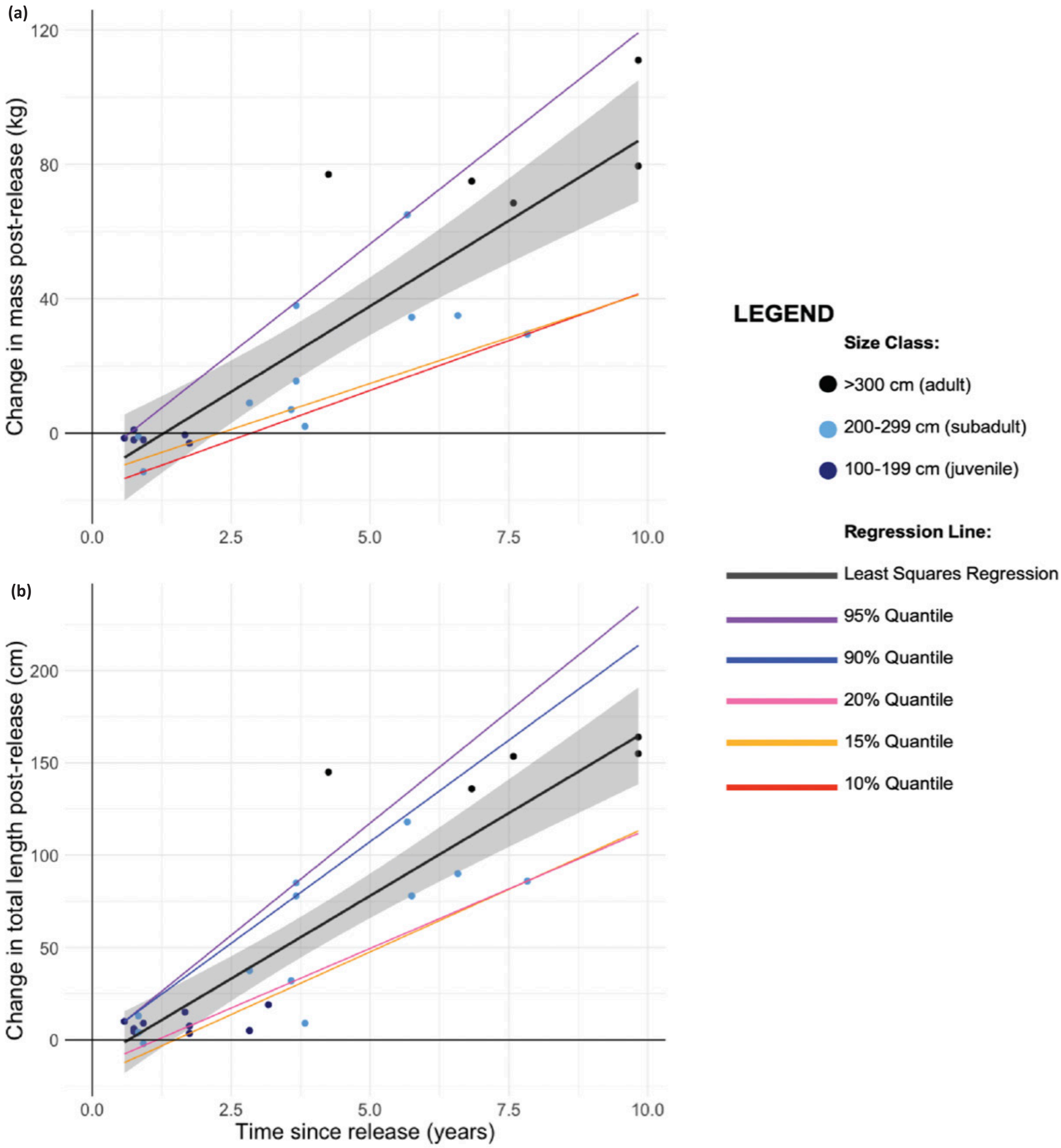

Figure 1. Change in (a) mass, n = 22 or (b) change in total length post-release, n = 26 for Gharial since release from captivity. Predictions are illustrated with the ordinary least squares (OLS) regression showing the mean growth trajectory, the 95% and 90% quantile regressions illustrate the estimated fast growth trajectory, with the 10%, 15%, and 25% quantile regressions illustrating the slow growth trajectory. The grey shaded area illustrates the confidence intervals of the OLS regression. Each data point is an individual Gharial colourised to show size-class at recapture.

no longer or heavier than younger Gharial upon release from captivity.

Mass change was positively correlated with time since release (Pearson's r = 0.86, n = 21, p = 0.01). However, all except one of the Gharials released less than two years ago had lost weight after release. The paired t-test estimated the mean change in mass for the two years following release was between -6 and +0.4 kg per year (t(7) = 2.36, p = 0.05, n = 8), considerably less than the OLS prediction of 10.18±2.80kg increase in mass per year.

Total length was positively correlated with time

Table 2. Measurements taken at both release and recapture of 26 captive-reared Gharial released into the Rapti River. Sex is stated as male (M), female (F) and indeterminate (I). Gharial for which the relationship mass = 1.3602*TL3.7175 was used to calculate mass (n = 4) or length at release (n = 1) are marked as so*. Four Gharial do not have a mass value and mass couldn't be estimated due to poor body condition.

Release				Recapture					
Release Date	Age (years)	Total Length (cm)	Mass (kg)	Sex	Capture Date	Age (years)	Age Difference Post-Release	Total Length (cm)	Mass (kg)
11-xi-2004		197		M	31-viii-2005	0.83	0.83	210	
02-ii-2013	5.67	172	16.5	M	25-viii-2013	6.25	0.58	182	15
02-ii-2010	5.67	162	10.5	F	06-x-2013	9.34	3.67	247	26
02-ii-2010	5.67	161	10	F	14-v-2014	9.92	4.25	306	**87***
05-iii-2014	7.75	173	12.5	F	09-v-2017	10.92	3.17	192	
07-ii-2017	6.67	205	29	F	08-i-2018	7.58	0.92	203	17.5
10-iii-2018	5.75	173	13	F	26-xi-2018	6.5	0.75	179	11
09-iii-2018	5.75	170	12	F	27-xi-2018	6.5	0.75	174.5	13
14-ii-2018	7.67	200	22	F	27-xi-2018	8.5	0.83	204	21
02-ii-2012	10.67	177	20	F	27-xi-2018	17.5	6.83	313	**95***
09-ii-2016	5.67	181	15	I	28-xi-2018	8.5	2.83	218.5	24
08-iii-2018	6.75	181	15.5	F	28-ii-2019	7.67	0.92	190	13.5
10-iii-2018	5.75	170	13.5	F	11-xi-2019	7.42	1.67	185	13
05-iv-2016	5.83	184	18	F	11-xi-2019	9.42	3.58	216	25
05-iii-2014	8.75	182	17.5	F	16-xi-2019	14.5	5.75	260	52
10-iii-2018	5.75	176	13	M	17-xi-2019	7.5	1.75	179.5	10
05-iv-2016	5.83	192	18	I	18-xi-2019	9.5	3.67	270	56
24-iii-2014	6.83	179	15	F	18-xi-2019	12.5	5.67	297	80
20-iv-2013	5.92	184	19	M	19-xi-2019	12.5	6.58	274	54
19-iv-2012	6.92	151	8.5	F	19-xi-2019	14.5	7.58	304.5	77
02-ii-2012	7.67	161	11.5	F	20-xi-2019	15.5	7.83	247	41
02-ii-2010	5.67	171	11	F	25-xi-2019	15.5	9.83	335	**122***
02-ii-2010	5.67	150	6.5	F	26-xi-2019	15.5	9.83	305	**86***
07-ii-2017	5.67	176	12.5	F	27-xi-2019	8.5	2.83	181	
09-iii-2018	5.75	181	14.5	F	09-xii-2019	7.5	1.75	188.5	
10-ii-2016	5.67	**200***	18	F	09-xii-2019	9.5	3.83	209	20

since release (Pearson's r = 0.91, n = 26, p = 0.01). All Gharials released less than years ago had shown only slight growth in TL, the paired t-test estimated the mean increase in TL for the two years following release was between 3.47 and 11.42 cm per year (t(10) = 2.26, p = 0.05, n = 10), less than the OLS prediction of 17.94±3.93 increase in TL per year, indicating that all Gharial grow slower (if at all) in the two years post-release.

DISCUSSION

Previous work (Singh 2018) found that TL growth in Gharial drops very suddenly around the 6th or 7th year, however we found that Gharials released from the GCBC continue growing post-release, despite their age (5.67–10.67 years old at release). This suggest there are factors limiting growth in captivity before release. The lack of correlation between age and TL or mass at the point of release suggests individuals are small for their age at release, especially the older Gharials. Singh (2018) found that when the TL growth rate dropped at the 6th or 7th year, the Gharial in his study had attained a near-adult length, and most facilities have found fast growth rates for captive Gharials, with them reaching over 200 cm in 3–4 years (Singh 1978, 2018; De Vos 1982). Growth rates at the GCBC in Nepal are slower, with Gharial reaching sizes of ~150 cm within four years of hatching (Khadka & Bashyal 2019). Gharials are therefore already 5+ years old when they are released from the GCBC, but a long way off mature size (300 cm for females, 400 cm for males). After release in suitable riverine habitat, the Gharials in this study resumed varying rates of growth, with some individuals reaching adult size at the time of recapture. The impact of this delayed maturity on the head started Gharial is unknown. The similar values for TL growth post-release of adult-sized Gharial at recapture, regardless of time since release, suggests that growth in length slows once Gharials reach adult size, likely indicating a shift in energy allocation from somatic growth to reproduction (Czarnołe'ski & Kozłowski 1998).

We found a large amount of variation in the growth rates of Gharials that had been released longer than two years. This variation is substantial – in the 5% quantile, mass change is estimated at a 5.93kg increase per year, whereas at the 95% quantile mass change estimate is as double this – at 13.01 kg per year. Most Gharials followed either a 'fast growth' or 'slow growth' trajectory. The underlying cause of this variation is not known, but it suggests there are key factors impacting post-release growth that we have not yet been measured. These differing growth rates will lead to some individuals reaching maturity much later than others – slow growing individuals could be close to 20 years old before reaching an adult size, which could have implications for reproduction. The differing lengths of time taken for Gharials to be recruited into the potentially reproductively active adult size class is also important for predicting population recovery, and should be incorporated into population models for Gharial management in Chitwan. Slow growing individuals will also spend a longer time in the smaller size classes, when they appear to be more vulnerable to threats such as net entanglement. Substantial variation in growth rates between individuals have also been found in captive studies of Gharials (Singh 1978; Khadka & Bashyal 2019), but the reasons underlying this variation are unclear.

Our results showed that Gharial lose mass in their first year or two after release, and gain mass after a 1–2 year acclimation phase, especially once they reach >300 cm. Gharial also appear to only increase TL very slowly in this acclimation phase. Singh (2018) also reported that Gharial growth rate will slow following a 'shift', such as to a new habitat or pen, for at least a year. This was suspected to be due to the shock of a shift to a new habitat, with time required to enable crocodilians to adjust and resume normal feeding.

One potential cause of the loss in mass and reduced growth rate is the new environment (Blake & Loveridge 1975; Singh 2018). This shift may lead to a difficulty or time-lag in shifting from eating dead fish to hunting live prey, increased activity related to adapting to riverine flow, and the need to find a suitable habitat to settle in and avoid new threats such as predators and entanglement in illegal fishing gear. The direct impact of these challenges, may cause chronic stress for the Gharials, and stress is thought to be a major cause of high mortality rates in reintroductions (Teixeira et al. 2007). Studies on crocodilians in captivity show a strong negative relationship between levels of corticosterone (stress hormone) and increase in body mass (Elsey et al. 1990; Morici et al. 1997; Turton et al. 1997), suggesting that crocodilians that lose weight are likely to also be physiologically stressed. Physiologically stressed crocodilians show elevated mortality rates (Morici et al. 1997), which could contribute to high mortality in the immediate two-years post-release that has been recorded by Ballouard et al. (2010). Gharials are currently released with a 'soft-release' approach: they are placed in in-situ grass enclosures at the river to acclimate to flow, and after some time break out themselves. The post-release loss of mass in Gharials from the GCBC suggests that this soft release programme could be further supported by supplemental feeding in the in situ release enclosure, ensuring Gharials do not deplete their resources during this period.

Another potential cause of this acclimation phase is that this is the lag-time required to overcome the impacts of chronic stress in captivity. High stress in captive crocodilians has been documented and is known to effect growth (Elsey et al. 1990), and can have a number of causes, including high stocking density, limited availability of a sufficient thermogradient, and fear due to high visitor numbers, or an inability to seek cover (Huchzermeyer 2003). Research into stress of Gharials at GCBC under different housing and husbandry conditions could help inform the programme.

It is also possible that Gharials have an elevated mass in the GCBC compared to wild Gharials of the same TL, due to the captive feeding regime and conditions, as this elevated mass of captive crocodilians is seen in many captive settings (Blake & Loveridge 1975; Elsey et al. 1992). Initial post-release declines may reflect a shift to a more 'natural' mass of Gharial. However, since the

Gharials were recaptured in this study less than two years post-release also showed poor conditions (thin body and tail) compared to observed Gharials of the same size in either captive collections or wild populations regardless of TL, we suspect that losses in mass reflected a decline of Gharial post-release to condition below the natural 'wild' state. Gharials recaptured after more than two years post release had more convex bodies and tails, suggesting a healthier condition.

The pre-monsoon and monsoon seasons are thought to be the best season for Gharials to hunt fish, due to murky water caused by high sediment load in the river. These seasons are also the time at which Gharials increase the most in both length and mass in captivity, due to high temperatures (Singh 2018; Khadka & Bashyal 2019). Warmer temperatures also lead to higher body temperatures in crocodilians, and these are therefore the seasons with the highest energetic costs (Lang 1987). The release of Gharials pre-monsoon, when energetic costs are high but they are attempting to catch live prey for the first time, could lead to the observed loss of mass. This may be compounded with high levels of corticosterone which is known to depress crocodilian growth regardless of resources (Elsey et al. 1990; Morici et al. 1997). This may lead to a 'missed' season of growth for released Gharials immediately after release, and they may enter their first winter without sufficient reserves. Release of Gharials in the post-monsoon or early winter, may enable them to adapt to the habitat earlier, and maximise opportunity for growth when the warmer season starts.

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