

**Headstarts and Setbacks:
an evidence-based approach to conservation of an
evolutionarily distinct and globally endangered
crocodylian, the gharial**



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This thesis is dedicated to:

Dr Tirtha Man Maskey, without whom there may not still have been gharial in Nepal,

Adrian Griffith, my father, without whom I may never have gone to Nepal,

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I wish I could thank you all.

Abstract

Crocodylians are instantly recognisable, large aquatic predators with the potential to act as flagship species in their freshwater habitats. Over half of crocodylian species are threatened with extinction and where threats to freshwater systems are particularly acute, such as Asia, all species are threatened. In order to mitigate multiple threats, effective conservation actions require a species-specific focus and site-specific solutions. Such an approach, combined with a broader understanding of critical differences and similarities of threatened freshwater crocodylians sets the stage for appropriate conservation actions elsewhere. This thesis provides a comprehensive assessment of specific conservation actions in a single case study, the conservation of the gharial in Chitwan, Nepal. It also aims to improve an understanding of extinction risk across Crocodylians by consideration of species-specific functional traits.

In this thesis, I begin by investigating the imperilled functional and phylogenetic diversity of crocodylians, identifying traits that may increase extinction risk, and identifying functional groupings and distinctiveness measures for all crocodylians. Based on this research, I propose the gharial is the highest priority crocodylian for conservation action, due not only to its highly threatened status, but also its unique contribution to functional and phylogenetic diversity.

I then focus on the case study of the Chitwan gharial population, a recovering gharial population in Nepal. Firstly, I compare the use of VHF and GPS telemetry data to estimate home ranges in wild gharial. I find that with appropriate processing of data and selection of analysis methods, estimates from GPS and VHF data are very similar, despite the higher spatial precision and temporal resolution of GPS transmitters. I recommend different systems in which GPS or VHF might be more suitable for studying gharial, according to the differing biases of the two technologies.

Using three different population estimation methods, I then provide the first estimates of population size for gharial in Chitwan, and identify the environmental covariates that influence gharial detectability in daylight surveys. I show that gharial are most abundant in rivers with high dry-season water levels and low seasonal variability. Gharial appear to be

more abundant further from a barrage, and in places with low human disturbance. Our results suggest there may be a threshold level of human disturbance, above which gharial do not occur. All methods found that the Chitwan population did not increase from 2018-2021, despite the release of hundreds of gharial from a headstarting (rear and release) programme, highlighting the need for an assessment of the programme.

I then conduct an analysis of the gharial headstarting programme in Chitwan. Using a range of data sources, I show that mortality remains high for headstarted gharial in Chitwan, primarily due to bycatch-related mortality, but also predation of naïve individuals.

Retrospective analysis of the headstarting programme shows it was a failure prior to 2004, but has had some success since. This is most likely due to improved survival in captivity and the shift of the release site to an upstream tributary of the initial release site, reducing downstream deleterious dispersal through a dam on the India-Nepal border. Movement patterns and mortality of headstarted gharial suggest that gharial should be released at a smaller size and younger age, and even further upstream of the dam. Successful recruitment observed in-situ, and continued high mortality and biased sex ratios in captivity, suggest more nests should be left in-situ in Chitwan, to promote natural population recovery. The priority moving forward must be tackling in-situ threats to reduce mortality to immature size classes and increase nesting site options. These results can inform other headstarting programmes, and I make predictions about when and where headstarting programmes are likely to fail or succeed.

Taken together, these results show that headstarting alone is insufficient for population recovery in gharial, although it may be a useful tool under some circumstances and specific conditions. This is because gharial are sensitive to in-situ threats such as habitat fragmentation, modification and over extraction. Gharial are particularly sensitive among the crocodylians due to traits such as their habitat specificity and lack of behavioural or physiological adaptations to cope with environmental extremes. I use my findings to suggest key areas of conservation action and ecological research for the future, both for the gharial in Chitwan and across its range, as well as for crocodylian headstarting globally.



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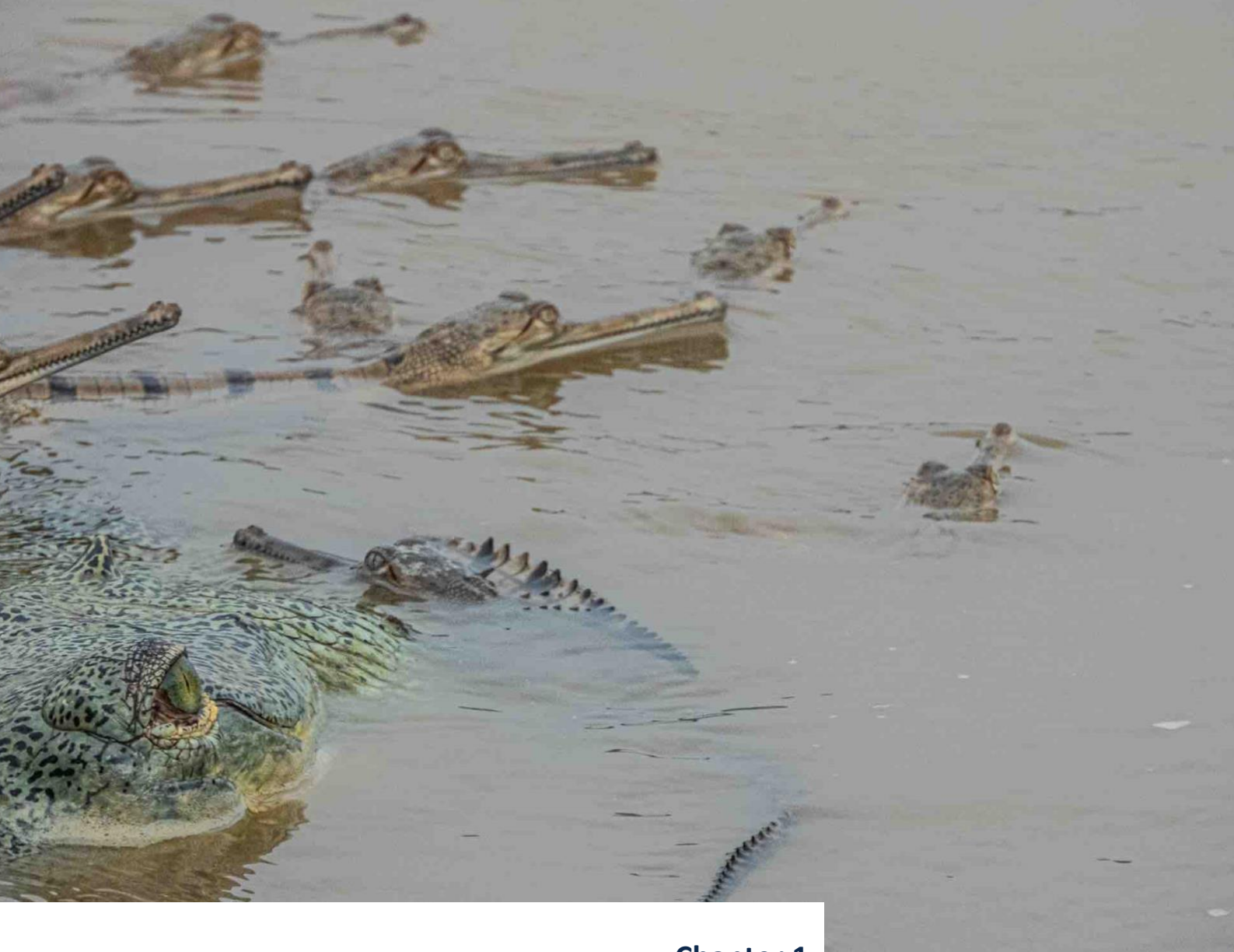
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Chapter 1

General Introduction

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1.1 The Ongoing Biodiversity Crisis

Human activity has such a profound influence on the world it is thought we are now in the 'Anthropocene', a new geological epoch where humans are the dominant cause of environmental changes (Lewis & Maslin 2015). One catastrophic feature of the Anthropocene so far is the loss of biodiversity: these losses are now so severe we are in an extinction crisis (Johnson et al. 2017), with extinction rates thousands of times above pre-human background rates (Ceballos et al. 2015). It is hard to conceptualise the scale of this biodiversity loss, because each generation has inherited a more depauperate world from their forebears. This dwindling inheritance results in a recent historical and personal experience of biodiversity that changes through time, shifting our perceptions of what we have lost: the shifting baseline syndrome (Jones et al. 2020).

The further back in time we look, the more concerning the current biodiversity crisis becomes through comparison. Since the evolutionary emergence of modern humans, our range expansion into different parts of the world has been associated with biodiversity declines, especially among megafauna (Johnson et al. 2017; Turvey & Cress 2019) with two thirds of megafaunal vertebrates lost by the end of the Pleistocene, and 23% of turtle and tortoise species (Rhodin et al. 2015). Although there is debate as to whether humans were the primary cause of, or a contributory factor to, these losses, the evidence of human-induced extinctions becomes stronger the nearer in time we move (Turvey & Cress 2019). Since the start of the recent historical period (1500 CE) these losses have been accelerating (Ceballos et al. 2015). European expansionism and colonialism, followed by industrialisation and the intensification of agriculture have led to cascading species losses (Johnson et al. 2017). The IUCN reports that 617 vertebrate species have become extinct, extinct in the wild or possibly extinct since 1500, primarily since 1900 (Ceballos et al. 2015). However, we have likely lost more, as we don't even know all that we have lost: as we collect paleontological and historical data we discover more and more past extinctions (Turvey &

Crees 2019). To take examples from one vertebrate Order – the crocodylians – there are multiple species disappearances associated with human arrival that are sequentially coming to light. Paleontological discoveries of crocodylian fossils on Pacific Islands have revealed the past occurrence of a number of crocodylian species called the Mekosuchids (Mead et al. 2002; Willis 2014). These include terrestrial crocodylians and possibly an arboreal species, with their extinction associated with the arrival of humans on their island homes (Mead et al. 2002; Willis 2014). More recently, in accounts by early European explorers, Malagasy peoples referred to two species of crocodile on the island of Madagascar. Sub-fossil material then indicated the recent occurrence of *Voay robustus*, a morphologically unusual ‘horned’ crocodile, the extinction of which was likely due to extirpation by humans (Hekkala et al. 2021). In 2022 a species of crocodylian was described that had been lost even more recently: *Hanyusuchus sinensis* (M. Iijima et al. 2022). Through accounts from Chinese historical literature combined with archaeological records, Iijima *et al* showed there had been an unusual Gavialid occurring in South China, with a mixture of physical and ecological characteristics of the only two remaining Gavialid species. This huge crocodylian reached at least 6m length and only went extinct a few hundred years ago due to hunting pressure, yet was only ‘rediscovered’ in modern times in 2022. The loss of awareness of disappearing wildlife can even occur within lifetimes: Turvey et al. (2010) found that younger fishers were less likely to have heard of or recognise the recently extinct baiji (Yangtze River Dolphin) or paddlefish than older fishers. Biodiversity loss is a catastrophic ongoing crisis, one that is subject to shifting baselines, and is therefore challenging to both conceptualise and measure. Consequently, it is a crisis most people likely underestimate.

The loss of a species is not an isolated event: it is the removal of that species and its role from an ecosystem, which may have ecosystem-level or cascading impacts (Raffaelli 2004; Estes et al. 2011; Ripple et al. 2017). The geologically recent disappearance of many species due to human actions have ongoing ecosystem-level consequences (Malhi et al. 2016; Galetti et al. 2018). It is not just extinctions that are cause for concern: species become increasingly rare as they decline, becoming functionally extinct across much of their range long before they approach extinction (McConkey & O’Farrill 2015; Valiente-Banuet et al. 2015). Evidence suggests that the loss of ecological interactions occurs before the

extinction of a species, therefore ecosystem functioning and ecosystem services are impacted faster than predicted by extinctions alone (Valiente-Banuet et al. 2015).

What is even more worrying is that the loss of biodiversity, measured by both extinctions and population decline, is accelerating. Since the 1960s, there has been 'The Great Acceleration', with unambiguous major anthropogenic global environmental impacts (Lewis & Maslin 2015). Modern extinction rates are not only exceptionally high, but increasing, with humanity destroying species at an accelerating rate (Ceballos et al. 2015). A million species of plant and animal are currently threatened with extinction, with monitored populations showing an average of decline of 69% since 1970 (WWF 2022). The immediate future is of even greater concern, as trends suggest extinction rates are about to accelerate to at least 5x those of the recent past (Johnson et al. 2017).

Across the earth, not all systems are impacted equally. The situation is worst in freshwaters, with populations of monitored freshwater vertebrates having declined by an average of 81% since 1970, compared to terrestrial (-38%) or marine (-36%) populations (McRae et al. 2017). Freshwater megafauna species (those able to reach >30kg as adults) are especially threatened (He et al. 2021). Global freshwater megafauna populations have declined by 88% since 1970, with megafishes showing the largest declines of 94% (He et al. 2019). Declines also show strong geographical patterns, for example freshwater megafauna populations in the Indomalaya region declined by a staggering 99% since 1970 (He et al. 2019). The loss of freshwater biodiversity is even more concerning if you consider the disproportionate richness of these habitats: over a third of vertebrate species are freshwater, including 50% of all fish, yet freshwaters cover just 1% of the Earth's surface and contain 0.01% of the Earth's water (Dudgeon et al. 2006; Balian et al. 2008; Carrete Vega & Wiens 2012). The concentration of biodiversity in a small area with tremendous anthropogenic pressures has led to the current dire state of freshwater biodiversity.

The intensified anthropogenic threats in freshwater systems are perhaps unsurprising: humans have always been closely associated with freshwaters (Macklin & Lewin 2015) as they affect every activity and aspiration of our societies, providing us with essential ecosystem services, from water and food to carbon sequestration and transportation (Aylward et al. 2005). As human consumption has increased in the last century, the threats

to freshwater systems and the species within them have intensified, and demand for water resources is predicted to intensify further (Vörösmarty et al. 2013). To meet human demands for water and energy, major infrastructure such as dams, barrages and levees have shattered river connectivity worldwide, with resulting range contractions, declines, and extinctions of freshwater species (He et al. 2021), with proposed hydropower projects threatening a further 260,000km of mainstem rivers (Thieme et al. 2021). Populations of freshwater migratory fish have shown an average decline of 76% from 1970-2016, although, since the study had insufficient data from Africa, Asia and South America, the situation could be far worse (Deinet et al. 2020; WWF 2022). Capture within inland fisheries has increased substantially over the last 70 years, leading to overexploitation both directly and through bycatch (Allan et al. 2005; Welcomme et al. 2010). Pollution continues to be a major threat to freshwater biodiversity and there are rapid changes in the contributing pollutants and processes, such as emerging issues of microplastics pollution (Dudgeon et al. 2006; Reid et al. 2019). Animal agriculture to provide animal-sourced foods to humans is a key cause of water pollution which is becoming worse with livestock industry intensification and increasing demand for animal products (Machovina et al. 2015). Additionally, agriculture is now thought to account for 70% of freshwater use (WWF 2022). A rapidly intensifying threat to rivers is Sand Mining, a term that refers to the extraction of aggregate encompassing sand, gravel, pebbles or cobbles (Koehnken et al. 2020). These aggregates are the most mined material in the world, with rivers being the preferred source (Koehnken et al. 2020). Aggregates are the basis of our modern society, from road bases to phone screens, yet their removal strips them from rivers where they conduct exceptionally important ecosystem services such as filtering water, supporting livelihoods such as agriculture and fisheries, flood protection and key habitat for river species (Bisht 2021). The problems of freshwater systems are compounded by the interconnected nature of freshwater systems, with the condition of rivers reflecting their catchments, as well as the impact of upstream processes on downstream assemblages (Dudgeon 2012).

It is hard to overstate the importance of healthy, functioning freshwater systems to humans: the degradation of these systems has cascading social and economic impacts. To choose just three examples: the pollution of freshwaters leads to international public health concerns (Jayaswal et al. 2018); inland fisheries are key for food and nutrition security,

providing a primary source of nutrition and animal protein to at least 200 million people, particularly vulnerable communities in Africa and Asia (Hughes 2021); and the sand mining industry is riddled with illegality and black markets, leading to social injustices such as intense violence, exploitative labour relations and livelihood precarity (Bisht 2021).

Deteriorating freshwater systems have led to one in three freshwater species being threatened with extinction (WWF 2022). Whereas past-extinctions were both geographically clumped and very size-biased (Turvey & Cress 2019), there is a lack of unifying life history traits associated with freshwater fish extinction risk (Duncan & Lockwood 2001), and evidence of freshwater species with dissimilar life histories collapsing on similar timescales (Turvey et al. 2012). This suggests freshwaters may be moving into a ‘field of bullets’ extinction scenario, where loss is non-selective and random (Raup 1992), which suggests overwhelming extrinsic threats. This is likely the case in much of the rivers of the human-dominated landscapes of monsoonal Asia, which are possibly the most endangered ecosystems on Earth (Dudgeon 2002). For example, in the Yangtze basin the freshwater system appears to already be in the ‘field of bullets’ scenario with species such as the Baiji, Chinese Paddlefish and Reeves Shad already lost, and others following closely (Turvey et al. 2012). In Chapter 2, I investigate correlates of extinction risk in crocodylians, and find that the Chinese (or Yangtze) Alligator is surprising, having intrinsic traits that should buffer it against extinction, yet also being the rarest crocodylian species. This makes more sense in the context of the Yangtze basin, where extrinsic threats are so severe that it is testament to the resilience of the species that it has not been long extinct.

1.2 Conservation Science and Prioritisation

All is not lost, however, and all has not been lost because people throughout the world have been tackling the biodiversity crisis. Conservation aims to maintain the diversity of life on earth (Akçakaya et al. 2018), and certainly without conservation action, things today would be much worse than they already are (Hoffmann et al. 2010). Conservationists do not aim merely to reduce extinction risk, but eventually to achieve recovery. Although recovery is challenging to define, the new IUCN Green Status of Species aims to do so, with a recovered species being defined as one that is ‘viable and ecologically functional in every part of its

indigenous and projected range' (Akçakaya et al. 2018). Although recovery may feel far away for most species and systems in the current crisis, there are success stories. The cessation of large-scale commercial whaling has seen some species recover strongly post exploitation (Clapham 2016), the switch from unregulated hunting of crocodylians such as the American Alligator and Australian Saltwater Crocodile, to a sustainable and well-managed system has led these species to become Least Concern where once they were Endangered (G. Grigg & Kirshner 2015), and the loss of mangroves has declined by an order of magnitude from the late 20th to early 21st century (Friess et al. 2020). Such cases are cause for optimism, although fundamental drivers of the unsustainable use of nature can only be tackled at the highest levels of economic and social planning (Johnson et al. 2017). A systemic shift to an economic, societal and cultural system that values nature is needed if we are to stem biodiversity loss (WWF 2020). Until then, conservation science aims to buy time and make conservation actions more effective (Johnson et al. 2017).

One of the major issues facing conservation science is that funding for global conservation is woefully inadequate and unevenly distributed, with the least spent in the developing world where threatened biodiversity is most concentrated (Johnson et al. 2017). Whilst the significant resources required to tackle the biodiversity crisis remain unforthcoming, those who engage in conservation are forced to allocate limited resources: the 'agony of choice' (Vane-Wright et al. 1991). The targeting of conservation resources using differing systems of conservation prioritisation is an area of considerable research, with prioritisation granted to different species, ecosystems or ecoregions by different statistical, ethical, financial or evolutionary considerations. There are many forms of conservation prioritisation. The flagship species approach uses popular species as a marketing tool to raise funds, awareness and support (McGowan et al. 2020). Schemes that aim to quantify the value of different species or areas usually do so by using some combination of irreplaceability, vulnerability and value (Brooks et al. 2006), usually based on a measure of species richness and endemism combined with threat, for example: the prioritisation of biodiversity hotspots (Myers et al. 2000), Alliance for Zero Extinction sites and Important Biodiversity Areas (Parr et al. 2009; Donald et al. 2019), or wilderness areas (Jones et al. 2018).

Increasingly, it is recognised that biodiversity conservation needs to focus on protecting all facets of biodiversity, including phylogenetic and functional diversity, not just measures of

species numbers (Pollock et al. 2017). Phylogenetic diversity (PD) is a measure of the total evolutionary history of a set of taxa (Faith 1992), the conservation of which aims to conserve feature diversity and therefore future options for humanity (Gumbs et al. 2018). Programmes such as the EDGE of Existence use a metric combining phylogenetic measures and extinction risk to try and maximise the conservation of PD by identifying priority species which are both Evolutionarily Distinct and Globally Endangered (Gumbs et al. 2022). Functional diversity aims to quantify the functional roles of species (Mouillot et al. 2013), and is generally measured through functional traits (Petchey & Gaston 2006), with metrics aiming to prioritise species that represent a key part of functional diversity (e.g. Hidasi-Neto et al. 2015; Griffin et al. 2020). By prioritising conservation using measures to preserve phylogenetic and functional diversity, scientists aim to better capture evolutionary heritage and the complexity of ecosystem processes, thereby maintaining the known and unknown contributions of this diversity to ecosystems and humanity (Faith 2015).

The issue of limited resources and overwhelming threat is most acute in freshwater systems. Freshwaters receive less conservation investment than other ecosystems (Darwall et al. 2011) and freshwater biodiversity is generally overlooked in environmental policy and by public interest (S J Cooke et al. 2013). The 'hidden' nature of freshwater species leads to a lack of public awareness and consequent conservation attention (Carrizo et al. 2017). Flagship species have been used very successfully to gain public attention of terrestrial and marine systems (Bowen-Jones & Entwistle 2002; Smith et al. 2012; McGowan et al. 2020), and it has been suggested that freshwater megafauna species could be employed in the same way for promoting the need for freshwater conservation (Carrizo et al. 2017; Kalinkat et al. 2017). Good flagship species have good marketing power, and occur in places of broader conservation value (Veríssimo et al. 2017; McGowan et al. 2020). By utilising species of high value for conserving phylogenetic and functional diversity as flagship species, conservationists can preserve multiple components of biodiversity at once.

1.3 Crocodylians: Flagship Species for Freshwater Conservation

Crocodylians make a strong candidate for flagship species in freshwater conservation. They are immediately recognisable, trigger strong emotions, and, although culturally and

geographically variable, are often associated with cultural value or interest (Lorimer 2007). They often grow to large sizes, have a fearsome and awe-inspiring appearance, and are amphibious – making many crocodylians much more visible than other freshwater species. Crocodiles can be popular: crocodiles and alligators are instantly recognisable to most people, feature prominently in modern and historic iconography and art, even the tv show ‘The Crocodile Hunter’ was the highest-rated Animal Planet series when it aired. Multiple crocodylians have been identified as potential flagships, being ‘popular with the target audience of potential donors in higher income countries’ (McGowan et al. 2020), but Crocodylians have been also been used successfully as flagship species with a local audience who live alongside them, for example in the Philippines (van der Ploeg et al. 2011), India (S. A. Hussain et al. 2013) and the USA (Mazzotti et al. 2009). Crocodylians are potential flagship species across much of the subtropics and tropics (Figure 1.1), occurrence which overlaps the areas of the earth with the highest biodiversity due to the latitudinal diversity gradient (Hillebrand 2004).

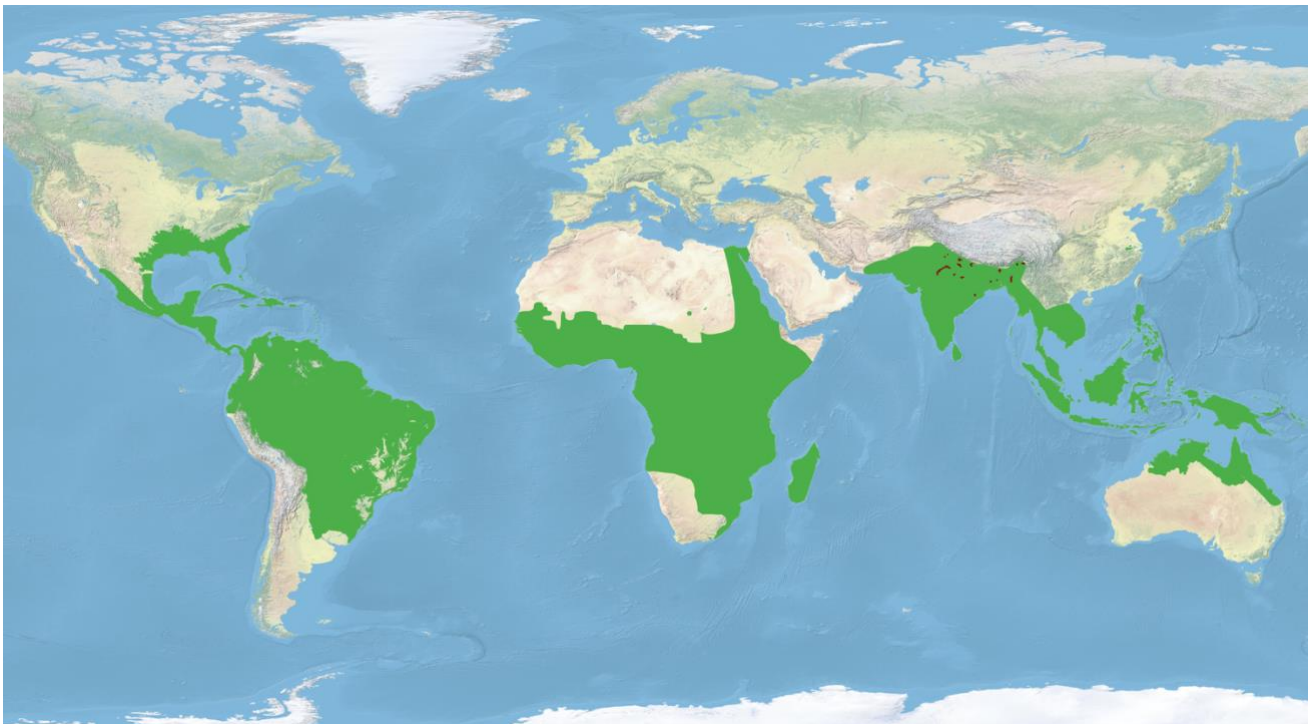


Figure 1.1: the combined global distribution of all extant species of Crocodylian (dark green) and current distribution of the focal species of this thesis, gharial (red). (Data from IUCN Red List for the Americas, Asia and Oceania, and from the Global Assessment of Reptile Distributions for Africa).

Crocodylian conservation is not just important for their potential role as flagship species in freshwater systems. They are a unique group thought to play important and irreplaceable ecological roles (Somaweera et al. 2020), yet more than half of crocodylian species are threatened with extinction (Chapter 2). In Chapter 2, I investigate the phylogenetic and functional diversity of this unique group of large vertebrates, determining priorities for crocodylian conservation at the global level, and predicting losses under current extinction scenarios. Using crocodylians as a case study, I also investigate the relationship between evolutionary and functional distinctiveness, and how well PD-based measures can act as a surrogate for the conservation of functional diversity. Finally, I investigate correlates of extinction risk amongst Crocodylia and quantify functional groupings, adding to the evidence base to inform conservation of crocodylians, and to inform the application and transfer of conservation actions between species.

1.4 Efficient Expenditure of Conservation Resources: the role of Conservation Evidence and Adaptive Management

Once the decision of where to target limited resources has been made, the subsequent challenge is how to spend those resources well: conservation of endangered species requires conservation managers to choose which conservation actions are most likely to lead to population stabilisation or recovery, usually with limited resources (Ferraro & Pattanayak 2006). For these actions to lead to effective conservation it is necessary for those making the decisions to know which actions are likely to work – or not work – in a given context, and subsequently evaluate whether actions have led to their stated objectives (Pullin et al. 2004). Such evaluation requires evidence to be collected, in a scientifically robust manner, on the impact and outcome of conservation actions, which can then inform decision making and lead to effective and cost-efficient conservation (Sutherland et al. 2004). However, conservation decision making is often based on anecdotal information rather than scientifically collected evidence, such as common sense, personal experience and rules of thumb (Sutherland et al. 2004). Conservation actions are often not evaluated, documented, systematically reviewed or actively disseminated (Sutherland et al. 2004). There has been an increasing call for more ‘Evidence-Based

Conservation' with the belief that decisions could be better made by accurately evaluating outcomes of management interventions, and making this easily accessible for decision makers (Sutherland et al. 2004; Adams & Sandbrook 2013; Gillson et al. 2019).

On the individual project level, an adaptive management approach is widely seen as one of the best available frameworks for structured decision making to underpin effective conservation or environmental management (Keith et al. 2011; Nicholls & Armstrong 2012). Adaptive management is an approach that allows for conservation implementation to account for high levels of uncertainty (Gillson et al. 2019). Adaptive management can be loosely categorised as 'passive' and 'active' (Gregory et al. 2006). In passive adaptive management historical data is typically used to develop an hypothesis for the outcome of a particular conservation action; in active adaptive management there are competing hypotheses as to the outcome of various conservation actions, and an experimental or quasi-experimental design is used to test these hypotheses. In both instances, monitoring and evaluation of outcomes is then carried out which is used to iteratively reassess the hypotheses, and subsequently the management action (Gregory et al. 2006).

Evidence-Based Conservation requires data to be collected by individual projects on the outcome of conservation actions, and then shared in some way, generally through a freely accessible database (Gillson et al. 2019). This data can then be used to inform the planning of a conservation project, which in itself could be designed based on an adaptive management framework. The monitoring and evaluation of outcomes of the project can be used both to reassess project hypotheses and design, and also to contribute to the Evidence-Base which can inform other projects. This framework of the interaction between adaptive management and Evidence-Based Conservation is illustrated in Figure 1.2 (Gillson et al. 2019).

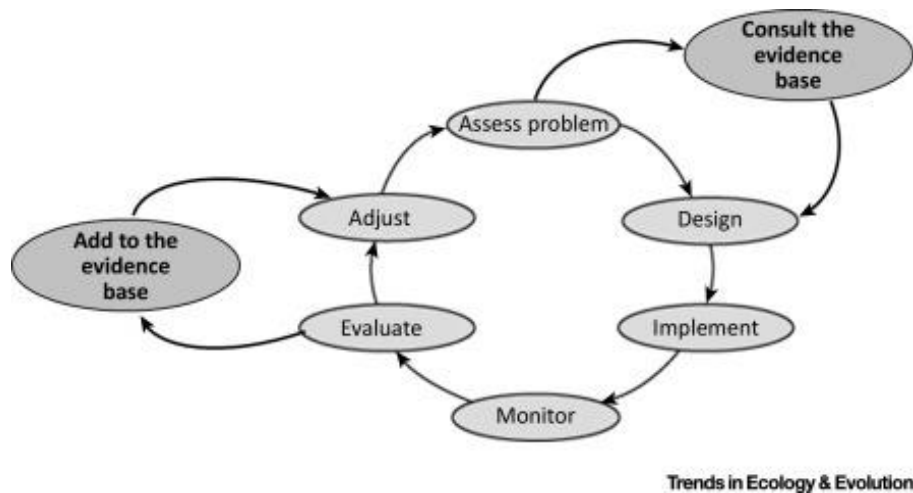


Figure 1.2: the adaptive management cycle (inner loop), with potential interaction with the evidence base illustrated through a connection to both the design and evaluation phases.

After Gilson *et al.* 2019

Both adaptive management and Evidence-Based Conservation therefore require the collection of useful data on conservation outcomes. Although conservation funding increasingly involves a ‘monitoring and evaluation’ component (Ferraro & Pattanayak 2006), funding allocation remains biased towards maximising the expected conservation outcome rather than scientifically monitoring the outcomes, meaning many conservation programmes have a limited, or absent, monitoring component (McCarthy & Possingham 2007). Furthermore, monitoring of the outcomes of grants is often difficult within the short timeframes of conservation or research grants (POST PN, 2011), or it is carried out without appropriate application of scientific methods. In scientific theory, quality and accuracy of evidence depends on how rigorously experiment design is adhered to. A study which would provide the strongest evidence for causality in an experimental approach would randomly assign subjects to experimental and control groups (Margoluis *et al.* 2009). However, in real-life conservation situations, good experimental design can be challenging, especially as these are often highly complex and dynamic systems, although there are still a range of ways of carrying out effective evaluation (Margoluis *et al.* 2009).

One of the critical questions being considered when evaluating the impact of a conservation intervention is: what is the counter-factual? That is, what would the outcome have been

with no intervention, or an alternative intervention (Ferraro 2009)? The counter-factual can be inferred indirectly through causal inference, with the strength of the inference depending on the quality of the available data and its interpretation. In an ideal situation, the counter-factual can be determined by using experimental or quasi-experimental design from the first stage on conservation planning to randomise treatments (i.e. conservation actions) between different sites, groups or populations (Ferraro 2009). Where this is not possible, a non-experimental design can draw correlative inferences about the impact of actions by identifying 'control' subjects that have been incidentally exposed to different conservation actions (Margoluis et al. 2009). If a quantitative evaluation is not possible, qualitative evaluation can be carried out, usually focusing on the knowledge, perceptions, attitudes and behaviour of a range of people associated with the conservation issue (Margoluis et al. 2009): however, it is important that the qualitative approach is used very cautiously unless the links between human behaviour and target conservation are well understood.

An issue which many conservationists attempting evaluation face is that they often enter into a project that has already been failing to achieve certain indicators of success only once the project has been ongoing for some time. Estimates of the counter factual using the approaches detailed in the last paragraph can then be brought in at this later stage, but an improved understanding of the past can be difficult to extrapolate. What is the historical counter factual? This can be estimated through a retrospective evaluation using casual inference (Law et al. 2017), with the available data used to inform a 'best guess' counter-factual. Improved design of future evaluation can then be used to reinform these hypotheses as more data becomes available for more accurate causal inference.

1.5 Conservation Evidence and Adaptive Management for Headstarting Crocodylians

A widely used conservation intervention, which could benefit greatly from the application of the frameworks of evidence-based conservation and adaptive management, is headstarting. Headstarting is commonly used in reptile conservation programmes, such as for turtles and crocodylians. Headstarting involves the collection of eggs from the wild, or captive breeding, with animals reared in captivity for a particular length of time before they are

released into the wild (Heppell et al. 1996). The underlying theory is that larger reptiles have a higher probability of annual survival than smaller ones, mostly due to reduced predation pressure (Ferguson et al. 1982). Headstarting is also called 'rear-and-release', 'ranching' and 'restocking'. Headstarting is a form of conservation reinforcement when the 'headstarts' are released into an established population, and reintroduction when they are released into an area where the species formerly occurred but has been extirpated (IUCN/SSC 2013). Crocodylians are generally seen as good candidates for headstarting because female crocodylians lay many eggs per annum, which have high natural mortality in the small size classes. This mortality can theoretically be circumvented by rearing them to a 'predator proof' size before release. However, other aspects of crocodilian biology, such as parental care and importance of learned behaviour, could cause issues that are not generally considered in herpetofauna reintroductions. Despite being used in dozens of crocodylian species, and drawing a substantial proportion of crocodylian conservation investment, headstarting in crocodylians has received relatively little evaluation and the evidence of its success rests almost entirely on anecdotal data and 'common sense'. The majority of data in favour of headstarting is from comprehensive work on a single species in a single system: the American Alligator in Louisiana (e.g. Elsey, 2013).

Head-starting has been more comprehensively reviewed in turtles, where it appears that headstarting is of limited utility compared to the protection of adult animals, since the lifecycle of turtles (as for crocodylians) is based upon long-lived adults having repeated reproductive opportunities over very long lifespans, after a long immature period (Frzer 1992; Heppell et al. 1996; Burke 2015). Therefore the key area for conservation intervention must be adult protection (Frzer 1992; Heppell et al. 1996). However, these studies have suggested that for extremely small populations head-starting can be useful if conducted with high enough numbers to impact overall survival rate of a cohort, so long as headstarted animals have a statistically higher chance of recruitment than non-headstarted individuals. These modelling approaches have emphasised that head-starting cannot compensate for the loss of adult animals (Heppell et al. 1996), therefore any head-starting programme that diverts attention or funding away from conserving adult animals in-situ is likely to be undermining its own aims. Despite this, headstarting is often a much more

feasible and affordable option which makes it attractive for conservation managers (Burke 2015) and it remains widely used though under-evaluated in turtles and crocodilians.

1.6 Conservation of the Gharial in Chitwan, Nepal

The issues of using an anecdote and belief informed headstarting approach are exemplified by conservation of the gharial crocodylian (*Gavialis gangeticus*) in Chitwan, Nepal, the major case study of this thesis. The gharial (*Gavialis gangeticus*) is a highly evolutionarily distinct crocodylian that is endemic to the Indian subcontinent. Gharial are the most aquatic and piscivorous of the crocodilians, and are the crocodylian with the most conspicuous sexual dimorphism: the males have a bulbous outgrowth of irregular connective tissue on the end of their snout (Martin & Bellairs 1977). This ghara – meaning small clay pot in Hindi – is what gives the gharial its name, and likely performs a function in acoustic communication (Jailabdeen, unpublished data). The gharial is Critically Endangered due to declines of at least 94% occurring in the mid-20th century (Lang et al. 2019). These declines are believed to have been principally due to dams and barrages disrupting river hydrology, mortality in fishing gear, water control and extraction activities, as well as unregulated hide-hunting (Whitaker 2007; Lang et al. 2019). Populations remain low due to a lack of reversal, or intensification, of the above threats (other than hunting which has largely been prevented), compounded by anthropogenic river-bank disturbance and sand and rock mining on the river banks (Lang et al. 2019). The gharial now only occurs in 14 largely isolated subpopulations in India and Nepal, of which only 6 exhibit recent reproduction, and only one subpopulation – in the National Chambal Sanctuary in India – is in a large protected area on an unobstructed river with a self-sustaining gharial population (Lang et al. 2019; Bashyal et al. 2019). In Nepal, the gharial once occurred in all lowland river systems. Now there are only two wild, remnant populations in river stretches within the Bardia and Chitwan National Parks (Lang et al. 2019). An extensive description of the gharial can be found in Chapter 3.

Gharial conservation efforts in Nepal have focused on the Chitwan National Park, where an intensive head-starting approach has been carried out for the past forty years (Acharya et al. 2017). Despite forty years of this intervention, which is expensive and requires

considerable manpower and resources, the adult gharial population has not recovered. Very limited monitoring and evaluation have meant that the reasons behind the lack of success are not understood, and an adaptive-management approach has therefore been precluded. Calls have been made for gharial conservation funding in Nepal (which is limited and irregular) to shift to in-situ protection (Stevenson 2015; Lang 2016; Acharya et al. 2017), but there are concerns that if this shift is not evidence-based it may lead to the extirpation of the gharial. It is anecdotally thought, although there is no evidence to support this, that the counterfactual could have been much worse in the absence of headstarting, with the species being extirpated (J. M. Ballouard et al. 2010; Acharya et al. 2017). Conversely, however, the lack of evidence means we do not know whether the counter-factual could have been much better, with the gharial population naturally recovering if the head-starting programme had not removed all the eggs from the wild for so many decades. Adaptive management requires adequate models to provide not only an understanding of the past, but also useful comparative projections of the future, in order to compare management options (Williams 1997). At the commencement of this project in 2017, the absence of population estimates in Chitwan, a lack of any quantitative understanding of post-release survival and dispersal of gharial, and limited data on reproduction meant such models could not be created.

This thesis aims to fill that knowledge gap in order to provide sufficient data to begin a cycle of adaptive management of gharial in Chitwan, and to contribute to the evidence base so that such management can be commenced for other headstarting programmes too. By providing a retrospective analysis of the ongoing programme, combined with preliminary data on variables influencing – and causes of – mortality and dispersal, I have provided a suitable evidence base for stakeholders to come together and reassess the programme moving forward. I aim to provide actionable suggestions and useful hypotheses to improve management, which can be tested through an active management approach to compare actions. The utility of selecting Chitwan as a case study for investigating headstarting is that there is sufficient baseline data from the 1980s, and sporadic counts of both populations and nesting, to understand general population trends through time, as well as data on annual releases and nest collections. Furthermore, Chitwan is in the furthest upstream reaches of gharial range, so there is no potentially contributing upstream gharial population.

The threats to gharial in Chitwan – a dam on the Indo-Nepal border, fishing by-catch and sand mining – are representative of the general threats to gharial found more broadly in South Asia. Finally, there were substantial management changes between 2004 and 2010 which correlate with improving success in the programme, helping to disentangle different hypotheses for why headstarting has had such variable success. I hope that the identification of past and ongoing failures will not be disheartening, but act as a catalyst for change in practice, and innovation and experimentation on exciting new potential avenues for gharial conservation which build on the wonderful success of gharial conservation so far: that the species is not extinct.

1.7 Thesis Outline

This thesis begins with the current introduction, and there is secondary introduction to the primary study species and site in Chapter 3. Chapters 2 and 4-6 are data chapters, and Chapter 7 is a general discussion, with considerations for future research and conservation action. In all data chapters I use the third person plural ‘we’ as I had extensive assistance with all data collection. Although I conducted all analyses and writing of these chapters myself, I continue to use ‘we’ throughout. Supporting information to Chapters 2 and 5 is included in the Appendices, as well as a published paper, which I wrote as a co-author, which is an output of the broader research project of which this thesis is one part.

In Chapter 2, I compiled a database of functional traits for all 28 extant crocodylian species. Using this database and published phylogenies, I investigated the functional and phylogenetic diversity of crocodylians, projected how this diversity is threatened, and made recommendations for the prioritisation of conservation of species to best preserve this threatened diversity. I investigated the relationship between evolutionary and functional distinctiveness, and how this relationship may influence the ability of measures of phylogenetic diversity to act as surrogates for functional diversity, which is challenging to measure. Additionally, I used the functional trait database to identify functional groupings of crocodylians and traits associated with extinction risk, to help inform conservation action and ecological research. This chapter was published in *Functional Ecology*, and is formatted as such, and includes the necessary section on author contributions.

Chapter 3 is a secondary introduction, introducing the biology, ecology and conservation status of the focal species of this thesis, the gharial (*Gavialis gangeticus*) and the primary study site, the Narayani and Rapti rivers of Chitwan, Nepal

In Chapter 4 I compare Home Range estimates for gharial derived from two different types of technology: GPS-GSM tags and VHF tags. By comparing the Home Range estimates I investigate the differing biases of the two technologies. I then compare different methods of processing VHF data to obtain more accurate Home Range estimates. By comparing the two technologies, I aimed to inform analysis of data collected using VHF telemetry in Chitwan, the Chambal and other parts of gharial range, and to provide a straightforward way of processing low spatial resolution VHF data to create Home Range estimates which prove useful for ecological studies and conservation management.

In Chapter 5, I use data from large-scale repeat surveys conducted 2018-2021 to investigate gharial abundance and population trends in Chitwan. These surveys were conducted by the DNWPC, NTNC, Himalayan Nature and ZSL with funding from the Darwin Initiative. I employ three different techniques for estimating the population size in Chitwan, and compare results, producing the first population estimates for the entire Chitwan gharial population. Using N-mixture models, I identify correlates of varying detectability in gharial surveys, and also factors influencing abundance of gharial in Chitwan's major rivers.

In Chapter 6 I conduct a retrospective analysis of the headstarting programme for gharial conservation in Chitwan 1983-2021. I tagged twenty-five gharial upon release from the Gharial Conservation Breeding Centre in Chitwan 2019-2020, and twenty free-living gharial captured in the Rapti river in 2018-2019. I then conducted extensive monitoring of all gharial to determine causes of mortality, and investigate movement patterns and reproductive ecology. I collated data from multiple sources on unadjusted population and nest counts, causes of mortality in untagged gharial, long-distance dispersal events and yearling sightings. I use these data to parameterise a deterministic model of the gharial population in Chitwan, with stochastic mortality and dispersal estimates. I conduct retrospective population projections for gharial total population, male and female adult population and annual nesting effort, and compare these to the dynamics of observed data. This allows me to draw conclusions about the apparent survival of past cohorts of gharial,

time to maturation, reproductive frequency and sex ratios in the adult population. Using the results, I make recommendations for adaptive management in the future, both in Chitwan, and for headstarting programmes in other sites and for other species.

Chapter 7 is a brief synthesis of the findings, which have been primarily discussed in detail in the discussion of each chapter. I use this chapter to consider the future of conservation management of the gharial in Chitwan, the gharial across South Asia, and of headstarting crocodylians globally.

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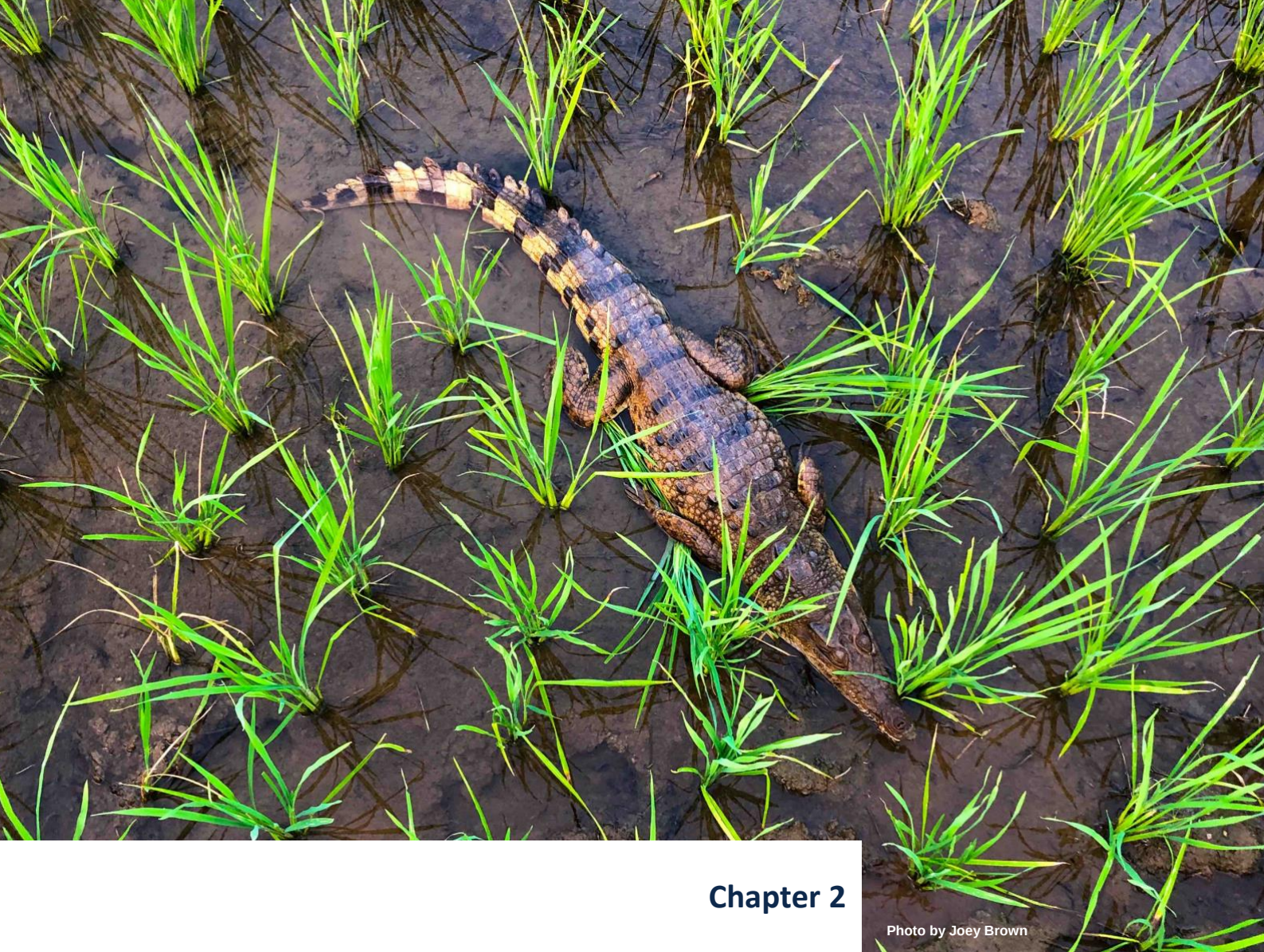


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Chapter 2

Using functional traits to identify conservation priorities for the world's crocodylians

RESEARCH ARTICLE

Animal Functional Traits

Using functional traits to identify conservation priorities for the world's crocodylians

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Abstract

1. Over half of all crocodylians are threatened with extinction, with many species considered to be functionally extinct throughout their historical ranges. How such losses impact crocodylian functional diversity (FD) and its conservation is poorly understood, but can be investigated using measurable traits. Where such information is unavailable, phylogenetic diversity (PD) has been proposed as an effective strategy to capture FD, but this assumption is not well tested.
2. We constructed a global trait database for 28 extant crocodylians, and quantified their FD, distinctiveness and groupings. Functionally distinctive crocodylians include the most and least evolutionarily distinct species, likely because unusual trait values and combinations can be produced by both evolutionary isolation and evolutionarily recent adaptive radiation. We identified four functional groups of crocodylians, within which species have similar ecological roles. Highly distinctive species, such as the gharial, fit less well into functional groups.
3. We combined functional distinctiveness (FUD) and threat ranking scores to prioritise species for conservation. The metric is termed Ecologically Distinct and Globally Endangered (EcoDGE), and we tested how well it operates to preserve crocodylian FD among extant Crocodylia. Under current extinction scenarios, 32%–38% of crocodylian FD might be lost within 100 years. Losses increase to 48% if all threatened species go extinct, with Asia identified as a hotspot of threatened FD in crocodylians. Highly threatened distinctive species lead this extinction scenario, exacerbated by clusters of species sharing traits linked to high extinction risk. These traits include habitat specialism and lower reproductive investment; in contrast, species that tolerate climate extremes are more resilient.
4. Prioritising at-risk species based on FUD and threat status appears to be an effective way to conserve present-day crocodylian diversity. Conservation prioritisation based on the EcoDGE metric performed well to minimise projected losses in FD. Among extant crocodylians, FD losses are projected when high diversity overlaps with high extrinsic threats.
5. We then examined the utility of PD-based measures as surrogates for FD conservation, to better understand the advantages and drawbacks of such substitutions.

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A PD-based measure such as the Evolutionarily Distinct and Globally Endangered metric provided an effective tool to capture FD in our tests.

KEYWORDS

conservation prioritisation, Crocodylia, EDGE, extinction risk, functional distinctiveness, functional diversity

1 | INTRODUCTION

Human impacts on global ecosystems have led to major losses of species worldwide (Dirzo et al., 2014; Hull et al., 2015). As species become increasingly rare and decline towards extinction, they cease to perform functional roles in their ecosystems, which has the potential to cause cascading ecological impacts (Valiente-Banuet et al., 2014). Indeed, species often become functionally extinct in most of their range long before final extinction (McConkey & O'Farrill, 2015; Valiente-Banuet et al., 2014). Conservation of the global pool of biodiversity is vitally important, and should consider all facets of biodiversity, including functional diversity (FD; Pollock et al., 2020).

The functional role of a species can be estimated through an assemblage of functional traits, that is, traits which impact fitness via their effects on growth, reproduction and survival (Violle et al., 2007). Traits can be used to determine FD in a taxonomic group (e.g. Pollock et al., 2017) or in an ecological community (e.g. Murgier et al., 2021). Functionally distinctive species are those which have unusual or unique trait values and/or trait combinations (Dee et al., 2019), and are therefore likely to be ecologically irreplaceable (Violle et al., 2017). Irreplaceability and vulnerability are key concepts for prioritisation in biodiversity conservation (Brooks et al., 2006), and prioritising conservation of multiple components of global biodiversity is also important for maintaining future options for natural systems to support human society (IPBES, 2019).

Understanding the functional roles of species in their ecosystem can inform ecological studies and conservation planning. However, populations of many species are now so depleted that field studies in most or all sites of former occurrence are often impossible. Transferring knowledge from one species to others is therefore of increasing importance, and can be informed by patterns of FD within a taxonomic group, such as functional groupings (Blaum et al., 2011); species with similar sets of traits may respond similarly to environmental change or to conservation interventions, whereas functionally distinctive species are likely to have a more idiosyncratic response.

Measures of diversity other than a direct metric of FD can also be employed in conservation prioritisation (Petchey & Gaston, 2006). For freshwater species, large population declines and limited extinction risk data (He et al., 2018), coupled with difficulties of obtaining functional trait data (Schmera et al., 2017), necessitate alternative measures. One such metric is phylogenetic diversity (PD), which captures the unique evolutionary history of a set of species (Faith, 1992). PD may also play a dual role as a useful surrogate for capturing FD (Redding & Mooers, 2015; Tucker et al., 2019). PD-based estimates measure feature diversity (not

just function) across the tree of life, with the aim to conserve current and future unknown benefits that biodiversity can provide (Molina-Venegas et al., 2021). PD has been integrated directly into practical conservation priority setting through the Evolutionarily Distinct and Globally Endangered (EDGE) metric, which ranks species based on their Evolutionary Distinctiveness (ED) and IUCN Red List status (Isaac et al., 2007).

Crocodylia is an order comprised of just ~28 extant species exhibiting a broadly similar within-order biology, distinct from that of other extant vertebrates (Colston et al., 2020; Uetz, 2022). This unusual biology means that crocodylians are functionally unique within their freshwater ecosystems and, as ectotherms, can reach higher densities than equivalently sized endothermic apex predators. This suggests that crocodylians play important and irreplaceable ecological roles (Somaweera et al., 2020). As a first step to understanding the identity and diversity of these roles, functional similarities and differences across crocodylian species could be determined using traits specifically selected to capture the distinct ecological functions of the group.

Traits can also be used as a quantitative metric to identify conservation priorities for threatened FD (e.g. Hidasi-Neto et al., 2015), and therefore to inform decisions on how to conserve this diversity as a key facet of global crocodylian biodiversity. This need is urgent as over half of the assessed crocodylian species are threatened, with several Critically Endangered species already extirpated or functionally extinct across most of their ranges (IUCN, 2021). A trait-based approach can also be used to understand the mechanisms of extinction risk (Cardillo et al., 2008). Traits that correlate with extinction vulnerability include low rates of reproduction (Pincheira-Donoso et al., 2021; Purvis et al., 2000) and habitat specialisation (Böhm et al., 2016; Isaac, 2009), and if such traits are found in functionally distinctive species and/or in groups of functionally similar species, the loss of FD will be accelerated as such species go extinct. Taken together, these considerations indicate that a functional approach should improve conservation planning for crocodylians.

The highly derived biology of crocodylians means that a narrow taxonomic approach is likely to be more precise for both comparative models and functional comparison (Cardillo et al., 2008); in multi-taxa analyses investigating extinction risk, the signal of the species-poor order of crocodylians is swamped (e.g. Colston et al., 2020). Additionally, broad functional traits (e.g. 'terrestrial/aquatic') could mask subtler functionally important differences which are relevant for understanding differing susceptibilities to similar threats. For example, in South Asia, the gharial *Gavialis gangeticus*, a highly aquatic specialist with limited terrestrial capabilities, is Critically

Endangered, but the sympatric mugger crocodile *Crocodylus palustris*, a habitat generalist that moves considerable overland distances, is merely Vulnerable (IUCN, 2021).

Using crocodylians as a case study, we constructed a novel database of specific functional traits of extant species. Using simulations of extinctions on functional trait dendrograms, we predict losses of FD, and how patterns of functional distinctiveness (FUD) and clustering may lead to these projected losses. We investigate the associations between extinction risk and traits, to determine which intrinsic traits influence susceptibility to threats. Using a metric that combines FUD and extinction risk data [Ecologically Distinct and Globally Endangered (EcoDGE); Hidasi-Neto et al., 2015], we determine whether prioritisation of species with this metric is effective for conserving FD. We compare this metric with other prioritisation schemes, in particular the EDGE metric as a stand-in estimator for imperilled FD when comprehensive trait information is lacking. Our results not only suggest useful functional groupings and patterns within crocodylians, but also provide wider insights into the value of using various diversity estimators to understand and prioritise conservation actions.

2 | MATERIALS AND METHODS

2.1 | Crocodylian phylogeny

For this analysis, we included 28 crocodylian species (Supporting Information, Methods). For phylogenetic analyses, we used the dated phylogenies from Colston et al. (2020) (Figure 2), which contain 27 of the species in our dataset and provided a distribution of phylogenetic hypotheses, allowing us to incorporate phylogenetic uncertainty. The 28th species, the recently described Hall's New Guinea crocodile *Crocodylus halli*, was excluded from analyses requiring phylogenetic information. For extinction risk analyses, we used the maximum-likelihood tree, which is suitable as this phylogeny does not use taxonomic imputation (Rabosky, 2015). For all other analyses, we used 100 unique trees randomly selected from the posterior distribution of 10,000 dated phylogenies in Colston et al. (2020).

2.2 | Traits database

We compiled a database of 12 traits considered to be functionally relevant from the published literature, grey literature and personal communications with experts (Griffith et al., 2022). These are traits related to diet/foraging strategy (skull shape, bite force), reproduction [nest type, relative clutch mass (RCM)], body size (largest male size, female size at maturity), habitat type (generality, salt tolerance, terrestriality), tolerance of extreme climates (aestivation, brumation) and potential to act as ecosystem engineers (ability to dig burrows). Many traits will have impacts across functional categories (e.g. terrestriality will influence diet and foraging strategy, as well as habitat type and factors such as dispersal ability).

2.3 | Statistical analysis

2.3.1 | Construction of functional trait dendrograms

We carried out all statistical analyses in R version 4.0.5 (R Core Team, 2021). We constructed functional trait dendrograms to investigate functional groups and diversity, following Pollock et al. (2017). We calculated pairwise species dissimilarity with a generalised Gower's distance matrix, which accommodates continuous, ordinal and binary variables. We then generated a dendrogram for all species including all traits, using an unweighted pair group arithmetic mean hierarchical clustering method. We repeated the dendrogram construction, dropping random combinations of 1–3 traits from the analysis each time to capture uncertainty and reduce the impact of specific traits on the results. We repeated this process to generate a distribution of 100 trees. Dendrograms produce similarity hierarchies comparable to phylogenetic trees, enabling us to use analogous techniques for investigating functional and PD in our analyses.

2.3.2 | Calculating functional and evolutionary distinctiveness

To estimate the contribution of each individual species to crocodylian FD, we calculated 'FUD' using the fair proportion metric (Redding, 2003). This metric determines the contribution of each individual taxon to a tree (i.e. functional dendrogram tree) by apportioning the length of each branch equally between all species tips of that branch (Redding, 2003). We calculated the FUD for each tree in the distributions of 100 unique trees, and took the median value for each species as the representative FUD score. We then repeated this method using phylogenetic trees to obtain an 'ED' score to estimate the contribution of each species to crocodylian PD. We used the fair proportion metric for both distinctiveness measures as it is used to calculate ED, which is the most widely used measure of evolutionary uniqueness (Faith et al., 2018) and for comparable scores of FUD (Hidasi-Neto et al., 2015).

The EcoDGE metric is derived from the EDGE metric, and combines the FUD of a species with a measure of threat based upon Red List status, to rank species for conservation priority with the aim of conserving FD (Hidasi-Neto et al., 2015). We calculated EcoDGE scores for each crocodylian species, multiplying our FUD scores by 100 to balance the weighting of the two component values of the EcoDGE metric:

$$\text{EcoDGE} = \ln(1 + \text{FUD}) + \text{GE}^* \ln(2),$$

where GE is the Red List category weight [Least Concern (LC) = 0, Near Threatened (NT) = 1, Vulnerable (VU) = 2, Endangered (EN) = 3, Critically Endangered (CR) = 4 (Purvis et al., 2000)].

We also calculated the EDGE score for each crocodylian species to provide an updated EDGE score for each species and scores for new species, based on the Colston et al. (2020) phylogeny. For

recently split species, we contacted Red List evaluators to request the probable Red List status from draft evaluations.

2.3.3 | Relationship between functional and evolutionary distinctiveness

To determine the relationship between ED and FUD scores, we ran two regression models. We transformed ED and FUD to achieve normality, and after visual inspection used a quadratic and a piecewise regression approach (using the R package *SEGMENTED*, in which a single breakpoint in the model is estimated used a maximum likelihood approach; Muggeo, 2008), and selected the model which best fit the data. To examine the relationship between FUD or ED and extinction risk, we conducted generalised linear models where Red List status (categorised as 0–4) was regressed against FUD or ED as the predictor variable, and where a binary measure of CR/not CR was regressed against the same predictor variables, since high probabilities of extinction of CR species drive extinction scenarios.

2.3.4 | Correlates of extinction risk

To assess traits correlated with extinction risk, we used Red List categories as response variables and all traits from our database as explanatory variables in a phylogenetic generalised linear model (pGLS), which controls for shared ancestry (Böhm et al., 2016) and is appropriate for our ordinal, continuous and binary trait data (Symonds & Blomberg, 2014). RCM, male and female size, and skull measures were log-transformed to achieve normality. As in the previous analyses, extinction risk was quantified using Red List status categorised as 0–4. Not evaluated species were treated as in (b). We implemented analyses in the R package *CAPER* (Orme et al., 2013). We first ran univariate pGLS of all predictors on extinction risk, and then carried out stepwise multiple regressions to produce minimum adequate models (MAMs) to explain extinction risk, following Purvis et al. (2000). To reduce the possibility of Type 1 errors due to testing multiple hypotheses on non-independent data subsets, we used Bonferroni corrections of *p*-values during stepwise regression (Böhm et al., 2016).

2.3.5 | Extinction projections

To estimate the percentage of functional and PD of crocodylians likely to be lost under current levels of threat, we simulated extinction scenarios using probabilities of extinction $p(\text{ext})$. We randomly selected a tree from the existing distribution of 100 trees (either functional dendrograms or phylogenetic trees) and ran a scenario where each species had a probability $p(\text{ext})$ of going extinct, and determined the change in FD or PD in the tree before and after extinctions (Faith, 2008). We repeated this process 1,000 times for both sets, and determined the median change.

For $p(\text{ext})$ we followed Davis et al. (2018), who extrapolated this value from Red List criterion E; for CR species, $p(\text{ext}) = 0.999$, EN = 0.6723, VU = 0.1, NT = 0.0141 and LC = 0.0017. As these are extrapolations of 'minimum risk of extinction' to qualify for each threat category, and the within-category range could be substantial (e.g. a VU species could be close to either NT or EN), we also ran a 'pessimistic' scenario in which EN species were assigned $p(\text{ext}) = 0.8991$, VU = 0.605, NT = 0.09 and LC = 0.01269 (i.e. up to within 10% of the classification of the higher threat category). We also ran a scenario that assumed all threatened species would go extinct.

To determine if more functional and PD was currently threatened than if extinction risk was randomly distributed across the functional and phylogenetic trees of crocodylians, we created a null comparison in which we randomly assigned species to threat categories, but retained the same total number of species in each category. We then repeated the same process as above. We calculated effect size using Hedges' *g* (Hedges, 1981). A large effect size means more diversity is lost than if extinction risk is distributed at random, suggesting threatened species are clustered and/or highly distinctive.

2.3.6 | Gain of FD under different conservation schemes

We investigated the gain in FD under different currently used conservation prioritisation schemes, by sequentially protecting 1–16 species (16 = all threatened species) under the following methods:

1. EcoDGE ranking
2. EDGE ranking
3. Red List status, from most to least threatened (with random sampling within category)
4. Random draw from the 16 threatened species, to create a conservative null model (after Redding & Mooers, 2015)

For each method, we ran the extinction scenario 100 times for each number of species protected (i.e. 1,500 total runs per prioritisation measure) and calculated the median change in FD per species protected. We repeated this for each prioritisation measure, and compared the accumulation curves at the 25%, 50% and 75% quartiles. The use of the 'optimistic' and 'pessimistic' extinction scenarios from (d) gave similar results when comparing between methods, so we report only the 'optimistic' scenario.

3 | RESULTS

3.1 | Functional and Evolutionary Distinctiveness, EcoDGE and EDGE

The gharial is the most functionally distinct crocodylian (Figure 1), with a score 100% more distinct than the next species, and the highest distinctiveness score in all functional dendrograms. Since the



Skull attributions: top row and bottom row centre © The Trustees of the Natural History Museum, London; others © Matthew Shirley

FIGURE 1 Clockwise from top left: *Gavialis gangeticus* skull and male and female; *Crocodylus porosus* skull and individual in ocean; *Alligator sinensis* skull and male and female; *Osteolaemus afzelli* skull and adult; *Tomistoma schlegelii* skull and adult; and *Mecistops cataphractus* skull and adult, showing convergent evolution with *Tomistoma*.

gharial was so distinct, we repeated all analyses with the gharial excluded; although this impacted some effect sizes, it did not alter any overall conclusions, so we retained gharial in final analyses. The gharial also ranked highest for conservation prioritisation based on EcoDGE scores, followed by the Chinese alligator *Alligator sinensis* and Orinoco crocodile *Crocodylus intermedius*. ED and EDGE scores calculated in this study (Table 1) had similar scores and rankings to previous studies on ED and EDGE in reptiles (Gumbs et al., 2018).

3.2 | Functional groupings

The clustering of species within the functional dendrograms showed four functional groupings (Figure 2, groupings A, B, C and D). Grouping A are fecund species with short, broad skulls, which are tolerant of environmental extremes but mostly lack physiological tolerance of saltwater. Grouping B are fairly terrestrial species, which are known to hunt on land and spend periods of time away from the water. Grouping C are 'classic crocodiles', mostly extreme habitat and dietary generalists that will take terrestrial prey at the water's edge, and are physiologically tolerant of saltwater. Grouping D are highly aquatic, mostly freshwater species that predominantly consume aquatic prey. Groupings A and B appeared to be the most similar, being unresolved in 39% of trees (Supporting Information, Results).

3.3 | Relationship between functional and evolutionary distinctiveness

A linear function with a breakpoint just above the median ED value (Figure 3) best describes the relationship between ED and FUD, accounting for 30% of variation ($T = -2.097/2.845$, $df = 23$, $p < 0.05$).

This suggests the most functionally distinctive crocodylian species are those with the highest and lowest ED. The GLM for ED did not find a significant relationship between Red List category and either FUD or ED of crocodylians. The GLM for CR/not CR against FUD found a positive relationship that is nearly significant at $\alpha = 0.05$, with functionally distinctive species more likely to be CR (estimate = 2.9, $df = 26$, $p = 0.09$); limited significance could be due to our small sample size ($n = 27$).

3.4 | Correlates of extinction risk

The MAM accounted for 56% of total variance (Table 2), suggesting that species have a greater threat of extinction if they are habitat specialists and have a lower RCM. Models with additional covariates had significant multicollinearity, with habitat generality being positively correlated with burrowing ability, and with ability to aestivate being positively correlated with burrowing ability.

Several other variables were also significantly correlated with extinction risk in univariate pGLS (Table 3). Species are more at risk if they do not dig burrows, are not known to aestivate, are habitat specialists and have a lower RCM.

3.5 | Extinction projections

Our models project that 32%–38% of crocodylian FD is likely to be lost within 100 years, compared to an estimate of 20%–30% if threat status was randomly distributed among species. This increases to 48% of FD if all threatened species were to go extinct (Figure 4; Table S4), compared to 41% if threat was allocated randomly. Our models also project that 19%–31% of

TABLE 1 Functional distinctiveness (FUD), evolutionary distinctiveness (ED), Ecologically Distinct and Globally Endangered (EcoDGE) and Evolutionarily Distinct and Globally Endangered (EDGE) scores and IUCN Red List categories for all 28 crocodylian species, ordered from most to least functionally distinct.

Species	Species code	FUD	ED	EcoDGE	EDGE	IUCN
<i>Gavialis gangeticus</i>	GG	23.55	41.26	5.97	6.52	CR
<i>Crocodylus porosus</i>	CPo	10.73	14.78	2.46	2.76	LC
<i>Caiman latirostris</i>	CaL	10.57	25.99	2.45	3.3	LC
<i>Alligator mississippiensis</i>	AM	9.18	47.42	2.32	3.88	LC
<i>Alligator sinensis</i>	AS	9.13	47.42	5.09	6.65	CR
<i>Crocodylus johnsoni</i>	CJ	8.92	19.65	2.29	3.03	LC
<i>Crocodylus intermedius</i>	CI	8.76	11.52	5.05	5.3	CR
<i>Crocodylus rhombifer</i>	CR	8.38	13.3	5.01	5.43	CR
<i>Crocodylus mindorensis</i>	CMi	8.02	16.4	4.97	5.63	CR
<i>Crocodylus siamensis</i>	CSi	7.85	14.78	4.95	5.53	CR
<i>Crocodylus acutus</i>	CA	7.43	11.52	3.52	3.91	VU
<i>Crocodylus niloticus</i>	CNi	7.29	15.68	2.12	2.81	LC
<i>Melanosuchus niger</i>	MN	7.22	29.26	2.80	4.1	NT
<i>Crocodylus moreletii</i>	CMo	7.11	13.3	2.09	2.66	LC
<i>Crocodylus palustris</i>	CPa	6.55	19.1	3.41	4.39	VU
<i>Crocodylus suchus</i>	CS	6.52	17.98	3.40	4.33	VU
<i>Caiman crocodilus</i>	CaC	6.20	21.95	1.97	3.13	LC
<i>Caiman yacare</i>	CaY	6.16	21.95	1.97	3.13	LC
<i>Tomistoma schlegelii</i>	TS	6.04	41.26	3.34	5.13	VU
<i>Paleosuchus trigonatus</i>	PT	5.74	33.66	1.91	3.55	LC
<i>Paleosuchus palpebrosus</i>	PP	5.68	33.66	1.90	3.55	LC
<i>Crocodylus novaeguineae</i>	CNo	5.24	16.4	1.83	2.86	LC
<i>Crocodylus halli</i>	CH	5.20		1.83		LC
<i>Osteolaemus osborni</i>	OO	4.98	22.79	3.18	4.56	VU
<i>Osteolaemus afzelli</i>	OA	4.78	20.4	3.83	4.45	EN
<i>Osteolaemus tetraspis</i>	OT	4.72	20.4	3.13	4.45	VU
<i>Mecistops cataphractus</i>	MC	4.62	22.51	4.50	5.93	CR
<i>Mecistops leptorhynchus</i>	ML	4.62	22.51	3.81	5.93	EN

Abbreviations: CR, critically endangered; EN, endangered; LC, least concern; NT, near threatened; VU, vulnerable.

crocodylian PD is likely to be lost within 100 years, compared to an estimate of 17%–26% if threat status was randomly distributed among species (Figure 4; Table S5). This represents the loss of 115–191 MY of PD, or a loss of 271 MY of PD and 44% of PD if all threatened species went extinct, compared to 35% if threat was allocated randomly.

3.6 | Gain of FD under different conservation schemes

FD was recovered most quickly and consistently by prioritisation using the EcoDGE metric. Prioritisation of species for protection using Red List category alone, or using the EDGE metric, both recovered FD more quickly than the random allocation of protection among threatened species (Figure 5). Until protection of the 8th species, the lower quartiles of FD recovered by EDGE prioritisation

(‘worst case scenarios’) recover more FD than the equivalent quartiles of using Red List category alone.

4 | DISCUSSION

In this study, we found that crocodylian FD is highly threatened under current extinction scenarios, attributable to phylogenetic clustering of species possessing traits associated with increased extinction risk (e.g. habitat specialisation, low fecundity, reduced ability to withstand climate extremes), and to the high proportion of Critically Endangered functionally distinctive species. Multiple facets of crocodylian diversity are highly threatened, with projected losses of 19%–31% of PD of crocodylians, and 32%–38% of FD, rising to 44% of phylogenetic and 48% of FD if all threatened species were to go extinct. When investigating how to recover maximal FD within the Crocodylia, we found that prioritising species based on

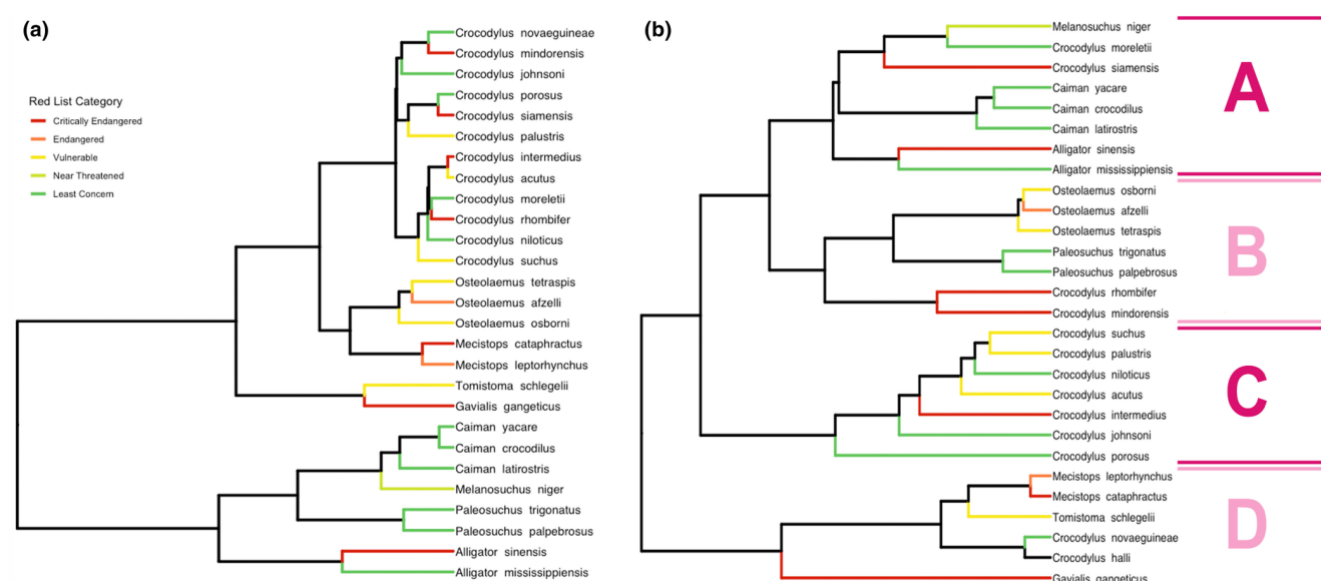


FIGURE 2 (a) Phylogeny of 27 species of extant crocodylian, from the maximum likelihood tree of Colston et al., 2020. Branch tips are coloured by the Red List category of each species, with species that have been split since the most recent assessment awarded their current draft Red List category. (b) Functional trait dendrogram illustrating functional groupings of 28 species of extant crocodylian, branch tips coloured as in the left panel. Functional groupings of crocodylians are illustrated (A–D).

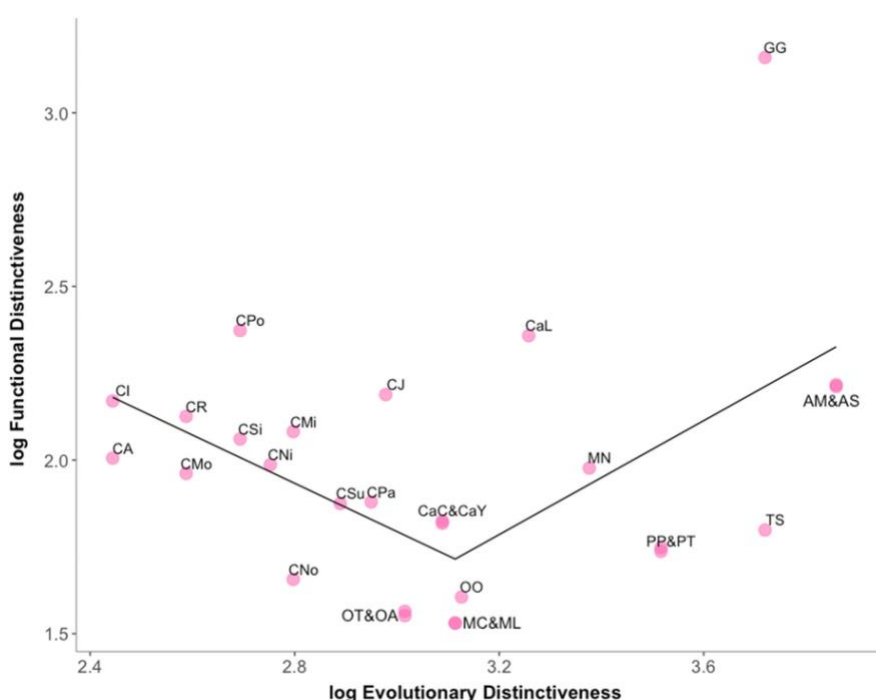


FIGURE 3 Linear relationship with breakpoint at 3.11 between (log) functional distinctiveness and (log) evolutionary distinctiveness ($R^2 = 0.30$). Species abbreviations in Table 1

FUD and risk of extinction was the most successful strategy; in the absence of adequate data on functional traits, prioritising species based on their ED and extinction risk provided a more suitable surrogate for conserving FD than extinction risk alone.

The gharial is the most functionally distinctive crocodylian species (Figure 1), and has extreme values for many traits, including an exceptionally long, thin skull (associated with a diet almost exclusively comprised of fish), a highly aquatic nature, and very large adult sizes (Griffith et al., 2022). Other highly distinctive species include

the saltwater crocodile *Crocodylus porosus*, the largest extant species, which is saltwater tolerant; the Chinese alligator and American alligator *Alligator mississippiensis*, which both occur at high latitudes and overwinter at cold temperatures; and the broad-snouted caiman *Caiman latirostris*, which has the broadest of all crocodylian snouts. These distinctive species also exhibit other unusual features that we did not measure, such as the gharial's sexually dimorphic 'ghara' (Figure 1), the long-distance movements of saltwater crocodiles (Campbell et al., 2013), and the communal mound nesting of

TABLE 2 Minimally adequate model explaining extinction risk in crocodylians using dated phylogeny. Uncorrected (p) and Bonferroni adjusted (p corr) values are shown.

	Coefficient	SE	t-value	p	p corr	Model R^2	λ
Intercept	8.94537	1.20950	7.3958	<0.001		0.5608	0.000
Generality	-0.42579	0.13168	-3.2336	<0.01	<0.01		
RCM	-2.05438	0.45887	-4.4770	<0.001	<0.001		

TABLE 3 Results of univariate pGLS on extinction risk (as defined by Red List category) for the global crocodylian dataset ($n = 27$), with *Crocodylus halli* excluded. Phylogenetic signal (Pagel's λ) of biological traits correlated with extinction risk is reported if λ was significantly different from zero.

Variable	λ	p -value of λ	R^2	t-value	df	p -value
Burrow			0.1526	-2.0793	24	<0.05
Aestivation			0.1656	-2.1827	24	<0.05
Generality	0.393	<0.05	0.2641	-2.9344	24	<0.01
Relative Clutch Mass			0.3611	-3.6830	24	<0.01

Abbreviation: df, degrees of freedom.

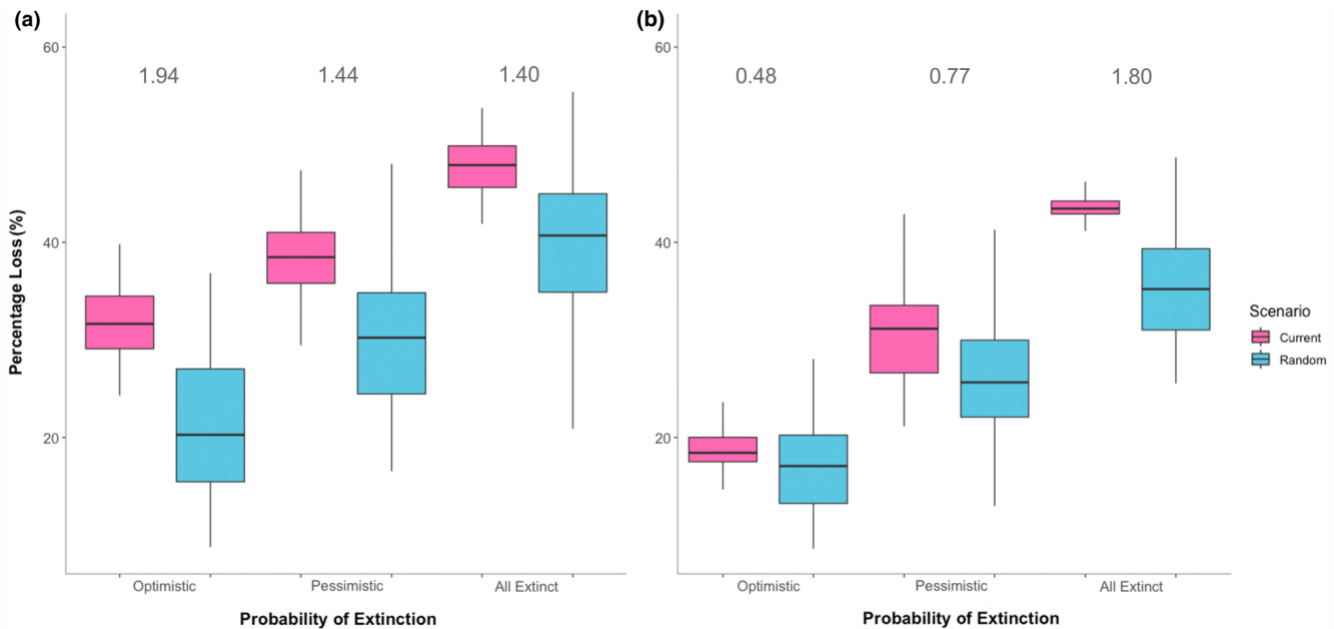


FIGURE 4 Projected percentage loss of (a) functional diversity and (b) phylogenetic diversity in crocodylians. Projections are either optimistic (O) scenarios (where species are assigned the lowest probability of extinction $p(\text{ext})$ for their Red List category), pessimistic (P) scenarios (where the highest $p(\text{ext})$ for the category is used), or all extinct (a) scenarios (where all threatened species are projected to go extinct). Scenarios labelled 'current' assign species to their current Red List category, and 'random' scenarios provide a null model in which the same proportion of threat categories are randomly assigned across species. Numbers above box plots give Hedge's g values, indicating effect size for difference between observed and random results, with values over 0.8 indicating a large effect size.

broad-snouted caimans (Larriera, 2002). These additional characteristics suggest that our nominal traits also captured unmeasured functional differences among species.

Of the 10 most functionally distinctive species, six occur in the Asia-Pacific region. Asia is the only continent where all three crocodylian families occur, and is also thought to be where the speciose *Crocodylus* genus originated (Oaks, 2011). We found that this region contained species with the highest and lowest ED. Long periods of unique evolutionary history can drive high FUD, especially through phylogenetic conservatism of traits and trait combinations (Faith, 2015). Rapid diversification in evolutionary radiations can also drive evolution of distinctive trait combinations (Cadotte

et al., 2013), and several species of *Crocodylus*, which radiated pan-tropically over the last ~10 MY (Oaks, 2011), exhibit surprisingly high FUD.

In the Asia-Pacific region, the saltwater crocodile is sympatric with a number of smaller-bodied *Crocodylus* species that are also highly distinctive, which is perhaps an indicator of niche differentiation driven by competition with this large, highly aggressive species. Spatial sorting, the concept of evolutionary change arising as a result of differential dispersal rates at expanding range edges (Shine et al., 2011), may also drive higher rates of diversification in the Crocodylidae (Nicolai & Matzke, 2019). It has been proposed that species with good dispersal ability (e.g. saltwater crocodile) and

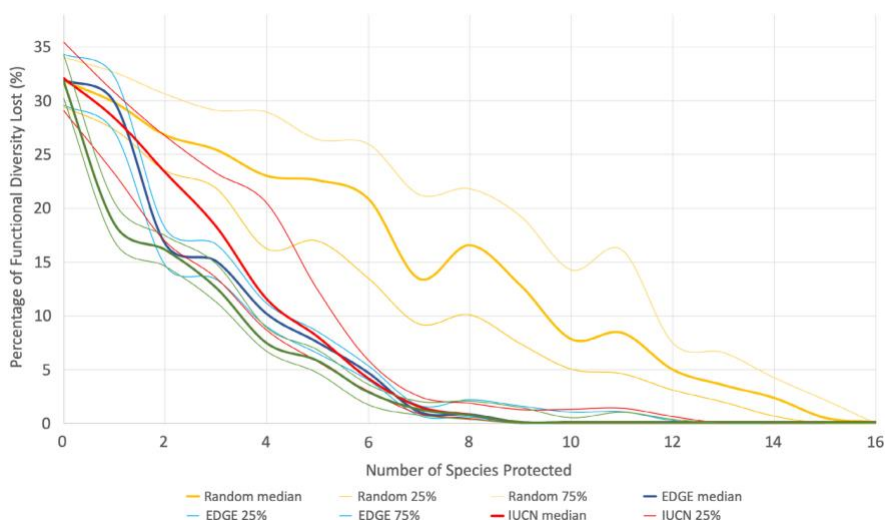


FIGURE 5 Percentage of functional diversity (FD) recovered as species are sequentially protected from extinction according to: Random protected of threatened species (yellow); Evolutionarily Distinct and Globally Endangered (EDGE) metric (blue); Red List alone (red); (d) Ecologically Distinct and Globally Endangered (EcoDGE) metric (green). Steeper accumulation curves indicate more FD recovered per species protected.

species that are no longer at the range expansion front (e.g. Siamese crocodile *C. siamensis*) could speciate from each other due to increased and reduced range expansion potential in these different populations/species (Nicolai & Matzke, 2019).

Of the 10 highest scoring functionally distinctive species, six are Critically Endangered and are sufficiently depleted as to be functionally extinct in most, if not all, of their range. The EcoDGE scores, based on species' FUD combined with their extinction risk, further highlights Asia as a hotspot of extrinsic threats. Crocodylians occupy inherently fragile habitats such as freshwaters and coastlines, and Asia has exceptionally high human pressures. The average percentage of an Asian crocodylian species' range under high human impact is 18%, versus an average of 0.1%–3% for other continents (2009 Human Footprint Index; Supporting Information, Results). The highest ranked EcoDGE species, the gharial, was historically abundant at very high densities (Lang, 2018), but the species is now absent from over 94% of its historical range, with a concomitant decline in abundance (Lang et al., 2019). The Chinese alligator, Siamese crocodile and Philippines crocodile *Crocodylus mindorensis* are also high priority EcoDGE species; indeed, in Asia north of Wallace's Line, all populations of crocodylians are threatened (Colston et al., 2020).

However, sympatric species also show different responses to similar threats. In crocodylians, we found that threat resilience was linked to fecundity, habitat generalism and ability to withstand climate extremes, relationships also found in other taxonomic groups (Böhm et al., 2016; Lee & Jetz, 2011; Liow et al., 2009). RCM (a measure of fecundity) was the strongest predictor of extinction risk that, when combined with habitat generality in our models, explained 56% of the variation in crocodylian extinction risk. Population recovery following declines will be at slower rates for low-fecundity species, which are less able to compensate for ongoing mortality (Purvis et al., 2000). Depleted populations will remain small for longer, and therefore remain vulnerable to processes including demographic and environmental stochasticity (Caughley, 1994).

These concerns are particularly relevant for crocodylians, as many populations were substantially reduced in the 20th century. While

some populations have recovered, others have not. Expensive and intensive reintroduction efforts have met with hugely varying success rates (Grigg & Kirshner, 2015). For example, differences in fecundity may be a key factor in differential establishment of the Cuban crocodile *Crocodylus rhombifer* and invasive brown caiman *Caiman crocodilus fuscus* in the Lanier Swamp, Cuba. Whereas the Cuban crocodile was locally extirpated by hunting following a dedicated reintroduction attempt, the brown caiman established a viable population from a few introduced individuals (Ramos & Rodriguez-Soberon, 2020). Habitat specialisation may also be a factor; our analyses reveal that habitat specialists are at higher risk of extinction, a trend observed across multiple animal taxa (Böhm et al., 2016; Owens & Bennett, 2000). Habitat specialists are more sensitive to habitat loss and degradation, and may be less able to move through marginal habitats to new suitable areas in response to habitat or climate change.

The univariate pGLS also identified that crocodylians that dig burrows and/or aestivate periodically had a lower extinction risk. Such 'sleep-or-hide' traits have been associated with reduced extinction risk in mammals (Liow et al., 2009). These traits may enable such species to persist despite habitat degradation and/or climatic changes by weathering intermittent unsuitable environments, or by enabling individuals to survive long enough to shift to another environment. Understanding traits associated with extinction risk can be useful for conservation. For example, low-fecundity species may respond well to 'headstarting' programmes that boost population levels, whereas species lacking sleep-or-hide traits are likely to respond better to habitat restoration and linkages. Similarly, habitat specialists may serve as good indicator species, as they are more sensitive to environmental changes.

We identified four 'functional groupings' of extant species (Figure 2). Each grouping represents a cluster of crocodylians in trait space with either similar responses to, or effects on, their ecosystem (Hooper et al., 2002). This functional clustering may explain the surprisingly low distinctiveness values for the evolutionarily distinct Sunda gharial *Tomistoma*, which has convergently evolved similar traits (e.g. longirostrine skull, aquatic nature) with the African slender-snouted crocodiles *Mecistops* (Figure 1). Our groupings can

inform the potential for knowledge transfer between species, including ecological research methods or conservation interventions, and in understanding similarity of ecological roles and broader patterns of convergence in vertebrate evolution.

We found species that shared high extinction risk traits tended to cluster together in functional groupings. For example, grouping D comprises mostly habitat specialists with low fecundity and few sleep-or-hide traits (Figure 2). This clustering of at-risk species, combined with the CR status of many highly distinctive species, leads to our predictions that 32%–38% of crocodylian FD is likely to be lost within 100 years. This is a substantial loss of ecological function for extant crocodylians, and the remaining FD might be even more precarious. Such clustering could lead to ‘tipping points’ where a functional group persists despite successive species losses, then collapses following the loss of the last remaining group member (Faith, 2015). These results thus support the use of priority-setting approaches that account for co-extinction of similar species, which would prioritise highly threatened clusters of similar species as well as distinctive threatened species, thereby addressing functional complementarity (Faith, 2015).

Uneven distribution of threat status between functional groupings suggests that we may lose whole sets of similar species before we are able to collect empirical data to fully understand their functional roles. Unfortunately, extant crocodylian species already constitute a functionally impoverished subset of pre-human crocodylian diversity. In particular, the arrival or activities of humans is associated with the extinction of the morphologically unusual South-China gavialid *Hanyusuchus sinensis* (Iijima et al., 2022), the endemic Madagascar crocodylian *Voay robustus* (Hekkala et al., 2021), and the Mekosuchinae, an Australasian clade of fully terrestrial crocodylians including a possibly arboreal species (Grigg & Kirshner, 2015). We will likely never know the ecological importance of these species, or whether their geologically recent disappearance has ongoing ecosystem-level consequences (Galletti et al., 2017).

It is vital to prioritise species conservation efficiently in response to ongoing biodiversity declines. However, measuring FD and distinctiveness is challenging, as results depend strongly on initial trait selection, which can be subjective and biased by data availability (Bernhardt-Römermann et al., 2008). Consequently, EcoDGE determinations may not be feasible. Instead, PD, designed to recover evolutionary features across the tree of life, may also capture FD. However, PD assumes that shared traits are due to shared evolutionary histories, whereas convergence can obscure the linkage between PD and diversity of traits; where convergence is high, PD may not necessarily capture the most diverse sets of measurable traits (Kelly et al., 2014), leading some researchers to refer to use of PD to conserve traits as the ‘phylogenetic gambit’ (Mazel et al., 2018). Our results with crocodylians suggest that using ED to conserve FD would be partially complementary: both metrics prioritise functionally distinctive species that lack close relatives on the tree of life. However, measures of ED will fail to capture those species that are distinct due to rapid evolution.

To assess their usefulness as an aid in real-world assessments, we tested the gain in FD under simulations of four different conservation prioritisation scenarios (EcoDGE ranking, our updated EDGE scores, Red List status alone and random selections of threatened species). The EcoDGE metric swiftly recovered FD, indicating that a ranking based on FUD is a suitable measure for capturing FD within the Crocodylia. Conserving species based on the EDGE metric, or by Red List status alone, also recovered more FD than random allocation. Notably, the ‘worst case scenario’ (lower quartile and lower limit) of FD recovered by EDGE prioritisation recovered more FD than using Red List status, suggesting that the EDGE metric is a more reliable method for conserving FD than extinction risk alone. In our analysis using crocodylians, the EDGE metric thus appears to perform well at capturing FD, even though the measure is designed to preserve PD.

It is important to recognise that PD-based prioritisation aims to capture the diversity of evolutionary features of species, both measurable and unmeasurable, and so maximise future ‘option values’ (Faith, 1992). FD is just one part of this diversity. Crocodylian blood has been found to contain antimicrobial peptides which may have future medical uses through anti-tumour and anti-bacterial ability (Chook et al., 2021). These potential uses of crocodylian peptides provide a clear example of an unanticipated value, and conserving crocodylians thus enhances our chances of having such options in the future (Molina-Venegas et al., 2021). Therefore, as has been found in other studies, considering both FD and PD for biodiversity conservation will capture more of the ecological differences, measured and unmeasured, between species (Cadotte et al., 2013; Hidas-Neto et al., 2015).

Our results show that patterns of FD and extinction risk traits can exacerbate predicted losses of FD. This pattern may, however, also provide a guide to identifying trait differences which could underlie differential responses to threats and conservation actions. Beyond crocodylians, this suggests a functional trait approach could be a useful first step when evaluating conservation actions, and their transferability between species or contexts, and for identifying species that could be utilised as indicator species. These results also suggest that developing an understanding of functional traits and their association with extinction risk may help in predicting threat status of Data-Deficient species. In particular, we found fairly subtle differences in trait values and combinations can lead to different responses to threats. Therefore, conducting such analyses with a narrower taxonomic focus and directed selection of traits may lead to more accurate predictions when aiming to predict species responses to threats or interventions.

Finally, we found that the use of a metric based on FUD and threat status (EcoDGE) to prioritise species for conservation helped recover predicted losses in FD, which supports studies employing similar useful measures such as FUSE (Functionally Unique, Specialised and Endangered; Pimiento et al., 2020). Within the Crocodylia, we identified key species which should be prioritised to maintain global crocodylian FD, with the gharial and Chinese alligator having the highest EcoDGE scores; these two species also have the highest EDGE scores,

highlighting their importance for global crocodylian biodiversity. However, given the challenges associated with collection of functional trait data, our results suggest that PD-based prioritisations can also capture significant amounts of threatened FD, and continue to be a useful tool in the urgent need to maintain multiple facets of biodiversity.

AUTHOR CONTRIBUTIONS

Phoebe Griffith, Rikki Gumbs and Samuel T Turvey conceived the ideas and designed the methodology; Phoebe Griffith compiled the database; Phoebe Griffith analysed the data with input from Rikki Gumbs and Jeffrey W Lang; Phoebe Griffith led the writing of the manuscript with input from all authors. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

Data available from the Zenodo repository <https://doi.org/10.5281/zenodo.6645415>, Griffith et al. (2022).

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SUPPORTING INFORMATION

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Chapter 3

An introduction to the study species and study site: the gharial in Chitwan National Park

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Figure 3.1: an adult female gharial basking in the shallows of the Budhi Rapti river in the Baghmara Community Forest within the buffer zone of Chitwan National Park. This image illustrates the incredibly elongated snout of the gharial. This is an extreme adaptation to moving its head swiftly through the water to catch aquatic prey, but unfortunately also a snout easily entangled in fishing gear, leading to high bycatch mortality.

3.1 Species Description

3.1.1 Taxonomy and Phylogeny

The gharial (*Gavialis gangeticus*) is one of 2 extant members of the family Gavialidae, alongside the tomistoma (*Tomistoma schlegelii*) of South East Asia (Oaks 2011). The third species within this family was recently discovered using historical specimens and accounts, *Hanyusuchus sinensis*, which was found in China until its extinction just a few hundred years ago, almost certainly caused by persecution by humans (M. Iijima et al. 2022). Gavialids are characterised by slender snouts relative to other crocodylians, with this anatomical feature taken to the extreme by the gharial (Figure 3.1). The extreme morphology of the gharial, compared to the more typically crocodylian *Tomistoma* has meant the placement of *Tomistoma* in the Gavialidae family has been contentious, with studies using morphological and fossil data suggesting that *Tomistoma* should be placed in the Crocodylidae (or ‘true

crocodiles') family with Gavialidae forming a monospecific sister family to all other extant crocodylians (Piras et al. 2010; Groh et al. 2020). However, the molecular data now strongly supports the placement of the two species in the family Gavialidae (Oaks 2011; Colston et al. 2020; Pan et al. 2021) with new morphological trees supporting this decision (Ristevski et al. 2020; Rio & Mannion 2021). Furthermore, the discovery of *Hanyusuchus sinensis* provides a morphologically intermediate species comprised of a mosaic of features of the other two species, lending even more support to the molecular evidence (M. Iijima et al. 2022).

3.1.2 Conservation Prioritisation

The family Gavialidae is a whole family on the tree of life which is in crisis due to anthropogenic activity: *Hanyusuchus* is already extinct, the tomistoma is soon to be uplisted to Endangered on the IUCN Red List (Shaney, pers. comm.) and the gharial is Critically Endangered. If we prioritise species for conservation action according to phylogenetic diversity and current extinction risk, such as through the EDGE metric, the gharial is a species of high conservation priority, second only among the Crocodylians to the Chinese Alligator (Chapter 2). However, revisions of the EDGE metric to EDGE2 will update the system of phylogenetic prioritisation of species (Gumbs et al. 2022). The inclusion of the extinction risk of a species' closest relatives on the tree of life, combined with the uplisting of the tomistoma, will push the gharial to the top of the list of EDGE priority crocodylian species. Combined with our recommendation for the gharial to be the highest priority crocodylian for conservation action based on functional distinctiveness (Chapter 2), the need to recover this species from its Critically Endangered status is acute.

Beyond practical recommendations to conserve gharial due to their contribution to the phylogenetic and functional diversity which make up biodiversity, the gharial is a species of cultural importance. The role of the gharial in the culture of South Asia goes back over 4000 years – to the Harrapan Civilisation of the Indus Valley (Parpola 2011). The gharial appears to have been important, appearing on tablets, seals and on pots, although the hieroglyph-like script of this civilisation has not yet been deciphered (Parpola 2011). Depictions of gharial often show them in the process of catching fish, no doubt important as catching fish

remains a major source of protein in South Asia today. Interestingly, these images of gharial were often depicted above animals, as if in the sky, suggesting the gharial was one of the Harappan constellations, perhaps with the tip of its tail being the Pole Star (Parpola 2011, 2014). The gharial also seems to have had a fertility role. Images show a gharial using its long snout to impregnate a human woman, and a potential translation of the hieroglyphic-like script suggests that the gharial is called an ‘embryo maker’ (Parpola 2011). This link with fertility could be related to the excellent parenting and creche guarding of gharial with thousands of hatchlings, or perhaps a view that the long snout of the male gharial had a phallic appearance. Links between gharial and fertility were also reported by pioneering Nepalese conservationists Tirtha Man Maskey and Hemanta Mishra who reported beliefs in Chitwan (Nepal) that the ghara of a male gharial, placed below the pillow of a pregnant woman, would relieve labour, and that the eggs were an aphrodisiac (Maskey 1989).

An important creature in Hindu mythology, the Makara, is thought to have been inspired by the gharial and other crocodylians (Gupte 1956; Wibowo 2020). Makara depictions are found widely across Asia, especially in Hindu and Buddhist temples, where it often guards thresholds, as well as regularly being a spout for springs (Gupte 1956; Wibowo 2020). Some Hindu gods are known to use the Makara as their Vahana – or vehicle – including the river goddess Ganga who is the personification of the sacred river Ganges, the basin of which is the only place gharial can still be found in the wild today. Many images of Ganga show her upon a crocodile, gharial or Makara. The Makara has many associated beliefs. It is a symbol of water and fertility (much like the gharial symbolism in Harappan culture), but also fearsome enough to be a protector of temples and gods. It is a creature that is both terrestrial and aquatic, symbolising the combination of earth and water (Gupte 1956).

3.1.3 Range and habitat

Gharial are known to have occurred historically in the Indus, Gangetic and Brahmaputra drainages, as well as the Mahanadi-Brahmini system, although they may well have occurred in other drainages in India and as far east as the Irrawaddy drainage (Lang et al. 2019). Their historical widespread occurrence and abundance can be determined due to the frequent

mention of the species in colonial Indian narratives, and because their distinctive skull morphology allows them to be readily distinguished from other crocodilians (Lang 2018).

The gharial is a riverine species, occurring Nepal and northern India in perennial lowland rivers which are highly seasonal, monsoonal rivers. They are particularly associated with large rivers with deep pools or channels, although they will migrate into smaller rivers and tributaries during monsoon season. Gharial preferentially bask on sand banks, although will also bask on rocky, muddy or grassy banks directly adjacent to rivers (Hussain 1991; Maskey et al. 1995; Rajbhandari & Acharya 2015). Their particularly preferred habitat appears to be deep pools of rivers with an island or peninsula (pers. obs.). High sand banks are necessary for nesting, into which females will dig holes in which to nest.



Figure 3.2: the remaining distribution of gharial: widely spaced locations in the Ganges Basin, with key populations labelled. Populations are coloured according to potential viability: the green population is viable and self-sustaining, orange populations have evidence of regular, recent breeding effort but are not currently viable, red populations are either a small population with sporadic or absent breeding, a population of a few individuals (possibly from another population) or recent reintroductions (after Lang et al. (2019))

The gharial is now restricted to 14 largely isolated subpopulations in localities in India and Nepal (Figure 3.2), having been extirpated in Pakistan, Bangladesh, Bhutan and Myanmar (Lang et al. 2019). Only 6 of these subpopulations have evidence of regular recent reproduction, and only one subpopulation – the National Chambal Sanctuary in India – is in a large protected area on an unobstructed river with a self-sustaining gharial population (Lang et al. 2019; Bashyal et al. 2019) (Figure 3.2, green). The Chambal population contains at least 500 mature adults, and ~86% of the global nest total: the importance of this population to gharial persistence as a species cannot be understated (Lang & Kumar 2018; Lang et al. 2019). The other key sites are the Katarniaghat, Gandak and Corbett subpopulations (India) and the Chitwan and Babai subpopulations (Nepal).

3.1.4 Ontogeny

Gavialids lay the largest eggs of all crocodylians, with gharial hatchlings emerging from the egg at over 30cm long (Gordon C. Grigg & Kirshner 2015a). The main ontogenetic changes in proportional morphology are that the skull becomes slightly less gracile and more robust over development, although this is not as pronounced a change as it is in the tomistoma (Piras et al. 2010). Adult male gharial can reach up to, and probably considerably exceed, 6m in length. Hunting records from early 20th century Nepal and India regularly report specimens over 7m length (e.g. Mishra 2014). In the wild in India, sexual maturity in females is attained at >3m length, and in males at ~4.5m length (Lang & Kumar 2018). In captivity and in higher latitudes these sizes may differ. Adults are sexually dimorphic, with adult male gharial developing a 'ghara' (Martin & Bellairs 1977). The ghara, meaning a small earth pot in Hindi, is what gives the gharial its name. It is a bulbous outgrowth on the end of the snout overlying the nares formed of dense and irregular connective tissue surrounding an internal cavity (Martin & Bellairs 1977). The function of the ghara is not well understood, but appears to be key to amplifying or producing the key audio signal male gharial use for communication, in particular 'popping' and hissing (Jailabdeen et al. 2019).

3.1.5 Behaviour

Gharial are the most aquatic of all crocodylians, and do not have the capability to move long distances overland like most crocodylians, so they are very much restricted to connected river systems and the immediate bank of the river. Being ectothermic, gharial regulate their body temperature behaviourally through basking and movement in the water column, as well as physiologically through control of peripheral blood flow (Gordon C. Grigg & Kirshner 2015b). Gharial are gregarious, basking in groups of adults and sub-adults in preferred basking habitats (although adult males will exclude other males during breeding season).

Telemetry studies on migration in gharial on the Chambal River in India show that adult gharial will migrate 200+ km to find suitable mating, nesting and feeding sites throughout the year (Lang & Kumar 2016). Sub-adult animals appear to be much more sedentary, remaining in a home range of <20km (GEP, pers. comm.). Animals released from captivity have been seen to travel 1000+km (Khadka 2020) but this is likely aberrant behaviour related to captive rearing and release. In the National Chambal Sanctuary adult gharial predominantly consume fish during the monsoon, when they migrate to the confluence of two large rivers to continually catch and consume fish in the turbulent waters, and return back upstream post-monsoon (Lang & Kumar 2016). Further telemetry studies are necessary to determine if these migrations are typical of gharial as a species or peculiar to the Chambal river system. Recent telemetry research in Chitwan shows that some - but not all - adults show migratory behaviour in this system, but opposite to that of the Chambal, with females moving upstream in the monsoon (Griffith, unpublished data). Gharial can be observed catching and consuming fish throughout the year, although in the cold months of winter such observations are unusual, suggesting they rarely eat in the cold weather since digestion and metabolic processes in crocodilians are temperature-sensitive.

Gharial mate from January to March (with variation depending on position of the population within species range). Male gharial will patrol areas of river which correspond to favoured female basking sites. Males will then exclude other males from this area of river, using audio, visual and physical challenges. Males will court females using a courtship routine which involves making a 'popping' noise, hissing, blowing bubbles and a physical display involving rolling the back and tail above the surface of the water (Jailabdeen, in prep). Females will then choose whether or not to mate with a particular male. Whether females

mate with multiple males in one breeding season, and whether nests have multiple paternity are particularly interesting questions currently being investigated (Singh pers. comm.). Following mating, females will nest communally in high sand banks adjacent to the river in March/April. Following an incubation period lasting 2-2.5 months (varying according to temperature), nests hatch fairly synchronously in late May and early June. Before hatching, young gharial will call out, with communication pre-hatching occurring between adults and young, and also between nests (Jailabdeen pers. comm.). Following the calling of hatchlings, female gharial will excavate the nests, although unlike all Crocodylidae and Alligatoridae they do not carry hatchlings to the water in their jaws because of the nature of the skull and dentition (Gordon C. Grigg & Kirshner 2015a). Gharial young will then form a creche of a single nest up to 1000+ hatchlings, which is guarded by a guardian female and additional satellite guardian females, and in parts of the range, especially the Chambal River, a guardian male (Lang pers. comm. and pers. obs.). These adults (or in some instances sub-adults) will guard the hatchlings from predators from hatching until the arrival of the monsoon floods, a period of 2-10 weeks. During this time hatchlings aggregate around, and on, the adults and adjacent shoreline. Following the arrival of the monsoon, adults then switch to predominately showing feeding behaviour, which may involve a migration to monsoon feeding grounds, and hatchlings will alter their behaviour to become highly cryptic. Yearlings will continue to associate with adults (in particular the guardian male) after monsoon (Lang pers. comm.).

3.1.6 Diet

Diet can be inferred from skull morphology, dentition and opportunistic observation. Gharial are the most longirostrine crocodilians, with a large proportion of skull length allotted to a long, slim snout, and a rostral proportion (ratio of the width of the snout at the anterior margin of the orbits to the length from there to the tip of the snout) of 0.10 (Erickson et al. 2012). Studies suggest the adaptation of a long snout allows for a swift strike suitable for catching agile aquatic prey, with the concurrent narrowing of the snout to reduce drag in the water also leading to reduced strength for the capture of larger prey (McHenry et al. 2006; Pierce et al. 2008). The homodont dentition of conical, sharp teeth and straight jawline of gharial, as well as the remarkable convergence in skull shape of river

dolphins and gharial, most likely driven by feeding ecology, further support the hypothesis that gharial are primarily piscivorous (McCurry et al. 2017). Opportunistic observations also suggest they are the most piscivorous of all crocodilian species, however this does not mean gharial exclusively eat fish. Gharial will eat invertebrates (pers. obs.), amphibians (Lang pers. comm.), softshell turtles (Kumar pers. comm.) and birds (Griffith & Chaudhary unpublished data) when they have the opportunity, so it seems likely that they opportunistically eat animals available in the river which do not pose a risk of damage to their slender snouts.

3.1.7 Co-occurrence with the mugger crocodile (*Crocodylus palustris*)

The gharial co-occurs throughout its range with the Marsh Mugger, *Crocodylus palustris*, a large 'true' crocodile with a brevirostrine snout. The co-occurrence of the mugger and the gharial is likely to be an example of niche differentiation in crocodilians, with the extreme differences in snout shape reducing competition (Pearcy 2011). However, interactions between the two species, in particular antagonistic interactions, are likely to be important to the ecology and occurrence of gharial. Mugger are known to kill and eat juvenile gharial (Lang and Kumar pers. comm., Khadka pers. comm.) and large mugger will displace juvenile and subadult gharial during basking (pers. obs.). Conversely, large gharial will also displace mugger crocodiles from basking sites, particularly large adult male gharial (Lang pers. comm., Griffith pers. obs.)

3.2 Conservation Status and Action

3.2.1 Conservation Status

The gharial is classified as Critically Endangered on the IUCN Red list (Lang et al. 2019). As stated above, the gharial occurs in just a few isolated sub-populations in the Ganges drainage, with an overall adult population of just 650 (median) concentrated mostly within the single Chambal population (Lang et al. 2019). Exponential declines occurred in the mid-20th century, with declines of at least 94% inferred, and contraction of area of occupancy conservatively estimated to be also 94%. Declines are believed to have been due principally

to dams and barrages disrupting river hydrology, mortality in fishing gear, and water control and extraction activities, as well as unregulated hide-hunting (Whitaker 2007; Lang et al. 2019). Populations remain low due to a lack of reversal or intensification of the above threats (other than hunting which has largely been prevented), compounded by anthropogenic river-bank disturbance, especially sand mining on the river banks (Lang et al. 2019). Sand mining (the removal of river aggregate for the construction industry) is an emerging major threat to rivers in Asia (Koehnken et al. 2020), and of particular concern to future gharial conservation. Gharial are very sensitive to human disturbance, although they can become habituated to certain human river uses in certain areas (pers. obs, Lang pers. comm). Threats are only likely to continue to intensify as demand for the extraction of river resources intensifies in South Asia.

3.2.2 Conservation Action in India

Surveys showed that by the 1970s gharial populations throughout the species' range had collapsed and it was realised that the species was at risk of extinction (Whitaker & Basu 1982). In India, in response to these declines (and also to concurrent collapses in the *Crocodylus palustris* and *C. porosus* populations) 'Project Crocodile' was begun by the Indian government in 1975 (Whitaker 2007). The establishment of several sanctuaries, especially the National Chambal Sanctuary, was particularly important for preserving the species. However, effective enforcement of the protection of gharial and engagement of communities for gharial conservation has rarely occurred, as the focus of conservation from the 1980s was on rear-and-release or 'headstarting' strategies (Whitaker 2007). A number of rearing and breeding centres were established throughout the country. Through captive breeding and egg collection from the wild, the rearing centres were populated with juvenile gharial which began to be released in the wild in the 1980s when they had reached about 1m in length.

India had released over 5000 gharial into Indian rivers by 2004 (Whitaker 2007). By surveys in the late 1990s it appeared that gharial populations in areas of release were recovering, but a lack of systematic monitoring made it impossible to tell whether this was 'true' recovery, or merely a counting of recently released gharial before they succumbed to the

threats that had previously decimated gharial numbers (Stevenson 2015). Since gharial reintroductions in areas where the species had been extirpated were not successful in any instance except one (Hastinapur: discussed below) this suggests that effective protection of gharial and their habitat is the key to effective conservation of the species. Project Crocodile ceased in 1996. Within ten years, the gharial population had plummeted again – and was thought to have fallen to fewer than 200 adults (Whitaker 2007). The gharial was listed as Critically Endangered on the IUCN Red List in 2007. Then in 2007 there was a mass die-off of gharial in the National Chambal Sanctuary (Lang et al. 2019), with over 100 gharial (mostly subadults) found dead. The mass mortality was most likely to have been caused by a toxin, probably the result of a point-pollution event (Lang pers. comm.). The many questions raised by this event could not be answered due to a lack of fundamental understanding of gharial behaviour and ecology. In response, the Gharial Ecology Project (GEP) of the Madras Crocodile Bank Trust (MCBT) was formed, the data from which forms most of the understanding of gharial described above. Using telemetry, direct observation, remote observation, audio recording, and genetics, the data of the Gharial Ecology Project forms the basis of most of the biological data known about the gharial that will be discussed and used in this thesis.

Outside the Chambal, breeding populations are still surviving in India in the Katarniaghat Wildlife Sanctuary, Corbett National Park and the Gandak River. Conservation on the Gandak River involves promoting the protection of nests by local communities and efforts to designate the Gandak a gharial sanctuary, with some degree of protection (Sinha pers. comm.). However, due to the Gandak Barrage on the Indo-Nepal border it is possible that the Gandak population is a sink population related to the Chitwan population as a source (Chapter 6). Conservation efforts on the Ghaghara River, Bhagirathi-Hooghly River, Son River, Ken River and Mahanadi River have not led to population recovery. Hastinapur is a site at which gharial were historically extirpated, but a reintroduction programme that released animals between 2009 and 2017 has led to the establishment of a population of subadults in a wildlife sanctuary, which is an exciting first step towards a successful reintroduction (S.K. Yadav & Khan 2016). In general, the issues across India for gharial conservation are that gharial conservation does not work without sufficient habitat protection, removal of threats, and support by local communities, and that the majority of

gharial conservation has simply focused on headstarting (Whitaker 2007; Stevenson 2015). This issue of restocking without mitigation of limiting factors has been described in wildlife translocation as ‘literally pouring new animals down the sink’ (Seddon 2010).

3.2.3 The Status and Conservation of the Gharial in Chitwan, Nepal

In Nepal, the gharial once occurred in all lowland river systems. Now there are only two wild, remnant populations in river stretches within the Bardia and Chitwan National Parks (Lang et al. 2019). Nepal’s gharial population had fallen dangerously low by 1978, just 50-60 individuals (Tirtha Man Maskey 1989) which seems to correlate with the building of the Gandak/Tribeni Barrage on the Indo-Nepal border in 1964, before the construction of which ‘hundreds’ of gharial were seen by local people in the Narayani (Maskey 1989). The 1960s is also the decade in which gill-nets became a dominant form of fishing gear throughout the Indian subcontinent (Radhalekshmy & Gopalan Nayar 1973; Karunaharan 2006), after the establishment of synthetic fibre industries in the 1950s, and gill nets are a major cause of gharial mortality in Nepal (Khadka pers. comm.) and in South Asia more widely. The population crashes in Nepal led to the establishment of the Chitwan Gharial Breeding Centre (GCBC) (Maskey 1989). The aim was to supplement the wild population with captive-bred individuals, and also to rear and release gharials from eggs that are collected annually from the wild. Legal protection measures were also taken, making it illegal to kill gharial and limiting fishing to permit-only on the river stretches adjacent to the National Park (Maskey 1989). Although purposive killing of gharial for hides or medicine has been prevented, enforcement of other habitat protections (preventing bycatch, limiting substrate mining, preventing habitat degradation) has been weak, with the funding and focus almost entirely on headstarting. I will conduct a retrospective analysis of the impact of the headstarting programme in Chapter 6. Although gharial have not been extirpated in Chitwan, the population of mature wild gharial has not increased since 1981, although numbers of adults are higher than at their lowest ebb in the early 2000s. This is most likely to be due a failure to address ongoing threats, such as barrages disrupting river hydrology, overfishing/bycatch, sand mining and other human disturbances. Post-release monitoring, when it has occurred, has suggested that there is a low survival rate for headstarted individuals (Maskey 1989, Ballouard et al 2010). This perceived failure of the restocking

programme has led to multiple suggestions that the focus of gharial conservation in Nepal must move away from restocking and towards conservation of wild populations (J. M. Ballouard et al. 2010; Lang 2016; Acharya et al. 2017), which would be best achieved by directly addressing threats and extinction drivers, and by building up a better understanding of gharial ecology and behaviour (Stevenson 2015; Lang 2016; Acharya et al. 2017). These suggestions, however, have led to few practical management changes for gharial in Chitwan, suggesting institutional inertia for gharial conservation in the National Park.

It is likely that the very small population sizes, low rates or absence of breeding, biased sex ratios and the naturally low recruitment rates of crocodylians combine into an 'extinction vortex' which can trap small populations into a series of positive feedback loops that can make it difficult to break out of a negative growth trend (Caughley 1994). It is possible that the gharial population in Chitwan is struggling due to the inherent issues of having reached such small population sizes. A particular type of issue that can cause small populations to show a negative growth rate are 'Allee effects' which deterministically enhance extinction risk for small populations (Dereced & Courchamp 2007). An Allee effect is 'a positive relationship between any measurable component of individual fitness and population size or density' (Berec et al. 2007). The very small gharial population in Chitwan may also be subject to multiple Allee effects. For example, the creche guarding behaviour of adults involving hatchlings from multiple nests suggests there is some evolutionary benefit to this group nesting and guarding, probably that risk of predation per individual tends to be reduced with increasing aggregation size (Spieler 2003; Wood & Ackland 2007). It is plausible that reduced size of hatchling aggregations may lead to reduced hatchling survival rates. Another consideration is the role of adult male gharial: adult males are seen to drive off mugger crocodiles, a known predator of juvenile gharials, from basking sites. Interestingly, yearling gharial often continue to associate with guardian adult males (Lang, pers. comm.) suggesting the role of the male could remain important for gharial survival beyond the creche phase, and the absence of adult males in Chitwan may also therefore be another Allee effect.

The other major issue in Chitwan is a lack of adult males (Acharya et al. 2017). The sex-ratio of adult gharial under natural conditions seems to be female biased 6:1 (Lang & Kumar 2018), however in the Chitwan population the ratio is currently 20:1, and in 2017 there was

just 1 adult male (Bed Bahadur Khadka & Kandel 2020). This led to the introduction of a wild-caught male from the Babai population in West Nepal (Bed Bahadur Khadka & Kandel 2020), and following the maturation of a sub-adult there are now three adult males in Chitwan, with two more likely to mature either in 2023 or 2024 (pers. obs.). This may also lead to Allee effects in reproduction due to an inability of females to find suitable mates, or severe inbreeding depression in the future if all Nepalese gharial are descendants of one or two males (Berec et al. 2007). The potential cause of the incredibly low number of males in the Chitwan population is investigated in Chapter 6.

3.3 Study Site description

3.3.1 Narayani and Rapti Rivers and their tributaries

The study area consists of approximately 300km of linear river, made up of the Narayani River and Rapti River and their tributaries which fall in the Chitwan District of southern Nepal (Figure 3.3). The Rapti forms the northern park boundary of the Chitwan National Park, and the Narayani forms the western boundary except in the far south where the Narayani runs through the National Park on both sides and in the far north where the river itself forms the northern extension of the Park, with internal forested islands falling under National Park protection. The tributaries of the Narayani and Rapti tend to flow through agricultural land, although the lower reaches of some are well protected within community forests, and the Rue flows mostly through the National Park. The Narayani, Rapti and Rue have a confluence at Gholaghat Dhoban, after which they flow as the Narayani to the Indo-Nepal border. On the border is the Gandak (Tribeni) Barrage, after which the river is called the Gandak and eventually joins the main channel of the Ganges.

The Rapti River rises at Chisapani Garhi in the Mahabharat Range, which are the southernmost mountains of the Himalayan system. The Rapti is fed by ground water and springs, and flows for about 120km until its confluence with the Narayani (Shanker 1985). The Narayani forms where the Trishuli and Kali Gandaki rivers converge. The Kali Gandaki rises in the high Himalaya of Nepal at the Nhubine Himal Glacier. The Trishuli is formed from five major tributaries which also rise from upper glacierised catchments in the high Himalaya and Tibetan Plateau (Racoviteanu et al. 2013).

The Chitwan District has a sub-tropical monsoon climate (Gurung 1983). There are three main seasons: the pre-monsoon (February-May), monsoon (June-September) and post-monsoon or winter (October-February) (Mishra 2014). The pre-monsoon begins cool, and warms up to temperatures of over 40 °C by May. Although primarily dry, with low humidity, there are usually sporadic pre-monsoon storms. During the monsoon season almost 90% of annual rainfall falls, causing sporadic but severe floods (Mishra 2014). In the winter, minimum temperatures can fall as low as 4 °C, relative humidity approaches 100% at night, but rainfall is very rare (Maskey 1989). Mean annual rainfall in Chitwan is 2,214 mm (1995-2006, Rampur Weather Station).

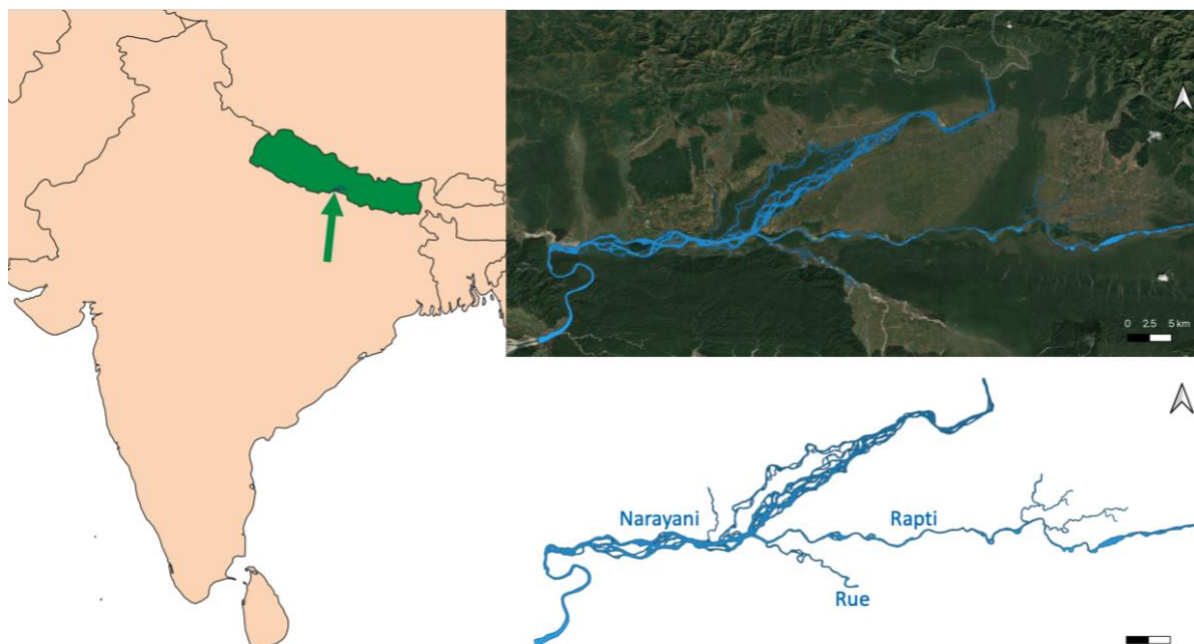


Figure 3.3: the location of the Narayani basin study site in Chitwan. Clockwise, from left: the location of Nepal in South Asia, with the central location of Chitwan indicated; satellite imagery of Chitwan with the river system superimposed for clarity, forested areas south of or within the river are the Chitwan National Park; the river system with the major rivers labelled.

3.3.2 Chitwan National Park

The Chitwan National Park is Nepal's first National Park, established in 1973, and a World Heritage Site. The park is under the jurisdiction of the Department of National Parks and Wildlife Conservation (DNPWC) within the Government of Nepal's Ministry of Forest and

Environment. It lies in the Terai-Duar savanna and grasslands ecoregion. The vegetation of the National Park is mostly Himalayan subtropical broadleaf forest, dominated by Sal trees. However, the monsoon flooding of the rivers creates a riverine forest (dominated by *Acacia*, *Dalbergia*, *Bombax* and *Trewia*) and grassland mosaic. These grasslands are some of the tallest in the world. *Saccharum* sp. are quick to colonise sand banks deposited or de-vegetated by floods (Mishra 2014), and make them less suitable for gharial basking and nesting, with the scouring action of monsoon floodwaters required to clear potential nesting sites of vegetation (Vashistha, Mungi, et al. 2021).

3.3.3 Co-occurring Wildlife

The rivers, and the grasslands they create, are vital for much of the Chitwan wildlife, including some of the world's largest remaining terrestrial megafauna species: the tiger (*Panthera tigris*), greater one-horned rhinoceros (*Rhinoceros unicornis*), gaur (*Bos gaurus*) and wild Asian elephant (*Elephas maximus*). Megafauna diversity is also high in the rivers of Chitwan, not just on land. Freshwater megafauna are freshwater species that reach over 30kg, and are often underrepresented in both science and conservation efforts despite high levels of threat (He et al. 2017). The famous Golden Mahseer (*Tor putitora*) once widely sought for sport fishing and food, is now Endangered. Still persisting in the Narayani, it migrates to upstream tributaries to breed, including the Rapti river which is a very important spawning ground of the species (Shrestha 1997). Just like the gharial, the Mahseer is threatened by the impacts of barrages and dams, and habitat destruction, especially sand and gravel mining (Shrestha 1997). The Gounch catfish (*Bagarius bagarius*) is the largest fish in Chitwan, able to reach a mass of over 100kg (Shrestha 2008). It is known to make noises similar to those of a crocodile (Shrestha 2008), widely reported to pick human remains from funeral pyres and occasionally thought to prey on humans (Mishra 2014). Gounch are migratory, and large individuals of this Vulnerable fish are getting rarer (Shrestha 2008). The Rohu (*Labeo rohita*) and Catla (*Catla catla*) are both members of the carp family, and these megafishes are considered a delicacy, the preferred species for many local dishes. It is not just the megafauna beneath the surface of Chitwan's freshwaters: at least 125 fish species can be found in the Chitwan National Park (Mishra 2014).

Other freshwater megafauna species of note are the two softshell river turtles, the mugger crocodile and Gangetic river dolphin. The Indian Narrow Headed Softshell turtle (*Chitra indica*) is an Endangered species of turtle which can reach over 1m in carapace length, an ambush predator that waits for prey on the sandy river beds of Chitwan, and the Ganges Soft Shell Turtle (*Nilssonina gangetica*) is only slightly smaller, similarly Endangered, and frequently encountered basking alongside gharial in the Chitwan rivers. The mugger crocodile (*Crocodylus palustris*) is a large 'true' crocodile, with catholic dietary and habitat requirements. Although the mugger is found in rivers alongside the gharial, it will inhabit any body of water, primarily lakes and ponds, but also artificial tanks and canals. Unlike the gharial, it does occasionally prey on humans and there is an emerging human-mugger conflict in Chitwan, as mugger population density is rising within the buffer zone (Shakya *et al*, in prep). The river dolphin is an important species in both Hinduism and the folklore of the local Tharu and Bote indigenous communities. In Hinduism it is believed the dolphin emerged from the god Shiva's locks when the goddess Ganga became the sacred Ganges river, and flowed through the Lord Shiva's hair to control her fall as she fell to Earth (Shrestha 1995). Locally in Chitwan, indigenous tales hold that the dolphin was once a woman transformed into a fish, and should be treated as a fellow human. As such, the species is highly regarded and the subject of many folk beliefs. Despite this, the construction of the barrage at Tribeni has fragmented the dolphin habitat so severely (Paudel et al. 2015) that evidence suggests the population is now just a single individual (Griffith and Chaudhury, unpublished data). The habitat requirements of dolphins for large rivers with deep pools are similar, but even more specific, than those of the gharial. The dolphin has been suggested as an indicator species of the state of its riverine ecosystems, so its disappearance from Chitwan is cause for concern (Shrestha 1995; Turvey et al. 2012).

3.4 The gharial as a flagship and umbrella species in South Asia

Freshwaters may cover less than one percent of the world's surface, but they contain a disproportionate amount of the world's biodiversity (Dudgeon et al. 2006). These systems are also disproportionately threatened, with far less conservation attention than terrestrial systems (Carrizo et al. 2017). There is a need for more flagship and umbrella species to try

and catalyse action and interest in conserving these freshwater systems, and capture conservation funding (Kalinkat et al. 2017). The gharial is a charismatic and popular species, more visible than most aquatic animals due to its large size and amphibious nature, and has been used as a 'hook' for conservation funding with comparatively more success than for many aquatic species. This suggests the gharial could be a good flagship species for the conservation of its riverine habitats, if its conservation was focussed on in-situ efforts, and not on headstarting. The results of this thesis support the assertions of others (e.g. Lang 2016; Acharya et al. 2017) that the future of gharial conservation is to shift away from a headstarting approach, and towards the in-situ protection of the gharial's riverine environment. If this shift could be achieved, the gharial could also be a useful umbrella species due to its overlap in occurrence with areas of high biodiversity, large range size and complex habitat requirements to cover all life stages (Kalinkat et al. 2017).

I hope this thesis provides a useful assessment of our understanding of gharial conservation in Chitwan to date, with insights to catalyse a change in the way gharial conservation is approached in Nepal and throughout South Asia, and tools to help managers investigate the status and ecology of gharial as a flagship and umbrella species to protect the river systems in which it lives.

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Chapter 4

Comparing use of GPS and VHF telemetry for home range estimates of a Critically Endangered crocodylian, the gharial

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4.1 Introduction

As the habitats of many species become progressively fragmented and degraded, studying how animals use their habitats is increasingly important for designing effective conservation measures (Silva et al. 2022). The need for biological data to inform conservation planning is especially acute in freshwater systems (Steven J Cooke et al. 2013), as freshwater species are at greater risk of extinction than are species in terrestrial and marine realms (WWF 2022) due to extensive impacts to meet human needs, on the limited area of freshwater habitat (Dudgeon et al. 2006). Despite this, even large and charismatic species like freshwater megafauna receive little conservation attention (He et al. 2019). One such species is the gharial (*Gavialis gangeticus*), a Critically Endangered obligate riverine crocodylian species found in South Asia. Conservation efforts have not led to the recovery of most populations, and the only gharial population thought to be viable is found on the Chambal River, which is the only river in the species' distribution that remains free-flowing (Lang et al. 2019). The strong association between river fragmentation by major infrastructure and gharial population decline is typical for freshwater megafauna (He, Thieme, et al. 2021), and highlights a need to investigate gharial space use and habitat requirements to inform conservation efforts.

For crocodylians, VHF (Very High Frequency) tags have been used successfully for determining location in multiple species, including gharial (Lang & Whitaker 2010; Balaguera-Reina et al. 2016; Kouman et al. 2021). VHF tags emit a high frequency radio pulse signal that is detected with a receiver and antenna, and is the first technology used for wildlife telemetry (Moses et al. 2023). Satellite tags collect positional data using satellites, and may then transmit this data using satellites or non-satellite means depending on the chosen technology (Moses et al. 2023). Satellite tags are also being used more frequently on crocodylians (Read et al. 2007; Baker et al. 2019; Caut et al. 2019).

The challenges of tracking freshwater species differ from those of terrestrial species: when locating an individual in murky rivers it can be impossible to sight an underwater animal regardless of proximity (Steven J Cooke et al. 2013) and technologies typically used for terrestrial species may function less effectively or not at all when underwater. Radio waves attenuate greatly underwater, and are reflected and refracted below and at the water surface, leading to reduced positional accuracy of VHF telemetry when animals are shallowly submerged, and no signal at depths greater than three metres (Lang & Whitaker 2010; Sendra et al. 2013). For GPS and satellite tracking technologies, the challenges of water are even greater: these tags cannot fix under water of any depth due to the dampening in water of the electromagnetic signals from the satellites (Taraldsen et al. 2011). Passive Acoustic (PA) systems are widely used for monitoring many aquatic species (Steven J Cooke et al. 2013), including crocodylians (Dwyer et al. 2015), as each tag emits a unique sound that is detected by underwater acoustic receivers (Moses et al. 2023). The requirement of an array of receivers precludes their use in systems where it is not possible to set receivers.

VHF technology has multiple limitations, including the cost and challenges of manual tracking, inability to detect animals in inaccessible locations or on longer forays, low temporal resolution and low spatial precision, especially when the animal cannot be sighted (Frair et al. 2010; Skupien et al. 2016). Satellite tags can collect location data with greater temporal resolution and spatial precision than VHF, by determining location using the Global Positioning System (GPS) (Frair et al. 2010). Furthermore, GPS tags can determine location even in inaccessible locations, at night, or across vast distances (Hebblewhite & Haydon 2010). Although GPS overcomes many of the issues of VHF, the major challenge is that taking a positional fix relies on the fact crocodylians are air-breathing, and spend time either at the water's surface or basking on-land. If this time is insufficient, such as during monsoon for gharial, location may not be determined. This could lead to biased missing data, which in turn can bias analyses such as home range estimation (Frair et al. 2010). The other problems with satellite tags are they generally still prohibitively expensive for most conservation research programmes, and generally have shorter battery lives than PA or VHF transmitters (Steven J Cooke et al. 2013). With limited funds, it has been argued that the priority should be on tagging more study animals, and on tagging animals for a longer

period, rather than on having temporarily frequent data on a few individuals for a short time (Luca Börger et al. 2006). This is because small sample sizes lead to poor population level inference, and finer scale data does not necessarily lead to better inferences relevant to understanding ecology or conservation management of a species (Hebblewhite & Haydon 2010). One concept for which this trade-off between technologies is relevant for conservation planning is animal home range.

Conceptually, an animal's home range is the spatial area required by that animal to complete the behaviours required to survive and reproduce (Burt 1943; Noonan et al. 2019). Ecologists attempt to quantify this concept using a range of statistical approximations, resulting in a 'home range estimate', the selection of which depends on the research question (Powell & Mitchell 2012). A home range estimate can be useful for addressing multiple behavioural and ecological questions (Luca Börger et al. 2008), but the most pressing application for threatened species such as the gharial is for conservation planning: understanding home ranges can inform understanding of the amount of and type of habitat required for the persistence of a population (e.g. Block et al. 2011; Nicholson et al. 2013) and informing design and assessment of protected areas (e.g. Knapp & Owens 2005).

The importance in selecting the method of home range estimation is to quantify the space use of an animal based on genuine ecological processes, rather than biases in the method of estimation (Noonan et al. 2019). Comparing data from different types of transmitter on the same individuals is a methodology that has been recommended to enable researchers to quantify various biases (e.g. Skupien et al. 2016), and thereby to develop methodologies for minimising them (e.g. Dwyer et al. 2015). GPS tag location data has a high temporal resolution and spatial precision, and thus is more likely to lead to accurate home range estimates than VHF, from which location is estimated following data processing (Frair et al. 2010). By comparing the results of GPS home range estimates to that of different methods of processing the VHF data, we can quantify the best method during seasons when GPS units have high fix rates. Conversely, in monsoon season when gharial are underwater for considerable periods of time and GPS tags rarely fix, we can use the VHF location data to determine how this missing data can bias home range estimates (Frair et al. 2010). Therefore, we can investigate the biases of both types of tag to investigate whether futures

studies could use a small number of expensive, short-lived GPS tags in conjunction with a larger number of cheaper, longer-lived VHF tags to optimise the advantages of both methods.

In this study, we determine the biases of these two telemetry methods – VHF and GPS – on gharial by comparing the patterns, size and position of home range estimates derived from the two technologies. By comparing home range estimates from GPS data to VHF data processed using differing methodologies, we determine if analytical methods can be used to obtain home range estimates from the cheaper VHF tags that are equivalent to the estimates obtained from the GPS tags, for investigations of gharial space use to inform conservation planning and management.

4.2 Materials and Methods

4.2.1 Gharial capture and transmitter attachment

In winter 2018 and 2019, gharial were captured in the Rapti river and its tributaries in Chitwan National Park, Nepal, using one of two methods. The first was setting of curtains of mesh netting (50-100m length) at the deep-water approaches to favoured basking sites, generally by attaching a float to one end of the netting, and drifting this past the site where gharial were basking or expected to bask. Gharial would then be flushed towards the nets by a team member approaching from the land, and nets were immediately checked and any entangled animals removed. The second method was utilised in clear water, where it was possible to see submerged gharial. In this method, gharial were captured using cast nets deployed from traditional dugout canoes approaching alongside the submerged gharial.

After capture, gharial were brought alongside the canoes and immediately blindfolded, then brought ashore. Physical restraint was used to initially restrain animals, whilst they were removed from the net, and then they were fully immobilised using ropes to attach the gharial to a ladder. Attachment of the gharial to a ladder ensures the animal is fully immobile for the radio attachment procedure, enables easier collection of morphometric measures, and facilitates movement of the animal for a swift release (Gharial Ecology Project, pers. comm., Brien and Manolis, 2016). Restrained gharial were protected from overheating by placing damp sacking on the gharial. The maximum holding time of any individual was 60 minutes, although most gharial were released within 30 minutes. Gharial

are known to tolerate being restrained in this method for 15+ hours (Khadka pers. comm.), so we were considerably within safe timescales for restraint.

Whilst the gharial was restrained, the radio transmitter(s) were attached to the tail, just anterior to the junction of the single/double tail scutes, following the protocol of the Gharial Ecology Project (Lang & Whitaker 2010). Kevlar fishing line was anchored to the transmitter's epoxy casing, and was then passed through small holes drilled in the base of up to three tail scutes, then inserted underneath the dorsal skin immediately below the base of the transmitter using a trocar-style needle as a guide. The Kevlar line was then passed back thorough small holes in tail scutes symmetrical to those on the other side. The loose ends of the line were anchored to the transmitter using metal swages which were crimped and fixed with epoxy.

Morphometric measurements were collected (mass, snout-vent length, vent-tail length, total length, snout width, head length, head width, mid-body circumference and sex), and it was recorded if the gharial had any scute cuttings to compare to the release record from the National Park, which has records of the year of hatching and release, and morphometrics at time of release for each individual. Following transmitter attachment and collection of measurements, gharial were released. Each gharial was oriented so the head was close to the water, to facilitate their immediate return into the river. A wooden stick was buried in the sand on the side of the gharial away from the water. All limb and snout bindings were removed except the blindfold, and all handlers then moved away onto the side of the buried stick (which prevents the gharial turning in that direction). When everyone was at a distance, the blindfold was removed using a cord.

All capture, handling and transmitter attachment was conducted with permission from the Department of National Parks and Wildlife Conservation and following ethical review from the University of Oxford (APA/1/5/ZOO/NASPA/WildCRU/Gharial).

4.2.2 Transmitter Deployment and Tracking

Two types of transmitter were attached to each gharial included in this analysis. The first was a solar-powered GPS-GSM transmitter (Movetech Telemetry, UK), which attempted to

locate gharial position using GPS at equal intervals four times per day, on a duty cycle of 23 hours. This duty cycle ensures that fixes are taken at a slightly different time each day, to minimise the risk of a positional fix to the satellite repeatedly being attempted at a time of day when gharial behaviour makes successful fixing unlikely (e.g. times of day when they are underwater). Once per day, the unit attempted to upload all positions to the GSM network for remote download: if the GSM upload failed, all locations were saved on the device to attempt upload the next day. The second was a VHF transmitter (151 MHz; Advanced Telemetry Systems, USA). Tracking of the VHF transmitters was using a 5-element hand-held antenna and a portable field receiver (ATS R410). Our tracking protocol followed that of Lang and Whitaker (2010), with listening posts on high ground every 2-3km along the riverside, at which we listened for each individual gharial to determine the strength, direction and patterns of the VHF signal. We attempted to locate each gharial at least once per week, which was the highest frequency of relocation practically possible over the large size of the study site with the available resources. We conducted VHF tracking at the times of day gharial were most likely to be basking to maximise chance to detection and sighting, which varied according to season. There were some gaps in our data (April-May 2020, January and May 2021) due to Covid19 precautions. Some sections of the river were challenging to reach, so could only be accessed occasionally, and similarly some sections of river were inaccessible at certain times, such as if a male wild elephant took up residence in an area. If the gharial could be sighted (e.g. when basking) the location was recorded. More often gharial were submerged, or triangulated to sites that couldn't easily be accessed, in which case the signal strength and direction were recorded, allowing us to determine that the gharial was in a particular stretch of river. To determine the relationship between strength/direction of signal and listening points, we 'tracked' stationary VHF transmitters on beaches, transmitters in boats of team members following a GPS track, and signal from gharial currently under observation by one team whilst another team conducted tracking. This data was used to construct the detection polygons (Data Processing, below).

4.2.3 Data Processing

The high temporal resolution and spatial precision of GPS is likely to lead to more accurate space use estimates than VHF (Frair et al. 2010), so long as the GPS is taking regular fixes,

indicating the gharial is not spending substantial periods of time underwater. We therefore compared home range estimates from GPS data to different methods of processing VHF data in order to determine the best way to process VHF data.

To facilitate comparison, data was pre-processed to ensure that the GPS and VHF data were for the same length of time, as VHF battery life was generally longer. GPS locations with a satellite dilution of precision error that led to a precision accuracy of $>100\text{m}$ were removed (0.8% of all locations), although precision was good, with 96% of locations having a precision error of less than 20m. GPS location was a specific point location.

If gharial were observed basking, a GPS unit was used to take a specific point location. However, more often the gharial was not directly observed. Instead, the signal could be triangulated to a particular stretch of river based on direction and strength of signal. Using the stationary tests and tests of known-location transmitter described above, we were able to use GIS software to construct polygons which were ascribed to sections of the river that corresponded to particular strengths and directions of signal. Polygons were restricted to the river and its immediate banks, as gharial do not use habitat beyond this. Therefore, gharial location could be triangulated to within a specific pre-defined polygon. Since the precise location of a gharial at detection was unknown, other than we were confident it was within the polygons we defined, we generated three sets of pseudo-positional data to facilitate comparison with GPS data. Firstly, we assumed that any gharial detected within a polygon was located at the same single fixed location within that polygon. This location was a beach within the polygon at which we had observed gharial basking during the study, which had then been used for stationary tests. Secondly, we allocated each detection to a random point within the polygon, assuming that the gharial could feasibly be anywhere inside the polygon. Thirdly, if the gharial had not been detected the day before or after detection, we assumed the gharial was in the polygon for three days, and for each day we randomly allocated the gharial a location within the polygon. We will refer to these randomly allocated locations as pseudo-locations. Specific point data is necessary for all home range estimation methods, so pseudo-locations are necessary for further analysis.

4.2.4 Home Range Quantification

To calculate home range estimates we used the method of least cost kernel utilisation distribution (lcUDs) (Laffan & Taylor 2013) following Baker et al. (2022), with adaptations to ensure the method was suitable for VHF data. Locations (either GPS detections, or estimated pseudo-locations) were converted into one centre of activity (COA) per day (Udyawer et al. 2018), which was then adjusted, if that COA occurred outside the river channel, to the nearest point within the river system, since gharial are restricted to the river channel and its banks. COA were calculated using the VTrack (Campbell et al. 2012) and sf (Pebesma 2018) packages in the R statistical environment (R Core Team 2021). One location per day was selected as we rarely had VHF detections more regularly than this, and to reduce bias of days with more detections. Since telemetry data often has inherent temporal autocorrelation, the lcUD method accounts for this by incorporating the estimated trajectory between consecutive detections into the kernel utilisation estimates (Laffan & Taylor 2013). The trajectory is estimated as the shortest possible route between consecutive detections, whilst being constrained by the river channel (i.e. assuming a gharial has swum the most parsimonious route within the river from point A to point B). Kernel utilisation methods have also been shown to be preferable to other home range estimators in data sets with few locations (Luca Börger et al. 2006). We followed the method outlined by Baker et al. (2022) for estimating the lcUDs, which essentially uses a raster of the river system to constrain estimated paths to within the river channels. The lcUD used was the combination of the two utilisation distributions: one for the detected locations and one for their connecting pathways, then clipped to the boundary of the river system and the adjacent 100m of riverbank. This method is appropriate for species where their potential location is constrained by a known habitat feature, in this case, a river (Laffan & Taylor 2013; Slaght et al. 2013).

We determined the 95% and 50% volume contour lcUDs, using a smoothing parameter (h) of 300 which we found to be the most appropriate parameter in sensitivity analyses, as the minimum value at which the extent of activity space of tagged gharial began to plateau.

4.2.5 Home Range Size and Overlap

For each individual, the size of the home range was estimated as the area within the 95% contour of the lCUD and the core range was that within the 50% contour (Laver & Kelly 2008). The linear river home range size for each individual was also determined for the 95% contour of the lCUD by determining the distance of river between the furthest upstream and downstream locations of the home range (calculated as the least cost pathway between these locations in the river raster). For both the home range size and linear river home range size we compared each of the three methods of processing the VHF with the GPS-based estimate using a paired Wilcoxon signed rank test (Dwyer et al. 2015).

For each gharial, we compared the overlap of its home range estimate calculated from GPS data, to the three estimates from the three VHF processing techniques. We calculated two measures of overlap for each comparison. Firstly, we used the Volume Intersection (VI) method following Dwyer et al. (2020) carried out in VTrack (Udyawer et al. 2018). VI provides a measure from 0 (two home ranges have no overlap) to 1 (two home ranges are identical, and could be perfectly superimposed). VI accounts for variation in probability of uses of areas within the selected lCUD contour (Seidel 1992). Secondly, we determined a measure of the conditional probability of a point in the home range estimate from GPS data being located within the home range determined from the VHF measures, $PHR_{VHF,GPS}$ (Fieberg & Kochanny 2005). This measure did not compare the variation in use within the lCUD, just the total size and shape of the home ranges being compared. $PHR_{VHF,GPS}$ provides a measure from 0 (no locations from the GPS home range estimate fall within the VHF estimate) to 1 (all locations from the GPS estimate fall within the VHF estimate).

To investigate the impact of contour selection and method of VHF data processing on the overlap scores we used mixed effects models. The aim of this analysis was to compare overlap scores (either VI or $PHR_{VHF,GPS}$) of home range estimates from different VHF data processing methods, nested by individual gharial. Therefore we used gharial as a random effect (random slope for each gharial considered), with lCUD contour (50% and 95%), and method of VHF data processing (fixed, SRand and TRand) as the two fixed effects. Models were conducted in the package lme4 (Bates et al. 2015).

4.3 Results

4.3.1 Captured Gharial and Location Data

Nine gharial were captured and tagged with both VHF and GPS tags. Two GPS tags failed within a month of deployment, leaving seven gharial which were included in the final analysis. The gharial in this study ranged between 204 and 335cm total length. Three were adult females, a fourth female matured over the duration of the study, two were subadult females and one was a subadult of indeterminate sex. We recorded a mean of 1306 (SE=288, range 784-3010) GPS locations per gharial covering a duration of 8-15 months, a mean of 132 GPS locations per gharial per month. We recorded a mean of 37 (16-81) VHF locations per gharial over the same duration, a mean of 4 VHF locations per gharial per month.

4.3.2 Home Range Size

There was no evidence that estimated home range size differed significantly between estimates based on type of data and processing method (Figure 4.1) for area at the 95% or 50% lcUD (Wilcoxon signed rank test, $n=7$, all $P>0.05$) or linear river distance at the 95% lcUD (Wilcoxon signed rank test, $n=7$, all $P>0.05$). There was a slight trend in home range size estimates, with mean estimates from GPS being $\sim 1\text{km}^2$ (area) or $\sim 3.5\text{km}$ (linear river distance) smaller than the estimates from VHF at the 95% lcUD (Figure 4.1, A).

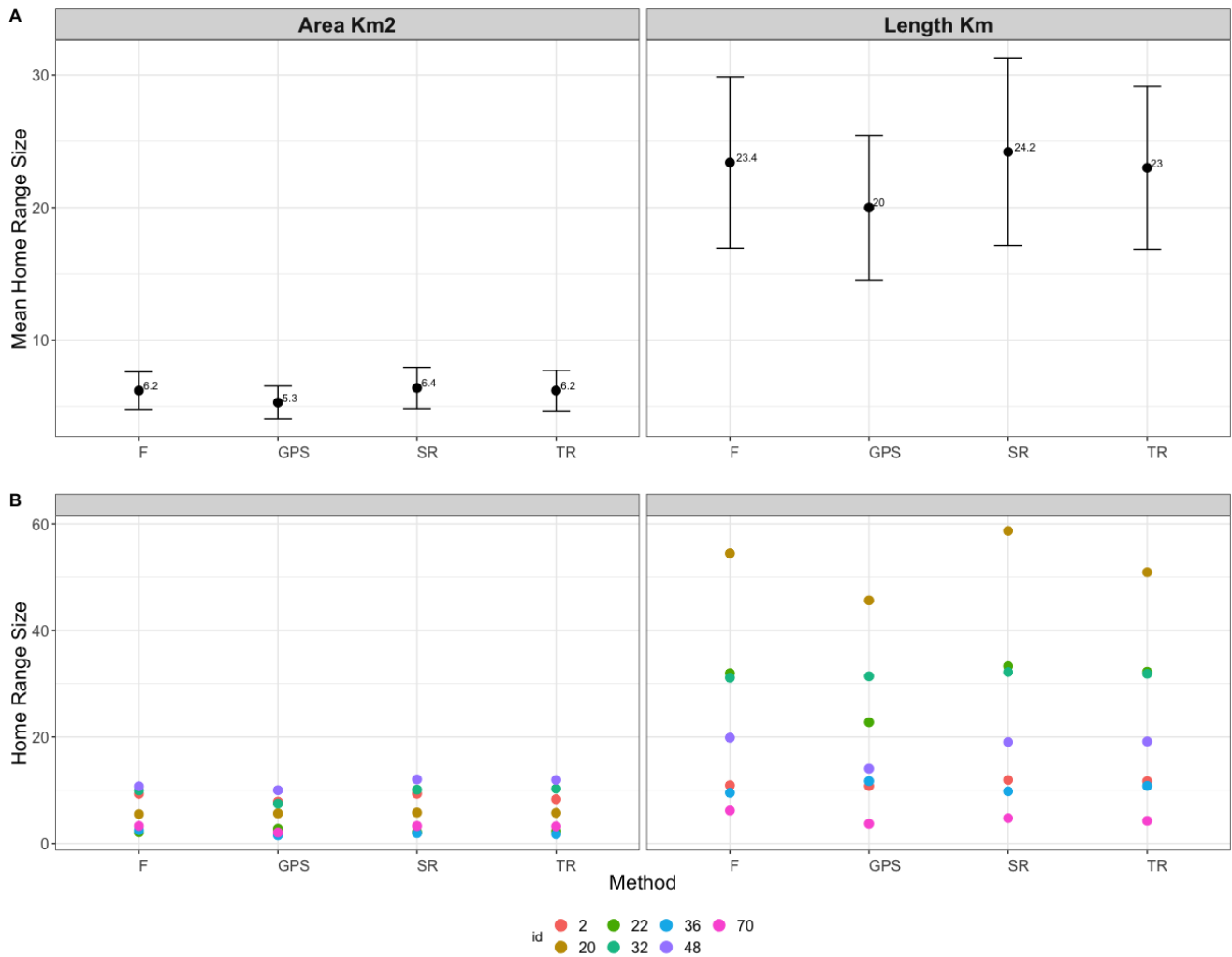


Figure 4.1: home range estimates for area (km²) and length (km) at the 95% lcUD for four different data collection or processing methods GPS, F (fixed point within a detection polygon), SR (single random location within a polygon) and TR (three random locations within a polygon). (a) mean home range size per method, with bars indicating the standard error. (b) home range size for each individual gharial using each method, coloured by gharial id

4.3.3 Home Range Overlap

We used a mixed effects model, nested by individual gharial, to compare Volume Intersection (VI) overlap scores between home range estimates from GPS location data and three different methods of processing VHF location data, at the 95% and 50% lcUD contours. Contour had a strong effect on VI scores ($F=33.83$, $p<0.01$), with estimates showing higher overlap at the 95% than 50% contour (Figure 4.2). Method of processing had a weaker effect on VI scores ($F=3.17$, $p=0.056$), with this effect driven by lower VI scores

when using the fixed location method, which had significant contrasts with the SRand ($t=2.15$, $p = 0.04$) and TRand ($t=2.21$, $p=0.03$) methods (Figure 4.2). The fixed location method had the least overlap with the GPS estimate at both the 50% and 95% lcUD contour. Therefore, we repeated the mixed effects model with the fixed receiver data excluded. We found the impact of method was no longer significant ($F=0.008$, $p>0.1$), with no significant contrasts between SRand and TRand.

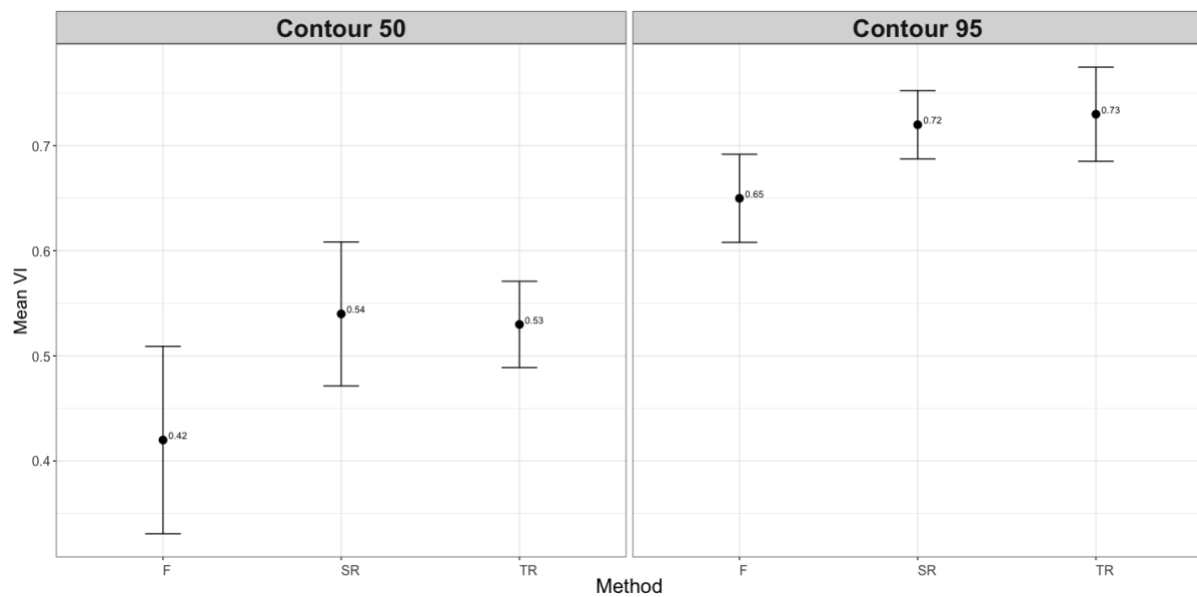


Figure 4.2: plots showing mean Volume Intersection (VI) values (points) with their standard error (bars), comparing overlap of home range estimates from GPS location data with those estimated using three methods of processing VHF location data fixed location (F), Single Random Pseudolocation (SR) and Triple Random Pseudolocation (TR). Home Range estimates were least cost utilisation distributions at the 50% (left) and 95% (right) contour.

We then used a mixed effects model, nested by individual gharial, to investigate $PHR_{VHF, GPS}$ overlap between GPS and SRand or TRand home range estimates, at the 95% and 50% lcUD contour. As for VI, contour had a strong effect on $PHR_{VHF, GPS}$ ($F=6.67$, $p=0.02$), with estimates showing higher overlap at the 95% contour (Figure 4.3). We did not find a significant impact of processing method on $PHR_{VHF, GPS}$ score ($F=0.39$, $p>0.5$). However, TRand estimates generally had higher scores, especially at the 50% lcUD contour, and had smaller

standard error values (Figure 4.3). Similarly, the minimum score of TRand was 0.43 at the 50% contour, compared to 0.28 for SRand, indicating a better ‘worst-case’ score for TRand.

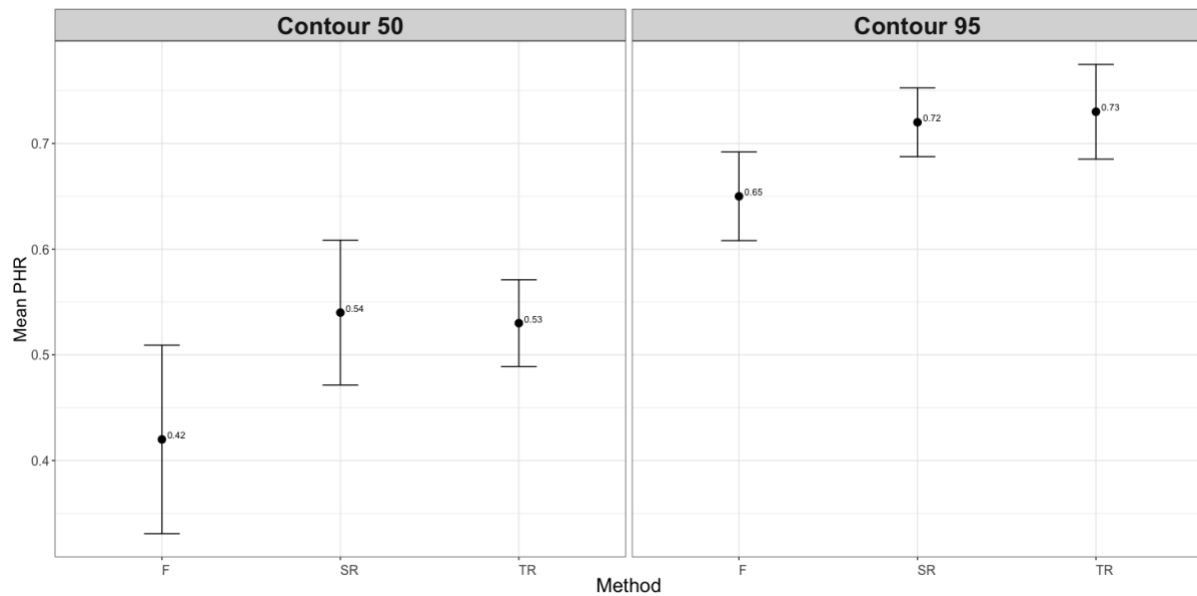


Figure 4.3: plots showing mean $PHR_{VHF,GPS}$ overlap values (points) with their standard error (bars), comparing overlap of home range estimates from GPS location data with those estimated using three methods of processing VHF location data fixed location (F), Single Random Pseudolocation (SR) and Triple Random Pseudolocation (TR). Home Range estimates were least cost utilisation distributions at the 50% (left) and 95% (right) contour.

Inspection of maps of the home range estimates from the gharial with the lowest overlap scores revealed the discrepancy between estimates from GPS and VHF data were primarily attributable to three types of bias: VHF methods underestimating use in areas challenging to reach for tracking, GPS methods underestimating use in areas used at times of year when the gharial is primarily underwater, and reduced precision using VHF methods. Figure 4.4 illustrates the gharial with the lowest home range (95%) VI score, which illustrates two of these biases: the upstream river use in the monsoon is not detected by the GPS, which cannot record a fix when the gharial spends the entire time underwater, and the probability of downstream river use is underestimated by the VHF methods, as this was an area difficult to access for tracking. Figure 4.5 illustrates the gharial with Core Range (50%) estimate with the lowest combined VI and $PHR_{VHF,GPS}$ scores.

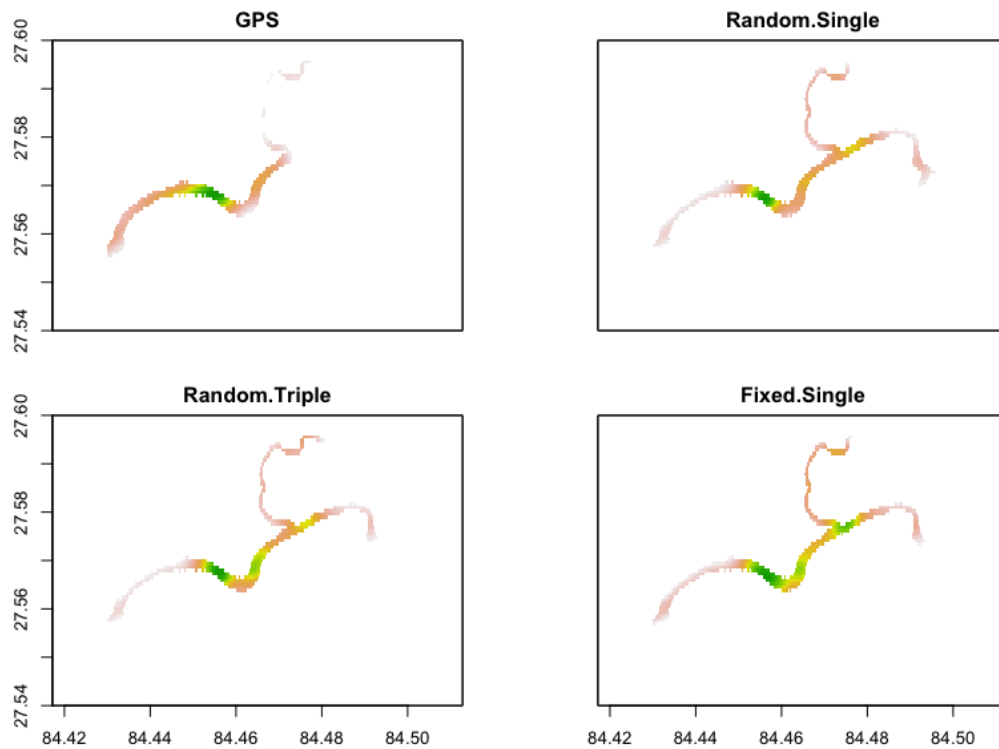


Figure 4.4: Home Range (IcUD 95% contour) estimates for gharial 02 in the Rapti River and its tributaries. Female 02 had the lowest VI score due to the large section of river use not detected by the GPS method (upstream) and the underestimated probability of the downstream river section being used in the VHF method, as this area of river was difficult to reach for tracking.

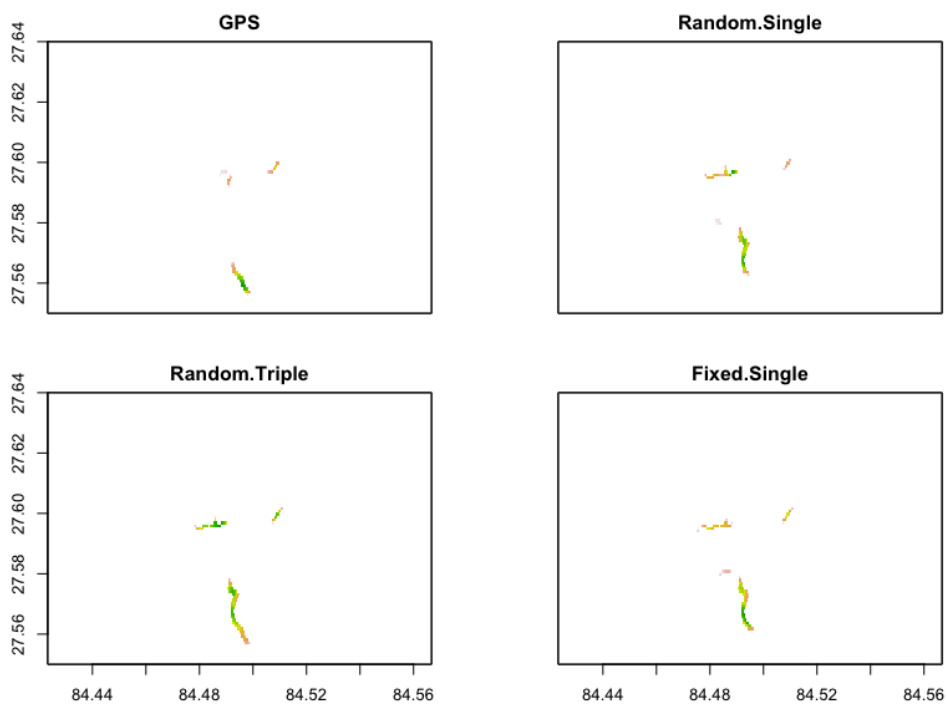


Figure 4.5: Core Range (IcUD 50% contour) estimates for gharial 20 in the Rapti River and its tributaries. Gharial 20 had the lowest VI and PHR scores for the core range estimate due to the

greater area of the estimates from VHF tracking, and very specific use of small sections of the river, captured by GPS but not by the larger detection polygon

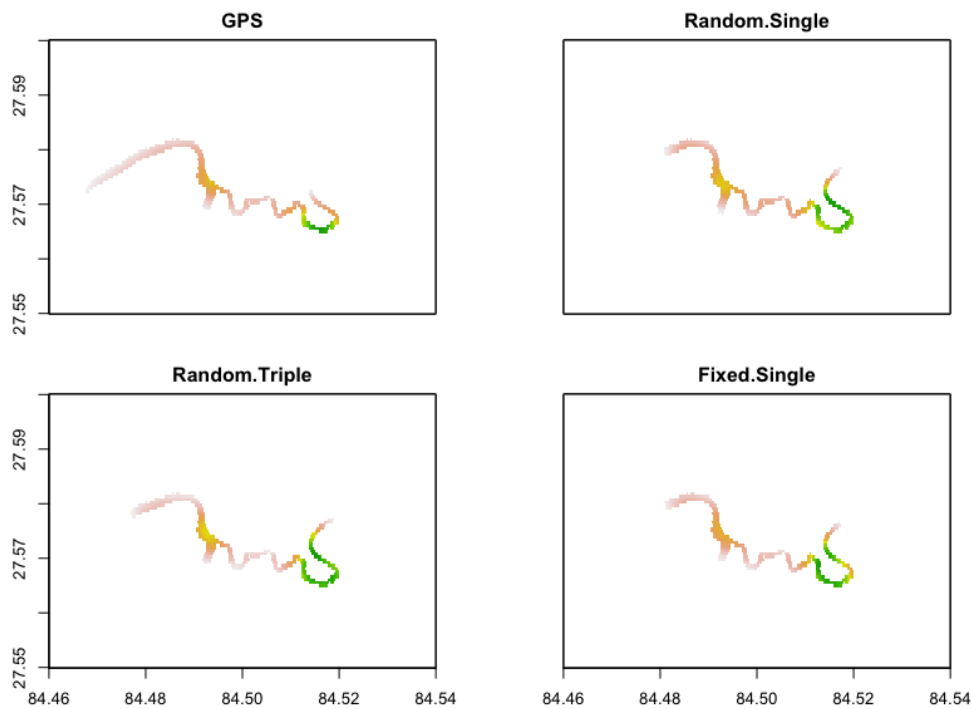


Figure 4.6: Home Range (IcUD 95% contour) estimates for gharial 22 in the Rapti River and its tributaries. Gharial 22 had the lowest PHR scores due to the VHF estimates underestimating the probability of use of a downstream section, difficult for trackers to access. Green indicates the Core Range: the GPS more precisely highlights her preference for basking on a small section of a meander

4.4 Discussion

We found home range estimates of gharial tagged with both GPS and VHF transmitter were surprisingly similar, both in terms of size and overlap, despite there being an average of 35 times more location fixes from the GPS units. Differences between GPS and VHF estimates were primarily driven by predictable biases: GPS devices failed to record location during seasons when individuals spent most of the time underwater, and VHF tracking tended to underestimate use of areas that were inaccessible. We found patterns of overlap could be improved by different methods of pseudo-sampling from the VHF data, and although precision of space use within the home range was lower for VHF estimates, similar understanding of the biological use of home ranges could be enabled by supplementation with the observational data that the manual tracking regime ensures.

When tracking VHF-tagged gharial, our major issues were that the gharial were often submerged and could not be seen, locating each gharial was time and resource intensive,

large stretches of the river could not easily be accessed for safety reasons (for example, only accessible on foot, which required a team of three people because of dangerous megafauna and often a full day's travel), and at times during the study we were not able to track due to Covid19 lockdowns. This meant our VHF data were temporally sparse, and generally were a labelled stretch of river we knew the gharial was in, rather than a precise location.

Locations from GPS telemetry tended to be at least once a day, and generally accurate to <20m. Therefore, the GPS data is likely to be very accurately identifying areas which gharial are using regularly for basking, onshore activities, and when swimming at the surface. By comparing home ranges estimates from the VHF data to estimates from GPS data, we were able to determine the best method of processing the VHF data to obtain estimates similar to, and encompassing, estimates from GPS.

When estimating home ranges, our estimates based on random pseudolocations performed better than assigning a single fixed location within a polygon, shown by significantly higher overlap with GPS home range estimates using both VI and $PHR_{VHF,GPS}$. There were not significant differences between the performance of a single or triple random pseudolocation identified by our mixed effect model, however the triple random pseudolocation method buffered the risk of very low scores as shown by a higher 'worst-case' probability of 0.43 for the core range estimates, compared to 0.28 for the single random pseudolocation method. This is because the single random location could perform very well – randomly landing exactly where the gharial occurred as determined from the GPS location – or it could fall at the furthest possible point in the polygon from the 'true' location. In contrast, the three random locations are likely to give a broader spread across the polygon, spreading out the risk of missing the 'true' location of the gharial, but also making any estimate less precise.

We found all VHF methods had considerably higher overlap scores for Home Range estimates (95% IcUD) compared to Core Range estimates (50% IcUD). Core Range estimates use a more precise estimate of space use within the high probability space use by gharial, and differences between the GPS and VHF estimates were driven by two different issues. Firstly, if the gharial had a low VI score but high $PHR_{VHF,GPS}$ score, this meant the GPS core range was encompassed within the VHF range (Figure 4.4), in which case we think this is not an issue due to the biases of the GPS technology discussed below. Secondly, if the gharial had both a low VI score accompanied by a low $PHR_{VHF,GPS}$ score, this meant that the core

range estimates were mismatched, and the VHF estimate had not captured the full core range of the GPS estimate (Figure 4.5). This was either caused by insufficient VHF detections meaning certain core areas were not included in VHF core range estimates, or because the gharial only used a small, specific section of the polygon. The GPS estimate could pick up on this specific small use with precision, however the VHF estimate could be slightly too far up or downstream within the overall polygon, depending where the random locations happen to fall. Therefore, we suggest more caution should be exercised when interpreting of core range results when there are few VHF detections in a space of time e.g. just 1 per month vs 1 per week.

We used a kernel method for estimating home ranges, as these have been shown to be the most unbiased home range estimator when low numbers of locations are available (Luca Börger et al. 2006), and our results lend support to this, as the VHF data (with very low fixes) brings about similar estimates to the GPS data with its substantially larger sample size. Although precision and accuracy would no doubt improve with a greater number of VHF fixes per month (Luca Börger et al. 2006), we suggest that even as few fixes as 4 per month can provide accurate home range estimates, although accurate core range estimates may require a greater numbers of fixes. The ability of our VHF data to perform similarly to the GPS data is likely due in part to the use of lcUDs, rather than other kernel methods. lcUDS have been used to accurately describe space use in crocodylians (Baker et al. 2022), overcoming previous issues identified from use of Kernel Estimation methods that do not incorporate least coast paths, which miss river sections crocodylians are known to use (Dwyer et al. 2015). The incorporation of the least cost paths means the use of the river stretch between fixes is also considered, counteracting the biases caused by GPS not detecting underwater locations, and of the low temporal resolution of the VHF rarely detecting a gharial during a movement event. This method also limits estimates to gharial habitat (the river and its banks) which is important for accurate home range estimates for species constrained to specific habitats (Slaght et al. 2013). Our results lend further support to the use of lcUDs in movement studies of crocodylian – or other riverine species – as the similarity of estimates from the GPS and VHF data suggest that this method is providing a realistic measure of space use.

Home range (IcUDs 95%) size estimates did not differ significantly between GPS estimates and the different VHF estimates, either by home range area or linear river length within home range. However, in some individuals the pattern of the home range area differed, as indicated by a low score of either VI or $PHR_{VHF,GPS}$ which allowed us to investigate the causes of bias of both types of technology. An example of the potential biases of both types of technology is Gharial 02 (Figure 4.4), where a considerable upstream portion of the river used by the gharial in the monsoon was missed entirely by the GPS technology. This occurred in the monsoon in August, when the gharial rarely bask as it is very warm: the GPS did not record a location for 25 days, whereas the VHF was able to indicate the gharial had made an upstream foray in this time (probably motivated by feeding, as gharial do throughout the monsoon). Conversely, GPS was better at detecting the use of the downstream stretches of this gharial's home range, as the GPS still fixes in areas that were difficult to reach for VHF tracking, or on short-term movements that occur between VHF tracking days. This has been found for other crocodylians, such as use of difficult-to-access marshes by alligators being picked up by satellite but not VHF tags (Skupien et al. 2016).

The GPS IcUDs were able to identify key areas of onshore habitat used with high precision, whereas the VHF estimates instead had a spread of pseudo-estimates around the river area used. It is not possible to know if this difference reflects a biological reality of the GPS detecting more precise space use for the Home Range, as in terrestrial species loss in the detail of patterns of animal space use has been found when using VHF rather than GPS (Fieberg & Kochanny 2005). However, since gharial cannot be detected underwater by GPS, this may instead reflect a specificity in basking site selection, with the GPS units underestimating the use of the surrounding river. Similarly, the generally smaller GPS home range estimates may be an underestimate if gharial use particular sections of their home range in which they do not bask, and usually remain submerged. Gharial remain at depth for much of the time when thermoregulating in deep pools, or when feeding, except when surfacing to breath or swallow prey, when the tag (on the tail) may not break the water surface. Therefore, the larger home ranges estimated by VHF may partially reflect the biases of the GPS technology. This is contrary to findings on both crocodiles and alligators, where satellite tracking found larger home ranges because more movement events and use of difficult to reach habitat were better determined with GPS (Skupien et al. 2016;

Beauchamp et al. 2018). The relatively good accessibility of the river (compared to marshes, mangroves or swamp) in our study is potentially the reason for this, combined with our tracking protocol of focussing on locating gharial 'missing' after a movement event.

The precision of the GPS, although not necessarily important for home range estimates, does indicate that GPS data was able, by location data alone, to identify the key onshore habitats used by gharial. This has been found in other studies, with GPS able to achieve a higher resolution of patterns of animal space use (Fieberg & Kochanny 2005). However, the importance of the key areas identified by GPS were generally also identified by the tracking team. The usefulness of VHF is often that it means direct observation becomes part of the essential tracking routine, so a good understanding of animal behaviour, environment and other factors is gained through fieldwork, whereas it is easier for field observation and GPS data to become divorced (Hebblewhite & Haydon 2010). It has also been found that VHF is better at identifying key habitats in which GPS cannot fix, such as burrows which are widely used by most crocodylians, although not gharial (Skupien et al. 2016). For example, the GPS quantified the fact that gharial 22 primarily used one small river bend to bask (Figure 4.6). However, our regular sightings of gharial 22, combined with discussion with local boatmen and tour guides who would report seeing her, meant this meander was identified by direct observation, combined with additional information on use of the bend by conspecifics and other crocodylians. Gaining a biologically relevant understanding of home range ideally allows for all pertinent data to be considered, not just location (Powell & Mitchell 2012), and we suggest the use of VHF helps ensure an understanding of what the gharial is doing and why they are there, whereas with GPS it is possible to just collect location data. However, our field observation were only possible because gharial in this area of Chitwan are habituated to humans and boats, whereas the GPS data would be very useful in other areas where gharial are very shy of humans.

Due to the differing biases of the two technologies, we think it is most important to consider the $PHR_{VHF,GP}$ overlap scores when determining the usefulness of the different VHF techniques. This ensures that the smaller GPS-estimate home ranges are mostly captured by the VHF estimates. For the ecological questions relevant to conservation management of gharial, it is most important for the entire home range to be captured, as underestimates of home range can lead to less effective conservation recommendations (Noonan et al. 2019)

for example, protected areas which are too small (Brashares et al. 2001). $PHR_{VHF,GP}$ overlap scores also do not penalise VHF home ranges which have managed to capture river use missed by GPS technology (in-river movements without basking, especially in the monsoon), unlike VI scores. Furthermore, VI has been shown to tend to be biased too low when considering utilisation distributions with a high degree of overlap (Fieberg & Kochanny 2005). The triple random pseudolocation method performed best on both size and overlap and had the best ‘worst-case scenario’ scores. Therefore, we recommend using repeated pseudo locations for processing VHF data when unsighted riverine animals can only be assigned to a stretch of water when tracking. Because of the different biases in estimates when using GPS and VHF data, caution should be exercised when comparing home range estimates derived using the two different techniques. In particular, the data should be inspected to identify when differences are likely to be due to the biases in the two technologies that we have discussed above, and ideally compared to data from double-tagged individuals.

Our results suggest that VHF and GPS telemetry have differing advantages in the study of space use by gharial, and therefore other amphibious or aquatic species. For most conservation and ecology applications VHF is likely to be preferable, as we have shown the data produced, when processed in a certain way, can create home range estimates very similar to GPS in terms of both size and overlap, and therefore for the same cost a much greater number of units can be purchased, improving replicability, and allowing greater generalisations to the population level. Furthermore, the much longer battery life of VHF (with current GPS technology) means that questions about interannual variability can also be investigated. The GPS unit solar panels tended to lead to a shorter battery life, mostly lasting less than one year, primarily driven by sedimentation on the solar panel. However, in a large, braided river system, one that is very inaccessible to trackers, or for individuals thought to travel a very long distance, or across international borders (such as the headstarted gharial in Chapter 6) GPS technology may be more useful. GPS may also be of use for ecological questions that require very fine-scale habitat use data, especially for on-land habitat use such as basking-site or nest-site selection, especially when animals are in inaccessible areas, highly disturbed by human presence or carrying out behaviours at night, such as nesting.

In conclusion, we found that VHF data, even at temporally low resolution, are effective at identifying gharial home ranges. Identified biases were relatively small in terms of real-world conservation or ecological application, and estimates can be improved by using multiple pseudo-locations to account for the uncertainty of each location. This is reassuring, as the collection of data on rare, threatened and aquatic species such as the gharial is very challenging, and high-resolution data or a fixed sampling regime is often impossible to collect with available resources. For investigating the ecology of gharial and other crocodylians to inform conservation planning, we suggest the use of VHF telemetry remains a useful and more affordable technology, so long as most of the system is reachable for manual tracking. The use of a small number of higher resolution satellite devices can be a useful addition for quantifying and accounting for specific biases in a new species or system.

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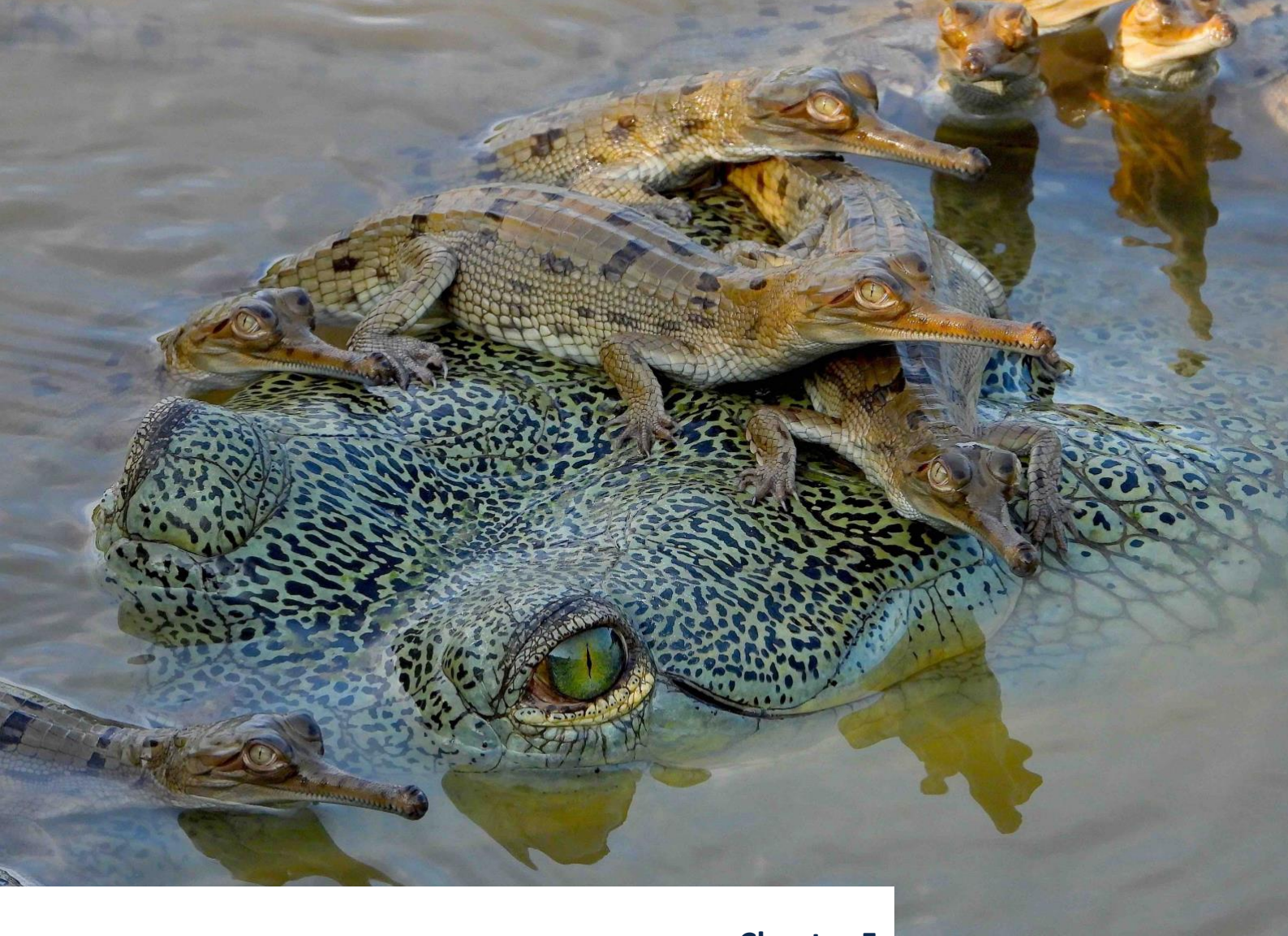
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Chapter 5

**Estimating detectability, abundance and population trends
of a reinforced population of gharial in Chitwan
National Park, Nepal**

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5.1 Introduction

Many wildlife populations have experienced substantial recent population declines, with such declines most acute in freshwaters, where populations have fallen by 83% since 1970 (WWF 2022). One intervention utilised by conservationists to try and reverse such declines is reinforcement: the releasing of supplemental individuals into a population, with the aim of increasing population size (IUCN/SSC 2013). In order to know whether a population is progressing toward the objective (population recovery), it is essential to determine the trend in the population. Population growth should be the main target of reintroduction programmes (Armstrong & Seddon 2008). Such monitoring is a key aspect of an adaptive management approach, providing one piece of evidence that helps managers to assess this invention against other management alternatives (McCarthy et al. 2012). This is especially urgent for Critically Endangered species, for which there is the imminent threat of extinction if the population declines, or even if it just remains stable at a low level.

However, measuring abundance of, and trend in, populations of wildlife are key challenges in conservation biology, and ecology more broadly (H. Iijima 2020). The major issue is detectability: not all individuals present in a given area will be counted (Royle & Nichols 2003). Many population monitoring programmes depend on unadjusted counts, or indices of relative density that assume there is a constant relationship between absolute and relative density (Yoccoz et al. 2001). However, when detection probabilities have not been estimated, such programmes cannot draw strong inferences about trends in a population, as the heterogeneity caused by varying detectability is not accounted for (Yoccoz et al. 2001).

Crocodylians are a group of amphibious predators, which are often the subject of conservation management since more than half of extant species are threatened with

extinction (P. Griffith et al. 2022). However, since crocodylians are cryptic, amphibious and wary of humans, detectability is likely to be low during surveys that count individuals, either by daylight or spotlight (Bayliss et al. 1986; Balaguera-Reina et al. 2018; Mazzotti et al. 2019). Therefore, surveys monitoring crocodylians usually seek to measure the relative density of a population e.g. crocodiles encountered per km (Bayliss 1987). Such studies assume there is a linear relationship between relative and absolute density that is stable over time (Bayliss 1987). However, this assumption is likely to be violated when it is impossible to standardize all covariates that influence detectability (e.g. weather) and when a population is experiencing rapid demographic turnover (e.g. a rapidly recovering or declining population, a reintroduced population) as different demographic classes of crocodylians are thought to have differing detectability (Webb & Messel 1979; Bayliss et al. 1986; Nair et al. 2012). Therefore, to make inferences about trends in populations it is necessary to investigate variation in detectability before trusting indirect indices.

The gharial is a Critically Endangered species of crocodylian found in South Asia, which suffered exponential declines in the last century, and now remains in just a few isolated populations in the Ganges basin (Lang et al. 2019). The majority of gharial monitoring uses daylight boat-based surveys, and unadjusted count data as an index of underlying population size, assuming that the majority of gharial are visible and basking during winter months (e.g. Hussain 1999; Acharya et al. 2017). However, unadjusted count data was shown to not detect a mass mortality event on the Chambal River, and use of individual marking for capture-recapture showed heterogeneous detectability (Nair et al. 2012), suggesting the relationship between unadjusted count data and population is highly variable. It is important to monitor trends in gharial populations, because the species has seen multiple population crashes following supposed conservation ‘success’ (Whitaker 2007). Furthermore, the majority of gharial populations are subject to – or have been subject to – reinforcement (aka headstarting) programmes which release juvenile or subadult gharial to reinforce the resident population, none of which have been monitored sufficiently to be able to determine if the releases have contributed to population recovery (J. M. Ballouard et al. 2010; Stevenson 2015).

The National Chambal Sanctuary in India is by far the largest remaining gharial population in the world, thought to have over 500 adults and over 86% of the world’s gharial nesting

effort (Lang et al. 2019). There are just five additional gharial populations believed to have more than 50 individuals remaining: at least 90 gharial were counted in the Corbett Tiger Reserve in 2015 (Chowfin & Leslie 2016), 72 gharial were counted in Katarniaghat Sanctuary in 2020 (Vashistha, Mungi, et al. 2021), 111 non-yearling gharial were counted in the Gandak in 2020 (Sinha et al. 2020) and approximately 100 gharial have settled post-reintroduction in Hastinapur Sanctuary (Yadav & Khan 2016). The fifth population is in the Chitwan National Park, Nepal, and is the world's second largest population of gharial according to unadjusted count data of non-yearlings.

The Chitwan population has been supplemented by a headstarting programme that started in 1978 (Tirtha Man Maskey 1989). Although by 2016 the population showed signs of recovery (Acharya et al. 2017), recent work has suggested such recoveries are variable across sites (Khadka *et al* 2023), and that detectability is variable within seasons (Yadav et al. 2022). Furthermore, nest counts for Chitwan remain low. For example, in 2020 the nest count in the Katarniaghat gharial population was 36, compared to an unadjusted population count of 72 (Vashistha, Mungi, et al. 2021), whereas in 2020 the nest count in Chitwan was 22, compared to an unadjusted population count of 204 (data from this study). This suggests the proportion of adults in the Chitwan population is low, and counts could be inflated by large annual releases of headstarted gharial from captivity. Therefore, an assessment of the gharial detectability, as well as underlying abundance and population trends, is required to understand the current status of the population and the impact of headstarting.

In this study, we use three different analysis methods to estimate detectability and abundance using count data from within-season surveys of unmarked individuals. Firstly, we will use two methods developed specifically for crocodylians, which aim to estimate the fraction of the population which has been sighted using variability between repeat counts (Messel et al. 1981; King et al. 1990). Secondly, we will use an N-mixture modelling approach, which estimates both detection probability and abundance, utilising repeat counts with varying values of covariates (Royle & Nichols 2003). The inclusion of these covariates allows for further investigation of what is driving both detectability, allowing better survey designs, and abundance, informing understanding of species ecology. Overall,

we provide the first estimates of total population size of gharial in Chitwan, and a simple and replicable methodology that can be repeated in other sites.

5.2 Methods

5.2.1 Study area

The study was conducted in Chitwan National Park, Nepal, which is thought to hold the world's second largest remaining gharial population (Lang et al. 2019). Gharial are obligate riverine species, primarily occupying deep free-flowing rivers with sandy banks (Whitaker & Basu 1982). In Chitwan, gharial occur predominantly on the two major rivers that make up the north and west borders of the National Park: the large, glacier-fed Narayani River, and its smaller rain-fed tributary, the Rapti River. During the monsoon gharial will move outside these major rivers, however during the winter, when we conducted surveys, gharial are limited to these rivers and short stretches of the most downstream portion of their tributaries.

5.2.2 Secondary data collection

To investigate general patterns in survey data in Chitwan, we collated all unadjusted count data from 2002-2022 in the published literature, in journals, student theses, Chitwan National Park annual reports and Crocodile Specialist Newsletter Articles. Survey results were only included if they were conducted on the whole of either the Rapti or Narayani river, and if daylight-boat based visual survey methods were used. Only total count data were used, due to the high risk of inconsistencies in size estimation between inexperienced observers (Griffith, unpublished data). The survey results were collated to see if any general trends could be surmised, and to determine if there was high variability between surveys in the same year.

5.2.3 Survey data collection

Surveys were conducted from above the uppermost limit of recent gharial sightings, to the downstream point at which the Narayani crosses the Nepal-India border, through the Gandak Barrage. Although short stretches of two tributaries of the Rapti were also surveyed (Dungre Khola and Budhi Rapti) they were excluded from this study due to our inability to obtain certain covariates for abundance estimates.

The rivers were divided into 12 transects (Table 5.1). Each transect was surveyed concurrently by 12 survey teams thrice over three consecutive days. Each survey team was comprised of at least two observers, and two boatmen, moving downstream using a traditional dugout canoe or inflatable raft. Observers scanned both sides of the river, and the main channel, for any gharial either on the river surface or the river bank. Location of each sighting was recorded with a GPS, as were the weather conditions (sunny, sunny intervals, overcast, cloudy, or raining) and time. Temperature and rainfall data were taken from the local meteorological office station.

Surveys were conducted in the winter because gharial spend a greater proportion of the day basking during winter and therefore detectability is improved. Each year referred to throughout is the year in which the winter season ended. Surveys were conducted on 23-25 February 2018, 26-28 February 2019, and 2-4 December 2020 ('Year 2021'). The irregular survey occurrence was due to Covid-19 pandemic lockdowns.

Gharial were classified after Acharya et al (2017), adult males (>350cm + ghara), adults (>280cm), sub-adults (220 – 280cm), juveniles (100 – 220cm) and yearlings (<100cm), although we recommend categorisation after Lang & Jailabdeen (2018) for future surveys to better reflect maturity transitions. We have used total count data only where possible, as the selected size classes will give an inflated estimate of adults (females are only thought to mature at 300cm, Chapter 6), and because tests with observers for size estimation on known-size animals showed a huge variation within and between observers. Adult males are easily identifiable by their enormous size, and the presence of a cartilaginous growth on the tip of their snout called a ghara (Whitaker & Basu 1982). However, within the 'adult' category, adult females and subadult males cannot be distinguished.

River	Transect	Start Coordinates	End Coordinates
Rapti	Lothar – Jindagani Ghat	27.56147 / 84.70923	27.56172 / 84.58078
	Jindagani Ghat – Sauraha	27.56172 / 84.58078	27.57388 / 84.49316
	Sauraha – Kasara Bridge	27.57388 / 84.49316	27.55142 / 84.32554
	Kasara Bridge – Golaghat	27.55142 / 84.32554	27.56356 / 84.15929
Narayani	Golaghat – Amaltari	27.56356 / 84.15929	27.55567 / 84.08851
	Amaltari – Arunkhola (outer branch)	27.55567 / 84.08851	27.55249 / 83.96418
	Amaltari – Arunkhola (inner branch)	27.55567 / 84.08851	27.55249 / 83.96418
	Arunkhola – Triveni	27.55249 / 83.96418	27.47130 / 83.94278
	Gidddeni – Confluence	27.66952 / 84.26642	27.55614 / 84.11926
	Ganjipur – Golaghat (outer branch)	27.65561 / 84.28331	27.56356 / 84.15929
	Tinbhangalo – Golaghat (inner branch)	27.66822 / 84.27341	27.56356 / 84.15929
	Devghat – Gidddeni	27.73965 / 84.42313	27.67362 / 84.28093

Table 5.1: start and finish points for transects on the Rapti and Narayani Rivers, which were concurrently surveyed for gharial in this study

5.2.4 Covariates of Abundance

We investigated eight covariates which we thought were likely to influence the abundance and distribution of gharial in Chitwan. These were: human disturbance (number of roofs of buildings within 1km of transect, counted using satellite imagery in QGIS), distance to the headstarting programme release site (km from the centre of each transect), distance to the Gandak barrage (km from the downstream point of the transect), length of transect (km). Measures of river coverage and persistence were taken from the Global Surface Water Explorer dataset for each transect (Pekel et al. 2017). These were low and high-water coverage (number of 30x30m pixels identified as water within the river transect in 2020 in the dry and wet seasons), seasonality (number of months an average pixel from a transect

was identified as water) and water occurrence (percentage of time between 1984-2021 that an average pixel from a transect was identified as water).

5.2.5 Abundance Estimation

Juvenile gharial are annually released on the Rapti river as part of an ongoing headstarting programme led by the National Park. To avoid inflated gharial counts of recently released individuals, we excluded counts of juveniles within the mean maximum distance known to be travelled by released headstarted gharial in their first month post-release (15km, Chapter 6, this thesis). Yearlings were also excluded from all analyses, as is usual for crocodylian surveys due to the low recruitment rate of crocodylians in their first year of life (Messel et al. 1981; Balaguera-Reina et al. 2018). Transect start and finish points were adjusted slightly in post-processing to ensure each transect started and finished at logical locations on the river (e.g. did not break up areas gharial are known to move between regularly). All sightings with potential errors (e.g. inconsistent time) were excluded.

5.2.6 Sighting Fraction Abundance Estimates

We first estimated gharial abundance using two sighting fraction methods developed for visual surveys of crocodylians (Messel et al. 1981; King et al. 1990).

Messel et al (1981) defined sighting fraction (p) as:

$$p = \frac{\bar{X}}{\sum_{i=1}^{SC} max}$$

where $\bar{X}$ is the average count of crocodilians, and max is the maximum count of crocodilians seen in each size class (SC). The assumption is that the data fit a binomial distribution. King et al (1990) defined sighting fraction (p) as:

$$p = \frac{\bar{X}}{(2SD + \bar{X})1.05}$$

where SD is the standard deviation. The assumption is that the data fit a normal distribution. To compare between years, we conducted a one-way repeated measures ANOVA to determine if there were any significant differences in counts per transect between years.

We also calculated estimated encounter rates (gharial per kilometre (Bayliss 1987) based on the results of each sighting fraction method. Encounter rate data were log-transformed to achieve normality. We then used a linear mixed-effects model, with transect as random effect, to investigate if there was a change in encounter rate over time (Khadka *et al* 2023).

5.2.7 N-Mixture Model Abundance Estimation

We used binomial N-mixture models to estimate gharial population size in Chitwan from repeated count data, accounting for imperfect detection (Royle & Nichols 2003). An N-mixture model is a hierarchical model made up of two linked generalized linear models: an ecological process that distributes individuals in space, and an observation process that accounts for an imperfect detection leading to the collected count data. We chose to use a Negative Binomial (NB) distribution to describe the ecological process, after investigating goodness of fit of candidate models with a Poisson, Negative Binomial (NB) and Zero-Inflated Poisson distribution through parametric bootstrapping (100 simulations) using the parboot() function (Fiske & Chandler 2011). Since we had count data from multiple years, we used a year-stratified model. This approach regards each year as an independent data set, and fits separate parameters for each year (Kéry & Royle 2020). Therefore our N-mixture model can be summarised as the two following linked processes:

1. Ecological process: $N_{i,t} \sim \text{Negative Binomial}(\lambda_{i,t})$
2. Observation process: $C_{i,j,t} \sim \text{Binomial}(N_{i,t}, p_{i,j,t})$

where $N_{i,t}$ is the latent abundance state at site i in year t , λ is the expected abundance, $C_{i,j}$ is the count at site i during survey j and p is the per-individual detection probability.

We fitted all N-mixture models in the unmarked package (Fiske & Chandler 2011) in R (R Core Team 2021). We investigated the following environmental covariates for lambda: transect length, human disturbance, distance to headstarting release site, distance to barrage, number of pixels of water in transect in high and low water seasons, water seasonality and water occurrence. To identify any trends in abundance, we also investigated time as a continuous variable, and cumulative number of gharial released from captivity. Weather, time of day of survey start, season, year, temperature and day of survey within season were used as covariates of p. An integer value specifying the upper index of integration (K) was set to 200 for all models after we determined this was large enough to have no effect on model results (Fiske & Chandler 2011).

We used the Akaike's Information Criterion (Akaike 1974) to identify the best-supported models. For these models, we calculated a dispersion parameter ($\hat{c}$) which was the ratio of the observed fit statistic to the mean of the simulated distribution, as determined through parametric bootstrapping (Kéry & Royle 2020). To understand the relationship between covariates and λ and p in our best models, we used the predict() function in unmarked, and plotted these relationships using ggplot2 (Wickham 2016).

We estimated total population size using the predict() function, and reported the predictions with 95% confidence intervals. We then calculated gharial per km for each transect by dividing the number of gharial estimated for each transect by the length of that transect.

5.3 Results

5.3.1 Combination of Secondary Survey Data and Our Data

Between 2002 and 2008 surveys found fairly constant numbers of gharial in both the Narayani and Rapti rivers, although there were rarely two counts in the same year, so it is not possible to determine variability (Figure 5.1). Counts in the Narayani River between 2011-2013 were generally higher, although this increase is hard to determine in the Rapti River. There is a sudden jump between 2013 and 2016, with counts then remaining high

(Figure 5.1). However, variability in unadjusted counts within years is so great, it is impossible to determine a post-2016 population trend in either river.

5.3.2 Unadjusted Counts from Our Survey Data

Across all three years, with triple surveys in each transect annually, there were a combined total of 1664 gharial detections, of which 203 were excluded as yearlings or juveniles close to the release site. Maximum number of detected gharial per transect per year ranged between 32 and 46, and total number of detections per survey varied from 125-189 (Table S5.1, Appendix 2).

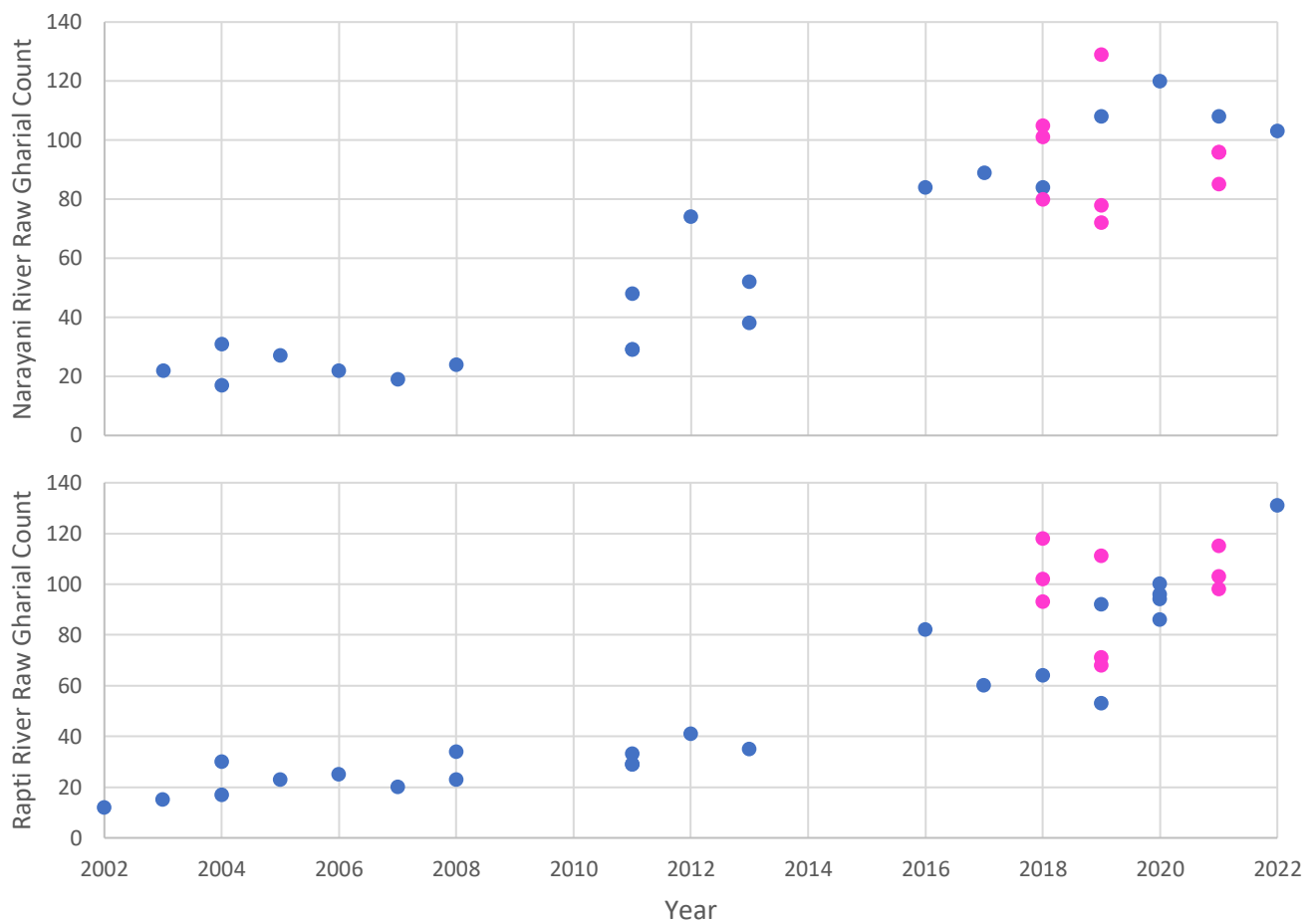


Figure 5.1: combined counts of all gharial surveys conducted on the Narayani (top) and Rapti (bottom) Rivers, Chitwan, between 2002 and 2022, collated from both secondary data (blue) and this study (pink). Figures are total counts excluding yearlings.

5.3.3 Sighting Fraction Methods to Estimate Abundance

Sighting fraction estimates were on average 0.69 for Messel's equation, leading to estimates of 202 ± 33 (2018), 177 ± 43 (2019) and 219 ± 28 (2021) gharial in Chitwan (Table 5.2, Figure 5.2). The transects with the highest estimated population were those between Sauraha and Golaghat on the Rapti, and the transect immediately downstream of Golaghat on the Narayani (Table S5.2, Appendix 2). The one-way repeated measures ANOVA showed was no statistically significant difference in estimated gharial counts between years $F(2,20)=1.91$, $p=0.19$. The linear mixed-effects models showed that, once transect was controlled for, there was no significant trend in the gharial encounter rate from 2018-2021 ($p>0.1$). Therefore, using data from all years, there was an overall population density of 0.94 gharial/km² in Chitwan.

	Sighting Fraction			Estimated Population Size			Estimated Encounter rate		
	2018	2019	2021	2018	2019	2021	2018	2019	2021
Messel 1981	0.69	0.76	0.68	202 ± 33	177 ± 43	219 ± 28	0.97	0.86	1.05
King 1990	0.65	0.59	0.73	207 ± 36	228 ± 54	198 ± 28	0.99	1.11	0.95

Table 5.2: Abundance estimation of Gharial in Chitwan using the sighting fraction methods of Messell (1981) and King (1990)

For King's equation, sighting fraction estimates averaged 0.65 (Table 5.2), with population estimates of 207 ± 36 (2018), 228 ± 54 (2019), 198 ± 28 (2021) gharial (Figure 5.2), with the highest estimated populations on the same transects as for Messel's equation (Table S5.3, Appendix 2). The one-way repeated measures ANOVA showed was no significant difference in estimated gharial counts between years $F(2,20)=1.41$, $p=0.27$. The linear mixed-effects models showed that, once transect was controlled for, there was no significant trend in the gharial encounter rate from 2018-2021 ($p>0.1$). Therefore, using data from all years, there was an overall population density of 1.00 gharial/km² in Chitwan.

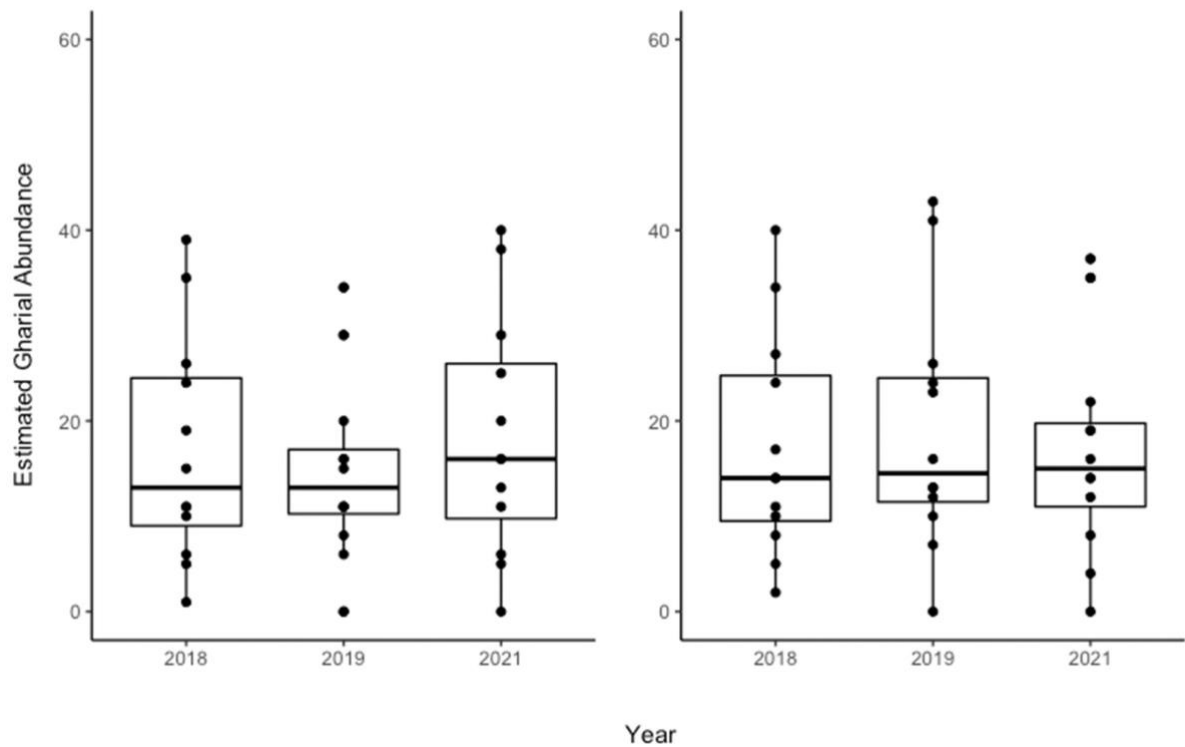


Figure 5.2: box plots illustrating the per-transect counts estimates of gharial abundance in Chitwan using Messel's equation (left) and King's equation (right). A one-way repeated measures ANOVA for each method showed there were no significant differences in abundance between years.

5.3.4 N-Mixture Model

Following all possible combinations of covariates (of which those with the lowest AIC and highest R^2 are reported in Table 5.3), the best supported model was [p(time of day + weather + temperature), λ (disturbance + distance to headstart release site (Kasara) + low water coverage + seasonality)]. This model is supported with a very high R^2 which indicates this model explains 95% of variation in gharial abundance, and lowest AIC value indicating the best trade-off between fit and complexity. Goodness of fit bootstrap statistics found that this model had an adequate fit to the data (SSE: $p = 0.37$; Chi-square: $p = 0.13$; Freeman–Tukey: $p = 0.16$), although, the dispersion parameter $\hat{c}=1.22$, indicates some over-dispersion.

Model Covariates	nPars	AIC	ΔAIC	R^2
Detection Probability				
p(time + weather + temperature), λ (.)	9	614.06	0	0.66
p(time + weather + survey day), λ (.)	9	619.60	5.54	0.60
p(time + weather), λ (.)	8	621.30	7.24	0.55
Detection Probability + Abundance				
p(weather + time + temperature), λ(disturbance + distance to Kasara + low water level + seasonality)	13	549.14	0	0.95
p(weather + time + temperature), λ (disturbance + distance to Kasara + low water level + seasonality + decreasing linear trend)	14	550.53	1.39	0.96
p(weather + time + temperature), λ (disturbance + distance to Kasara + low water level + distance to dam)	13	564.62	15.49	0.93

Table 5.3: Abundance N-mixture models of gharial in Chitwan National Park, with the covariate combinations that gave the lowest AIC scores for detection probabilities and detection probabilities and abundance. The best supported model is highlighted in bold.

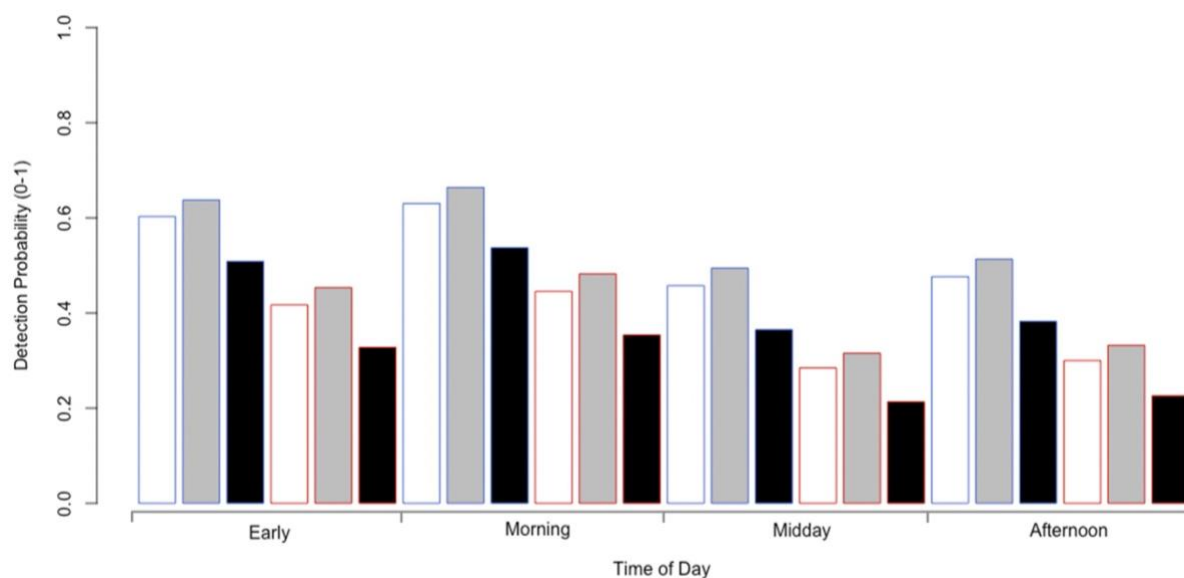


Figure 5.3: Gharial detection probability estimates as a function of different starting time of day of the survey, weather conditions (white = sunny, grey = partly cloudy, black = cloudy), and temperature (blue border = cool, red border = warm), as calculated through our Negative Binomial N-Mixture model. Probabilities shown here are for the third consecutive day of a survey.

Based on the best supported model, gharial detectability was higher in cold temperatures, sunny or sunny-interval conditions, and during the morning survey (starting between 10 and 11.30am) (Figure 5.3). Gharial detectability was lowest on warm, cloudy days after midday (with lowest probabilities of 0.21 ± 0.05).

The covariates that were included in the best-fit model indicated that gharial abundance was higher in transects where there was lower human disturbance, which were closer to the headstarting release site, and where the river was less seasonally variable and there was a lower low water pixel count (Figure 5.4). Abundances generated through this model estimated transect abundances that varied from one gharial in the Devghat transect, to 61 in the Gholaghat transect. This led to an overall estimate of 309 (204-468) gharial in Chitwan from 2018-2021 (Table 5.4), or an average of 1.46 gharial per km of river (0.96 - 2.21 gharial/km).

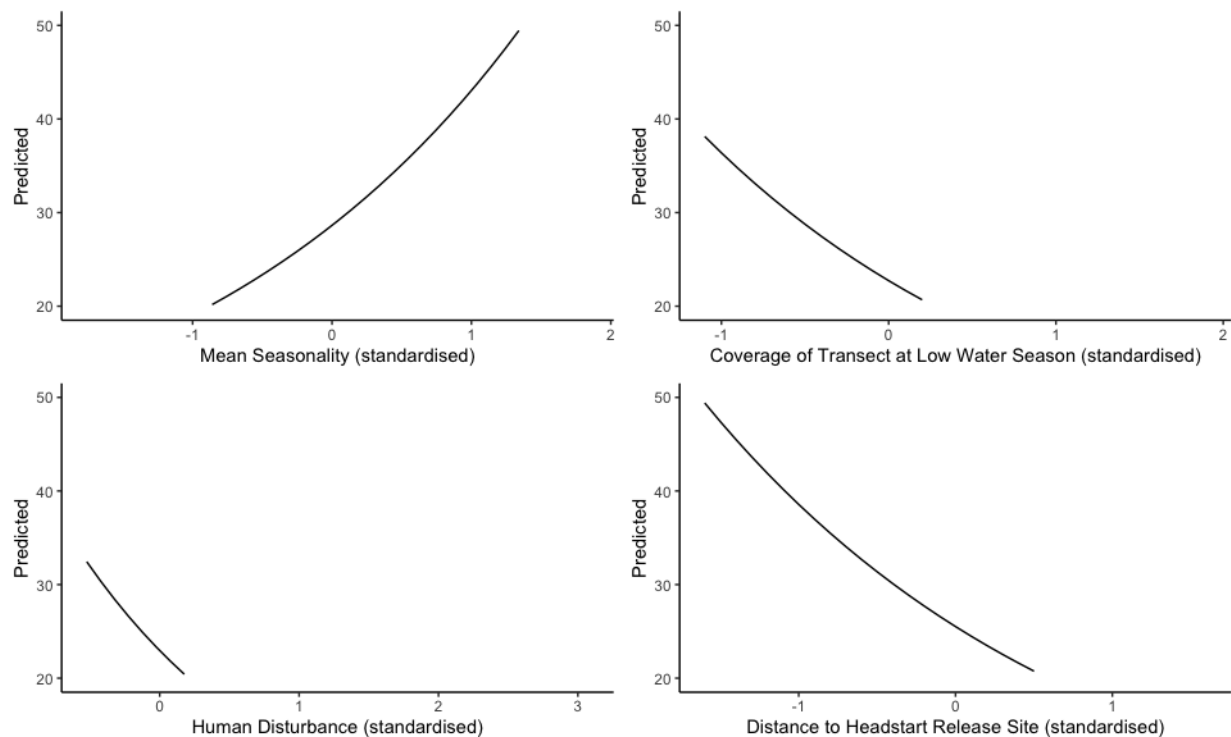


Figure 5.4: Generalised linear models showing the relationship between gharial predicted abundance and different covariates calculated through the best fit N-mixture model.

	<i>Estimated Gharial Population</i>	<i>SE</i>	<i>Lower 95% CI</i>	<i>Upper 95% CI</i>
<i>Lothar</i>	13	2.85	9	20
<i>Jingagani ghat</i>	24	5.16	16	37
<i>Sauraha</i>	52	10.80	34	78
<i>Kasara</i>	48	10.10	32	72
<i>Arunkhola</i>	16	3.46	10	24
<i>Amaltari (outer branch)</i>	11	2.37	8	17
<i>Amaltari (inner branch)</i>	21	4.21	14	31
<i>Gholaghat</i>	61	13.43	40	94
<i>Giddeni (western branch)</i>	24	4.69	17	35
<i>Ganjipur (central branch)</i>	17	3.23	11	24
<i>Tinbhangalo (eastern branch)</i>	20	4.64	13	32
<i>Devghat</i>	1	0.55	0	3
<i>Total</i>	309		204	468

Table 5.4: per transect estimations of gharial population size in Chitwan National Park, Nepal, 2018-2021, estimated from N-mixture model with 95% confidence intervals. Maximum number of gharial counted in one day of survey (raw count), in that transect in any year also reported.

5.4 Discussion

Our study provides the first population estimates for gharial across the entirety of Chitwan National Park, Nepal. Estimates were conducted using three alternative methods of analysing count data from repeat within-season surveys, over a span of four years. The N-mixture model, which accounts for both uneven patterns of gharial abundance and imperfect detection, estimated gharial abundance in Chitwan to be ~309 (204-468) from 2018-2021. This model found gharial detectability to be highly variable under different conditions, with the highest probability of detection (0.66 ± 0.28) on surveys conducted on

cold days with sunny intervals, starting between 10 and 11.30am. Gharial population estimates using methods that do not utilise covariates of abundance and detectability made lower estimates of abundance, just 177-228 gharial (Table 5.2). These findings suggest the Chitwan population is higher than estimates based on unadjusted counts. When combined with historical survey data, it appears there was a substantial increase in the total Nepal gharial population between 2008 and 2016. However, our results also suggest this population is no longer increasing despite ongoing release of gharial from captivity, which is of concern for conservation of this Critically Endangered Species. It is also worth noting this increase is driven by increasing numbers of immature gharial, and although the adult population has grown slightly from a few individuals in the early 2000s, adult counts remain as low in 2022 as when the project started in the 1970s (Maskey 1989).

Our results support the widely proposed predictors of gharial detectability (temperature, weather, time of day) (Whitaker & Basu 1982; Hussain 1999) as having a substantial influence on detectability, along with day of survey having an additional minor effect, likely due to improving ability of observers with practice (Mazerolle et al. 2007). Therefore, we strongly recommend that surveys should take place on cold, sunny days in the mid-morning, especially when surveys cannot be repeated. If this is not possible, these covariates should be accounted for in the analysis and discussions of relative density results. Ideally, gharial surveys should be repeated at least thrice to be able to replicate our models to obtain population estimates, and to take advantage of improving detectability over multiple days. One previous study used N-mixture modelling for gharial on the Rapti river, Chitwan (Yadav et al. 2022), however this study did not consider the environmental factors generally thought to be key for gharial detectability.

Our N-mixture model gave higher estimates of gharial abundance than the sighting fraction methods. These methods have also been found to underestimate population size in crocodylians when compared to more accurate methodologies such as mark-release-recapture (Webb et al. 1989; Balaguera-Reina et al. 2018). An issue with both sighting fraction methods is the requirement for data to fit a theoretical underlying distribution (Balaguera-Reina et al. 2018) which is challenging to achieve in nature, and an additional issue with the method of Messell *et al* (1981) is the requirement for counts that accurately delineate crocodylians into size classes, which is very difficult for observers unless they are

highly experienced. The lower estimates of both these methods is driven by fairly high estimated sighting fractions (0.59-0.76) compared to the individual detection probabilities of the N-mixture model (0.21 ± 0.05 - 0.66 ± 0.28), although these are not directly comparable since the sighting fraction is an estimate of the proportion of all members of the population that were detected (Barão-Nóbrega et al. 2022) whereas the latter is the probability that an individual will be detected under certain conditions (Royle & Nichols 2003). Some previous studies using N-mixture models on crocodylians have suggested that detection probabilities resulting from the model were implausibly low, leading to overestimates in abundance (Mazzotti et al. 2019; Cartagena-Otálvaro et al. 2020), whereas others have provided accurate estimates (Farris et al. 2021; Barão-Nóbrega et al. 2022). Unmodeled sources of variation in detection probability (such as gharial habituation, gharial size class, or observer experience) could also lead to biases in the results, however, since our detection model (no abundance covariates) accounts for 66% of the variation in count data, and our complete model accounts for 95% of the variation, we think this impact is minimal. Furthermore, given the individual detection probability of seeing specific known individuals (three adult males) in these surveys was 0.37, it seems our N-mixture model estimates are reasonable, as we would expect adult males to be on the lower end of detectability at this time of year. Therefore, we concur with other studies that the sighting fraction methods are likely an underestimation, and cautiously propose our N-mixture model is giving a more accurate estimate: a population of around 300 gharial in Chitwan.

It is possible the estimate of around 300 is an overestimate, and certainly it appears that the upper 95% confidence interval is too high, primarily due to additional disturbance factors impacting abundance we failed to quantify, and therefore couldn't include in the model. This was fishing pressure and sand mining occurrence in areas with low building density (leading to high estimates in the two Amaltari branches). Measures of illegal fishing are very challenging to determine and quantify spatially, and sand mining is similarly challenging to map due to sporadic occurrence and limited monitoring. These factors may impact both detectability and abundance.

Alternative methodologies to our N-mixture modelling approach include mark-release-recapture (MRR). Such studies are thought to provide accurate population estimates for crocodylians (Briggs-Gonzalez et al. 2017; Balaguera-Reina et al. 2018), however these are

not appropriate for gharial due to individually varying trap shyness, and the near-impossibility of catching individuals twice (Gharial Ecology Project pers. comm), as well as the high costs and extensive catcher experience required. The methodology of Nair *et al* (2012) utilised individual-specific scale patterns to conduct non-invasive MRR, which is another promising methodology for gharial population estimates at certain sites: however, the combination of vegetated riverbanks and gharial wariness in Chitwan makes capturing sufficient clear photos for this methodology very challenging.

If the population remains at ~300 gharial, then survey results from secondary data suggest counts have been within the variation expected from this population size since 2016 and until 2022. When considering the unadjusted counts from all past surveys we could find, it seems there was a substantial increase in gharial numbers between the period 2008-2016. Prior to 2008, all counts were less than 60 individuals suggesting a worryingly small gharial population. The increase seen from 2008-2016 appears to have now levelled off, leading to the lack of population trend we found from 2018-2021: this is cause for concern. 334 juvenile gharial were released from captivity into the Rapti river during this period which, despite our adjustments of the raw data to try and exclude the juvenile gharial released the same year as each survey, should still have led to detectable increases in the population. The continued low nest counts in Chitwan relative to population size suggest the fraction of the population which is adult remains low, likely around 30 adult females and three adult males (Chapter 6). The high population estimates we have found, primarily comprised of subadults and juveniles, and lack of increasing population trend despite releases, suggest that many headstarted gharial are not becoming an established part of the wild resident population. This highlights the requirement for an assessment of the headstarting programme, which I will address in Chapter 6. The covariates of gharial abundance identified in the best supported N-mixture model are useful to help identify the threats to the recovery of gharial in-situ, and therefore which interventions may be most important.

Firstly, the sharp cut-off in gharial occurrence above a certain number of houses close to the river suggests a threshold for generalised human disturbance, above which gharial cease to occur. This fits with the current distribution of gharial, found only in areas of their range with lower human disturbance (Lang et al. 2019) (few and far between in the Ganges Basin), and highlights the importance of conservation of those stretches of river with lower rate of

human disturbance, and to protect them from riverbank development and urbanisation. The impact of disturbance was such that even though the upstream section of the Narayani was highly suitable habitat according to other covariates, it was rendered uninhabitable, despite this being an important stretch of both occurrence and nesting in the 1970s-80s (Maskey 1989).

The preference of gharial for stretches of river less seasonally variable is well reported, as these tend to be the stretches with greatest water depth in the dry season when surveys take place (Whitaker & Basu 1982; Hussain 2009, Bashyal et al *in prep*). The low water pixel count primarily describes river width, since it is a count of pixels of river in transects of similar length. The relationship here seems to be driven by the higher abundance of gharial on the narrower Rapti River, as compared to the Narayani, and reflects other studies that find the gharial population on the Rapti river is doing comparatively better (Acharya et al. 2017, Khadka et al. 2023). The Rapti is a well-protected river, on which fishermen report higher average fish catches than on the Narayani, as well as increasing fish numbers (Raj Giri et al. *in review*). It is also the site of the release programme, which leads to the other correlation observed – that transects closest to the release site have higher overall gharial abundances. Since the majority of Chitwan gharial are headstarts released on the Rapti (Khadka et al. 2022) it is unsurprising this is an important covariate. It has also been suggested that gharial released from captivity may have different habitat preferences to wild gharial, driving increasing gharial numbers in the slow, seasonally stable and warmer Rapti (Khadka et al 2023).

Our results – across all three methods – highlight the issues of using an index of relative density for determining population trends in a sensitive cryptic species that has varying detectability with different environmental and survey conditions. The results of single surveys during the period 2016-2022 could lead to conclusions of gharial population increase, decrease or stability, depending on which survey results were used. This is of serious concern for a population that remains seriously depleted, where understanding population trends is critical for determining whether conservation interventions are working or not, and to implement new management plans when the population is declining. In the history of gharial conservation, catastrophic population collapses have regularly followed supposed conservation success (Whitaker 2007), so timely understanding of population

trends is essential to allow swift interventions. This is especially vital as these results suggest that the Chitwan gharial population is now the second largest remaining gharial population, after the Chambal River population (which is substantially larger, and both viable and self-sustaining, which the Chitwan population is not). Furthermore, it is the only population which has not had gharial introduced from elsewhere in their range, so is a vital 'baseline' population for future genetic work.

Future work on gharial in Chitwan should focus on conservation interventions: the results of this study show that conservation is at a key moment, in-situ conservation efforts must capitalise on previous population increases to allow for population growth to continue. Since the priority of effort and funding should be on interventions, new monitoring methodologies that are more accurate – but not substantially more expensive – are needed. The issues identified by this study raise challenges for monitoring gharial populations. If single surveys are insufficient, they are not useful, however multiple repeat surveys may be prohibitively expensive and require a substantially increased allocation of staff time. Drone surveys may provide a solution: especially as commercial drones become both cheaper and better equipped. Drone survey methodologies (e.g. Thapa et al. 2018; Aubert et al. 2022) should be trialled and compared to conventional boat-based surveys. These surveys would also allow for more accurate delineation of size class, since drone images can be used to accurately measure gharial (Griffith and Jailabdeen, unpublished data) and therefore provide added accuracy to population estimation models, such as by allowing size-class specific individual detection probabilities. In terms of improvement in this N-mixture approach, the next stage would be to investigate the accuracy of individual detection probability estimates by running a single-season model with a substantial increase in the number of within-season replicates.

In conclusion, our results suggest that N-mixture modelling is a useful technique for estimating gharial populations, and that surveys without replication or considering detection covariates can lead to counts that are decoupled from underlying population trends. This is relevant for managers monitoring gharial across South Asia, and highlights the need for population estimates in order to be confident of population change or stability. Furthermore, the results provide additional evidence that headstarting alone is insufficient for gharial population recovery, and broader in-situ conservation that considers the entire

riverine ecosystems of gharial should be the priority. A cause for cautious optimism is that, in Chitwan, even our lowest population estimates for gharial are substantially higher than raw counts for gharial in 2003/2004 when the population teetered on the brink of extinction. However, the apparent stalling of population recovery and the continued low adult proportion of the population shows there is still a long way to go to secure this important gharial population, and new in-situ conservation interventions are necessary if we want to ensure gharial are found in Chitwan in the future.

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Chapter 6

Retrospective analysis of the gharial headstarting programme in Chitwan National Park 1983-2021

Chapter 6

Retrospective Analysis of the Gharial Headstarting Programme in Chitwan National Park, 1983-2021

6.1 Introduction

Once a wildlife population has catastrophically declined, conservation translocations are one of the tools conservationists may attempt to use to try and help that population recover. One type of conservation translocation is reinforcement, ‘the intentional movement and release of an organism into an existing population of conspecifics’ (IUCN/SSC, 2013). The aim of reinforcements is to enhance population viability, such as by increasing population size above a critically low threshold, or increasing genetic variation or demographic representation (Seddon, 2010). Translocations have been widely used in herpetofauna (Germano and Bishop, 2009), and within crocodylians reinforcement has been the most widely used conservation translocation technique. Typically, the type of reinforcement used in long-lived reptiles such as crocodylians and turtles is headstarting, ‘the practice of protecting especially vulnerable life stages of a species to increase the likelihood of survivorship for conservation purposes’, giving individuals a literal ‘headstart’ in life (Burke, 2015). However, the success of such programmes has been very variable (Dodd and Seigel, 1991; Germano and Bishop, 2009). Understanding of why translocations succeed or fail in their objectives is vital to improve management of ongoing and future translocations. An essential component of any translocation is post-release monitoring (Sutherland *et al.*, 2010; IUCN/SSC, 2013). If a translocation fails, it is not possible to understand which of a suite of possibilities caused the failure without tailored and appropriate monitoring (Parker *et al.*, 2013). Appropriate monitoring allows for adaptive management or informed cessation of the programme (Parker *et al.*, 2013), whereas inadequate monitoring can lead to a programme being incorrectly classed as ‘successful’ (Fischer and Lindenmayer, 2000). Despite this, many programmes have failed to conduct adequate monitoring, although an increasing volume of reintroduction literature suggests this is changing (Griffith *et al.* 1989; Bubac *et al.* 2019; Berger-Tal *et al.* 2020).

6.1.1 Gharial Headstarting Programmes

A reintroduction programme once described as “among the most successful conservation projects in the world” in 1982 illustrates the importance of sufficient monitoring, as this same programme was shown by 2006 to have primarily been a failure (Whitaker, 2007). This programme was the headstarting of the gharial in India under ‘Project Crocodile’. The gharial is one of the world’s largest crocodylians: found only in South Asia, the species was once widely distributed in the lowland rivers of the Indus, Ganges and Brahmaputra basins (Lang, Chowfin and Ross, 2019). Following exponential declines in the mid-20th century, the species was extirpated from all of its range except 14 small, isolated populations in the Ganges basin in India and Nepal (Lang, Chowfin and Ross, 2019). Both the governments of Nepal and India set up headstarting programmes which carried out both population reinforcements and reintroductions across multiple sites, almost exclusively using eggs collected in the wild and reared to a larger size class in ex-situ facilities. By 2005 over 5000 gharial had been released in India (Whitaker, 2007), and to date over 1700 gharial have been released in Nepal (DNPWC, 2022).

Most of the headstarting programmes did not work, and there has been insufficient monitoring of most of these programmes to understand why. Only one gharial population has recovered to a viable level – in the National Chambal Sanctuary (Lang, Chowfin and Ross, 2019) – and to what extent the headstarting programme contributed to this is impossible to determine due to a lack of monitoring. Hussain (1999) showed there was a substantial population growth rate following the initiation of the headstarting programme, and it is probable the release of 1792 gharial from 1979-1990 did indeed give the population the intended ‘headstart’ to reach over 874 non-yearling individuals by 1992 (Hussain, 1999). However, it is also possible that the majority of this recovery was driven by natural population recovery, as there were still ~30 adults before the programme commenced. These confounding factors make it impossible to determine, retrospectively, the importance of the headstarting programme. Similarly, the release of 257 gharial in 1985-1994 into the Corbett Tiger Reserve probably contributed to the establishment of an adult population of ~80 gharial by 2015 (Chowfin and Leslie, 2016), however, the lack of monitoring and the presence of at least some adult gharial in the reserve prior to the headstarting programme once again provide limited evidence either way. The reestablishment of a small population

in Hastinapur entirely from reintroduced individuals shows promise of being the first, and only, evidenced success of establishment using headstarting (Yadav, Nawab and Khan, 2013; WWF India, 2017) with maturation and reproduction of this population in-situ being the next key step.

Other programmes have a clearer outcome: that the headstarting did not work. On the river Mahanadi 700 gharial were released prior to 1999 (Singh 1999), but surveys in 2005 found just two gharial (Mohanty *et al.*, 2010). Other sites include the Ken River, where 142 gharial were released, but surveys in 2013 could not find a single gharial (Nair and Katdare, 2013), and 164 were released in the Son Gharial Sanctuary, yet surveys 1996-2010 found a declining population trend to just 20 animals (Nair and Katdare, 2013). The release of >1800 gharial into the Katarniaghat Sanctuary has similarly been shown to have no impact on population increases, with releases of substantial numbers of juvenile animals not being reflected in population counts the following year (Vashistha *et al.*, 2021). In Nepal, prior to 2021, 95 gharial were released in the Koshi River, none of which survived and established (Khadka & Khatiwada 2022). In the Babai river 110 gharial have been released but the 2019 population was just 19 individuals (Bashyal *et al.*, 2021).

Other, more recent, headstarting programmes such as on the Beas River in the Punjab, have been initiated too recently to comment on their potential success or failure, especially since many of the failed headstarting programmes showed promising signs of ‘success’ in the initial years of the programme (Whitaker, 2007), which likely reflected re-sightings of reintroduced gharial which would eventually die. The continued use of headstarting for gharial throughout India and Nepal, and the uptake of this technique across new sites, shows there is an urgency to understand why these programmes fail and if failure can be avoided.

6.1.2 Gharial Headstarting in the Chitwan National Park

To investigate gharial headstarting, the Chitwan National Park in Nepal provides the most useful candidate study site. The Narayani River and its tributaries in the Terai lowlands of Nepal, principally the Rapti River, contain the largest remaining gharial population in Nepal,

and would have once formed the most northerly part of the range of the gharial on the Gandak tributary of the Ganges River. The remaining population is primarily within the Chitwan National Park and its buffer zone. In the 1960s the gharial population collapsed, leading to a wild population of just 50-60 adults and no immature gharial by the 1980s, so Gharial Conservation Breeding Centre was established at Kasara in Chitwan in 1978 (Maskey, 1989). From 1980-2017 the majority of gharial nests that could be found were brought to the centre, and gharial were reared in captivity. Since 2017 proportionally fewer nests have been collected, but still at least nine per annum. The captive-reared gharial are released annually from the GCBC at an age of 5-7 years, usually in February and March.

There are good baseline data on the population in the 1980s from the pioneering work of Tirtha Man Maskey (Maskey 1989), and sufficient unadjusted count data from 2003-2021 to show that the population continued to decline 1986-2003 despite the headstarting programme, and then began to increase after 2010 (Chapter 5), with sufficient survival to adulthood of these headstarted gharial that the nest count has also been increasing since 2010. As such, the Chitwan headstarting programme has been both a failure and – more recently – a partial success, hence why it is a useful study site to investigate causes of gharial headstarting success and failure. This change correlates with two major alterations in the programme: a change in release site, and an increase in annual releases. Prior to 2010 an average of 19 gharial were released per annum, increasing to 71 from 2010-2021. Prior to 2004, all (except 17 individuals) were released in the large Narayani river, whereas after this date the majority of gharial have been released on its smaller tributary, the Rapti.

However, despite the improved population counts in Chitwan, the overall retention and survival of released gharial is still low. By 2021 1369 gharial had been released in Chitwan, but the estimated population was just ~360 (Chapter 4). If we attribute all increases in wild gharial numbers in Nepal to the head-starting programme, over 1000 released gharial have still ‘disappeared’. Even if we optimistically interpreted this as a ~25% survival rate, this is still well below the survival rates seen in successful crocodilian release programmes, such as ~85% for alligator headstarts in Louisiana, USA (Elsey et al. 2001), an established population of over 280 Siamese Crocodiles from a reintroduction of just 60 individuals in Cát Tiên National Park, Vietnam (Chambert, Enot and Khanh, 2019) or the successful introduction of

a reproducing Philippines Crocodile population from 65 founders in Siargao Island (Binaday and Manalo, 2020; Binaday *et al.*, 2020)

6.1.3 Post-Release Monitoring of Headstarted Gharial in Chitwan

The population counts and estimates of gharial in Chitwan show there has predominately been failure in the reinforcement, but a lack of post-release monitoring to date makes it impossible to know what the causes of that failure are. In translocation programmes, continuing mortality due to unresolved threats may be an issue (Germano and Bishop, 2009), and these causes should be identified so they can be tackled. High post-release mortality could be due to predation, entanglement in fishing gear, intentional killing, disease, or failure of releases to adapt to the wild leading to starvation. Alternatively, the 'missing' gharial may have dispersed rather than died. The National Park authorities and past studies (Maskey 1989; Ballouard *et al.* 2010) have favoured the hypothesis that the majority of gharial are washed downstream over the barrage on the Indo-Nepal border which prevents their movements back upstream. This hypothesis is based on observations of a few individuals from the GCBC downstream on the Gandak and the fact of the increasing gharial population on the Gandak despite limited conservation action (Lang, 2016). To gain a better understanding on what might have happened to the 'missing' 1000+ gharial, survival rates must be determined in future releases. This can be achieved by monitoring a sample of marked individuals, with measures taken to avoid death and dispersal being confounded (IUCN/SSC, 2013). However, monitoring is often challenging, with a third of programmes in the IUCN global conservation translocation perspectives series reporting difficulties with post-release monitoring (Berger-Tal, Blumstein and Swaisgood, 2020).

Two studies have carried out post-release monitoring in Chitwan through the re-sighting of gharial with cut scutes by observers (the removal of vertical scutes on the tail is standard practice in crocodilian monitoring (Grigg & Kirshner 2015)). This led to conclusions that as few as 7% of gharial released prior to 1993 had survived (Maskey and Percival, 1994) and only 20% of released gharial survived and remained in Nepal over the 2 years before 2004 (Ballouard *et al.* 2010). However, observation of gharial with scute-cuts is very likely to lead

to inaccurate estimates of survival because gharial are very easily disturbed, often bask with their tail submerged, and spend a considerable portion of their time underwater.

Confidence in the correct individual identification of the released animal is therefore low.

Both of these studies recognised the limitations of the observer approach, and both attempted VHF telemetry (Maskey 1989; Ballouard & Cadi 2005). These studies yielded limited data due to technological failures and attachment difficulties. However, gharial telemetry techniques have subsequently been developed and utilised very successfully by the Gharial Ecology Project in India (Lang and Whitaker, 2010).

6.1.4 Telemetry for Post-Release Monitoring

Telemetry has been of great use for crocodilian biologists for overcoming some of the difficulties of working with these amphibious, camouflaged, cryptic reptiles (Lang and Whitaker, 2010), and is the only currently available method to confidently monitor gharial post-release for an extended period of time. VHF transmitters have been used in the majority of studies to date. Satellite tracking technology including Iridium, ARGOS, GPS-UHF and GPS-GSM have all been utilised with mixed success and generally high costs (e.g. Read et al., 2007, Brown pers. comm., Nestler pers. comm., Lang pers. comm.). The use of VHF transmitters allows tracking of released gharial for 2+ years, and for survival and movement patterns to be quantified. Tagged gharial are likely to be recovered in the event of death, and unlike GPS tags, VHF tags can still be detected when shallowly submerged, buried, or even swallowed by a predator (GEP unpublished data).

As well as the fate of recently released gharial, the other vital consideration of a translocation is reproduction (Seddon, 1999). Telemetry can reveal if gharial are reproducing by allowing specific individuals to be followed throughout the annual reproductive cycle. By recapturing and tagging individuals from the headstarting programme, it is possible to determine if headstarted individuals are surviving to reach reproductive maturity, and if so, whether they are showing normal reproductive behaviours. For gharial, normal reproductive behaviours involve mating, communal nesting and creche guardianship, although only a few females per communal nest site will act as guardians. Understanding if and when gharial from the programme reach reproductive size is vital for

both determining if the reinforcement is on the way to becoming a self-sustaining population (Seddon, 1999), and developing predictive models for population change following different interventions (Taylor *et al.*, 2017).

6.1.5 Modelling reinforced populations to understand past dynamics

Our understanding of a reinforced population can be outlined by a model of that system (Armstrong and Reynolds, 2012). In reintroduction biology, models are important for representing theoretical understanding of a system and also creating predictions on which to base management decisions (Armstrong *et al.*, 2021). Alternatively, when there is sufficient data, models can be used to determine how well our current understanding can explain past trends. Using data on survival and reproduction, we can generate retrospective population projections and compare these to past data on population dynamics (Armstrong *et al.*, 2021). Such models are a useful tool for identifying population rates that have driven past dynamics, such as survival rates, time to reproductive maturity and sex ratios, but cannot explain why these rates were seen (for example, causes of high mortality). A retrospective model which matches observed dynamics well can be used to develop predictive models (Armstrong *et al.*, 2021). Predictive models are key for adaptive management, informing future research and management decisions to project success and failure, and identifying areas of uncertainty requiring further research (McCarthy, Armstrong and Runge, 2012; Nichols and Armstrong, 2012).

In this study we determined the fate and quantified the post-release dispersal behaviours of a cohort of 20 tagged gharial released in March 2019 until July 2021. We also monitored the survival and reproductive ecology of a tagged cohort of 20 free-living gharial captured in winter 2018 or 2019, of which 18 were recaptures released from the headstarting programme 1-10 years earlier. To supplement the data from the tagged individuals, we collated additional mortality records and reports of long-distance dispersals of marked gharial from the Chitwan population Sept 2018-July 2021. We used the data from these small cohorts of tagged or marked gharial to parametrise a simple deterministic matrix

model to retrospectively investigate survival and recruitment over the last 40 years of the programme. We predicted annual gharial population, adult population of both sexes and nesting effort per year up until 2021, and compared this to population survey and nesting data to see how well our model reflected past dynamics.

6.2 Methods

6.2.1 Post-Release Monitoring

To better understand survival and dispersal of gharial for the first years after release from captivity, we tagged 20 individuals of a release cohort of 100 gharial in March 2019. The gharial were then released following standard procedure. This procedure involves releasing gharial in a soft-release enclosure made of elephant grass on the river bank, enclosing a section of both river and shore. Gharial are able to force their way out of the enclosure into the river, and exit becomes increasingly easy over the following weeks as the structure becomes more permeable in the river channel. VHF-transmitters (151 MHz; Advanced Telemetry Systems, USA) were attached to the dorsal surface of the tail, as outlined in Chapter 4. Morphometric measurements were collected (mass, snout-vent length, vent-tail length, total length, snout width, head length, head width, mid-body circumference and sex).



Figure 6.1: a gharial at the Chitwan Gharial Conservation Breeding Centre prior to soft-release into the wild, illustrating the attached VHF transmitter and cattle tag. The attachment of the transmitter allows for post-release monitoring, and the brightly coloured tag assists in sighting released animals.

After release, the VHF-tagged gharial were manually tracked using an ATS R410 scanning receiver and five element Yagi directional antenna. Tracking was carried out regularly, with the aim of locating each animal at least once per week, and those in more remote sites at least once per month. Tracking protocol followed the same protocol outlined in Chapter 4, and was carried out concurrently. The Wildlife Trusts of India, during their March 2020 gharial survey on the Gandak River, tracked for the VHF numbers of all gharial missing from Nepal (WTI, 2020).

6.2.2 Post-Release Dispersal and Settlement

We characterised and quantified post-release dispersal and time to settlement of the 20 VHF-tagged gharial released in March 2019 by comparing net squared displacement (NSD) with time since release (Ranc, Cagnacci and Moorcroft, 2022; Sievert *et al.*, 2022). NSD is a useful quantity for understanding animal movement, and was calculated as:

$$NSD_t = (x_t - r_0)^2 + (y_t - r_0)^2$$

where r_0 is the location of the release site, and (x_t, y_t) is the location of the animal at time t (Börger *et al.*, 2020). When a headstarted gharial is dispersing from the release site, NSD over time will continually change, whereas when (and if) it settles the change in NSD will approach an asymptote (Börger *et al.*, 2020). To convert raw detection data into dispersal distances, we firstly converted each raw detection (which identified the gharial was in a particular polygon representing a small stretch of river) into a point location by randomly sampling within that polygon (following Chapter 4). We then calculated the distance between each relocation and the release site, following least cost pathways constrained to the river channel using the R package *gdistance* (van Etten, 2017) following Baker *et al.* (2022). All analyses were conducted in the R statistical environment (R Core Team 2021).

From the pattern of the relationship between NSD and time since release, we categorised gharial into three groups: settlers (those that had an NSD that had plateaued, and remained at a fixed displacement (± 15 km) from the release site); non-settlers (those with a NSD that continued to increase or vary throughout the study period); and early-death/loss (those that died or were lost <250 days post release, when most gharial had not settled). We also

determined the maximum distance that each gharial had been detected from the release site and compared this with different probability distributions using the package *fitdistrplus* (Delignette-Muller and Dutang, 2015).

To determine if the gharial who followed the settlers or non-settlers movement patterns differed in size at release, we conducted three two-sample t-tests, comparing the length, mid-body girth and mass at release of gharial in the two movement categories. To test for an association between movement category and age we conducted a Fisher's exact test for gharial in each of the two movement categories.

6.2.3 Monitoring of Captured Wild Gharial

In addition to the 20 gharial tagged at the point of release in March 2019, we also captured and tagged wild gharial on the Rapti River and tributaries in winter 2018 and 2019 (following the methodology outlined in Chapter 4). Of the 20 wild gharial captured, 18 had permanent markings that showed they had originated in the GCBC headstarting programme. The post-release changes in mass and length of these gharial were investigated in Khadka et al. (2022, Appendix 3). Following re-release, we monitored the movement and behaviour of these gharial. We also monitored the reproductive behaviours of all gharial to determine which individuals were mating, nesting and performing guardianship behaviours. We closely monitored all creche sites in the Rapti River in 2019, 2020, 2021 and 2022 to determine if any (tagged or untagged) headstarted gharial were involved in creche guardianship. We also monitored the creche site on the Narayani in 2019. This was not possible in 2020 and 2021 due to Covid19 regulations, and in 2022 there was flooding following successful nest hatching which meant we were unable to observe creche guardianship behaviour.

Monitoring involved the installation of field cameras set to record one minute of footage every ten minutes, and modified action cameras taking continuous footage for up to 12 hours, as well as direct observations. This set up was used to monitor both nesting, hatching and creche guardianship. Direct observations only were used to monitor breeding behaviours.

6.2.4 Post-Release Fate and Mortality

To understand causes of mortality, all tagged gharial which were found dead underwent post-mortem with veterinarians of the National Trust for Nature Conservation (NTNC), when there was sufficient non-skeletal material remaining for this to be possible. Informal discussions were held with local communities from the areas in which dead gharial were found, to gather additional insights on cause of death. Mortality data included all dead tagged gharial (both the cohort released in 2019, and the wild gharial captured in 2018 and 2019).

For the 2019-release tagged cohort, at 24 months post-release we classed each gharial as either alive, alive (rescued), confirmed dead, missing (presumed dead), missing (presumed dispersed) and missing (unknown). Gharial were classed as alive (rescued) when they would have died due to entanglement or hooking in fishing gear, but were rescued by intervention from the National Park staff. Gharial were presumed dead when we suddenly stopped detecting them following regular detection, and this period did not correlate with a time of flooding when a live or dead gharial could be moved swiftly downstream. Gharial were presumed dispersed (crossed the border into India) when we stopped detecting them after their net squared displacement had been continually increasing and they had already moved further from the release site than the remaining distance to the border. Gharial were classed as missing (unknown) when they disappeared without any strong evidence of how they might have died, or without the above correlations suggesting they may have dispersed.

To determine if there was a difference in size at release of gharial that survived or died, we conducted two sample t-tests for three measures of size: total length, mid-body girth and mass. A t-test was run for each morphometric measurement, with data grouped by whether the gharial were alive or dead at the end of two years. Tests were run twice, firstly with gharial of unknown fate excluded, secondly with these included as dead gharial. The gharial thought to have dispersed out of the study area were excluded as the sample size was too small for any statistical test ($n=2$). To determine if there was an association between gharial survival and age, we conducted a Fisher's exact test for year of hatching (2011 or 2014) and whether the gharial survived to the end of two years. Tests were run twice, treating unknown fate gharial as we did for the t-tests.

We supplemented data on the mortality of tagged gharial with that of all gharial found dead in the National Park and suspected to have been killed by anthropogenic activities between September 2018 and February 2021, using records provided by Assistant Conservation Officer Bed Bahadur Khadka (unpublished data) and Chitwan National Park Reports (DNPWC, 2020). All gharial from the headstarting programme are permanently marked by the removal of tail scutes at the point of release, so it is possible to know the age and release year of each dead gharial. Additional observations by Park Staff or our field team of gharial being predated were also collated.

6.2.5 Resighting of Yearlings

To determine an estimate for mortality at the hatchling-yearling transition, we collated data from three sources on estimates of hatchlings in creches, and of resighting of these hatchlings the following winter. We collected data on creches and yearling surveys from Khadka (2020) and our own field data for 2019. To revise the estimates provided in this study, we used yearling counts from repeat surveys (Chapter 5) and our own observations of yearlings during tracking and adult surveys. Since our own yearling sightings were generally 10-100km downstream of nesting sites, we also included yearling counts from the Wildlife Trust of India Gandak Surveys (Sinha, Behera and Choudhury, 2020). We calculated a minimum percentage survival of hatchlings observed in the creche divided by hatchlings sighted the following year. We assumed that no more than 50% of the hatchlings observed in the Gandak could be from Nepal.

6.2.6 Retrospective Model: model structure

To improve our understanding of the headstarting programme, and see if our estimates of mortality, dispersal and maturation in small cohorts of gharial fit with observed population dynamics, we used a deterministic matrix model, with uncertainty incorporated in estimates of survival and dispersal. We compared these predictions with historical minimum population counts, our population estimate in Chapter 5, and annual nest counts.

The matrix was age structured by annual age classes (gharial have a single annual reproductive cycle), with three related matrices: an overall population matrix, an adult female matrix and an adult male matrix. Annual survival was estimated according to age and year post-release from the headstarting programme. Gharial that were released from the headstarting programme were assumed to be 5 years old at release, as gharial are predominantly released in their fifth year. Dispersal was incorporated for gharial in the first year post-release, and differed according to release location (Rapti or Narayani river). Gharial were assigned to either the adult male or female matrix at the point of maturity, according to the observed sex ratio. Estimated number of yearlings was determined by the number of adult females, reproductive frequency, average number of eggs per female and hatchling to yearling survival. These yearlings were then entered into the overall population matrix at one year old.

To incorporate stochasticity in our survival and dispersal estimates, we calculated the variance of our parameter estimates as $s(1-s)/c$, where s is the estimated probability of survival or dispersal, and c is the sample size upon which that estimate is based (Runge, 2013). In each run of the model, the survival and dispersal probabilities of each year in the matrix were sampled randomly from a logit-normal distribution defined by our parameter estimates, and the standard error of that estimate (White, 2000). We ran each model 100 times, and took the 5th and 95th percentile of each run to provide 95% confidence intervals of our estimates.

In addition to our estimates for the Chitwan population, we estimate the cumulative population in the Gandak that is from Nepal, according to our dispersal estimates, assuming the gharial have the same survival probabilities as the gharial in Nepal. We recognise this aspect of the model is a particularly strong oversimplification, as it does not account for continued downstream dispersal, and mortality may also be considerably higher on the unprotected Gandak. However, this provides a baseline for future investigation of cross-border dispersal and mortality rates in the Gandak.

To compare our model results to real world data, we compared the predicted populations from our model with (a) raw count data from surveys, as collated in Chapter 5; (b) our population estimates in Chapter 5; (c) nest count data of nests recorded in the wild (Khadka

et al. 2020), and our own data, and number of those nests collected in for the ex-situ programme, from the same sources. We also compare our Gandak estimates to population counts on the Gandak (Sinha, Behera and Choudhury, 2020; WTI, 2020).

All analyses were completed in Microsoft Excel to facilitate simplicity of discussing and sharing the results with managers, as well as transparency and ease of use for the model to be taken up in the management programme with minimal training and no requirement for any statistical education (Armstrong and Reynolds, 2012).

6.2.7 Retrospective Model: parameter estimation

In order to parameterise the retrospective model, we collected or estimated a number of key parameters which are summarised Table 6.1

The total number of gharial released per-annum was collected from National Park records (DNPWC, 2022). We used the initial adult population counts of Maskey (1989) from 1983-1986. Since we do not know the age of the adult population at this time (we only know the wild population had no sighted yearlings, juveniles or subadults), for the sake of simplicity we assumed all adult gharial were 15 years old in 1981.

We parametrised the model with the survival and dispersal probabilities for years 1-3 from our radio-tagged gharial (both releases and recaptures). Since we did not release gharial on the Rapti, we used the dispersal probabilities of gharial dispersing to India of those that had reached the Narayani River by their first monsoon. We assumed no dispersal after the first year, and that gharial survival was then the same for gharial released in either river after the first year. We followed a linear increase in probability of survival from year 4-6, to reach the annual survival probability equivalent to adults by year 7, when we assumed survival would be high based on the confirmed survival of all tagged gharial released 7+ years ago. We used a survival probability of 0.95 for adults, as has been used in population viability analyses on other crocodylians (Rodríguez *et al.*, 2017). For adult gharial prior to 2000, we used a lower survival probability, since declining adult counts between the 1980s and 2003 indicate adult mortality must have been higher during this period.

Gharial lifespan in the wild is unknown; it is suggested that gharial have a lifespan of 40-80 years, although some experts believe they can live to over 100 (Rao *et al.*, 1995). Rodríguez *et al.* (2017) use a crocodilian maximum lifespan of 70 for population models. To be conservative, we kept gharial survival probability at the adult probability until the gharial reached 40, then followed an exponential decay until 69, and fixed survival probability beyond age 70 at 0.

To determine when gharial from the release programme were recruited into the adult population, we used the predicted pre-maturation growth rates of gharial in Khadka *et al.* (2022), which found a divergence between 'fast growing' and 'slow growing' gharial post-release, which did not correspond with any measured trait, such as sex or size at release. Therefore, we assumed half our gharial would follow a slow growth trajectory, and the other half a fast growth trajectory. Time to maturity was estimated using growth rate data: if gharial are released at an average 175cm (Khadka *et al.* 2022), and females mature at ~300cm, and males at ~400cm (based on our own observational data of known-length wild gharial), we predicted it would take the females 6-10 years post-release to mature, and males 11-19 years.

We used an estimated sex ratio of 0.8 females, as the GCBC has historically had a sex ratio biased towards females, likely as the temperature of the nests at the GCBC has not been monitored regularly (Griffith *et al.*, in review) and gharial have temperature-dependent sex determination (Lang and Andrews, 1994). The exact ratio is unknown, as gharial juveniles are challenging to sex, and older recaptured gharial that can be sexed do not always correspond with the sex they were assigned at release. We used 0.8 as this was the sex ratio of recaptured gharial in Chitwan (Khadka *et al.* 2022).

We estimate the number of nests produced by adult females by using a reproductive frequency value of 0.8 nests per female per year, which is the frequency found in the radio-tagged adult females. Since we sporadically see gharial which we can confirm do not have cut scutes, and know that since 2018 some nests have been left in-situ leading to regular sightings of a small number of yearlings, there has been at least some – limited – recruitment in the wild. Therefore, we also estimated wild recruitment by subtracting the known number of nests collected by the GCBC from the estimated nests laid in our model,

and determining recruitment using an average of 39 eggs per gharial nest (Griffith et al. 2022), hatchling recruitment as 0.05 (Rao *et al.*, 1995), with subsequent gharial survival beyond the first year being given the same as for a released gharial (for both Nepal and India). Finally, we assigned a value of 0.8 as the probability the hatchling gharial will disperse to India during their first year, reversing to 0.2 once in a five-year cycle: this was based on the sporadic observations of gharial yearlings from 2018-2021 at least 35+km downstream of the nearest nesting site, although in 2022 when there were no monsoon floods on the Rapti, gharial yearlings have regularly been seen within 10km of the nesting site.

Vital Rate	Value	SSize	Estimate?	Source
Headstarted Gharial Released per Year	Variable		No	Chitwan National Park Records
Annual survival and retention in Nepal of gharial post-release	Year 1: Rapti: 0.6 ± 0.02 Narayani: 0.3 ± 0.07 Year 2: 0.63 ± 0.03 Year 3: 0.68 ± 0.1 Linear increase to Year 7	20 8 19 3	Yes	Telemetry Mortality and Dispersal Data
Annual Survival of adult gharial	0.95 ± 0.01	19	Yes	Rao et al. (1995); Rodríguez et al. (2017); Telemetry and known individual mortality data
Gharial longevity	40-70 years (exponential decay until probability of survival beyond 70 is 0)		Yes	Rao et al. (1995); Rodríguez et al. (2017)
First year dispersal of Gharial releases in Rapti	0.15 ± 0.03	20	Yes	Telemetry Dispersal Estimates
First year dispersal of releases in Narayani	0.45 ± 0.07	8	Yes	Telemetry Dispersal Estimates
Sex Ratio	0.8 females	26	Yes	Recaptured Gharial Sex Data
Gharial female time to maturity post-release	50% 6 years 50% 10 years		Yes	Khadka et al. (2022);

	Assuming maturity at 300cm			Breeding Observations in Chitwan (this study)
Gharial male time to maturity post-release	50% 11 years 50% 19 years Assuming maturity at 400cm		Yes	Khadka et al. (2022); Breeding Observations in Chitwan (this study)
Gharial Reproductive Frequency	0.8	8	Yes	Breeding Observations in Chitwan (this study)
Adult female reproductive senescence	Loss of 10% of the breeding population per year after 40		Yes	Chitwan Annual Reports (2015-2021)
Adult Survival Pre-2000	0.8		Yes	Maskey (1989); Ballouard et al. (2010)
Average Number of Eggs per Nest	39		Yes	Griffith et al. (2022)
Recruitment to 1-year age class	0.1		Yes	Rao et al. (1995)
Retention of Hatchlings in Nepal	0.2	65	Yes	Sightings of hatchlings usually 35-80km below nest sites

Table 6.1: vital rates and numbers used to parameterise our deterministic model of gharial population in Chitwan National Park and the Gandak River 1981-2021. SSize (sample size) is included for parameters which were estimated from our own data

6.3 Results

6.3.1 Post-Release Monitoring

Following release of 20 gharial on March 8-10 2019, most surviving gharial were relocated up to ~700 days post release, after which batteries began to fail, with one gharial tracked to 857 days post-release (July 2021). Due to Covid-19 lockdowns, tracking of gharial on the Narayani in 2020 was more sporadic, and after April 2021 it was impossible, so datasets for the Narayani gharial end slightly earlier. The GPS radios of the five gharial released in February 2020 were not tracked due to radio failure immediately post-release, although one of these gharial was found dead (Table 6.2).

6.3.2 Post-Release Fate and Mortality

Of the 20 gharial released in 2019, just 10 were confirmed to still be alive at the end of the monitoring period (Table 6.2). Five gharial were confirmed to be dead, all of which had strong evidence they had been killed due to human activities: four had evidence they were killed by people, and one appeared to have drowned following net entanglement. An additional gharial was thought to have been killed by a terrestrial predator: following release, this gharial showed aberrant behaviour, moving into the small stream near the breeding centre from which the centre pumps water for the tanks. Having been seen there regularly for over a month, one day she was missing. This is an area with many terrestrial predators, including tigers which have previously broken into the enclosures to take juvenile gharial. Two gharial are suspected to have dispersed to India, as we stopped detecting them after they had been continually moving downstream, although since these gharial disappeared during flooding time in the monsoon, it is also unlikely we would have recovered the bodies if they had died instead. A further two gharial disappeared, following regular detection: one in July 2019 during a period of high rainfall and flooding (and therefore possibly dispersed/washed downstream, or we could not find a body in the floods). The second gharial disappeared 521 days post-release, following regular redetection, having been relocated in the same stretch of river since release. Although this was during the monsoon (August 2020) it was not a time when the river was flooding or high. However, it was near the mouth of a small tributary, which the gharial may have travelled up, making finding the dead body challenging or impossible. This gharial was last detected in an area with considerable illegal fishing, where four gharial were found dead from fishing during the course of this study. Our tests for associations between the fate of gharial, and size (length $t(14)=0.01, p>0.1$, weight $t(14)=0.79, p>0.1$, and mid-body girth $t(14)=0.45, p>0.1$) and age ($X^2(1,18)=0.18, p>0.1$) found no statistically significant differences between alive or dead gharial, either with or without the missing gharial classified as dead.

As well as the tagged gharial deaths, an additional 18 gharial were recorded as killed due to human causes from September 2018-February 2021 (Khadka unpublished data, DNPWC 2020). Of these, the majority appeared to have been killed due to traumatic injuries (Table 6.3) after probable or confirmed net entanglement, with some having drowned after

Gharial	Fate	Settlement	Hatch Year	Length (cm)	Sex
4	Alive	non-settler	2011	185	F
8	Alive	non-settler: crossed barrage into India, then returned	2014	184	F
14	Alive	settler at 3 ± 5km	2014	175	F
38	Alive	non-settler	2011	185	M
40	Alive	non-settler	2011	191.5	F
44	Alive	settler at 22 ± 8km	2011	177	F
46	Alive	settler at 72 ± 3km	2011	174	F
68	Alive	non-settler	2011	183	F
72	Alive	non-settler	2014	173	F
76	Alive	settler at 58 ± 3km	2014	182	M
50	Dead: bones buried in forest	settler at 16 ± 4km	2011	173	F
42	Dead: killed by blow to head after gill-net entanglement	early death	2014	197	F
52	Dead: killed by blow to head after gill-net entanglement	early death	2014	181	F
66	Dead: probable drowning	settler at 38 ± 2km	2014	175	F
64	Dead: tail cut off and buried.	early death	2014	182	F
60	Missing (pres. dead): suspected predation	early death	2011	178	F
56	Missing (pres. dispersed)	early loss	2014	170.5	F
62	Missing (pres. dispersed)	early loss	2011	186	F
54	Missing (unknown)	settler at 11 ± 4km	2011	173	F
58	Missing (unknown)	early loss	2011	176.5	M

Table 6.2: the fate, movement pattern, and details of size, age and sex of a cohort of 20 VHF-tagged released from the Gharial Conservation Breeding Centre in Kasara, March 2019, and monitored for up to 2.5 years post-release.

Date of Discovery	Location	Age Class	Reported Detail on Cause of Death
24-Sep-18	Kasara, Rapti	subadult	head cut off
16-Oct-18	Khageri, Bagmara community forest	subadult	only tail recovered, cut off
16-Nov-18	Megghauli, Rapti	subadult	
17-Jan-19	Bayer Ghari, Rapti	subadult	drowned in set trap
27-Feb-19	Jayamangala, Rapti	subadult	
28-Feb-19	Rapti/Reu confluence	subadult	
18-Mar-19	Budhanagar, Rapti	subadult	snout broken, hidden under grasses
23-Apr-19	Ghatagain, Rapti	subadult	
10-Jun-19	Amaltari, Narayani	adult female	Breeding female
1-Jul-19	Tribeni Canal	subadult	floating in canal that is part of the Triveni barrage agricultural canals
13-Feb-20	Barahi lodge, Megghauli, Rapti	subadult	broken back
13-Feb-20	Khoriya, Narayani	subadult	injured on head
24-Feb-20	Ratanpur, Narayani	subadult	multiple traumatic injuries
25-Feb-20	Kumrose, Rapti	subadult	multiple traumatic injuries
26-Mar-20	Dhungrekhol, Rapti	subadult	multiple traumatic injuries
29-Mar-20	Baguban, Narayani	subadult	multiple traumatic injuries, net entanglement. (failed) GPS tag.
1-Apr-20	Gaindakhasa, Narayani	subadult	injured on head and stomach
13-Apr-20	Bayerghari Rapti	subadult	killed by harpoon
14-Apr-20	Hatidundi, Rapti	subadult	killed by harpoon

Table 6.3: reports of dead gharial suspected to have been killed by human activities, from records of Assistant Conservation Officer Bed Bahadur Khadka, and reports from our team and Chitwan National Park Annual Reports.

entanglement, and others having been cut into pieces, of which only some parts were found. Of the 23 gharial killed by human activities in the study period, 25% were found during the intensive riverbank tracking or surveys of the 2018-2022 project, or only because the dead gharial (buried or hidden) was wearing a tag. This suggests that dead gharial reported to the National Park, or recovered as part of routine National Park monitoring, is only a fraction of the number dying in Chitwan.

Multiple predation events were witnessed by the team or National Park staff, including two gharial headstarts killed by mugger crocodiles, and one adult female gharial killed by a tiger. The actual impact of unobserved predation events is likely to be higher.

6.3.3 Post-Release Dispersal and Settlement

The maximum recorded distance reached after release of the 20 gharial released in 2019 was 10-112km, with a mean distance of 51km. Maximum distance fit a normal distribution with a skew value of 0.56, indicating a positive skew (a long tail indicating the possibility of a small number of gharial dispersing a long distance) (Figure 6.2). Two very long-distance dispersals of headstart releases were also seen in the study period, although these individuals were not radio-tagged. On 17th May 2020 a gharial was captured by fishermen in the Hooghly River of West Bengal, India. This gharial had been released from the GCBC in March 2020, and had therefore travelled over 1100km in just 61 days (Khadka 2020a). A second gharial from the GCBC was captured in September 2020 in the Nagir River of West Bengal, India, also over 1000km from the release site (WTI, 2020). This gharial died a few days after capture.

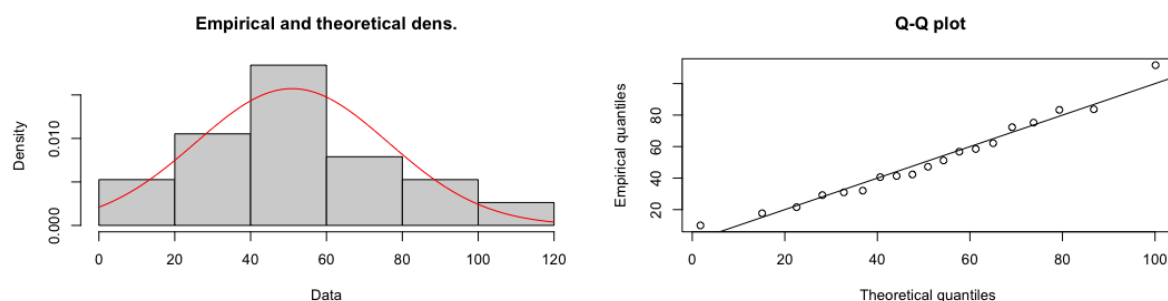


Figure 6.2: the fit of gharial maximum detected dispersal distance to a normal distribution.

Maximum dispersal distance fit a normal distribution with a skew value of 0.56 well, as indicated by the close match between the empirical and theoretical densities, and good fit in the Q-Q plot

Of the gharial released in 2019, seven were categorised as Settlers (Figure 6.3), six as Non-Settlers (Figure 6.3), and six as Early-Death/Loss. The Settlers settled at distances of 3-58km from the release site. Most gharial had a very variable NSD until after their first monsoon, and then settled into differing patterns of movement/settlement (Figure 6.3). We found that Non-Settlers were larger than Settlers, with this measure being statistically significant for all measures of size, length ($t(11)=2.89$, $p=0.01$) mid-body girth ($t(11)=2.42$, $p=0.03$) and mass ($t(11)=2.42$, $p=0.03$). The larger gharial tended to also be the older gharial; however, the Fisher's exact test found the association was not significant ($N=14$, $p=0.14$) but this was unsurprising given our small sample size.

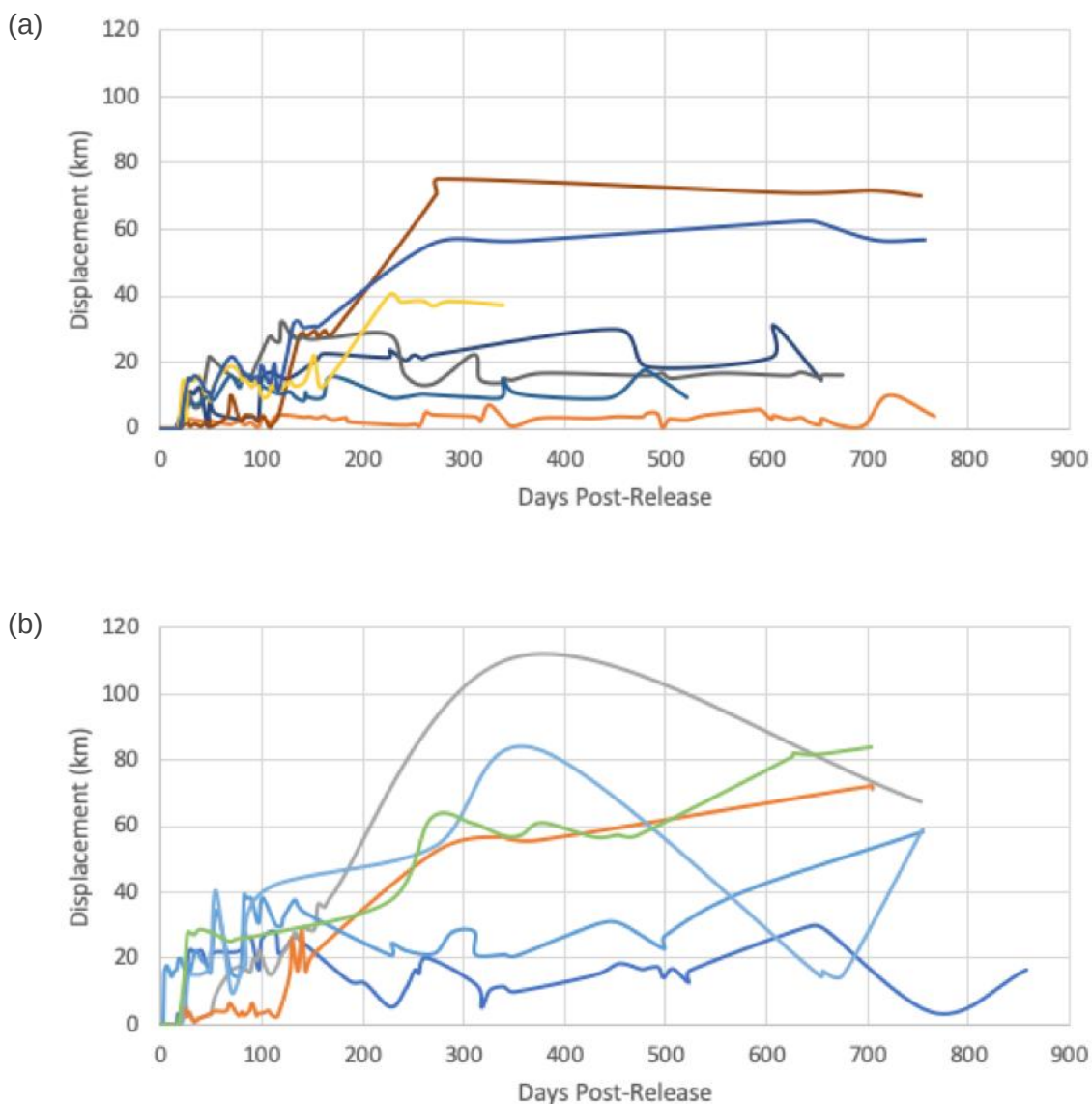


Figure 6.3: The displacement of gharial from their release site against days since release for: (a) gharial that eventually settled with a plateauing net-squared displacement value, NSD; (b) gharial

that did not settle throughout the study period, with a continually changing NSD. Although we used NSD to determine movement categories, we show displacement from release site here instead, so that the actual distance each gharial has moved is clear. The different colours indicate different individual gharial.

6.3.4 Recapture and Monitoring of Headstarts

Of the 20 gharial captured and tagged, 18 had scute cuts indicating they originated from the GCBC headstarting programme. The 18 recaptures had been released 0.75-10 years ago, averaging 4 years post-release (Table 6.4). Just two recaptures – one juvenile and one subadult – were male, although the sex of two further individuals could not be determined by physical examination. Six were adult females, aged 10-17.5 years. Of these adult females, one did not breed at all over three years of monitoring: notably, this female was the oldest individual at release (10.67 years), compared to an average of 6.5 years for other recaptures (Table 6.4). Most other females bred twice in the two years they were monitored: one female did not breed the first year, we suspect because she was still a subadult (this was the shortest and lightest tagged female in 2019). One female migrated 70+km to breed in the first year, and did not breed again in the second year. None of the tagged females which originated in the headstarting programme were seen performing creche guardianship behaviours. However, the primary guardian female at one nesting site in 2022 had scute cuts indicated she was from the headstarting programme.

Release Date	Capture Date	Years Post-Release	Age at Release	Length at Release (cm)	Length at Recapture (cm)	Sex	Reproductive Frequency
9-Mar-18	27-Nov-18	0.75	5.75	170	174.5	F	imm
10-Mar-18	26-Nov-18	0.75	5.75	173	179	F	imm
10-Mar-18	17-Nov-19	1.75	5.75	176	179.5	M	imm
7-Feb-17	27-Nov-19	2.83	5.67	176	181	F	imm

10-Mar-18	11-Nov-19	1.67	5.75	170	185	F	imm
9-Mar-18	9-Dec-19	1.75	5.75	181	188.5	F	imm
7-Feb-17	28-Feb-19	0.92	6.67	205	203	F	imm
14-Feb-18	27-Nov-18	0.83	7.66	200	204	F	imm
10-Feb-16	9-Dec-19	3.83	5.67	208	209	F	imm
5-Apr-16	11-Nov-19	3.58	5.83	184	216	F	imm
9-Feb-16	28-Nov-18	2.83	5.67	181	218.5	I	imm
9-Feb-16	27-Nov-19	2.83	5.67	181	218.5	I	imm
2-Feb-10	18-Nov-19	3.67	5.67	162	247	F	imm
2-Feb-12	20-Nov-19	7.83	7.67	161	247	F	imm
5-Mar-14	16-Nov-19	5.75	8.75	182	260	F	imm
20-Apr-13	19-Nov-19	6.58	5.92	184	274	M	imm
24-Mar-14	18-Nov-19	5.67	6.83	179	297	F	1/1*
19-Apr-12	19-Nov-19	7.58	6.92	151	304.5	F	1/2
2-Feb-10	26-Nov-19	9.83	5.67	150	305	F	2/2
2-Feb-12	27-Nov-18	6.83	10.67	177	313	F	0/3
2-Feb-10	25-Nov-19	9.83	5.67	171	335	F	2/2

Table 6.4: recaptured gharial that originated in the GCBC headstarting programme, and were released in the Rapti river, and recaptured in the Rapti or its tributaries. Sex is male (M), female (F) or indeterminate (I). imm designates immature gharial thought to be too small to breed, fractions indicate the number of monitored breeding seasons a mature female was observed breeding and nesting, with the female labelled * thought to be immature in her first year of observation.

6.3.5 Resighting of Yearlings

We found minimum estimates of hatchling to yearling survival were considerably higher than those in Khadka et al. (2020), especially after considering counts from the Gandak (Table 6.5). For the Rapti, counts by experienced observers that provide a baseline minimum number of yearlings were generally 2% higher than previous estimations, and estimates from river-wide population surveys were 17-21% higher than previous estimations. For the Narayani, our estimates of apparent survival within Nepal from population surveys were similar to those of Khadka et al (2020), however if Gandak yearling sightings are included, yearling estimates jump to remarkably high percentages. Detectability of yearlings was highly variable within the same season (e.g. 3-12 on consecutive days), so unadjusted counts risk being biased (Royle and Nichols, 2003).

	Creche Count	Khadka (2020b)	Population Surveys	Experienced Observations
Rapti River				
2017	34	0%	21%	NA
2018	59	2.9%	20%	5%
2019	170	0%	NA	2%
Narayani River				
2017	182	1.1%	0% (N) 14% (I)	
2018	63	14.3%	10% (N) 56% (I)	
2019	85	1.3%	87% (I)	1%

Table 6.5: estimates of number of individual gharial hatchlings in wild nests in the Rapti and Narayani rivers per year, and the estimated percentages of those creche populations resighted the following winter. Population surveys occurred in Nepal, denoted (N), and India, denoted (I) and were generally by large teams with a large variation in survey experience. Experienced observations were sightings by those with extensive experience working with gharial, but covered a small section of the river system without repeated surveys, and are therefore likely a very substantial underestimate.

6.3.6 Deterministic Model

Our model predicted a low but stable total population in Chitwan throughout the 1990s, despite the release of gharial from the headstarting programme. We predicted that the

population would have started to increase irregularly from around 2006, with a faster rate of population increase since 2015. We predicted adult female numbers would have collapsed before 1995, and then remained low and stable until after 2010. We predicted a steady increase in adult females after this date, slowing in recent years, and correspondingly low nest counts from 2000-2010, with an increase in the last decade. We predict that, given the female biased sex ratios in the GCBC, adult male numbers should still be very low in Chitwan, with additional males starting to mature from 2018.

The predictions of our simple model fit fairly well to the field data, suggesting that our estimates for the vital rates with which we parametrised it (Table 6.1) were accurate. In terms of overall population size, the model including headstarted releases (including those released that year) and wild recruitment fit best with the minimum counts per annum, as well as our population estimates in 2018 (Figure 6.4). All of the models in Figure 6.4 show that – to reach the low adult population counts of 2004-2006 – adult mortality must have been averaging at least 0.8 in the 1990s. Furthermore, the survival and retention of gharial in Nepal following release in the Narayani cannot have been higher than our predictions. Although improved survival rates in captivity led to higher annual releases since 2010, our model suggests that there has also been improved survival and retention of gharial in Nepal following the shift to releasing in the Rapti.

All our modelled population predictions give an increasing population trend for 2018-2020 that reaches a lower population estimate than those of Chapter 4 (Figure 6.4). Chapter 5 suggests that the population trend was stable from 2018-2021, rather than continuing to increase. Therefore, our model must overestimate mortality and/or dispersal during ~2010-2018, and underestimate mortality/dispersal from ~2018 onwards.

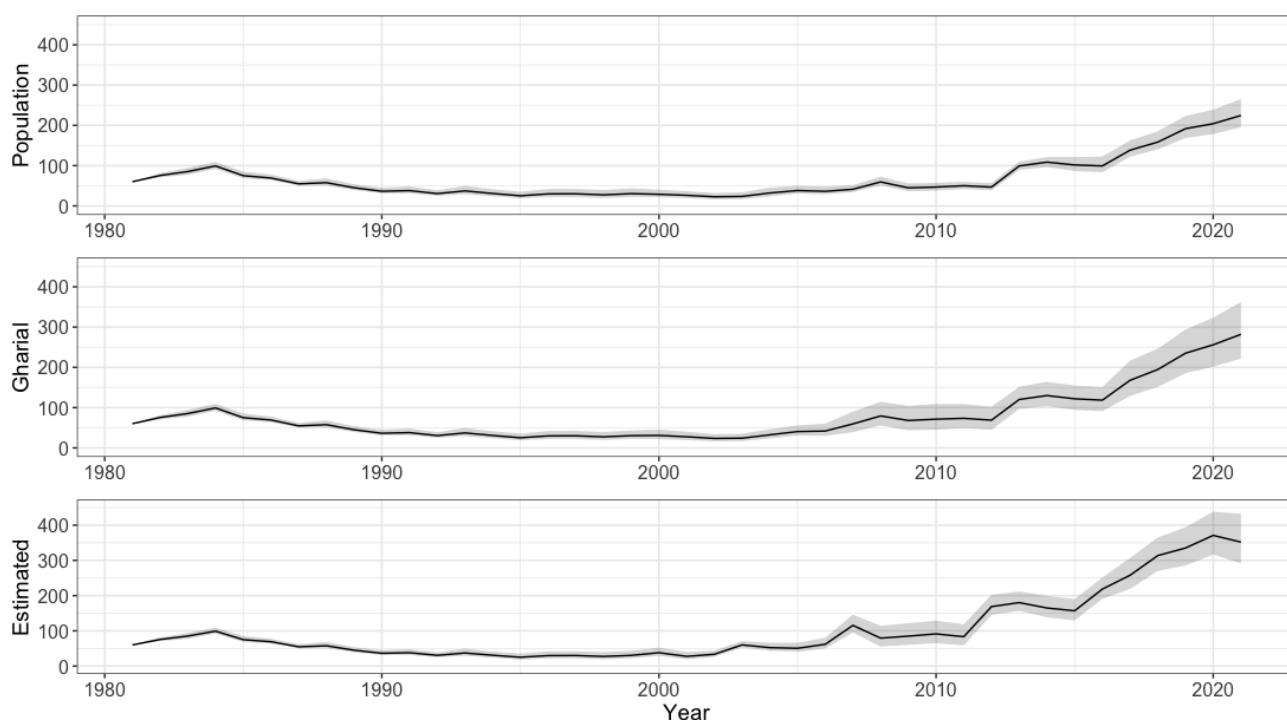


Figure 6.4: Estimates of gharial total population change in Chitwan from 1981-2021. (a) population change accounting for no recruitment in the wild, and excluding counts of headstarted gharial released that year; (b) population change including estimated wild recruitment, and excluding counts of headstarted gharial released that year; (c) population change including headstarted gharial released that year and wild recruitment. The black lines indicate median estimates, with shaded areas displaying the bootstrapped 95% confidence interval due to stochasticity in survival estimates.

A sex ratio at release of 0.8 females is sufficient to drive the very low number of adult males seen in the population in Nepal (Figure 6.5). The current number of adult males (excluding one individual translocated to Chitwan) is two, with three sub-adult males on the cusp of breeding size. Our estimates suggest a low number of females from the mid-nineties through until 2010, with an increasing trend that begins to level-off around 2019 (Figure 6.5). Based on a reproductive frequency of 0.8, this predicts a trend in nests that fits very well to the observed nest counts (Figure 6.5), although nest counts fall below the lower confidence interval of our nest estimates from 2005-2010. Based on breeding season observations, the estimate from our model of $27-30 \pm 7$ adult females in Chitwan from 2019-2021 is accurate (Bhatta, unpublished data). Our comparison of nests-collected to nests-predicted suggests there would be occasional yearlings recruited in the wild, at very low numbers (Figure 6.5).

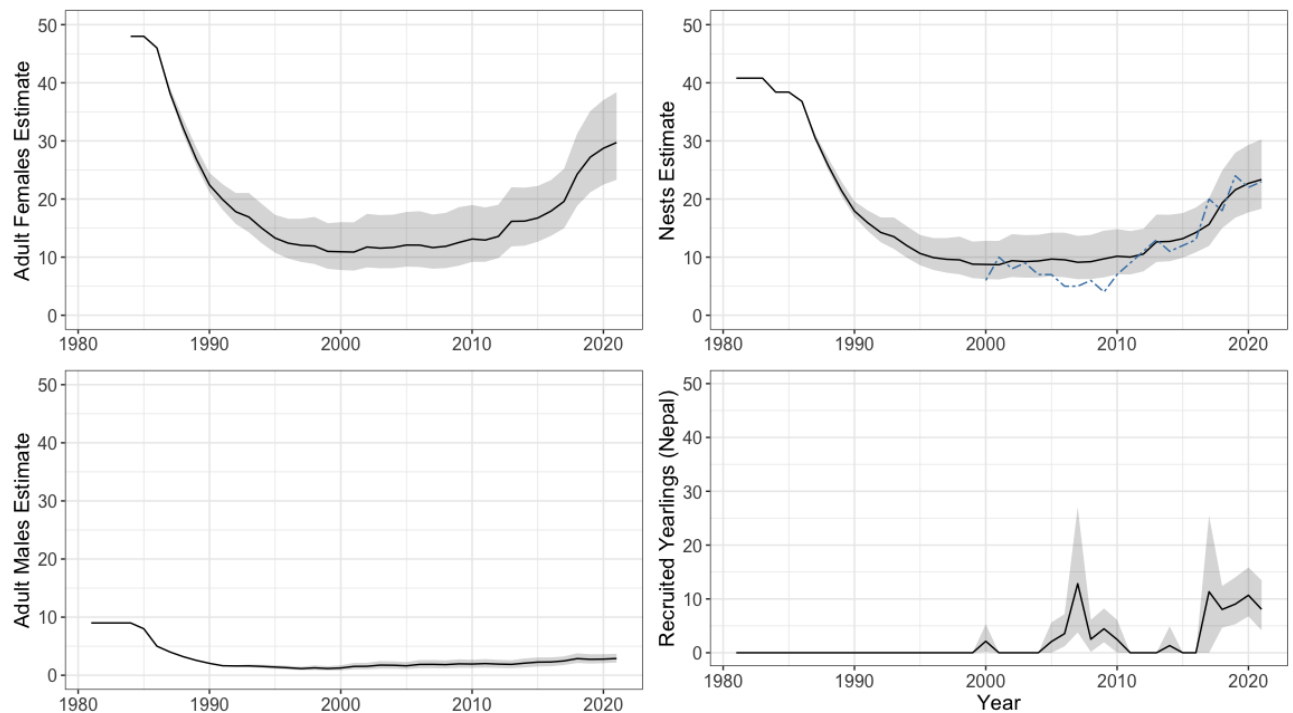


Figure 6.5: estimates for the gharial population in Chitwan National Park 1981-2020. (a) population change in adult female gharial; (b) nest counts per annum with actual counts overlaid (blue dashed line); (c) population change in adult male gharial; (d) hatchlings recruited into the yearling age class and remaining in Nepal. The black lines indicate median estimates, with shaded areas displaying the bootstrapped 95% confidence interval due to stochasticity in survival estimates.

Our dispersal estimates predict a growing population in the Gandak – one that substantially increased since 2010, and especially since 2015 (Figure 6.6), which is what has been seen on the Gandak (Table 6.6). However, surveys of the Gandak population in 2010 found just 15 gharial (Table 6.6), and our model of dispersal from Nepal predicted a population in that year of 40-133 non-yearling gharial (Figure 6.6). If the shift to releasing gharial in the Rapti – rather than the Narayani – had a positive impact on the population in Nepal because of reduced dispersal to India, as we strongly suspect, then this suggests that mortality of gharial that crossed the border into the Gandak was considerably higher than our estimates.

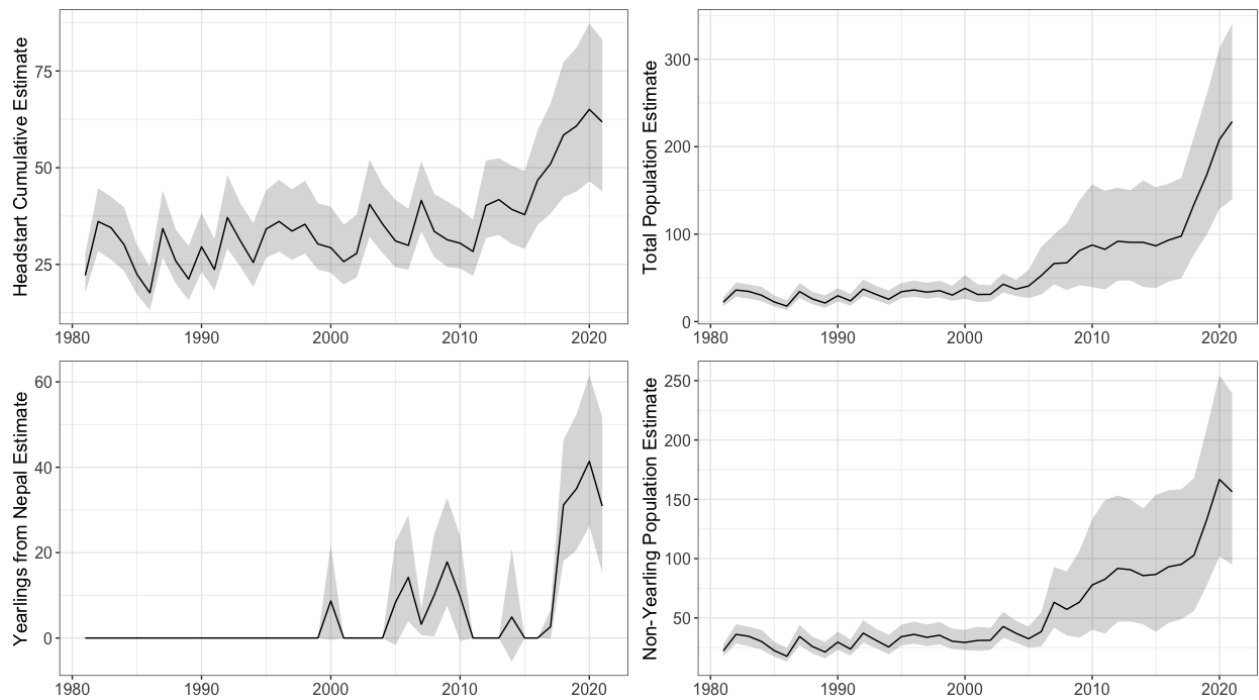


Figure 6.6: estimates for the contribution of gharial from Nepal to the Gandak gharial population 1981-2021. (a) cumulative number of gharial on the Gandak arriving from the Nepal headstarting programme, assuming the same survival rates as Chitwan; (b) total population estimate including both headstarts and wild recruitment from Nepal; (c) annual arrival of yearlings from wild recruitment in Nepal (d) total population estimate excluding gharial aged 1 or 2. The black lines indicate median estimates, with shaded areas displaying the bootstrapped 95% confidence interval due to stochasticity in survival estimates.

Our estimates of dispersal of hatchlings suggest a larger number of hatchlings/yearlings should be seen on the Gandak than in Chitwan, with high counts since 2018. Yearling counts for the Gandak have been high since 2018, with a remarkable 148 yearlings counted in 2020 (Table 6.6). We recorded very successful hatching and creche guardianship of over 300 hatchlings in Nepal in 2019: it appears that most of these ended up in the Gandak, and that our dispersal estimates of 0.8 are likely realistic, if not an underestimate, even if the 148 count in 2020 included both yearlings and two-year olds (as distinguishing size class is challenging for inexperienced observers). The high yearling counts on the Gandak suggests our recruitment to yearling estimate of 0.05 is pessimistic in combination with a high dispersal rate, and/or there is substantially more nesting in the Gandak than has been detected. Please note our predictions (Figure 6.6) are just for the contribution of gharial

from Nepal to the Gandak population: we do not account for the Gandak headstarting programme, and any initial population or wild recruitment.

Year	Non-Yearlings	Yearlings	Reference
2010	1	0	Choudhary (2010)
2014	48	2	Nair & Katdare (2014)
2015	54	0	Sinha et al. (2020)
2017	105	14	Sinha et al. (2020)
2018 (1)	143	5	Sinha et al. (2020)
2018 (2)	116	50	Sinha et al. (2020)
2019 (1)	180	24	Sinha et al. (2020)
2019 (2)	114	71	Sinha et al. (2020)
2020	111	148	Sinha et al. (2020)

Table 6.6: unadjusted count data of daytime boat-based surveys conducted on the Gandak, India, from 2010-2020. 2018 and 2019 had repeat surveys in the same season (Feb/Mar).

6.4 Discussion

We found post-release mortality of gharial is high in Chitwan, primarily due to bycatch-related mortality. Although mortality did not differ between gharial of different sizes or ages, we found the larger, older gharial were less likely to settle post-release, suggesting gharial should be released at a smaller size and younger age. There were some instances of long-distance dispersal of gharial, primarily in gharial that reach the Narayani river by their first monsoon, suggesting dispersal could be reduced by releasing gharial further upstream. We found some headstarted gharial are surviving to reach adulthood, leading to a growing number of in-situ nests despite lower reproductive frequency than in southern parts of the gharial's range. Preliminary evidence suggests survival of yearlings from nests left in-situ is much higher than previously thought. Our deterministic model, parameterized using data

from small cohorts of release gharial and the published evidence base, fit well to the observed data. Overall, this suggests apparent mortality is high post-release, and was considerably higher prior to 2004. Before 2004 it appears that the headstarting had no positive impact on the Chitwan population. However, our evidence suggests headstarting has contributed to population growth since 2010. The movement of the release site to the Rapti river and reduction of mortality in the centre leading to more releases per-annum appear to be the key factors in this improvement. Overall, these results suggest there should be a substantial reduction in the number of gharial nests collected from the wild, with a shift to focus on in-situ conservation and reproduction.

6.4.1 Post-Release Monitoring

The main identified cause of gharial mortality was direct killing by humans, generally following entanglement in fishing gear. Entanglement causing death – without deliberate killing – was another important cause of mortality, and would likely have had higher rates if the National Park GCBC staff did not rescue entangled gharial regularly. We shall refer to both death due to entanglement and death due to deliberate killing after entanglement as ‘bycatch-related mortality’. It seems that reported cases of bycatch-related mortality are an underestimate of the true scale of the threat: of the 23 gharial killed by human activities in the study period, 25% were found during the intensive riverbank tracking or surveys of the 2018-2022 project, or only because the dead gharial (buried or hidden) was wearing a tag.

Bycatch-related mortality is a major issue for gharial in much of their remaining range (Lang, Chowfin and Ross, 2019) and is also problematic for other co-occurring freshwater megafauna such as river dolphins (Behera, Singh and Sagar, 2013), and for other crocodilian reintroduction programmes, such as the Chinese Alligator (Lu *et al.*, 2014). Direct killing is a major issue for reintroduction of carnivores generally. Jule *et al.* (2008) found more than 50% of fatalities in mammalian carnivore translocations were due to direct killing by humans. Similarly, direct killing is a major threat to reintroductions of crocodiles including the Orinoco and Cuban crocodiles (Babarro, 2014; Ramas and Rodriguez-Soberon, 2020). Restoration programmes are unlikely to succeed if key threats to the population have not been dealt with prior to the commencement of population restoration, which for carnivores

– mammalian and crocodilian – is often human conflict. Given the introduction of the problematic monofilament gill nets corresponds in time (1950s-1960s) with declines of gharial across South Asia (Radhalekshmy and Gopalan Nayar, 1973) and was identified as a factor leading to the decline of gharial in Nepal (Maskey, 1989), bycatch related mortality was likely a major cause of initial declines in Chitwan. The failure to deal with one of causes of initial decline is a regularly identified problem for population restoration programmes (Fischer and Lindenmayer, 2000; Jule, Leaver and Lea, 2008; Bubac *et al.*, 2019; Berger-Tal, Blumstein and Swaisgood, 2020), and the gharial headstarting programme appears to be falling into this trap.

Additional causes of headstarted gharial mortality observed were inappropriate post-release behaviour leading to assumed predation, and directly observed predation by mugger crocodiles. Mugger crocodiles are a known predator of juvenile gharial, and studies on the Chambal have found headstarted gharial are much more prone to mugger predation than equivalently sized wild-recruited gharial (GEP, unpublished data). At least two separate instances of headstarted gharial being killed by mugger were directly observed, and conversations with local fishermen suggest such observations are not infrequent. Predator naivety leading to higher predation rates is an issue for multiple reintroduction programmes across a range of taxa (Tetzlaff *et al.*, 2019; Zhu *et al.*, 2022), as animals reared in captivity have no prior experience of predators for direct learning, or opportunity for learning from parents or conspecifics. Prey can be better conditioned to avoid predation through anti-predator training, which aims to improve predator avoidance behaviours in otherwise naïve captive-reared individuals, and has been shown to be effective in improving both survival and fitness, with results most pronounced in juvenile life stages and in fish (Tetzlaff *et al.*, 2019; Zhu *et al.*, 2022).

One radio-tagged gharial took up residence in a small stream from which the GCBC pumps water to fill the tanks in which gharial are reared. This gharial may have been expressing natal habitat preference induction (NHPI), a phenomenon where animals prefer new habitats with stimuli comparable to their natal habitat (Stamps and Swaisgood, 2007) in this case, the same water supply. The sudden disappearance of the gharial from this small stream – and failure to find the signal in the adjoining main river – suggested it was likely

taken by a terrestrial predator. Tigers prey on gharial and have even broken into enclosures in the centre to predate on gharial (Joshi, 2022) so are the likely candidate.

We did not find a significant relationship between size, age of sex and fate of gharial (alive/dead) at the end of the monitoring period. We had very small sample sizes (particularly of male gharial), but even so did not see any trend in these factors and mortality. In 2020 the GPS tagged gharial (which had radio failure) were selected to release individuals ~2m in the hope larger size would lead to greater survival, but within a few weeks of release one of these gharial was found killed following entanglement, suggesting larger subadults remain vulnerable to this threat. Even adult gharial have been killed by net entanglement, so increasing the size at release is unlikely to improve the situation, and our evidence so far supports this. Although larger gharial are more likely to break the nets and swim away, as found when catching wild gharial and by the observation of many adult gharial with broken nets on their snouts in the wild (Griffith, personal observations), the longer we do not release gharial the older and larger they will be at their first net entanglement. We contend that a person catching a subadult gharial in a net is more likely to kill it from fear in order to recover the net, compared to a person catching a yearling or small juvenile, which may appear less daunting to handle or cut free.

Although we believe mortality in Nepal is the primary cause of low success in establishment of gharial in Chitwan, dispersal is also an important area for improvement. High dispersal distance or rates are identified as a major cause of failure in translocations (Germano and Bishop, 2009; Berger-Tal, Blumstein and Swaisgood, 2020), and although we believe long distance dispersal distances are less common than when the releases occurred on the Rapti, we suspect they are still a significant cause of apparent mortality. We will first consider the implications of maximum distances of dispersal on the headstarting programme and downstream populations, and then consider observed patterns of movement and settlement.

Maximum recorded dispersal distance for tagged gharial (the location they reached furthest from the release site, regardless of subsequent return movement) were 10-112km distant. Two further gharial were thought to have dispersed further but were not detected, either reflecting mortality or considerably longer dispersal distances. The maximum dispersal

distances showed a skewed normal distribution with a long right-tail, suggesting most gharial will settle within a certain distance of the release site, with a smaller minority dispersing much longer distances. We caution that this distribution was observed with a very small sample size ($n=20$); however, it suggests that post-release dispersal at the population level may be predictable at the population level, and such predictability could be used to inform release location relative to habitat variations or threats, such as proximity to a dam or barrage, or edge of a protected area. The collection of further data on dispersal is key for improving predictions, and should be a key priority for all gharial headstarting programmes, as dispersal tendencies will be influenced by local environmental conditions.

Two remarkably long-distance dispersals of gharial from the GCBC programme were reported in 2020: two gharial were found in May and September 2020 in separate locations in West Bengal, over 1000km from the release site, one of which made this remarkable journey in just 61 days (Khadka 2020a; WTI 2020). The first gharial was taken into captivity, whilst the second was captured and subjected to highly stressful handling behaviour by crowds unused to gharial, which was likely the cause or contributing factor to its death a few days later. The bleak fate of the gharial documented travelling long-distances highlights a major problem: when gharial arrive in areas in which they have long been extirpated they are unlikely to survive. Unexpected coexistence between humans and large carnivores when these carnivores disperse outside of the release area is a repeated problem in translocations (Osborne and Seddon, 2012). Therefore, a dispersal outside of the targeted zones of the release programme is a failure, and every effort should be made to reduce risk of such movements.

The high connectivity of riverine habitats and excellent dispersal capabilities of gharial are intrinsic impediments to the success of their reintroduction or reinforcement. This is likely to be especially true where suitable habitat is small and fragmentary, and very distant from other sites. If linear river distance with suitable habitat (for example, protected areas without use of gill nets and other destructive fishing gears) is shorter than average dispersal distances, the majority of gharial will end up outside the protected area during their exploratory phase. Similarly, gharial released upstream of a dam will likely all be lost from the population if the release-site to dam distance is too short, and the dam provides a barrier or partial barrier to upstream movement. Our observed dispersal distances give a

preliminary suggestion that release sites should be at least 100km upstream of a physical barrier on the river, such as a dam. In Chitwan, a shift of the release site even further upstream on the Rapti may well lead to improved retention of releases in the area of National Park protection.

Our study of post-release movement patterns in twenty gharial released from the GCBC found that most gharial exhibited the classic behaviours of animal released in translocations: an exploratory phase with high propensity, distance and frequency of movement (Rittenhouse *et al.*, 2007; Osborne and Seddon, 2012; Hempel *et al.*, 2020), identified in this study by a highly variable or continually increasing net-squared displacement (NSD). Although generally downstream overall, likely reflecting a response to meeting flowing water for the first time, gharial moved erratically between river locations, thought in other species to reflect disorientation (Moehrensclager and Macdonald, 2003). This exploratory phase coincides with an acclimation period, defined as the period of elevated mortality post-release (Hamilton *et al.*, 2010). We similarly found that for six of the apparent mortalities, they were still in this exploratory phase. Exploratory phase and increased movement are associated with mortality in multiple studies. For example, translocated Box Turtles were found to move more and further than residents, increasing their mortality by increasing their risk of encountering threats (Hester, Price and Dorcas, 2008), and swift foxes that moved greater distances post-release were found to have lower probabilities of survival (Moehrensclager and Macdonald, 2003).

Following the acclimation/exploratory phase, movement can be categorised into an establishment phase where distances of movement from release location stabilise, but frequency of movement remains high, followed by a settlement phase where movements of translocated individuals reflect those of residents of the same demographic class (Moehrensclager and Macdonald, 2003). Such patterns are seen in multiple taxa with individual and sex-based variation reported in if and when settlement occurs (e.g. Hester *et al.* 2008; Sievert *et al.* 2022). We found released gharial in Chitwan tended to display this pattern of movement change too: seven released gharial eventually settled into a small area (<15km) where they remained until study-end or death. Occupancy of small areas of river is normal wild juvenile gharial behaviour in the well-studied Chambal population (GEP, personal communication). However, we also found six gharial remained in the exploratory

phase throughout the duration of monitoring (2+ years). Such movement is concerning because this does not reflect wild juvenile gharial behaviour. Gharial which did not show settlement post-release were significantly larger than settlers, they also tended to be older individuals, although this was not significant, likely due to our small sample size. Headstarting programmes tend to try and release larger individuals, so they suffer less size-related mortality (Germano and Bishop, 2009; Tetzlaff *et al.*, 2019), however our preliminary results suggest larger gharial may be less likely to settle post-release, suggesting a trade-off between size at release and time in captivity (Tetzlaff *et al.*, 2019).

Smaller, younger gharial may be more appropriate for release for a number of reasons. Differing behavioural predispositions have been found between crocodilian species, with differences found between exploratory behaviour and aversion to novelty (Reber *et al.*, 2021). Such behavioural predispositions are likely to be important for reintroduction success (Merrick and Koprowski, 2017; Kelleher, Silla and Byrne, 2018). Species-level behaviour predispositions have only recently begun to be investigated in crocodylians, but future research may also find ontogenetic shifts in risk-taking or risk-averse behaviour, as have been seen in other species (e.g. Roy & Arlinghaus 2022). Mortality in crocodilians is size-selected, and the niche of crocodilian shifts substantially through life as an adult can be 2-3 orders of magnitude larger than a hatchling. We might expect larger subadults to become bolder and more exploratory as predation risk diminishes with size. This could lead to these individuals showing reduced adversity to threats such as humans, fishing gear and mining. Secondly, these individuals may show more protracted exploratory behaviour and disperse greater distances from the release site, reducing the chance they will settle in suitable habitat, and possibly contributing to the lack of settlement seen in larger individuals.

Another key consideration underlying different post-release behaviours are different degrees of chronic stress in captivity. Studies on captive alligators have found a strong negative relationship between levels of stress hormones and body mass (Elsey *et al.* 1990; Turton *et al.* 1997). The older and larger gharials in the GCBC tend to be released later due to having grown slower to release size, therefore it is possible these larger gharial are more chronically stressed in captivity than those that are smaller at release due to having grown faster. Individual differences between levels of chronic stress in gharial in captivity may

relate to a whole suitable of between-individual variations, including in husbandry, enclosure positioning, individual personality or genetic predispositions. Stress may drive animals to disperse more (Dickens, Delehanty and Michael Romero, 2010) and may explain why the larger gharial were less likely to settle. Chronically stressed animals may show greater dispersal tendencies as a form of coping through avoidance behaviour, and show active searching as a way of exerting control over the stressful situation (Dickens, Delehanty and Michael Romero, 2010). Gharial are capable of long-distance dispersals, and historically occupied entire river basins across South Asia. Chronic stress could be a driver of movement which, in evolutionary terms, would have been beneficial as it would have taken the gharial to a new and more suitable habitat. Stress-triggered dispersal behaviours have been seen in juvenile birds (Korte *et al.*, 2005). However, a long-distance dispersal in the modern-day Ganges basin will almost certainly lead the gharial to unsuitable habitat.

6.4.2 In-Situ Reproduction

We found that most of the gharial from the headstarting programme and all the wild-origin gharial of a reproductive size showed regular breeding. However, the one female who did not breed suggests there may be long-term reproductive impacts of the stress and stunting of captivity if gharial are released at too late a stage. Reproductive frequency was lower than the annual breeding seen by female gharial on the Chambal River (Lang and Jailabdeen, 2018), this is not surprising, as crocodilians at the latitudinal limits of their range tend to reproduce less frequently (Thorbjarnarson, 1996). Not only did we find most headstarted females reproducing successfully, we also found headstarted females showing important natural reproductive behaviours, including migration between breeding/nesting and feeding sites involving travel of over 140km in one year, as well as headstarted females taking on the duties of creche guardianship, defending communal creches from predation. Although it took headstarted females a long time to reach a reproductive age and size (over seven years post-release), a substantial number of females are now maturing, leading to increasing annual nest counts.

One of the key reasons for starting the headstarting programme were concerns that nests and hatchlings were not surviving in the wild (Maskey, 1989). Luckily, our preliminary

evidence on hatchling survival suggests the situation in Chitwan is much more optimistic than either when the programme began, or even recent estimates (Khadka 2020b). Based on survey data, we estimated that survival of Rapti hatchlings would be ~20% in both 2017 and 2018. Given that inexperienced observers may class yearlings and small juveniles in the same category, we still had a minimum count of 2.4-5% in 2018-2019 based on our own observations of certain yearlings in very limited stretches of the river. As such, these are very much absolute minimal survival estimates. We suggest the discrepancy between our observations and low previous observations (Khadka 2020b) is due to the highly variable detectability of hatchlings combined with insufficient targeted monitoring effort for this specific age class. The small age classes of long lived reptiles are generally more challenging to find and investigate (Tuberville *et al.*, 2021), this is likely also true of gharial.

For the Narayani, Khadka et al (2020) report 1.1-14.3% survival in 2018-2019. We argue that, given the proximity of the Narayani nest sites to the Indian border, a consideration of survival probability needs to consider Gandak yearling counts. In 2018 apparent survival in Nepal according to our counts was 9.5%, and if Gandak counts are considered, apparent survival could be as high as 14-87% in 2017-2019. Even considering yearling counts in India – as for general surveys in Nepal – largely conflate the smallest size classes, this still suggests survival is markedly higher than previously thought. Even if the majority of the Gandak yearlings are from undetected Gandak nests, this suggests in-situ survival of hatchlings in the geographically adjacent Gandak (which is not a protected area) is high in this system. This is an excellent cause for optimism, and evidence in favour of leaving more nests in-situ. More data on hatchling survival is necessary, and targeted monitoring of yearlings annually is an important next-step for research in Chitwan. However, our preliminary results from the deterministic model suggest that survival in the wild may be even better than in the headstarting programme, once the post-release mortality of headstarts is considered. When considered alongside the many issues of ex-situ rearing, the high in-situ reproductive success suggests that the majority of nests in Chitwan should be left in-situ.

6.4.3 Retrospective Analysis 1983-2021

Our retrospective model suggests that adult mortality is currently low in Chitwan, with a slowly recovering adult population. Although our results suggest apparent mortality remains high for headstarted gharial after release, headstarting appears to have contributed to population growth since 2010.

Overall, these results are promising for population recovery. Population demographic models of turtles have found population recovery to be most sensitive to adult survival, such that headstarting has no impact on reversing population declines if adult mortality remains high, even if survival within the headstarting programme is 100% (Heppell, Crowder and Crouse, 1996; Mitrus, 2005). Crocodiles and turtles are both long-lived reptiles with a life history characterised by high egg and hatchling mortality, followed by longevity for survivors of these vulnerable stages (Briggs-Gonzalez *et al.*, 2017; Starking-Szymanski *et al.*, 2018). Such reptiles have evolved to have low and variable offspring survival, and thus for headstarting to augment a population the adult population must be stable or growing, and headstarted individuals should have a better chance of reaching adulthood than an equivalent egg left in the wild (Heppell, Crowder and Crouse, 1996). Our results suggest adult mortality has been reduced since gharial protection began in the 1970s, and been very low for the last 15 years. Maintaining this low adult mortality in-situ should be the primary goal of gharial conservation, given much greater importance than the headstarting programme – even if the headstarting programme is shown to be successful.

A recurrent identified problem with the Chitwan population has been the lack of adult male gharial (Acharya *et al.*, 2017) leading to the translocation of an adult male from the Babai in western Nepal (Khadka & Kandel 2020). However targeting of adult males for their ghara no longer occurs (Bhatta, 2009) and a mechanism for differential survival of males could not be identified (Acharya *et al.*, 2017). We show here that the female-biased sex ratio in the GCBC is sufficient to drive the very low adult male numbers seen in recent years. This ratio is likely due to a lack of monitoring or control of egg temperature in the centre, until the installation of an incubator in 2021 (Griffith *et al.*, *in prep*). Gharial have temperature-dependent sex determination (Lang and Andrews, 1994), so if eggs are consistently kept too cool there will be a female-biased sex ratio. If we estimate males take 11-19 years post-release to mature (Khadka *et al.* 2022), it is likely we have not yet seen an increased number

of maturing males corresponding to the maturing of adult females in 2015-2018. Sightings of at least three subadult males close to breeding age in recent years suggest an improving situation is close at hand. However, so long as the maturing male population remains small, there is a risk of extinction of all males due to demographic stochasticity (Armstrong and Seddon, 2008) resulting in a population unable to breed, as has occurred in other small gharial populations such as on the Son River (Jailabdeen, pers. comm.). As such, regulation of egg temperatures ex-situ to ensure a more balanced sex-ratio in release cohorts may reduce risks of male extinctions. Alternatively, leaving an increasing number of nests in-situ is likely to lead to a natural sex ratio by avoiding the re-burying of eggs taken to ex-situ facilities, which removes the maternal microhabitat decisions that likely influence natural sex ratios (e.g. Ewert et al. 2005; Mitchell et al. 2013).

Our model results suggest a substantial reduction in apparent mortality after the shift from the Rapti to the Narayani in 2004. Prior to 2004, the continuing decline of the gharial population means that post-release survival cannot be higher than our estimates of 6% probability of survival (release to adult), parameterised using contemporary telemetry data. We presumed gharial had dispersed (crossed the border into India) if we stopped detecting them after their NSD had been continually increasing and they had already moved further from the release site than the remaining distance to the border. Of the three gharial, one was then detected in India by the Wildlife Trusts of India (WTI, 2020), providing support that these gharial had dispersed.

It has long been suggested that the primary reason for the loss of individuals from the headstarting programme in Nepal is post-release dispersal through the barrage on the Indo-Nepal border, with return prevented by the barrage being a barrier to upstream movement (Maskey 1989; Ballouard et al. 2010; Acharya et al. 2017). For the purposes of a reinforcement programme that aims to restore the gharial population in Chitwan, the loss of gharial from the release area (the habitat where animals are hoped to remain in order to reinforce the population (Le Gouar, Mihoub and Sarrazin, 2012)) is a failure of establishment. We concur with previous analyses that suggest the loss of gharial to dispersal was very high from 1981-2004 when the releases happened on the Narayani (Maskey & Percival 1998; Ballouard et al. 2010). The very high volume and flow of water in the monsoonal Narayani may well flush the environmentally naïve new releases

downstream. Interestingly, we did not find any tagged gharial which were in the Narayani by their second monsoon disperse as far downstream as India, although two individuals did make substantial downstream movements during floods. The permeability of the barrage likely changes with seasons: the one gharial detected by WTI just downstream of the barrage was detected back in Nepal one year later, showing that upstream return movements are sometimes possible, when the gates of the barrage are open and the flow through the barrage is not too powerful. We suggest that the impact of dispersal into India has been greatly reduced since the programme shifted to releasing in the Rapti: likely due to reduced flow overall and seasonally and greater distance from release site to barrage. It appears that dispersal is a factor of reduced importance to programme success following change in release site, and this dispersal could be further reduced simply by shifting release site even further upstream.

We made equivalent retrospective projections for the Gandak based on our dispersal estimates from Nepal as a test for our partitioning of apparent survival in Nepal into mortality and downstream dispersal. For headstarted gharial, we predicted a growing Gandak population, especially since 2015, which is what is observed in count data. However, we predict much higher population counts than were observed on the Gandak in 2010. Given the difficulty in distinguishing between mortality and dispersal in our past data there are only two options for this difference: dispersal rates out of Nepal prior to 2010 were lower than predicted in our model, and therefore mortality in was is higher. Conversely, if dispersal rates are higher than predicted, mortality across the border on the Gandak is substantially higher than in Nepal, and the Gandak is primarily a sink population. Extensive transboundary monitoring is key for investigating this – for as long as a source population exports individuals the sink habitat will be occupied, giving the false impression that the sink is suitable habitat (Osborne and Seddon, 2012). These results highlight the need for more transboundary work: in the future there should be transboundary monitoring and interventions, and sharing of data, resources and results across borders in order to work out what works for gharial in-situ conservation, and to make it happen.

As well as the shift to release on the Rapti River, the other key change in the Chitwan headstarting programme was that from 2010 onwards considerably more gharial were released. Prior to 2010 an average of 19 gharial were released per annum, increasing to 71

from 2010-2021. An increased number of individuals being released has been repeatedly identified as a correlate of reintroduction success across species (Griffith et al. 1989; Fischer & Lindenmayer 2000; Germano & Bishop 2009). This link may be driven by larger founder populations being less likely to suffer Allee effects (reductions in individual fitness at low population size or density) and to have lower risk of demographic or stochastic extinction (Deredec and Courchamp, 2007). It appears in Chitwan the changing number of releases was driven by a reduction in egg and hatchling mortality in captivity (Khadka et al. 2013). Data from 1993-2003 show the average egg to yearling survival was less than 5% (Khadka et al. 2013). This led to a small number of releases annually. Our models suggest that the pre-2004 rate of survival from release to 10 years could not have been higher than 6%: in combination this is an egg-to-adult probability of survival for gharial in the headstarting programme of just 0.25%.

Exceptionally high historic mortality in breeding centre is almost certainly attributable to stress: High levels of chronic stress will lead to high rates of mortality in crocodilians as stress impacts on immune function (Morici, Elsey and Lance, 1997). Studies into the high hatchling mortality in Chitwan found pathogens causing mortality were opportunistic, presumably taking advantage of the impaired immunity of highly stressed individuals, many suffering from chronic anorexia (Gairhe, 2007). The slow growth of gharial in the breeding centre prior to release compared to other captive rearing programmes (Singh 1978; Khadka & Bashyal 2019) suggests chronic stress in captivity, as growth in both crocodilians and other reptiles is strongly linked to stress (Morici, Elsey and Lance, 1997; Shilton *et al.*, 2014). High stocking densities, stressors such as high visitor numbers or lack of ability to seek cover, and insufficient thermogradients and overhandling are all causes of high stress for crocodilians in captivity (Huchzermeyer, 2003); all these issues are present at the GCBC (Gairhe 2007; Khadka et al. 2022) alongside additional stressors such as force feeding (Gairhe, 2007). Although much improved in recent years, mortality in captivity is still high and conditions must be improved for both conservation and welfare benefit.

The results of this study show that prior to 2004 the headstarting programme in Chitwan was a failure, based on both measures of headstarting success identified in Heppell et al. (1996): a sustainable population was not established, and an egg-to-adult survival of 0.0025 is unlikely to be higher than survival in the wild. It seems that the assertion that the

headstarting programme prior to 2004 'maintains the population' (Ballouard et al 2010) was likely incorrect: the removal of almost all eggs from the wild to meet high mortality both in the centre and post-release may have had no positive impact on the population, and instead prevented the population from being able to make a natural recovery due to the removal of all wild nests. Further evidence of this lack of success is that the population declined from a count of approximately 76 adults in 1988 (Maskey, 1989), to approximately 17 adults in 2004 (Ballouard et al 2010).

However, our analysis suggests a much more promising interpretation of the headstarting programme since 2004. Egg-to-yearling survival improved to an average of over 20% during 2004-2012 (Khadka et al. 2013), and has improved further since (Khadka, personal communication). Our model suggests egg-to-adult survival now exceeds a minimum estimate of 2.36%, a substantial improvement. Comparative estimates to measure this against wild survival highlight the need for most nests left in-situ, with more substantial monitoring to estimate survival probabilities, but this headstarting figure may now be higher than wild survival.

The major diversion between our model and observed population dynamics was our population estimate did not show a levelling off to a stable population trend. This suggests our estimates of post-release survival are likely too optimistic since 2017. This is concerning, as we currently only estimate a 26% probability of gharial surviving to their fourth-year post-release. Demographic studies on multiple species of crocodylian have found that subadult survival is especially important for demography in increasing populations (Briggs-Gonzalez *et al.*, 2017), with a high sensitivity of population growth rate to juvenile/subadult survival. Continued high mortality of headstarts after release will therefore have a negative impact on population growth, even if adult survival is high. Identifying and tackling causes of mortality in Chitwan is therefore very important to ensure continued population growth.

6.4.4 Conclusions and Next Steps

Our results suggest that the impact of the headstarting programme in Nepal has been mixed. Prior to 2004, the headstarting programme is unlikely to have had a positive impact on the population, although since 2010 an accumulation of surviving and maturing gharial from the headstarting programme have led to a substantial population increase, and corresponding increase in nest counts. We recommend the next step is to begin a cycle of adaptive management. This next step would involve engaging stakeholders to agree on objectives for gharial conservation in Chitwan, with the results of this analysis informing decisions on potential management actions and developments from our model to project their impact (McCarthy, Armstrong and Runge, 2012; Nichols and Armstrong, 2012). The management actions we suggest should be tested are releasing gharial at much smaller sizes (younger ages) and at release sites further upstream, and the fate of these individuals should be monitored using telemetry. Most importantly, a greater proportion of nests should be left in-situ, with substantial research to determine the fate of hatchlings left in-situ across multiple years. Not only should a smaller proportion of nests be collected, a smaller absolute number of eggs should be collected, to substantially reduce stocking density in the centre and allow more flexibility in improving management. The nests collected just before (or hatchlings just after) hatching in-situ so natural sex ratios are maintained.

For headstarting or reintroduction of gharial and other riverine crocodylians beyond Chitwan, we suggest releases in small fragments of suitable habitat, or less than 80km upstream of a dam, are likely to fail. Animals released in large rivers, especially before flooding season, are more likely to show substantial downstream movement than those in smaller tributaries. Larger, older gharial appear less suitable for reintroduction, and animals stunted by captivity may show reproductive issues, suggesting a brief time in captivity is preferable. Removal of nests to ex-situ introduces additional issues, such as biased sex ratios and high mortality, therefore protection of nest sites and animals in-situ from anthropogenic threats is preferable for recovery where there is an established adult population. However, in-situ hatchling dispersal downstream is important to consider, and populations in reservoirs or just upstream of dams may have very limited recruitment.

More broadly, our results show that sufficient data on the impact of conservation actions is vital when trying to determine a counter-factual. Understanding the counter-factual (what would have happened in the absence of a management action) is increasingly important in conservation, especially for the new IUCN Green Status of Species (Grace *et al.*, 2021). This is the first use of a modelling approach to retrospectively evaluate a gharial headstarting programme. Although it is widely asserted that headstarting improves population persistence for gharial, including for Chitwan, the results of this case study show the impact of the intervention was historically negative or negligible. We therefore caution that logical argument or expert elicitation alone, without sufficient data and population modelling in support, may be misleading when inferring conservation success.

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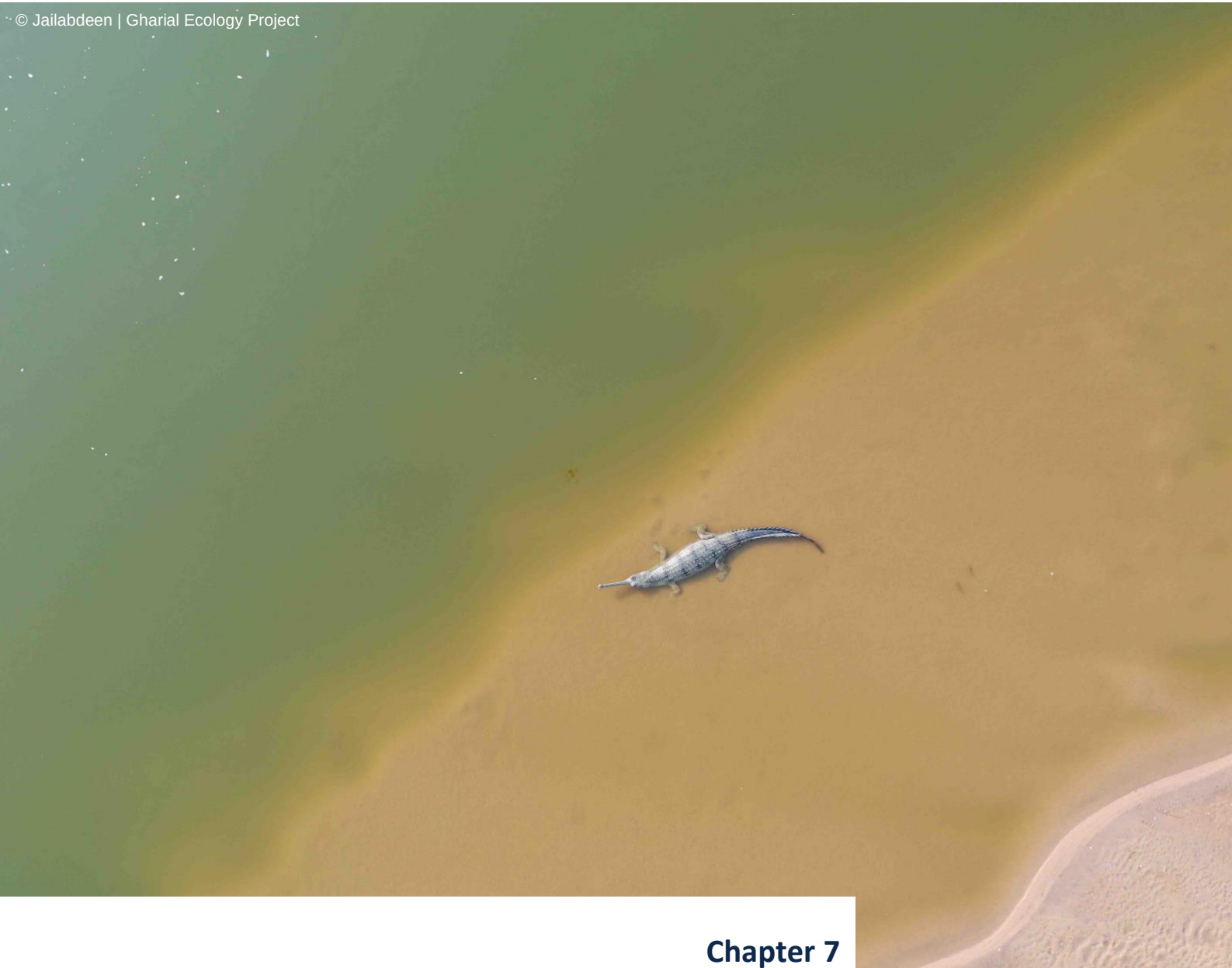
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Chapter 7

General Discussion

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7.1 Why conserve gharial?

Gharial are irreplaceable. Their evolutionary and functional distinctiveness, as shown in Chapter 2, indicate that there is no other species like them in the world. They differ substantially from even other crocodylians in terms of their functional role, and represent over 20 million years of unique evolutionary history. If this species was to go extinct, it would take a vital piece of biodiversity with it. The long-term ecosystem impacts of gharial loss are unknown, but their functional dissimilarity to other crocodylians, and of crocodylians to all other species, suggest that their loss is likely to be disruptive to their ecosystems (Violle et al. 2017; Dee et al. 2019)

Chapter 2 also highlights some of the reasons why the gharial is so threatened: it is a habitat specialist, with fairly low fecundity, which lacks the physiological and behavioural tolerances of other species of crocodylian to tolerate harsh environmental extremes. The gharial, among crocodylians, is therefore one of the species least likely to survive when its habitat is modified, disrupted or drained. As suitable patches of its riverine home in the Ganges dwindle, the gharial is left with nowhere to go, and cannot survive the changes. This has left the gharial Critically Endangered (Lang et al. 2019).

7.2 What would conservation recovery look like for gharial?

A fully recovered species is defined for the IUCN Green Status of Species as one that is 'viable and ecologically functional in every part of its indigenous and projected range' (Akçakaya et al. 2018). Gharial have been lost from at least 94% of their former range (Lang et al. 2019). The current situation for gharial across their remaining range remains precarious. Of the remaining populations, only one is currently both viable and functional, in the Chambal River of India. The other populations show varying degrees of viability: no adults, no reproduction, no recruitment, or limited recruited. Similarly, the density of gharial suggests high variation in the functionality of these populations, from a few gharial

likely to be having a negligible ecological impact, to a high density of gharial in a small protected area.

The first step towards recovery would be viable and functioning populations in the parts of the gharial distribution where populations are now small and fragmentary. Populations such as the one on the Chitwan-Gandak system currently lack substantial adult populations, and consequently have low rates of nesting. With sufficient protection from threats in-situ, gharial can bounce back from the brink, as evidenced by the recovery of the Chambal population. Population estimation tools such as we used in Chapter 5 can be implemented and refined to more closely understand population dynamics and keep track of when conservation action is working. New technologies such as drone-based individual ID provide exciting new tools to investigate population ecology, individual behaviour and inform conservation decision making (Desai et al. 2022).

The obvious next step to improve viability and connectivity of remaining populations is to geographically expand conservation efforts surrounding them. Given the substantial downstream movements of both headstarted and yearling gharial we observed in Chapter 6, the important sites to focus efforts are likely those immediately downstream of current populations, creating longer stretches of suitable habitat. Our results in Chapter 6 strongly suggest that the Gandak population and Chitwan population are in fact one linked population, primarily with movement from Chitwan to the Gandak. The movement of one tagged gharial back through the barrage also brings hope that the barrage has some permeability in the upstream direction: future research could investigate if there were ways to increase the likelihood of such movements. Restoration of habitat upstream of current gharial populations should also be considered. The gharial population on the Karnali-Girwa system across the India-Nepal border is now almost exclusively found in India due to anthropogenic disturbances on the upstream stretches in Nepal compounded by a shift in the primary channel of the river (Bashyal et al. 2021; Vashistha, Mungi, et al. 2021). Gharial move back into the Karnali during the monsoon annually, and in 2022 an adult female moved upstream into the Karnali to nest in Nepal's Bardia National Park. If the habitat could be better protected upstream, the overall viability of the Girwa-Karnali population, currently found in a tiny stretch of protected river in Katarniaghat Sanctuary, could be improved. Our findings of an increasing gharial population in the Rapti, far upstream of

historical sightings, suggest gharial have the capacity to recolonize suitable upstream habitats.

Future recovery would then see the return of gharial to parts of the Gangetic basin where they are no longer found, and also to the Indus and Brahmaputra basins, where the gharial has been extirpated. Occasional individuals have been reported on the Brahmaputra, including confirmed sightings of one gharial in the Kaziranga National Park in 2022 (Kaziranga NP 2022). It is unknown whether this individual is a long-distance disperser from the Ganges, or indicates a tiny remnant population in the system, but suggests the return of the gharial to this river system is not impossible. The Indus river system in Pakistan is perhaps is the most promising place for future reintroductions. Surveys have found Indus river dolphin numbers are rising substantially thanks to community-based conservation efforts (WWF 2017). If river dolphins are increasing due to successful protection, this is good news for gharial. River dolphin are susceptible to the same major threats as gharial, primarily river fragmentation by dams and bycatch mortality, so increasing dolphin populations suggests the system could be suitable for gharial again (Braulik et al. 2014; Kelkar & Dey 2020). A gharial reintroduction programme initiated on the Beas River in India, the only part of the Indus in India, suggests the return of gharial to Pakistan may already be being facilitated. As we found in Chapter 6, long distance dispersals of reintroduced gharial are not uncommon, and most gharial will disperse at least 40-60km downstream at some stage post-release. If patterns are similar for reintroductions in the Beas, gharial may have already reached the Pakistan border. A conservation action plan for gharial restoration in Pakistan should therefore be considered.

However, it is very important to not put the cart before the horse: past reintroductions in gharial have failed because the habitat was insufficiently restored, and threats that caused initial declines had not been removed. Gharial reintroductions should only be attempted in areas where these threats have already been tackled, as highlighted by our results in Chapter 6, showing that even on the comparatively well protected Narayani and Rapti rivers, mortality of released gharial remains high.

7.3 Insights on Headstarting Failure and Success

Taken together, our observations on post-release movement and mortality in Chapter 6 explain variation between headstarting programmes for gharial surprisingly well. Release sites less than 100km above a barrier such as a dam, barrage or weir are likely to see most gharial flushed from the system during their first monsoon, which would explain the failures of headstarting at Katarniaghat in India, and on the Koshi and Babai Rivers of Nepal.

Populations such as a small group of gharial below an irrigation weir on the Babai are likely doomed sink populations, although in a long lived species (and with sufficient sporadic headstarting) this may appear to be a suitable habitat due to continued occupation. Very small protected areas such as on the Mahanadi river are unlikely to protect sufficient habitat that the gharial can disperse into, similarly even the 45km of protected river in the Ken Gharial Sanctuary is likely to be insufficient, as this is lower than the median maximum dispersal distance we recorded in Chitwan, so most gharial would end up outside the protected area. Additionally, failure to remove threats – in particular bycatch-related mortality – in a sufficiently large area can lead to programme failure. The Ken, Son and Mahanadi Rivers have continued fishing pressures, which likely lead to high release mortality, as in Chitwan. The probable success story of gharial headstarting – the Chambal River – had a huge number of headstarts released early in the programme, in a very large river with no barriers to returning upstream following downstream exploratory dispersal (dams or barrages), good protection from threats such as fishing and an already established (albeit small) population to provide conspecific cues for settling. This suggests that headstarting may work as a tool for early-stage population recovery, but only in sites and systems with specific characteristics, high ex-situ survival, a well-chosen release site and removal of the threats that caused declines in the first place.

Post-release monitoring of gharial using VHF telemetry worked well in Chitwan, and we suggest continued use of this technology for post-release monitoring at this site, and at other sites where most of the river is accessible for tracking. However, for gharial that crossed the border into India, tracking became more challenging on the huge and highly braided Gandak river. Our results in Chapter 4 suggest that GPS-GSM telemetry would be a useful tool to understand long distance dispersal, as GPS tagged gharial can be located even in locations that are remote and difficult to reach.

In Chapter 6, by using location and behavioural data from radio or GPS tagged individuals, we were able to collect sufficient information on gharial movement, fate and reproduction to parametrise a model to retrospectively analyse the successes and failures of the Chitwan headstarting programme. We suggest this approach would be useful for other headstarting programmes, to understand site and system specific reasons for failure. Among 45 crocodylian reintroduction or reinforcement programmes in 14 species, there was insufficient results of post-release monitoring to estimate apparent survival or establishment rates in all except five projects (Griffith, unpublished database). This suggests improved post-release monitoring, and the use of that monitoring to develop simple models to evaluate success, is an important goal for most crocodylian reintroductions. We suggest our approach – population surveys, and telemetry of cohorts of both newly reintroduced and wild resident animals – could be applied to any other crocodylian species.

To improve outcomes in all conservation management of crocodylians, we strongly recommend that the results of population reintroduction and reinforcement in crocodylians are shared according to the principles of Evidence-Based Conservation (Sutherland et al. 2004; Gillson et al. 2019). The freely available sharing of data online on causes of success and failure, and the techniques used (either successfully or unsuccessfully) to monitor this would be hugely informative for other projects. Methods of analysing this data, and models used to evaluate interventions and project the impact of future action, should also be added to the Evidence Base. The details of the 45 crocodylian reintroduction or reinforcement projects described above were mostly found in the grey literature, primarily newsletters and proceedings of the Crocodile Specialist Group, with very few in the published scientific literature. This suggests there is a willingness of those working on crocodylians to share information and report on projects, but perhaps less capacity for writing up results as scientific articles. This may reflect a huge range of barriers, from funding to language barriers, to difficulty in presenting or publishing results of failures (Amano et al. 2016; Catalano et al. 2019). Furthermore, scientific papers which are not open-access are not contributing to the Evidence Base, and open-access publishing is often prohibitively expensive in conservation. The creation of an accessible database to share crocodylian headstarting information would vastly improve the evidence available to decision makers. The collated information could then contribute to an assessment of evidence in a larger

database, such as the Conservation Evidence database (<https://www.conservationevidence.com>), which already has a page for head-starting crocodilians, currently awaiting assessment.

7.4 How can the headstarting programme be improved in Nepal?

The headstarting programme in Nepal should shift to a system of adaptive management. Adaptive management is a form of structured decision making that helps incorporate uncertainty in making management decisions, and helps make those decisions in a structured and transparent way (Nichols & Armstrong 2012). It is a form of decision making that is designed for recurrent decisions with uncertainty (Nichols & Armstrong 2012), and therefore makes sense for a headstarting programme that operates on nest collection and juvenile release on an annual basis. We believe the results of this thesis provide sufficient information to begin the process of adaptive management, and hope to catalyse an interest in doing so. To begin the cycle, there must be a definition of the problem and identification of objectives by stakeholders (McCarthy et al. 2012). Following this, the impacts of various management actions can be modelled and predicted, and these models can then be developed and tested to help choose between competing management actions. Our deterministic model in Chapter 6 would be a useful place to begin with projections for the impacts of future actions (Armstrong & Reynolds 2012). Based on the results of the previous chapters, we recommend the following management actions are considered.

7.4.1 Leaving More Nests in-situ

The most important shift necessary in Chitwan is to leave a greater proportion of nests in-situ, as has been suggested previously (Acharya et al. 2017). This would avoid concerns over the multiple issues with the headstarting programme discussed in Chapter 6. Stocking density is currently very high in the Gharial Conservation Breeding Centre (GCBC), which is likely a contributor to high stress and resultant mortality. In-situ movement of nests could be trialled if necessary, for example moving nests to higher ground if they are at risk of flooding, or moving single nests into a larger communal group if it is at risk of predation or

human disturbance. If nests are still collected for the GCBC, egg collection could be trialled once the eggs are already ready to hatch (late May, early June) to avoid mortality related to egg transport, unsuitable conditions in man-made nests and to avoid the biased sex-ratios currently seen in the centre. A much-reduced captive rearing programme would also be much cheaper, freeing up funds for other aspects of gharial conservation. Collection of nests should be limited to a few single nests in highly disturbed area, or those at imminent risk of flooding. A continued programme at a smaller scale would allow the maintaining of education, awareness building and visibility that headstarting allows for (Burke 2015).

Keeping gharial in captivity has multiple issues. Captivity is often stressful for wild animals: they have restricted movement and ability to perform avoidance behaviours, and are kept at unnaturally high densities (Osborne & Seddon 2012). High mortality due to stresses in captivity may also lead to domestication selection. Domestication selection is a selection process that favours individuals that do better in captivity, but go on to have reduced fitness in the wild, and has been identified as an issue for captive rearing for supplementation, possibly in as few as one or two generations in salmonids (Fraser 2008). Headstarting and reinforcement programmes have found captivity to lead to differences in fitness, genetic diversity and morphology, with implications for both post-release survival and long-term population impacts (Fraser 2008; Drumheller et al. 2016; Ryerson 2020). For example, diet in captivity impacted garter snake head size and allometry (Ryerson 2020), or greater variation in skull shape in captive alligators (Drumheller et al. 2016). Multiple studies have found animals that are captive-born are less likely to successfully translocate than wild-caught individuals (Fischer & Lindenmayer 2000; Jule et al. 2008). As well as chronic stress concerns, captive-born individuals may be deficient in skills such as hunting and locomotion, or show unnatural confidence towards humans (Jule et al. 2008).

Leaving more nests in the wild should also be trialled as a measure to improve in-situ hatchling survival. Although there is not a consensus on the drivers of evolution of communal nesting in vertebrates, and whether there is an adaptive benefit (Doody et al. 2009), there are multiple proposed benefits, including reduction in predation risk, savings in energy or cooperative defence (Doody et al. 2021). If communal nesting does have an evolutionary benefit, this could be lost at low population densities when communal nests and consequent creche sizes are small. This may have a negative impact on individual

hatchling survival, acting as an Allee effect (Deredec & Courchamp 2007). If this is the case, the removal of nests from communal breeding sites annually may have a compounding negative impact on the remaining nests. This provides an additional theoretical basis to substantially reduce the number of nests collected from the wild per annum.

7.4.2 Changes to the Gharial Conservation Breeding Centre

In the GCBC staff are capable, careful and perform their duties very well, but they lack the time, resources and training to instigate substantial change, in facilities that will soon be fifty years old. The lack of change in the programme following recommendations (Ballouard et al. 2010; Lang 2016; Acharya et al. 2017) suggests institutional inertia that must be tackled. Straightforward changes that are possible in current facilities are to substantially reduce stocking density, especially in hatchlings and yearlings, completely remove visitor access to hatchlings and yearlings, and create greater thermoregulation gradients for hatchlings to avoid heat or cold stress. The feeding of live fish – at least to new hatchlings to avoid force-feeding, and to release-cohorts prior to release – may help improve survival in captivity and post-release. Feeding of live prey has been found to improve survival of other captive-reared species (Biggins et al. 1999; Tetzlaff, Sperry, & DeGregorio 2019). Environmental enrichment and anti-predator training have been found to improve survival and site fidelity across species (Tetzlaff, Sperry, & DeGregorio 2019; Zhu et al. 2022), and could be considered for gharial in future.

7.4.3 Changes to the release programme

Trialling alternative management recommendations for release procedure, followed by monitoring, is a component of the headstarting programme that lends itself particularly well to adaptive management. Each release cohort of gharial could be split between different release procedures, and the results compared as part of an active adaptive management system (McCarthy et al. 2012). Our results in Chapter 6 suggest that releases should be trialled further upstream on the Rapti. Since the majority of gharial tend to initially disperse downstream, release sites further upstream may improve retention within Chitwan as

animals will be released upstream of suitable habitat and areas of high conspecific density, which may encourage them to settle when they reach these areas. Furthermore, upstream release is further from the barrage, which appears to mostly prevent return of gharial once they pass through it. A more extensive soft release system may also work better, in which gharial are fed in the release pens for a number of weeks. Increased retention times in soft-release pens is associated with improved efficacy in reptiles (Tetzlaff, Sperry, Kingsbury, et al. 2019). I also support recommendations to trial releases in the post-monsoon season, when gharial have just completed a summer of being fed, and will therefore enter the winter season shortly after release (Khadka et al. 2022). In the current system, gharial are still in their exploratory acclimation phase when they reach their first monsoon, which may increase dispersal, or could increase mortality as warm weather leads to higher energy requirements in ectotherms, which gharial may not be able to meet when still learning to catch prey and before having settled into a home range.

I strongly recommend trialling releases of gharial at substantially smaller sizes and younger ages. Our results showed the smaller and younger gharial were more likely to settle into suitable habitat post-release. Conversely, the older and larger gharial did not show lower mortality, suggesting holding onto the gharial longer was not improving their chances of survival post-release. As discussed in Chapter 6, I also suggest that the release of gharial in much smaller size classes may also help, as the first-time gharial get entangled in a net they will be small enough to be disentangled. When catching gharial for the telemetry study in Chapter 4, we found gharial were very aware of what a net was and how to avoid being caught, suggesting free-living gharial in Chitwan can learn to better avoid capture. A large juvenile gharial caught for the first time in a net is a big animal to tackle, and it seems probable that a person would kill this animal rather than try to disentangle it. However, a small gharial may not be intimidating, a person could easily disentangle and release it, and the gharial may then learn to avoid fishing gear.

7.4.4 Individual and species predispositions

Differing behavioural predispositions have been found between crocodilian species, with American alligators found to show more exploratory behaviour and less aversion to novelty

than spectacled caiman (Reber et al. 2021), with implications for juvenile risk-taking and exploration. Such behavioural predispositions are likely to be important for reintroduction success (Merrick & Koprowski 2017; Kelleher et al. 2018). The combined evolutionary and functional uniqueness of the gharial (Griffith et al. 2022) makes it very challenging to infer behavioural predispositions from other species. The exceptional communal creche guardianship of gharial, and observed associations of guardian adult males with yearlings (Lang and Jailabdeen, pers. comm.) suggest an important parental care role that may differ from that of other crocodylians. Reber *et al* (2021) suggest that juveniles of larger crocodilian species may be bolder and more exploratory, due to reduced predation pressure as the parent itself has no predators: gharial fall into this category of care. For the headstarting programme, a species-level behavioural predisposition towards being exploratory or bold may make gharial less suitable for reintroduction than species which may be more cautious. As discussed in Chapter 6, such predispositions may change as gharial age and grow, and therefore certain size classes may be more or less suitable for reintroduction.

Individual personality may also be a factor that could influence patterns we observed, both in terms of settling and non-settling movement patterns, and differences in growth and mortality in captivity. Animal personalities are consistent differences in behaviour between individuals (Germano et al. 2017), and have been found in many species (Cabrera et al. 2021). Personality has been found to influence translocation outcomes in wildlife (Germano et al. 2017; Allard et al. 2019; Wilson et al. 2022), and is a candidate cause of variation in individual post-release behaviours we observed in gharial. Personality can also impact on stress levels in captivity (Cockrem 2007): such an interaction could also drive the differing patterns seen in gharial post-release. For example, studies on captive alligators have found a strong negative relationship between levels of stress hormones and body mass (Elsey et al. 1990; Turton et al. 1997). The older and larger gharials in the GCBC tend to be released later due to having grown slower to release size, therefore it is possible these larger gharials are more chronically stressed in captivity than those that are smaller at release due to having grown faster. Stress may drive animals to disperse more (Dickens et al. 2010) and may explain why the larger gharial were less likely to settle. If personality and stress do interact in gharial, then individual personality may also influence differential mortality in

captivity, and therefore the composition of release cohorts. Shy or reactive individuals tend to have a greater stress response (Cockrem 2013), and therefore may be more prone to chronic stress in captivity, and the consequent elevated mortality such individuals suffer. The surviving juveniles may therefore be disproportionately of the bold or proactive personality type. Captivity has been shown to promote boldness and risk-taking in fish, thought to be maladaptive post-release (Sundström et al. 2004; Roberts et al. 2011).

Gharial – like all crocodylians – are intelligent and display complex social behaviours (Grigg & Kirshner 2015; Doody et al. 2021): the simplistic headstarting approach used in reptile and amphibian translocations (Germano & Bishop 2009) is probably less appropriate than the more complex reintroduction efforts in mammals and birds (Jule et al. 2008; Tetzlaff, Sperry, & DeGregorio 2019). The systems of headstarting developed in the 1970s need radical and considerable updating to reflect our substantial shift in understanding of crocodylians and their behaviour in the last few decades.

7.5 What do we need to do for population recovery in Chitwan and beyond?

The conservation of gharial to date has focused on headstarting alone: this thesis adds to the body of evidence that this is insufficient. Achieving gharial population recovery will require use of a whole set of different conservation tools, of which headstarting is just one, and one that will only be useful in certain instances. Multiple conservation tools will be needed to tackle the current and emergent threats to gharial and their riverine habitats. In this section I will only consider in detail only the threats that this thesis has contributed additional understanding of: bycatch-related mortality, dams/barrages and sand-mining. I will also discuss two needs that are highlighted by our findings: the need for more ecological studies, and for more transboundary collaboration.

Most important, however, is to think of gharial conservation in the context of holistic river conservation. Conservation of the gharial should be part of an integrated approach that looks at the health of the river, the lives, livelihoods and cultural values of the people who live alongside it, and the biodiversity of the basin. Successful freshwater conservation will involve the restoration and safeguarding of flow and connectivity, the protection and

restoration of habitats throughout the system, improved water quality and sustainable exploitation of river resources (Tickner et al. 2020). Riverine systems are hugely interconnected: the river a gharial lives in is intimately connected to groundwater, tributaries hundreds to thousands of miles upstream and land use in catchment areas. Species of fish that gharial eat may spawn in Himalayan streams, the aquifers that recharge rivers in dry season may be drained by factories making clothes for markets on the other side of the world. Gharial are one small piece of this system. Gharial could be a useful flagship and umbrella species, playing a part in advocacy and protection of South Asia's rivers. Gharial need clean, free-flowing rivers with abundant prey, and so conservation measures that try to achieve this could have far-reaching positive impacts. However, if headstarting is the only conservation measure that the flagship gharial instigates, it will be a failure. Repeated restocking of rivers with gharial that do not survive long-term is neither ethical for gharial welfare, nor useful for river conservation. On the other hand, a gharial population with a recovering trend and increasing levels of natural recruitment is an exciting indicator of riverine system doing well.

Furthermore, gharial and river conservation can only succeed when riverside communities are included as key stakeholders (Whitaker 2007; Stevenson 2015). Freshwater conservation needs locally appropriate solutions that improve both ecosystems and the wellbeing of people, with active participation of stakeholders (Everard 2015; Sapkota et al. 2018). Gharial conservation more specifically requires education and awareness, and simply banning river use practices, such as fishing, without providing alternative livelihoods disempowers local people and removes the best potential collaborators in successful conservation (Whitaker 2007; Stevenson 2015). In Chitwan one of the most important emerging sections of gharial habitat outside the National Park is the Baghmara Community Forest, managed by nominated representatives from 1299 households. This community forest river stretch is used by dozens of gharial, including adult males and for nesting by females. Eco-tourism ventures to see the gharial and mugger crocodiles generate income. Following the inclusion of user group members in the tracking and nesting monitoring in Chapters 4 and 6, the committee is now considering how to conduct their own gharial conservation and management actions. This inspiring level of community interest, pride

and commitment to gharial and river conservation shows what can be achieved in community-led conservation.

7.5.1 Ecological Studies

Understanding the ecology and behaviour of species can assist conservation, whereas a lack of behavioural data may lead to programme failure (Oded Berger-Tal et al. 2011). The challenges of studying crocodylians can be best overcome using telemetry (Lang & Whitaker 2010; Kouman et al. 2021) data from which can inform management. For gharial, ecological studies have provided vital insights for conservation management, such as the discovery of adult migration on the Chambal (Lang & Kumar 2016) or the reduction in nesting following succession on sand banks (Vashistha, Mungi, et al. 2021). In Chitwan, telemetry shows gharial are extensively using tributaries in the urban-agricultural matrix of the buffer zone (Griffith, unpublished data).

My results from Chapter 4 show the potential biases and benefits of two different types of telemetry technology for determining gharial home range. Despite differing biases, the home range estimates of both methodologies were very similar using the data processing and analysis methods we propose, showing the cheaper VHF tags still provide high quality data that allows for useful ecological inference. This is particularly important as the cheaper cost of VHF tags also allows more animals to be tagged, which is important for both population-level inference and detecting behaviours that may be rare but nevertheless important for understanding space use (Powell & Mitchell 2012). Additionally, the long battery life of VHF transmitters allowed us to determine reproductive frequency in Chapter 6, informing predictions of nesting and therefore projections for population recovery targets. Gaining a better understanding gharial river use and response to threats will reduce uncertainty surrounding management decisions, both in Chitwan and other sites of gharial occurrence. Our results suggest GPS telemetry will be most useful for sites that are inaccessible, where gharial are very sensitive to human disturbance, or when gharial are likely to move large and unpredictable distances, especially across national or state borders.

7.5.2 International Transboundary Collaboration

Two of the key gharial populations are on the Nepal-India border: the Girwa-Karnali and the Chitwan-Gandak. Successful conservation in the long term will inevitably depend on cooperation and joint conservation efforts between Nepal and India. For example, our results on dispersal in Chapter 6 suggest that for headstarting to work in the Girwa, the gharial would have to be released on the Karnali. Similarly, yearling sightings suggest that successful nesting in the Narayani will lead to yearling dispersal into the Gandak, so these gharial need effective protection on both sides of the border.

7.5.3 Tackling key in-situ threats

Tackling the issue of high bycatch related mortality in Chitwan is important if the gharial population is to recover to a viable population size. However, it is interesting that most of the observed mortality was through the deliberate killing of gharial following bycatch – rather than just bycatch itself. Indigenous fishermen we spoke to, with experience handling gharial, were happy to disentangle gharial from nets and release them safely back into the river. This suggests the primary problem is likely people who are not from indigenous or fishing communities fishing illegally, casually and without traditional knowledge on gharial. Such illegal fishing mainly occurs at night, when gharial will be unlikely to see nets, and without monitoring of the nets since waiting by the riverbank at night is dangerous. This issue is not unique to Chitwan: traditional fishermen in the Vikramshila Gangetic Dolphin Sanctuary report of the issues of anyone being able to buy a net and put it in the river, leaving traditional fishermen without livelihood or, often, legal options to catch fish by traditional non-destructive methods (Colopy 2012).

Potential options for tackling this issue include training licensed fishermen in handling gharial, combined with increasing the number of fishing licenses to traditionally fish-dependent communities to reduce instances of illegal fishing in these groups. Gharial handling training has already been carried out for National Park staff in Chitwan (Khadka & Bhattarai 2022). However, this does not reach people doing casual or non-traditional fishing: environmental outreach and awareness programmes about gharial protection and importance could be attempted through social marketing, or environmental education in

schools or communities (Verissimo et al. 2019) for example as part of the new ‘Crocodile Festival’ initiative in Chitwan. Improving awareness of the risks of and illegality of using gill nets may also help, as interviews have found poor knowledge of the risks of gill nets to wildlife and dangers of human-wildlife conflict when riverbank fishing (Bhatta, unpublished data). Information on how to release crocodiles accidentally caught in nets has been successfully used to reduce mortality on other species, such as the Philippine crocodile (van Weerd & van der Ploeg 2012). Innovative and socially sensitive ways to tackle gharial bycatch-related mortality without undermining the traditional livelihoods of fishing communities is an important area of future management. Such actions should not be limited to Chitwan: tackling bycatch ethically has been and should be tackled across multiple sites, and results of both success and failures should be shared on the Evidence Base. Successful projects reducing bycatch in species such as dolphins may also provide vital evidence to inform the same for gharial.

The issue of the Gandak Barrage remains in Chitwan, and dams remain present throughout most of the gharial range, with new structures planned. Although gharial declines are strongly correlated with dam construction and presence, the mechanism behind this is poorly understood (Lang et al. 2019). The construction of the Gandak Barrage led to the decline in gharial (Tirtha Man Maskey 1989) and near-extinction of dolphins in Chitwan (Paudel et al. 2015). Evidence suggests dolphin declines are linked to low dry-season river discharge (Braulik et al. 2014) which fits with the situation in Chitwan (Paudel et al. 2015), whereas gharial are more resilient to low water levels. The sightings of yearlings very far downstream of nesting sites, as discussed in Chapter 6, suggests movement in juvenile size classes may be a major cause of decline for gharial populations upstream of dams, as well as a cause of failure in headstarting in these populations. We highlight that future research is needed on this, especially for populations that exist in the reservoirs of water control structures. The results in Chapter 4 suggest this type of study is where GPS telemetry will be particularly useful: able to track animals substantial distances, moving unpredictably, and potentially across borders and through politically sensitive water control structures.

Sand mining may well be the major emergent threat to gharial in the 21st century. In Chitwan this is already a major worry: there are already limited suitable nesting sites because of the occurrence of mining along much of the Narayani, and studies have shown

that gharial will not nest if there is insufficient nesting habitat (Vashistha, Mungi, et al. 2021). During the collection of the reproductive ecology data used in Chapter 6, we found gharial did not nest in areas that had been disturbed by sand mining, and that nests were threatened when sand mining began during the incubation period. Not only this, but key gharial prey species such as the mahseer are negatively impacted by sand mining, compounding the impact of dam construction on these migratory species (Tej Kumar Shrestha 1997). Worryingly, the Government of Madhya Pradesh has proposed to the relevant Ministry to legalise sand mining in multiple stretches of the National Chambal Sanctuary – the gharial’s last stronghold – although there is already substantial illegal mining (Gawade 2023). Like water resources (Carrizo et al. 2017), river aggregate resources are potentially challenging to engage public interest and awareness. The gharial is an obvious candidate as a flagship species: it is conspicuously negatively impacted by sand mining, and occurs (or should occur) in many of the rivers that are the new ‘sand frontiers’ of extraction (Bisht 2021). The future of river biodiversity in South Asia will increasingly depend on how governments deal with the sand mining industry: sustainable extraction from rivers to meet projected need will not be possible (Koehnken et al. 2020). The future of the gharial will no doubt depend on the future of sand, and time may be running out to find a solution.

7.6 Concluding remarks

On the recent extinction of megafauna species, Alfred Russell Wallace wrote that “we live in a zoologically impoverished world, from which all the hugest, and fiercest and strangest forms have recently disappeared” (Wallace 1876). I contend that some of the hugest and fiercest species remain – the crocodylians – and among them, as I show in Chapter 2, the strangest of these is no doubt the gharial. Conservation action to save this strange species is needed to preserve the evolutionary heritage and functional role the gharial provides. Effective conservation action based on robust science is needed to improve the status of gharial, and the results of this thesis provide tools for improved ecological research and conservation management of gharial in Chitwan, and across their range. I developed a simple method of processing VHF data, and applied techniques to analyse gharial home

range that will be useful for telemetry studies in gharial and other riverine species. I applied models to estimate gharial population size using repeat counts, illustrating a replicable method for populations estimates and highlighting the issues of unadjusted counts. Finally, I have shown how telemetry data, ecological observations and multiple forms of monitoring data can be combined to understand the impact of headstarting on a population. I recommend similar approaches for other crocodylian headstarting and conservation programmes, and to use this approach to begin a cycle of adaptive management. I recommend results should be shared freely according to the principle of Evidence Based Conservation, and therefore be available to inform management decisions to save not just the gharial, but other species too.

The failures of headstarting that we have identified show that a species-specific gharial programme of conservation doesn't work in isolation: there has to be a healthy, biodiverse river system with sufficient lengths of free-flowing in order to support gharial. Conserving rivers and freshwater biodiversity are major challenges for the future. To co-opt the words of Nepalese zoologist Professor Tej Kumar Shrestha when discussing the precarious status of the river dolphin and mahseer in Nepal: "there is still a vast amount to be done, and no time to be wasted" ... "we must preserve and rehabilitate our rivers one by one, with all the specific knowledge that is entailed in each case. We can do this." I hope this thesis has provided a little more of the specific knowledge that is needed to preserve the gharial, and with that knowledge, a little more confidence that we *can* do this.

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Contributions to Chapters

I declare that this thesis is primarily my own work. The conception and all data collection was collaborative, with additional contributions to the level of co-authorship listed below, although I recognise and thank you everyone who contributed to chapters at any level. All analyses and writing are my own. Since each chapter was collaborative I use the third person plural 'we' throughout.

Chapter 1:	Phoebe Griffith
Chapter 2:	Phoebe Griffith, Jeffrey W. Lang, Samuel T. Turvey, Rikki Gumbs
Chapter 3:	Phoebe Griffith
Chapter 4:	Phoebe Griffith, Bed Bahadur Khadka, Prakash Basnet, Alina Chaudhary, Basanta Mahato, Aitaram Bote, Ambika Chaudhary, Saroj Mani Poudel, Babu Ram Lamichhane, Tulsi Subedi, Prabin Shreshtha, Prakash Sigdel, Bhogendra Rayamajhi, Laxman Prasad Poudyal, Claudio Sillero, Samuel T. Turvey, Jeffrey W. Lang
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Chapter 6:	Phoebe Griffith, Bed Bahadur Khadka, Prakash Basnet, Alina Chaudhary, Basanta Mahato, Ambika Chaudhary, Aitaram Bote, Saroj Mani Poudel, Babu Ram Lamichhane, Tulsi Subedi, Prabin Shreshtha, Prakash Sigdel, Bhogendra Rayamajhi, Laxman Prasad Poudyal, Claudio Sillero, Samuel T. Turvey, Jeffrey W. Lang
Chapter 7:	Phoebe Griffith

Appendices



Appendix 1

Supporting Information for Chapter 2, Using functional traits to identify conservation priorities for the world's crocodylians

Authors: Phoebe Griffith, Jeffrey W. Lang, Samuel T. Turvey, Rikki Gumbs

Table S1: source of data for all museum specimens or published illustrations included in the skull analysis

Species	Museum Specimens Examined	Illustrations Examined
Alligator mississippiensis	MCZ 33949, MCZ 81457, TMM-M-67, TMM-M-253, TMM-M-983, TMM-M-4135, TMM-M-4863, TMM-M-4864, TMM-M-7388, TMM-M-7487, TMM-M-7488, TMM-M-7846, UCMP-131080, NHMUK 1868.2.12.6, NHMUK 1974.3010, NHMUK 2002.3, NHMUK 1873.2.21.2	
Alligator sinensis	MCZ 17550, SNHM 40071, SNHM 49685, SNHM 49685, SNHM 3219a, NHMUK X184, RBINS 3487	Cong, L., Hou, L., Wu, X., & Hou, J. (1998). The gross anatomy of Alligator sinensis Fauvel [in Chinese]. China Forestry Publishing House, Beijing.
Crocodylus acutus	AMNH 7121, AMNH 7856, AMNH 7857, AMNH 9659, AMNH 15175, AMNH 15182, AMNH 28653, AMNH 43299, AMNH 66392, AMNH 66637, AMNH 136528, FMNH 5775, FMNH 11038, FMNH 11041, FMNH 11042, FMNH 11043, FMNH 11057, FMNH 13220, FMNH 20159, FMNH 22934, FMNH 28847, FMNH 34563, FMNH 59070, FMNH 69884, FMNH 69886, FMNH 74923, FMNH 74924, NMNH 115353, NMNH 247943, NMNH 258735, NMNH 211273, NHMUK 1975.997	
Crocodylus halli	LSUMZ 44734, LSUMZ 44735, LSUMZ 44736, LSUMZ 44740, LSUMZ 44745, LSUMZ 44738, LSUMZ 44741, LSUMZ 44746, LSUMZ 44744, LSUMZ 44742	

<i>Crocodylus intermedius</i>	FMNH 75659, NMNH 21181, NHMUK 1862.10.19.1, NHMUK 1851.8.25.29, NHMUK 51-8-25-29, NHMUK 62-10-19-1, SNHM 28139, USNM 211281
<i>Crocodylus johnsoni</i>	MCZ 58790, TMM M 6807, NMNH 299810, TMM M 6807, SNHM 54425, SNHM 54426, USNM 299810, USNM 64091
<i>Crocodylus mindorensis</i>	SNHM 2888, SNHM 2889, FMNH 21904, Naturalis 37505, NMNH 228407, USNM 37435
<i>Crocodylus moreletii</i>	FMNH 4432, NMNH 52335, NHMUK 1861.4.1.4, SNHM 28169, SNHM 56317, TMM M 4980, TMM M 5491, USNM 52335
<i>Crocodylus niloticus</i>	NHMUK 1865.4.6.2, NHMUK 1889.9.20.1, NHMUK 1904.10.10.1, NHMUK 1927.4.23.2, NHMUK 1934.6.3.1
<i>Crocodylus novaeguineae</i>	FMNH 2854, FMNH 14039, FMNH 14040, FMNH 14043, FMNH 14044, FMNH 14047, FMNH 14048, FMNH 14050
<i>Crocodylus palustris</i>	MCZ 42174, SNHM 28168, SNHM 2915, NHMUK 1852.5.9.46, NHMUK 1868.4.9.11, NHMUK 1897.12.31.1, NHMUK 1929.8.6.2, NHMUK 1861.4.1.5, NHMUK 1845.1.8.204,
<i>Crocodylus porosus</i>	FMNH 15230, FMNH 15231, FMNH 15232, FMNH 28466, FMNH 28469, NMNH 37839, NMNH 210131, NHMUK 1847.3.5.33, NHMUK 1865.8.22.1, NHMUK 1867.4.2.188, NHMUK 1897.12.31.2, NHMUK 1952.12.9.2, NHMUK 1960.9.29.2, NHMUK 1847.3.5.33, NHMUK 1886.5.20.3
<i>Crocodylus rhombifer</i>	AMNH 6178, AMNH 6179, AMNH 6180, AMNH 6181, AMNH 6185, AMNH 57773, AMNH 77595, AMNH R154087, AMNH CF75033, NMNH 216197, SNHM 37081
<i>Crocodylus siamensis</i>	NHMUK 1920.1.20.1626, NHMUK 1921.4.1.168, NHMUK 1921.4.1.171, NHMUK 1929.11.23.1, NHMUK 1931.12.16.6, NHMUK 1931.12.16.7, NHMUK 1921.4.1.172, MNHN 198026, MCZ 17574, SNHM 28167, SMF 28167
<i>Crocodylus suchus</i>	NHMUK 1864.8.8.12, NHMUK 1904.9.9.1, NHMUK 1959.1.8.55, NHMUK 1974.3015

Caiman crocodilus	FMNH 6126, FMNH 69841, FMNH 69842, FMNH 69844, FMNH 69849, FMNH 69851, FMNH 69852, FMNH 69858, FMNH 69859, FMNH 69862, FMNH 69863, FMNH 73683, FMNH 73684, FMNH 73685, FMNH 73687, FMNH 73690, FMNH 73692, FMNH 73694, FMNH 73698, FMNH 73699, FMNH 73700, FMNH 73704, FMNH 73707, FMNH 73708, FMNH 73709, FMNH 73713, FMNH 73719, FMNH 53653, FMNH 69812, FMNH 69813, FMNH 69814, FMNH 69821, FMNH 69828, FMNH 69830, FMNH 69831, FMNH 73438, FMNH 73440, FMNH 73441, FMNH 73443, FMNH 73446,
Caiman latirostris	FMNH 9713, MACN 7375, MACN 30566, MACN I 7021, MAS W/N, MLP-R 5038, MLP-R 5043, MLP-R 5364, MLP-R 5802, MLP-R 5803, MLP-R 5804, MLP-R5806, MLP-R 5809, MLP-R 5810, MLP-R 5811, MLP-R5812, ZSM 298 1925, NHMUK 86.10.4.2
Caiman yacare	AMNH 97300, MACN 30531, MACN 30536, MACN 30542, MACN 30548, MACN 30552, MACN 30553, MACN 30555, MACN 30595, MACN 30602, MACN 30637, MACN 30638, MACN 43694, MACN-I 15144, MACN-I 8262, MACN-I 8265, MACN-I 8267, MACN-I 8268, MACN-I 8270, MLP 604, MLP-R 5041, MLP-R 5042, MLP-R 5044, MLP-R 5045, MLP-R 5046
Gavialis gangeticus	FMNH 82681, FMNH 98864, NHMUK uncat., NHMUK 1846.1.7.3, NHMUK 1935.6.4.1, NHMUK 1974.3009, NHMUK 704, NHMUK 96.7.7.4, NHMUK 1897.6.28.2, NHMUK 1845.1.8.203, NHMUK 2005.1601, MCZ 33950, MCZ 46551, TMM M 5485, TMM M 5486, TMM M 5487, TMM M 5490, TMM M 5490, SNHM 28133, SNHM 37951, SNHM 49529, UF 118998, UMZC R 5792
Mecistops cataphractus	AMNH R107634, MCZ R22483, NHMUK 86.12.30.2, NHMUK 1904.9.9.2, SNHM 54119
Mecistops leptorhynchus	AMNH R10075, AMNH R29300, AMNH R160902, MCZ R54251, Salamanca 971123, UAMZ R803, ZMA 13064

Melanosuchus niger	NHMMUK 45.8.25.125, NHMW 2024, NHMW 2025, SMF 28182, SMF 40171, ZSM 1/1906, ZSM 12/1911, ZSM 14/1911, ZSM 27/1911, ZSM 35/1911, ZSM 46/1911, ZSM 52/1911, ZSM 57/1911, ZSM 63/1911, ZSM 64/1911, ZSM 67/1911, ZSM 68/1911, ZSM 70/1911, ZSM 75/1911, ZSM 76/1911, ZSM 77/1911, ZSM 79/1911, ZSM 84/1911, ZSM 85/1911, ZSM 86/1911, ZSM 87/1911, ZSM 90/1911, ZSM 91/1911, ZSM 223/1925, ZSM 2416/2006, ZSM 3039/0	
Osteolaemus afzelli	MNHN 1991.4488, RBINS 10553, RMCA 97.67.R.1, NHMMUK 1957.1.4.42	
Osteolaemus osborni	RMCA 83.06.R.3, RMCA 83.06.R.4, RMCA 83.06.R.5, RMCA 83.06.R.6, RMCA 10984, RMCA 14999, RMCA ZooSkeleton1	
Osteolaemus tetraspis	RMCA 5401, UKNHM 62.6.30.5, UKNHM 62.6.30.6	
Paleosuchus palpebrosus	NHMMUK 45.8.25.126, UF 62509, UF 75020, UF 75023, UF 87980, Naturalis 37550, MNHN 197706, SNHM 43957, SNHM 40064,	Medem, F. (1958). The crocodilian genus Paleosuchus. Fieldiana Zoology, 39.
Paleosuchus trigonatus	NHMMUK 68.10.8.1, MCZ 84030, MNHN 197705, MNHN 1978, SNHM 36730, SNHM 50399, SNHM 51662, TMM M 11981, USNM 213705, USNM 234047, USNM 317555,	Medem, F. (1958). The crocodilian genus Paleosuchus. Fieldiana Zoology, 39.
Tomistoma schlegelii	FMNH 206755, GMZ X1227, NHMMUK 99.1.31.1, NHMMUK 48.10.31.19, NHMMUK 60.11.6.8, NHMMUK 94.2.21.1, NHMMUK W/N, MCZ 12459, SNHM 28135, TMM M 6342, TMM M 6820, WI 22 19, UCMP 81702 481, ZSM370	

Supporting Methods and Results

Supporting Methods

Crocodylian Species

For this analysis, we included 28 crocodylian species. These include the 26 currently recognised crocodylian species (Reptile Database 2021), as well as two additional species of *Osteolaemus*, *O. osborni* and *O. afzelli*, in addition to the currently recognised *O. tetraspis* (Shirley et al. 2015), as recommended by multiple phylogenetic studies (Oaks 2011; Colston et al. 2020). For phylogenetic analyses we used the dated phylogenies from Colston et al. (2020), which contain 27 of the species in our dataset. The 28th, *Crocodylus halli*, has been recently described by Murray et al. 2019. As the species is not in the Colston *et al.* trees, we excluded it from phylogenetic analyses, and for analyses comparing phylogenetic and functional information both datasets from this species were excluded.

Construction of Traits

Functional trait data was collected from peer-review literature, IUCN Red List Assessments, publications of the IUCN Species Survival Commission (SSC) Crocodile Specialist Group (newsletters, proceedings and action plans), crocodylian.com (hosted by the University of Bristol, the Florida Museum of Natural History and the IUCN SSC Crocodile Specialist Group, textbooks and theses. When values were not published, some values were decided based on personal communication with scientists working with the particular species, especially for terrestriality.

Where there were multiple values across studies or across geographical range, or where values were secondary data from studies that had themselves compiled various heterogeneous samples, we took the mean value for each trait for that species. Where species have been split we tried to use data with a geographical attachment so that values could be assigned to the correct species.

1. Male Max Size

- Size of crocodylians is a highly important determinant of life history traits and ecological role (O'Brien et al. 2019), and also has an important influence on other included functional traits which scale with size e.g. bite force (Erickson et al. 2012). Total length was the most widely documented size measure of crocodylians, so maximum male size was included for each species.

2. Female size at maturity

- Firstly, female length at maturity likely provides a more typical size of the species compared to the maximum male size as female crocodylians tend to reduce length growth rates after maturity (G. Grigg & Kirshner 2015).

Secondly, female size at maturity is important in determining the investment in growth before first reproductive event. The majority of crocodilian females mature between 6 to 15 years, although within-species variation in better-studied species is considerable (e.g. 6-18 years for female alligators, 9-24 years for *C. johnsoni*), with such variation thought to be driven by a number of factors, including climate, seasonality, habitat, food availability and social factors including density (Tucker et al. 2007). Since the values of age at reproduction are broadly overlapping for most species, our chosen value likely indicates a key aspect of investment in growth before first reproductive event and variation in this between species, as well as an informative size measure.

- However, certain Caimans, especially *Caiman crocodilus*, have been known to mature much young, as young as 3 years. Consequently, our simple female age at maturity value obscures this biologically meaningful difference, most notably between *Caiman crocodilus* which matures at ~120cm in 3-6 years, vs *Paleosuchus trigonatus* which may take up to 11 years to mature at ~110cm. A preferred measure of investment in reproduction would account for growth rate, or a combination of size and age at maturity and provide an index measure for investment in growth pre-reproduction. Such information is not currently available for most crocodylians, so currently female size at maturity is the best available measure: we recommend future functional databases of crocodilians to be updated if this data is published.

3. Relative Clutch Mass

- Relative Clutch Mass (RCM) was calculated after Thorbjarnarson (1996), where RCM was clutch mass divided by female mass. Clutch mass was either directly reported, or calculated as mean clutch size multiplied by mean egg mass for the species. Mean adult female mass was used as the value of female mass (Thorbjarnarson 1996), and where not available was calculated based on length-mass relationship. Relative Clutch Mass was selected as a comparable measure of reproductive investment between species.

4. Size-Standardised Bite Force

- Bite force measures were taken from Erickson et al. (2012) and were size-standardised using the same methodology as that study. Bite force is likely to impact on dietary ecology, with species able to tackle different prey, however it varies independently of rostral morphology, our other selected trait to reflect diet (Erickson et al. 2012). Size-standardised variation in bite force shows minimal variation between most species, instead scaling isometrically to body mass (Erickson et al. 2012). Crocodilians with low bite forces (Gavialids) are therefore likely to have different biomechanical constraints on prey selection compared to individuals of other species at the same size.

5. Skull Shape:

- Although all crocodylians have a generalist carnivorous diet, it is hypothesised that the shape of the skull is a key determinant of diet, as the

hydrodynamics and structural mechanics of the skull will influence prey acquisition and predation techniques (Busbey 1995; Pierce et al. 2008; Pearcy 2011). Given the paucity of study of crocodylian diet in the wild, we decided to use crocodylian skull as a proxy of diet.

- In order to have numerical values that summarise differences in skull shape between species, we examined cranial shape variation across all crocodylian species. We examined 367 cranial specimens using images collected for previous cranial variation studies (see Table S1). We only included adult or large subadult specimens of each species, with a minimum of three specimens per species. Cranial variation for each specimen was characterized using geometric morphometrics and subsequently generalised Procrustes analysis, after the method of Okamoto et al (2015). Eight landmarks were used that have been shown to quantify and characterise snout-shape variation across extant crocodylians, (Pearcy 2011; Okamoto et al. 2015) and for our trait database we used the median per-species value (Foth et al. 2018) of the first two Principal Components (since these explained 78.74% of the variance) of the skull analysis to quantify cranial shape variation among 77 species, weighted to create a single trait in analyses.

6. Nest Type

- Categorical classification of either building a mound nest from vegetation or hole nest in the substrate, or both. This trait was selected as it impacts the potential role of mound-nesting crocodylians to act as ecosystem engineers, for example by creating elevated structures or vegetation islands (Somaweera et al. 2020). This trait also strongly influences habitat selection for reproductive events, with occurrence of suitable nesting habitat influencing species occurrence, and reproductive effort (Vashistha, Lang, et al. 2021).

7. Communal Nesting

- Binary classification of either communal nesters or non-communal. Communal nesters were those species which have been recorded nesting communally, non-communal nesters were those species for which this behaviour has not been recorded.

8. Terrestriality

- Terrestriality was determined based on published records of overland movement (e.g. direct observations, telemetry data, news reports), and unpublished records through personal communication. For the small number of species with no available data on movement, terrestrial ability was estimated based on personal communication with experts, combined with inference using two proxies which have been suggested to reflect terrestrial ability.
- The first proxy was relative tail length (species with a longer relative tail length are thought to be more aquatic (Wermuth 1964; Pearcy 2011) as measured by Wermuth (1964). We adjusted Wermuth's ranking to account

for similar skull morphology (i.e. longirostrine, mesorostrine, brevirostrine) as skull hydrodynamics likely influence morphological evolution more broadly. The second proxy was humeral torsion, as measured by Meers (2000). As known highly aquatic species had very high humeral torsion, and known very terrestrial species had low humeral torsion values, values were used to gauge terrestriality relative to other species. Terrestrial ability was estimated by comparing species to others in the rankings of these two proxies, and placing them in categories with species with a similar relative tail length and/or similar values of humeral torsion.

- We used the following ordinal classification of terrestriality:
 1. Highly Terrestrially Capable: able to spend significant periods of time on land, including hunting terrestrial prey.
 2. Moderately Terrestrially Capable: evidence of large and/or regular overland movements, multiple records of movements >4km
 3. Terrestrially Capable: overland movements tend to be <4km
 4. Moderately Aquatic: overland movements trend to be <1km
 5. Highly Aquatic: only very short overland forays recorded
 6. Exceptionally Aquatic: incapable of even small overland forays due to the mechanics of movement and inability to high walk (only *Gavialis gangeticus* was placed in this category)

9. Burrow

- Binary classification of recorded ability to dig burrows vs no records of digging burrows. When species have been known to dig burrows in captivity but not the wild, we classed them as able to dig burrows, as we assumed this was a constraint of the wild habitat they are currently found in (as this discrepancy was found in species with highly reduced ranges). We included this trait as burrowing is a trait that influences survival (as a sleep-or-hide trait, (Liow et al 2009) and also the role of crocodylians as potential ecosystem engineers as burrows create a range of opportunities for other species including dry-season refuges, foraging sites and nesting sites (Somaweera et al. 2020).

10. Aestivation

- Binary classification of recorded ability to aestivate vs no records of aestivation. Aestivation is here defined as 'dormancy to survive periodic or seasonal episodes of drought, high temperatures, and low food availability' characterised in reptiles by inactivity and fasting (Secor & Lignot 2010). We included this trait as burrowing is a trait that influences survival and ability to persist in certain seasonal conditions (as a sleep-or-hide trait, (Liow et al 2009).

11. Cold Tolerance

- A single trait comprised of two equally weighted components: the first is maximum latitudinal occurrence of the species, determined through IUCN Red List distributions, or from historical records where current distribution is known to be reduced from recent historical distributions. The second

component is a binary classification of recorded ability to brumate in response to cold temperatures. Brumation is here defined as dormancy in response to cold temperatures (Grigg & Kirshner 2015). To distinguish from aestivation, crocodylians were only included that show high levels of cold tolerance i.e. ambient temperatures close to freezing (0°C). We included this trait as brumation is a trait that influences survival and ability to persist in cold conditions (as a sleep-or-hide trait, (Liow et al 2009), and can substantially influence the available habitat to crocodylians latitudinally, with alligators being the only temperate crocodylians.

12. Salt Tolerance

- Salt tolerance is likely to be important for oceanic crocodylian dispersal, movement between river basins through coastal dispersal, and connection between saline and freshwater ecosystems (Taplin & Grigg 1989; Nicola & Matzke 2019). Data used for determining salt tolerance were function of lingual salt glands and recorded occurrences of species in saltwater, brackish water and hyperosmotic water. We then used the following ordinal classification of salt tolerance:
 1. Species primarily or extensively associated with saltwater, highly functional lingual salt glands (average recorded lingual gland max Na secretion rate > 45 $\mu\text{mol}/100\text{g}0.7\text{h}$)
 2. Primarily freshwater but known to occupy brackish waters. Species which have also been shown to have functional salt glands (average recorded lingual gland max Na secretion rate 30-44 $\mu\text{mol}/100\text{g}0.7\text{h}$) and/or tolerance of living and feeding in hyperosmotic water.
 3. Primarily freshwater but known records from freshwater or brackish water. Includes alligatorids known to regularly use saltwater/brackish habitats but that are killed by hyperosmotic water. Species which have also been shown to have salt glands with low function (average recorded lingual gland max Na secretion rate 5-30 $\mu\text{mol}/100\text{g}0.7\text{h}$).
 4. Freshwater restricted. No (or very few) records of occurrence in saltwater or brackish waters (average recorded lingual gland max Na secretion rate <5 $\mu\text{mol}/100\text{g}0.7\text{h}$).

13. Generality

- Habitat generalism is crucial for determining how crocodylians may form links between different habitats, how they will respond to habitat loss or alternation, and to system changes such as climate change. A value for generality was constructed based on the IUCN Red List Habitats Classification Scheme (Version 3.1). Each species was assigned a value that
- corresponded to the number of habitat types within the Scheme they occurred in (IUCN code 1-17). As crocodylians predominantly occupy inland wetlands, in order to capture the more specific variation in generality in wetland use, the Wetlands (inland) class (IUCN code 5) was split into the second level of the hierarchy (i.e. code 5.1-5.18), leading to a theoretical

minimum score of 1 and maximum score of 35. We used the Red List Assessments to determine how many habitats these species occurs in. Where the Assessment contained insufficient detail, species were assigned to habitat categories based on available literature. If we found records in the literature of a species occurring in a habitat not included in its Red List assessment, we added that category.

Human Footprint Index values across crocodylian ranges

To compare human impact between the ranges of different crocodylian species, we used the 2009 Human Footprint Index (HFI). The range of each crocodylian species was taken from the Global Assessment of Reptile Distributions (GARD) (Roll et al. 2017), and using QGIS we determined the % of each species range which had a HFI impact value of >3. The value 3 has been shown to be a threshold value for extinction risk related to human impacts (Di Marco et al. 2018). We also determined the % each species range which was <6, >20 and >30, which are other suggested threshold values. Where species have been split after their inclusion in the GARD we split the distribution according to published information on new species distribution. The results are shown in Table S3.

Supporting Results

Functional Groupings

The clustering of species within the functional dendrograms showed a series of functional groupings (groupings A, B, C and D). These same groupings were seen in average trees generated from the 100 unique functional dendrograms; however, certain functionally distinctive species fit less well in the groupings. In particular, *Crocodylus porosus* was in Group A (or unresolved group A/B) in 52% of trees, rather than group C, and *Gavialis gangeticus* was outside of all groups in 77% of trees. *Crocodylus siamensis* was also not well positioned, being placed in Group A in 34% of trees, and Group D in 56% of trees. Repeating the analysis excluding measures of size did not change the general grouping of species, other than to closely ally *Crocodylus johnsoni* more often with *C. intermedius* and *C. acutus*. The fact that species with high functional distinctiveness scores do not 'sit comfortably' in these functional groupings suggests looking at both functional groupings and distinctiveness values will give the best indication of a species functional role.

Human Footprint Index values across crocodylian ranges

Species	Percentage of Species Range			
	>3	>6	>20	>30
Alligator mississippiensis	91.6	56.6	15.0	7.5
Alligator sinensis	98.9	98.9	43.0	12.3
Caiman crocodilus	39.3	23.0	1.1	0.3
Caiman latirostris	77.9	53.2	3.1	0.9
Caiman yacare	63.7	37.1	1.6	0.4
Crocodylus acutus	90.1	59.4	4.6	1.5
Crocodylus halli	47.3	29.2	0.1	0.0
Crocodylus intermedius	37.7	14.5	0.7	0.1
Crocodylus johnsoni	29.4	8.2	0.0	0.0
Crocodylus mindorensis	99.1	90.8	13.4	1.9
Crocodylus moreletii	90.2	49.7	4.0	1.4
Crocodylus niloticus	81.9	45.1	1.2	0.4
Crocodylus novaeguineae	64.8	31.7	0.2	0.0
Crocodylus palustris	98.0	89.2	20.1	2.8
Crocodylus porosus	77.2	58.5	8.4	1.4
Crocodylus rhombifer	97.4	81.9	6.6	1.4
Crocodylus siamensis	81.3	58.3	8.0	1.6
Crocodylus suchus	89.5	58.3	1.8	0.2

<i>Gavialis gangeticus</i>	98.7	88.3	17.7	2.3
<i>Mecistops cataphractus</i>	98.3	72.7	2.8	0.4
<i>Mecistops leptorhynchus</i>	75.3	29.1	0.3	0.0
<i>Melanosuchus niger</i>	23.5	9.7	0.2	0.0
<i>Osteolaemus afzelli</i>	99.0	71.5	2.8	0.4
<i>Osteolaemus osborni</i>	70.8	22.8	0.1	0.0
<i>Osteolaemus tetraspis</i>	68.5	24.0	0.3	0.1
<i>Paleosuchus palpebrosus</i>	39.5	22.8	0.8	0.2
<i>Paleosuchus trigonatus</i>	16.4	5.9	0.2	0.0
<i>Tomistoma schlegelii</i>	76.6	53.7	5.2	1.1

Table S3: the percentage of the range of each crocodylian species which is above or below the specified level of impact, according to the 2009 Human Footprint Index

Extinction Projections

Scenario	P(ext) values	Median FD loss	Median Percentage Loss	Lower Quartile	Upper Quartile
Current Threat Status	Optimistic	0.6470645	31.649	29.099	34.492
Current Threat Status	Pessimistic	0.7858939	38.447	35.804	41.014
Random Threat Status	Optimistic	0.4094697	20.274	15.460	27.018
Random Threat Status	Pessimistic	0.6184419	30.231	24.460	34.829
All Threatened Species Extinct		0.9793814	47.902	45.616	49.873
Random Species Extinct		0.8019847	40.694	34.892	44.963

Table S4: Outcomes of different extinction scenarios on FD (functional diversity). Current Threat Status represents the model where species are allocated their known IUCN threat category, whereas Random Threat Status models randomly allocate threat status to species.

Scenario	P(ext) values	Median PD loss	Median Percentage Loss	Lower Quartile	Upper Quartile
Current Threat Status	Optimistic	115.3566	18.43	17.52	20.02
Current Threat Status	Pessimistic	191.2073	31.15	26.61	33.54
Random Threat Status	Optimistic	105.3204	17.07	13.25	20.24
Random Threat Status	Pessimistic	161.9886	25.64	22.08	29.98
All Threatened Species Extinct		270.7018	43.45	42.90	44.22
Random Species Extinct		219.2966	35.21	31.02	39.34

Table S5: Outcomes of different extinction scenarios on PD (phylogenetic diversity). Current Threat Status represents the model where species are allocated their known IUCN threat category, whereas Random Threat Status models randomly allocate threat status to species.

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Appendix 2

Supporting information for Chapter 5: estimating detectability, abundance and population trends of a reinforced population of gharial in Chitwan National Park, Nepal

Table S5.1 unadjusted count data for surveys 2018-2021 conducted by the NTNC, Himalayan Nature and ZSL for the DNPWC in Chitwan National Park, Nepal. Counts of all yearlings, and juveniles possibly released that year have been removed, and the start/finish locations of some of the transects start/end locations have been adjusted from report publications to better encompass natural river features

	2018			2019			2021		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Lothar	3	4	4	8	4	5	3	4	6
Jingagani ghat	8	9	12	11	13	14	10	9	12
Sauraha	26	25	30	26	9	28	32	31	30
Kasara	20	19	22	7	12	19	18	18	18
Arunkhola	7	11	5	8	8	18	9	12	15
Amaltari (outer branch)	3	6	5	1	4	4	2	3	3
Amaltari (inner branch)	7	7	9	6	7	10	10	7	6
Gholaghat	25	30	34	25	22	34	32	32	28
Giddeni (western branch)	8	6	9	9	10	11	13	14	12
Ganjipur (central branch)	11	12	15	4	13	16	8	8	12
Tinbhangalo (eastern branch)	10	21	13	9	7	11	8	14	16
Devghat	1	0	0	0	0	0	0	0	0
Total	129	150	158	114	109	170	145	152	158

Table S5.2 Predicted sighting fraction, estimated population size and estimated density of gharial in Chitwan, estimated using the sighting fraction method of Messell (1981)

Messell's Equation (1981)

River	Transect Start	Sighting Fraction			Estimated Population Size			Encounter Rate		
		2018	2019	2021	2018	2019	2021	2018	2019	2021
Rapti	Lothar	0.73	0.71	0.72	5 ± 1.45	8 ± 2.85	6 ± 2.40	0.38	0.61	0.46
	Jindagani Ghat	0.51	1.15	0.65	19 ± 3.97	11 ± 1.50	16 ± 2.68	1.57	0.91	1.32
	Sauraha	0.77	1.40	0.82	35 ± 2.95	15 ± 3.23	38 ± 1.72	1.74	0.74	1.88
	Kasara	0.78	0.43	0.62	26 ± 2.21	29 ± 8.40	29 ± 0.00	1.37	1.52	1.52
	Devghat	0.33			1 ± 0.33	0 ± 0.0	0 ± 0.00	0.05	0.00	0.00
	Gidenni	0.70	0.91	0.81	11 ± 2.48	11 ± 1.54	16 ± 1.72	0.44	0.44	0.63
Narayani	Ganjipur	0.84	0.69	0.72	15 ± 2.39	16 ± 5.09	13 ± 2.96	0.89	0.95	0.77
	Tinbhangalo	0.61	0.56	0.51	24 ± 5.46	16 ± 3.52	25 ± 5.64	1.23	0.82	1.28
	Gholaghat	0.76	0.79	0.77	39 ± 3.91	34 ± 4.41	40 ± 2.78	2.26	1.97	2.32
	Amaltari (outer)	0.78	0.50	0.53	6 ± 2.22	6 ± 3.69	5 ± 1.99	0.32	0.32	0.27
	Amaltari (inner)	0.77	0.70	0.70	10 ± 1.96	11 ± 2.90	11 ± 2.90	0.72	0.79	0.79
	Arunkhola	0.70	0.57	0.60	11 ± 3.51	20 ± 5.94	20 ± 4.04	0.72	1.31	1.31
TOTAL		0.69	0.76	0.68	202 ± 33	177 ± 43	219 ± 28	0.97	0.86	1.05

Table S5.3 Predicted sighting fraction, estimated population size and estimated density of gharial in Chitwan, estimated using the sighting fraction method of King (1990)

King's Equation (1990)

River	Transect Start	Sighting Fraction			Estimated Population Size			Encounter Rate		
		2018	2019	2021	2018	2019	2021	2018	2019	2021
Rapti	Lothar	0.73	0.56	0.57	5 ± 1.45	10 ± 3.60	8 ± 3.04	0.38	0.77	0.58
	Jindagani Ghat	0.68	0.77	0.74	14 ± 2.99	16 ± 2.23	14 ± 2.33	1.18	1.35	1.15
	Sauraha	0.80	0.49	0.90	34 ± 2.84	43 ± 9.25	35 ± 1.56	1.67	2.13	1.71
	Kasara	0.83	0.48	0.95	24 ± 2.08	26 ± 7.25	19 ± 0	1.28	1.36	0.99
	Devghat	0.22			2 ± 1.5	0	0	0.07	0.00	0.00
Narayani	Gidenni	0.69	0.80	0.83	11 ± 2.51	13 ± 1.75	16 ± 1.69	0.44	0.50	0.62
	Ganjipur	0.73	0.46	0.65	17 ± 2.78	24 ± 7.65	14 ± 3.29	1.04	1.43	0.86
	Tinbhangalo	0.55	0.67	0.59	27 ± 6.09	13 ± 2.96	22 ± 4.88	1.37	0.69	1.11
	Gholaghat	0.74	0.66	0.83	40 ± 4.03	41 ± 5.29	37 ± 2.55	2.33	2.37	2.14
	Amaltari (outer)	0.59	0.45	0.67	8 ± 2.95	7 ± 4.06	4 ± 1.58	0.42	0.35	0.21
	Amaltari (inner)	0.74	0.63	0.63	10 ± 2.03	12 ± 3.22	12 ± 3.22	0.74	0.87	0.87
	Arunkhola	0.54	0.48	0.65	14 ± 4.52	23 ± 6.96	19 ± 3.76	0.93	1.53	1.22
TOTAL		0.65	0.59	0.73	207 ± 36	228 ± 54	198 ± 28	0.99	1.11	0.95

Appendix 3

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

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

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Abstract: Supplementation of wild populations of the Critically Endangered Gharial *Gavialis 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 prediction 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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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 genetic 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 and 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

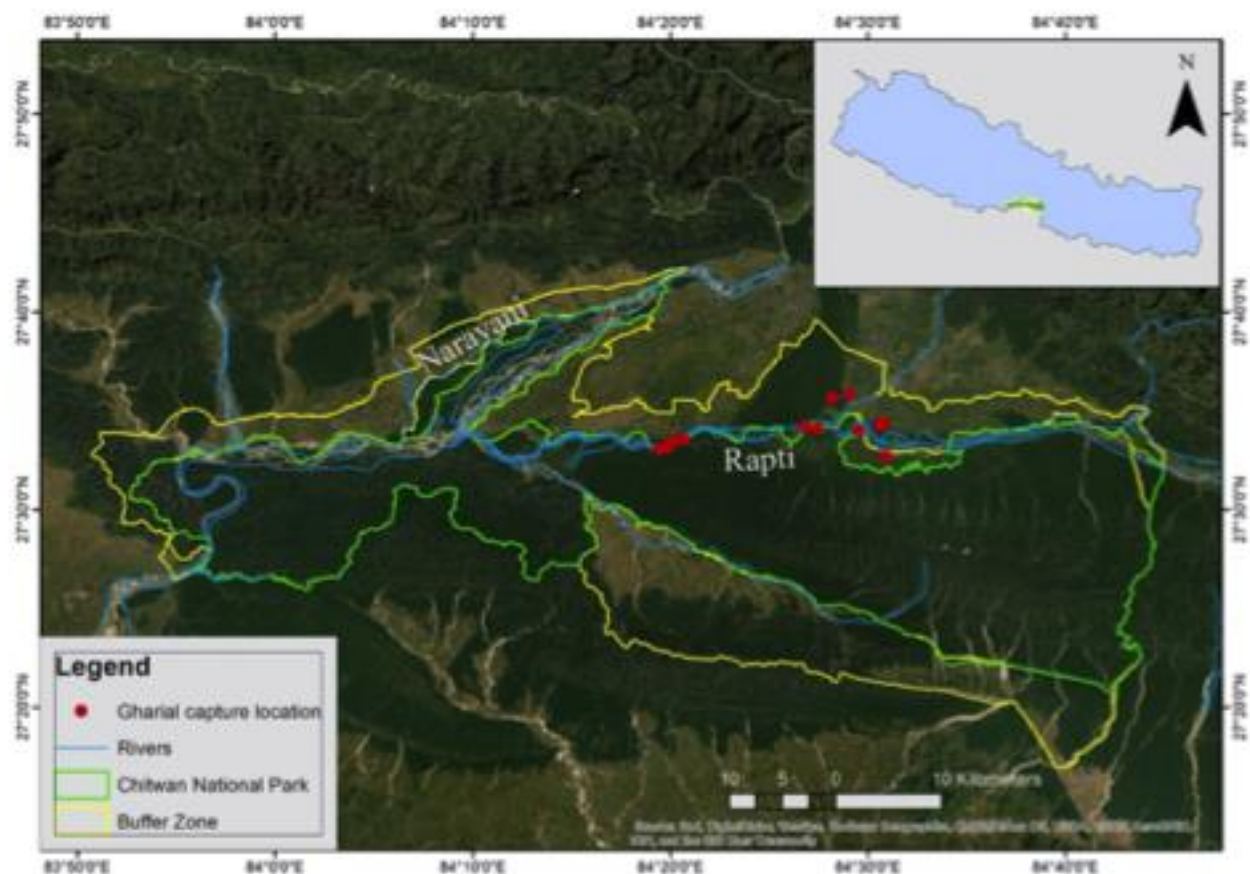


Image 1. Map of the Study Site in Chitwan National Park. The major rivers (Rapti and Narayani) are labelled, and locations of Gharial capture are indicated. Inset shows position of Chitwan National Park in Nepal.

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: $\text{mass} = 1.3602 \cdot \text{TL}^{3.7175}$ ($R^2 = 0.9824$, $n = 18$). 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 ($B = 10.18 \pm 2.80$, $t = 7.86$, $p < 0.01$) 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 ($B = 17.94 \pm 3.93$, $t = 9.42$, $p < 0.01$) 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.

Quantile	Value of Regression Coefficient	
	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

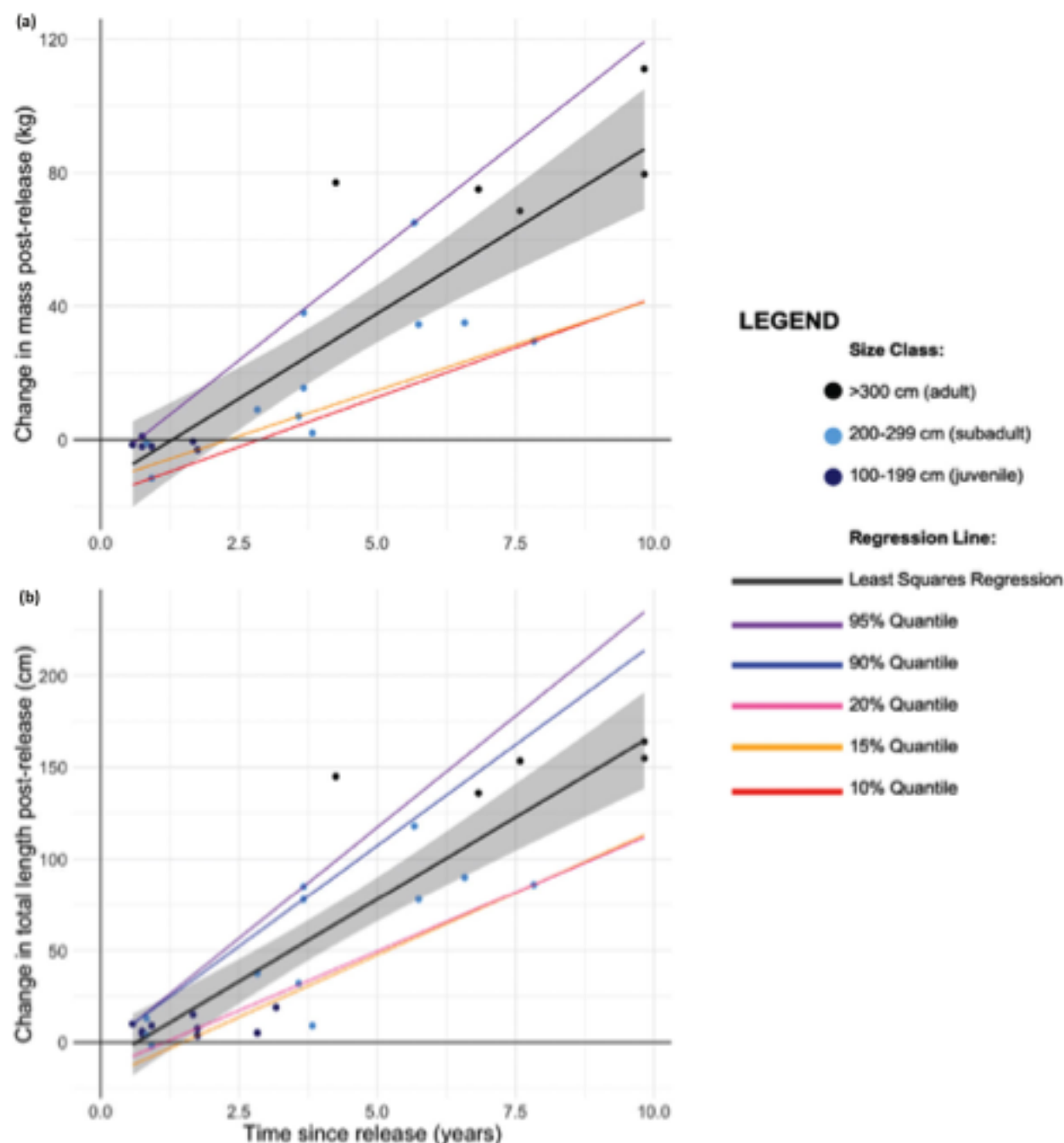


Figure 1. Change in (a) mass, $n = 22$ or (b) change in total length post-release, $n = 26$ for Gharial since release from captivity. Predictions are illustrated with the ordinary least squares (OLS) regression showing the mean growth trajectory, the 95% and 90% quantile regressions illustrate the estimated 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 coloured 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 ± 2.80 kg 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 $\text{mass} = 1.3602 \cdot \text{TL}^{3.7175}$ 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-vii-2005	0.83	0.83	210	
02-ii-2013	5.67	172	16.5	M	25-vii-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-ii-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-ii-2018	5.75	173	13	F	26-xi-2018	6.5	0.75	179	11
09-ii-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-ii-2018	6.75	181	15.5	F	28-ii-2019	7.67	0.92	190	13.5
10-ii-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-ii-2014	8.75	182	17.5	F	16-xi-2019	14.5	5.75	260	52
10-ii-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-ii-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-ii-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 (Czarnolewski & 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 (Else 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 (Else 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 (Huchermeyer 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; Else 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 (Else 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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