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Production Implications of Trace Element Concentrations in Crocodile Eggs and Tissues

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Production Implications of Trace Element Concentrations in Crocodile Eggs and Tissues

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Foreword

This research provides baseline comparative information on the concentrations of 24 trace elements in wild and captive-laid eggs and crocodile meat. In the case of crocodile meat, the data forms preliminary standards on which industry can now build.

Viability of the eggs of captive-laid Saltwater crocodiles is typically lower than that of their wild counterparts. Although some causes of low egg viability are known, the relationship between environment (including diet), egg production, and egg viability in captive crocodilians, is poorly understood. Recognised as good bio-indicators of their environment, research is now slowly shedding light on the potential effect of chemicals and heavy metals on crocodilian reproduction. This has implications not only for farm production, but also on the quality of meat produced for human consumption. Crocodile meat is a valuable by-product of crocodile farming in Australia, and few standards are available on its composition.

The project was funded from RIRDC core funds, which are provided by the Australian Government. This report is an addition to RIRDC's diverse range of over 2000 research publications and it forms part of our New Animal Products R&D program, which aims to accelerate the development of viable new animal industries.

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Craig Burns
Managing Director
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What the report is about

This research establishes baseline information on trace element concentrations in a food product, and examines factors that affect captive breeding of long-lived reptiles (crocodiles).

Who is the report targeted at?

The report is targeted at the Australian crocodile farming industry. However, farming enterprises elsewhere in the world may also benefit.

Where are the relevant industries located in Australia?

The crocodile farming industry is located in northern Australia, and comprises 15 crocodile farms: two in Western Australia (Broome, Wyndham), six in the Northern Territory (Darwin, Noonamah, Elizabeth Valley, Middle Point, Victoria River), and seven in Queensland (Rockhampton, Innisfail, Cairns, Mareeba, Palm Cove, Cooktown, Edward River).

Some farms grow crocodiles to a certain size and on-sell to other farms (including an Aboriginal enterprise in Queensland); some transport crocodiles to other farms for processing, and others grow crocodiles all the way through to culling size and process them onsite. Processing facilities are located at seven of the farms, and at least two are AQIS-approved for export.

Currently, the main markets for Australian Saltwater crocodile skins are in France, Singapore and Japan. Crocodile meat is mostly traded domestically, as are other by-products.

All farms will benefit from this research, although operations that carry out captive breeding may have a greater interest.

Background

Viability of the eggs of captive-laid Saltwater crocodiles is typically lower than that of their wild counterparts. Although some causes of low egg viability are known, the relationship between environment (including diet), egg production, and egg viability in captive crocodilians, is poorly understood. Recognised as good bio-indicators of their environment, research is now slowly shedding light on the potential effect of chemicals and heavy metals on crocodilian reproduction. This has implications not only for farm production, but also on the quality of meat produced for human consumption. Crocodile meat is a valuable by-product of crocodile farming in Australia, and few standards are available on its composition.

Aims/objectives

The aim of this study is to provide baseline data on the trace element composition of captive-laid and wild Saltwater crocodile eggs and crocodile meat, and to assess the effect of egg composition on survival and growth of hatchlings.

Methods used

Captive breeding was examined in a battery of 20 standardised pens designed to hold one male and one female Saltwater crocodile. The impact of supplement feeding on indices of reproduction was examined using linear regression analysis. Egg incubation and rearing of hatchlings followed established procedures. Concentrations of 23 trace elements in wild and captive crocodile egg contents (yolk, albumen, eggshell) and crocodile meat were quantified using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

Results/key findings

Crocodiles are good bio-indicators of their environment, and tissues and eggs reflect the diet and habitat in which animals are living. Analysis of concentrations of trace elements at two key crocodile meat-producing farms revealed some differences, but nonetheless provides baseline information on which the industry can build with other variables (for example, assessing the impact of pesticide use).

Lead ingestion by wild-caught adults, or adults with access to wild prey, is a potential factor that may reduce embryonic survival. However, all levels of heavy metals in crocodile meat were well below acceptable limits, and there is no reason for concern. Notwithstanding recent disease outbreaks that severely hampered the subsequent raising of crocodiles, the myriad of factors that profoundly affect post-hatching growth and survival overshadowed the potential benefits of mineral supplements. Analysis of reproductive success for known female Saltwater crocodiles will allow future manipulation of diet and/or other variables, in an effort to improve hatchling production per female. The effect of stress in captivity is also poorly understood, and may be a contributing factor to poor egg quality in captivity.

Implications for relevant stakeholders

The main beneficiary of this research is the crocodile farming industry and associated industries (meat wholesalers and retailers) and, to a degree, researchers looking to expand our knowledge of the species.

In the face of increasing competition from saltwater crocodile farming operations in other countries (for example, Thailand, Philippines, Papua New Guinea, Indonesia), improved efficiency of egg and hatchling production from captive production is important to the Australian crocodile industry. Remote indigenous communities and private landowners benefit economically from the farming industry, and improvements at the farm production level will invariably lead to increased benefits to those landowners. The ability to ensure compliance of crocodile meat with food safety standards may assist policy makers.

Recommendations

Recommendations are directed at private and government researchers and the crocodile farming industry. A more extensive assessment of meat from different farms/abattoirs in Australia would be beneficial, as the industry has changed since this study was initiated (for example, greater interstate trade in live animals). Comparative studies on crocodile meat from overseas producers would also provide useful comparative data. Collaboration with Northern Territory researchers investigating the utility of using isotopes to quantify the origin of crocodiles is recommended, as this methodology has the potential to distinguish between wild and farmed animals, and serve as a more effective enforcement tool for different types of crocodile products, including meat. A detailed assessment of captive breeding at different crocodile farms may extend our knowledge of the effect of different pond designs, husbandry and other factors on egg and hatchling quality over a long period of time (some breeding farms have now been in operation for over 30 years).

Introduction

The Australian crocodile farming industry relies heavily on the production of captive-bred Saltwater crocodile (*Crocodylus porosus*) hatchlings. In Queensland, all hatchling production is currently from captive breeding. Although ongoing research may result in some wild egg harvesting in the future, captive breeding is likely to remain the major form of hatchling production in the foreseeable future (QEPA 2007; Britton 2012). In the Northern Territory and Western Australia, captive production is boosted by hatchlings produced through wild egg ranching programs (Leach et al. 2009; DEC 2009). Around 25 per cent of hatchling production in the Northern Territory is derived from captive breeding, which represents a significant investment by the industry to infrastructure (NT Department of Primary Industry Fisheries, pers. comm.). Relative to ranching, the costs of producing a hatchling from captive breeding are generally high, but captive breeding offers insurance against years where wild nest/egg production is reduced (for example, by up to 50 per cent) due to natural environmental factors. Security of tenure over wild resources is also a factor taken into account by farmers when considering whether to invest in captive breeding.

Infertility and early (<1 day) embryonic death are generally higher in captive-laid eggs than in wild eggs, and incubation success of live fertile eggs, is invariably lower in captive versus wild eggs. The types of breeding enclosures and housing arrangements for adult Saltwater crocodiles vary greatly between farms, within and outside of Australia. But, almost without exception, overall hatchling production (per female) in large, communal breeding pens is lower than that from enclosures containing one male and one female. Social interactions between adult crocodiles are a significant factor that can affect the extent of egg infertility (for example, through asynchrony of mating), and may play a more significant role on egg quality through the effect of stress on breeding females (Lance 1987).

The effect of raising conditions, including diet, on the chemical composition and "quality" of captive-laid crocodilian eggs, has received little attention. Joanen and McNease (1987) examined the effect of diet on hatching success in a group of captive-raised American alligators (*Alligator mississippiensis*), and provided some insights into the importance of certain vitamins (for example, vitamin E) in cases where particular diets were used. Elsey and Kinler (2012) reported that captive breeding of American alligators is not widespread in the USA, in part because of the cost of captive breeding, but largely due to the extensive egg ranching programs that operate in Florida and Louisiana. But captive breeding is also not favoured because of species-specific traits that appear to limit the reproductive life of female alligators (Manolis, pers. obs.).

In terms of post-hatching growth and survival, there is also considerable inter-clutch variation in post-hatching survival and growth of farmed *C. porosus* hatchlings. This is generally assumed to be genetic, although it could equally reflect environmental factors. The diet of adult females probably does affect the composition of their eggs, and environmental contaminants also appear to be passed through females to the egg, and ultimately to the hatchlings (Manolis et al. 2002b; Packard et al. 2004). Preliminary data indicate that the mineral composition of the yolk of *C. porosus* eggs varies between areas in the wild, and between wild and captive-laid eggs (Manolis et al. 2002b).

Wild crocodiles are long-term bioaccumulators of heavy metals, and these elements provide signatures for specific environments (rivers) in which an animal has been living (Jeffree et al. 2001; Twining et al. 1999). With the recovery of the Saltwater crocodile population in the Northern Territory to pre-European colonization levels, there is increasing movement of crocodiles into upstream freshwater areas, and around the coast between rivers (Leach et al. 2009; Fukuda et al. 2011). The use of heavy metals to determine the "origin" of crocodiles moving between areas may be complicated by these types of movement, as crocodiles may spend varying amounts of time in different habitat types and locations. The use of isotopes to quantify origin of wild crocodiles is currently under investigation (Fukuda et al. 2012).

That crocodiles are bioaccumulators is of relevance to the crocodile industry, as meat is an economically significant by-product of production. Most meat is produced from captive-raised crocodiles on farms, but some meat in the Northern Territory is obtained from wild harvested crocodiles, including those taken through the Northern Territory Problem Crocodile Program (Leach et al. 2009). Outside of the industry, crocodiles and their eggs are a traditional food source for Aboriginal people. The crocodile industry is relatively new compared to conventional livestock industries, and so there are few food standards available with respect to crocodile meat, although this situation is slowly changing (for example, Beilken et al. 2007). The benefits of having standards on which to confirm the quality of crocodile meat are clear.

Many factors are known to impact directly on the viability and subsequent hatching success of crocodilian eggs, including flooding and overheating during incubation. But the impact of more subtle factors that are passed from the female to the egg during folliculogenesis and which may potentially reduce egg viability are poorly understood for crocodilians. The relationship between exposure of wild American alligators to organochlorine pesticides and low clutch viability has been documented (Rauschenburger et al. 2007). The experience with alligators has inspired research on the impact of "Roundup", a commonly used herbicide, on *Caiman latirostris* hatchlings in Argentina (Lopez et al. 2012). In Australia, a preliminary assessment of pesticide concentrations in *C. porosus* eggs from the Northern Territory and Queensland confirmed some low levels of pesticide in those eggs, even in locations with little or no agricultural activity associated with them (Packett et al. 2004).

That contaminants were passed from the female to her eggs raises the possibility that contamination could affect egg quality, but also indicates that the female herself is likely to contain levels of contaminants in her tissues. That environmental contaminants such as heavy metals may reduce crocodile egg viability has also been raised by Lance et al. (2006). Huchzermeyer (2003) recently summarised the limited amount of information on heavy metal contamination in the meat and tissues of crocodilians. For a young emerging industry, knowledge on the extent to which environmental contaminants accumulate in crocodile meat is of importance to maintaining and/or addressing issues of food safety. In addition, the relationship between contaminants in the environment (for example, use of copper sulphate to control algal growth) and egg viability, is of relevance to production efficiency.

From a conservation point of view, an understanding of the potential impact of contaminants on reproductive performance and hatchling fitness may assist reintroduction efforts with endangered species such as the Chinese alligator (Xu et al. 2006; Ding et al. 2001).

Objectives

The objectives of the study were:

1. To provide baseline data on trace element composition of captive-laid and wild *C. porosus* eggs.
2. To quantify the effect of the diet of captive female *C. porosus* on chemical composition and hatching success of their eggs.
3. To quantify the effect of egg composition on the growth and survival of hatchlings of known parentage to one year of age.
4. To provide baseline data (standards) on the chemical constituents of crocodile meat, which could be used to monitor changes over time and which can be used for risk assessment.

Methodology

Captive Breeding

The battery of 20 breeding pens were constructed at Crocodylus Park (Berrimah, Northern Territory) in 1994, each of which was designed to house one male and one female Saltwater crocodile, with standardised conditions in each pen for experimental purposes. The facility comprises a concrete channel with a concrete dividing wall down the middle, and a covered wooden walkway running lengthways on the dividing wall (Fig. 1). The channel is v-shaped, with maximum water depth of 80cm at the deepest point at the dividing wall, decreasing to 0cm at the water/land interface (Fig. 2).

Figure 1. Walkway across battery of 20 breeding pens (10 on each side) at Crocodylus Park. The battery lies in a north-south alignment.

Figure 2. One of 20 standardised breeding pens at Crocodylus Park. Note the dividing wall protruding above the water surface to eliminate visual contact between the male and female when separated on either side. Nests are laid in the back corner (upper left).

Pens were separated by concrete walls in the water area and with 1.8 m high chain mesh at the land areas (Fig. 2). Conveyor belt rubber on the lower part of chain mesh fences between pens served to eliminate visual contact between pairs in each pen, particularly males. Each individual breeding pen is 5.5m wide and 10m long, with water and land areas each comprising 50 per cent of the total pen area.

An internal dividing wall in the water created two separate areas (4.0m and 1.5m in width respectively). Palms planted in raised planter boxes constructed of treated pine longs provided additional shade and created a barrier to visual contact between animals on the land area (Fig. 2). This design allowed adults to be separate from each other if they chose, and when at the water surface of the water or basking on land to not be able to see each other and thus reduce the likelihood of aggressive interactions.

The breeding enclosure was stocked with wild Saltwater crocodiles captured in the downstream portion (km 22-55) of the Adelaide River (all females, 10 males; 30 May to 15 June 1994) and freshwater sections of the Mary River (10 males; June-August 1994), in the Northern Territory. All crocodiles captured by harpoon were selected on the basis of the minimum size for maturity (males >3.5 m, females >2.4 m) (Webb et al. 1978). Prior to introduction into breeding pens, all crocodiles were scute-clipped for subsequent identification (Richardson et al. 2002).

In the case of the wild females it was not known whether they had nested previously or not. An average of 11.3 nests per season had been recorded in the four years prior to the captures, and an average of 11.0 nests were located in the two years after the harvest (WMI, unpubl. data). It is thus likely that some of these females may never have nested in the past. This was probably due to social factors associated with the increase in the *C. porosus* population due to protection in 1971; or, they were simply immature (Webb & Manolis 1992; Webb et al. 1978). By the end of 1994, all of the breeding pens had been filled with crocodiles assumed to be mature adults and capable of breeding.

Adults were fed mainly whole chickens, at a rate of 2-3kg for males and 1-2kg for females per week, depending on body size. The amount fed on a particular day varied according to the frequency of feeding, but generally animals were fed 2-3 times per week. During the cool part of the dry season (June-August), crocodiles were sometimes reluctant to eat, but this varied greatly between individuals. They were still offered food.

In October each year, 2-3 bales of mulch hay were placed at the back of each pen for females to use for nest construction. Breeding performance was monitored through: numbers of nests produced; clutch size; egg infertility; numbers of eggs dead before collection; numbers of eggs dead at collection; number of eggs dead during incubation; and numbers of hatchlings.

Supplement

Egg yolk deposition in Saltwater crocodiles occurs evenly and simultaneously in all enlarging follicles (Astheimer et al. 1989). The timing of follicular development in the American alligator is well documented, but this is not the case for most other species of crocodilian, including *C. porosus* (Lance 1987). In order to maximise the chances of trace elements being delivered to adult females through the entire period of follicular development, a commercial mineral supplement was offered with the food to adult females between July and December 2004, leading up to the 2004/05 nesting season. Nesting mainly occurs in December and January each year.

Initially, a pre-determined amount of supplement was packaged in sausage-skin, based on the estimated bodyweight of the adult female being fed. Bodyweight was predicted using published morphometric relationships for wild *C. porosus*, and increased by 10 per cent to account for the heavier mass per unit length of captive crocodiles at Crocodylus Park (Webb & Messel 1978; Bates et al. 2004). Sausages were "hidden" inside chicken fillets and fed to individual females twice per week, using a pole and string (Fig. 3).

Figure 3. **Saltwater crocodile being fed individually using the "pole and string" method from the covered walkway over the pens.**

On the basis of feeding records in the first few weeks of the trial, "individual" sausages for females were no longer prepared. Instead, to streamline the process, females were grouped into three size classes, and fed chicken with a sausage containing 14, 18 or 24g of supplement. Records were maintained on whether females were able to be fed (that is, the male did not interrupt the feeding event), whether they definitely swallowed the meat or not, or whether the food was later found floating in the water. Females would often take the meat into their mouth and go underwater immediately, and later release the food, and not swallow it. The amount of dietary supplement likely to have been consumed by individuals females was calculated on the basis of the feeding records.

Egg Collection

Captive nests were checked as soon as indications of female behaviour (nest guarding), and/or the state of the nest, suggested that egg-laying may have occurred. Wild nests were checked for eggs once they had been located from the air (helicopter) or at times from a boat (in case of tidal rivers).

The procedure once eggs were confirmed was the same for both captive and wild eggs. Once it was considered safe to do so, the eggs were uncovered sufficiently to allow temperature around the eggs to be measured using a calibrated mercury thermometer. Eggs were then marked on the uppermost surface using a pencil, and then removed from the nest and placed amongst nesting material within insulated eskies for transport to the incubation facilities. Eggs were maintained in the same position, with the mark uppermost, throughout transport. At all times, eskies were handled carefully to avoid jolting that could affect egg viability.

At the laboratory, eggs were washed in tap water (typically around 26-27^{o}C) to remove mucous (in the case of freshly laid eggs) and/or nesting material, and 10 eggs from each clutch were measured (egg length and egg width using electronic callipers; $\pm$ 0.1 mm) and weighed ($\pm$ 0.1 g; electronic balance). Eggs not located within 24 hours of being laid were aged on the basis of the opaque band (Webb et al. 1987a). Translucent eggs were candled, and if deemed to be infertile (that is, no subembryonic fluid was visible), were not placed in the incubators.

Egg Incubation

Eggs were incubated without nesting media, within plastic trays (Manolis & Webb 1991). Clutches were maintained in separate trays, and covered with a lid around 3-4 weeks prior to hatching, so that hatchlings could be identified with their clutch. At the beginning of the season (late November-early December), eggs were placed in water-jacketed incubators (0.6 x 0.5 x 0.9m), set at 32°C (± 0.2°C) and 99+ per cent relative humidity. By mid-late December, when sufficient eggs were available from both captive breeding and the wild harvest, eggs were transferred from the water-jacketed incubators or placed directly into a large walk-in incubator (4.65 x 2.66 x 2.30m), also set at 32°C (± 0.2°C) and 99+ per cent relative humidity. Oxygen supply to the incubators was maximised, but not measured.

Incubation at 32°C during the temperature-sensitive period results in maleness being assigned to the developing embryos. Males have a higher probability of surviving after hatching and grow faster on average relative to animals incubated at female-producing temperatures (≤31°C and ≥33°C) (Webb & Cooper-Preston 1989; Webb et al. 1987b).

Eggs were checked throughout incubation, and dead eggs removed and opened to ascertain the stage at which the embryo had died (Webb & Manolis 1987; Webb et al. 1987b). Where possible, dead embryos were also examined for signs of visible abnormalities that could have explained mortality.

Hatching

At the first signs of hatchlings pipping (snouts protruding from the egg), eggs were left for at least 24 hours to enable hatchlings to hatch by themselves. Where animals that had not hatched within 24 hours, eggs were opened by hand to assist hatchlings to emerge. Hatchlings were washed in tap water, and 10 animals measured using a clear ruler (snout-vent length; ± 1 mm) and weighed (± 0.1 g).

Hatchlings were considered to be "normal" if there were no signs of physical abnormalities (for example, twisted spines and tails, bump on head), and the yolk scar was less than 5-7mm wide. Where yolk scar was greater than 7mm, this was invariably associated with a large internalised yolk. Hatchlings which had not internalised their yolks or which had serious physical abnormalities were humanely euthanised (NRMMC 2009).

Hatchlings were "marked" by removing a sequence of vertical tail scutes (scales) with scissors, allowing the animals to be identified to clutch and season (Richardson et al. 2002).

Raising

Hatchlings were maintained within plastic trays in the incubator for up to four days after hatching, to allow them to begin absorption of residual yolk, and for general observation. Following this period they were moved to raising facilities, which consisted of two types of enclosure.

Most hatchlings were reared in fibreglass tanks (4.0 x 1.7 x 0.5m), which were subdivided into four smaller, equally-sized pens with land (40 per cent) and water (60 per cent) areas, and water depth of around 8cm. Water temperature was maintained at around 32°C through the recirculation of electrically-heated water from a small water sump. This system was later modified such that electrically-heated hot water (55-60°C) was injected directly into the pens, and excess water taken to waste via an overflow system. Further refinements have since been made, through the conversion to more efficient gas heating.

Attempts were not made to control ambient temperature precisely, but heat lamps (100 watt) provided an area of warmth on the land area. Both water and ambient heating were both thermostatically controlled. Wooden hideboards in each pen provided cover for the hatchlings. During the day, lids over each tank were open, but closed at the end of each day. All fibreglass tanks were housed within a covered building, such that sunlight was available through the open sides. During the cool parts of the year, roller blinds were lowered at night to reduce heat loss from the sides of the building.

The second raising facility comprised large block pens (36 x 4m), divided into eight sections (9 x 2m), each of which was divided into six smaller pens (each 1.5 x 2m; maximum water depth 20-25cm, water comprised approximately 65-70 per cent of pen area). A water sump (1kl) at the end of each block was heated using electric heaters (32°C), and water pumped into each enclosure; that is, it comprised a recirculating system. Covers over the top of the blocks were opened each morning to facilitate feeding and cleaning, and closed at the end of each day. Wooden hideboards in each pen provided cover for hatchlings. A thermostatically controlled "Spitfire" gas blower forced warm air into the block at night during the cooler times of the year, with a target ambient temperature of 30-32°C.

Feeding was carried out seven days per week for the first month, and gradually reduced until animals were being fed three days per week by around six months of age. Food consisted of a mixture of minced horse meat (75 per cent) and chicken heads (25 per cent), to which di-calcium phosphate (two per cent) and multivitamin (one per cent) was added just before feeding. Food was placed on aluminium trays in each pen between 4.00pm and 5.00pm on feed days, and uneaten food removed the next morning (8.30am to 10.00am), after which the water was discarded, pens washed, and refilled with clean water.

Hatchlings were inventoried at around 12 months of age, at which time snout-vent length and bodyweight were measured.

Samples

Samples were obtained from: captive-laid *C. porosus* eggs at Crocodylus Park (wild females, since 1994; captive-bred females, since 1998), Crocodile Farms NT Pty Ltd (wild females, since 1980), Janamba Croc Farm (wild females, since 1982) and Lagoon (single wild pair, since 1992); wild *C. porosus* eggs (Adelaide, Liverpool and Tomkinson Rivers, Melacca Swamp). Crocodile meat was obtained from two of the largest producers of crocodile meat (Crocodile Farms NT Pty Ltd and Lagoon Crocodile Farm). Meat samples obtained from farms in Queensland and Western Australia were lost in an extensive office fire that also destroyed other data associated with this project.

Samples of yolk, albumen and eggshell were obtained from infertile eggs, which were assumed to be representative of all follicles produced by the female in a season. Eggs were opened with scissors (see Webb and Manolis 1987), contents carefully removed using a sterile syringe, and frozen in sterile specimen jars. Care was taken to avoid small pieces of eggshell that invariably flaked off as the egg was opened, and which sometimes fell into the open egg. Meat was obtained from different crocodiles after culling, and during subsequent processing in registered abattoirs (see ARMC 1998).

Chemical Analyses

Trace element (metal) concentrations in samples were quantified using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS), at the Australian Nuclear Science and Technology Organisation (Lucas Heights, NSW).

Concentrations of 23 metals [aluminium (Al), calcium (Ca), iron (Fe), potassium (K), magnesium (Mg), strontium (Sr), zinc (Zn), chromium (Cr), manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), arsenic (As), selenium (Se), molybdenum (Mo), cadmium (Cd), tin (Sn), antinomy (Sb), barium (Ba), mercury (Hg), lead (Pb), thorium (Th), uranium (U)] were provided as mg/l or µg/l. Some preliminary data were obtained using egg contents that had been stored since the 1987/88 and 2000/01 nesting seasons, and obtained from the Finniss, Reynolds and Adelaide Rivers, and Melacca Swamp (Manolis et al. 2002b).

Experiment conditions and observations

Breeding Pens

The breeding pens at Crocodylus Park have now been established for 18 years. The majority of adult pairs placed in the pens have been compatible, and 70 per cent of females have been in the facility since at least 1998.

Supplement

Breeding females varied with regard to the amount of supplement that they were known to have definitely consumed. Most of the 20 females consumed between 300 and 700g of supplement over the 21 week period (20 July to 20 December 2004); 0-100g (1), 200-300g (1), 300-400g (4), 400-500g (6), 500-600g (2), 600-700g (6). Two females were reticent to feed, perhaps due to the proximity of males to them most of the time.

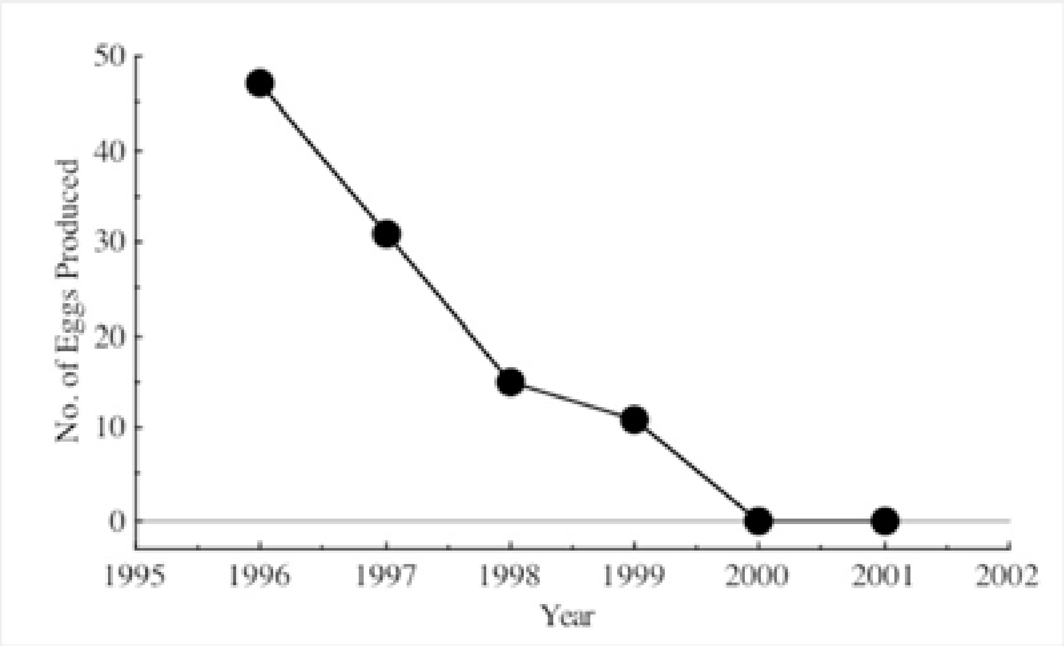
Of the 12 females that had been in the breeding pens the longest (since 1994), the majority consumed between 500 and 700 g of supplement; 200-300 g (1), 300-400 g (1), 400-500 g (4), 500-600 (1), 600-700 (5). This is equivalent to a dosage rate of 0.03-0.04 per cent of bodyweight per week. By way of comparison, hatchling crocodiles, growing rapidly and with a high requirement for minerals for skeletal growth, consume vitamin and di-calcium phosphate supplements at a rate of up to 1-2 per cent of bodyweight per week. This is in addition to bone that may be contained in the food already (Manolis et al. 1989; Manolis 1994).

Breeding Performance

The battery of 20 breeding pens at Crocodylus Park provides an opportunity to monitor "known" adults in a standardised environment, with reduced social interactions that are common in large communal enclosures containing large numbers of adult Saltwater crocodiles. To a certain degree, the use of this breeding facility for tourism at Crocodylus Park also allows adult crocodiles to become accustomed to people and the associated disturbance, but also introduces a variable that may affect crocodiles in some way.

The first 20 wild female crocodiles were placed in the breeding pens on 30 May 1994. One nest was produced in the following nesting season (1994/95), but consistent nesting did not occur until the next (1995/96) nesting season. By 2011/12, 12 of the 20 initial wild females were still in the breeding pens. Seven of the other eight original females had been either killed by the male, or removed to other enclosures because of aggressive interaction with the male. One female (BP1) was culled in 2001 as her egg production had declined significantly over time to nil, suggesting that she had reached senescence (Fig. 4). Autopsy revealed the presence of a single follicle within the ovary.

Figure 4. Egg production of 3.03m long female Saltwater crocodile (BP1) over time. The animal is around the maximum size of an adult female *C. porosus* (Webb et al. 1978), and assumed to have reached senescence. 96=1995/96 season, etc. She was introduced into the breeding pens on 30 May 1994.



Using only those 12 females that have spent the entire 1994-2012 period in the breeding pens, a relatively high proportion of the females nested each year in the nine years leading up to the experimental treatment with supplement (mean= 90.7 per cent, SE= 5.46, N= 9). In the eight years following treatment, the proportion of females nesting each season declined (mean= 80.2 per cent, SE= 2.70, N=8), although not significantly so.

It is unclear why there was such a low proportion (50 per cent) of females nested in the 1996/97 season. Numbers of wild female Saltwater crocodiles nesting in the wild can be reduced by up to 50 per cent in years where the preceding wet season has been short, where there has been reduced rainfall relative to normal years, and/or these factors have resulted in drier conditions during the following dry season (that is, leading up to the nesting season for Saltwater crocodiles). These conditions impact on prey availability and water levels, such that females may simply not undergo follicular development that season, or do not ovulate and reabsorb any follicles that may have developed.

In captivity, environmental conditions that affect the reproductive effort of wild crocodile populations do not appear to be as significant, as water levels and food availability can be maintained at optimum levels at all times. Long-term nesting data from a tidal river (Adelaide River) and a spring-fed swamp (Melacca Swamp), about 60km east of Darwin, showed no such perturbation in nesting effort in the 1996/97 nesting season, suggesting that ambient conditions *per se* were not responsible for the reduced nesting effort observed in the breeding pens at Crocodylus Park.

McClure and Mayer (2003) provided data indicating that a very low Southern Oscillation Index (SOI) in the preceding August was correlated with a low proportion of female *C. porosus* nesting at a crocodile farm in Cairns, Queensland. But SOI in June-August was ruled out as a contributing factor to the low nesting frequency at Crocodylus Park in 1996/97, as the published monthly SOI was not low during this period (see www.bom.gov.au).

Closer examination of the nesting patterns for individual females provided some additional insights:

- Female BP15 did not nest for the first three seasons after being brought to Crocodylus Park. As there were compatibility issues between the female and the first male provided, he was replaced in 1995. The new male did not eat for almost one year, and it was at least another year before he would bask during the day whilst people were present. It is possible that the lack of nesting for the female reflected a lack of courtship and mating behaviour on the part of the male. Since he settled in, nesting has occurred every year (Table 1).

- Female BP17 nested six times in nine years prior to 2005 (66.7 per cent of seasons), but has only nested once in eight years (12.5 per cent of seasons) since that time (Table 1). Her male was changed in 2003/04, and her lack of nesting may reflect an incompatibility with him. That senescence may also be involved cannot be excluded (see above).

- Eleven females that nested annually between 1998/99 and 2003/04 (six years) became more erratic with regard to the frequency of nesting after treatment. Four females continued to nest annually, but the remaining seven females have not nested.

- There was no significant correlation between the change in nesting pattern and the amount of supplement ingested by females. One of the four females that continued to nest annually received a “low” dose of supplement, and the others received “medium” (two) and “high” doses (one) respectively.

Table 1. **Seasons in which 12 female Saltwater crocodiles in the battery of breeding pens since 1994, and two females since 1997 and 1998 (BP11 and BP18 respectively), have nested (non-nesting is indicated by coloured squares). 96=1995/96 season, etc.; X=females were not present in those years.**

Female No.	Non-Nesting Seasons	96	97	98	99	00	01	02	03	04	05	06	07	08	09	10	11	12
18	7	X	X	•				•	•	•	•			•		•	•	
11	2	X		•	•	•	•	•	•	•	•		•	•	•	•	•	•
12	0	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
16	0	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
8	1	•		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
13	1	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	
20	1	•	•	•	•	•	•	•	•	•	•	•		•	•	•	•	•
3	2	•	•	•	•	•	•	•	•	•		•	•	•	•		•	•
5	2	•		•	•	•	•	•	•	•	•		•	•	•	•	•	•
15	2			•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
10	3	•	•	•	•	•	•	•	•	•	•	•	•		•	•		
7	3	•		•	•	•	•	•	•	•	•	•		•	•		•	•
4	4	•			•	•	•	•	•	•		•	•	•	•	•	•	
17	10	•			•	•		•	•	•			•					

Clutch Size

There was no significant relationship between mean clutch size and year up to the time of treatment (r^2= 0.02, p= 0.69, N= 9); Fig. 5; Table 2). After the treatment there was significant decline in mean clutch size (r^2= 0.59, p= 0.04, N= 7) (2010/11 was excluded as it was clearly an extraordinary year, with all clutches being generally large). With female BP17 excluded from the dataset as she nested intermittently during the period (see Table 1), there was still no significant relationship between mean clutch size and year before treatment (r^2= 0.01, p=0.81, N= 9), but the relationship after treatment, showing declining clutch size, just reached significance (r^2= 0.58, p= 0.05). The reasons for the decline are unclear. Mean clutch size for 2004/05, after supplement feeding, were consistent with the trend for the preceding period, but thereafter it has declined.

Figure 5. **Non-significant linear regression relationships between mean clutch size and year, before and after treatment, for 12 adult Saltwater crocodiles housed in breeding pens since 1994. Data for 2010/11 were excluded from the analysis.**

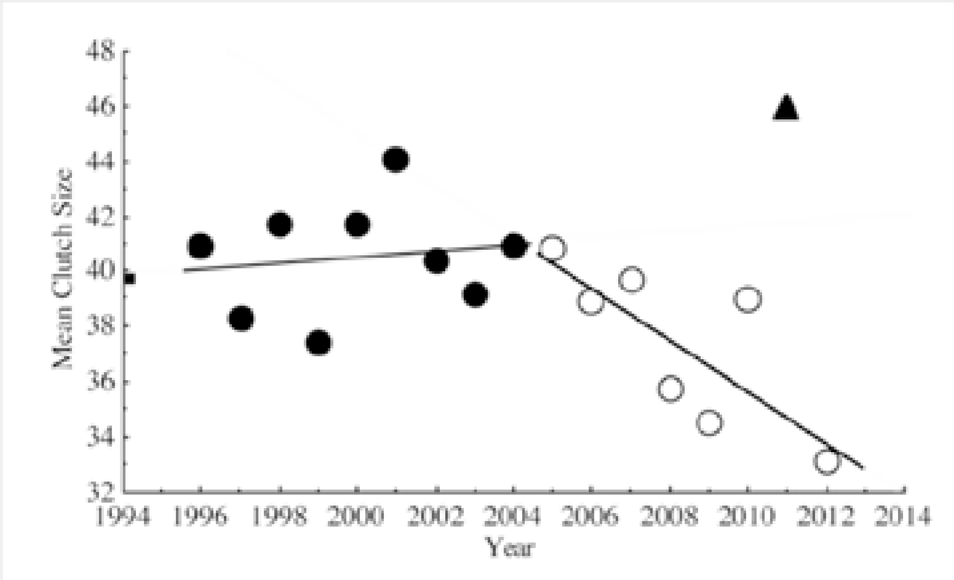


Table 2. **Mean clutch size and mean percentage infertility for nests produced in each season by 12 female Saltwater crocodiles housed in breeding pens since 1994. A=all clutches, including those considered not to represent a females full complement of eggs; C=clutches considered to represent a female's full complement of eggs; AC=only complete clutches produced in that season.**

Season	Complete Clutch?	No. of Females	No. of Nests	Mean CS	Range (CS)	SE (CS)	% Infertile Eggs	Range
95/96	A	12	11	38.1	10-54	3.99	12.7	2.6-44.1
95/96	C	12	10	40.9	27-54	3.12	11.0	13.0-44.1
96/97	A, C	12	6	38.3	33-50	2.58	15.7	2.6-26.0
97/98	A, C	12	12	41.7	29-55	2.16	8.9	0.0-45.0
98/99	A, C	12	10	37.4	29-48	1.84	10.3	0.0-39.6
99/00	A, C	12	12	39.5	15-56	2.99	13.6	0.0-67.5
99/00	C	12	11	41.7	31-56	2.18	12.4	0.0-67.5
00/01	A	12	11	41.3	13-56	3.37	18.8	0.0-88.6
00/01	C	12	10	44.1	32-56	2.04	20.7	0.0-88.6
01/02	A, C	12	12	40.4	30-51	1.99	9.1	0.0-30.6
02/03	A	12	12	36.5	7-56	4.20	20.7	0.0-95.0
02/03	C	12	11	39.2	20-56	3.55	22.6	2.2-95.0
03/04	A	12	12	38.8	16-57	3.12	15.1	0.0-49.1
03/04	C	12	11	40.9	26-57	2.55	16.4	0.0-49.1
04/05	A, C	12	9	40.8	32-50	1.77	15.0	0.0-40.6
05/06	A, C	12	10	38.9	17-52	3.07	38.2	5.6-91.4
06/07	A	12	10	34.0	1-49	4.50	22.2	0.0-100.0
06/07	C	12	7	39.7	26-49	3.07	16.2	0.0-31.8
07/08	A, C	12	10	35.8	24-49	2.26	23.0	0.0-100.0
08/09	A, C	12	11	34.5	19-42	1.89	23.3	0.0-68.4
09/10	A, C	12	9	39.0	30-48	2.30	18.5	0.0-61.7
10/11	A	12	10	41.5	1-53	4.91	22.7	2.6-100.0

10/11	C	12	9	46.0	35-53	2.01	14.1	2.6-42.9
11/12	A	12	8	29.1	1-45	5.04	34.8	2.6-100.0
11/12	C	12	7	33.1	20-45	3.51	25.5	2.6-69.6

Egg Size

Mean egg size increased between 1995/96 and 2003/04, in terms of both egg width (r^2= 0.09, p= 0.005, N= 81) and egg weight (r^2= 0.06, p= 0.02, N= 81), but between 2003/04 and 2011/12 it stabilised (r^2= 0.02, p= 0.21, N= 64 and r^2= 0.005, p= 0.59, N= 64 for egg width and egg weight respectively). The change in trend is considered to reflect the different mix of females that nested in a particular year (see Table 1), but the decline in mean clutch size (Fig. 5) may also be implicated, and may reflect a more physiological reason for the trend.

Egg Infertility

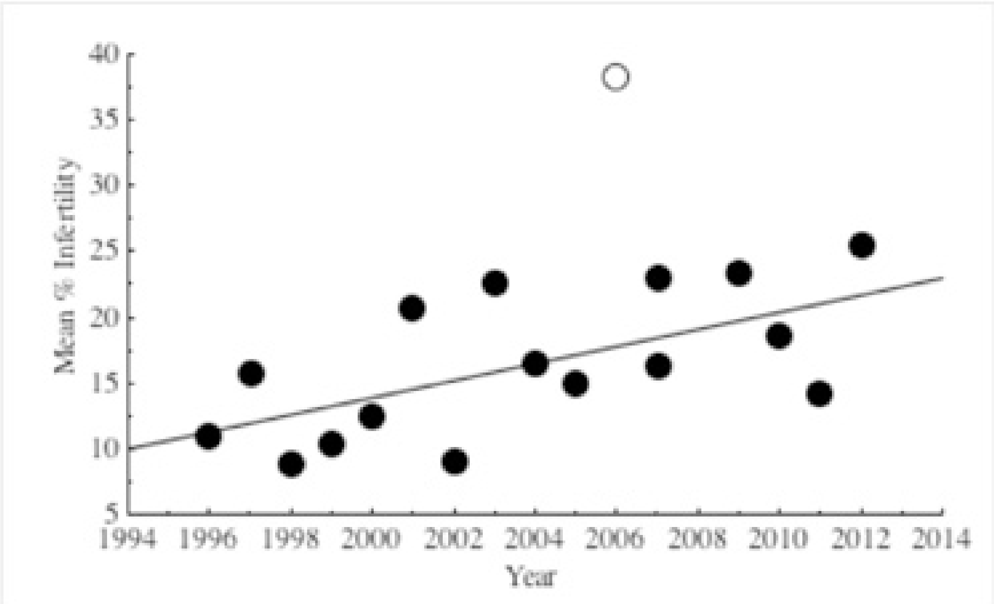
Infertility prior to 2004/5 was stable over time (r^2= 0.23, p= 0.19, N= 9), at a mean of 14.1 per cent (mean of annual means). Likewise, infertility since 2004/05 was stable (r^2= 0.02, p= 0.72, N= 8), at a higher mean of 21.7 per cent. Infertility in 2005/06 was relatively high due to two clutches with >90 per cent of eggs being infertile, but even with this year excluded mean infertility was higher, at 19.4 per cent.

Eggs that have not been fertilised are clearly recognisable by candling (Webb et al. 1987a), but eggs in which the embryo has died before subembryonic fluid has formed will also appear as “infertile”. It is not possible to distinguish this very early mortality, which presumably occurs immediately after fertilisation, within the female and before the eggs have been laid. Temperature has a profound effect on embryonic development (Webb et al. 1987b). Results from temperature data loggers fed to females in the breeding pens and retained in the stomach for around one year, indicated that adult females are able to bask efficiently and maintain their body temperature within optimal limits (WMI, unpubl. data). High body temperatures can be ruled out, to a large degree, as a cause of very early embryonic mortality.

Egg infertility in breeding pens containing only one breeding pair of Saltwater crocodiles is typically less than that from communal pens with multiple males and females. The male-male and female-female social interactions in communal pens often result in asynchrony between mating and ovulation, and completely or almost completely infertile clutches are commonplace. The exception to this rule is very large communal enclosures, where adults have sufficient space to establish territories and avoid each other, and where the situation mirrors that of the wild.

Relative to wild clutches, where infertility averages about five per cent (WMI, unpubl. data), the results here were clearly higher. Combining data for all years indicated a significant, albeit highly variable, relationship between percentage infertility and year (r^2= 0.05, p= 0.002, N= 175). Figure 6 shows the significant linear regression relationship between mean percentage infertility and year (r^2= 0.38, p= 0.01, N= 16). The introduction of treatment (coded as 1 and 0) with year in a multiple regression rendered both year and treatment as non-significant.

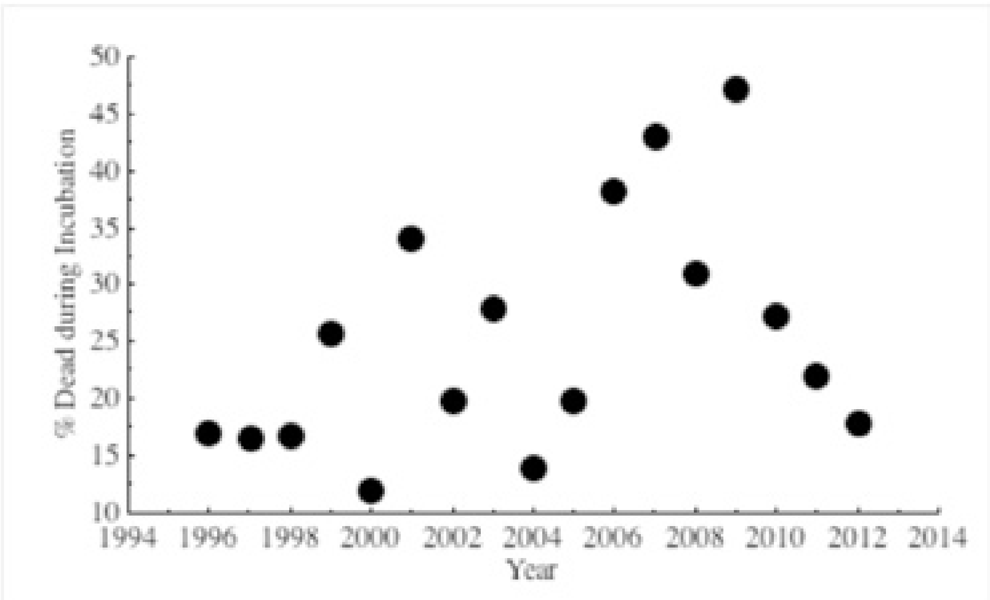
Figure 6. Significant linear regression relationship between mean percentage infertility and year (with 2005/06 season excluded).



Incubation Success

Excluding mortality caused by physical damage to eggs (for example, crushed during laying), most mortality during incubation for captive-laid eggs occurs in the first 7-10 days of incubation, which differs from the situation for wild-collected clutches, where early embryonic mortality is typically associated with factors such as flooding and overheating. Up to 2004/05, mean incubation mortality (dead during incubation) varied from year to year, around a mean of 20.4 per cent (SE= 2.42; N= 9) of live eggs incubated. Eggs that died as a result of collection or were sacrificed for research purposes were excluded. After 2004/05, mean percentage incubation mortality was more variable, and generally higher until 2009/10 to 2011/12 (Fig. 7).

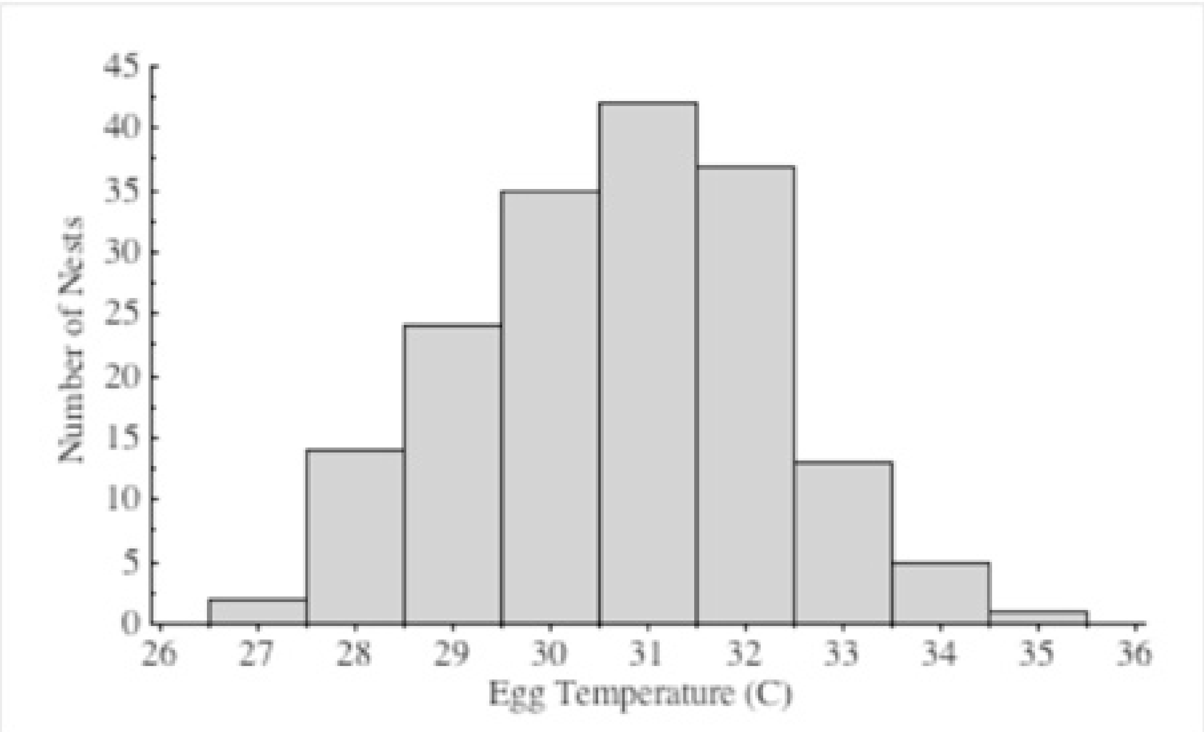
Figure 7. Mean annual percentage of eggs that died during incubation against year.



Nest temperature has a profound effect on embryonic development and, if at lethal levels early in incubation, can cause significant mortality. In the case of captive breeding at Crocodylus Park, most eggs are collected within 12 hours of being laid (<0.5 d, 68.9 per cent; 1-2 d, 24.3 per cent, 2-3 d, 4.1 per cent, 3-4 d, 1.8 per cent, >4 d, 0.9 per cent), such that exposure to high temperatures was minimised. But embryonic death can occur within hours of the eggs being laid if the nest environment is too hot (Manolis, pers. obs.), and embryonic mortality throughout incubation is a characteristic of nest overheating.

The nesting hay offered to females was selected because it does generate much heat during decomposition. For this reason, egg temperature for the great majority of eggs at the time of collection was within optimal levels (29-32°C; Fig. 8), and was not a significant variable affecting mortality during incubation (r^2= 0.04, p= 0.41, N= 173 all females; r^2= 0.01, p= 0.37, N= 122 for 12 females). It was, however, a significant variable with respect to the proportion of eggs in a clutch that were dead before collection (r^2= 0.04, p= 0.01, N= 174); this was due to the inclusion of two clutches that reported exceptionally high mortalities before collection (71.8 and 82.8 per cent respectively). Interestingly, these two clutches were not located until 36 and 60 hours after being laid, and their exclusion renders the linear regression relationship non-significant (r^2= <0.001, p= 0.87, N= 172).

Figure 8. Distribution of egg temperatures for nests produced in Crocodylus Park breeding pens (N= 20), from 1995 to 2012.



High egg temperatures can occur when females lay their eggs close to the upper surface of the nest mound, with subsequent overheating from exposure to the sun. Overheating within the nest prior to collection is not considered to be a problem at Crocodylus Park, and other factors are considered to be responsible for the relatively high proportion of early embryonic deaths.

The proportion of hatchlings that are considered "abnormal" has remained stable over time (using only clutches with 20 or more hatchlings; r^2= 0.03, p= 0.06). There was no physical sign to suggest that captive-bred hatchlings were less robust than hatchlings produced from wild eggs.

Raising

There were no significant differences between captive-bred and ranched hatchlings (derived from the wild egg harvest) reared under the same conditions, in terms of either survival or growth. Clutch effects explained most variation in growth and survival between captive-bred hatchlings. With the benefit of hindsight, it is now clear that other factors that profoundly influence the performance of hatchling Saltwater crocodiles in captivity overshadowed the potential benefits of mineral supplements.

The aggressive and territorial nature of adult Saltwater crocodiles is well known, but current studies have confirmed that similar behaviours occur between hatchlings, almost as soon as they emerge from the egg (Brien 2012). Thus, regardless of variables such as the quality of housing, diet, supplements, and so on, the effect of the behaviour of dominant individuals on conspecifics (from the same or different clutches) can overshadow and negate those benefits. The initiation of feeding by some individuals is now known to be affected by aggressive interaction with dominant individuals, although a genetic influence is probably also involved (Manolis et al. 1989).

Of particular significance, the ability to assess the performance of hatchlings at Crocodylus Park was affected significantly by a disease outbreak that claimed over 95 per cent of hatchlings in mid-2006 (see Jerrett et al. 2008), and which re-emerged in subsequent years (up to 2009/10) with serious effect on survival and growth of hatchlings and juveniles.

The difficulties with raising Saltwater crocodile hatchlings have long been recognised by farmers in Southeast Asia, to the extent that a much less valuable species (Siamese crocodile; *C. siamensis*) is preferred over *C. porosus*, even though raising costs are the same for both species (Nga, pers. comm.).

Egg Composition

Concentrations of minerals in the yolks of captive-laid and wild eggs are in Table 3. Generally, the results for all of the elements showed considerable variation, and no clear differences were apparent between eggs from farms and those from the wild.

Table 3. Concentrations of metals in the yolk of captive-laid and wild Saltwater crocodile eggs. Sources: wild-caught adults at Crocodylus Park (BP; 1M:1F), Crocodile Farms NT (CF), Lagoon Crocodile Farm (Lag), Janamba Croc Farm (Jan); captive-bred adults at Crocodylus Park (LB; communal pond); and, wild nests [Liverpool-Tomkinson Rivers (LT), Adelaide River (Ad), Melacca Swamp (Me)]. Numbers in brackets indicate sample sizes.

	Al (mg/L)	Ca (mg/L)	Fe (mg/L)	K (mg/L)	Mg (mg/L)	Sr (mg/L)	Zn (mg/L)	Cr (mg/L)	Mn (mg/L)	Co (µg/L)	Ni (µg/L)	Cu (µg/L)
BP (11)	0.03	296	2.12	242	18.7	0.341	1.56	0.012	0.020	*	*	121
LB (7)	0.04	275	1.69	210	15.8	0.268	1.32	0.010	0.009	*	*	105
CF (4)	0.03	300	2.15	245	18.2	0.419	1.60	0.012	0.017	*	2	123
Jan (4)	0.02	335	1.58	268	19.2	0.199	1.49	0.014	0.017	*	*	114
Lag (1)	0.11	242	1.82	219	18.6	0.353	1.57	0.019	0.021	*	6	102
LT (11)	0.04	314	2.15	242	20.7	0.477	1.64	0.012	0.018	*	2	145
Ad (3)	0.10	330	2.75	265	21.4	1.003	1.58	0.019	0.022	*	7	186
Me (3)	0.07	278	2.27	217	18.1	0.782	1.51	0.010	0.017	*	11	178

	As (µg/L)	Se (µg/L)	Mo (µg/L)	Cd (µg/L)	Sn (µg/L)	Sb (µg/L)	Ba (µg/L)	Hg (µg/L)	Pb (µg/L)	Th (µg/L)	U (µg/L)
BP (11)	3.1	66.3	3.3	1.6	1.7	*	222	*	22.9	6.8	*
LB (7)	1.9	64.5	3.8	2.0	-	*	151	*	15.8	6.6	*

Jan (4)	*	81.2	4.4	2.7	-	*	228	*	9.0	6.0	*
CF (4)	*	54.1	5.0	2.8	-	2.60	267	*	6.5	5.7	*
Lag (1)	*	39.0	4.0	3.0	-	*	116	*	79.0	5.0	*
LT (11)	<1	63.2	3.9	2.7	-	*	157	*	30.0	5.9	*
Ad (3)	2.9	52.7	4.7	2.4	-	*	258	*	12.3	5.2	*
Me (3)	<1	50.7	4.7	2.4	-	*	102	*	88.9	5.8	*

* levels similar to blanks tested at the same time (below detectable levels)

The generally higher levels (>130 per cent) of strontium (Sr) in the wild egg yolks merit mention. The Liverpool, Tomkinson and Adelaide Rivers are all under tidal influence. Although Melacca Swamp is a freshwater, spring-fed swamp, it runs into the tidal Melacca Creek of the Adelaide River (Webb et al. 1983). Increases in nesting in Melacca Swamp since 1980 has occurred mainly in the downstream portion of the swamp, close to the creek, and attributed to females that reside in the tidal Adelaide River coming into the swamp for nesting. Other female Saltwater crocodiles in Melacca Swamp most likely reside there all year round (WMI, unpubl. data).

Crustaceans utilise calcium, strontium and magnesium in their shells (for example, strontium carbonate), and mud crabs (*Scylla serrata*) are a common food item for large Saltwater crocodiles living in tidal, saline habitats. The elevated levels of strontium and magnesium in the egg yolks may reflect the diet of females from these habitats. This notion is also supported by the higher levels of copper in wild eggs (around 50 per cent higher than captive eggs), which may reflect the copper-based blood of crustaceans. Captive Saltwater crocodiles at all Northern Territory crocodile farms, some of which have been in captivity for up to 30 years, do not receive food such as crustaceans.

Lead concentrations in the yolk varied greatly. Saltwater crocodiles are known to ingest lead bullets and shotgun pellets that are lodged in prey or carrion and, like stones, they can be retained in the stomach for long periods of time (for example, Manolis et al. 2002b; Hammerton 2002; Hammerton et al. 2002; Webb and Manolis 1989; Webb et al. 1982). Lead levels in the blood can remain elevated without the animal showing any adverse symptoms of lead toxicity (Hammerton *et al.* 2002). The high levels of lead in the yolk of both captive and wild eggs reflects the previous ingestion of lead by the nesting female.

Examination of the relationship between hatching success and concentration of lead in the yolk revealed a significant trend, however this trend was rendered non-significant by the exclusion of an outlier (r^2= 0.0003, p= 0.94, N= 20) where the cause of low hatching success was considered to have been caused by another factor. The effect of lead passed onto eggs at the time of development within the female, and then presumably to the embryo, remains unclear. On the basis of the recorded levels and available data, there would not appear to be a significant effect on lead on hatching success, but a possible effect with very high levels of lead cannot be discounted completely.

The relatively high concentrations of calcium in the yolk reflect the importance of this element in many physiological functions, as well as bone development. The only data available for a crocodilian (New Guinea Freshwater crocodile; *C. novaeguineae*) suggest that 30-40 per cent of the hatchling's calcium is obtained from the egg contents (Jenkins 1975).

The albumen of crocodile eggs is mainly a store of water (96.4 per cent water content for *C. porosus*; Manolis et al. 1987), which is utilised during embryonic development. The albumen is laid down over the ova within the oviduct, just before the eggshell and shell membrane. The trends with strontium, copper and magnesium are not evident as they were with the yolk (Table 4), perhaps reflecting the different process involved in the formation of the albumen. The variation in lead was however apparent in the albumen. Indeed the concentration of in the albumen of one egg from the Liverpool River was higher than that recorded in the yolk of the same egg (353 µg/L and 56 µg/L respectively).

Table 4. **Concentrations of metals in the albumen of captive-laid and wild Saltwater crocodile eggs. Sources: wild-caught adults at Crocodylus Park (BP; 1M:1F); captive-bred adults at Crocodylus Park (LB; communal pond); and, wild nests [Liverpool River (L), Adelaide River (Ad), Melacca Swamp (Me)]. Numbers in brackets indicate sample sizes.**

	Al (mg/L)	Ca (mg/L)	Fe (mg/L)	K (mg/L)	Mg (mg/L)	Sr (mg/L)	Zn (mg/L)	Cr (mg/L)	Mn (mg/L)	Co (µg/L)	Ni (µg/L)	Cu (µg/L)
BP (4)	0.04	6.16	0.05	42.0	2.82	0.028	0.05	0.001	*	*	23	78
LB (1)	*	5.93	*	29.7	2.97	0.019	0.30	*	*	*	*	67
Ad (3)	*	7.45	0.07	39.7	3.33	0.062	0.02	*	*	*	5	42
Me (3)	*	5.05	0.07	33.1	3.07	0.020	0.26	*	*	*	9	114
L (3)	*	6.04	0.07	38.4	3.90	0.018	0.39	*	*	*	*	104

	As (µg/L)	Se (µg/L)	Mo (µg/L)	Cd (µg/L)	Sn (µg/L)	Sb (µg/L)	Ba (µg/L)	Hg (µg/L)	Pb (µg/L)	Th (µg/L)	U (µg/L)
BP (4)	1.0	16.8	3.9	1.7	2.0	*	6.4	*	14.0	5.8	<1
LB (1)	*	9.0	3.0	2.0	-	*	3.0	*	3.0	4.0	*
Ad (3)	1.6	8.5	3.3	2.5	-	*	8.8	*	13.0	4.6	*
M (3)	*	8.0	3.2	2.7	-	*	3.5	*	56.5	5.2	*
L (3)	*	5.7	3.3	2.8	-	*	6.8	*	129.7	4.4	*

*levels similar to blanks tested at the same time (below detectable levels)

Concentrations for eggshell are shown in Table 5. As expected, calcium is the dominant element (as calcium carbonate). Of the remainder of the elements, copper was slightly higher in shell of wild eggs, possibly reflecting the diet in tidal rivers. The much higher concentration (>350 per cent) of nickel in wild eggs is perhaps the most striking difference.

Table 5. **Concentrations of metals in the eggshell of captive-laid and wild Saltwater crocodile eggs. Sources: wild-caught adults at Crocodylus Park (BP; 1M:1F); captive-bred adults at Crocodylus Park (LB; communal pond); and, wild nests [Liverpool River (L), Adelaide River (Ad), Melacca Swamp (Me)]. Numbers in brackets indicate sample sizes.**

	Al (mg/L)	Ca (mg/L)	Fe (mg/L)	K (mg/L)	Mg (mg/L)	Sr (mg/L)	Zn (mg/L)	Cr (mg/L)	Mn (mg/L)	Co (µg/L)	Ni (µg/L)	Cu (µg/L)
BP (4)	0.03	3729	0.01	1.60	21.1	2.84	0.01	*	0.003	*	3	25
LB (1)	0.06	3999	*	0.85	23.0	2.14	0.06	*	0.007	<1	4	25
Me (3)	0.06	3493	0.05	1.08	22.9	3.68	0.17	*	0.009	<1	18	41
Ad (3)	0.14	4470	0.09	1.69	25.7	7.31	0.05	*	0.015	<1	20	56
L (3)	0.05	3399	0.06	2.08	23.8	2.39	0.07	*	0.002	<1	12	33

	As (µg/L)	Se (µg/L)	Mo (µg/L)	Cd (µg/L)	Sn (µg/L)	Sb (µg/L)	Ba (µg/L)	Hg (µg/L)	Pb (µg/L)	Th (µg/L)	U (µg/L)
BP (4)	*	51.0	3.2	2.0	1.9	*	474	*	4.6	5.8	*
LB (1)	*	49.0	2.0	2.0	-	*	231	*	3.0	4.0	*
L (3)	*	42.9	3.0	3.2	-	*	268	*	7.6	4.4	*
Ad (3)	*	51.4	3.3	3.4	-	*	576	*	50.8	5.2	*
Me (3)	*	40.7	3.6	3.2	-	*	281	*	11.3	5.1	*

* levels similar to blanks tested at the same time (below detectable levels)

The role of the eggshell of the crocodile egg is to provide a physical structure to the egg, facilitate gas exchange into and out of the egg, act as a barrier to water loss, and provide the embryo with a significant portion of its calcium requirements (63-70 per cent; Jenkins 1975). The provision of calcium in the diet of adult crocodiles is well recognised by farmers. There is no evidence to suggest that, in cases where there is a lack of calcium in the eggshell, it is related to diet. Where reported, thinly shelled eggs are invariably associated with young females nesting for the first time, and where the eggs appear to have been laid before they have been adequately shelled whilst in the oviducts.

Meat Composition

The data from crocodile meat from two major meat-producing farms in the Northern Territory provide baseline information on the product, and allow an assessment to be made with regard to heavy metals (Table 6).

Table 6. Concentrations (mg/l or µg/l) of trace elements in crocodile meat from two major meat-producing farms in the Northern Territory. SE=standard error; N=sample size; *=means were significantly different from each other.

		Farm 1			Farm 2			
Element		Conc.	SE	N	Conc.	SE	N	
Al	mg/l	0.14	0.020	13	0.06	0.006	6	*
Ca	mg/l	376.3	15.89	13	8.5	1.02	6	*
Fe	mg/l	0.23	0.026	13	0.20	0.026	6	
K	mg/l	79.9	8.48	13	269.1	29.79	6	*
Mg	mg/l	9.73	0.582	13	19.6	2.27	6	*
Sr	mg/l	0.22	0.011	13	0.01	0.001	6	*
Zn	mg/l	0.88	0.065	13	0.44	0.043	6	*
Cr	mg/l	0.009	0.001	13	0.013	0.002	6	
Mn	mg/l	0.005	0.001	13	0.006	0.001	6	
Co	µg/l	<1.000	-	13	<1	-	6	
Ni	µg/l	27.35	14.485	13	7.3	0.66	6	*
Ni ^	µg/l	13.0	2.21	12	7.3	0.66	6	
Cu	µg/l	20.22	2.248	13	47.3	3.44	6	*
As	µg/l	<1	-	13	<1	-	6	
Se	µg/l	22.7	1.15	13	16.0	2.09	6	*
Mo	µg/l	3.2	0.82	13	4.0	0.31	6	
Cd	µg/l	1.8	0.34	13	2.8	0.10	6	*
Sn	µg/l	34.8	1.77	13	-	-	-	
Sb	µg/l	<1	-	13	<1	-	6	
Ba	µg/l	30.2	1.79	13	5.3	2.60	6	*
Hg	µg/l	<1	-	13	<1	-	6	
Pb	µg/l	52.3	18.07	13	6.8	2.85	6	*
Th	µg/l	7.3	0.16	13	6.1	0.62	6	
U	µg/l	<1	-	13	<1	-	6	

^ outlier excluded (see text)

Of the 24 elements quantified, 12 were significantly different between the two farms (non-overlap of 2 standard errors). One sample had an exceptionally high reading of nickel (199 µg/L) compared to all of the other samples (2-29 µg/L). Where farms are also involved with tourism, there is a possibility of ingestion of foreign objects by crocodiles such as coins, cans, and cameras, potentially leading to elevated levels of certain metals in the blood and tissues. For example, ingestion of soft drink cans is known to have caused mortality in large, adult *C. porosus,* due to the rapid absorption of large amounts of aluminium into the blood. With respect to this high reading for nickel, an error at the laboratory cannot be ruled out (Wong, pers. comm.), and exclusion of the reading resulted in no significant difference in nickel concentration between farms.

As with egg contents (see above), lead ingestion is known to cause elevated concentrations in the tissues. In this case, a number of elevated levels were apparent in three samples from one farm. The Northern Territory crocodile industry is based mainly on ranching, but a wild harvest of juveniles, sub-adults and adults is permitted. As crocodiles are classified as a game animal, the meat of wild crocodiles can be utilised for human consumption, as long as they are processed in an appropriate manner (Leach et al. 2009).

In addition to the wild harvest, some 300 problem Saltwater crocodiles are also removed from Darwin Harbour and other areas each year, and this number is increasing (Leach et al. 2012; PWCNT 2012). Some of these crocodiles are utilised as breeding stock, but most are now processed soon after capture into meat, skins and other products. It is possible that wild crocodiles with higher than normal lead levels in their tissues may enter the farms, but this source of meat is estimated to make up less than five per cent of the crocodile meat produced annually in Australia. Still, even the highest value recorded in this study (around 0.03ppm) is lower than the maximum concentration permitted in food (6ppm). Levels of cadmium (Cd), chromium (Cr), mercury (Hg) and tin (Sn) were also well below acceptable maximum levels for meat for human consumption.

Results

New research results are rapidly expanding our knowledge on factors that affect post-hatching growth and survival of hatchling Saltwater crocodiles. Clutch-specific preferences for different types of food, size of food particle, density and available space are just some of the variables that can have profound effects on growth and survival in the first few months of life (Manolis et al. 1989; Manolis 1994; Webb et al. unpubl. data; Webb et al. 1990). Because of these, the ability to test the efficacy of mineral and vitamin supplements, provided to nesting females, on hatchlings performance, remains somewhat constrained. That individual growth rates of crocodiles are highly variable, both within and between clutches, is an additional variable that potentially overshadows the effects of egg composition on post-hatching performance.

More recently, the aggressive behaviour of dominant individuals, from the time of hatching, has been identified as an important factor to consider when raising *C. porosus* hatchlings (Brien 2012). Thus, although quality of hatchling produced, through captive breeding or ranching, is an important consideration, it may be somewhat subservient to factors such as social interaction between individuals. Why some individuals of a clutch become dominant is unknown, but there are clearly good reasons for such a trait to be selected for from an evolutionary point of view. That Saltwater crocodiles are far more difficult to raise has been recognised by crocodile farmers in Southeast Asia, where the less valuable Siamese crocodile is preferred for that reason.

Clutch effects remain as the most significant variable explaining variation in growth and survival, as is the case for most raising experiments with crocodiles. There are no published data for other crocodilian species on differences in post-hatching performance between captive-bred and wild/ranched hatchlings. Often, management programs are entirely based on captive breeding (for example, Thailand, Cambodia, Vietnam, China, Colombia, Mexico, Philippines, Singapore) or ranching (for example, Argentina, USA), and so comparative information is generally not available. In cases where both ranching and captive breeding are carried out together (for example, Australia, Zimbabwe, Madagascar, Papua New Guinea), hatchlings are often not marked in such a way that performance can be monitored and any differences, if evident, may simply go unnoticed.

With this in mind, research attention could focus on providing the most appropriate vitamin and mineral supplements for hatchlings after they have hatched. Joanen and McNease (1987) developed a vitamin premix that was used at a rate of one per cent of food weight, for American alligators of all sizes, including adults. However, the composition of the premix was not determined on the basis of research, but rather was a “guesstimate” of what the animals required. Smith and Coulson (1992) also recommended vitamin supplements for hatchlings, growers and breeding Nile crocodiles (*C. niloticus*), and provided a list of ‘minerals which would be required by crocodilia’, which appears to be based on the requirements of an egg-laying hen (Smith & Coulson 1992). Again, these supplements do not appear to be based on the specific requirements of captive crocodiles, but rather attempts to provide more that what is considered to be required as “insurance” against deficiencies.

Generally, unless there is clear evidence that vitamin supplements being used are inadequate (for example, symptoms suggesting vitamin deficiencies are apparent), farms generally adhere to established procedures, using commercially available premixes (for example, “Petvite”, “Monsoon Crocodile Premix”). In the absence of more detailed information, this appears to be a suitable approach, certainly for raising stock.

In the case of breeding stock, the reasons for reduced egg viability of captive-raised relative to wild eggs remains unclear. The battery of standardised breeding pens at Crocodylus Park was designed with long-term research in mind. Seventy per cent of the adult females have been in the facility since at least 1998, and with the benefit of reproductive history now available for these females over a long period of time (18 years), a platform to perform more detailed assessments on the effect of diet and supplements is available. Previous attempts to assess captive breeding data on farms have been confounded by an inability to identify females nesting in communal enclosures, or to separate the effects of social interactions. Interestingly, the pattern of nesting at Crocodylus Park changed in 2004/05, after females were provided with a mineral supplement. Whether this reflects the treatment itself, or other factors that are now affecting reproductive output, is also unclear.

That the battery of pens is also used for tourism purposes (including feeding during tour) is a factor to consider. However, breeding pens at Crocodylus Park that are out of bounds to visitors have not performed any better, suggesting that "tourist" disturbance is not significant. That these crocodiles feed in front of large numbers of people throughout the year does not necessarily imply that there is no underlying "stress". The effect of stress on male and female adult crocodilians is poorly understood. Stress is known to reduce levels of male sex hormones in American alligators, which may in turn affect reproductive performance (Lance & Elsey 1986). That the male may be implicated in infertility and/or lower viability of eggs during incubation is usually not considered, and the female is assumed to be responsible. Some pairs of Saltwater crocodiles have a long history of producing good eggs, with low infertility and good incubation and hatching success, but adjacent to them can be another pair with greatly reduced success. Yet conditions are exactly the same, including diet.

Saltwater crocodiles are highly territorial, and in the wild are generally very intolerant of other adults (Lang 1987). In captivity, the proximity of adult Saltwater crocodiles to each other may result in levels of stress whose effects may be more subtle. Lance et al. (2004) reported that high corticosterone levels in the blood, an index of stress, were associated with suppression of testosterone secretion in adult male American alligators, but no research has been undertaken to assess the situation with Saltwater crocodiles, a far more aggressive species of crocodilian.

The nutrition of adult breeding crocodiles has received little attention generally (Huchzermeyer 2003). Joanen and McNease (1987) reported that alligators fed fish showed declines in clutch size, fertility and hatchability relative to animals fed red meat. Levels of Vitamin E were considered to be implicated in this reduced performance (Lance 1987). Farms in Australia tend to use the most readily available diet for adult crocodiles, and whole chicken is the most commonly used food. Growth certainly does not appear be affected in a detrimental manner with a diet of whole chickens, but the potential effect on vitamin requirements is unknown. The frequency of feeding in captivity is much higher than in the wild, resulting in captive crocodiles that are more obese per unit length. This has been suggested as a factor affecting reproduction of captive American alligators, but definitive data have not yet been provided.

Currently, most of Australia's crocodile meat is consumed domestically. Exports are constrained to a degree due to less expensive meat being produced in developing countries where producers have the advantage of a much cheaper labour supply than Australia. Proximity to the markets such as China also places many of these producers in a more favourable position. Imports of crocodile meat into Australia have occurred in the past (for example, Nile crocodile from Zimbabwe, and Saltwater Crocodile from Papua New Guinea), and importers must adhere to the Australian standards on processing crocodiles for human consumption (ARMC 1998). But regulations that govern the use of antibiotics and/or other chemicals on crocodiles in Australia are not necessarily applied elsewhere to the same degree. That imported crocodile meat may not be subject to the same rigid regulations as meat produced in Australia is a concern for the crocodile industry (Crocodile Farmers Association of the Northern Territory, pers. comm.). The reported concentrations of trace elements in crocodile meat provide a basis on which imported crocodile meat can be assessed to some degree, but clearly standards need to be extended to other potential contaminants, including antibiotics.

Crocodiles have been identified as good bio-indicators of their environment (Manolis et al. 2002a), and the results here attest to the fact that some elements in their environment can be reflected in their tissues (meat), and passed on to their eggs (in the case of females). It is likely that crocodiles raised in a particular farm may have certain element characteristics that are indicative of their environment. For example, farms vary with respect to geographic location, proximity to urban and/or agricultural areas, sources of water, and so on.

The effect of agricultural practices of wild crocodiles merits further investigation. In Florida, the widespread use of pesticides and their impact on alligator reproduction is of concern to authorities, and heavy metals such as mercury have also been detected in alligators from polluted areas (Huchzermeyer 2003). Pesticides are not considered an area of concern for crocodile farms in Australia, albeit little work has been undertaken on this issue. Packett et al. (2004) selected wild Saltwater crocodile eggs from the Adelaide River (Northern Territory) to serve as a control against which he could compare eggs collected from areas exposed to pesticide, and noted that their controls also registered levels of pesticide.

That there were few significant differences in element concentrations between wild and captive eggs was a welcome result, suggesting no major deficiencies between the two sources of eggs. However, of particular interest was lead. Camus et al. (1998) considered lead consumption to be implicated with mortality of farmed American alligators fed wild-caught nutria. Lance et al. (2006) cited lead as a "probable cause" of embryonic mortality in alligators. Hammerton et al. (2003) on the other hand reported that in juvenile Saltwater crocodiles fed lead pellets, high levels of lead were noted in the blood, but the animals did not show any adverse physical effects. Although there was no significant relationship between lead levels in eggs and hatching success in this study, lead ingestion remains a potential contributor to embryonic mortality in *C. porosus*, particularly in cases a high proportion of breeding stock is from the wild where exposure to lead through ingestion of prey occurs.

In the Northern Territory, lead shot has now been replaced by bismuth, but the former is still used in remote communities such as Arnhem Land, and remains in habitats where it is eaten by geese and other waterbirds as they forage. Some clutches, from all areas examined, showed high lead levels, suggesting that lead is possibly widespread in the environment, or at least in those wetlands where hunting of waterbirds occurs (for example, magpie geese). Lead levels in crocodile meat were at very low levels, and are not considered to be a health risk in any way. Likewise levels of mercury and cadmium were very low, and were well within acceptable limits.

Implications

The outcomes of this research allow industry to continue to build industry standards for crocodile meat, an important by-product of the processing of crocodiles. This research also establishes baseline information on the concentration of trace elements in wild Saltwater crocodile eggs, against which captive-bred eggs can be compared. The long-term monitoring of nesting of a group of known females will allow further experimental manipulation on other aspects of husbandry such as diet, with a view to understanding the factors that affect reproduction in captive Saltwater crocodiles.

Recommendations

A more extensive assessment of meat derived from different farms or abattoirs in Australia would be beneficial, as the nature of the industry has changed since this study was initiated. For example, many crocodiles are produced in the Northern Territory and raised for varying amounts of time, but are then transferred to different Queensland crocodile farms for the final stages of growth and processing. This introduces another variable when examining the composition of meat from particular sources (note: This recommended activity has now been initiated).

Comparative studies using crocodile meat imported into Australia would be useful. Attempts to obtain imported *C. porosus* meat from Papua New Guinea in this study were unsuccessful. However, given concerns on the quality of meat imported into Australia, analysis of imported meat could be important to ensure that standards are being met.

Collaboration with Northern Territory researchers investigating the utility of isotopes to quantify the origin of crocodiles is recommended, as this methodology will most likely be applicable to farmed crocodiles as well. This method has been used to distinguish between wild and farmed sea turtles (Moncada *et al.* 1998), and could serve as a more effective enforcement tool for different types of crocodile products, including meat.

A detailed assessment of captive breeding at different crocodile farms may extend our knowledge of the effect of different pond designs, husbandry and other factors on egg and hatchling quality over a long period of time (some breeding farms have now been operation for over 30 years). The potential reluctance of farms to divulge this type of 'confidential' information may be a constraint to advancing this type of initiative, but it could perhaps be pursued through the newly established Crocodile Farmers Association of the Northern Territory.

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Production Implications of Trace Element Concentrations in Crocodile Eggs and Tissues

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This research provides baseline comparative information on the concentrations of 24 trace elements in wild and captive-laid eggs and crocodile meat. In the case of crocodile meat, the data forms preliminary standards on which industry can now build.

The ability to monitor the reproductive performance of known breeding Saltwater crocodiles over a long period is important to our understanding of how crocodiles perform in captivity, in response to different environmental and husbandry variables. There is scope for improvement in hatchling production through captive breeding.

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