Improving the quality of Australian crocodile skins

A report for the Rural Industries Research and Development Corporation

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Foreword

There is no doubt that the demand for crocodilian skins that existed in the mid- to late 1980s is a thing of the past. Grading standards have become and will remain much stricter than they ever were before. Attempts to improve the quality of skins have largely been restricted to varying diet and husbandry techniques to minimise obvious external problems (e.g. scars, scratches, stretching). There has been no attempt to assess the structural correlates, and feedback in this area is a matter of simply waiting to see what happens when tanners and manufacturers deal with the results.

Preliminary discussions with some of the world's best tanners of crocodilian skins (in France, Japan, Singapore) indicate that they are largely ignorant of the morphological characteristics that dictate skin quality. But they do know that wild skins are invariably of higher quality than farm-raised skins.

This project, like the Australian research carried out on crocodilian egg incubation, takes a largely unknown field of critical commercial significance, and attempts to develop a sound understanding of the processes involved in skin development, that can be applied to existing problems. In many conventional animal industries (e.g. beef, pork, chicken), the emphasis is on increased size (for meat). This project directs research at the key marketable product of crocodile farming (the skin), rather than at what is essentially a byproduct (meat/size) of the industry.

This report, a new addition to RIRDC's diverse range of over 450 research publications, forms part of our New Animals Products R&D program, which aims to accelerate the development of viable new animal industries

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Peter Core
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Executive Summary

The number of crocodile farms around the world has increased greatly over the last decade. As a result of increasing numbers of farmed crocodile skins on the international market, grading standards have become very strict. Saltwater Crocodiles (*Crocodylus porosus*) have held their reputation as having the best skin of all of the crocodilian species, but this counts for little if skin quality is compromised.

The presence and/or extent of physical damage (eg scratches, bites, processing cuts) contributes significantly to the downgrading of skins, and is usually related to husbandry regimes. In some cases it may be related to disease (eg brown spot, pox virus), the effects of which sometimes may not be apparent until after processing (tanning). Farmed skins invariably are wider per unit length than wild skins, the result of fast growth rates and types of food in a captive situation. The relatively high fat contents of captive diets, coupled with the highly efficient crocodilian system for storing fat, causes farm animals to be more obese and 'wider' than their wild counterparts.

The relatively high value of *C. porosus* skins exists because of the characteristics of the species, namely: there is no bone in the belly scales; the ventral scales are of similar size; and, there is a high number of scale rows on the belly surface (i.e. more scales). We found considerable variation in the number of scale rows in *C. porosus*, ranging from 27-37 rows (mean = 31.2 rows). From the product manufacturers point of view, the more scales on the belly, the better the skin for many high quality fashion products. However, a very low proportion (<2%) of animals examined possess the highest numbers of rows (35-37), and so a skin with this characteristic does not have a premium price. Some manufacturers indicated that a regular supply of skins with higher numbers of rows would be very well received.

Manipulation of the incubation environment was considered a way to increase the number of scale rows on an animal (the number of rows is set by the time of hatching). There was a significant effect of incubation temperature on the mean number of scale rows, with higher temperatures producing a higher mean number of rows. Scale formation is visibly evident by 30-35 days (at 30C), about one-third the way through incubation, and so pulse and switch experiments were undertaken to determine the sensitive period at which scale formation could be affected. These experiments revealed that the sensitive period was between 26 and 30 days for high temperature (33C) incubation, and 10-30 days for cool temperature (30C) incubation.

A genetic component was also identified as affecting the number of scale rows. Examination of the progeny of known nesting pairs of adult *C. porosus* indicated that the female was not significant, but the male was. That is, there was a significant (although highly variable) relationship between the number of scale rows on the male and that on his progeny. With farms having an increasing reliance on captive-bred hatchlings (there is no other option in Queensland), this result indicates that selection of male breeding stock is more important than is currently thought.

The embryonic development of the skin of *C. porosus* occurs in four stages. The first stage (14-21 days) is characterised by a single layered epidermis, below which lies a poorly defined dermis. Stage 2 (22-34 days) is characterised by a thickened multilayered epidermis, and the dermis consists of loose fine fibers. Both epidermis and dermis thicken during this period, to 11 μm and 50 μm respectively. Small eleveations in the eopidermis correspond to integumentary sense organs of older embryos. By Stage 3 (35-66 days) the dermis appears as two dicrete types - loose dermis underlying the epidermis, and dense dermis. The dense dermis thickens rapidly at a steady rate, and continues to do so until hatching. The loose dermis decreases in thickness,

from about 38 to 18 μm. The epidermis does not change thickness between 26 and 40 days, remaining at about 11 μm. This was different to the situation observed in Australian Freshwater Crocodiles (*C. johnstoni*), where the epidermis grows steadily throught the incubation period. The significance of this difference is unknown, but the period of stable epidermal thickness corresponds to the sensitive period indicated by incubation experiments.

Stage 4 (67 days to hatching) is characterised by a thick dermis, the presence of birefringent fibers/granules in the loose dermis and the formation of a definite keratin layer. The periderm becomes progressively thinner after about 60 days, and is totally lost before hatching. At hatching the epidermis is about 28 μm thick, and consists of four strata (s. germanitivum, s. spinosum, s. granulosum, s. corneum). The dense dermis from one scale traverses below the interscalar cleft to embed into the dense dermis of the adjacent scale. The development of all the major skin components was quantified. The growth of the skin as a whole (to about 250 μm at hatching) proceeds in a linear fashion.

The skin continues to grow in a linear fashion after hatching, with general thickening of the epidermis and dermis. As a high proportion of the skin thickness is attributable to dermis (generally greater the 80%), the development of the dermis reflects that of the total skin. At hatching skin thickness from different sites on the body was very similar, but differences become apparent by one year of age. The skin on the ventral surfaces (e.g. belly, tail) was thicker than sites elsewhere (e.g. neck, wrist), a difference more apparent after one year of age. In addition, keratin made up a higher proportion of the epidermis in the ventral surfaces. For a given sized crocodile, from the wild or farmed, skin thickness was highly variable. The skin from the chin of wild crocodiles was thicker (33%) than that of farmed crocodiles, confirming the information from skin buyers and tanners that wild skins tend to be thicker than farmed skins. Interestingly, the increase in overall thickness was the result of thicker dermis and thinner epidermis. In the wrist the wild skin was slightly thinner than farmed ones, but the epidermis was still relatively thinner (by about 50%).

Within different types of skin, four patterns of dermal architecture were identified. These varied on the basis of the arrangement of the collagen bundles making up the dermis. These longtitudinal bundles tended to be narrower and more compact than in farmed animals, and it is felt that changes in these orthogonal layers may reflect differences between wild and farmed animals. Growth rates on crocodile farms far exceed those of wild crocodiles, and together with a fattier diet, may alter the dermal structure of the skin.

Certainly, skin buyers and tanners were largely ignorant of the morphological characteristics of crocodile skins. Saltwater Crocodiles are widely distributed and extensively farmed and/or harvested (e.g. Malaysia, Thailand, Papua New Guinea, Indonesia, Palau, Solomon Islands, Singapore, India) throughout their range. There appears to be a high degree of variation between *C. porosus* from different countries and farming operations, and there are probably interaction of factors influencing the quality of skins from them.

Introduction

With the reduction in most populations of crocodilian species worldwide, as a result of uncontrolled hunting for their skins, crocodile farming increasingly became an avenue for the production of skins for the fashion leather industry. In Australia, crocodile farming began on a large scale in the early 1980s, mainly in the Northern Territory and Queensland. During those early years, there was a high demand for skins, particularly those of *Crocodylus porosus* (Saltwater Crocodile), and grading standards were not strict. Skin quality was really not a major problem faced by Autsralian crocodile farmers, and high prices were being achieved. However, in the 1990s, with increasing numbers of crocodile farms around the world (Groombridge and Luxmoore 1992), and subsequent increases in the numbers of classic skins [mainly Nile Crocodile (*C. niloticus*) and American Alligator (*Alligator mississippiensis*)] on the international market, grading standards (eg Van Jaarsveldt 1987) became more strict. It was now a question of skin quality.

Many skins which previously would have passed as first grade no longer did so. Even though *C. porosus* skins are considered the best crocodilian skin in the world, and the species retained its position relative to other crocodilians, the emphasis was on production of high quality (first grade) skins. Some of the problems affecting skin quality were already known, such as physical damage (scars, scratches, bites). Thus attempts to improve the quality of skins were aimed at reducing physical damage, through improved raising facilities, reduction of stocking densities and improved efficiency of raising. Given the nature of the skin industry, there was usually little feedback to farms on the quality of their skins, other than grading standards.

There were indications from skin buyers and tanners that wild skins were considered to be of higher quality than farmed skins, but there were simply no criteria on which to quantify any differences. Our knowledge on the development of the skin was essentially non-existent. In the early 1980s, Australian research on crocodile egg incubation (eg Webb *et al.* 1987; Webb and Cooper-Preston 1989) led to significant advances in crocodile farming generally. It was felt that the understanding of the basic processes involved in skin development, within the egg, could provide a baseline from which improvements could be made to the quality of crocodile skins from Australia.

Objectives

The objectives of the project were: to describe the morphological structure of the skin of farmed *Crocodylus porosus*; to determine the problems associated with skin quality as perceived by skin buyers, tanners and product manufacturers; to develop assessment criteria, based on skin structure; to describe in captive *C. porosus*: the embryological development of the skin (incubation effects); the development of skin structure with age and size; the extent of natural variation in skin pattern; and, the level of heritability of skin structure and pattern; and, to compare the structure of the skin of wild *C. porosus* with that of farmed *C. porosus*.

Methodology

Embryological development of skin

Crocodylus porosus and *C. johnstoni* eggs were incubated under constant temperature conditions (32C and 30C respectively) in 'Labec' water-jacketed incubators, and embryos removed at various stages throughout incubation (from 14 days to hatching). Following euthenasia, samples of skin were removed from different parts of the body (belly, trunk, tail, neck), and fixed in 10% neutral buffered formalin for standard histology. Histological examination of 5 μm sections of the skin was undertaken after treatment with various combinations of stains [eg haematoxylin and eosin (general morphology), PAS-alcian blue (muco-polysaccharides), Gordon and Sweet's reticulin (reticular fibers), Masson's trichrome (collagen and muscle), peripheral nerve stain (myelin), performic acid alcian blue (keratin), Shikata's orcein (elastin), perl's reaction (haemosiderin), Oil Red O (fat), potassium permangenate removal and oxalic acid clearing (melenin). Some skin samples were fixed for examination using electron microscopy (see Richardson *et al*. 2000a).

Skin samples

Skin samples were taken from farm-raised crocodiles produced from wild or captive-laid eggs. In the case of the former, in the Northern Territory eggs are collected during an annual egg harvest (December-April), carried out under the regulation of the Parks and Wildlife Commission N.T. All eggs, regardless of their origin, were incubated at constant 32C, 99+% humidity and maximum oxygen availability, without nesting media (ie on open racks; see Webb *et al.* 1987). Captive-laid eggs tended to be collected within one day of laying, whereas wild eggs varied in age, from freshly-laid to near hatching. At the time of hatching, most hatchlings were scute-clipped, and their age could therefore be determined.

Raising facilities varied between and within facilities for different sized (aged) animals. Hatchlings (0-1 year of age) were generally raised under cover, in small enclosures (1.5-3.0 m^2) constructed of fibreglass or concrete, and with some type of heating of the water (around 32C). Diet consisted of a minced mixture of 75-80% red meat (kangaroo, horse, feral pig) and 20-25% chicken heads, with added multivitamins and di-calcium phosphate powder. Animals were fed 6-7 times per week for the first 3-6 months of age, and less regularly with increasing size, until by one year of age they were typically being fed 3-4 times per week.

After one year crocodiles are usually large enough to be housed in outdoor pens. These are concrete-lined, with varying degrees of shading, water depth and density of crocodiles, depending largely on the size of the animals. Additional covers are placed over pens during the cooler times of the year (June-September) to maintain warmth within the pens, but otherwise no other control of the environment is attempted. Diet for juveniles consisted predominately of chicken heads, from one year of age up to the time of slaughter. Frequency of feeding was usually 2-4 times per week. All pens were

cleaned after each feed, and the water replaced completely.

Initially, attempts were made to take skin samples from immobilised crocodiles (using “Flaxedil”), but this was found to be unsuitable due to the nervous response of the animals as the skin was being cut. Even with the use of local anaesthetic in conjunction with the immobilising agent, samples were generally of little value for histological purposes. In crocodilians the nervous response remains after death (a typical reptilian trait due to their anaerobic metabolism).

All crocodiles were shot from close range in the back of the cranial platform using a 0.22” (short calibre) rifle. The spinal cord was then severed and the animal left to bleed, before being processed. Most skin samples were collected from farm-raised *C. porosus* at Janamba Croc Farm (Middle Point, N.T.) and Crocodylus Park (Darwin, N.T.), soon after they were slaughtered and being prepared for skinning. Similarly, skin samples were taken from wild *C. porosus* collected from Darwin Harbour by the Parks and Wildlife Commission N.T., and from crocodiles caught by WMI staff in collaboration with landowners during harvest operations in the Liverpool River (Arnhem Land) and the Mary River (Swim Creek Station and Carmor Plains Station). Crocodiles were caught using a harpoon (see Walsh 1987), pulled alongside a boat, and shot in the back of the cranial platform.

Skin samples (3-5 mm x 1-2 mm x 3-4 mm) were taken from various parts of the body of different-sized farm and wild animals (eg chin, belly mid, belly pelvic, thoracic belly, belly caudal, vent, ventral tail, dorsolateral tail, flank, occipit, nuchal, posterior-nuchals, interscute, dorsal wrist, ventral wrist). As the value of crocodilian skins is greatly reduced if the belly region has even the smallest hole, sampling was restricted to areas away from the valuable portion of the skin. All samples were fixed in 10% neutral buffered formalin for standard histology (see above). A calibrated eyepiece micrometer was used to measure all tissue thickness.

Variation in skin pattern

The numbers of scale rows on the belly skin is an index of scale size, and can vary greatly between individuals. The number of scale rows for individual hatchlings was determined by counting the ventral scales down the midline of the belly, from the collar (neck) to the anterior margin of the cloaca (vent). This was undertaken on the animals themselves or from photocopies of the belly surface, which provided a hard copy of the belly scale pattern. The scale row counts of hatchlings derived from the annual wild egg harvest in different areas, were quantified over three seasons. In some cases complete clutches were counted, but in others a random sample (at least 10) of the hatchlings produced from a clutch were examined.

Level of heritability and effect of incubation temperature on skin pattern

During the 1996/97 nesting season, the effect of incubation temperature on the number of scale rows was determined by incubating freshly-laid (<1 day old) *C. porosus* eggs at constant incubation temperatures of 31, 32 and 33C. The number of scale rows was determined for each animal at hatching.

From these preliminary results, experiments in the 1997/98 nesting season were aimed to identify the period during incubation where scale row formation was effected. The period from 22-45 days is when the dorsal and ventral body scales become evident to the eye (Ferguson 1985). Eggs were ‘pulsed’ at one particular incubation temperature, after starting and finishing incubation at another temperature. Specifically, eggs were initially incubated at 32C for 10 days, and then placed in either 30, 31, 32, 33 or 34C for an amount of time equivalent to 27 days of incubation at 30C (real times of incubation at each temperature were calculated using development rate coefficients derived by Webb *et al.* 1987). Following this pulse, eggs were returned to 32C to complete incubation. The number of scale rows

were determined for each animal at hatching.

During the 1998/99 nesting season, switch experiments were carried out throughout the sensitive period (identified previously during the 1997/98 season) to determine the actual age at which scale formation (number of rows) is fixed. Eggs from freshly laid clutches were split between 30C and 33C. Eggs started at 30C were shifted to 33C after 18, 20, 22, 24, 26, 27, 28 or 30 days (ie 2 day intervals). Eggs started at 33C were shifted to 30C either after 9, 13, 17, 22, 26 or 30 days (ie after 5 days at 30C equivalents), or after 14, 16, 17, 19, 21, 22, 24 or 26 days (ie 2 days at 30C equivalents). The number of scale rows were determined for each animal at hatching.

Captive-bred hatchlings were obtained from individually marked pairs of adult *C. porosus* held at Crocodylus Park (Darwin) since 1994. Adults were housed in 20 identical unitised pens, allowing the progeny to be assigned and compared to known parents. Eggs were collected within one day of laying and incubated at 32C.

Industry perceptions

It was initially proposed to develop a questionnaire in order to collect information on what problems the crocodile industry considered important with regard to skin quality. However, preliminary discussions with some skin buyers revealed that this was probably not the best option for gathering information. The industry involved with *C. porosus* is small, and interchange of ideas is based on existing relationships. There is also still a level of confidentiality in the industry, and many of the traders are in commercial competition with each other. A level of 'trust' must be developed, and so the approach taken was to discuss the issues face-to-face. Opportunities were taken to do so at various fora where people involved in the industry were present. This included attendance at the Hong Kong Leather Fair (April 1998, 1999) and meetings of the IUCN-SSC Crocodile Specialist Group (Singapore 1999) and its Steering Committee (Zimbabwe, June 1997). Contact was made with key industry people during annual visits to Japan, and during other activities in Papua New Guinea (1998) and Zimbabwe (1997). Skin buyers visiting Australia were interviewed.

The key organisations from which information was gathered included the following: Nam Heng Leather Dying, Kwanpen Reptile Traders, Heng Long Leather Co., (Singapore), Italhide (Italy), Mainland Holdings, Bush Developments (Papua New Guinea), Australian Crocodile Traders, Janamba Croc Farm (Australia), Inoue and Co., Horiuchi Trading Co., Japan Leather and Leather Goods Industries Association, Kataoka Corporation (Japan), Gordon-Choisy, Etienne Droulez (France), Lake Crocodile Park, Crocodile Farmers Association of Zimbabwe (Zimbabwe).

Interviewees varied greatly with regard to their experience and involvement in the industry. For this reason questions were usually tailored for the particular person and their experience with *C. porosus* skins. Questions were mainly aimed at identifying the problems people encountered with skin quality, and possible causes for such problems.

Results

Embryological development of skin

Four stages of development of the skin were identified in both *C. porosus* and *C. johnstoni* (Richardson *et al.* 2000a, b, c). Here, only *C. porosus* will be described, following Richardson *et al.* (2000a). Ages are days of incubation at 30C, the standard temperature used by Webb *et al.* (1987).

The first stage, from 14-21 days, is characterised by a single layered epidermis (stratum germinativum) of flattened cells. Immediately below the basement membrane, the presumptive dermis, hypodermis and muscle are poorly defined. The presumptive dermis consists of individual basophilic fibroblasts and few thin collagen strands separated by large intercellular spaces.

Stage 2 (22-34 days) is characterised by a greatly thickened, multilayered dermis. Throughout most of this stage the dermis consists of loose fine fibers, but towards the end of the period an inner zone of wide fibers can just be seen. The epidermis thickens from about 2.2 to 11 μm (Fig. 1), and the dermis from about 20 to 50 μm (Fig. 2). The periderm cells form a squamous surface layer which is laying down large amounts of neutral mucopolysaccharides. Indications of scale formation are apparent between 31 and 35 days, and appear as shallow epidermal grooves. Small epidermal elevations correspond to the integumentary sense organs apparent in older embryos.

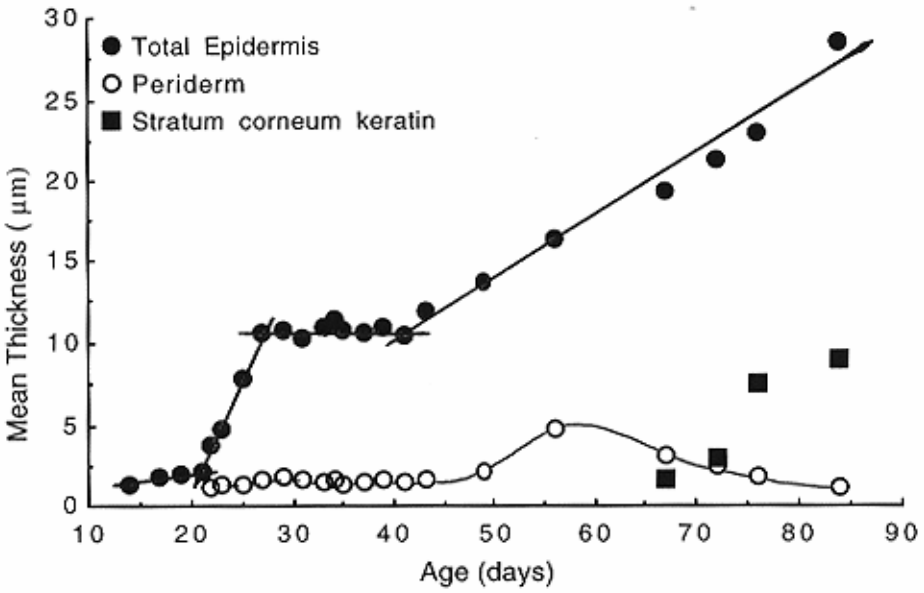

Figure 1. Mean thickness of the periderm, stratum corneum keratin and total epidermis in the skin of *Crocodylus porosus* embryos incubated at 30C, from 14 days to 1 day post-hatching. Lines indicate regressions.

By Stage 3 (35-66 days) there is an rapid increase in the thickness of the dermis (Fig. 2). Scales are clearly seen by 44 days. At the beginning of this stage the peridermal cells are simple squamous cells with large numbers of mitochondria, but by the end of the stage they lose their cytoplasmic organelles and become difficult to see. The underlying dermis has the same appearance as that of other maturing vertebrates. During this stage the loose dermis layer immediately beneath the epidermis decreases in thickness, from about 38 to 18 μm. It is uniformly distributed beneath the scales but virtually absent at the interscalar clefts. In contrast, the dense dermis increases in thickness at a steady rate (Fig. 2). Melanophores are found at the junction of the loose and dense dermis, in the lateral and dorsal aspects of the body, but not in the belly skin.

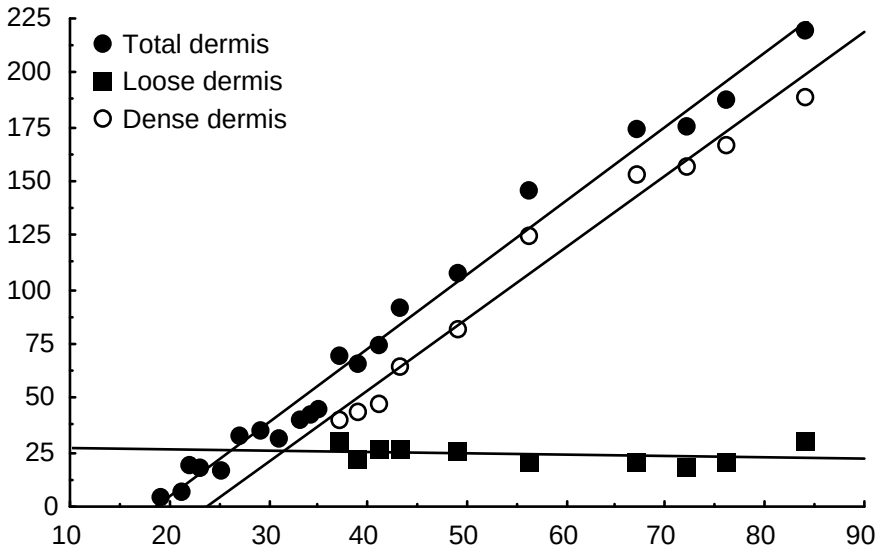

Figure 2. Mean thickness of loose, dense and total dermis in the skin of *Crocodylus porosus* embryos incubated at 30C, from 14 days to 1 day post-hatching. Up to 30 days, the total dermis is composed of loose dermis. Lines indicate linear regressions.

Stage 4 (67 days to hatching) is characterised by a thick dense dermis, the presence of birefringent fibers/granules in the loose dermis and the formation of a definite keratin layer. The epidermis is well established and is accompanied by an increase in both cell number and density. The stratum corneum gives rise to an amorphous, translucent layer of keratin which is eosinophobic over the scale and eosinophilic in the interscalar clefts. The periderm becomes progressively thinner and is totally lost (Fig. 1). The structure of the dermis is similar to that in Stage 3, except that a network of arborising fine fibers associated with globules of brown pigment are found in the loose dermis early in the stage. Under polarising light these cells have a strong silver reflection (birefringence).

At hatching, the epidermis (about 28 μm in thickness) consists of a basement membrane surmounted by four strata (s. germinativum, s. spinosum, s. granulosum, s. corneum). The dermis consists of an outer loosely arranged collagen fiber zone (loose dermis) above an inner densely packed collagen layer (dense dermis). The birefringence of the loose dermis is pronounced. As in older embryos, the dense dermis from one scale traverses below the interscalar cleft to embed into the dense dermis of the adjacent scale. Integumentary sense organs are similar in structure to those found in Stage 4 embryos.

The development of the different components of the skin was quantified. The periderm increases in thickness from around 1.1 mm at 22 days, to a mean thickness of 1.5 μm (SD= 0.12, N= 11) between 25 and 43 days. At some stage between 43 and 48 days it begins to thicken, reaching its maximum thickness (4.8 μm) at around 55-60 days (Fig. 1). From 56 days onwards it decreases in thickness (P) rapidly in a linear fashion (P= 12.2 - 0.13A; r^2= 0.99, p= 0.0002, N= 4; A= days at 32^{o}C), to around 1.1 μm at hatching (Fig. 1). During the period where the periderm is decreasing in thickness, the stratum corneum keratin is forming, increasing from around 1.5 μm at 67 days to about 9 μm at the time of hatching (Fig. 1).

The epidermis (E) grows in four distinct stages. The initial period is characterised by a slow linear increase from 14-21 days [E= -0.52 + 0.13A; r^2= 0.97, p= 0.015, N= 4), from 1.2 μm to 2.2 μm thickness. Between 21 and 27 days the epidermis thickens much more rapidly, from 2.2 μm to about 11.0 μm (E= -27.4 + 1.41A; r^2= 0.998, p= 0.0001, N= 5), a 5 fold increase. Following this period of rapid growth, from 27 and 41 days, the thickness of the epidermis remains stable (mean= 10.8 μm,

SD= 0.34, N= 9). After 41 days it again begins to thicken rapidly, to about 28 μm at the time of hatching. Epidermal thickness from 42-84 days is described by a linear regression (E= -4.8 + 0.38A; r^2= 0.97, p= 0.0001, N= 7) (Fig. 1).

Around 32-35 days, it was difficult to distinguish loose dermis from dense dermis, and it was considered likely that "early" dense dermis (even before this time) was mis-identified as loose dermis. Data relating to both loose and dense dermis in this period were thus excluded from any analyses. From 18-30 days, the total dermis is composed mostly of loose dermis, which from 35-70 days of age does not change significantly in thickness (mean= 24.4 μm, SD= 4.37, N= 10) (Fig. 2).

The dense dermis (DD) on the other hand thickens rapidly (DD= -79.2 + 3.31A; r^2= 0.98, p= 0.0001, N= 10; linear regression) from 36 days to hatching (Fig. 2). In the latter part of incubation the thickness of the total skin (TD= -63.3 + 3.41A; r^2= 0.99, p= 0.0001, N= 21; Fig. 3) reflects the growth of the dense dermis component (Fig. 2). At the time of hatching, the skin is around 250 μm in thickness (Fig. 3).

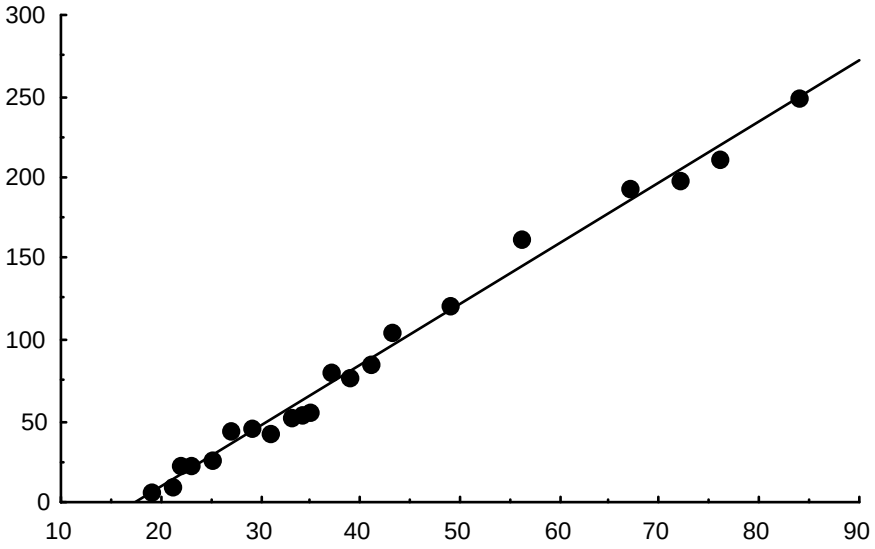

Figure 3. Mean thickness of the total skin of *Crocodylus porosus* embryos. Age is time of incubation at 30C.

Table 1. Distribution (%) of scale row counts for *Crocodylus porosus* hatchlings from 64 clutches, in areas from which 5 or more clutches were represented. Numbers in brackets indicate the numbers of clutches. Mean scale row counts (means of means) are also shown.

No. of Rows	All Areas (64)	Adelaide (11)	Arafura (8)	Finniss (18)	Melacca (5)
27	0.2	0.6	0.0	0.2	0.0
28	1.6	2.1	1.9	1.6	0.5
29	8.3	7.4	7.5	6.5	12.1
30	21.8	25.5	20.6	17.7	31.0
31	25.6	26.3	29.6	22.7	26.8
32	24.1	23.3	22.0	26.2	20.1
33	12.5	11.3	15.3	13.9	7.8
34	4.1	3.4	2.5	6.6	1.6
35	1.5	0.0	0.6	3.4	0.0
36	0.3	0.0	0.0	0.9	0.0
37	0.1	0.0	0.0	0.2	0.0
Mean	31.2	31.1	31.2	31.6	30.8

Variation in skin pattern

The number of scale rows on individual hatchlings produced from the wild egg harvest varied between 27 and 37 rows (Table 1). The mean scale row counts from the harvest areas (Melacca Swamp, Adelaide River, Arafura Swamp, Finniss River) from which the majority of hatchlings are derived for Northern Territory crocodile farms were similar (30.8 to 31.6; Table 1). Taking these four areas together, most hatchlings (84.0%) examined had between 30 and 33 scale rows; few (1.9%) possessed 35 or more rows (Table 1). Compared with other areas from which eggs have been harvested, but for which smaller numbers of clutches were available for examination (Blyth, Cadell, Fitmaurice, Victoria, Goomadeer, King, Roper, Liverpool, Mary, Reynolds and Tomkinson Rivers, Nungbulgarri Creek), the trend was similar, with 83.0% of hatchlings possessing 30-33 rows, and only 2.0% having 35 or more rows. The mean scale row count for 1849 hatchlings from all areas was 31.2 rows.

Within individual clutches, the difference between the hatchling with the highest number of scale rows and that with the lowest number varied between 2 and 7 rows. Most clutches (84.7%) showed variation of 4-6 rows (Table 2).

Table 2. Difference between the highest and lowest scale row counts within 85 clutches of wild *Crocodylus porosus* eggs.

Difference	No. of Clutches	% of Clutches
2	1	1.2
3	7	8.2
4	27	31.8
5	25	29.4
6	20	23.5
7	5	5.9

Level of heritability and effect of incubation temperature on skin pattern

Constant temperature

For 8 of 9 clutches incubated at 31, 32 and 33C, there were significant linear regression relationships between mean scale row count and incubation temperature (r^2= 0.10-0.38; p= 0.0001-0.03); all indicated increasing mean scale row counts with increasing temperature (Fig. 4; Table 3). Although the relationship for the one remaining clutch was not significant (r^2= 0.03; p= 0.39), it still indicated an increasing (positive) trend.

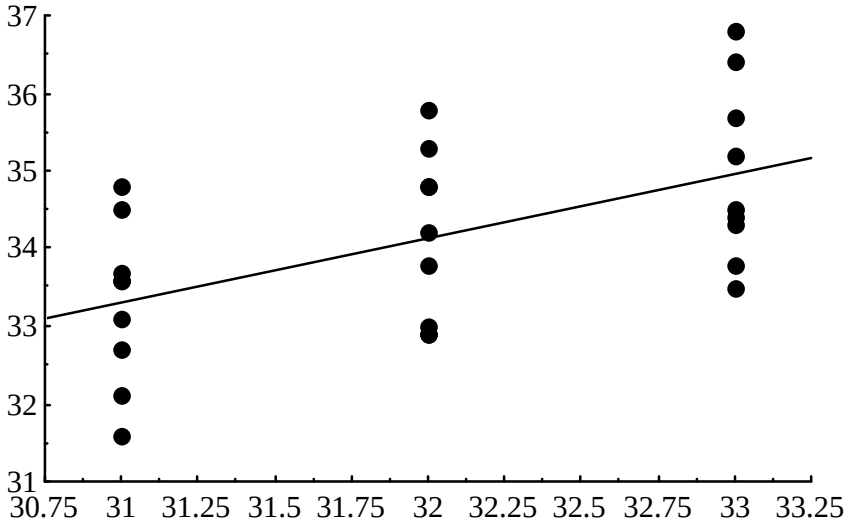

Figure 4. Relationship between mean scale row count and constant incubation temperature for hatchlings from 8 clutches of *Crocodylus porosus* hatchlings. See Table 3 for data.

Table 3. Mean scale row counts for 9 clutches of *Crocodylus porosus* eggs incubated at constant 31, 32 and 33C. Numbers in brackets are range, sample size and standard error respectively.

Clutch	31C	32C	33C
A	32.7 (31-35; 11; 0.36)	33.8 (32-35; 13; 0.32)	34.5 (33-36; 13; 0.22)
B	34.8 (33-37; 14; 0.28)	35.8 (32-38; 13; 0.47)	36.8 (30-40; 12; 0.71)
C	33.6 (30-36; 12; 0.45)	34.2 (32-36; 11; 0.33)	36.4 (35-40; 9; 0.56)
D	33.1 (30-35; 12; 0.40)	34.8 (33-38; 10; 0.51)	35.2 (33-38; 10; 0.59)
E	31.6 (30-33; 11; 0.28)	32.9 (31-35; 16; 0.27)	33.5 (31-35; 15; 0.26)
F	33.6 (31-36; 13; 0.40)	33.0 (31-34; 7; 0.44)	34.3 (31-37; 9; 0.73)
G	32.1 (30-35; 15; 0.34)	32.9 (31-34; 12; 0.29)	33.8 (32-35; 17; 0.22)
H	33.7 (32-35;14; 0.27)	34.8 (32-37;17; 0.37)	34.4 (31-37;18; 0.39)
I	34.5 (32-37; 15; 0.32)	35.3 (35-36; 4; 0.25)	35.7 (34-37; 15; 0.25)

Pulse experiments

Mean scale row count (SRC) increased significantly with increasing "pulse" temperatures (PT; 30-34C) (r^2= 0.43, p= 0.0001) (Fig. 5). However, as mean scale row count varies between clutches incubated at the same temperature, this regression relationship can only apply to the data for those particular clutches. To overcome the significant clutch effects, 32C was chosen as the standard incubation temperature, and mean scale row counts at this temperature were treated as zero. Counts from other temperatures were expressed as the difference from this standard. (Note: 32C is used as the standard incubation temperature for all wild and captive-laid eggs by crocodile farms).

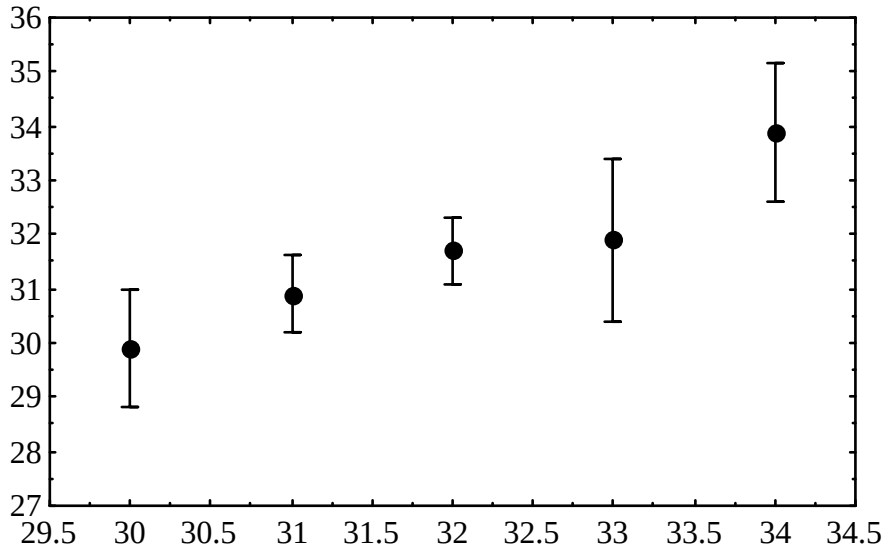

Figure 5. Mean scale row counts for clutches incubated at 32C for 13 days, 'pulsed' at different temperatures for the equivalent of 20 days of 30C development, and then returned to 32C to complete incubation. Means are means of clutch means (N= 3); error bars indicate 2 standard errors.

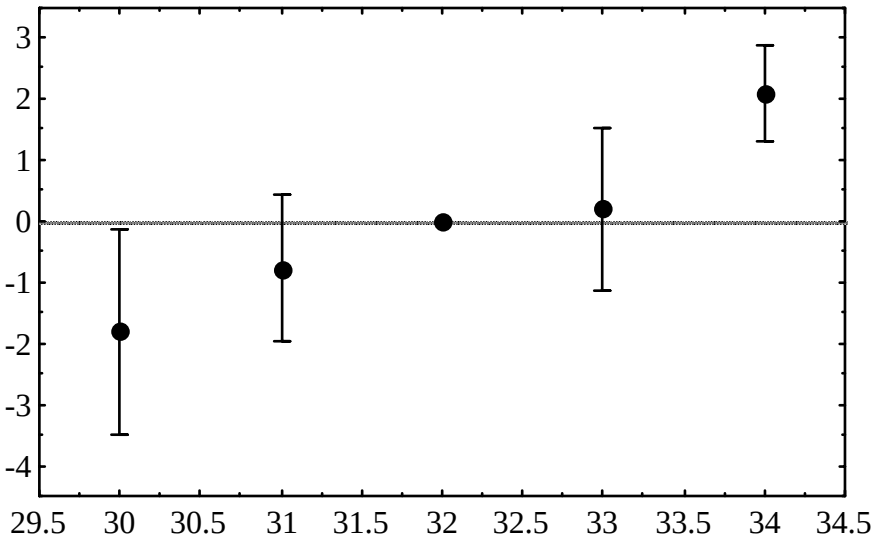

Figure 6. Difference between mean scale counts at different pulse temperatures and that at 32C (standard). Eggs were pulsed for the equivalent of 20 days aof 30C development. Means are clutch means; error bars indicate 2 standard errors. The regression relationship was highly significant (see text).

With the standardised data, the relationship between the difference in scale row counts from the standard (32C), and pulse temperature, was highly significant (SRC.Diff = 0.873.PT -27.99; r^2= 0.66, p= 0.0003, N= 15) (Fig. 6). The mean difference between the highest (34C) and lowest (30C) pulse temperatures was around 4 scale rows. Eggs pulsed at temperatures below 32C produced hatchlings with fewer scale rows on average than did eggs pulsed at temperatures higher than 32C (Fig. 6).

Switch experiments

Preliminary switch experiments in the 1997/98 nesting season (using 5 day switch intervals) indicated that for 30C to 33C switches, the sensitive period for scale row development was from 20-30 days. More detailed experiments in the 1998/99 season using 2 day switching intervals indicate that eggs switched from 30C to 33C between 18-26 days showed similar mean scale row counts, but from 26 days onwards the mean count decreased in a somewhat linear fashion (Fig. 7). Thus the stage at which high incubation temperatures affect scale row counts occurs between 26 and 30 days.

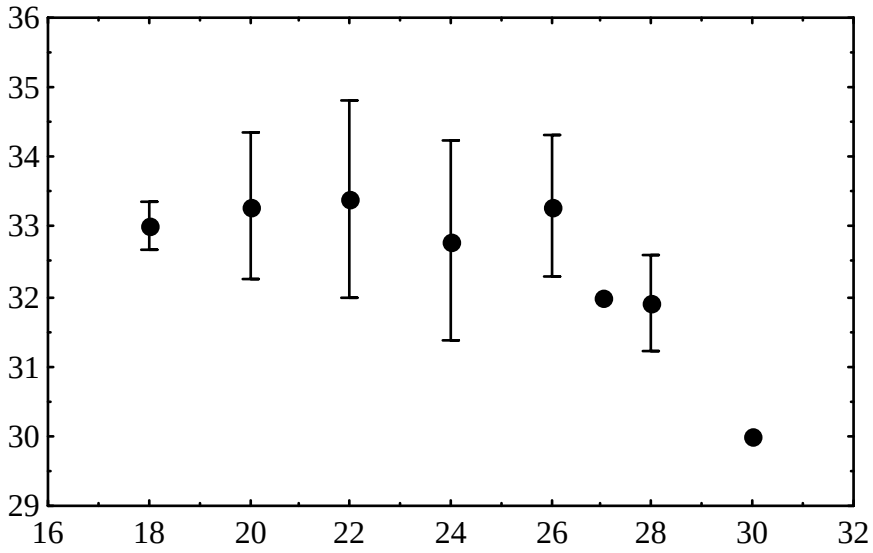

Figure 7. Mean scale row count for eggs shifted from 30C to 33C at different ages (2 day intervals). Data are means of clutch means (N= 3); errors bars are 2 standard errors.

For the reverse switch (33C to 30C), preliminary results using 5 day intervals indicated that the period between 10 and 30 days was critical; by 30 days there is no effect of the low temperature. Later results, concentrating on the 10-30 day period, were similar to the preliminary experiments, showing a gradual increase in the mean count (Figs. 8 and 9) with increasing age of shift.

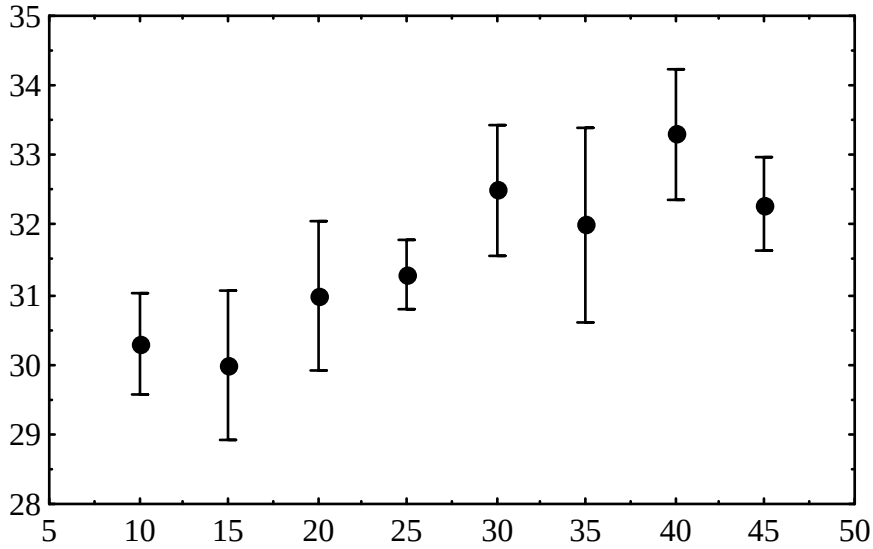

Figure 8. Mean scale row count for eggs shifted from 33C to 30C at different ages (5 day intervals). Data are means of clutch means (N= 2); errors bars are 2 standard errors.

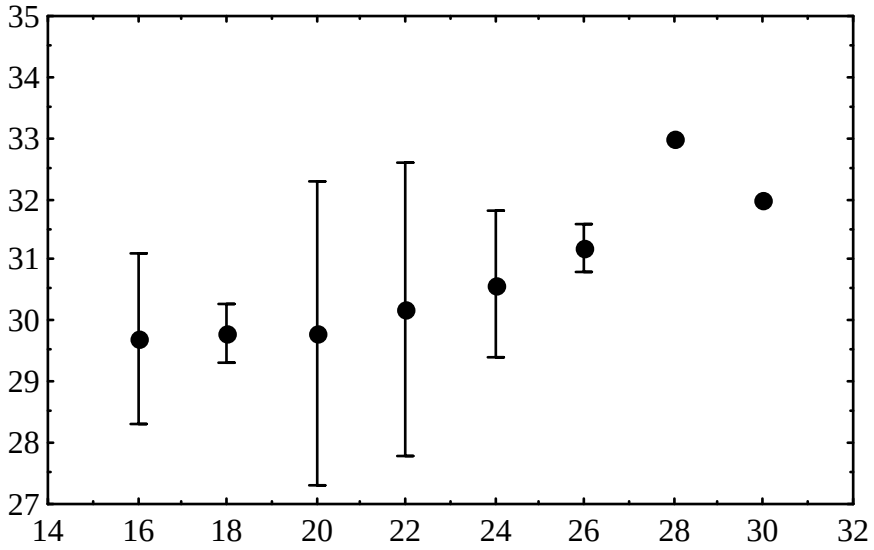

Figure 9. Mean scale row count for eggs shifted from 33C to 30C at different ages (2 day intervals). Data are means of clutch means (N= 2); errors bars are 2 standard errors.

Genetic effects

The contribution of the parents to an offsprings scale row count was examined over two nesting seasons (1997/98 and 1998/99); there were insufficient data from the 1996/97 season. There was no significant relationship between mean scale row count of hatchlings (32C incubation) in a clutch and that of their mother in either the 1997/98 or 1998/99 season (r^2= 0.001, p= 0.90, N= 14 and r^2= 0.004, p= 0.74, N= 14 respectively).

However, the situation with the father (male) was very different, and indicated a genetic relationship. In the 1997/98 season, the relationship between mean scale row count of the hatchlings and that of their father was significant (Fig. 10; r^2= 0.42, p= 0.01). In the 1998/99 season, with essentially the same males, the relationship was just outside the level of significance at the 5% level (r^2= 0.28, p= 0.053; Fig. 11), but was significant at the 10% level; the trend was clearly in the same direction as that in the 1997/98 season.

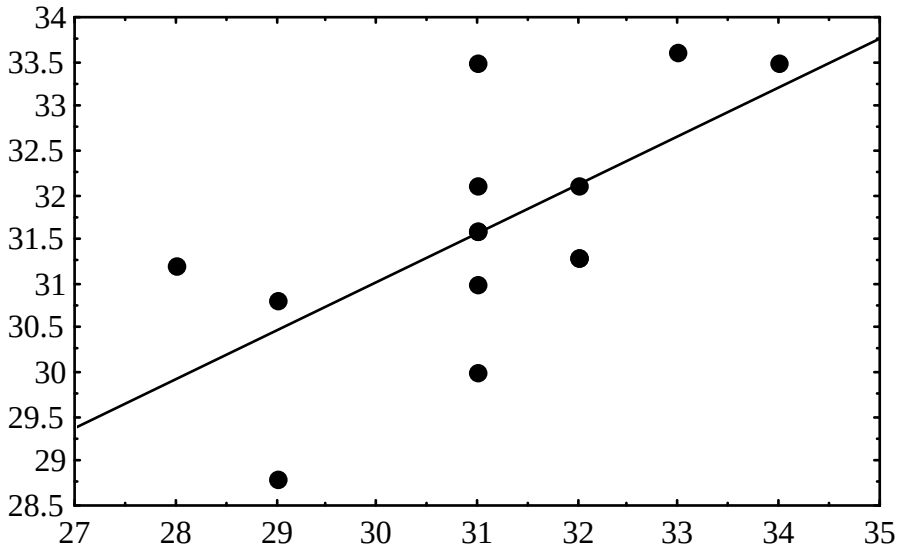

Figure 10. Mean scale row count of hatchlings against that of their father, in the 1997/98 nesting season. Line indicates significant linear regression.

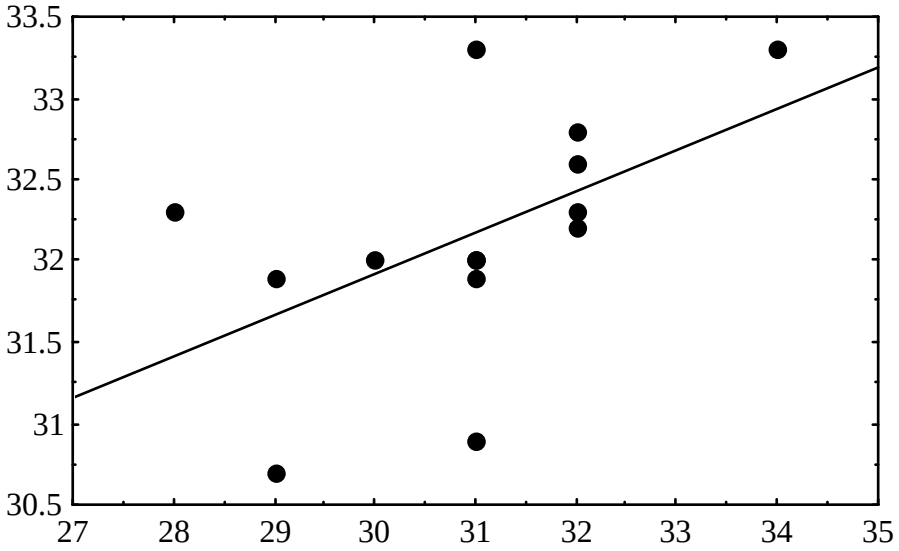

Figure 11. Mean scale row count of hatchlings against that of their father, in the 1998/99 nesting season. Line indicates linear regression which was just outside the level of significance (see text).

Data on the progeny of known males (and females) over three nesting seasons (Table 4) indicate the degree of variation in mean scale row counts from year to year at 32C incubation. Generally the mean count for a particular male was fairly constant over the limited time period. For clutches with sample sizes of 7 or more hatchlings, the mean maximum difference between clutch means for any particular male varied between 0.5 and 0.9 in different years, with a mean difference of 0.73 (N= 6) scale rows.

Table 4. Mean scale row count of the progeny of adult *Crocodylus porosus* males for which two or more clutches were produced over three nesting seasons (1996/97 to 1998/99). N= sample size; SE= standard error. Incubation temperature = 32C.

Male No.	---- 1996/97 ---- Mean (N)	SE	---- 1997/98 ---- Mean (N)	SE	---- 1998/99 ---- Mean (N)	SE
1	-	-	31.3 (11)	0.24	32.3 (9)	0.56
2	-	-	28.8 (9)	0.22	30.7 (3)	0.88
3	-	-	33.5 (10)	0.31	33.3 (6)	0.21
4	31.7 (13)	0.27	-	-	32.2 (26)	0.22
5	31.7 (13)	0.33	31.2 (6)	0.48	32.3 (15)	0.27
6	-	-	31.0 (7)	0.38	31.9 (7)	0.34
7	-	-	30.8 (6)	0.31	31.9 (16)	0.30
8	32.7 (13)	0.43	33.5 (8)	0.57	33.3 (32)	0.23
9	-	-	30.0 (4)	0.67	32.0 (5)	0.45
10	32.8 (10)	0.51	31.3 (4)	0.25	32.6 (5)	0.51
11	-	-	31.6 (8)	0.42	30.9 (7)	0.46

Farmed crocodiles

With a normal culling size (1.6 m) crocodile as the point of reference, the 19 sites from which skin samples were examined could be divided into two broad groups, largely based on the thickness and relative contribution of the eipidermis to the total skin thickness. The first group (A) consisted of most of the ventral sites (belly caudal, belly thoracic, belly mid, belly pelvic, tail ventral), facial and dorsolateral tail. The second (B) consisted of chin, vent, thoracic flank, occipit, nuchals, caudal-nuchals, interscute, wrist dorsal, wrist ventral, ankle dorsal, ankle ventral and cheek.

At the time of hatching, there was little difference in mean skin thickness between the two groups. The dermis made up a similar proportion of skin thickness (83%; Table 5), as did the epidermis (17%). A significant part of the epidermis consisted of keratin (31-34%). Thus the skin samples from different parts of the body could not be distinguished from each at the time of hatching on the basis of morphometric measurements. By one year of age the skin had thickened considerably, and differences were apparent between the two groups of sites with regard to this growth. The contribution of the epidermis to the total skin thickness had decreased in both groups to about 9.3% (Table 5), but in absolute terms the epidermis of the ventral scales (A) had increased by around 50% in thickness, whereas in Group B it had increased by about 20%. In the latter group, the proportion of keratin in the epidermis had changed to a lesser degree relative to the former group (Table 5). The dermis now comprised a slightly greater (compared to that at hatching) but similar proportion of the skin in both groups (about 91%; Table 5).

The most significant changes were apparent in two and three-year-old crocodiles. The skin had continued to thicken with increasing age, but differences in the relative increases (growth) in the skin were far more obvious. In the three-year-old animal, the epidermis of the ventral scales (A) was almost three times as thick as that in the other scales (Group B) (Table 6). Likewise, the keratin layer was substantially thicker (90 μm compared with 21 μm), and was around 62% of the epidermis thickness (compared with 43% in Group B). Although mean total skin thickness of the two groups was similar at two years of age, it was very different by three years (Table 6). The dermis continued to be the major component of the skin (89 and 94% in A and B respectively).

Table 5. Mean thickness of skin components and their percentage contribution to the total skin, from 19 sites (N) which have been divided into two groups (A and B; see text), for *Crocodylus porosus* at hatching (28 cm total length) and 0.7 m total length (1-year-old). ‘Total Epidermis’ includes the keratin layer.

Component		Total Length = 0.3 m				Total Length = 0.7 m			
		Mean Thickness (μm)	SE	% of Skin	N	Mean Thickness (μm)	SE	% of Skin	N
Keratin	A	9.8	0.9	5.9	7	18.7	3.3	4.0	7
	B	7.8	0.7	5.3	12	10.7	1.2	3.3	12
%Epidermis as keratin	A	-	-	34.4	7	-	-	44.2	7
	B	-	-	31.3	12	-	-	35.3	12
Total Epidermis	A	28.3	1.7	16.9	7	41.4	4.0	9.4	7
	B	24.7	1.1	16.9	12	30.1	2.9	9.2	12
Total dermis	A	139.9	6.5	83.1	7	418.7	33.1	90.6	7
	B	131.3	11.5	83.3	12	331.4	30.2	90.8	12
Total skin	A	168.1	6.5	-	7	460.1	85.5	-	7
	B	156.0	11.5	-	12	361.5	29.4	-	12

Table 6. Mean thickness of skin components and their percentage contribution to total skin thickness, from 19 sites (N) divided into two groups (A and B; see text), for *Crocodylus porosus* near (1.1 m; 2-year-old) and at normal culling size (1.6 m; 3-year-old). 'Total Epidermis' includes the keratin layer.

Component		Total Length = 1.1 m				Total Length = 1.6 m			
		Mean Thickness (mm)	SE	% of Skin	N	Mean Thickness (mm)	SE	% of Skin	N
Keratin	A	39.2	3.0	5.7	7	89.9	24.8	7.0	5
	B	15.3	2.0	2.6	12	20.6	2.1	1.3	10
%Epidermis as keratin	A	-	-	49.3	7	-	-	61.7	5
	B	-	-	37.4	12	-	-	43.0	10
Total Epidermis	A	80.2	3.3	11.7	7	141.7	35.3	11.1	5
	B	40.4	4.2	6.9	12	48.0	4.3	6.2	10
Total dermis	A	626.9	53.7	88.3	7	1106.9	37.6	88.9	5
	B	578.9	57.0	93.1	12	817.1	98.4	93.8	10
Total skin	A	707.0	54.7	-	7	1248.9	55.6	-	5
	B	619.3	58.6	-	12	865.1	98.6	-	10

Structurally, the epidermis and its keratin component appeared the same between the different sampling sites for different aged (sized) animals (Fig. 12). As described previously for hatchlings, the keratin of the interscalar region is composed of alpha-keratin, and that of the scale itself is of tougher beta-keratin (Fig. 12). The superficial dermis of the lateral and dorsal skin areas contained many melanophores and free melanin granules (Fig. 13). The large melanophores have a globular cell body from which elongate arms extend towards the dermal/epidermal junction. Ventral (belly) scales were devoid of melanocytes, and lack any dark pigmentation.

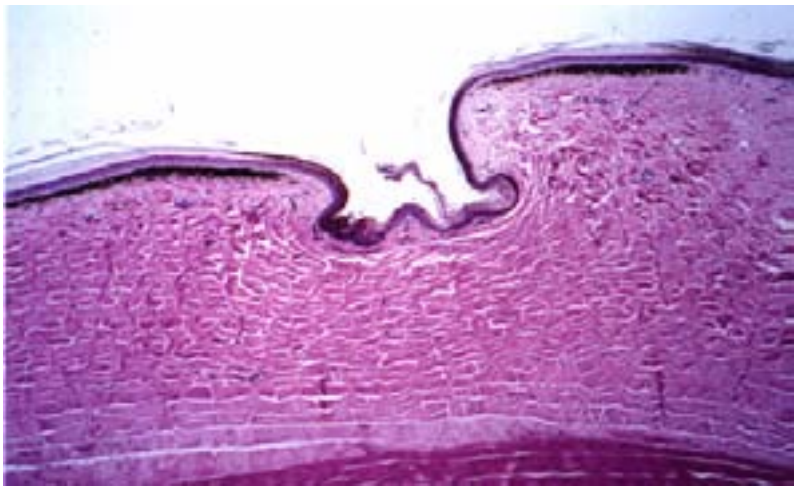

Figure 12. Interscalar zone of the skin from a three-year-old *Crocodylus porosus*. Note the different staining of the scalar (clear) and the interscalar (dark) keratin, the extensive pigment of the loose

dermis in the scalar zone, and the layering of the dense dermis. [H & E].

In both hatchling and large crocodiles there is an extensive arborising meshwork immediately beneath the epidermis, in the same zone as the melanophores. This network consists of thin intertwined cellular processes which have a light brown slightly granular appearance with haematoxylin and eosin staining. Under polarising light these processes (iridocytes) fluoresce strongly (Fig. 13).

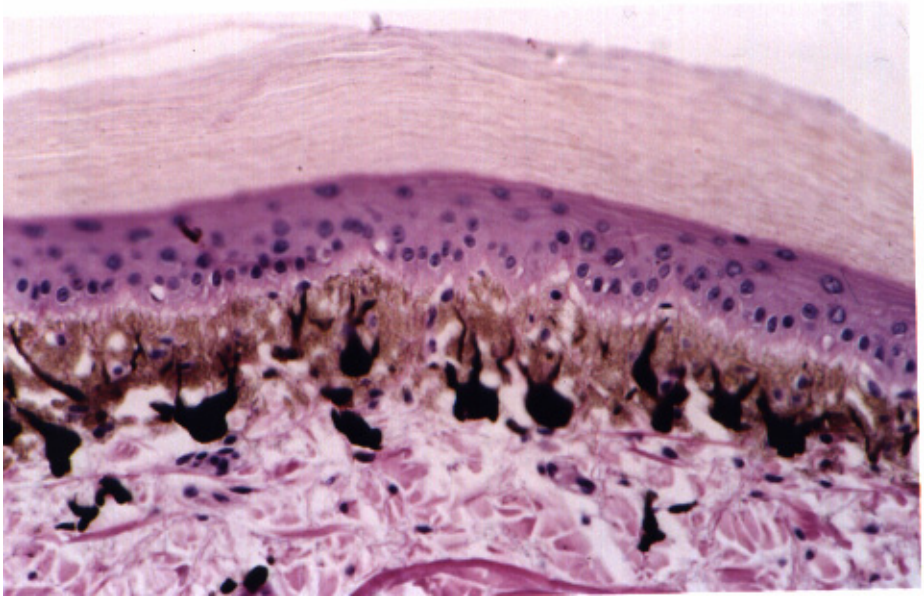

Figure 13. The clearly defined epidermis is topped by a broad amorphous keratin layer. Note the large darkly staining melanophores, and the diffuse light brown appearance of the extensive iridocyte processes within the loose dermis. [H & E].

It was the arrangement of the dense dermal collagen which varied between sites, and the dense dermis could be divided into four ‘architectural’ patterns (shown diagrammatically in Figure 14).

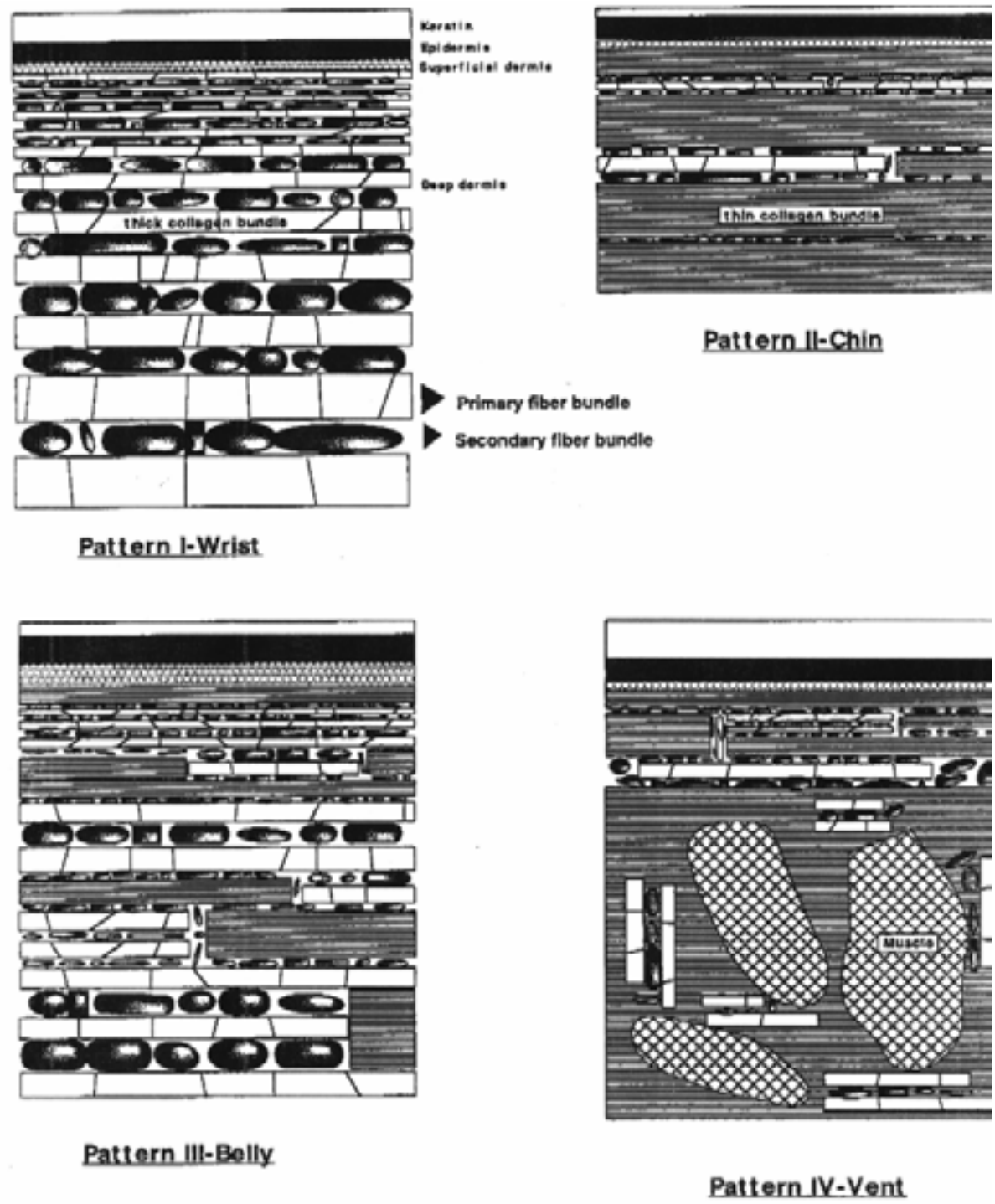

Figure 14. Stylised representation of the four patterns of collagen arrangements identified in the skin of juvenile/subadult *Crocodylus porosus*.

The first (Pattern I) is seen in the dermis of the wrist, ankle and dorsolateral tail skin. Collagen fibres run in sequential pairs of orthogonally arranged bundles. In the vertical plane there are primary bundles running in the direction of the axis. Between sequential primary bundle layers are secondary bundle layers which are oriented orthogonally to them. The number of rows and their widths varies, possibly depending on age/size. The row width is greatest in the deep layers and least in the superficial layers. In the wrist of a three-year-old *C. porosus* the number of primary rows was 14-15. These were narrowest in the interscalar region (35-50 μm) (Fig. 12), and widest in the deeper layers bellow the scales (90-110 μm). Similarly the collagen bundles in the primary rows varied from 2-70 μm in

thickness. In a one-year-old animal, the number of primary rows was 12-13; 11-17 μm wide in the interscalar region, and 25-40 μm in deeper layers. The arrangement of collagen fibres is clearer in the ventral skin samples than in the dorsal skin samples.

The second arrangement (Pattern II) was seen in nuchal, chin and nasal skin. Here, fibre bundles are mostly narrow and run primarily in the axial direction. These layers each consist of narrow, thin fibre bundles (3-10 μm) resembling slender, long threads. Although a few thick bundles are present, the collagen fibres do not form thick rows as in Pattern I. The arrangement of the collagen fibre bundles is very clear in chin skin.

Pattern III is a composite arrangement of Patterns I and II, and varies greatly from site to site. Adjacent the superficial dermis are a number of thin parallel bundles as in Pattern II. Deeper lie defined axially directed large bundles. The primary bundles (both narrow and broad) run in an axial direction. Secondary bundles lie orthogonally between each pair of sequential primary bundles. This pattern is seen in midflank, thoracic belly and caudal belly. The skin of the vent (cloaca) is characteristic of Pattern IV. There is an irregular arrangement of collagen bundles, which are both thick and thin, and have no specific orientation to the skin surface. Many large muscle bundles are distributed irregularly throughout the deep dermis.

Wild versus farmed crocodiles

As the value of a crocodile skin is determined by the quality of the belly area (including tail and throat), it was prohibitive to sample extensively from the ventral surfaces of either farmed or wild crocodiles. Such sampling, with even the smallest of holes, would have rendered the skin as a ‘reject’. Difficulties were encountered when taking skin samples, and many of the samples taken were found to be of limited (if any) use after histological processing. In particular, the dermis was found to tear and was therefore not complete. This problem was further exacerbated by the anerobic metabolism of crocodiles (see Methods), making sampling difficult from even freshly-dead animals. Nonetheless, samples from the ventral and dorsal aspects of the wrist or ankle were consistently of better quality than from other areas, and were found to be easier to take. The area behind the cranial platform (occipit) and the flank were also considered to be suitable.

The skin thickens with increasing body size. As an independent variable, age is not very good, due to the highly variable growth rates exhibited by crcoodilians generally. Such variability can be apparent between individuals from a particular clutch, or can result from the type of husbandry, incubation temperature and possible genetic factors. For example, a 1.2 metre long *C. porosus* could reach this size in 1.5 years or more than 5 years, depending on these various factors.

There was high variability in skin thickness between farmed crocodiles of similar sizes, and data were lumped in size categories (in 10 cm increments). The skin increases in thickness in a linear fashion with increasing size (Figs. 15 and 16), as indicated by representative sample sites. These trends mirror the growth of the dermis, which represents a high proportion of the skin’s structure. With hatchlings excluded, the dermis made up a mean of 94.5%, 88.0%, 88.9% and 82.6% for flank, chin, dorsal wrist and ventral wrist respectively, regardless of the size of the crocodile. Although there was a trend towards a decreasing proportion of keratin in the epidermis in these four sampling sites, none of the relationships was statistically significant. For animals greater than 1.5 m total length there was clearly no significant relationship with size, and keratin comprised a mean of 26.4%, 27.6%, 32.9% and 33.5% for flank, chin, dorsal wrist and ventral wrist skin respectively.

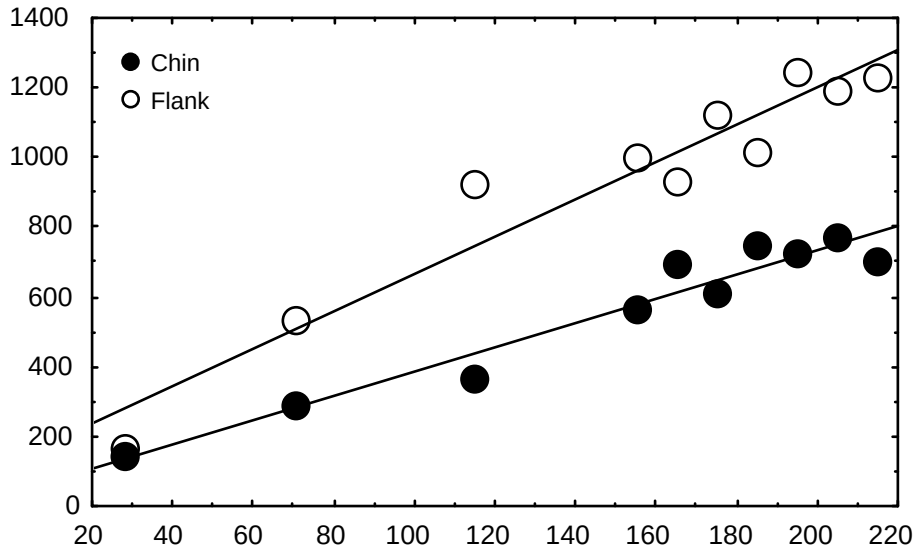

Figure 15. Change in mean total thickness of flank and chin skin of farmed *Crocodylus porosus*. Lines indicate significant linear regressions (r2= 0.93, p= 0.0003 and r2= 0.94, p= 0.0001 respectively).

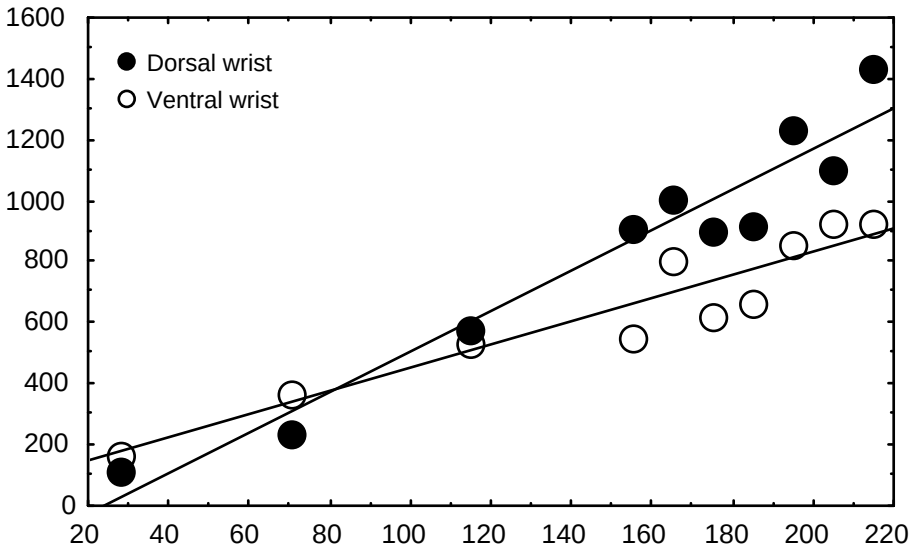

Figure 16. Change in mean total thickness of wrist (dorsal and ventral surfaces) skin of farmed *Crocodylus porosus*. Lines indicate significant linear regressions (r^2= 0.94, p= 0.0001 and r^2= 0.89, p= 0.0001 respectively).

The low numbers of suitable skin samples from wild crocodiles makes comparisons difficult. The general pattern of growth of the skin was similar in wild crocodiles. Where samples could be compared, with similar-sized animals, some differences were apparent. For example, the skin from the chin of wild crocodiles was 33% thicker than that in farmed animals. This difference was the result of an increase in the thickness of the dermis (41%), and a decrease in the thickness of the epidermis (including keratin; -35%). The keratin layer in the epidermis of wild crocodile skin was also slightly thinner (9%) than that of farmed counterparts.

Similar relationships were evident with ventral wrist skin. In farmed crocodiles less than 1.5 m total length, the dermis comprised about 74.8% of skin thickness, lower than that of larger crocodiles

(89.5%). In larger animals this was accompanied by a decrease in the relative proportion of epidermis. The mean proportion of keratin in the epidermis was similar (about 32.5%) in all sizes of farmed crocodiles. Thus, the thicknesses of keratin (31.7 μm), epidermis (including keratin; 96.2 μm) and dermis (902 μm), although variable in individuals over 1.5 m in length, did not change significantly with size in the ventral wrist skin.

The wrist skin of wild crocodiles also had a thinner epidermis (48% and 49% thinner for dorsal and ventral wrist respectively). In this case however, the dermis of wild crocodiles was less thick than that in farmed crocodiles, resulting in thinner skin overall [this was more pronounced in the dorsal wrist (21%) than the ventral wrist (2%)].

Industry perception

The main market for Australian Saltwater Crocodile skins is in Asia (Singapore, Japan) and Europe (France), with negligible quantities being exported elsewhere. These centres are where the highest quality fashion goods are produced. Interviewees were usually associated with only one aspect of the crocodile skin industry, which can be considered to be made up of skin producers (farmers), skin buyers, tanners, product manufacturers and product retailers.

The main problems identified by skin buyers related to physical damage to the skin. Where such damage may have been 'acceptable' in the past, when limited numbers of *C. porosus* skins were available and there was less competition from other species (eg Nile Crocodile, American Alligator), the situation now is very different, and grading will continue to be very strict. No buyers could see this situation changing in the foreseeable future.

Physical damage is usually caused by fighting among the animals (bite marks) or by rough floor surfaces of pens (scratches). Bite marks usually go through the keratin layer and into the dermis, and if in the belly portion of the skin, lead to instant downgrading of the skin. If scratching is superficial, and is limited entirely to the upper keratin layer, then it may be considered acceptable. However, it is often difficult to ascertain the ultimate effect of scratches on a tanned skin, and more often than not downgrading is the option taken by buyers. Knife cuts during skinning were considered a problem that could be rectified easily, as was skin preservation (salting).

A more difficult problem to identify on raw skins is bacterial infection (eg *Dermatophilus*; Buenviaje 2000). Although the brown spots associated with some of these bacteria indicate their presence, often there are no visible signs, and the effects of the infection are not seen until tanning and shaving of the skin. At this time small holes may appear on the skin surface. As it is difficult for buyers to pinpoint the exact skins with bacterial problems at the time of inspection, prices of skins from particular farms or areas are usually reduced to account for the probability that skins will be affected.

The presence of worm trails (caused by *Capillaria* spp.) in the belly scales was not considered a problem for farmed skins, but they are prevalent in wild *C. porosus* skins taken in Papua New Guinea and Indonesia. As very few *C. porosus* skins are produced from wild harvesting in Australia, it was not considered a key issue relating to Australian skins.

Wild skins were generally considered better than farmed skins due to their shape. This was mainly because wild skins are narrower per unit length than their farmed counterparts. The main use of *C. porosus* skins is in the manufacture of handbags, and wild-shaped skins have less wastage associated with them after cutting. The belly portion forms the panels of the bag, and gussets, bottom panels and straps are usually derived from the neck and tail areas.

Buyers with considerable experience identified feeding regime as a key factor affecting the skin shape of farmed crocodiles. It was also considered a factor affecting the appearance of the scale pattern of

the belly skin, as very obese individuals often had stretching between the ventral scales. This greatly affects the visual appearance of the belly skin, and limits where a skin can be used. Scale pattern is important in assessing the final use of a skin in a particular product.

Another husbandry-related problem was referred to as ‘double scaling’, which affects the lateral body scales on the flanks. The flank scales are generally round-oval in shape, and are poorly keeled (Brazaitis 1987). Double scaling appears to reflect changes in growth of the scale, such that the inner portion becomes raised and defined from the lower part of the scale. It may occur only on a few scales or it can be be more extensive. The condition may indicate sudden changes in growth rate after crocodiles are disturbed (eg after grading) or as a result of fighting, and subsequently not feeding for a time, or may be due to changes in food or feeding regime which affect growth rates dramatically.

Some product manufacturers indicated that skins with more scale rows are more sought after, but the limited numbers of them currently in trade did not warrant a premium price. Regularity of scale pattern was also considered to be important. This relates to the degree to which scale rows are divided into additional rows. For example, a row of scales in the middle of the skin may divide into two rows as it proceeds to the flanks. Manufacturers involved with the highest quality (and value) products did agree that if a regular source of skins with high numbers of scale rows and regular scale pattern was available, such skins could command a higher price.

Wild skins were considered by some skin buyers to be of higher quality due to their ‘structure’. However, few interviewees could actually qualify what they meant by this. It could not be translated into a tangible property of the skin. A large tannery in Singapore, with a very high turnover of skins, did not perceive a difference between wild and farmed skins with regard to skin thickness, but could tell from the ‘feel’ of the skin whether it was farmed or wild. On the other hand, an Australian and Japanese buyer considered wild skins to be thicker than farm skins. The ability to distinguish between wild and farm skins (by whatever means), and to identify the origin of skins (farm or locality), was linked to experience in the industry. A number of variables, including habitat type for wild skins and husbandry practices for farmed skins were identified as important factors, although reasons for differences were not known.

The way in which buying and selling of crocodile skins within the industry means that players often only see one aspect of it. For example, in Japan, a trading house will buy raw skins from a farmer, and sell to the tanner, who will sell to the product manufacturer, who will sell to a retailer. Thus the product manufacturer has no contact with the trader (skin buyer) or the raw product itself, and has little understanding of it. Few organisations or individuals are involved in all stages. This system was also apparent in Singapore, although not to the same degree.

Discussion

The sequence of developmental changes in *C. porosus* embryos and hatchlings was similar to that reported for Australian Freshwater Crocodiles, *C. johnstoni* (Richardson *et al*. 2000b). The four stages of skin development were similar in both species, but the development of the epidermis differed during one stage of incubation. In *C. porosus* the epidermis does not change in thickness between 27 and 41 days (Fig. 1), remaining at around 10.8 mm, whereas in *C. johnstoni* it thickens in a linear fashion throughout incubation (Richardson *et al*. 2000b). The significance of this 'plateau effect' in *C. porosus* is not clear, although it is perhaps significant to note that it is during this period that the scale pattern becomes visible. It is possible that disrupting of the development of this layer at this time, by changing the development rate (incubation temperature), could bring about changes in the scale pattern.

The periderm, which is lost by the time of hatching, may have a nutritional or osmotic role in the embryo (Manolis *et al*. 1987), allowing nutrients and salts to pass through the outer layers of the epidermis. With hatching, the periderm becomes redundant, as the keratin layer takes on the protective role. The stratum corniem is an impervious layer shielding the newborn from dessication and osmotic tensions, and affording physical protection. This layer in *C. porosus* is similar to that described in adult *Alligator mississippiensis* and *Caiman crocodilus* (Baden and Maderson 1970; Maderson and Sawyer 1979; Maderson 1985; Landmann 1986).

The presence of a birefringent layer, possibly of guanine crystals, in hatchling *C. porosus*, but its absence in *C. johnstoni* is perplexing. Similar membrane-bound crystals have been described in lizards (Morrison and Frost-Masson 1991), and their function remains unknown.

The high value of *C. porosus* on the international market, relative to other crocodilian species, is largely due to: the lack of osteoderms (bone) in the ventral scales; the relatively high number of scale rows; and, the regularity of the scale pattern on the belly. The more scale rows that are present, the smaller the scales. Although C. siamensis (Siamese Crocodile) lacks osteoderms, and has a high number of scale rows, the pattern of scales (small ones in the pectoral and pelvic areas, and larger ones in the middle) results in a skin of less value than that of *C. porosus*.

The results from this study indicate that crocodile farms are producing *C. porosus* skins with variable numbers of scale rows (from 27 to 37), and a mean of 31 rows (Table 1). Few hatchlings (<2%) were found with 35 or more rows. It was clear from discussions with skin buyers that skins with higher numbers of rows would be more sought after, but there are insufficient in the market to allow them to be allocated a premium price. There are simply too few being produced.

The study identified two areas with the potential to influence the types of skins being produced, through manipulation of the number of scale rows on hatchlings. The first is through incubation temperature. Typically, farms incubate their eggs at constant 32C, the temperature at which mostly males are produced (Webb *et al*. 1987), and from which hatchlings with greater post-hatching growth and survival (Webb and Cooper-Preston 1989) are produced. Our results indicated that above and beyond any genetic effects (see later), there was a significant relationship between temperature of incubation and the mean number of rows on hatchlings, with increasing numbers of rows with increasing temperatures. However, incubation at high temperatures (>33C) for higher mean scale row counts would also result in higher embryonic mortality during incubation (Webb *et al*. 1987; Webb and Cooper-Preston 1989), and lower post-hatching survivorship (Webb and Cooper-Preston 1989).

Pulse and switch experiments allowed the stage of embryonic development at which incubation temperature affected scale row development to be identified more precisely. On average, eggs pulsed at higher temperatures produced hatchlings with more scale rows. High temperatures affected scale row development between 26 and 30 days of age (30C age), but at low temperatures the period extended for longer, from 10 to 30 days. Similar experiments to elucidate the sensitive period for

temperature-dependent sex determination in *C. porosus* and *C. johnstoni* indicated that the effect of high temperature (assigning maleness) was delayed relative to that of low temperatures (femaleness) (Webb *et al*. 1987). The preliminary results obtained here suggest that a similar type of situation may exist, and like sex determination, it may be the effect of embryonic development rate of different tissues, rather than temperature per se, causing it.

Crocodile farms in the Northern Territory rely mainly on a wild harvest of eggs for hatchling stocks. In this case, the effects of incubation may already be over by the time eggs are located and collected for artificial incubation. Nonetheless, alteration of the incubation environment for younger eggs may be an option to pursue. The reliance on captive breeding of *C. porosus* in Queensland and Western Australia, and increased importance of captive-bred hatchings in the Northern Territory, allows a wider window of opportunity, as captive-laid eggs are typically collected within a few days of laying and the incubation environment is able to be controlled through to hatching. However, for captive-laid laid eggs, a second option to influence the scale pattern may be potentially available.

A significant result of the study was identifying a genetic component affecting the mean number number of scale rows in a clutch of hatchlings. Captive breeding of *C. porosus* usually focuses attention on the female, and as the one producing the eggs, this is rightly so. The results here showed no influence on a clutch by the female parent, but a significant effect of the male parent (see Figs. 10 and 11). Males with high numbers of scale rows tended to produce offspring with high numbers of rows. Thus selection of males, particularly for unitised breeding pens (1 male, 1 female), could take into account the scale count of the male, together with other attributes (eg size). Greater control may thus be exerted on the type of hatchlings being produced. Additional data from more known pairs of *C. porosus* would expand our knowledge on the genetic effect on scale row development.

Wild crocodile skins are still keenly sought by international skin buyers. To date, this market has been serviced by skins of Nile Crocodiles, American Alligators, Saltwater Crocodiles (Papua New Guinea, Indonesia and now Australia) and New Guinea Freshwater Crocodiles (Papua New Guinea, Indonesia). (Some wild Australian Freshwater Crocodiles are taken in Western Australia). For each species, farmed skins are also available. Some characteristics of wild skins which make them ‘better’ than farmed skins do not necessarily involve the structure of the skin.

The shape of a skin is considered important by product manufacturers. Long, thin skins are preferred over short, wide ones, as there is less wastage after cutting for the former. In crocodile farms, feeding and patterns of growth largely determine the body shape. In addition, larger skins are generally available from the wild - growing crocodiles to large sizes in farms is simply not economically viable. Thus, farms will probably never compete with the large skins available from wild populations, but certainly can investigate ways to produce a ‘slimmer’ crocodile at a particular length. Types of feed coupled with rate of feeding are two variables to consider in this regard.

The results of skin sampling were disappointing, and we were unable to confirm whether there were clear structural differences between the skins of wild and captive crocodiles. In both, the skin thickens with increasing size, but there is high variability in skin thickness in similar-sized individuals, and there were significant differences in the dermis from different parts of the body. It is these differences in the structure of the collagen in the dermis which may explain why wild skins are sometimes considered of better quality than farmed skins by skin buyers.

The differences between wild and farmed animals with regard to the thickness of the epidermis, was reflected in both the keratin and other epidermal layers. The significance of this thinner epidermis in wild crocodiles is unclear. Removal of the keratin layer is one of the first steps in the tanning process, and the epidermis contributes little to the total thickness of the final tanned skin. It is difficult to imagine that the epidermis alone could affect the tanning characteristics of the skin. Still, that farmed skins have a thicker epidermis implies that the cells within it are larger. Fat may also be deposited in the epidermis, but it is difficult to identify this histologically, and we were unable to do so.

It is more likely that changes in the dermis would be more significant in determining the 'quality' of a skin, and may explain why some industry players noted that the skin of wild animals is thicker. (This may in part reflect the fact that wild skins tend to be much larger than farmed ones). Notwithstanding the four patterns of dermal structure identified, the patterns of collagen in the dermis may be affected by the conditions under which crocodiles are raised in captivity. The longtitudinal bundles of collagen in the dermis of wild crocodiles tended to be more narrower and more compact relative to farmed animals, which had a loose architecture. The density of these orthogonal layers may reflect the slower growth rates experienced by wild *C. porosus*. In addition, the diet of farmed crocodilians tends to be much higher in fat than in the wild, and fat within the dermis may affect its structure and subsequent tanning. Again, the histological techniques used here were unable to determine whether the levels of fat in the dermis differed significantly. Examination of tanned wild and farmed *C. porosus* skins side by side did not result in any obvious, visible differences between the two. Tanners with considerable experience note that skins of a species from different areas may vary, and this perhaps reflects different growing conditions in different populations.

The relatively fatty diet of farmed crocodiles (Manolis *et al* 1989; Webb *et al.* 1991).also leads to the production of a 'short, wide' skin, and may also affect the quality of the skin in another way. The distribution of keratin over the scales, and particularly in the interscalar region, allows the skin to be flexible. The soft interscalar region is however prone to stretching, and this can be considerable if crocodiles are growing wide rather than long. This is a clear effect of husbandry on the structure and quality of the skin produced. Even after tanning such skins may retain their stretchiness, although this was not perceived as a major problem by people in the industry.

An area of skin other than the belly, which could be used to monitor the 'quality' of the belly skin, could not be identified readily. Wrist or ankle skin produced good skin samples, but did not adequately represent the belly skin. Thus skin assessment criteria could not be developed or tested. In retrospect, concentrating on skin from areas away from the belly area was not the best option. Any future work should focus more on the belly skin itself, and specifically examine the structure of the dermis in more detail throughout the body. This would require more intricate histological and histochemical techniques, and examination of large sample sizes to overcome the great variation between individuals.

At a broader level of resolution, there are no doubt a great variety of factors affecting the quality of skins, between crocodile farms and from different geographic localities in the wild. Specific habitat types may actually reflect other factors, such as food availability and growth rates. Thus tidal areas may produce better skins than freshwater habitats, but this may not be due to the differences in water, but rather in the different associated vegetation types which support the animal's food supply. A species like *C. porosus*, which has a wide geographic range, occurs in a variety of habitats, and is farmed under various conditions throughout its range, will therefore exhibit a range of differences in skin quality.

Implications

Results from this study indicate that the number of scale rows on the ventral belly area can be manipulated to a certain degree by varying the incubation environment during a critical stage of embryonic development. In addition, identification of a genetic component in determining the number of scale rows allow selection of adult breeding stock to take this into account. There is no doubt that this is a significant step towards the production of Australian crocodile skins with particular characteristics for the international skin market.

Recommendations

Additional research could refine these initial results on the effects of the incubation environment on scale rows and patterns, and possibly identify other important factors to be considered. More importantly, the complex nature of the skin, particularly the collagen layers of the dermis, suggest that this is an area which should be investigated further in its own right. Quantification of scale patters and general characteristics for Saltwater Crocodiles skins within the species' geographic range could uncover genetic differences, and could be undertaken at major tannery (e.g. Singapore) where large numbers of *C. porosus* skins are available at any one time.

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