Caiman. The objectives of this Sub-Program are:

- Make the local residents obtain knowledge to be involved direct or indirectly with the Management Plan of the Caiman.
- Change of the local population's attitude, not only with the species subject to harvest, but also with the wildlife in general, through the understanding of the importance of the conservation and the sustainable development.
- Transmit the results of the activities developed inside the management of this species to the local communities direct and indirectly involved in the program.

Conclusions

The improvement of the organizational structures of the communities that harvest Caimans will allow to improve the social control on the resource management and to assure a more transparency in the processes, giving the necessary sustainability to the process of harvest of the Caiman.

The social control exercised by the communities can, without a doubt, strengthen the control of the program, in charge of authorities, as much National as Departmental. Likewise, an appropriate coordination with national police and armed forces will allow to reduce the impact of the furtive hunters.

In the measure that the responsibility of the administration (from the registration of the animals to the commercialization of the product) will fall in the same communities that harvest Caimans, it is possible to achieve a fairer and more equal distribution of the benefits of the program.

Through the diffusion and training programs (permanent along the administration), it is possible to strengthen the information that should reach to the producers, concerning their rights and obligations on managing a resource.

The establishment of a sufficiently sensitive monitoring program, with participation of local people and the support of the scientific entities, will offer reliable information about the population status of the species and the population dynamic, which allow to take appropriate management decisions.

Nevertheless the elements considered in this proposal, it is necessary to have the political support of the responsible authorities to be able to carry out this model of management that can enrich and improve a National Program of Conservation and Harvest of the Spectacled Caiman in Bolivia.

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The Increasing Role of the Internet in Crocodilian Conservation

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Abstract

The Internet is a communications revolution that enables over 750 million people worldwide to transfer information instantly. Given that conservation relies on effective communication, the Internet is a powerful tool if used correctly. However, its scope and limitations must be appreciated for it to be used effectively. Different audiences must be recognized, and activities and interactions appropriate. For example, a conservation working group benefits in quite different ways to communities involved in conservation and management plans, and not all members of each group have access to the Internet. We examine the increasing role of the Internet in crocodilian conservation, highlight specific case studies, and look at the wider implications and possibilities that this tool presents.

Introduction

The Internet is a revolution in communication. It has built on the infrastructure established by the telegraph, the telephone, radio and the computer, and now provides the means for over three quarters of a billion people to communicate, collaborate and interact regardless of their geographic location. Access requires little more than a personal computer and a telephone, and the abundance of "Internet Cafés" in many countries even obviates the need for those. Nearly everyone knows what an "e-mail address" is (or has one) and phrases like http://www.google.com are instantly familiar to many.

Brief History of the Internet

The Internet's origins can be traced to the 1960s (Kleinrock 1961, 1964) where plans for a worldwide "Galactic Network" (Licklader and Roberts 1962) seemed like science fiction. Yet before the end of the decade ARPANET - essentially a transatlantic academic and military communication project - went online. E-mail was first demonstrated to the public in 1972, and was responsible for the first massive increase in network traffic during the 1970s (Leiner *et al.* 2003). The "Internet" spawned from ARPANET in the 1980s, its "open architecture" philosophy (ie anyone can contribute to its development) ensuring exponential growth. Once the commercial sector tapped into the potential of the Internet, there was no turning back - task forces were deployed to advance key areas, common protocols were developed, and possibilities expanded. The World Wide Web first appeared in 1991 and was responsible for bringing the Internet out of the research community and into the global one. The World Wide Web Consortium (W3C) continues to evolve protocols and standards on the Web, and in 1995 the Web became the number one service available on the Internet (after FTP and Telnet).

The Modern Internet

The Web enables anyone to present information in an easily accessible and readable format using a suitable browser interface. Billions of pages (Nielsen 2004) are now available, from amateurish personal messages to professional corporate services. In recent years the Web has become largely a commodity service, selling data, information and products. The biggest challenge for a Web user is finding quality information amongst the sea of pages.

In 2004, an estimated 785 million people have Internet access (Nielsen 2004; InternetWorldStats 2004). All major companies, governmental and non-governmental institutions have permanent or regular access to the Internet, plus a significant percentage of the population in both rich and - increasingly - poor countries. English-speaking users form the greatest online presence (36%) followed by Chinese (14%), Japanese (10%), Spanish (9%) and German (7%) (Global Reach 2004). Worldwide, people spend an average of 26 hours a month "surfing the Web", but only an average 50 seconds on each web page (Nielsen 2004). Faced with so much information, most users stick to familiar sites and skip fleetingly through unfamiliar ones. The challenge of capturing most visitors' attention is a significant one.

The Internet will continue to change with technology, the most imminent of which is real-time transport of audio and video information, bringing Internet telephony and Internet TV to the masses. The ability for anyone to make and instantly distribute their films to anyone in the world is already reality. Phones and other portable devices are becoming increasingly "wired" for the Internet. More people are going "online" in every country of the world, and access will become faster and cheaper. The Internet is no passing fad, but a paradigm change in the way the world communicates. Such a change raises interesting questions: Who owns information? Should you pay for information? Can anyone offer information and opinions? What information can you trust?

The Internet as a Conservation Tool

A basic premise of successful conservation is communication at any level, which makes the Internet a powerful conservation tool. To realise its potential, the requirements and expectations of different users and targets need to be appreciated.

A conservation working group such as the CSG uses the Internet in fairly obvious ways: e-mail is used to exchange information and coordinate action, web pages are used as an archive of information, to target a wider audience, and as a fund raiser. Other web pages on the Web represent the world's largest library, although finding quality information often takes skill. The working group can target users directly or indirectly. The policies of political groups may be influenced directly by e-mail, or indirectly through information on web sites. Effective messages require visitors, and there is an art in encouraging "links" to your website.

Other targets include businesses and private sector individuals, both potential sources of support and funding. Educators and the media will actively promote the group and its issues if they are sympathetic to the message. Even those affected by the conservation problem are viable benefactors, ranging from prosperous landowners in Florida to extremely poor village communities in Cambodia. Many in this group have limited or no Internet access, but for those that do the Internet represents an opportunity to promote appropriate management and conservation solutions.

Case Study 1 - Chinese Alligator Fund

Original Goal

The goal of the Chinese Alligator Fund (CAF) was to raise awareness of the status of *Alligator sinensis* in the wild, and to raise funds that would contribute to its conservation.

History and Motivation

The decision to establish the CAF was based on the report made at the 15th Working Meeting of the CSG in Varadero, Cuba (Thorbjarnarson *et al.* 2000a) where it was clear that the remaining wild *A. sinensis* population was in serious trouble. While the principal conservation efforts involved a combination of crocodile specialists, international conventions and the Chinese Government, there was a similar desire by non-professional enthusiasts and hobbyists to contribute towards this effort. To tap this resource, Adam Britton coordinated the creation of the CAF with assistance from several key hobbyists (including Tim Wiegmann, Billy Heinbuch, Ragnar Lonn). A website was quickly established (http://www.flmnh.ufl.edu/alligatorfund) to serve as an information resource, to encourage donations, and to chart the progress of the fund.

Raising Awareness

The creators of the CAF believed that a lack of awareness of the conservation status of *A. sinensis* impaired fund-raising efforts, and that enthusiasts often felt powerless to assist in conservation efforts. Hence a primary goal of the CAF was to bring this conservation problem to the attention of as many potentially interested parties as possible, and to make those parties feel capable of actively contributing to *A. sinensis* conservation. This was facilitated by the creation of a web-site containing the following elements:

- A brief description of *A. sinensis*, its habitat, and its status in the wild, with links to more detailed information if desired.
- Details on how to take part in conservation efforts through:
    - downloading leaflets for distribution, detailing the CAF and its aims
    - donating money directly to the CAF through the CSG

    - donating money by purchasing high quality photographic prints
    - donating money by purchasing clothing and other small items from an independent online store
    - raising awareness via school projects
- A list of every person who donated money and purchased prints, with a ranking "reward system" depending on the amount donated.
- Regular news updates on progress with *A. sinensis* conservation in general, progress of the CAF, total money raised to date and where this money was going

Location and web space were provided by the CSG at the Florida Museum of Natural History (FLMNH) which lent credibility and reassured visitors that donations would reach the right place. The web-site was promoted by the CSG, Internet discussion groups, and by word of mouth. The popularity of the hosting site crocodilian.com (1.5 million visitors a year directly and via the CSG) ensured that awareness of the CAF was relatively high within the "community". David Kirshner, an artist based in Brisbane, donated a characteristic logo for the CAF (Fig. 1) which was used on the web-site, leaflets and clothing.

Figure 1. Chinese Alligator Fund logo, designed by Brisbane artist David Kirshner.

Overall, the website was very successful once news of its existence had spread through the appropriate segments of the Internet community. The updated news pages were popular with visitors, as were the ranking pages where donors felt a degree of achievement. The website also provided an easy means of directing people to download appropriate information and leaflets.

Use of Leaflets

Single page, US letter-sized leaflets bearing the CAF logo, the CSG logo and a short description of the CAF were made available in two popular formats (Microsoft Word and Adobe PDF) on the website. These leaflets described the CAF, encouraged readers to visit the web-site to learn more, and contribute to conservation efforts by spreading the word, purchasing merchandise and so on. The format was kept simple and easy to print out. This encouraged motivated people to print out multiple copies to distribute with permission at zoos, reptile shows, reptile group meetings etc. Although their effectiveness was difficult to measure, they appeared to generate significant interest and many people downloaded them. With improvements in server technology, it is now very easy to obtain a direct measure of the number of times a particular file was downloaded and hence obtain a rough index of success. Given the small investment in time required to create them, and the fact that no effort by the organisers was required to distribute them, they were a successful addition to the website.

Direct Donations

Cash donations made directly to the CAF were highest in the year following its establishment (Fig. 2), the result of concerned private individuals wishing to play a small role in helping conservation efforts. When high-quality photographic prints were made available, smaller private donors preferred to purchase permanent reminders of their contribution and hence simple cash donations became rare from this sector. The bulk of cash donations then came via fund-raising drives made by larger groups (eg fund-raising days at zoos, raffles by state reptile groups in the USA), and the majority of CAF funds were obtained this way.

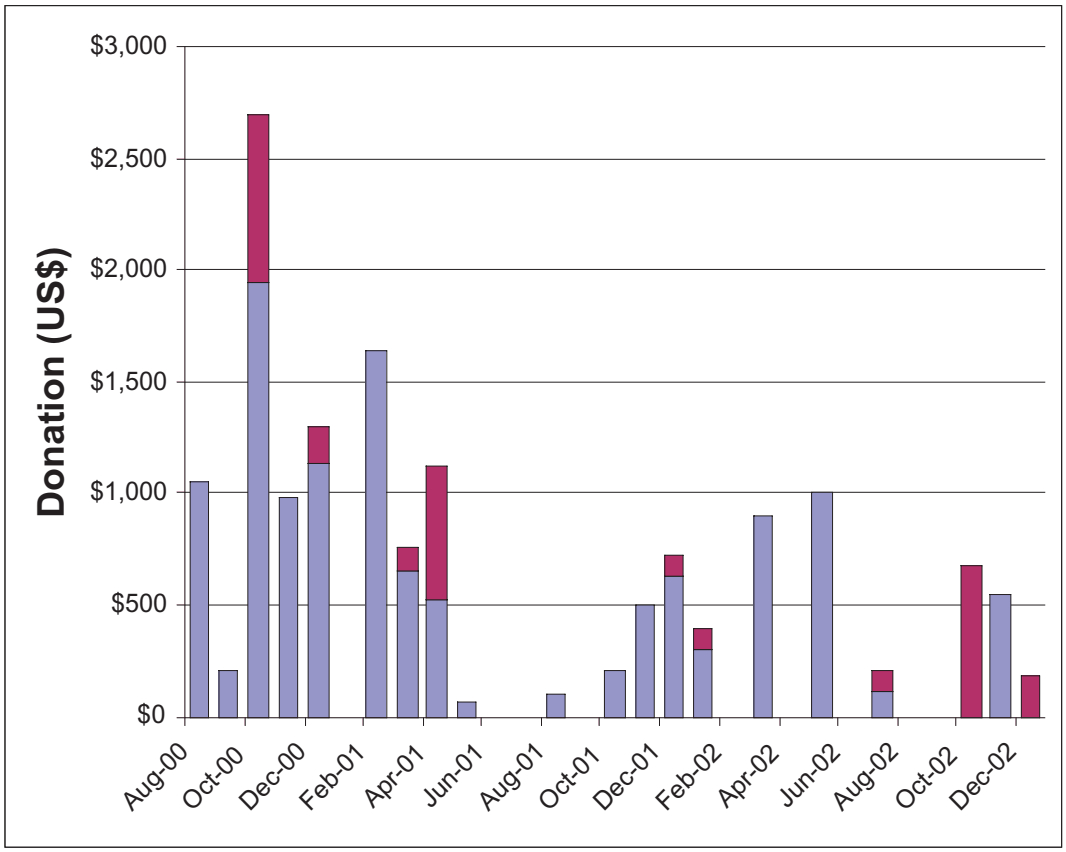

Figure 2. Main donation history of the CAF. Light bars represent cash donations. Dark bars represent profits from sales of photographic prints.

Direct donations are obviously a part of any fund. It was felt at the outset that the CAF would reach a particular target audience (private enthusiasts) better if it offered incentives to donate, particularly if those were collectable (eg prints). This ultimately proved a successful approach, yet did not deter societies from wishing to help through direct donations. Such societies benefited from downloadable materials available through the website (eg essays, photographs for use in talks, etc.).

Photographic Prints

High quality photographic prints were made available three months after the inception of the CAF. These were manufactured using the Ilfochrome printing process that uses high resolution paper and inks to create professional prints that do not fade over time. Although more expensive, we believed it was more important to offer archive quality prints that attracted larger donations. We also believed that purchasers would select high quality photographs over images specifically of Chinese alligators. This proved to be the case, with the two most popular images being of American alligators and saltwater crocodiles respectively, as we lacked similar spectacular images featuring Chinese alligators.

Prints were offered as “limited edition” only, each signed by the photographer and numbered out of 25. This seemed effective in encouraging rapid uptake by buyers. Each print was accompanied by an A4 letter of thanks to each supporter, bearing the CAF and CSG logos. Although standard prints were not offered, we believed that anyone

motivated enough to support the CAF would go the extra mile and pay more for a significantly higher quality print. Overall the strategy was a successful fund-raising component (22%) of the CAF (Fig. 2).

Offering prints created the greatest logistical overhead for the CAF, and relied on voluntary efforts by key organisers with limited resources. Prints were made to order, with a minimum of three orders collected before the order was made. This often meant a slight delay between placing the order and sending the print, but no buyer complained about this process. Although costs decreased slightly with larger orders, the volume of orders was rarely sufficient for large orders to accrue in a reasonable timeframe. Adam Britton bore the cost of 10 advance prints in the beginning, but this quickly ceased to be a realistic option without financial support.

The average production cost of each print was $US37 plus $US3 for postage and packaging. Prints were sold at a minimum of $US90, which ensured a minimum of $US50 was donated to the CAF with each print sold. Although some buyers provided an extra donation by paying up to $US150 per print, most paid the minimum asking price. Some people felt that $US90 was quite expensive, others felt that the quality of the prints justified the final cost. There were no complaints from buyers and everyone expressed satisfaction with the purchase.

Prints were made in Darwin, later Canberra. The buyer sent orders directly to the CSG Executive Officer (Perran Ross) in Florida and money was paid to the FLMNH in US dollars. The Executive Officer then informed the CAF (Adam Britton) of the order, and the print was dispatched when available. FLMNH later repaid Adam Britton $US40 per print to cover production costs. By asking buyers to deal directly with FLMNH, money was saved on exchange rates, a degree of security was added to the transactions, and an appropriate degree of legitimacy was conferred upon the CAF.

Prints were also sold successfully at a number of venues including reptile shows and presentations given to reptile groups and zoos. For example, a raffle of one unique print at a talk in the US raised almost $US800 in one night. Prints were sent to motivated supporters keen to sell prints at such venues. The vast majority of prints were sold to buyers in the USA.

New prints were occasionally introduced to encourage more sales, although with limited success. Assuming later prints were as appealing as earlier prints, this suggests that the majority of print sales occur when the fund-raising process is still relatively novel. These prints were only made to order, so no cost was incurred through their introduction later.

Overall, sales of photographic prints can contribute significantly to fund-raising efforts, although most sales occur early in the promotional campaign. Offering new incentives to purchase prints later in the campaign (eg new images, special offers) had limited success with the CAF, but this may have been a shortcoming in the ability to reach a new audience. The majority of orders were made on the basis of one or two “must have” photographs, with other photographs becoming essentially superfluous. However, a greater range of prints with wider applications (eg for education, competitions, awards, publicity, etc.) may have been a solution, but the organizers lacked the resources to pursue this.

In terms of logistics, virtually all print sales originated in the USA. Using a US dollar account, preferably based in the USA, was a key factor in reducing logistical costs. Close communication between the person distributing the orders and the person taking the orders was also essential, and worked well for the CAF. However, difficulties were encountered when a local photographic lab closed down, and the only cost-effective alternative was found in another state. This added a further layer of complexity to a process that ideally needs to remain simple and streamlined, but could have been avoided by having a liaison in close contact with the new photographic lab. At the time, the lab needed to be sent negatives with each print order, but now modern digital techniques mean that negatives can be scanned and stored at the lab until more prints are required. This minimizes the need to order large volumes of prints, which can be a serious problem when resources are low.

Other Merchandise

Once the CAF logo was completed, it was obvious this appealing design could be used on hats, T-shirts, mugs and other merchandise to assist fund raising. Although inquiries were made in the USA and Australia to print the logo onto t-shirts, the costs proved prohibitive without additional financial support, and the quality was always in doubt.

Eventually the Internet company Cafepress was found, which offered a cheap means of providing a wide variety of

merchandise bearing the CAF logo through a convenient web-based design and ordering system. Initial tests indicated an acceptable quality for the merchandise, and the CAF page announced the new merchandise.

The result was disappointing, with less than $US200 raised in total after several months. While a number of orders were received, the amount made after Cafepress deducted their royalties meant that little cash was actually donated to the CAF. However, considering the low cost of setting up the web page (one day) and no overhead cost of maintaining the online "store", the experiment proved an inexpensive lesson.

The main reason for the failure of the Cafepress store was likely its timing - it was introduced after the initial "excitement" of the fund-raising process had died down. Had it been introduced at an early stage it is likely far more sales would have been generated. The alternative approach of investing in a stock of T-shirts can be costly when demand is low, and solutions like Cafepress appear to be a better alternative if the timing is right and the targeting is appropriate.

Feedback to Donors

Two systems were created to provide donors with feedback and incentive. The first was a regularly updated news page on progress with *A. sinensis* conservation efforts in general, plus the role the CAF was playing in those efforts - all donors were keen to know whether their donations were actually making a difference. The second was a donor ranking system. All donations, no matter how small, were listed on the website next to the donor's name, and were placed into one of several categories based on the total donation to date by that person or organisation. This was very effective in encouraging donors to keep donating to see their name in lights under higher ranks - several emails were received that mentioned this specifically.

It was clear that CAF donors wanted feedback - to know that they had played a small part in the conservation efforts. Although occasional newsletters sent to donors was considered, the website proved the most effective method of providing feedback to existing and new visitors.

School Projects

Only one school project was initiated, but proved to be a success. Bruce Shwedick (Crocodile Conservation Services) worked with Garrisonville Elementary in Garrisonville, Virginia, USA. Each year the school holds a "Gator Day" and CCS introduced the theme of the Chinese alligator to the students. Workshops and competitions were organised and proved very successful (Appendix I). John Behler and Adam Britton judged winning essay and painting entries and the results were displayed on the CAF website.

There is considerable scope to raise awareness of conservation activities through public education if approached correctly. In this case not only were the students introduced to an unusual species and conservation problem, but awareness spread into the local community. Such actions clearly depend on the motivation of individuals to organise appropriate events, but making materials and activities available through the website greatly assists those individuals.

Parallel Activities

Somewhat independently of the CAF, Terry Cullen (USA) and others working under the banner of the CSG were responsible for raising significant funds at major reptile shows. These were very much hands-on activities at popular annual events attended by thousands of people - something of a regional phenomenon, but an example of how a key sector of the public interested in reptiles can be harnessed to raise funds for a group they had not heard of before walking through the door.

Overall Result

Overall, the CAF raised in excess of $US15,000 (Fig. 3). Given the unconventional distribution model for the CAF, and the relatively small amounts donated by most donors, the amount raised was far in excess of expectations. Funds have been used to assist in the organization of an international conference on Chinese alligator conservation in Guangzhou ($US8000) and the remainder will likely be used on specific alligator projects under the direction of the Chinese Government. Moreover, the achievements of the CAF were brought to the attention of the Chinese Government, and proved to be politically advantageous. The CAF encouraged matching donations from larger organizations such as the Wildlife Conservation Society, and succeeded in bringing *A. sinensis* conservation to a

much wider audience including the international media.

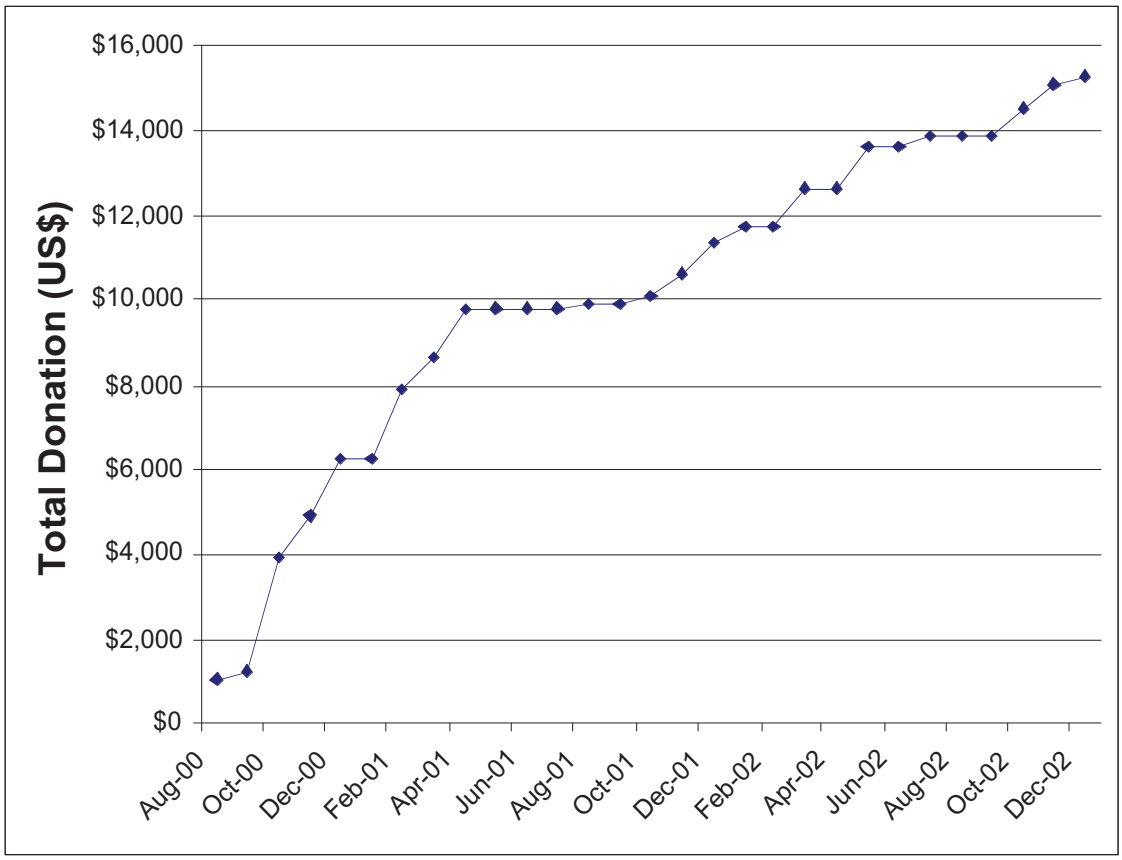

Figure 3. Total money raised for the CAF over time. Over two-thirds of the total was raised in the first 9 months.

Case Study 2 - Tomistoma Task Force

About TTF

CSG's Tomistoma Task Force (TTF) is a group that is dedicated to quantifying the status of *Tomistoma schlegelii* (False Gharial, classified as Critically Endangered under the IUCN Red List) in the wild, identifying the threats to which they are exposed, and to promote conservation action.

History and Motivation

The decision to establish the TTF was based on the meeting made at the 16th Working Meeting of the CSG in Florida, USA. A TTF Committee was established, comprising the key players and other interested CSG members concerned with Tomistoma conservation.

The TTF Trust Fund was established under the auspices of the CSG to collect and distribute funds raised for Tomistoma conservation. The contents of the TTF Trust Fund are assigned to projects by the TTF Committee.

Expected activities and current stage of TTF web-site

The TTF expects to raise funds for field research, to analyse threats to crocodiles and their habitat, to raise awareness and promote public education, to understand the social background of local societies and advance conservation of Tomistoma in Indonesia and Malaysia. Use of the Internet was considered vital to facilitate these efforts.

Development of the TTF website was undertaken voluntarily, and roughly three quarters of the site's intended features were online by January 2004. Activities outside the website were confined to communication between the TTF committee and various attempts to raise both minor and major sources of funding. Some of these activities were reported on the TTF website.

As future TTF activities (eg field research, conferences) take place, reports will be made available on the website. Eventually the website will include a registration zone where members and supporters can get more involved in TTF activities, and efforts to raise the profile of TTF can take off. At present, the TTF website is still at an early stage.

Development of TTF website

Basic elements and a rough structure of the website were discussed shortly after the formation of TTF. A website subcommittee was established to handle the technical issues.

1. Conceptual Structure of TTF Website

TTF Website was designed as three-layered structure by Akira Matsuda (Fig. 4). The 1st layer was designed for visitors and named "Public Area". It contains Visitor Zone and TTF Charity Shop. Visitor's Zone offers general information regarding TTF and conservation of *Tomistoma schlegelii*. TTF Charity Shop offers charitable merchandise of TTF. All donations and profits are put into TTF Trust Fund.

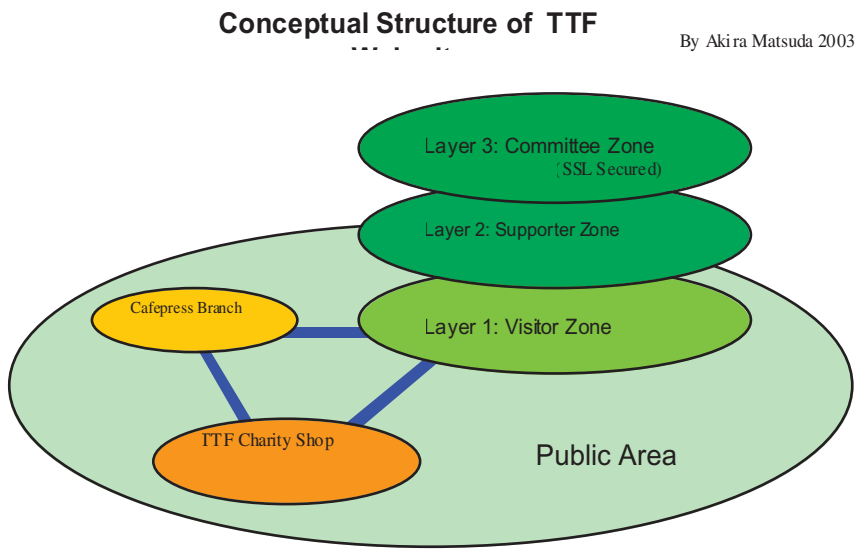

Figure 4. Conceptual diagram of TTF Website (Version 2004).

The 2nd layer was designed for registered users and named "Supporter Zone". This area will not open until TTF activities have developed further, and suitable content can be offered to registered users.

The 3rd layer was designed for TTF Committee members. It aims to facilitate communication among members who are scattered all over the world. It has the potential to greatly facilitate discussion, build consensus among active members, and to share both data and unconfirmed information. The Committee Zone identifies individual members and is protected by passwords and high level SSL security. It enables safe communication within the browser, in contrast to standard e-mail that can be eavesdropped and spoofed easily. To date, communication in this secure area has been very limited, primarily due to members' familiarity and comfort with e-mail, and partly because the security features take some getting used to.

2. History of the TTF Website

The Committee Zone was initially developed in April 2003. It was used to gather materials from TTF members for construction of the Visitors Zone.

The Visitor Zone was opened in May 2003 with principal pages only. A total of 35 web pages were planned as menu items. Several of these were developed by TTF members and further refined by the Committee. By May 2004, 20 web pages were available. Manuscripts for remaining pages are open to TTF members, and should be complete by the end of 2004.

The Free Download Section, where leaflets can be downloaded, was started in August 2003.

The TTF Charity Shop was opened in January 2004 for a test run with minimum merchandise. The future addition of appropriate merchandise is open to the TTF Committee. Various issues with the sale of merchandise remain to be solved, such as minimizing the cost of international shipping. As with the rest of the website, the shop is run on a voluntary basis by TTF members.

The TTF Charity Shop Cafepress Branch is an extension of the TTF Website, and was established in April 2004. It offers various sundries bearing the TTF logo, and although Cafepress claim a significant royalty on each item sold, it operates completely independently of the efforts of TTF members.

3. Major elements of Visitor Zone of TTF Website

The Visitor Zone, which is the public face of the TTF on the Internet, contains the following major elements:

- A brief introduction to the TTF.
- A brief description of *Tomistoma schlegelii*, its habitat, significant threats and its status in the wild, with links to further information on other web-sites.
- A brief description on how to get involved with TTF and conservation efforts through:
  - Becoming a "Friend of Tomistoma" to support TTF.
  - Becoming a "Partner" to support TTF more directly.
  - The advantages of becoming a registered user.
  - Donating money by purchasing high quality photographic prints
  - Donating money by purchasing clothing and other sundries.
- A page to download leaflets about *T. schlegelii* and the TTF (currently in English and German).
- A list of facilities that hold *T. schlegelii* (currently Europe only).
- A list of supporters who register as a "Friend of Tomistoma" or "Partner".
- A list of person who donate money to TTF.
- Regular news updates regarding TTF activities and on *T. schlegelii* conservation in general. This includes an article about the 2002 False Gharial Workshop held in Indonesia.
- An inquiry form.

Contents of the Visitor Zone will be enriched and enhanced as development on the TTF website continues. Web space and all costs of development of the TTF website were provided by Akira Matsuda who runs a small Internet service as a part of business providing benefit to crocodilian conservation groups.

Raising Awareness

TTF believes that a lack of awareness of the conservation status of *T. schlegelii* will impair both conservation and fund-raising efforts. The following methods are used to raise awareness.

1. Development of the website

The most significant aspect of the TTF's public profile is the website, described above.

2. Holding of local events

Local events are held that introduce TTF to appropriate groups about conservation of Tomistoma. These are held voluntarily by TTF members (see below).

3. Leaflets

Leaflets are available for download from the website. Initially produced in English, they are also available in German and will be translated into other languages. The aim of the leaflets is to provide an easy means of introducing people to the need for Tomistoma conservation. The leaflets are available in Adobe PDF (Portable Document Format) - a standard distribution method for electronic documents around the world.

Additional leaflets will be produced on a voluntary basis by TTF members. Printed versions of key leaflets will

also be factored into the TTF budget.

4. Use of mass media

Exploiting the mass media to promote Tomistoma conservation has been considered, especially once field activities commence and conservation action can be demonstrated. However, securing the interest of the media will rely upon selling an interesting story. The website has the potential to reach a wide audience with a message that is under the control of TTF.

Fund raising

1. Fund raising through Internet

Internet fund-raising has been implemented through the TTF Charity Shop and Cafepress Store, and funds have started to trickle in. However, serious fund-raising will not be possible without significant media exposure and publicity of the website - an area that TTF needs to address in the near future.

2. Fund raising through local events

A number of key events are held each year by the hobbyist herpetoculture community, and the opportunity to set up a table and promote TTF and Tomistoma conservation has been taken by specific TTF members on a voluntary basis - Bruce Shwedick in the USA, Ralf Sommerlad in Germany, Colin Stevenson in Australia. The TTF Website announces the details and schedule of all fund-raising events, although at this stage most visitors to these events are unaware of the TTF. Given that a significant percentage of the hobbyist herpetoculture community has Internet access, there is real potential to promote TTF's presence at these events. The Internet can also be used to advertise educational lectures, arrange booking for events, issue special coupons, and provide follow-up services following an event. These actions depend on the motivation of specific TTF members, but providing a framework under which these actions are possible will help.

3. Charitable merchandise

A number of items of merchandise are available for sale through the TTF Web-site and at fund-raising events. The prestige items are a number of high quality photographic prints donated by TTF Committee members, similar to the approach offered by CAF. The most popular were selected within the committee using an online voting system in the Committee Zone, and offered as "limited edition" prints primarily through the website.

Postcards, T-shirts and other sundries bearing the TTF logo have been organized by members, with all profits going to the TTF Trust Fund. The biggest challenge has been finding ways of making these items available at a modest cost to ensure the greatest benefit to the TTF. As TTF gains exposure, these items should sell in modest numbers and provide a modest but useful source of funds.

Program for supporters

A "supporters program" was established to reward supports of the TTF. The concept is an attempt to encourage enthusiasts who often feel powerless to assist in wildlife conservation efforts.

1. Categories of supporters

Two categories (Table 1) were created under which to list supporters. "Friend of Tomistoma" is for private individuals who want to feel involved, and "Partner" is intended for organizations, companies or individuals who provide significant financial support for Tomistoma conservation activities.

2. Services for supporters

The newsletter and activity reports are planned as feedback for supporters and donors. These will be distributed through the TTF Website (to reduce printing costs), although printed copies will be available for public events and to send to key individuals and organizations. However, a regular and long-term commitment is required by TTF members if these are to be produced in a timely and productive manner. The benefits of these services need

Table 1. Principal features of the program for supporters.

Features	Friends of Tomistoma	Partners
Annual contributions	$US50 (rate negotiable)	Over $US1000
Certificates	Certificate of 'Friendship'	'TTF Partner' plaque
Newsletter	Yes	Yes
Reports	-	Yes
Login to the registrant zone	Yes	Yes
Discount on TTF merchandise	Yes	Yes

to be assessed. The Supporter Zone on the TTF Website will host forums where supporters can interact with TTF Committee members by sending comments and asking questions. More qualified supporters could play important roles in field studies. These services are yet to be implemented.

Major differences between CAF and TTF

1. Difference of principle aims

CAF concentrates on raising awareness and raising funds that can be applied to Chinese alligator conservation. In contrast, TTF is a working group that includes raising awareness and funds within its remit, but whose members work together to promote Tomistoma conservation, organize surveys, allocate funds and advance political issues of relevance. However, public perceptions about CAF and TTF may be similar on the surface - they are methods through which conservation goals of each species can be achieved.

CAF
1. To raise awareness of the status of *Alligator sinensis* in the wild.
2. To raise funds that would contribute to its conservation.

TTF
1. To quantifying the status of *Tomistoma schlegelii* in the wild.
2. To identify the threats to which they are exposed.
3. To promote conservation action.

2. What's in a name?

The name "Chinese Alligator Fund" clearly describes the principal aim of the initiative - a fund-raising body for the Chinese alligator that seeks charitable donations. "Tomistoma Task Force" is less clear - the name "Tomistoma" is unfamiliar to the majority of people, and the goals of a "task force" are not necessarily obvious. It does not invoke the image of a fund or a body seeking donations. However, it was felt that using the common name "False Gharial" was unwise in some countries where "false" carries negative connotations. Given the more active nature of the TTF members, the implications of the name may be less relevant.

3. Use of media

The Chinese Alligator Fund was created in direct response to publication of the WCS report on the critical status of wild populations (Thorbjarnarson 2000b). Significant publicity arose from this in the world's media, which helped to bring the Chinese alligator situation (and hence fund-raising efforts such as CAF) to a wider audience. No doubt the CAF's exposure benefited significantly from this.

The CAF also benefited from exposure through an existing, popular crocodilian website (crocodilian.com), which led to a significant number of donations from an active section of the Internet community interested in crocodilians.

Key factors such as timely, multi-sector involvement, use of mass media including paper media, and use of an existing high-traffic website contributed to the success of the CAF in a relatively short time-frame.

In contrast, TTF has a lot of development ahead and is a harder "sell" to create significant conservation benefits for Tomistoma. The status of Tomistoma in the wild has still not been satisfactorily determined, and proving its critical status would lend power to the TTF's mission. To date, TTF Website and TTF Charity Shop have had relatively few visitors - TTF needs to get the word out, and promote a compelling conservation message, for its public fund-raising efforts to take off.

Success of TTF Committee Zone

The Committee Zone of the TTF Web-site was created using dynamic content technologies to make the website interactive, in contrast to a "read only" website. It facilitates communication among the committee members who are scattered all over the world, and enhance discussions compared with e-mail. Interactive items include the ability to submit news, use threaded discussions, participate in surveys, assist in the creation of a glossary and other public content, upload files and photos of interest, add dates to a calendar, etc. In effect it turns the site into an interactive newsletter for its members.

However, these items have not been well used by the TTF Members, particularly those who have limited access to or experience with interactive web-sites. E-mail is a familiar and relatively simple technology that is widely used, and switching to a more complex - if more powerful - alternative that takes longer to access and is less intuitive is not always easy. It is clear that interactive websites need to offer significant advantages over e-mail, but not at the cost of being easy and convenient to use. E-mail will remain the primary means of communication for key issues, but certain member activities can benefit from interactive features on the website that are not possible by e-mail, particularly as activities increase and communication / interaction with the general public plays a greater role.

Overall Result

The TTF is an ongoing and evolving group that is still in its infancy. In 18 months since its inception, the TTF has brought together key players in Tomistoma conservation, established regular communication, created a detailed web resource and interactive database, begun to collate data on Tomistoma from different countries, and investigated a number of promising sources of funding for field studies. During that time, the group has raised several thousand $US with relatively little exposure outside of the crocodilian community, with a species that has had very little media exposure.

From an Internet perspective, the TTF website is very ambitious and perhaps ahead of its time for a group unfamiliar with its potential. In many ways the website is overkill for the TTF at its current level of development, but if the TTF grows into a larger group with greater public exposure, the benefits of establishing such a powerful website will become clear. At present, without the incentive to expand beyond simple e-mail communication, the growth of the website's content will be slow. Greater efforts need to be made to increase exposure of the website and the fund-raising mechanisms to the general public.

The cost of developing these websites is hard to judge, as they were developed voluntarily in spare time. The CAF website took one person a few days to complete, but the TTF website took several people several months to reach a functioning stage. No cost-benefit analysis has been done on these websites, but they have the potential to expand exponentially beyond their original scope once the community gets involved, as has happened many times within Internet communities.

Future Role of the Internet in Crocodilian Conservation

Internet technologies continue to develop, both in terms of what websites can offer and what users can experience. We are still exploring the potential of the Internet for crocodilian conservation, but the two case studies highlighted here are effectively pilot studies of the medium's potential.

The most imminent advances in technology will be the speed at which information can be transmitted and downloaded via the Internet. Real-time delivery of high quality video and audio are not far off, enabling Internet Television and live online broadcasts to nearly a billion people simultaneously. The power to communicate ideas will expand exponentially as more people have access to these technologies, and large online communities will become an effective means of coordinating conservation action.

Even with existing technologies, we have barely scratched the surface of what is possible on the Internet. Most

crocodilian websites are effective in offering an archive of information on general crocodilian science and natural history, but there is considerable scope for improvement. The CSG website has the potential to become the central hub for its conservation activities, starting with its Task Forces which are the focus for priority species. Not only will this improve communication and conservation action within the group, it will communicate the need for conservation to a wider group of people that are becoming increasingly detached from the natural world. Such actions are vital if awareness is to be increased and funds raised for conservation efforts.

There is a need for a research-quality archive of species on the Internet - something that is of benefit to scientists working in the field on a variety of issues. The website offers the means by which such information archives can be updated as new information becomes available, and new findings can be presented to the community for analysis. However, to be effective members will have to embrace this new medium and contribute towards its success.

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Appendix I

Program of school fundraising activities by Bruce Shwedick:

Gainesville Elementary School Celebrates "Gator Day" 2001

The students and teachers of Garrisonville Elementary in Garrisonville, Virginia, USA celebrate the gator, their school's mascot on the first Friday of every March. This event is not only about school spirit, it also is utilized to promote reading.

Bruce Shwedick has been the guest speaker at Gator Day for over a dozen years now and in 2001 he thought it was appropriate to emphasize the plight of the Chinese Alligator. He invited the school's third, fourth and fifth graders to create art and to write essays about the Chinese Alligator and it's struggle for survival.

The students were invited to participate in the first "Help Save the Chinese Alligator Art and Essay Contest". The students were given two months to create their art and write their essays. The school was provided with a copy of John Thorbjarnarson's recent article about the Chinese Alligator featured in Wildlife Conservation magazine and the students were encouraged to visit www.crocodilian.com to learn more about the Chinese Alligator.

The student's work was judged during the AZA Crocodile Biology and Captive Management School held at the St. Augustine Alligator Farm. Judges included John Behler, the Curator of Herpetology at the Wildlife Conservation Park/Bronx Zoo, Dr Adam Britton and Bruce Shwedick.

The winner of the art contest is Cara Lawn. Jennifer Park and Beth Van Briesen won honorable mention. The winner received a copy of "I Didn't Know That Crocodiles Yawned to Keep Cool". Jennifer and Beth received copies of "The Legend of Gomek" and "The Missing Gator of Gumbo Limbo". A tie was declared in the essay contest between Cara Lawn and Courtney Cook and each winner received a copy of "Alligators and Crocodiles" by J. Behler and D. Behler.

The students of Garrisonville Elementary have learned a lot about this unique and endangered crocodilian. We encourage you and your students to read more about the Chinese Alligator and to utilize the "Earth Dragon Classroom Activity Worksheet".

Earth Dragon Classroom Activity Worksheet

GRADES K - 6

I. Introduce the Chinese Alligator using books, articles, websites and videotapes related to crocodilians followed by classroom discussion.

A. General characteristics of a crocodilian: aquatic reptile, cold-blooded, covered in scales, long jaws with sharp teeth, four strong limbs, toes on hind feet slightly webbed, elliptical pupil, powerful tail, egg-laying.
B. Crocodilian groups: alligators, caimans, crocodiles and gharial.
C. Chinese Alligator: geographical range, size and diet.

II. Chinese Alligator ecology. Classroom discussion.

A. Habitat - where do Chinese Alligators live and with what other animals do they share their environment?
B. Burrows - why do Chinese Alligators dig burrows? What impact do these burrows have on people?
C. What has caused the Chinese Alligator to become an endangered species?

III. Activities.

A. Art - students can create artwork that could feature the Chinese Alligator, its habitat and the native plants, animals and the people that share the habitat.
B. Creative writing - imagine that you lived near the Chinese Alligator, what would you want to tell your friends and parents about it? Imagine that you spent the day visiting wild Chinese Alligators and write a story about your adventure.

C. Geography - how many miles do Chinese Alligators live from your home?

GRADES 3-6

I. Natural History.

A. Review the general characteristics of crocodilians and the specific characteristics of alligators, caimans, crocodiles and the gharial.
B. Discuss Chinese Alligator reproduction including nest construction, egg-laying and maternal care.

II. Conservation.

A. Discuss what is being done to save the Chinese Alligator from extinction. Examples: captive breeding, protection by laws, establishing protected reserves and changing people's attitudes about alligators.

III. Activities.

A. Written and oral reports - what is the difference between the Chinese Alligator and the American Alligator? Consider the differences in their appearance, size, habitat and the ways they are utilized by people.
B. Find out if there is a Chinese Alligator in a zoo near you. Take its photograph and collect more Chinese Alligator facts to share with your class.
C. Find out what you can do to help the Chinese Alligator and other endangered species at your zoo or in the wild. Share what you find with your class.

VOCABULARY

1. crocodilian or crocodylian - any member of the taxonomic group "Crocodylia".
2. caiman - a diverse group of tropical alligators from Central and South America.
3. gharial - an extremely slender-snouted crocodilian from India.
4. T'u-lung - the "Earth Dragon". A traditional name for the alligator in China.
5. Anhui - the Province in China where most wild Chinese Alligators remain.

World Trade in Crocodilans: an Update

John Caldwell

Data are presented showing that global trade in crocodilian skins peaked at just over 1.3 million in both 2000 and 2001 but fell back to 1.1 million in 2002. Exports of caiman from Colombia peaked in 2000 and their decline the following year was replaced by a massive increase in exports of American alligator. Trade in both declined in 2002, although caiman appears to have been more seriously affected. Exports of Nile crocodiles increased steadily during the late 1990s with steady or increased production in Madagascar, South Africa, Zambia and Zimbabwe. Exports from other African countries show a general decrease. Exports of *Crocodylus novaeguineae* from Indonesia have been increasing steadily while those from Papua New Guinea appear stable. Estuarine crocodile exports from Australia peaked in 2000 and appear to have declined slightly since while exports from Indonesia and Papua New Guinea appear to be stable. Of the other crocodile species, *C. johnstoni* has not been traded since 1996, there are low levels of exports of Morelet's crocodile from Mexico and a few thousand skins of *C. siamensis* are exported annually from Thailand. Trade in wild-caught caiman from Venezuela has declined steadily to under 20,000 yearly while exports of Yacaré from Bolivia, Brazil and Paraguay have been increasing steadily since 1999.

Major Issues Affecting Sustainable Trade of Crocodilians

Don Ashley (Chair)
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Twenty-six CSG members from 12 countries participated in a trade workshop to identify major issues affecting the sustainable trade of crocodilians. Ten CSG Steering Committee members attended, including all three officers of the Trade Committee.

The consensus was trade workshops were useful during CSG meetings, provided an opportunity to review and discuss major trade issues and engaged a wider cross-section of CSG interests involved with sustainable trade. More interest in sustainable trade issues has evolved since the Singapore CSG meeting and the Gainesville trade workshop with clear support in Darwin for more frequent interactions on trade issues that should include industry updates, inquiries to clarify timely issues, general trade information on market trends and more venues for enhancing the business and conservation benefits of sustainable trade. A series of Sustainable Trade Forums is recommended.

The rhetorical question: **"Would world trade in crocodilians have been restored without the conservation benefits of sustainable use?"** was generally agreed as **"no",** at least not at the near historical peak levels of classics and increasing supplies of caiman. The important point is profits are enjoyed because of conservation benefits to crocodilian sustainable use and a responsibility to enhance conservation is inherent to the economics of sustainable trade. Since the best conservation engages and benefits local people, the emphasis on community-based conservation that benefits people and wildlife should be a priority.

The workshop group felt the major issues should be stated simply and the focus be on achievable results. The major issues are generally grouped in four categories: CITES, CSG, Range States and General:

1. CITES

   A) Compliance

      The issues of complying with the pre-conditions of downlistings as well as annual reports, infractions, illegal trade and inadequate enforcement are urgent priorities. As stated originally in Singapore, CSG must insure its own house is in order if we expect wider acceptance of sustainable use as a conservation strategy. The clear standard for all crocodilian trade must be it is <u>legal, sustainable and verifiable.</u>

   B) Personal Effects Exemption

      The harmonization of CITES Range states to allow unrestricted import and export of up to four legal, non-commercial crocodilian products is very important to enhance trade. The USA in fact will accept up to 8 personal effects products, the EU is adopting the CITES standard of 4 products, Japan will implement the same and China will propose in Bangkok that "harmonization" is expected of CITES Parties unless they notify the Secretariat. Addition of new species to the Personal Effects list (currently crocodilians, caviar, rainsticks and queen conch) are expected to be dried seahorses, coral and perhaps other marine products. The standard for all species listed under the Personal Effects exemptions is that the trade is legal, sustainable and verifiable.

   C) Trade Fair Permits (Carnets for product samples)

      The continuing effort of CITES and range states to simplify and streamline permit requests is important to trade.

   D) Source Code Amendments

      The USA proposal to redefine CITES source codes was a concern to the trade workshop group and immediate clarification will be requested through the Chair.

2. CSG

A) Economic Analysis (McGregor Reports)

Concern by some trade group members was expressed concerning this Report. While the overall Report was judged as good, the usefulness to the trade remains a question to some. On the other hand, the original goal of economic analysis projects was to monitor trade data compiled through the IACTS Report and initiate case studies to review issues that could impact conservation benefits. The only economist in attendance at the workshop suggested the information collected for economic review should be data that could "change management decisions" and that strategic or trend data was important to ongoing economic analysis. The decision was to await review of the newly completed draft of Phase Two of the project coordinated by TRAFFIC and drafted by McGregor before recommending a course of action to the CSG.

B) Captive Breeding

The issues of captive breeding and its relationship to conservation benefits merits continued monitoring and analysis. A CITES review is in order.

C) Live trade in crocodilians out of native ranges for commercial farming

This issue merits immediate action by CSG, particularly with *C. siamensis* and live exports to China from Cambodia, Vietnam and other critically endangered populations. The illegal capture of wild specimens for live trade must stop.

D) Trade Representation

The CSG trade group has been under represented from Latin America and more representation from the primary Range States of Colombia, Venezuela, Brazil, Argentina, Mexico and others is needed. Six representatives attended the Darwin trade workshop from South America and participated in the discussions.

3. RANGE STATES

A) USA

Concerns with the Endangered Species Act (ESA) and its potential impact on restored trade of some species (ie *C. latirostris* from Argentina or captive breeding restrictions) needs to be clarified. In addition, difficulties with exports of legal *C. porosus* from Thailand to US needs to be addressed. The Chair will provide an update on these issues. Efforts by Louisiana and CSG to initiate a repeal of the unenforceable California law prohibiting sales of crocodilian products should continue.

B) European Union

Slow import procedures, particularly in Italy and perhaps other countries must be addressed. Permit processing times up to four weeks are not acceptable to business and does not enhance sustainable use. The issues of tagging and retagging also needs clarification.

C) Mexico and Central America

Concerns with the sustainable use of *C. moreletti*, *C. acutus* and *Caiman crocodilus* require review, particularly in Mexico, Panama, Honduras and Guatemala. The impact of the USA (ESA) needs to be addressed.

4. GENERAL

A) Airport Displays

Continued effort to remove negative or misleading airport displays should continue. In addition opportunities to provide better conservation education to the public on sustainable use should tell a more balanced story about the value of crocodilians to people and wildlife.

B) Trade and Market Information

While the trade data from IACTS derived from CITES Annual Reports is essential, the report is two years dated (2004 report compiles data through 2002). More current trade information is important, particularly to range states without direct access to current market trends.

C) Directory of Farming and Ranching Operations

An updated directory of farming and ranching operations would be useful and UNEP-WCMC is willing to undertake it once funding is secured.

D) Authenticity of Trade

The relationship of scientists to traders is sometimes skeptical of commitments to conservation benefits. Credibility must improve to foster better cooperation in telling the conservation success story of crocodilians with benefits to people and wildlife through sustainable use.

The six most urgent recommendations were to address:

1) Live trade of critically endangered *C. siamensis* and captive breeding issues.
2) Compliance issues with CITES and Range States.
3) Maintain high standards for CITES personal Effects exemptions.
4) Slow import procedures of some European Union countries.
5) Concerns with US Endangered Species Act and California legislation.
6) Airport and other negative public displays that mislead people about crocodilian products and conservation benefits of sustainable use.

In summary, the three primary conclusions of the trade workshop were to initiate some trade forums between the two-year CSG meetings, improve credibility of traders and explore ways to enhance community-based conservation that benefits people and wildlife.

CrocPLAN: A Genetic Improvement Program for Saltwater Crocodiles

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Abstract

By implementing an improvement program based on reproductive performance, juvenile growth and juvenile survival, superior juveniles will be selected as future breeding animals to increase the profitability of crocodile farms. It was, therefore, the aim of this study to create a plan for a practical genetic improvement program at a commercial crocodile farm (Janamba Croc Farm), thereby establishing an industry-wide genetic improvement program to be called CrocPLAN. Data were collected from Janamba Croc Farm and analysed to obtain relevant genetic and phenotypic parameters (heritability, repeatability and correlation) for breeding objectives and selection criteria. Relative economic weights were estimated and combined with estimated crocodile breeding values (CBVs) into a crocodile economic selection index ($CESI). Each $CESI value was expressed as a dollar ($) deviation from the herd average. Using the breeding pairs from Janamba, the highest-ranked pair had a $CESI value of +$4748, whereas the lowest-ranked pair had a $CESI of -$5257. The response to selection was predicted to be $324 increase in profit per breeding pair per annum. This result reinforces the potential for implementing CrocPLAN on saltwater crocodile farms in Australia. It also illustrates the potential for similar selection programs to be implemented using other crocodilians.

Introduction

To date, research in crocodilian industries has concentrated on improving management, for example in relation to nutrition and housing, to optimise production efficiency. No research has yet been conducted into the possibility of genetic improvement in captive breeding populations. The aim of a genetic improvement program is to improve the total economic value of the herd, and consequently maximise farm profit, for a given set of inputs. Identifying and breeding from pairs, or preferably individuals, that are genetically superior will increase the average genetic merit of the herd and aid in improving farm profitability.

Isberg *et al.* (2003) defined breeding objectives for the Australian crocodile industry. They included skin grade, number of hatchlings per female per year, survival rate, food conversion efficiency, age at slaughter and potential skin "quality" traits (scale row number and regularity, shape and thickness). Subsequently, Isberg *et al.* (2004) presented results from genetic analyses of these breeding objectives with the exception of the skin grade and food conversion efficiency breeding objectives. A summary of these results are presented in this paper. Resultant crocodile breeding values (CBVs) have been combined with estimated economic values into a crocodile economic selection index ($CESI) for candidate selection. The application of CrocPLAN, using Janamba Croc Farm as a model, is also presented.

Methods and Materials

All data (reproduction, production and survival) were collected on the Janamba Croc Farm (Middle Point, Northern Territory) using information from 30 known breeding pairs between 1994 and 2002 (Isberg *et al.* 2004). Restricted maximum likelihood (REML) procedures were used to obtain the required (co)variance components for estimating the relevant parameters (repeatability, heritability, genetic and phenotypic correlations) for each breeding objective and relevant selection criteria. A Cox's proportional hazards model was used for the survival analysis. Only repeatability and phenotypic correlation could be estimated for the reproductive data due to the unknown pedigree structure. Heritability and genetic and phenotypic correlations were estimated for the breeding objectives age at slaughter and scale row number, whilst heritability only was estimated for juvenile survival. Data were not available for food conversion efficiency or the other skin-quality traits. In addition, estimated crocodile breeding values (CBVs) for

each pair were obtained.

Economic values for each breeding objective were estimated by Gray (2003), and were also presented in Isberg *et al.* (2004). The economic values were estimated on a 'per breeding pair' basis using a Cobb-Douglas type production function.

Results and Discussion

Number of hatchlings per female per year

The reproduction breeding objective investigated was the number of hatchlings produced per female per year (NoHatch). Possible selection criteria also included in the analysis were initial clutch size, number of viable eggs, hatchability, average hatchling snout-vent length and time of nesting. Only repeatability could be estimated since no pedigree structure was available. All repeatability estimates were high, ranging from 0.24 (hatchability) to 0.68 (initial clutch size and time of nesting). Phenotypic correlations between the traits ranged from negligible (0.03) to high (0.86). All traits were kept in the multivariate analysis for the estimation of CBVs for NoHatch shown in Figure 1.

The CBVs shown in Figure 1 range between -8.75 hatchlings (B20) and 15.09 hatchlings (UB03), a difference of 23.84 hatchlings per female per year. These CBVs are expressed as deviations from the average herd CBV. Specifically, mating of a pair of offspring from parents both with a CBV of 15.09 like the pair UB03 will produce 15.09 hatchlings per annum greater than a pair whose parents have average breeding value, whilst progeny from a pair like B20 will have a reproductive performance of 8.75 hatchlings less than the herd average.

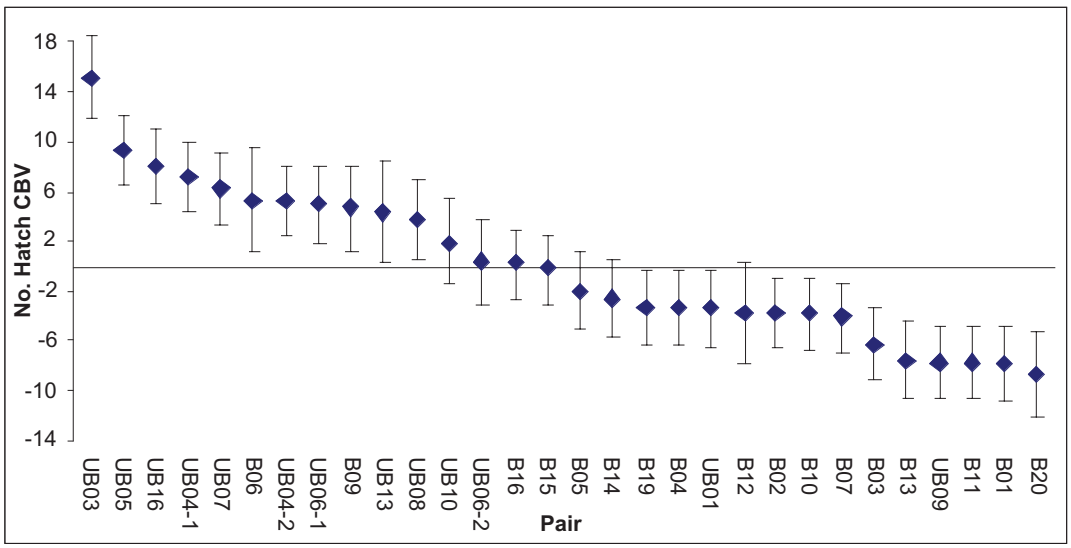

Figure 1. Pair CBVs (± SE) for the reproduction breeding objective, number of hatchlings produced per clutch per year (NoHatch).

Age at slaughter

The age when an animal is slaughtered directly influences the cost of production on crocodile farms. An investigation of this breeding objective, age at slaughter, and possible selection criteria including measurements taken at hatching and inventory (average age ~ 9 months) showed heritability estimates to be high. Heritability for age at slaughter was 0.40 (SE 0.10), whilst for hatchling snout-vent length, it was 0.60 (SE 0.15). The genetic (-0.81) and phenotypic (-0.82) correlations between slaughter ages and inventory head length were high, whereas the correlation estimates between hatchling snout-vent length and the other traits were either low and/or unreliable due to large standard errors. The resulting CBVs (± SE) for slaughter age are shown in Figure 2.

The range of CBVs shown in Figure 2 is between -158 days (B16) and 144 days (B01), with a difference of 302 days. These results indicate that future offspring from pair B16 will be predicted to reach slaughter size 158 days before the herd average, whilst offspring from pair B01 take an additional 144 days to reach the marketable size.

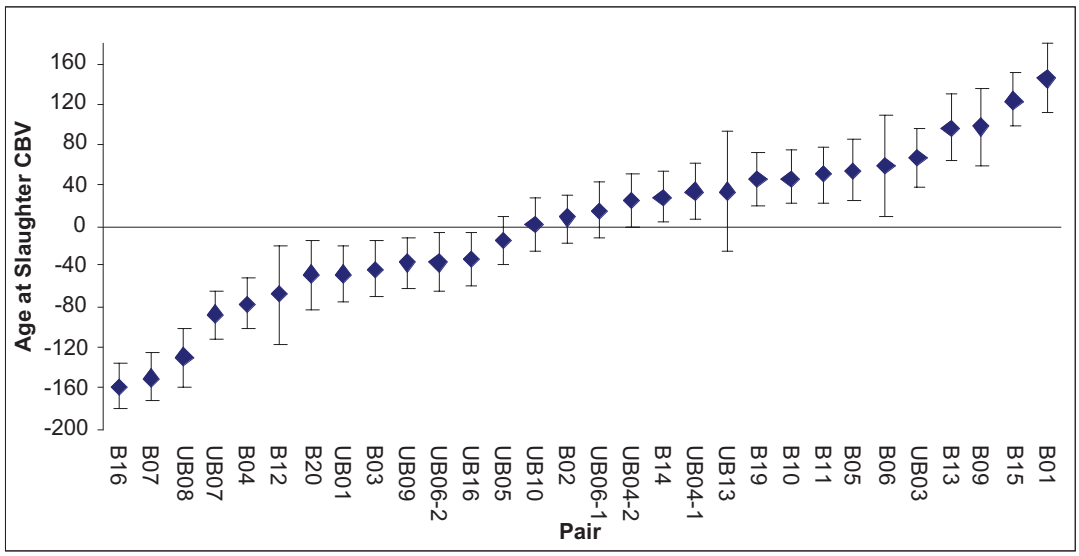

Figure 2. Pair CBVs (± SE) for the breeding objective, age at slaughter (days).

Juvenile survival

The number of juveniles that reach slaughter size from each clutch directly influences farm income. This trait was investigated using a Cox's proportional hazard analysis. The heritability estimate for log juvenile crocodile survival time was 0.15 (SE 0.04). The log hazard pair estimates are shown in Figure 3a. However, since the average age at slaughter is three years, CBVs were calculated based on a juvenile surviving to 1095 days (or three years). These CBVs are presenting in Figure 3b.

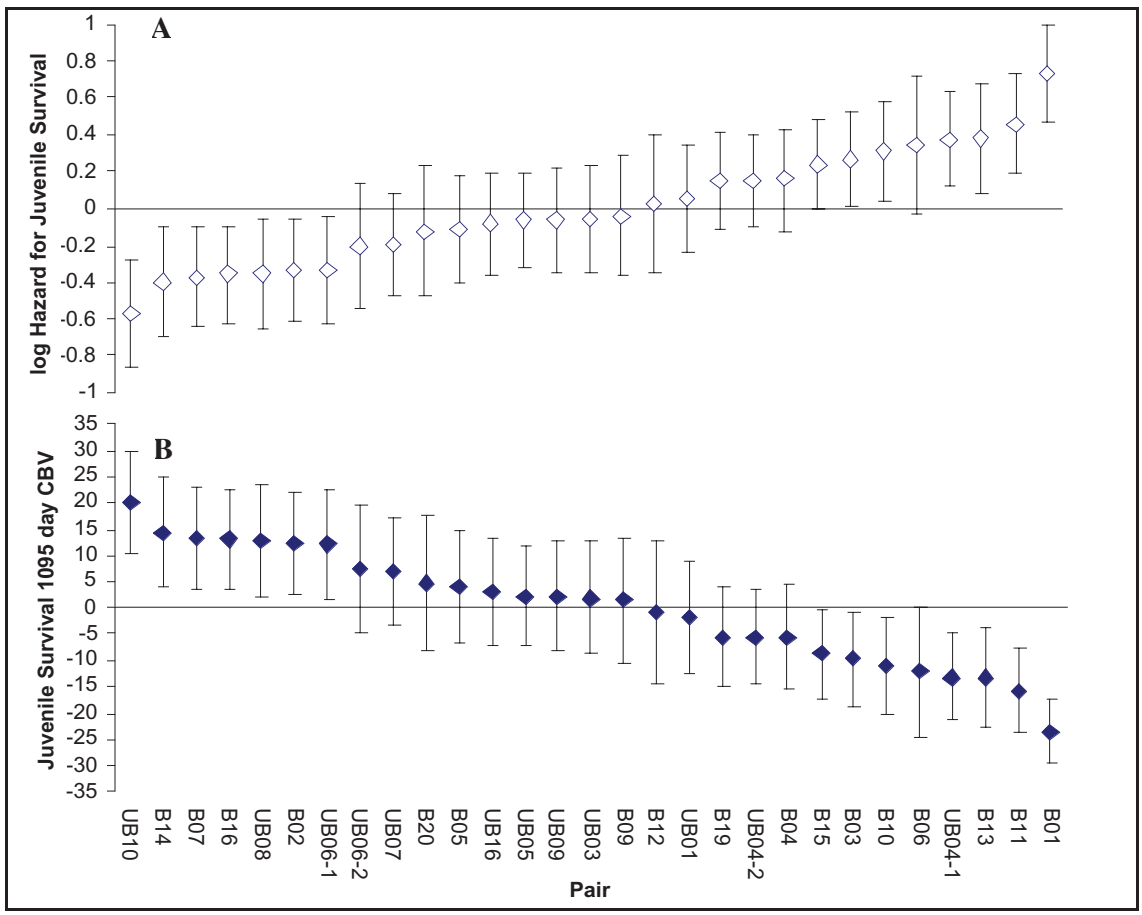

Figure 3. A) Log hazard pair estimates (± SE) of juvenile survival produced using the Cox's proportional hazards model. B) Pair CBVs (± SE) for juvenile survival (%) at 1095 days (or three years).

UB10 had the lowest log hazard estimate of -0.57 (antilog estimate ($e^{-0.57}$) = 0.57), whilst B01 had the highest estimate of 0.74 (antilog estimate = 2.09). This means that a juvenile from a clutch produced by UB10 has a higher chance of surviving to slaughter than a juvenile produced by the pair B01. More specifically, if we denote $S_0(t)$ as the baseline survival function, that is the probability that an individual survives to age t, averaged across the population, then the survival function for offspring of UB10 will be $[S_0(t)]^{0.57}$ (increased survival) whereas those from B01 will have a survival function of $[S_0(t)]^{2.09}$ (reduced survival). So in general, the survival function for offspring of a particular pair will be $[S_0(t)]^R$, where R is the hazard ratio for a particular pair, being the antilog of the BLUP estimate on the log hazard scale. A further description is given in Isberg *et al.* (2004).

Since the hazard of mortality changes with time, it was decided that the most appropriate time to approximate breeding values was at day 1095 (or three years) since this was the average age at slaughter (Isberg *et al.* 2004). Juvenile survival CBVs were then expressed as a percentage difference in survival to 1095 days, relative to the population average. Thus, from the CBVs (Fig. 3b), offspring from pair UB10 have a 20.3% reduced risk of mortality compared to the herd average, whilst offspring from B01 have a 23.5% greater risk of dying before cull age.

Number of scale rows

The price received for an individual crocodile skin is currently determined by i) its width, and ii) its grade (determined by the number and severity of blemishes). Additional skin-quality traits have been identified by Manolis *et al.* (2000) that could, in the future, become important when marketing skins. Possible quality traits include: number of scale rows, regularity of scale pattern, and skin thickness. Currently, there is no premium received for any of these traits. Only data on the number of scale rows were collected. The heritability estimate using data from Janamba Croc Farm was 0.37. The CBVs (± SE) for number of scale rows are shown in Figure 4. The range of CBVs was between 0.92 rows (UB13) and -1.20 rows (B12).

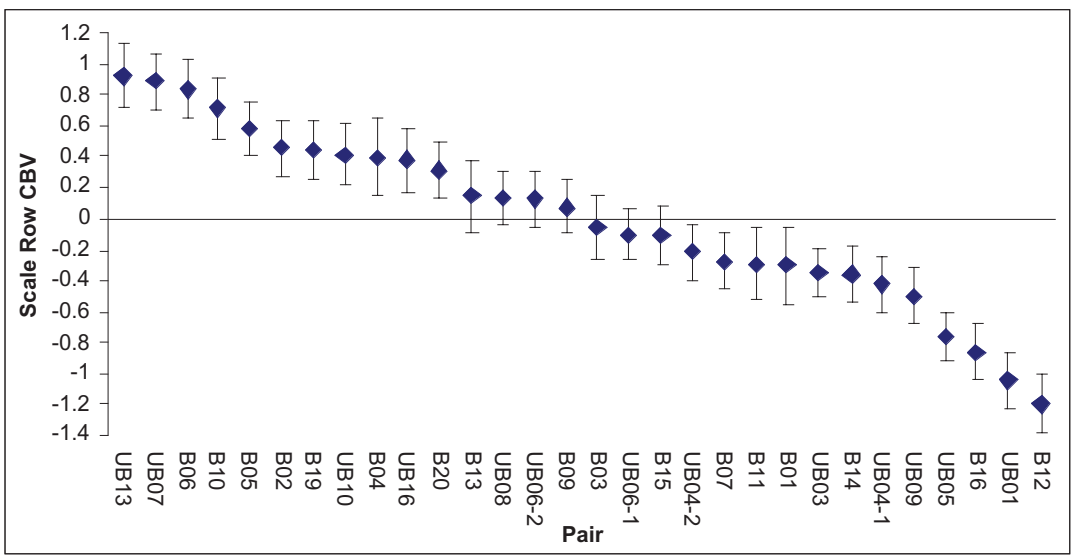

Figure 4. Pair CBVs (± SE) for number of scale rows.

Economic values

Economic values for unit improvements in each breeding objective are presented in Table 1. An economic value could not be obtained for the number of scale rows since there is currently no premium price received for a greater number of scale rows on a belly skin.

The crocodile economic selection index ($CESI)

The aggregate breeding value for profitability is: $H = v_1BV_1 + v_2BV_2 + ... + v_mBV_m$

where v_i = the economic value for the i^{th} breeding objective, BV_i = the true breeding value for the i^{th} breeding objective, and m = the total number of breeding objectives in the selection index. Candidates are selected after ranking based on an estimate of their aggregate breeding value, H, which is calculated as the crocodile economic

Table 1. Economic values for crocodile breeding objectives. "Abb." is the abbreviated term for the respective breeding objective.

Breeding objective	Abb.	Increment	Economic value ($AUD)
Number of hatchlings per year	NoHatch	Increase by one hatchling per year	41.95
Age at slaughter	CullAge	Increase time to reach slaughter size by one week	-25.68
Juvenile survival	Surv	Increase survival by 1%	52.37
Number of scale rows	SR	Increase by one row	0.00

selection index ($CESI)

$$\$CESI = v_{NoHatch}CBV_{NoHatch} + v_{CullAge}CBV_{CullAge} + v_{Surv}CBV_{Surv} + v_{SR}CBV_{SR}$$

After substituting the economic values from Table 1, the $CESI becomes

$$\$CESI = 41.95(CBV_{NoHatch}) - 25.68(CBV_{CullAge}) + 52.37(CBV_{Surv}) + 0(CBV_{SR})$$

A $CESI value for each breeding pair was estimated and is shown graphically in Figure 5. Each pair is expressed as a dollar ($) deviation from the pair average. Pair B16 has the largest $CESI value (+$4748), whilst B01 has the lowest value (-$5257). Note that because covariances between the component CBVs could not be determined, it was not possible to determine standard errors for these $CESIs.

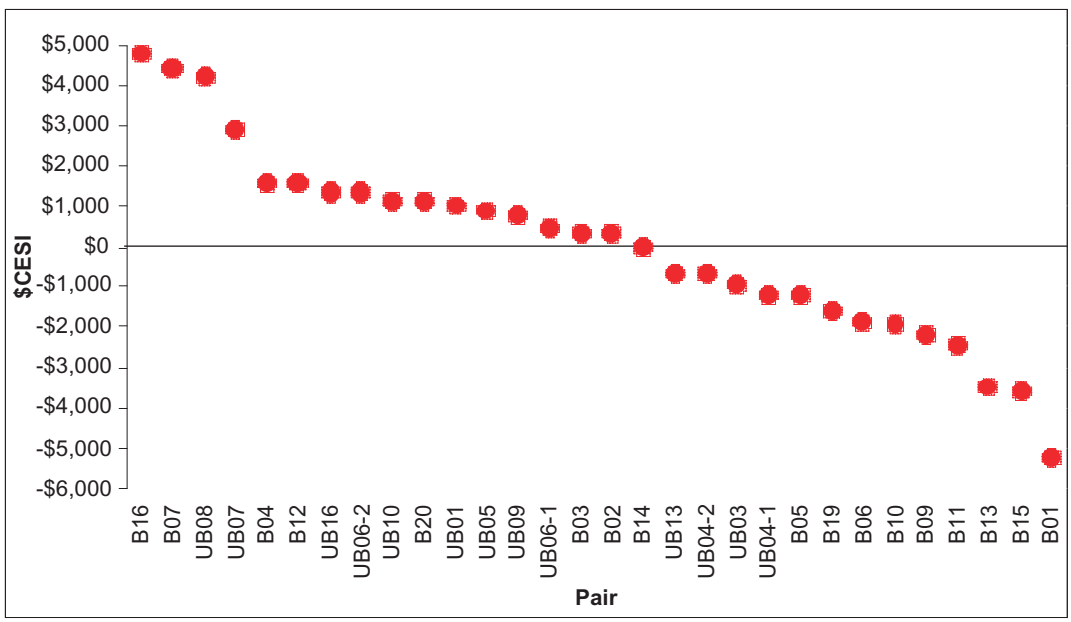

Figure 5. Crocodile economic selection index ($CESI) values for each breeding pair at Janamba Croc Farm used in this study. The CESI value is expressed as a dollar ($) deviation from the herd average, expressed on a per annum basis.

Recommendations for implementing CrocPLAN

Isberg *et al.* (2004) provided recommendations for implementing CrocPLAN using Janamba Croc Farm as a model. Briefly, the selection of replacement breeder animals will occur when the animals reach slaughter size (approximately 2.8 years). Juveniles will be selected using their own $CESI value, using individual performance records integrated into an index with records from relatives. Ten per cent of adult breeding pairs will be replaced each year, with selection intensities (*i*) of 2.88 and 2.05, for males and females respectively. Generation interval was estimated to be

13 years for both males and females, using the following assumptions: sexual maturity will occur at eights years of age for both males and females (Elsey *et al.* 1993); replacement rate is 10%; and, the oldest six pairs are replaced each year (that is, each animal breeds for ten years after sexual maturity).

Using these parameters, the response to selection was predicted to be a $324 increase in profit per annum per breeding pair, assuming no genetic and phenotypic correlation amongst breeding objectives. This result, in conjunction with the large variation in CBVs reinforces the potential for implementing a genetic improvement program (CrocPLAN) on Australian crocodile farms.

A recommendation for maximising genetic variation is the industry-wide adoption of CrocPLAN allowing between-farm (or across-herd) selection of replacement animals. With a functional crocodile economic selection index, $CESI-values can be obtained at any time, allowing across-herd trade of genetically superior (fertile) eggs, hatchlings, juveniles, and adults. The occasional inclusion of wild-harvested animals may also provide additional variability. Additionally, if the level of genetic variation is consistent between crocodilian species, these results provide evidence that genetic selection programs may also provide a method of improving farm profitability in these industries.

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Outbreaks of Mycoplasmosis in Farmed Nile Crocodiles in South Africa

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Abstract

Since July 2002 several severe outbreaks of mycoplasmosis with polyarthritis and pericarditis have occurred on South African crocodile farms. The affected crocodiles became unable to move and had to be slaughtered. A *Mycoplasma* sp. isolated from the lesions was different from *M. crocodyli* previously known from outbreaks in Zimbabwe. In more recent outbreaks of polyarthritis in farmed Nile crocodile hatchlings and juveniles *M. crocodyli* was isolated. The affected hatchlings had been imported from Kenya. The epidemiology of the outbreaks still needs to be investigated further.

Introduction

Outbreaks of mycoplasmosis are associated mainly with polyarthritis and occasionally with pericarditis as well. Such outbreaks are known to have occurred in American alligators *Alligator mississippiensis* and in Nile crocodiles *Crocodylus niloticus* (Mohan *et al*. 1996; Clippinger *et al*. 2000) and two species of crocodilian mycoplasmas have been described: *Mycoplasma alligatoris* (Brown *et al*. 2001) and *Mycoplasma crocodyli* (Kirchhoff *et al*. 1997). In June 2002 a severe outbreak of polyarthritis occurred in juvenile farmed crocodiles on a farm in the Limpopo Province of South Africa and this was followed by further outbreaks on other crocodile farms. This paper reports on the investigations into these outbreaks.

Materials and Methods

Juvenile Nile crocodiles from several crocodile farms in South Africa suffering from outbreaks of polyarthritis were killed humanely and dissected either before or after skinning. Whole legs affected by arthritis and pericardial sacs containing the heart were cut out and taken to the laboratory for *Mycoplasma* isolation. The legs and pericardial sac were dissected aseptically and swabs taken from the joints and the pericardial fluid. These were plated onto Columbia blood agar (Oxoid, Basingstoke, England) containing 5% horse blood, Hayflicks mycoplasma (Hayflick 1965) and Chalquest (Chalquest 1962) agars and incubated in 5% CO_2 at 37°C and in air at 22°C. Both of the agars contained 10 mg ampicillin to inhibit Gram-positive bacteria. Small colonies (<1mm diameter) with a so-called "fried egg" appearance were purified and proven to be mycoplasmas by repeated subculture onto Hayflick's agar without antibiotics. Basic biochemical characterization was also done.

Results

Between December 2002 and April 2004 crocodiles from nine farms were examined. Clinically most of the crocodiles had swollen leg joints and were unable to move. The swollen joints were filled either with excessive quantities of turbid fluid or with dry fibrinous exudate and were surrounded by edematous tissue. Some of the crocodiles also had an exudative pericarditis. Lung lesions were not seen. In some cases the pharyngeal tonsils were found to be swollen.

Mycoplasmas were isolated from specimens from five of the nine farms (Table 1). The *Mycoplasma* isolates showed several different features. One group of mycoplasmas showed all the characteristics of *M. crocodyli* while the most notable feature of the earlier mycoplasmas cultured was their ability to only grow at 22°C and not at 37°C. They differed from *M. alligatoris* by being able to hydrolyze arginine and from *M. crocodyli* by being able to hydrolyze arginine and not growing at 37°C.

The brief histories of the disease on the nine farms are as follows:

Farm 1: High incidence of polyarthritis in 2-year-old crocodiles in one pen where water from a river with wild crocodiles had been used. The other pens received borehole water.

Table 1. Pattern of *Mycoplasma* isolations from farmed Nile crocodiles in South Africa. * Preferred temperature at which the mycoplasmas were cultured. The mycoplasmas that grew at 37°C also grew at 22°C, whereas those that grew at 22°C did not grow at temperatures above 25°C.

Date submitted	Farm	Laboratory Number	Result (Temperature*)	Site
17 Dec 2002	1	B898/02	+ (22°C)	Heart and peric. fluid
27 Jan 2003	2	B85/03	+ (22°C)	Joint
1 Apr 2003	3	B283/03	+ (22°C)	Joint
27 Jun 2003	4	B424/03	-	Percardial sac
3 Jun 2003	4	B442/03	-	Pericardial sac
26 Jun 2003	5	B510/03	-	Lung and peric. fluid
10 Nov 2003	6	B910/03	+ (37°C)	Heart and joints
27 Nov 2003	7	B988/03 (1)	-	Joint and heart
		B988/03 (2)	+ (37°C)	Heart
25 Mar 2004	8	B230/04	-	Heart and joints
1 Apr 2004	9	B252/04	-	Joint
25 Mar 2004	3	B272/04	2 + (37°C) 12 -	Joints
3 Apr 2004	3	B317/04	+ (37°C)	Joints

Farm 2: High incidence of polyarthritis in 2-year-old crocodiles, probably triggered by temperature stress. The outbreak started in July 2002, while the senior author was overseas. It was first investigated in September of the same year after his return but mycoplasma cultures were undertaken only later, in January 2003.

Farm 3: High incidence of polyarthritis in two- and three-year-old crocodiles, probably first triggered by temperature stress. This farm had purchased crocodiles from several sources.

Farm 4: Hatchlings with pericarditis and peritonitis later found to have been caused by Adenovirus and salmonellosis.

Farm 5: Mortality in 3-year-old crocodiles with pericarditis later found to have been caused by salmonellosis.

Farm 6: High incidence of polyarthritis and pericarditis in hatchlings which had been imported from Kenya. The outbreak started after the hatchlings had been purchased from the original importer and transported by road for ± 1500 km, triggered by transport stress.

Farm 7: Polyarthritis and pericarditis in hatchlings imported from Kenya.

Farm 8: High incidence of polyarthritis in one group of 3-year-old crocodiles. There may have been a connection with farm 3. In spite of negative culture results the farm is believed to be infected. This will have to be confirmed by serological testing.

Farm 9: Mortality in 3-year-old crocodiles later diagnosed as stress septicaemia. Only one of the examined animals had arthritis.

Discussion

Mycoplasma species were not cultured from all suspect cases. Even in confirmed cases not every joint or pericardial sac submitted yielded an isolate. Although there may have been other unidentified causes of this disease, it is more likely that since culturing is a relatively insensitive method that these were false negative results. Mycoplasmas are sensitive to overgrowth by putrefactive bacteria and it was found that in spite of using aseptic sampling methods many joints had a mixed growth of walled bacteria which grow more rapidly than mycoplasmas and were are not inhibited by the penicillin in the media. Tetracycline therapy of affected animals will inhibit most mycoplasmas. The causative mycoplasmas may also have been eliminated by the host's immune system by the time crocodiles with chronic disease were slaughtered.

All of the outbreaks appeared to have been triggered by stress - preceding stressful events or conditions. As the crocodiles became immobilized and unable to feed, they had to be slaughtered in an attempt to harvest their skins and thus to save at least some value. Particularly on farms 2 and 3 the losses were quite catastrophic.

There had been two outbreaks of polyarthritis in crocodiles in preceding years which at the time had been interpreted as stress-induced septicaemia. Those outbreaks, however, were very limited in extent whereas the more recent ones, the subjects of this paper, appeared to be grouped or linked. Another difference is that in the present outbreaks group treatment with tetracycline after the removal of clinical cases appears to be suppressing the infection for a limited time only, approximately two months, and then new cases appear, more clinically affected crocodiles have to be slaughtered and more antibiotic treatment has to be administered.

Two species of *Mycoplasma* have been isolated from the outbreaks, first a new species yet to be described and subsequently *M. crocodyli*. While the epidemiology of the outbreaks still is confused, it appears that the new species may be of local origin, from a local population of wild crocodiles, whereas *M. crocodyli* was at least in one case brought in with the importation of hatchlings from another country.

Mycoplasmosis is thought to be transmitted via the water with the mycoplasmas originating from either the oral cavities or faeces of carrier crocodiles. There appears to be a silent carrier stage, while outbreaks of clinical disease appear to be triggered by stressful events or conditions. Antibacterial treatment, mainly with tetracycline, did lead to an improvement of clinically ill crocodiles. However, it suppressed further appearance of new cases, but only for a limited period after which waves of new cases tended to appear. Extreme care should be taken to prevent spreading the infection from pen to pen and when considering the purchase of stock from a farm of unknown disease status.

Conclusion

More work needs to be done to clarify the epidemiology of the recent outbreaks and to develop diagnostic tools, in particular serological tests, which could be used to monitor imported crocodiles or even those traded between farms.

Acknowledgements

This paper is dedicated to the memory of Prof. K. Mohan, the discoverer of *Mycoplasma crocodyli*, who passed away in 2003.

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Ultrasound as a Tool to Evaluate Reproductive Structures of Female *Caiman crocodilus fuscus* in Closed Cycle Conditions

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Abstract

To select reproductive groups of crocodilians in farms, ultrasound is a tool that allows us to evaluate reproductive structures of females. Between December 2002 and February 2003, reproductive animals at three farms located in Atlántico, Colombia, were classified. During the period when courting and mating begin for *C.c. fucus*, gravid females are not normally found. Of 8435 females (mean TL of 136.2 cm, mean weight 8.5 kg), 3590 were evaluated with ultrasound on both flanks. We diagnosed: 3170 "empty" females; 320 with vitellogenic follicles in the ovary to ova in the oviduct (32 mm); 73 with ova of different sizes and calcified eggs (32 to 72 mm); and, 27 with eggs in the abdominal cavity (32 to 66 mm). The transfer mechanism of the eggs from the oviduct to the abdominal cavity is unknown, where they stick to the intestine to be reabsorbed. According to what we observed in 15 females that were sacrificed to confirm the diagnosis, we examined the oviducts macroscopically and passed a catheter inside without finding evidence of any ruptures, also we analyzed egg samples, oviducts and abdominal liquids to isolate microorganisms, all of which were reported as negative.

Para seleccionar grupos reproductores en granjas de crocodílidos el ultrasonido es una herramienta, que permite evaluar estructuras reproductivas de las hembras. Entre diciembre 2002 y febrero 2003, se clasificaron los grupos de reproductores en tres granjas ubicadas en el departamento de Atlántico, Colombia; época donde comienza el cortejo y apareamiento para C.c.fuscus, y normalmente, nunca deben encontrarse hembras ovadas. De 8435 hembras totales, se evaluaron por ultrasonido en ambos flancos 3590, con 136.2 cm (LT) promedio y 8.5 kg (W). Se diagnosticaron 3170 hembras vacías; 320 presentaron desde folículos vitelogénicos en ovario, hasta óvulos en oviducto 32 mm; 73 hembras con óvulos de diferentes tamaños y huevos calcificados desde 32 mm hasta 72 mm; 27 tenían huevos en cavidad abdominal desde 35 mm hasta 66 mm. Se desconoce el mecanismo de traspaso de huevos desde el oviducto a cavidad abdominal, donde se adhieren al intestino para ser reabsorbidos, de acuerdo a lo observado en parte de 15 hembras sacrificadas para confirmar diagnósticos, se examinaron los oviductos macroscópicamente y se pasó un catéter por dentro sin hallar evidencia de rupturas, también se analizaron muestras de huevos, oviductos y líquidos abdominales para aislar microorganismos, siendo negativo el reporte del laboratorio.

Introduction

The success of the majority of the animal production system is based on the selection of the reproducers, Being this a managing practice that is normally used. To accomplish this it has been very important the identification of determinate anatomic and physiological characteristics of the individuals that lead to establish efficient biological standards, to be able to accomplish the optimization of these production systems.

Nowadays we have implemented technologies that allow us predict, diagnose and correct critical points of the processes already mentioned. Case of many species of the domestic fauna, in which to monitor the estrus cycle, detect pathologies, determine pregnancy, establish development state during the gestation and detect anomalies or malformations in the reproductive structures, the ultrasound technique is used. Ones the specimen fulfills the protocol where the pathologies are discarded, we evaluate the uterus and the increasing of the follicular through ultrasound and the professional decides the hour of artificial insemination of the females to obtain high quality genetic breeding.

The breeding of crocodilians in closed cycle, like any other industry of livestock production, must adjust to the use of new technologies that allow the increasing of the efficiency to meet the goals of production.

Materials and Methods

A ultrasonography was done to 3590 females of the *C. c. fucus* species coming from three farms (DEL CARIBE

COLOMBIANO, SAN FRANSICO e INVERSIONES SALAZAR DIAZGRANADOS) located in the “Departamento del Atlàntico, Colombia”.

For this procedure we used: special gel for ultrasounds, Pie Medical equipment, lineal transducer of 5Mhz., and basic equipment for the procedure.

Each animal was ultra sounded in the left and right flank and we used special gel for the ultrasound to improve the image quality.

Each animal previous to the ultrasound was marked, weighed, and measured. The females have a double marking system; a general one that consists of an amputation of the first dorsal crest of the tail, an the other one is an individual marking with a numbered plastic band in the base of the tail. For the statistic analysis we used the SPSS 9.0. program. The variables included were:

1. Ultrasound date
2. Specimen identification
3. Length
4. Weight
5. Left and right flank

The characteristics found in the flanks were the following:

* Empty: The animal that didn’t present any particular internal characteristics when viewed by the ultrasound machine.
* Gemma in formation: These are defined as structures like small black circles generally located in the ovary level like a cluster of grapes.
* Gemma: They are black round circles, that were observed generally a long side the flank, we found them in different sizes or very similar inside the animal.

In case of finding different sizes we measured the gemmas that size was more abundant, we established an average and it was given a rank of calcification. The gemmas were classified in the following way.

Gemmas under 10 mm
Gemmas 10-20 mm
Gemmas 21-30 mm
Gemmas 31-40 mm
Gemmas over 40 mm

Masses: These structures were presented with shape of a gemma but in the ultrasound they were observed in color gray, its size was variable and in many cases very difficult to diagnose. These masses also were classified by size in the following way:

Masses like so
Masses under 10 mm
Masses 10-20 mm
Masses over 20 mm

Masses and Gemmas: Likewise some specimens showed in their flanks mixed masses and gemmas and they were classified in this category.

Eggs: In some females we could appreciate eggs perfectly delimitated and with shell formation in the majority of the cases. The sizes like the gemmas that varied inside the animal in the position of the egg or by being in different grades of formation. In these cases we averaged the size presented with more frequency and it was categorized in the following ranks:

Eggs under 40 mm
Eggs 40-50 mm
Eggs 51-60 mm

Eggs over 60 mm
Eggs without shell: In this rank we classified the females that presented eggs of great size without evidence of a shell.
Gemmas, Eggs and Masses: Some females showed in their flank 3 types of structures and they were classified in this group.

Eggs in apparent abdominal cavity: In our discoveries we found that some females out of the laying period presented one or many eggs, generally observed in either one of the flanks and located in the abdominal cavity. It called our attention and some specimens were sacrificed to corroborate our diagnostic.

These eggs were found like we expected out of the oviduct, additionally we evaluated the oviduct to try to find the way out to the abdominal cavity passing through a flexible pipette for horse insemination and we didn't observe in any of the cases rupture of the oviducts wall or scar, that would allow us assurance that the reason of this so called exit was a breach of the oviduct tissue. For us this characteristic is still a mystery, we couldn't find any mechanic explanation for the localization of these eggs in the abdominal cavity, but we could find how they were reabsorbed.

The process begins with the extension of the intestine, through the small fimbrias and catches the egg and hinge to it. There we think the re-absorption begins, with time the shell gets weak. By mechanic action, fights between animals or manipulation of de man, the liquid of the egg is spilled in the abdomen absorbing it directly, the rest of the egg's structures are absorbed reducing themselves to small masses found in the intestinal walls.

As something curious I add, a female diagnosed with an abdominal egg died 6 months later of the diagnostic and it still persisted in the abdominal cavity.

Eggs in apparent re-absorption: Here we classified the animals that showed eggs in the abdominal cavity, but with the particular characteristic that the ultrasound viewed deformations, not round or oval.

Liquids in the abdominal cavity: In some cases we observed in the flanks of some specimens the presence of liquids, these are seen in the ultrasound as black stains. Probably in some cases specially at the beginning of the job and due to inexperience, the observed liquids were coming from the stomach of the babilla (*C. c. fuscus*) when these weren't submitted to a fasting.

Fat presence: This fat could be observed at the level of the flank in previous or posterior localization, generally when it was present it didn't let the ultrasound penetrate at it made any diagnostic impossible.

Characteristics: This variable included all those particular phenotypic aspects of the species, this would help us in some cases to recognize corporal conditions, mutilations or abnormalities that would permit us recognize even more the animal or change in the corporal condition to relate it with the reproductive state.

Results

Descriptive statistics

With the use of the transducer of e Mhz. It was impossible to observe from the belly or from the back any structure or particular characteristics due to the presence of the osteodermus that don't allow the penetration of sound. The average of ultrasounds were 142 females per day. The speed of the diagnostic depended on the disposability of the animals in the tanks., the size of the animals and the experience of operators. Each animal was measured, weighed previously to the ultrasound diagnostic. Registering the data of length and weight, the minimum and maximum sizes, which average analysis and standard deviation are presented in Table 1.

In Table 2 the weight and the length are related to obtain information that allows us predict in three farms, with the data of the length the approximated weight of each female. The females under 128 cm in their majority were selected as replacements of a group of reproducers in three farms in which we also found reproductive activity. Each animal was ultrasounded in its left and right flank, in some of them were found different types of structures, those animals in which wasn't observed any particularity were diagnosed as empty. The results shows us that the 88.3% of the monitored animals didn't present any type of structure that allow us assure that for those dates when the ultrasounds were done that the females were presenting some type of ovary or reproductive activity (Table 3).

Table 1. Ranges, averages and standard deviations of the length and weight of 3580 female *C.c. fuscus.*

Parameter	N	Minimum	Maximum	Average	Std. Deviation
Length (cm)	3590	110.0	186.0	136.2	18.0
Weight (kg)	3590	4.0	27.0	8.5	4.64

Table 2. Standards deviations (SD) for the length and weight of 3580 female *C.c.fuscus*.

Length (cm)	Weight (kg)	N	SD	Length (cm)	Weight (kg)	N	SD
100.0	2.919	41	0.338	144.0	9.445	141	1.313
102.0	3.184	57	0.346	146.0	9.946	118	1.432
104.0	3.338	92	0.351	148.0	10.196	139	1.318
106.0	3.515	76	0.336	150.0	11.092	108	1.465
108.0	3.740	68	0.400	152.0	11.188	120	1.569
110.0	3.802	34	0.383	154.0	11.941	77	1.548
112.0	4.060	19	0.659	156.0	12.543	86	0.866
114.0	4.125	11	0.760	158.0	13.294	78	1.647
116.0	4.263	18	0.643	160.0	13.704	101	1.925
118.0	4.543	15	0.615	162.0	13.854	78	1.988
120.0	4.927	230	0.640	164.0	14.220	53	1.882
122.0	5.287	190	0.740	166.0	15.109	43	2.188
124.0	5.801	169	1.472	168.0	15.586	35	1.837
126.0	6.250	145	1.028	170.0	16.346	38	2.067
128.0	6.708	177	1.033	172.0	16.108	22	2.276
130.0	6.990	161	1.072	174.0	17.562	28	2.966
132.0	7.345	160	1.031	176.0	17.625	16	2.362
134.0	7.691	147	0.872	178.0	18.869	13	2.214
136.0	8.083	107	1.356	180.0	19.875	8	3.226
138.0	8.125	102	1.191	182.0	17.000	1	
140.0	8.580	111	1.180	184.0	13.000	1	
142.0	10.261	117	1.713	186.0	20.500	2	3.535

Conclusions

The industry of caimans breeding has to adopt diagnostic and production systems completely updated, to detect unfertile specimens with reproductive pathologies. These animals take space, consume food and hand craft, and they'll stay in the breeding place for long periods of time with out being really productive.

Acknowledgements

We specially thanks the croc farms: From the DEL CARIBE COLOMBIANO, SAN FRANCISCO, INVERSIONES SALAZAR DIAZGRANADOS, located in the Atlantico department Colombia for the unconditional cooperation for the development of this investigation, Thanks to croc farm REPTICOSTA for you're support and Miguel Rodriguez for his comments.

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Table 3. Reproductive structures in the left oviduct of female *C.c. fuscus* in three farms on the Caribbean coast of Colombia.

Parameter	Frequency	Percent	Cumulative Percentage
Empty	1143	88.9	88.9
Gemmas in formation	4	0.3	89.3
Gemmas >10 mm	21	1.6	90.9
Gemmas 10-20 mm	55	4.3	95.2
Gemmas 21-30 mm	11	0.9	96.0
Gemmas and Masses	1	0.1	96.1
Masses	1	0.1	96.2
Masses >10 mm	1	0.1	96.3
Masses 10-20 mm	6	0.5	96.7
Masses <20 mm	1	0.1	96.8
Gemmas and shelled eggs	1	0.1	96.9
Eggs >40 mm	4	0.3	97.2
Eggs 40-50 mm	1	0.1	97.3
Eggs 51-60 mm	9	0.7	98.0
Eggs <60 mm	4	0.3	98.3
Eggs Aparent abdominal cavity	3	0.2	98.5
Eggs abdominal cavity	1	0.1	98.6
Fluids Abdominal cavity	1	0.1	98.7
Flank fat	7	0.5	99.2
Gemmas 11-20 mm and Masses	1	0.1	99.3
Gemmas 10-20 mm & Masses 10-20 mm	2	0.2	99.5
Gemmas 21-30 mm & Masses 11-20 mm	2	0.2	99.6
Masses <20 mm and Abdominal. Fluid	1	0.1	99.7
Eggs without shells 40-50 mm	1	0.1	99.8
Eggs Aparent Reabsortion 50-60 mm	2	0.2	99.9
Gemmas 10-20 mm & Masses 10-20 mm & Eggs 40-50 mm	1	0.1	100.0

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Development of ManufacturedFeeds for *Crocodylus porosus*

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Abstract

Potential exists to meet the nutrient requirements for the growth and production of *Crocodylus porosus* using manufactured semi-moist feeds, thus presenting an opportunity to reduce overall production costs and improve the efficiency of feeding. Research completed to date has demonstrated that juvenile crocodiles can achieve growth rates approaching 16 g/d with feed conversion ratios of 3:1 when fed semi-moist pellets (420 g/kg crude protein; 50 g/kg crude fat; 630 g/kg dry matter) at a rate of 3% of liveweight per day. Manufactured diets do not require the inclusion of fresh meat co-products (kangaroo mince, beef mince, chicken heads, etc.) to achieve adequate intakes, and attractants appear to have little influence on feeding intensity or total feed intake. A staged introduction of manufactured feeds is required to ensure newly hatched crocodiles are successfully weaned onto manufactured feeds as their primary source of nutrients, but once weaned, no difference in growth or feeding efficiency is observed between feeding every 3, 4 or 5 days. Further research will investigate improved weaning procedures, ingredient characterisation, feeding delivery strategies, feed production methods, and nutrient requirements with a view to reducing the proportion of production costs attributable to feed.

Introduction

Commercial crocodile production in Australia relies primarily on combinations of fresh meat for the supply of nutrients to all production phases. Not only is fresh meat unlikely to represent an optimal supply of nutrients for efficient crocodile growth, but supply, storage and handling can be difficult and costly. In contrast, the composition of manufactured feeds can be manipulated to match diet specifications to the nutrient requirements of the crocodile for a particular production phase, manufactured feeds can be formulated to reduce the content of fresh meat thus improving shelf life and reducing the need for refrigerated storage, and they are generally easy to handle. In addition, the nutrient density of manufactured feeds is often greater than fresh meat and hence the cost per nutrient supplied in every kilogram of manufactured feed is usually less than via fresh meat.

When defining the most appropriate approach for the development of manufactured feeds for crocodiles, it is important to maintain a focus on the primary nutritional drivers of profitability in any intensive animal production system (Fig. 1). By maintaining this focus, it becomes clear that the primary nutritional challenges for *Crocodylus porosus*, where development of manufactured feeds is still in its infancy, include:

- Identification of the most suitable diet form taking in to account acceptance by crocodiles in various production phases, feeding habits, digestive anatomy and physiology, and the shelf-life and handling characteristics of the feed and the most efficient means of manufacturing the feed;
- Selection of the most appropriate ingredients for inclusion in manufactured feeds;
- Matching diet specifications to the nutrient requirements of crocodiles in various phases of production;
- Initiation of feeding and the weaning of juvenile crocodiles onto manufactured diets as their primary food source;
- Identification that influence feed intake of manufactured feeds by crocodiles and the most appropriate feeding strategies.

The aim of this paper is to describe the attributes of manufactured feeds and feeding strategies developed for *Crocodylus porosus* and results of an extensive research program that underpins this development. Overall, we are seeking to develop manufactured feeds that are:

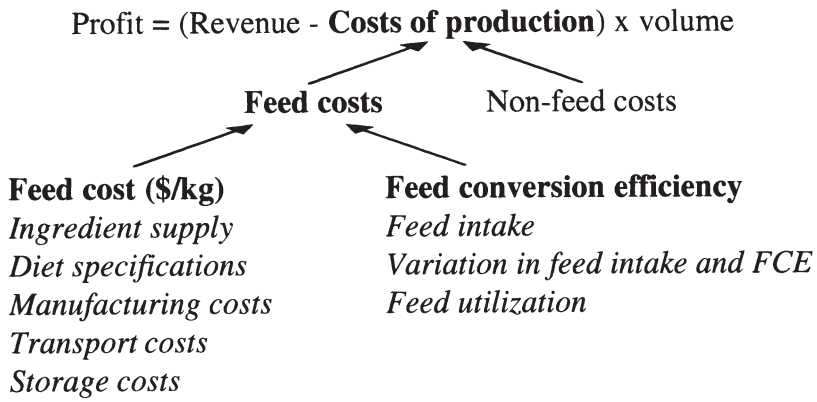

Figure 1. Primary nutritional drivers of profitability in intensive animal production systems.

- Acceptable to crocodiles;
- Cost-effective;
- Capable of promoting efficient production;
- Optimal in terms of final product quality;
- Easy to manufacture, store and handle.

Diet Form

Based on research undertaken previously in Zimbabwe and local experience, a semi-moist pellet (ie 25% moisture) was deemed to be the most appropriate initial diet form for manufactured crocodile feeds in Australia. Jansen-Van Vuuren (1993) reported that dry diets were poorly accepted by crocodiles, but acceptance was improved when moist pasta-like products were offered. As more experience is gained with feeding manufactured feeds to crocodiles, it is possible that the need for a semi-moist pellet will be reduced and that dry pellets could have application with specific production phases. This was certainly the experience of the salmon industry worldwide that now relies totally on low moisture, high protein, high fat extruded feeds, but started as an industry feeding fresh bait fish and semi-moist feeds (Fig. 2).

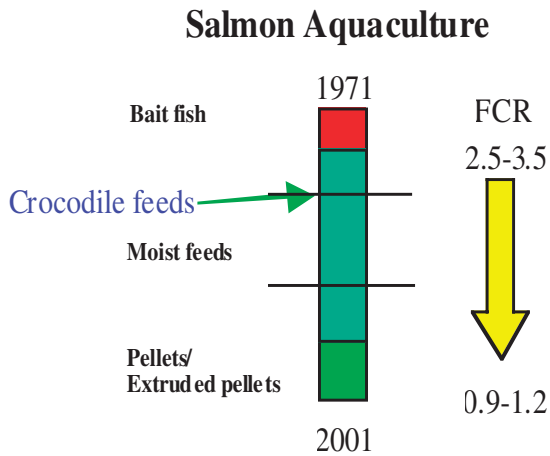

Figure 2. Schematic representation of chronological development of manufactured feeds in the salmon industry.

As well as acceptance, diet form must be matched to the digestive anatomy and physiology and the feeding habits of the target animal. Crocodiles are generally opportunistic feeders and can go for long periods without food with frequency of feeding influenced by body size and environmental temperatures. In addition, crocodiles do not extensively chew their food, have a highly acidic and mechanically active stomach environment and comparatively short intestines and colon. As a consequence, the diet form needs to be such that it can be maintained in the stomach environment for a period of time ensuring a constant flow of nutrients over this period to the intestine, but as there is limited chewing, it cannot be so well bound that it will not break down in the stomach.

The current semi-moist pellet used for crocodiles in Australia is produced with wheat gluten as the binder. A significant amount of trial and error has resulted in definition of the optimum balance between wheat gluten, oil and water so that pellet binding is optimal. Pellet sizes vary depending on the age of crocodiles being fed, ranging from a mince during the weaning period to pellets exceeding 5 cm in diameter for adult breeding crocodiles. The semi-moist pellets produced are generally sinking, but potential exists to produce floating pellets, but this may be at the expense of water stability in the first instance.

Semi-moist pellets are produced using a modified mixer/mincer with a range of tube die attachments (Fig. 3). A gearbox allows the auger speed to be varied, so that the amount of mechanical energy imparted on the feed can be controlled. Ideally, if the temperature of the mix can reach approximately 52°C through friction, the semi-moist pellet binding will be optimal.

Figure 3. Modified mixer/mincer with pipe die used to produce semi-moist pellets for crocodiles.

Ingredient Selection

Ingredients used in manufactured diets for crocodiles must match the capacity of the crocodile to digest these components as well as being acceptable. While diets consumed by crocodiles in the wild are some guide to potential ingredients in manufactured feed, this is generally more likely to reflect the feed that is available rather than the most appropriate ingredients for use in the supply of nutrients in an intensive production system.

Crocodiles have a high capacity to digest protein from both animal and vegetable sources (Coulson and Hernandez 1983; Staton and Vernon 1991; Manolis 1993), and fats (Manolis 1993). It also appears that crocodiles have some capacity to utilise carbohydrates as a source of dietary energy (Staton *et al.* 1990b). As a consequence, a wide range of feed ingredients can be utilised in manufactured crocodile feeds, resulting in significant flexibility in formulations, and potential to closely match diet specifications to the nutrient requirements of the crocodiles.

While we have a basic understanding of the types of food constituents that can be digested by crocodiles, further refinement of manufactured feeds will be achieved through a better understanding of the enzymic and microbiological profiles in the gut, and how manufactured feeds can influence these factors. For example, while some enzymes have been identified (including pepsin, intestinal protease, trypsin, chymotrypsin, carboxypeptidase, aminopeptidase and a-amylase) their activity and relative proportions have not been identified. Digestive enzymes, whether of microbial or endogenous origin are the key to understanding digestive processes in the intestinal tract. However, not only is the total digestive profile important, it is also essential to understand the regional localisation of specific digestive enzymes so that the structure of feed pellets can be matched with digestive capabilities. In order to maximise the digestibility and absorption of nutrients, we need to understand which digestive enzymes have the greatest activities, where they are located and whether they change in level with changes in feed.

In addition to enzyme activities and profiles, microbial populations also play an important role in the efficiency of digestion and manufactured feeds composition and form. Microorganisms play an important role in digestive processes in most terrestrial species, and there is good evidence that microorganisms also exist in the digestive tract of aquatic species and reptiles. However, it is not clear what role these bacteria play. They could play a passive role in restricting

the establishment of significant numbers of pathogenic bacteria in the gut, or a more active role by promoting or enhancing digestion of the normal diet. When crocodiles are fed a manufactured diet containing components not normally found in the wild, the action of microbial populations may be essential in achieving optimal digestion and growth response. Integration of the microbial and enzyme components of the farmed crocodilian gut could provide useful information about the capability of crocodiles to digest manufactured feeds, and help in the structural and compositional design of a feed pellet.

Manufactured diets for crocodiles that have been used to produce acceptable growth rates in crocodiles consist primarily of:

- Proteins including wheat gluten, meat and bone meal, feather meal, blood meal, poultry meal and fish meal;
- Water;
- Oils or tallows;
- Vitamins and minerals;
- Preservatives and anti-microbials such as potassium sorbate, phosphoric acid and propylene glycol to manipulate water activity and confer increased shelf life;
- Anti-oxidants and mycotoxin binders.

While fresh meat is used as a basal diet in many crocodile production systems, it is not necessary to include it in manufactured diets to achieve acceptable intakes. Fresh meat is not currently used as a ingredient in manufactured diets in Australia and this does not effect the efficacy of the resulting diet. In addition, it appears that crocodiles do not have distinct preferences for particular ingredients in manufactured diets. Research undertaken by the Queensland Department of Primary Industries in Townsville compared the influence of fresh meat content (0 or 50%), fat level (10 or 15%), fat type (chicken tallow or canola oil) and an attractant (0 or added chicken flavour). All combinations of these factors were tested (Table 1) through a total of 16 different diets, using paired preference tests to reveal no significant preference for any combination in crocodiles of different sizes.

One characteristic of crocodile nutrition in the wild is the fact that they are known to consume a significant proportion of indigestible material and to retain proportions of this in the gut as gastroliths. This is similar to the consumption of shell grit by poultry to enhance the action of the gizzard. Previous research undertaken in Australia had demonstrated that diets with increasing levels of kaolin (a fine clay used in the production of porcelain) as an indigestible filler and decreasing levels of fat promoted superior performance in growing crocodiles (Fig. 4). It was hypothesized that the kaolin may be acting as a gastrolith and could be an important addition to manufactured feeds. Subsequent research however, demonstrated that addition of dietary kaolin has no influence on crocodile performance, nor do other clays such as bentonite (Fig. 5).

Table 1. Diet combinations used to test preference for fresh meat, fat level, fat type and attractant in manufactured semi-moist pellets.

Diet	Fat Content (%)	Fresh Meat (%)	Fat type	Attractant
1	10	0	Chicken tallow	No
2	15	0	Chicken tallow	No
3	10	25	Chicken tallow	No
4	15	25	Chicken tallow	No
5	10	0	Canola oil	No
6	15	0	Canola oil	No
7	10	25	Canola oil	No
8	15	25	Canola oil	No
9	10	0	Chicken tallow	Chicken flavour
10	15	0	Chicken tallow	Chicken flavour
11	10	25	Chicken tallow	Chicken flavour
12	15	25	Chicken tallow	Chicken flavour
13	10	0	Canola oil	Chicken flavour
14	15	0	Canola oil	Chicken flavour
15	10	25	Canola oil	Chicken flavour
16	15	25	Canola oil	Chicken flavour

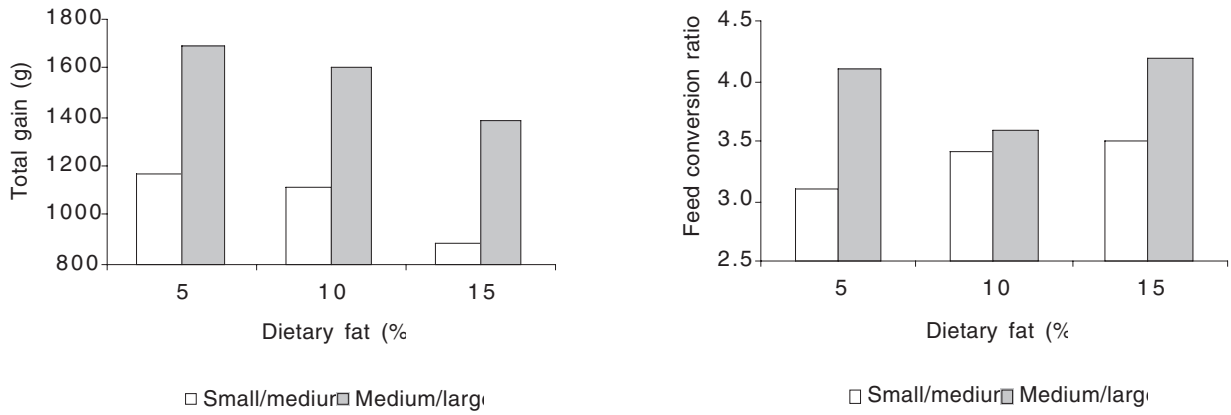

Figure 4. Response of growing crocodiles to increasing levels of dietary fat and dietary kaolin.

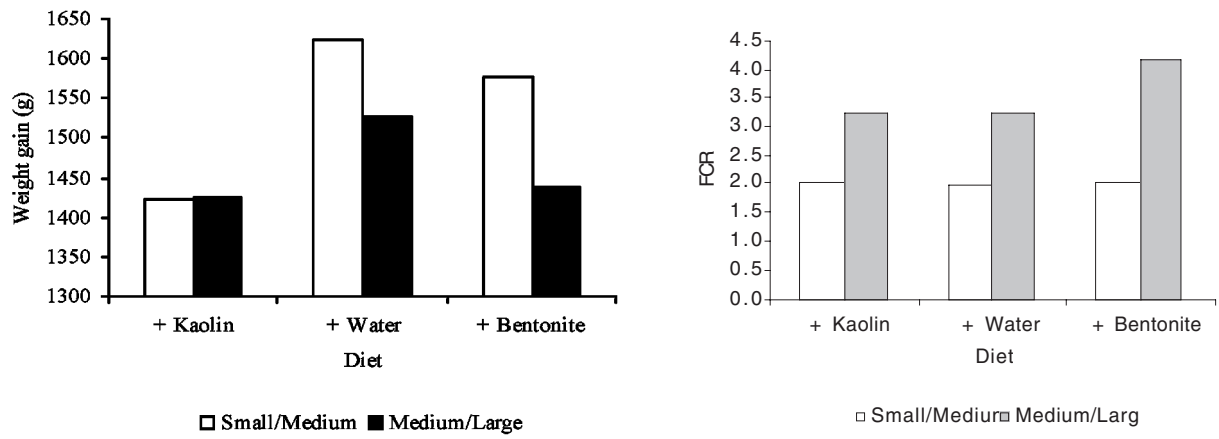

Figure 5. Influence of dietary kaolin on the growth and feed conversion efficiency of growing crocodiles.

Diet Specifications

There is limited information available on the nutrient requirements of crocodiles. Studies comparing the performance of crocodiles fed different forms of fresh meat clearly demonstrate that farmed crocodiles can respond to differences in nutrient intake, but they have done little to define the actual requirements of specific nutrients. Garnett (1985) made some progress in the assessment of fatty acid requirements showing that saturated fatty acids were apparently digested less efficiently by *C. porosus* than longer chain, unsaturated fatty acids and that C20:5 (eicosapentaenoic acid, EPA) and C22:6 (docosahexaenoic acid, DHA) are essential in diets for these crocodiles. Staton *et al*. (1990a) suggested that a dietary source of arachidonic acid may also be required for maximum growth of alligators (*Alligator mississipiensis*). Staton *et al.* (1990b) went further and estimated optimum digestible energy:dietary protein ratios for young alligators to be 1.96-2.60 kJ/g protein which was similar to the requirements of other aquatic ecotherms of equal size.

Despite some basic information on the nutrient requirements of crocodiles, there are a large number of factors that influence our capacity to accurate define diet specifications. First and foremost is the fact that individual crocodiles farmed in groups have a large variation in daily intake and crocodiles also have a high capacity to vary their metabolic rate. In addition, it has been shown that environmental temperature can have a significant influence of the rate of intake and the feed conversion efficiency. As a consequence, definition of generic nutrient requirement information for crocodiles will be difficult and the need for highly specified diets is diminished. Instead, diet specifications based on broad parameters such as crude protein, crude fat and digestible energy are likely to be sufficient.

The composition of the first manufactured diets produced in Australia were based on available literature (eg Staton and Vernon 1991) and local experience, and resembled the following:

Dry matter content:	60%
Crude protein content (as fed)	35-40%
Crude fat content (as fed)	16%
Fresh product:	20-50%
Binders:	Nil
Cost:	$1.00-$1.50/kg

Subsequent research, such as that examining dietary fat content (Figure 4), and growth studies has resulted in a significant revision of these specifications for growing crocodiles, with diet composition now reflecting the following:

Dry matter content:	63%
Crude protein content (as fed)	42%
Crude fat content (as fed)	5%
Fresh product:	0%
Binders:	0%
Cost:	$0.70-$0.80/kg
Other additives:	Shell grit Mycotoxin binders, acidifiers, Vitamin D and Fe supplements

Feeding Initiation

Manolis (1993) suggested that one of the most important steps in the development of manufactured feeds involved ensuring rapid initiation of feeding. Unless feeding initiation is completed rapidly, long-term performance of the crocodiles can be compromised. Manolis *et al.* (1989) also demonstrated that within the first month of life, *C. porosus* hatchlings showed clutch-specific preferences for certain foods, and in general, there was an avoidance of "smelly" foods. The primary drivers of initial preference are poorly understood, but it appears that weaning management rather than particular feeds has the greatest impact on the rate of acceptance and feeding initiation.

Research undertaken by the Queensland Department of Primary Industries has assessed the use of a wide range of attractants for juvenile crocodiles. Some of the attractants assessed as part of a structured initiation trial include:

- Fresh meat;
- Chicken head digest;
- Fresh blood;
- Liver digest;
- A range of proprietary digests and attractants;
- A variety of oil and meat combinations.

These experiments revealed a high level of variability in response, and all demonstrated minimal "attractive properties". Responses to these attractants was so poor that the experiment had to be prematurely terminated. In the absence of attractants, it appears that the best approach to ensuring a rapid initiation of feeding on manufactured feeds is via "staged weaning". This process involved the gradual replacement of fresh meat with manufactured pellets. Staged weaning processes currently under investigation are aimed at reducing the time required to progress from fresh meat to 100% semi-moist pellets and resemble the following over a 6 week period:

90% meat : 10% pellets	7 days
80% meat : 20% pellets	7 days
70% meat : 30% pellets	7 days
60% meat : 40% pellets	7 days
50% meat : 50% pellets	7 days
100% manufactured pellets	

Feeding Frequency

Crocodiles are opportunistic feeders and as a consequence, their digestive systems and metabolism may be unsuited to frequent feeding. This is further supported by general observations of daily feed intake of farmed crocodiles (Fig. 6). It can be seen that feed intake drops after each large feed suggesting that daily feeding may not be required.

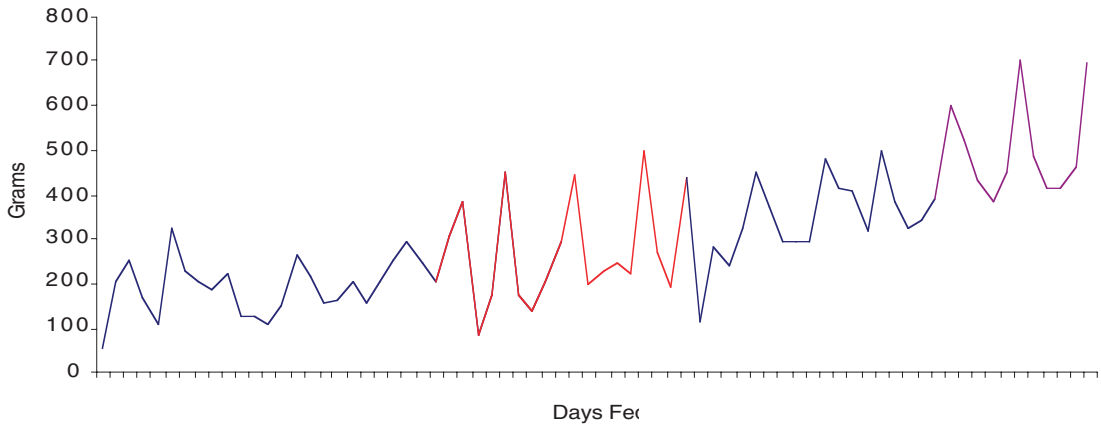

Figure 6: Variation in daily intake of manufactured feeds by growing *C. porosus*.

Initial research has shown that there is significant improvement in feed conversion efficiency of crocodiles are not fed daily. Subsequent research using fifteen individually caged animals balanced across five clutches undertaken by the Queensland Department of Primary Industries examined feeding frequencies of 3, 4 or 5 days. It was demonstrated that feeding every five days had no negative influence on final body weight, and that there were numeric improvements in feed conversion efficiency (Fig. 7). Further research is required to examine the effects of infrequent feeding on carcase quality.

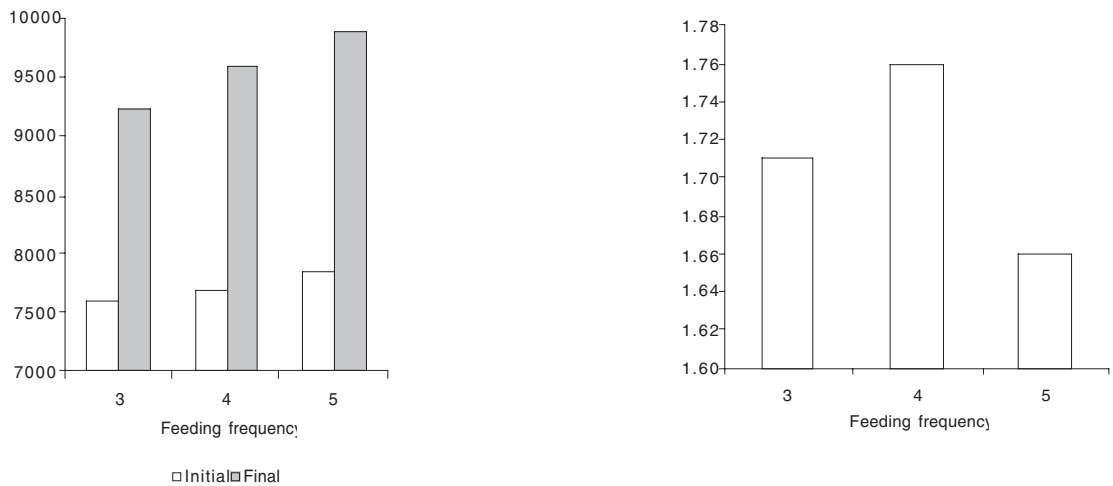

Figure 7. Influence of feeding frequency of growth and feed conversion of *C. porosus*.

Overall Feeding Management

While significant progress has been made in the development of manufactured feeds for crocodiles in Australia, it is important to remember that the diet itself is only half of the equation. Using the "salmon model", successful feeding of manufactured diets to crocodiles is going to depend equally on the feeding management. It is unlikely that a suitable manufactured diet will be developed that promotes acceptable levels of intake, growth and feed conversion in the absence of appropriate management.

As diet development progresses, additional factors that need to be considered include the impact of manufactured diet use in crocodile health and overall production hygiene, the capacity of manufactured feeds to manipulate body composition and overall product quality, and the role of different diets for different crocodile production phases.

Conclusions

In summary, crocodile nutrition research conducted to date has resulted in the development of manufactured diets with no fresh product needed that will support growth of crocodiles at an acceptable rate. A research program is now needed to capitalize on these initial investigation with a view to reducing the nutritional costs of producing crocodiles.

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The Crocodilian Model in Current Developmental and Evolutionary Studies

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Abstract

Living crocodilians are the only present day descendants of basal archosaurs. Because of their archaic appearance and their Triassic origin, they have been predicted to be biologically more primitive than other modern reptiles. Crocodilians, however, possess many advanced anatomical, physiological and behavioral patterns. These well illustrate the evolutionary potential of the Archosauria, a group which has produced the most diversified and competitive vertebrates in evolutionary history: actively flying feathered creatures, warm-blooded reptiles, and animals with parental care. The Crocodilia have undergone incomplete adaptation to an aquatic life style with only minor alterations in their morphology. Conservative uniform morphology is typical also for the other living archosaurs, birds. Once they evolved the power of flight, they did not change the aerodynamic adaptations of their body, even when becoming flightless. The crocodilian and avian model (CAM) thus represents an unique case for the reconstruction of evolutionary novelties in the morphology of extinct non-avian dinosaurs, which are phylogentically bracketed with recent archosaurs. The unique role of the crocodilian morphotype in the CAM is apparent, and more detailed crocodilian developmental mechanisms and regulatory gene expression patterns, comparable to those in the chick model, are needed. In relation to this need, the dynamics and migratory pathways of the cephalic neural crest cells of the crocodilian embryo are described for the first time.

Introduction

Living crocodilians and birds are the only survivors of the highly diversified Mesozoic archosaurs, which passed through the selective mass extinction at the Cretaceous/Tertiary boundary. The crocodilians thus represent a model for a primitive archosaur morphotype, while the birds reflect an opposite, highly modified, archosaur morphotype. Both morphotypes differ from each other in several important features, which correspond with the possession of highly variable locomotor behaviors, and which are among the major evolutionary novelties in vertebrates. However, these neotypic structures evolved in non-avian archosaur ancestors, among theropod dinosaurs, which are phylogenetically bracketed with recent archosaurs. The fact that the crocodilians and birds are the two most-proximal living outgroups of the non-avian theropods allows us to apply the Extant Phylogentic Bracket Approach, not only for functional reconstruction and interpretation of the fossil soft tissue patterns (Witmer 1995), but challenge us to reconstruct a developmental scenario of different molecular signalling pathways amongst others. These signalling pathways produce homologous but diferently adapted structures in both crocodilians and birds, such as neotypic integumental structures (Widelitz *et al.* 2003; Kundrát 2004), craniofacial transformation, including loss of the teeth, and the inclusion of digit reduction patterns (Kundrát *et al.* 2002).

Detailed comparisons of developmental mechanisms and programs in crocodilian and avian model system (CAM) would permit the elucidation of how evolution has worked through developmental mechanisms. Application of new molecular techniques combined with experimental surgical techniques on the crocodilian-avian chimaeric system may represent a way forward in answering some of these questions. To effect this, we need to define signalling molecular pathways, establish *Hox* gene expression patterns, as well as study transcriptional regulation at the genomic level in the crocodilian model. Studies focusing on the molecular evolution of crocodilians (eg Janke and Arnason 1997; Dessauer *et al.* 2002) creates the base on which to define the crocodilian embryo as a unique model for a universal component of a CAM experimental system for investigating relations between developmental mechanisms and evolutionary morphogenetic pathways.

Because of the complex behavior of the cephalic neural crest cell populations, which include intricate cell-cell and cell-environment interactions involved in molecular signalling patways, as well as the potential evolutionary significance of the interspecies differences in migratory cell populations (Kulesa *et al.* 2004), staining patterns of the cephalic neural crest migratory routes in crocodilian embryos were obtained with HNK-1 marker, and are described below by developmental day-stages.

Material and Methods

Crocodilian eggs (*Crocodylus niloticus*) were obtained in the La Ferme aux Crocodiles (Pierrelatte, France) during March and May 2003. Clutches were collected from nesting areas and incubated at 28-31°C in a mixture of vermiculite and sand. Embryos were retrieved by methods of Webb and Manolis (1987) and fixed to get a representative pattern. In the case of this study, embryos were collected each day within period of 1 through 11 days after egg-laying. Embryos were fixed and refixed again within 24 hours with two kinds of fixatives, each for a different procedure (see below): 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PFA/PBS) with diethyl-pyrocarbonate, and Bouin´s fixative. These embryos of *Crocodylus niloticus* were compared with embryonic stages of *Alligator mississippiensis* according to the scheme of Ferguson (1985).

Whole-mount immunostaining

After fixation with PFA/PBS the embryos were kept at 4°C. Then embryos were dehydrated in graded series of methanol solutions and stored at -20°C. After treatment with Dent´s fixative and MeOH (diluted 4/1), in order to block endogenous peroxidase activities, the embryos were washed in Tris-HCl-buffered saline-0.01% Triton X/100/ dimethyl sulfoxid (TST/DMSO) and then blocked with 5% non-fat dried milk in TST/DMSO (TST/M/DMSO). The monoclonal antibody, HNK-1 (Leu-7, CD 57, Becton Dickinson, San Jose, CA) was used to label early migration of the cephalic neural crest cells, as well as their neurogenic subpopulations. The primary antibody was diluted 1/50 in TST/M/DMSO containing 0.1% sodium azide, and applied to the embryos for 3 days at room temperature. After washing with TS1/3T/DMSO, secondary antibody, HRP-anti mouse IgM (ZYMED Lab. Inc.) diluted 1/200 in TS1/ 3T/M/DMSO, was applied to the embryos for 1 day. After final washing in TST/DMSO, the embryos were preincubated with 3,3´-diaminobenzidine (25mg/ml DAB:distiled water) in TST/DMSO (diluted 1/100) for 1 h. Then the embryos were allowed to react with hydrogen peroxide 0.01% (v/v) hydrogen peroxide at 0°C. The reaction was stopped by several washings in TST/DMSO.

Immunostaining on sectioned specimens

Specimens fixed in Bouin´s at 4°C were embedded in paraffin and sectioned at 7 to 10μm. They were deparaffinized and treated with MeOH with 1% (v/v) hydrogen peroxide for 30 minutes at room temperature, followed by washing in TS1/3T/DMSO. Anti-HNK-1 mAb was used to label both the migrating cephalic neural crest cells and the developing peripheral nervous system on the paraffin-sectioned specimens. Primary Ab was diluted 1/50 in TS1/3T/M/DMSO and applied to the sections for 1h at room temperature. After washing in TS1/3T/DMSO, the secondary Ab IgM was diluted 1/200 in TS1/3T/M/DMSO and applied on the sections for 1 h. Then the samples were finally washed in TS1/ 3T/DMSO, and preincubated using a kit: DAB/Metal Concentrate:Stable Peroxide Substrate Buffer [Pierce]. After the peroxidase reaction, the sections were counter-stained with hematoxylin.

HNK-1 (Leu 7 antigen, CD 57) was raised originally as a membrane fraction of the human T cell line (Abo and Balch 1981). This is a membrane antigen, expressed on human natural killer cells and some other lymphoid cells (Lainer *et al.* 1986). It is also present on approximately 15-20% of normal peripheral blood mononuclear cells as well as on subsets of NK lymphocytes and T lymphocytes (Fitzgerald-Bocarlsy *et al.* 1989). The HNK-1 epitope is demonstrated to be the sulfated trisacharide, which is shared with glycolipids and glycoproteins, acting as an adhesive. The expression of this carbohydrate epitope is observed in rhombomeres, in subpopulations of the cephalic neural crest cells since their earliest emigration from the neural tube folds, as well as temporally and spatially regulated in development of the nervous system (data shown here).

Despite it not being a pan-specific marker for neural crest cells in all vertebrates, positive HNK-1 immunoreactivity, visualizing the neural crest migration routes, has been demonstrated in many vertebrate species: lampreys (Hirata *et al.* 1997; Morikawa *et al.* 2001; *Petromyzon marinus* - Kundrát, unpubl. data), skate (*Raja erinacea* - Kundrát unpubl.data), teleost fish (Sadaghiani and Vielkind 1990; Hirata *et al.* 1997), frog (Olsson *et al.* 2002), turtles (Hou and Takeuchi 1994; Clark *et al.* 2001; *Pelodiscus sinensis* - Kundrát, unpubl. data), chick, quail (Bronner-Fraser 1986; Newgreen *et al.* 1990; Heath *et al.* 1992; *Struthio camelus* - Kundrát, unpubl. data), rat (Erickson *et al.* 1989). Negative HNK-1 immuoreactivity was reported for opossum by Vaglia and Smith (2003)

Using immunohistochemistry on whole mount embryos and sectioned material, I observed the localization and expression of the HNK-1 epitope in crocodilian embryos at different developmental stages. Based on HNK-1 expression patterns, I can present the first description of migration and colonization patterns of the neural crest cells in crocodilians, namely in embryos of *Crocodylus niloticus.*