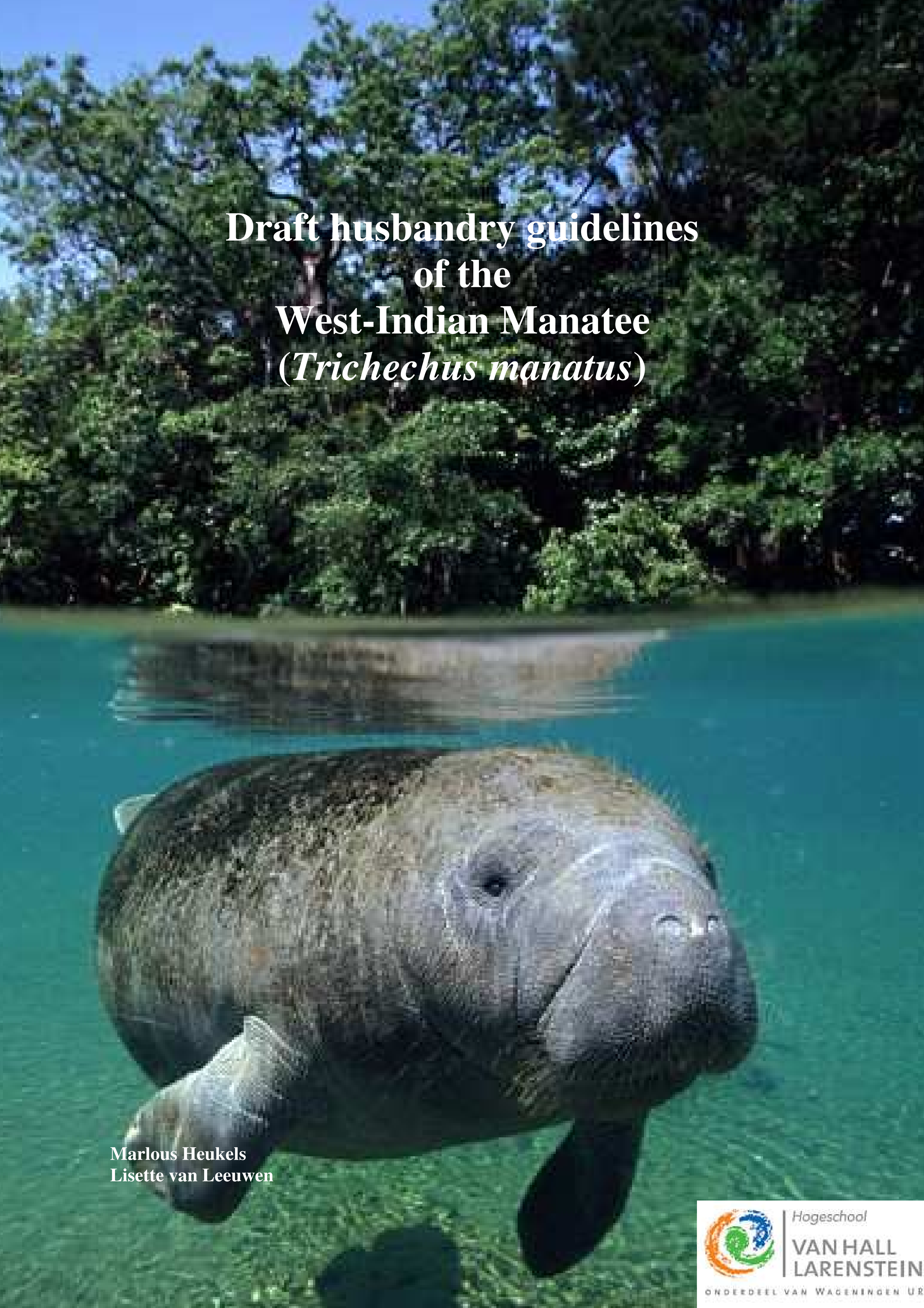

Draft husbandry guidelines of the West-Indian Manatee (*Trichechus manatus*)

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ONDERDEEL VAN WAGENINGEN UR

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of the
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Leeuwarden, June, 2008

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Source photograph front page “Manatee under water”: BLTC Research, 1995

Preface

This study is carried out as a final thesis at the Van Hall Larenstein Institute in Leeuwarden, the Netherlands, from February till June 2008. These husbandry guidelines will be reviewed and edited by the Marine Mammal TAG to a final version and used to improve the husbandry of manatees. We hope this report will be useful for all EAZA member zoos that keep manatees or are planning on keeping them in the future.

We would like to thank the following experts for their time and enthusiasm to help: Christiaan Luttenberg and Niels Anderson, keepers of the manatees in Burgers zoo, Bjarne Klausen and Kirstin Andersen Hansen of Odense Zoo and Allan Mæland of Randers Regnskoven for giving a tour and much useful information about their manatees and enclosures. Also thanks to Joep Wensing and Henrik Herold. We are very grateful for the help of the many experts worldwide that took the time for filling in a manatee husbandry questionnaire and shared their knowledge and experience. They made it possible to develop these husbandry guidelines.

Special thanks go out to Tine Griede and Martijn Weterings from the Van Hall Larenstein Institute and Gerard Meijer from Ouwehands Zoo and Chair of the Marine Mammal TAG, for all the help and good advice they have given us while being our supervisors. Their support, criticism and suggestions during our thesis are greatly appreciated.

Last but not least we would also like to thank our family and friends, for their moral support and patience during a period of hard work and pressure.

Marlous Heukels and Lisette van Leeuwen

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Abstract

EAZA has as goal to facilitate and promote cooperation between zoos for regional collection planning and wildlife conservation in Europe. One way this is done is by the Taxon Advisory Groups. TAGs are responsible for the exchange of information on how zoos and aquaria should best take care of the animals. Such information is collected by the TAG and put together in so-called husbandry and management guidelines

The Marine Mammal TAG is responsible for compiling husbandry guidelines for marine mammals kept in captivity, in this case for West-Indian manatees.

The aim of this research is to give an overview of literature and experts opinions regarding responsible management of captive West-Indian manatees and to give an overview of the information relevant for husbandry available of this species in the wild.

The main question which has to be answered in this report is:
What are the optimal conditions for the husbandry and care of West-Indian manatees (*Trichechus manatus*) in EAZA zoos according to the experts?

This concept for husbandry guidelines for captive West-Indian manatees is compiled by studying the biology and field data, as well as the management in captivity of manatees. Information was gathered through a literature study and by consulting experts. This was done by means of a questionnaire, which has been sent to 13 institutions worldwide that currently keep manatees. Of these institutions five were based in Europe, one in Malaysia, one in Venezuela and six in the United States. Seven experts that work in these institutions answered the questionnaire. The structure of the husbandry guidelines is set up according to the standard index of EAZA husbandry guidelines.

The husbandry guidelines are divided in two sections, section A and section B. Section A covers the situation of the West-Indian manatee in the wild. In this part the biology and the field data of the manatee are described. Biology covers the taxonomy, morphology and anatomy of manatees. The field data covers the geography, distribution, feeding behaviour and the reproduction of manatees. Section B covers the aspect concerning the captive situation of manatees. This section covers the topics enclosure, feeding, behavioural and social structure, enrichment, breeding, handling, health and welfare, population management and legislation concerning manatees in captivity.

All information is based on expert’s experiences and scientific knowledge. Additional research is necessary to improve the quality of these husbandry guidelines.

Contents

Introduction

Nowadays it is harder to get animals from the wild to replenish the animal collections of zoos, because of the public opinions and the regulations of CITES (CITES, 1963). Therefore zoo populations should be self sustainable. In order to achieve a genetic viable population zoos should work together by exchanging specimen from one zoo to another and breed with these animals. This cooperation should not only happen nationally but also internationally, because the national population of some species is too small for a genetic viable population. (EAZA, 1988)

European zoos went beyond political barriers when they joined their forces and established a multinational zoo organisation within the European Community in 1988, the European Association of Zoos and Aquaria (EAZA). The main goal of this organisation is to facilitate and promote cooperation between these zoos for regional collection planning and wildlife conservation in Europe. (EAZA, 1988)

In order to regulate cooperation so-called Taxon Advisory Groups (TAGs) and European Endangered species Programmes (EEPs) have been established. The latter is the most intensive programme of population management for a species kept in EAZA zoos.
One of the main tasks of TAGs is to develop Regional Collection Plans that describe which species are recommended to be kept, the reason for this and how the genetic management of these species should be in zoos and also identify which species need to be managed in European breeding programmes. Each TAG focuses on a specific group of animals, such as penguins, bears, hornbills, cats or marine mammals. TAG members are professional zoo and aquarium people who work in EAZA member institutions, have specialist knowledge and a keen interest in the group of species covered by the specific TAG. (EAZA, 1988)
Another task of which the TAGs are responsible is the exchange of information on how zoos and aquaria should best take care of the animals, also known as the responsible management of the animals. Such information is collected by the TAG and put together in so-called husbandry and management guidelines (EAZA, 1988).

The information needed to compile the husbandry guidelines for a particular species are most of the time not available from scientific research, especially the information about the captive situation of the species. This is the main reason why most of the husbandry guidelines are based on the experiences of skilled people who work with the species concerned (pers. comm. T. Griede, 2008).
To coordinate cooperation between zoos EAZA has made a standard index for husbandry guidelines, this will make sure that all husbandry guidelines made in Europe have the same format (EAZA, 1988). The husbandry guidelines should also serve as a reference tool based on natural history to in situ and ex situ research information (Jaguar Species Survival Plan, 2000). These guidelines cover several topics about wild and captive animals of a species. The information given about the wild species covers morphology, anatomy, physiology, longevity, geography, ecology, diet, reproduction and behaviour. The information given about the captive situation covers the topics housing, diet, behaviour, social structure, enrichment, breeding, handling, health and welfare, population management and legislation.
The guidelines are living documents which means that they should be up-dated frequently when new information is available.

The Marine Mammal TAG is responsible for compiling husbandry guidelines for marine mammals kept in captivity. The Marine Mammal TAG covers both native and exotic marine mammal taxa including the following taxonomic groups: Otariidae (fur-seals, sea-lions), Delphinidae (dolphins) and Dugongidae (dugongs) (ARAZPA, 1990). Also Trichechidae (manatees) belong to this TAG.

The Chair of the Marine Mammal TAG wants a draft for the husbandry guidelines for manatees to be compiled (pers. comm. G. Meijer, 2008). This especially for the West-Indian manatees (*Trichechus manatus*), because this is the only species of the family Trichechidae kept in zoos worldwide. In Europe the total amount of manatees kept is 23 animals, 12 males and 11 females, spread over seven zoos. In the United States this species is kept in seven zoos and the total amount of West-Indian manatees is 49, 18 males, 30 females and one of unknown sex. (ISIS, 1973).

West-Indian manatees are large, grey or brownish aquatic mammals which are distributed in the Atlantic Ocean at the West coast of North and South America. They eat mostly waterplants. Manatees are protected by law. (Powell, 2002) Internationally manatees are protected by Cites (CITES, 1963).

Aim

The aim of this research is to give an overview of literature and experts opinions regarding responsible management of captive West-Indian manatees and to give an overview of the information relevant for husbandry available of this species in the wild.

Research questions

Main question:

What are the optimal conditions for the husbandry and care of West-Indian manatees (*Trichechus manatus*) in EAZA zoos according to the experts?

Sub-questions:

A. 1) What is the biology of the West-Indian manatees, concerning:
- Taxonomy
- Morphology
- Anatomy and physiology
- Longevity

2) What are the field data* in the wild of the West-Indian manatees, concerning:
- Geography and ecology
- Diet and feeding behaviour
- Reproduction
- Behaviour

B. How is the responsible management* for captive West-Indian manatees, concerning:

1) Enclosure
2) Feeding
3) Behaviour and social structure
4) Enrichment
5) Breeding
6) Handling
7) Health and welfare
8) Population management
9) Legislation

* terminology usually used in husbandry guidelines, for precise definition see Appendix 1: Glossary

Report structure

The report is divided into two sections, section A and section B, section A covers the in-situ biology and field data of the West-Indian manatee. Section B covers the ex-situ management for keeping manatees in captivity which includes information about the subjects Enclosure, Feeding, Behavioural and social structure, Enrichment, Breeding, Handling, Health and welfare, Population management and Legislation.

Material and Method

Preconditions
In order to make this report a concept of the EAZA husbandry guidelines for the West-Indian manatee, a framework was made in consultancy with the Marine Mammal Tag:

- It needed to be based on the standard index for EAZA husbandry guidelines
- It needed to be the same format as the husbandry guidelines of the True Seals and the Eared Seals.
- The research population was chosen by G. Meijer, Chair of the EAZA Marine Mammal TAG.

Research population
The research population consists of a number of EAZA member experts that keep West-Indian manatees (*Trichechus manatus*). The experts were chosen by the Chair of the TAG using the following criteria:

- Experience with keeping manatees
- Good cooperation in the EEP

This resulted in five experts from four different countries in Europe namely, Bjarne Klausen (Odense Zoo), Henrik Herold (Randers Regnskoven), Francoise Delord (Zoo Beauval), Joep Wensing (Burgers Zoo) and Bernhard Neurohr (Zoo Nurnberg).

Also experts from the United States, Singapore and Maracaibo were appointed by the Chair of the TAG to gather the necessary information. The following criteria were used to select the experts:

- Experience with keeping manatees
- Member of Isis

This resulted in six experts from the United States, one from Malaysia and one from Venezuela. The persons contacted were: Mike Dulaney (Cincinnati Zoo and Botanical Garden); Mike Brittsan (Columbus Zoo and Aquarium); Daryl Richardson (Dallas World Aquarium); Art Yerian (Homosassa Springs Wildlife State Park); John Kerivan and Brad Andrews (Sea World Orlando); Biswajit Guha (Singapore Zoological Gardens); Lee Ann Rottman (Tampa's Lowry Park Zoo) and the director of Parque Zoologico Sur de Maracaibo.

The 13 experts were asked to fill in a questionnaire containing questions about keeping this species in captivity.

Data collection methods
To gather the information needed to answer the sub research questions of section A and section B, a literature study was performed. Literature about the biology and field data of the West-Indian manatee was gathered with the use of the Internet, institutions, articles and books.

These guidelines are meant for the daily use in zoos. Also for zoos that do not have a large collection of books about housing etc. of manatees. Many books are used to complete this document. The following books that are mainly used for this draft are; Dierauf and Gulland, 2001, *CRC Handbook of Marine Mammal Medicine;* Brook van Meter, 1989, *The Florida Manatee*; Standards of USDA and IATA.

The key words used to find the information for the husbandry guidelines on Internet were: Manatee(s); *Trichechus manatus;* West-Indian manatee; Caribbean manatee, Sirenians, physiology, morphology, feeding, legislation, enclosure, diseases and combinations of these. During the literature study in the following databases was searched for the information needed:
Science direct, U.S. Fish and Wildlife Service, Interscience, Zoo Biology, Blackwell publishing, National Center for Biotechnology Information (NCBI), PUBMED, Jstor, WUR library and United States Department of Agriculture (USDA).

The information that was found during the literature study was carefully selected in relevancy and in reliability, by the following conditions:

- Information should be about the *Trichechus manatus*
- Information should cover one of the topics mentioned in research questions
- Information should come from reliable source (With reliable sources it is meant information from organisations like IUCN, EAZA, Zoos that keep manatees and (scientific) universities. Also known experts and articles published in scientific magazines and on scientific websites.)

A questionnaire was set up to gather information from the experts about the sub questions of section B, the captive situation, of the manatees. This questionnaire consisted of 70 questions, divided in the subjects General, Husbandry, Feeding, Group structure, Training, Enrichment, Breeding, Catching animal and Veterinary care.

The questionnaire was based on the standard index for EAZA husbandry guidelines and consists of questions about all possible aspects related to husbandry of manatees. Closed questions were used as much as possible, in order to simplify analysing the answers. Because of the fact that zoo-curators receive a lot of questionnaires it is important that they are relatively easy and that they do not take a long time to answer (pers. comm. T. Griede, 2008). But because of the extent of information needed for the husbandry guidelines this was not always a possibility. The questionnaire was made on the internet website www.thesistools.com, which provides tools for making a professional questionnaire (Thesis tools, 2006). The questionnaire was sent to the selected experts and could be answered on this website by opening a link that was sent to them individually. The answered questionnaires were collected and saved by www.thesistools.com. A deadline four weeks after sending the questionnaire was determined in order to receive all replies in time.

The results of the questionnaire are incorporated in the husbandry guidelines. This is done by indicating how many respondents gave that answer. As an example 3/7 indicates that three respondents of the total 7 respondents gave that answer.

Husbandry Guidelines for West-Indian Manatees

Section A: Biology and field data

1 Biology

The knowledge about the biology of the West-Indian manatee is important for the good management of the husbandry of these species in captivity. To make the captive situations adjusted as much as possible to the needs of the animal.

1.1 Taxonomy

An overview showing the taxonomy of the West-Indian manatees is represented in figure 1.

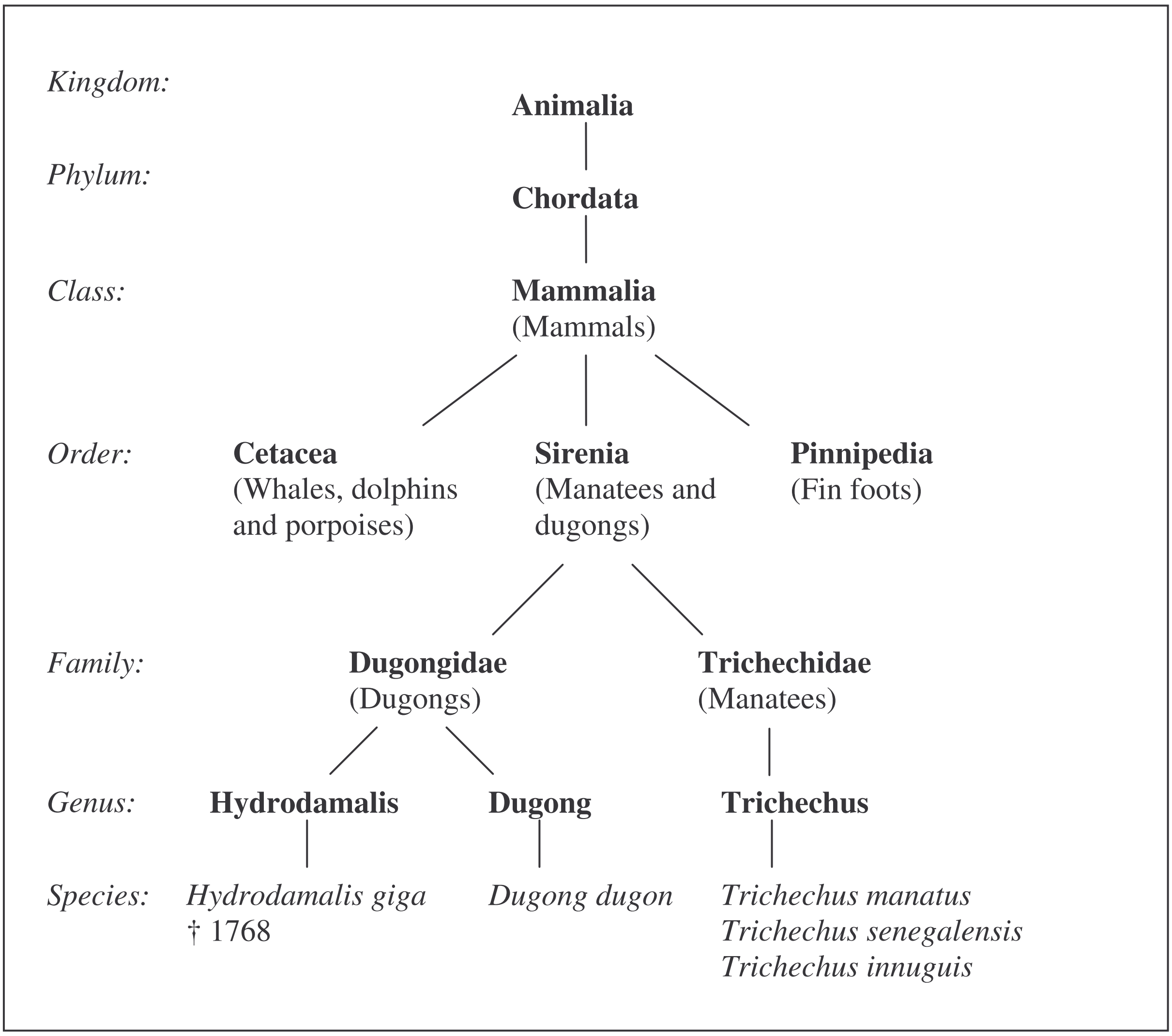


Figure 1 Classification of the manatee species and the dugong. (Arkive, 2003; IUCN, 1986; Powell, 2002; U.S. Fish and Wildlife Service, 1999)

Subspecies

The West-Indian manatee (*Trichechus manatus*) has two subspecies, the Florida manatee (*Trichechus manatus latirostris*) and the Antillean manatee (*Trichechus manatus manatus*). Neither the West-African manatee nor the Amazonian manatee are currently recognized to have subspecies. (Powell, 2002)

1.2 Morphology

Like the more familiar whales, dolphins, walruses, sea lions and seals, manatees and dugongs are marine mammals. Even though they share many of the same characteristics, such as body shape, flippers and nostrils that close, they are not related to other marine mammals. (Powell, 2002)
Manatee and Dugongs are the only herbivorous mammals living in the sea. The dugong is entirely and strictly marine, some manatee species can move between the sea and fresh water systems while other manatee species are wholly restricted to freshwater (Reeves *et al*., 1992).

The families Trichedae and Dugondidae differ in a number of external features shown in table 1 and figure 2 (Reeves *et al*., 1992).

Table 1 External differences between the Trichedae family and the Dugondidae family. (Reeves *et al*., 1992)

Trichedae	Dugondidae
Tail beaver-like	Tail whale-like
Forelimbs with rounded tips, nails present (except on *Trichechus inuguis*)	Forelimbs pointed, nails absent
Skin heavily pleated, surface rough and often covered in algae and barnacles	Skin unpleated, surface relatively smooth
Hairs on body long and flexible	Hairs on body short and rigid
Ear openings small and indistinct	Ear openings large and easily visible
Nostrils at muzzle tip	Nostrils behind muzzle tip
Incisor teeth absent (in adults)	Incisors tusk-like (up to 18cm in length)

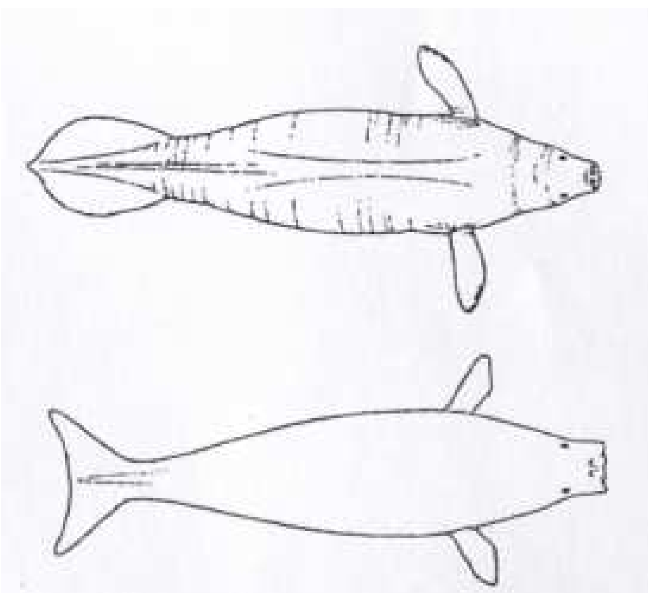


Figure 2 The differences between the morphology of a Manatee (upper) and a Dugong (lower) seen from above. There are differences in the snout, paddles and tail. (Reeves *et al*., 1992)

Trichedae

Manatees are slow moving animals that do not show the athletic abilities so characteristic of dolphins and seals. The dorsal fin is lacking and also the hind limbs are absent. The front limbs (or paddles) move from the elbow and the upper arm is being enclosed within the flank. The tail is flattened for propulsion and the tail lobes are supported only by a ventral row of caudal vertebrae as in whales. The nostrils open at the top of the muzzle as a pair of circular nasal openings. (Reeves *et al*., 1992)

The teats are located at the 'armpits', just behind the paddles of the female. The skin is tough but not entirely naked; there are hairs sparsely distributed on the body. The head is distinctly flat-faced with a densely bristled appearance. The two enlarged lobes of the upper lip hang down, one on each side of the mouth, in a manner similar to the pendulous lips of a bloodhound. There is a prominent and bristly chin. There are no external ear flaps and the ear canal opens at the skin surface behind the eye. The eyes are relatively small without well defined eyelids, and are protected by a heavy tear secretion. (Reeves *et al*., 1992)

Manatees have molariform teeth only and lack canines and incisors. This makes them efficient at chewing vegetation (Reep and Bonde, 2006).

The difference between males and females is visible by the different location of the urogenital opening (See figure 3).The urogenital opening of a male is located just below the umbilicus (or navel), while the female urogenital opening is located just above the anus (Xavier university, 2001).
Also a female has mammary glands located at each armpit, though not readily apparent under all viewing circumstances (Bush entertainment Cooperation, 1994*b*).

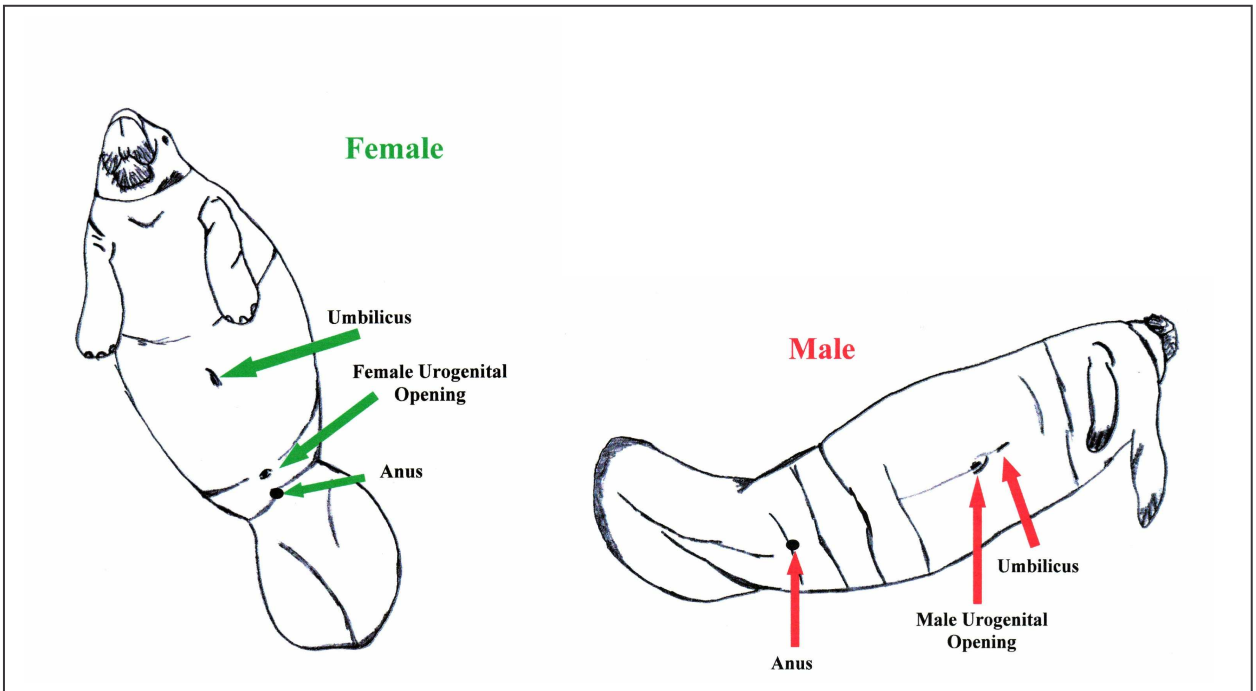


Figure 3 Visible differences between manatee gender (Xavier university, 2001)

Species Trichedae

Amazonian manatee (*Trichechus inunguis*)
The Amazonian or Amazon manatee (see figure 4) is a completely aquatic mammal (Powell, 2002). The maximum documented length for this manatee is slightly less than three meters, this is therefore the smallest of the three manatee species. It is nevertheless the largest

mammal on the South American continent, with some individuals weighing as much as 400-450 kg. The birth length of this species is 85 to 105 cm with a weight of 10 to 15 kg. The body of the Amazonian manatee is somewhat less cylindrical and more fusiform than that of the West-African and West-Indian manatees. This is probably because it has fewer ribs and thus a shorter rib cage. (Waller *et al*., 1996)

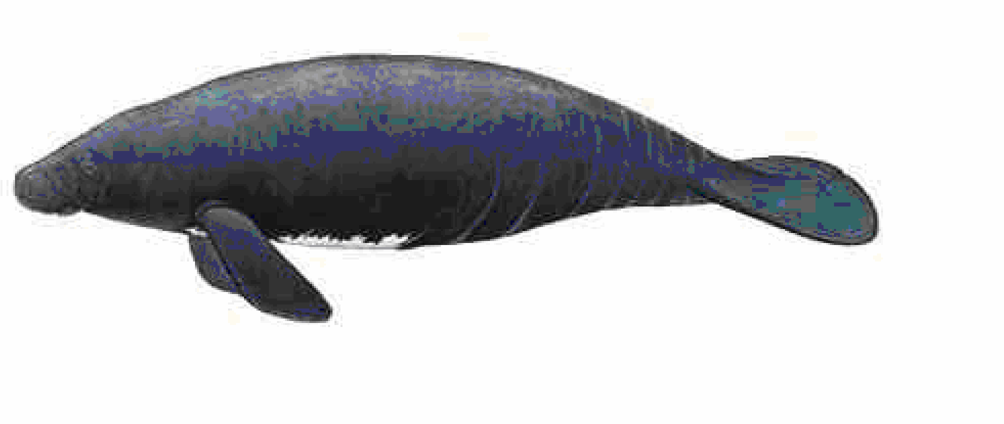


Figure 4 Amazonian manatee (Baidu, 2008)

The skin of this manatee is smooth with some sparse hairs. (Powell, 2002) Newborns have a heavily wrinkled outer layer of skin that peals off in the first few weeks after birth (Waller *et al.,* 1996).

The colour of the skin of this species is dark grey to black with white of pinkish blazes on the ventral surface (Powell, 2002). It is usually centred on the chest and abdomen but can extend forward onto the throat or backward onto the tail (Waller *et al*., 1996). Unlike the other two manatee species the Amazonian manatee does not have fingernails on the tips of its fore flippers (Powell, 2002).

West-African manatee (*Trichechus senegalensis*)
The West-African manatee (see figure 5) is the least studied of all the manatees (Sirenian International, 2000). This manatee species weighs less than 500 kg, adults are generally 3 - 4 meter long (Animal info, 1999*a*).

Figure 5 West-African manatee (Animal info, 1999b)

The skin of this species is rough with some spare hairs on it, the colour is dark grey to brown depending up on the types of algae growing on its back. This species has fingernails on its

fore flippers (Powell, 2002). The West-African manatee is more slender with a blunter snout than the West-Indian manatee (Powell, 2002).
The West-African manatee inhabits coastal areas, estuarine lagoons, large rivers that range from brackish to fresh water, freshwater lakes and the extreme upper reaches of rivers above cataracts. This manatee species is dependent on emergent or overhanging, rather than submerged, vegetation. Populations in some rivers depend heavily on overhanging bank growth, and those in estuarine areas feed exclusively on mangroves. (Animal Info, 1999*a*)

West-Indian manatee (*Trichechus manatus*)
The West-Indian manatee (see figure 6) is also a completely aquatic mammal, inhabiting both fresh, brackish, and marine waters (U.S. Fish and Wildlife Service, 1999).

The adults are 3 to 3.5 meters long and have an average weigh of 500 kg. But large individuals can be close to 4 meters and weigh more than 1600 kg. Newborns may be one meter or more long and weigh nearly 30 kg. Males and females are similar in size and appearance (Reeves *et al*., 1992).

Figure 6 West-Indian manatee (Bush entertainment Cooperation, 1994d)

The skin of the West-Indian manatee is rough with the colour grey, brown or even greenish depending on the types of algae growing on their skin. The skin has no markings unless the animal was scarred from injuries caused by watercraft collisions. Also the West-Indian manatee has fingernails on the tips of its fore flippers. (Powell, 2002)

1.3 Anatomy and physiology

In this paragraph the anatomy and physiology of the manatee is described, this involves the integument, respiratory system, digestive system, cardiovascular system, genital system, kidney, thermoregulation, osmoregulation and the senses of the manatees.

<u>Anatomy</u>

Integument
The integument of the West-Indian manatee consists of three layers; the outer layer (the epidermis), the middle layer (the dermis) and a deep blubber layer (the hypodermis). The integument is an organ system that defines the animal’s boundary with its aquatic environment. It forms a protective and dynamic isolative layer, adds buoyancy, and comprises propulsive structures such as the flukes of sirenians. The skin of the manatees is distinguished

by the absence of hair, except for the vibrissae especially dense around the mouth of the manatees. (Romero, 2005)
The skin of manatees is very thick, sometimes over 2,5 cm thick (Reep and Bonde, 2006).

The structure of the epidermis of marine mammals is made of multiple strata of squamous epithelium consisting of 3-5 layers. Mostly three layers namely; stratum basale, stratum spinosum, and stratum corneum. The outmost layer of the epidermis, the stratum corneum, consists of a layer of flattened, solid, keratinized cells that make the skin waterproof. (Romero, 2005)

The dermis layer contains hair follicles, sebaceous and (in most animals) sweat glands. The sweat glands are absent in sirenians. There is a rudimentary sebaceous gland associated with the snout hairs of the sirenians. The dermis is composed of dense irregular connective tissue. It is well vascularised and contains fat cells which increase in number with depth as it becomes continuous with the hypodermis. In the sirenians, dermal papillae and a sub-papillary layer form a very thick dermis. Dermal papillae often penetrate the epidermis and may even extend into the horny layers, so that they lay only a few cell rows below the surface. They may therefore have a tactile function. (Romero, 2005)

The hypodermis or blubber is loose connective tissue composed of fat cells interlayered with bundles of collagen. It is loosely connected to the underlying muscle layer. The fatty hypodermis of sirenians is a substitute for the absence of hair and functions as an insulator. Blubber is thinner in sirenians than in most other marine mammals. (Romero, 2005)

The vibrissae on the manatees are loosely scattered over the body, but become denser and very robust on the muzzle and around the mouth (Romero, 2005).
Vibrissae send signals to the brain indicating that contact has been made with something in the environment and helping to localize it for further information. The oral disk, the region between the mouth and nose, contains about 600 vibrissae that are brought into contact with novel objects in the environment. Also on the rest of the body vibrissae are found, about 3000 hairs are found sparsely distributed on the body, all of these hairs are vibrissae. The manatee system of body vibrissae appear to represent a tactile array capable of detecting and localizing water displacement. (Reep and Bonde, 2006)

The West-Indian manatee has rudimentary nails which are present on the second, third and fourth digits (Romero, 2005).

Respiratory System
Manatees need to hold their breath for a long time when under water. A large manatee can stay below the surface for up to 20 minutes. Typically manatees tend to stay submerged for around two or three minutes before surfacing to breath. They stay submerged longer when resting or will surface more when they are active. When manatees breathe they usually surface with just the tip of their two nostrils showing above the surface. (Powell, 2002)

Manatees replace about 90 percent of the air in their lungs with each breath. A lot more than humans with replacing 10 percent of air in their lungs with each breath. (Brook van Meter, 1989)

The lungs of a manatee are unique. Unlike other mammals, their lungs are flattened and elongated and extend horizontally along the back almost to the anus. (Save the manatee club, 1981*a*) The manatee’s lungs lie along its backbone instead of along its rib cage as is found in most mammals. The lungs are long (1 meter or more in adults), wide (20 cm), and thin (5 cm or less). Besides breathing, the lungs help the manatee with buoyancy control (Save the manatee club, 1981*b*). The manatee's elongated lung distributes the buoyant forces along more of the body, thus helping the manatee to float horizontally. This orientation also minimizes the pressure differences between different parts of the lung. (Save the manatee club, 1981*a*)

An unusual thing about the lungs of a manatee is that each lung has its own diaphragm, called hemidiaphragms, and if one lung or diaphragm is damaged, say by a boat strike, this anatomy may allow each lung to act semi-independently. (Powell, 2002)

Resting manatees are able to ascend and descend effortlessly through the water column, for example when surfacing to breathe. This movement is achieved partly through a modification of the lung cavity of these mammals. The lung cavity of the manatees extends almost along the whole length of the animals back instead of a forward position in front of the gut as seen in most mammals. The lungs themselves have large air spaces within them. It is thought that the lung volume can be reduced to cause the manatee to sink or increased to cause it to rise in the water column, using voluntary muscular compression and relaxation. Compression of gas decreases the lung volume and therefore buoyancy is reduced. (Waller *et al*., 1996)

Digestive System

Manatees’ only teeth are molars for grinding vegetation (Bush entertainment Cooperation, 1994*d*; Powell, 2002).

To compensate for excessive tooth wear caused by the tough vegetation matter manatees feed upon, they replace old, worn-down teeth for new ones (U.S. Fish and Wildlife Service, 1999). As the animal ages the molars migrate forward in the jaw. The oldest teeth and those closest to the front have their roots absorbed. Eventually these older and worn teeth fall out. While the worn teeth are falling out in the front of the jaw, new ones are being formed and emerge at the back of the jaw. (Powell, 2002)

This replacement process occurs at a rate of about one mm per month. Manatees may use 30 or more molars in a lifetime (U.S. Fish and Wildlife Service, 1999).

The manatee digestive system (see figure 7) is similar in structure of that of the horse; both are hindgut fermenters with bacterial digestion of cellulose occurring primarily in the hind part of the gut (i.e. the large intestine). This digestive system is adapted to processing large amounts of high-fibre, low-protein food. The intestines of adults can measure up to 39,5 meters in length. Because of these factors manatees are highly efficient herbivores capable of extracting up to 80% of the digestible materials in the plants that they eat. (Brook van Meter, 1989)

Manatees exhibit a combination of unusual traits rarely seen in other mammals. One of these, called the accessory digestive gland (or cardiac gland), is associated with the stomach. This gland produces hydrochloric acid, digestive enzymes, and mucus that coats swallowed food and protects the lining of the digestive system from abrasion. Isolating these cells in a separate gland may protect them from the abrasive effects of ingested sand. (Reep and Bonde, 2006)

The duodenum, that portion of the small intestine located just below the stomach, contains large out-pockets that appear to serve as storage sites for ingested food arriving from the stomach. It appears that food passes rather quickly through the small intestine and collects in the large intestine. At the junction of the small and large intestines, the enlarged, muscularised, paired cecum is a major site of volatile fatty acid production and absorption and of cellulose digestion by micro-organisms. (Reep and Bonde, 2006)

Manatees absorb most of their nutrients in the large rather than the small intestine. Manatees also consume relatively low quality food source, meaning that it is high in fibre, low in protein, and low in caloric value. They appear to maximize the efficiency with which they extract nutrients by utilizing an extremely slow gut transmit time for ingested food. The gut transit time is about six or seven days. Species that appear to come in within the same range of gut transit time are highly specialized herbivores and include the koala, three-toed sloth and the two-toed sloth. (Reep and Bonde, 2006)

Manatees are very efficient at digesting cellulose compared to other herbivores, including the horse, and this is probably attributable to their slow gut passage time, large body size, and lower lignin content of aquatic versus terrestrial vegetation. (Reep and Bonde, 2006)

Digestion is accompanied by the formation of large amounts of gas. Oddly enough, the manatee diaphragm may compress gas in the intestines to permit manatees to control their buoyancy without apparent muscular movement. (Brook van Meter, 1989)

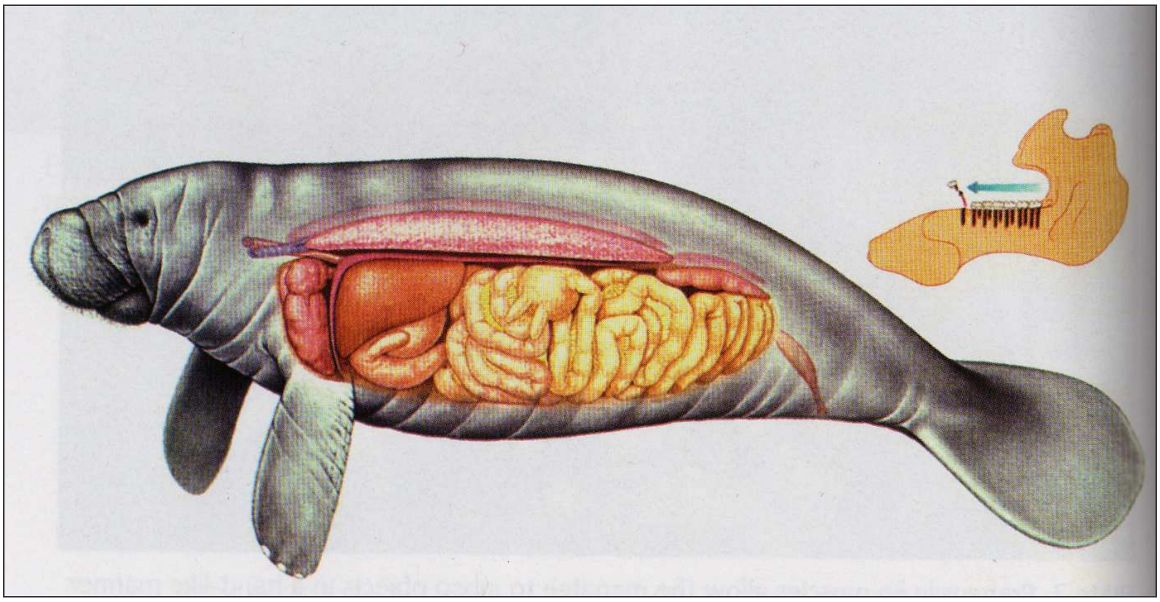


Figure 7 Digestive system manatee (Reep and Bonde, 2006)

Cardiovascular System

Manatees at rest have relatively low heart rates compared to those of terrestrial mammals. A large resting manatee can stay submerged for 20 minutes. Smaller and active manatees cannot stay under for that long and need to breathe every two or three minutes. (Brook van Meter, 1989)

A manatee's heart beats at a rate of 50 to 60 beats a minute (Save the manatee club, 1981*b*). Manatees slow their heartbeats when submerging or if they need to stay submerged (Powell, 2002). The heart rate slows down to 30 beats a minute during a long dive (Save the manatee club, 1981*b*).

Genital System
The aquatic environment presents a thermal challenge to manatee reproductive tracts, but its exact character is opposite of what one might expect. Like many marine mammals, male manatees have their testes and associated structures located deep inside their bodies rather than in scrotal sacs. This makes manatees more streamlined during movement through the water, but presents a potential problem of heat build-up. Maturing sperm need a thermal environment that is not too warm, and this deep location beneath fat and muscle is subject to warming beyond the tolerable limit, even during winter. To get around this, manatees possess an extensive blood vessel network between surface structures and the testes. This network brings cooler surface blood to the testes, keeping them in the correct temperature range. Interestingly female manatees have a corresponding arrangement which may protect their reproductive tracts from hypothermia. (Reep and Bonde, 2006)
Male manatees possess enlarged seminal vesicles, which may compensate for smaller testes through production of large volumes of seminal fluids. This trade-off could be related to the low metabolic rate of manatees, if production of semen is less expensive metabolically than production of sperm. (Reep and Bonde, 2006)

Kidney
The anatomical structure of the kidney in sirenians differs from that of pinnipeds and cetaceans. The kidney of the West-Indian manatees is considered superficially lobulate since it lacks true reniculi and has several large lobes with a continuous cortex. The striking differences in renal anatomy among the various species of sirenians suggest that these variations in kidney morphology may be related to the variations in habitat of each species. (Ortiz, 2001) For the location of the kidney and other organs see figure 8.

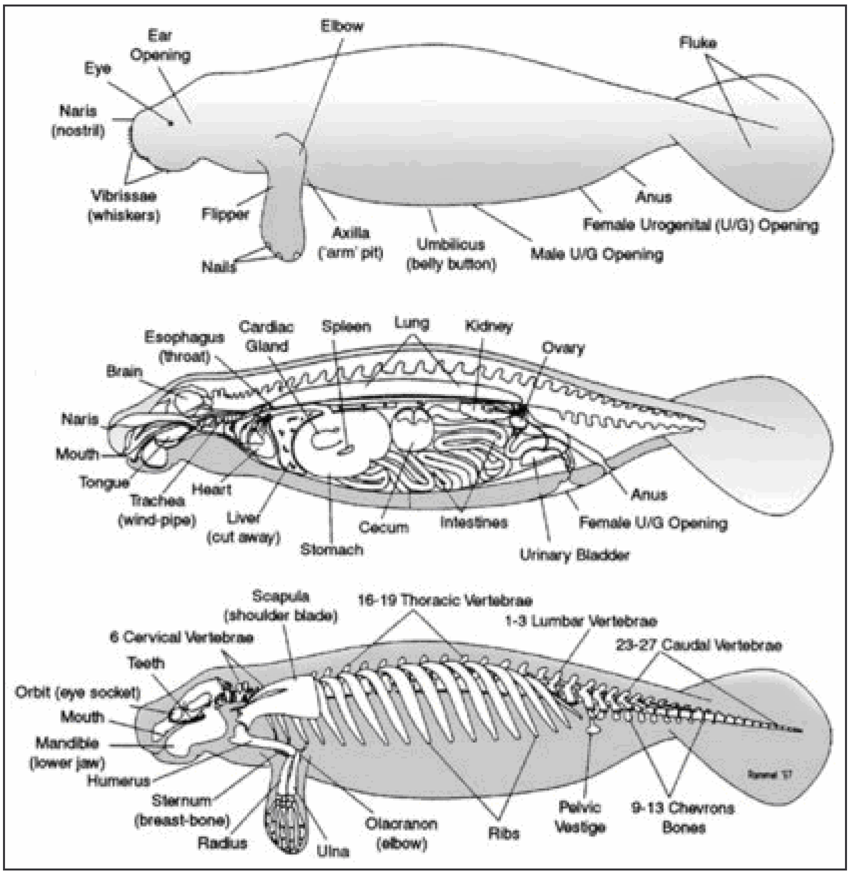


Figure 8 Anatomy of manatee (Xavier university, 2001)

Homeostasis

Thermoregulation

Manatees are tropical animals not well suited for life at relatively cold temperatures that can occur in their habitat, this is reflected in its adaptation to warm environments. An animal's thermoneutral zone is that range of temperatures in which it does not need to use active metabolism to keep warm at the low end or to throw off excessive heat at the high end. For West-Indian manatees, the lower end of the thermoneutral zone is 20-23ºC, in contrast to 8 - 15 ºC for bottlenose dolphins, sea otters, and sea lions. This corresponds to the observed low tolerance of manatees for cold water, so that they actively seek out warm water locales in the winter months. Adults are able to respond to brief, moderate cold (19-20 ºC) by increasing their metabolism; younger animals, however, are apparently unable to do so. (Reeves *et al*., 1992)

The skin of manatees may have properties that help manatees regulate body temperature. By sending the blood through vessels located near the body surface this can help these tropical animals release body heat during warm months. Conversely, in the winter, manatees are often observed exposing their backs above the water surface to the warming rays of the sun. West-Indian manatees are larger than their counterparts elsewhere in the tropics. This may help them deal with temperature extremes, because larger animals lose internally generated heat less rapidly to the environment than smaller mammals do. The internal body temperature of West-Indian manatees at rest is 36ºC, and they begin to display signs of cold stress with prolonged exposure to water less than 20ºC. (Reeves *et al*., 1992)

Osmoregulation

West-Indian manatees move between fresh water and a marine environment with no deleterious effect. Manatees do not consume sea water voluntarily and during periods of food deprivation their water needs are met from metabolic water production, as in pinnipeds and cetaceans. Consumption of fresh water can be high, leading to lower osmotic and ionic concentrations. Plasma osmolality and electrolyte concentrations in wild freshwater manatees are similar to those of wild and captive animals in salt water, suggesting that wild animals in fresh water have occasional access to salts either by temporarily residing in a marine environment or by consuming aquatic vegetation with a sufficient salt water content. Therefore, captive manatees held permanently in fresh water may be susceptible to hyponatremia. (Ortiz, 2001)

An examination of the cortical: medullary thickness of the kidney of the West-Indian manatees suggest that these animals possess the ability to concentrate their urine to an osmolality greater than that of sea water and, in fact, urine osmolaties of approximately 1200 mosmol 1^{-1} have been determined from samples taken, from wild animals in salt water. When manatees that had been held in fresh water were exposed to salt water for 4 days, the sole urine sample collected was only 217 mosmol 1^{-1}, 29% lower than the animal's plasma osmolality. Although the animals was in salt water, it continued to feed on its normal diet of lettuce (which has a high water content), so as a source of fresh water was maintained and there was no requirement to concentrate its urine. (Ortiz, 2001)

Senses

Hearing

The hearing of manatees is similar to other marine mammals. They can detect sounds from a hundred or more meters. (Powell, 2002)
The auditory components of the central nervous system are well developed. This includes the peripherally located auditory nerves, which convey signals from the inner ear, and the brain regions that process that information. (Reep and Bonde, 2006)
Also do manatees have large earbones that also suggest that they have a keen sense of hearing (Powell, 2002).

Manatees can hear the frequencies of 0,5 kHz – 38 kHz, with greatest sensitivity at 16-18 kHz and rapid declines in sensitivity below 2 kHz and above 25 kHz. (Reep and Bonde, 2006)

Sight

The eyes of manatees are small and covered by a circular eyelid. The optic nerves, which convey visual signals to the brain, are also reduced in size. The external eye muscles and the nerves that control movement of the eyeball are small, as are the brain regions that process visual information. All of this suggest that manatee vision is rather poor, which makes sense for a species adapted to turbid-water environments. (Reep and Bonde, 2006)
Manatees exhibit a rather rudimentary condition where there is no localized grouping of ganglion cells and the overall concentration is low. This may be associated with adaptation to short-distance vision in turbid water. Behavioural observations also suggest that manatees are nearsighted. (Reep and Bonde, 2006)
The internal structure of the manatee eye reveals the existing of two types of cones as well as rods, suggesting that manatees have colour vision (Powell, 2002).

Smell/Olfaction

Manatees have olfactory tissue on small internal nasal bones, and probably have some sense of smell. The capability for scent discrimination is largely unknown. (Bush entertainment Cooperation, 1994*c*)

Taste

The tongue of manatees contain a few taste buds, there is no evidence for the presence of taste buds in more exotic locations (Powell, 2002; Reep and Bonde, 2006). Field observations have shown that manatees seek out fresh water to drink, and some of the animals food preferences may be based on taste (Reep and Bonde, 2006).

Touch

Manatees are tactile animals (Powell, 2002). They have vibrissae that send signals to the brain indicating that contact has been made with something in the environment and helping to localize it for further investigation (Reep and Bonde, 2006). The oral disk, the region between the mouth and nose, contains about 600 vibrissae hairs that are brought into contact with novel objects in the environment. Manatees prepare to "scan" or feel an object by first broadening and flattening the oral disk. This causes the bristle-like hairs to protrude and prepare for contact. Normally these hairs are hidden between fleshy folds of the oral disk when it is loose and relaxed rather than flattened. Then, the manatee moves the oral disk back and forth, scraping the hairs against the object. (Reep and Bonde, 2006)

1.4 Longevity

Wild situation

Manatees constantly replace their old tooth with new ones (Powell, 2002). A consequence of this tooth replacement is that scientists cannot use the teeth to determine the age of individual manatees, which is a procedure commonly used to determine the ages of many other marine mammal species. Scientists use a different method of aging based on studying the growth patterns on the ear bones. (Bush entertainment Cooperation, 1994*a*)

Studies of the manatee's ear bones indicate that the bones have growth layers that may persists throughout the animals life and can therefore be used as an indicator of age. Using this technique, ages of over 50 years have been documented. (Brook van Meter, 1989)
Because of the findings of the aging techniques used it is estimated that manatees live a maximum of about 60-70 years in the wild (Powell, 2002).

Captive situation

Because many of the animals kept in captivity come from the wild it is hard to give an exact age of those animals. In captivity ages over 40 years occur (Brook van Meter, 1989).

2. Field data

Knowledge about geography and ecology, diet and feeding behaviour, reproduction and behaviour is important to keep the manatees in an enclosure that resembles their natural habitat as much as possible. Information about their behaviour in the wild can be used as a reference for the behaviour of the manatees in captivity.

2.1 Geography and ecology

2.1.1 Distribution

The Amazonian manatee can be found in the Amazon River and is restricted to fresh water. The West-African manatee can be found in the coastal waters of West Africa (Powell, 2002; Animal info, 1999a).

The West-Indian has two subspecies. One, known as the Florida manatee is found in Florida and occasionally ventures as far north as Connecticut and possibly west along the Gulf of Mexico to Texas. Waifs are also known to reach to the Bahamas. The other, called the Antillean manatee, ranges along the coasts of Central and South America and may reach as far south as Recife. They are also found in the Greater Antilles, including Cuba, Jamaica, Hispaniola and Puerto Rico, with ephemeral stragglers to the smaller islands. (Powell, 2002) Figure 9 shows the distribution of all manatee species.

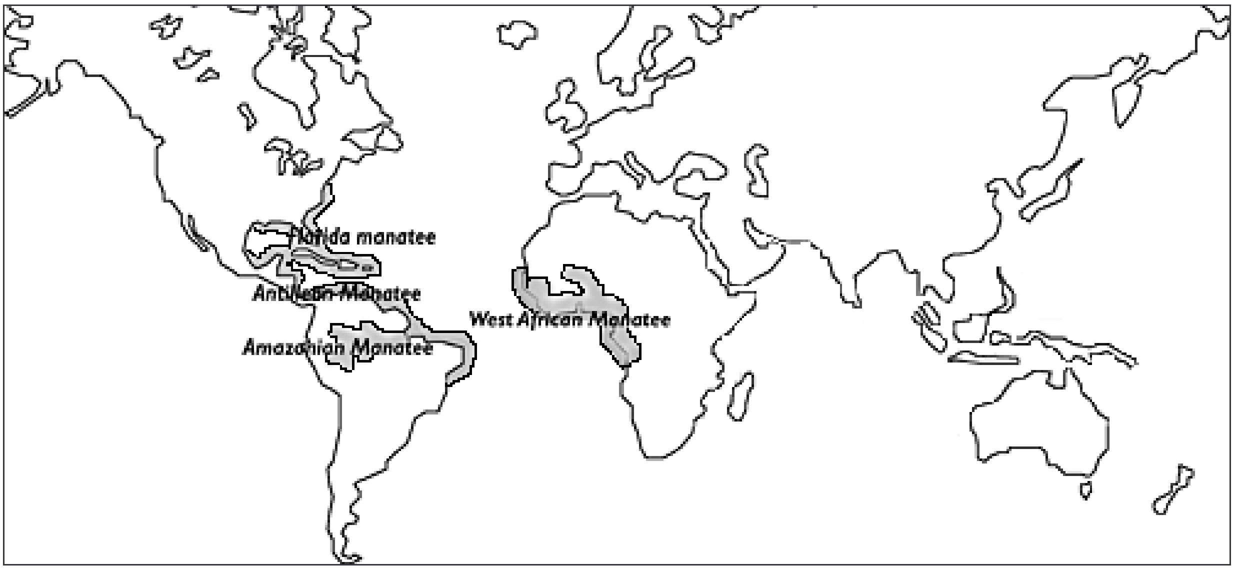


Figure 9 Distribution of the three manatees species (The Wild Ones, 2002)

2.1.2 Habitat

The Amazonian manatee is restricted to the fresh water Amazon Basin and may occasionally descend into brackish water. Amazonian manatees occur through most the Amazon River drainage, from the headwaters, in Colombia, Ecuador and Peru to the mouth of the Amazon (close to the Marajó Island) in Brazil over an estimated seven million km^2. However, they are patchily distributed, concentrating in areas of nutrient-rich flooded forest, which covers around 300,000 km². Most of the waters inhabited by Amazonian manatees are very murky, and, probably as an adaptation to past and ongoing hunting pressure, Amazonian manatees are extremely secretive. (IUCN, 1986)

By contrast, the West-African manatee can move freely between saltwater and freshwater habitats. They can be found in the coastal waters of West Africa. (Powell, 2002; Animal info 1999a)

West-Indian manatees are capable of withstanding large changes in salinity and move freely between freshwater and marine habitats. They are commonly found in shallow coastal areas, but can also be found in shallow rivers, estuaries, and canals. Because of their extremely low metabolic rate and absence of a thick layer of insulating body fat, they are restricted to tropical and subtropical waters. In the United States this species is concentrated around Florida in the winter months and migrates as far north as Virginia and as far west as Louisiana in summer months. The range of this species extends through the Caribbean, along the eastern coast of Central America and the northern coast of South America as far as northeastern Brazil. (University of Michigan Museum of Zoology, 1995)

This species may inhabit clear or muddy waters. Because of their large size, manatees prefer water reaching at least 1 to 2 meters in depth. These animals are most commonly found traveling in waters 3 to 5 meters deep and waters over 6 meters are generally avoided.
If the water is deep enough and the currents are not too strong (under 5 kilometers per hour), manatees are capable of traveling large distances upstream on inland rivers. In St. John's River, manatees live up to 200 km away from the ocean. Manatees found in the Gulf of Mexico are rarely more than a kilometer from the mouth of a river. (University of Michigan Museum of Zoology, 1995)

2.1.3 Population and conservation status

Exploitation of manatees by native peoples has occurred throughout history and probably had little effect on manatee populations until the introduction of modern hunting methods and equipment. In recent times, manatees have been netted, trapped, harpooned and dynamited in many parts of their range for meat, skin and oil. The shallow riverine and tropical coastal habitats of manatees are suitable as prime development sites and the future commercialisation of manatee habitat will place further pressure on their populations. This has occurred in Florida with serious consequences for the local manatees; there is a high mortality rate in manatees from a variety of causes of which powerboat collisions are perhaps the most well known. (Reeves, 1992)
Although the small manatee population is legally protected by the State (Florida Manatee Sanctuary Act of 1973), pressure on their habitats from boats continues to increase. Registrations of boats rose nearly seven-fold between 1960 and 1990 in Florida (Waller *et al.,* 1996). West-Indian manatees are legally protected in most countries where they occur: in Guyana since 1956, Honduras since 1959, Brazil since 1967, Venezuela since 1978 and Panama since 1967. However poaching has continued in many countries, since there is little enforcement. (Reeves *et al*., 1992)

Amazonian manatee
Estimated population size: less than 10,000
IUCN's Red List: Vulnerable
CITES: Appendix I

Trichechus inunguis is listed as Vulnerable based on a suspected population decline of at least 30% within the next three generations (assuming a generation length of 20 years, based on what is known for *T. manatus*) due primarily to ongoing levels of hunting, sometimes involving new and sophisticated techniques, coupled with increasing incidental calf mortality, climate change and habitat loss and degradation. (IUCN, 1986)

West-African manatee
Estimated population size: less than 10,000
IUCN's Red List: Vulnerable
CITES: Appendix II

The level of threats, particularly hunting and incidental catches, appears to be continuing to increase throughout range with locally high rates and near extirpation in some regions. Lack of protein and continued poverty, and limited enforcement of national laws, are expected to drive increasing hunting levels. Destruction of coastal areas from mangrove harvesting, siltation and dams are resulting in reduced habitat. There is an estimation of a high probability that a 30% or greater reduction in population size will result within a 90 year three generation period. (IUCN, 1986)

Using survey information from Cote d'Ivoire, Guinea-Bissau, the Gambia, portions of Senegal and Cameroon, and inferring what is known about manatee habitat in other range states and manatee density data for T. manatus, it is estimated that there are fewer than 10,000 manatees in West Africa. A population decline of at least 10% is anticipated based on continuing and increasing anthropogenic threats. (IUCN, 1986)

West-Indian manatee
Estimated population size: less than 10,000
IUCN's Red List: Vulnerable, Endangered
CITES: Appendix I

The West-Indian manatee is listed as Vulnerable because the number of mature individuals is currently estimated to number less than 10,000 (based on combined population estimates for the Florida and Antillean subspecies) and is expected to decline at a rate of at least 10% over the course of three generations (given a generation time of ~20 years) as a result of both habitat loss and anthropogenic factors. (IUCN, 1986)

Trichechus manatus latirostris:
The Florida manatee subspecies is listed as Endangered on the basis of a population size of less than 2,500 mature individuals and the population is estimated to decline by at least 20% over the next two generations (estimated at ~40 years) due to anticipated future changes in warm-water habitat and threats from increasing watercraft traffic over the next several decades. (IUCN, 1986)

Trichechus manatus manatus:
The Antillean manatee subspecies is listed as Endangered because the current population is estimated at less than 2,500 mature individuals and is predicted to undergo a decline of more than 20% over the next two generations (estimated at ~40 years for an unexploited population, based on *T. m. latirostris* data) without effective conservation actions, due to current and projected future anthropogenic threats (habitat degradation and loss, hunting, accidental fishing-related mortality, pollution, and human disturbance). (IUCN, 1986)

2.1.4 Migration

The seasonal distribution of the manatee is affected by water temperatures. Waters colder than 20˚ Celsius increase the manatees' susceptibility to coldstress and cold-induced mortality. Because of this temperature restriction, manatees seek out warm water refuges to help reduce energetic maintenance costs. The manatee occurs throughout the south-eastern United States. The only year-round populations of manatees occur throughout the coastal and inland waterways of peninsular Florida and Georgia. (U.S. Fish and Wildlife Service, 1999)

In the summer, finding warm water is not a problem, but as winter approaches, manatees living in areas north of Florida Bay, Biscayne Bay, and the Everglades often travel to sources of warm water (see figure 10). Those that roam outside Florida's borders in the summer and fail to return by winter may not survive. During extreme drops in temperature, manatees usually remain close to a warm-water source and, during breaks in the weather, travel to nearby feeding areas. Sometimes when the weather remains very cold, manatees may not feed for days at a time. (Brook van Meter, 1989)

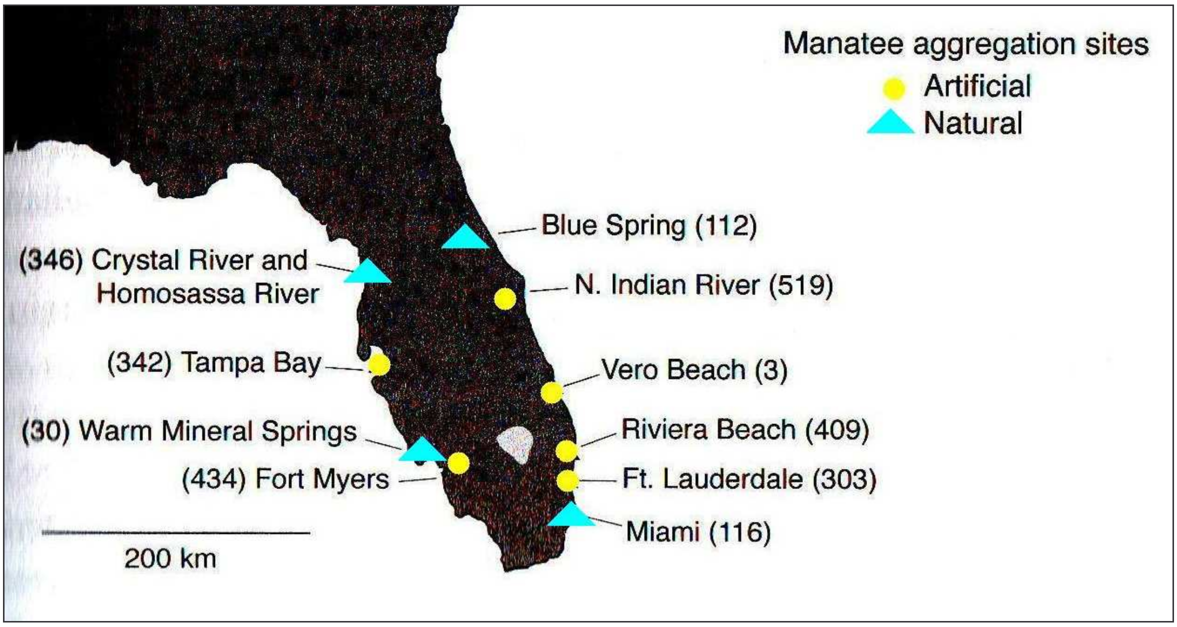


Figure 10 Manatee aggregation sites in Florida (Hartman, 1979)

North-western manatees

North-western Florida manatees typically range from the Apalachicola River south to Tampa Bay. The number of manatees seen in the panhandle section of Florida, as well as in Louisiana and Mississippi, has increased as the Northwest Florida manatee population has expanded. During the summer months, manatees are regularly observed throughout most of the west coast, from the Everglades to Citrus County and the rest of the Big Bend area. Several natural springs are used on the west coast during the winter. The most important springs are at the headwaters of the Crystal and Homosassa Rivers in Citrus County. Natural springs on the west coast provide sources of warmth and an abundance of food. Over three hundred manatees regularly use the Crystal and Homosassa River area as winter aggregation sites (see figure 11). (Brook van Meter, 1989)

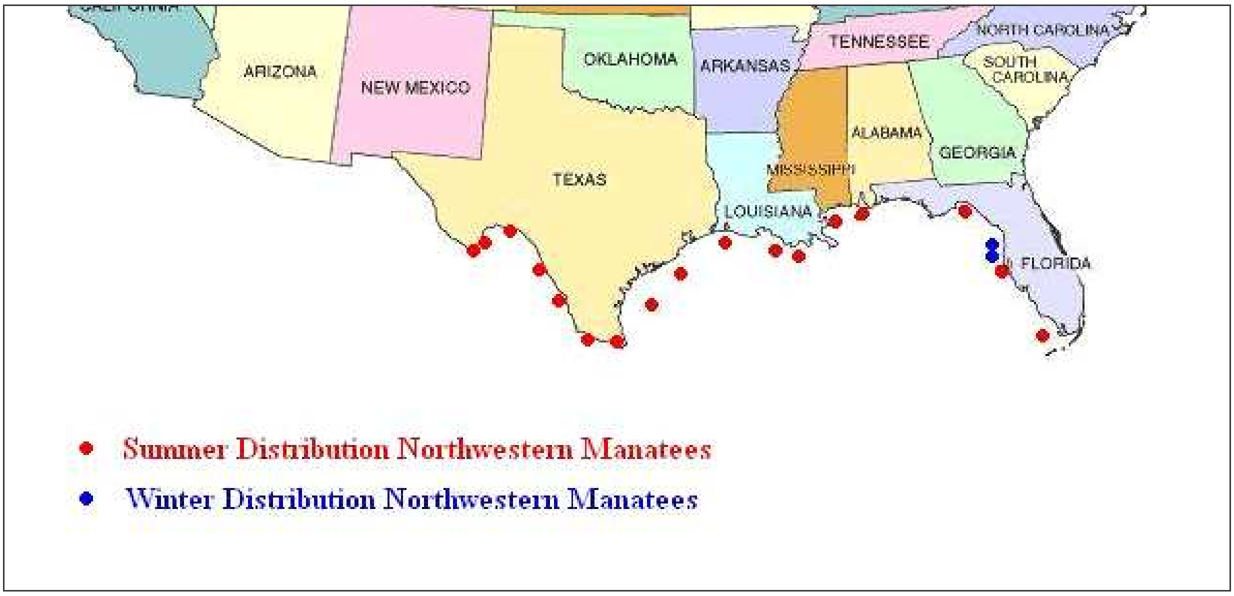


Figure 11 Summer and winter distribution of the subpopulation North-western manatee (Brook van Meter, 1989)

South-western manatees

Hundreds of south-western manatees also rely on warm-water sources in Lee County. The largest manatee aggregations occur at industrial sites near Ft. Myers and in various manmade canal systems, such as those at Matlacha Isles and Port of the Islands. (Brook van Meter, 1989)

Manatee usage of the upper St. Johns River has increased substantially over time. The number of manatees gathering at Blue Spring has increased at a rate of 8% per year over the last 28 years due largely to reproduction, a high rate of adult survival, and immigration from outside the St. Johns River (including the release of captive manatees). Well over one hundred manatees have been documented using the spring during the winter and most of these animals are believed to stay in the upper St. Johns River area during the warmer months. During the winter, many manatees congregate at Blue Spring State Park on the St. Johns River and at artificial warm water sources farther south (see figure 12). (Brook van Meter, 1989)

Atlantic coast manatees

Atlantic coast manatees can range from the St. Johns River south to Miami and the Florida Keys, but some have been known to travel up the eastern seaboard. One animal travelled as far as Rhode Island. Travelling occurs mostly seasonally as manatees move between summer dispersal areas and winter gathering sites, but manatees may also shift wintering locations during a season. (Brook van Meter, 1989)

Manatees are also found abundantly on the east coast in Florida’s Intracoastal Waterway and are occasionally sighted offshore in coastal waters. The Indian River Lagoon, which stretches from Brevard County to northern Martin County, is known to be important to manatees for a variety of reasons, including for feeding and travelling. Brevard County seems to be preferred year-round manatee habitat. During the winter months, hundreds of manatees aggregate near industrial warm-water discharges on the Indian River and farther south in Palm Beach and Broward counties. During the spring and summer months, manatee use seems to be heaviest in the Banana River. The upper Banana River is used extensively by manatees; more than 360 manatees have been observed in the area during surveys conducted in the spring. This area provides manatees with abundant foraging habitat; it is also a protected area for manatees and is designated by the federal government as an area in which motorboats are prohibited (see figure 12). (Brook van Meter, 1989)

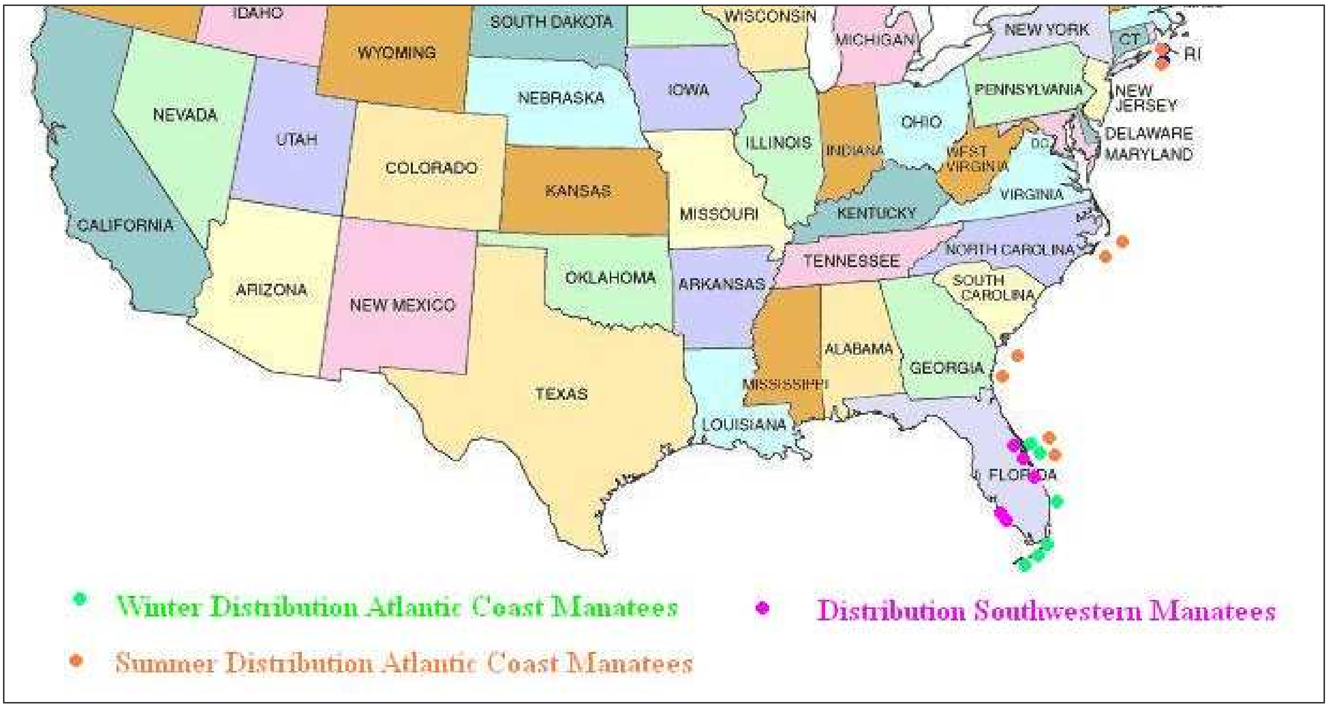


Figure 12 Summer and winter distribution of the subpopulations Atlantic coast manatee and South-western manatee (Brook van Meter, 1989)

It is believed that the historical winter range of the manatee was once centred in southern Florida, with small groups spending the winter at a few natural springs in northern Florida. For more than 50 years, industrial sites that discharge warm water, perhaps coupled with the loss of natural habitats, have caused a shift in manatee winter distribution. Today, manatees congregate at about a dozen warm-water sources during the winter. The number of manatees observed daily at each of these sites ranges from 30 to 500 depending on the severity of the winter. (See figure 13) (Brook van Meter, 1989)

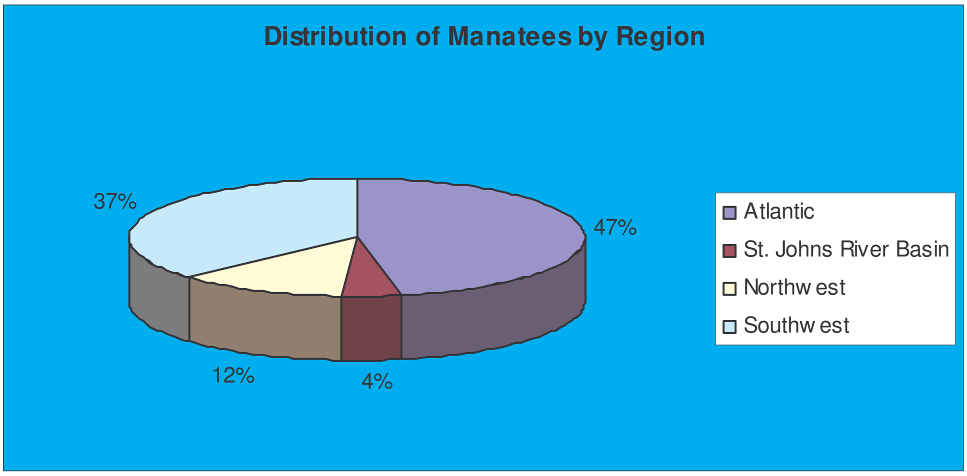


Figure 13 Four subpopulations of the Florida manatee with percentage estimates based on winter survey counts in each region between 1996-2000. (Smithsonian Marine Station at Fort Pierce, 1969)

2.2 Diet and feeding behaviour

Manatees eat on a wide variety of seagrasses and freshwater aquatic vegetation. They are not strict herbivores, it is observed that manatees eat fish that is caught in nets and also the snails that live on the seagrasses. (Powell, 2002)

2.2.1 Diet

West-Indian manatees are non ruminant herbivores and eat mainly on submerged vegetation, but they also eat floating and emergent plants. They feed mostly on the following six dominant species of submerged plants: hydrilla (*Hydrilla verticillata*), eelgrass (*Vallisneria neotropicalis*), rigid hornwort (*Ceratophyllum demersum*), spiked water-milfoil (*Myriophyllum spicatum*), widgeon grass (*Ruppia maritima*) and shoal grass (*Diplanthera wrightii*). (Hartman, 1979) In Florida freshwater habitats also waterhyacinth (*Eichhornia crassipes*) is consumed, along with a great variety of other plants (Waller *et al.,* 1996), like manatee grass (*Syringodium filiforme*), turtle grass (*Thalassia testudinum*), sago pondweed (*Potamogeton pectinatus*), waterweed (*Elodea densa*) and alligatorweed (*Alternanthera philoxeroides*). It is for sure that at different places in Florida, the manatees have different preferences when it comes to eating waterplants (Hartman 1979). Also tapegrass (*Vallisnera americana*), parrot feather (*Myriophyllum aquaticum*) and smooth cord grass (*Spartina alterniflora*) are eaten by the West-Indian manatee (Reep and Bonde, 2006).

The West-Indian manatee is recorded to eat about sixty species of plants in Florida waters and seems to avoid those species that contains toxins (blue-green algae). They sometimes partially beach themselves to reach desired food items, such as forbs and grasses growing on the banks of tidal rivers and creeks (Waller *et al.,* 1996). Manatees regularly eat marsh grass in the salt-marsh areas of north-eastern Florida and south-eastern Georgia. Animals in the St. Johns River forage on live oak acorns during the winter. In marine waters, sea grasses and epiphytic algae are consumed. These manatees feed on sea grasses either by grazing only the blades or by rooting to consume the entire plant. Numerous invertebrates are ingested incidentally and they may constitute an important supplement to the manatees' mainly vegetarian diet. (Reeves *et al.,* 1992) It is seen that manatees sometimes prefer consuming invertebrates that are attached to a dock. Manatees were heard crunching invertebrate shells and seen pulling animals, such as tunicates, into their mouths. These observations suggest that manatees actively, rather than incidentally, consume invertebrates in some cases. (Courbis and Worthy, 2003) Manatees can sometimes be seen feeding on vegetation overhanging the water or fruits and nuts that have fallen into the water (Powell, 2002).

2.2.2 Feeding behaviour

Manatees feed in discrete sessions during which they concentrate on one species of plant. Feeding sessions normally last between 30 and 90 minutes but continue for more than two hours if animals are exceptionally hungry (Hartman, 1979). They spend about six to eight hours a day feeding and in that time may consume about four to nine percent of their body weight in wet vegetation. Vegetation is grasped and torn by the lips, which are strengthened with lateral, horny pads, and then passed back to the grinding molars. Stomach content analyses show that food is well chewed. Manatees feeding in seagrass beds either crop the seagrass leaves or dig into the sediment with their flippers to eat the entire plant. Much of the biomass and carbohydrates in seagrass are concentrated in the underground portion of the plant. In areas where manatees congregate, digging up seagrass roots can result in the temporary disruption of seagrass beds. Manatees are often seen feeding at the edge of seagrass beds, possibly because nearby deeper areas offer an escape route if disturbed. Protection from

wind and currents seems to be preferred, so manatees often choose feeding sites behind barrier islands. (Brook van Meter, 1989)
Manatees do not strongly compete with other herbivores, likely due to their flexibility in making food choices based on availability. (Smithsonian Marine Station at Fort Pierce, 1969)

Sirenians use their thick movable lip pads to grasp and move vegetation toward their mouth. They have a marvellous mechanism for transporting the food with their movable lip pads. On the underside of the muzzle, the lips have several rows of stiff bristles or hairs called vibrissae. Each vibrissa resides in a small cavity with only the tip of the bristle exposed above the skin. While eating, they can extend and retract these specialized whiskers so they act somewhat like the legs of a centipede marching and guiding the bits of food toward the mouth. The tongue then takes over and manoeuvres the food to the teeth for mastication. (See figure 14) (Powell, 2002)

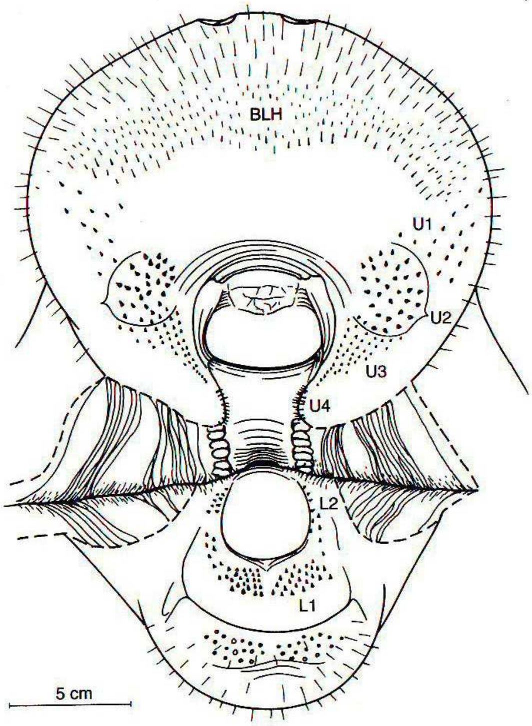


Figure 14 Hair on the manatee face, including groups of thick bristles on the upper lips (U1-U4) and lower lips (L2-L2). Bristle-like hairs (BLH) are present on the oral disk. (Reep and Bonde, 2006)

2.2.3 Water intake

The freshwater requirements of manatees are not well known, but they are frequently spotted drinking freshwater from outfalls and culverts in salt and brackish water areas. Research on the structure of the manatee kidney suggests that the animals may survive for extended periods without freshwater. (Brook van Meter, 1989)
Manatees in fresh water drink large volumes of water (Ortiz *et al.,* 1999). In Florida West-Indian manatees are often been seen drinking fresh water emanating from storm drains or even gulping water from running hoses dangling from a dock. (Powell, 2002; Hartman, 1979)

The distribution patterns of sirenians suggest that they may possess a range of physiological capabilities relating to water balance and osmoregulation. Dugongs and manatees possess kidneys which, on the basis of their anatomy, should theoretically enable them to deal with a marine environment for prolonged periods. It is suggested that West-Indian manatees were capable of drinking salt water since urine osmolality varied with salinity. (Ortiz *et al.,* 1999)

2.2.4 Calves

Manatee calves drink mostly milk from their mother, but can eat vegetation, like algae and grass, when only a few weeks old, but usually nurse up for one to two years (Powell, 2002, Hartman, 1979). Manatee milk contains more fat, proteins and salt than cow's milk and does not contain lactose. (Brook van Meter, 1989)

If calves feed on grass, they eat with less concentration and for shorter periods than adults. They often have to wait for their mothers to finish feeding (Hartman, 1979). It is suggested that nursing calves do not drink salt water, but they do drink fresh water when nursed. (Ortiz *et al.,* 2006)

2.3 Reproduction

2.3.1 Sexual maturity

Manatees have an approximately 1:1 sex ratio and low reproductive rates. Male manatees mature at two to three years of age (Smithsonian Marine Station at Fort Pierce; 1969, Brook van Meter, 1989). Female manatees sexually mature when they are three to four years old (Powell, 2002). They may produce calves as early as four to five years (Hartman, 1979; Reeves *et al.,* 1992) of age but at such an early age may be unsuccessful at raising calves. Most females breed successfully by six to ten years of age. (Brook van Meter, 1989)

2.3.2 Seasonality

Behaviour interpret to be mating has been observed in all seasons and there is no clear peak season for calving (Reeves *et al.,* 1992).

However, peak sperm production (as analyzed in recovered carcasses) occurs primarily from March through November. Only 20% of adult males showed evidence of sperm production from December to February. (Smithsonian Marine Station at Fort Pierce, 1969)

In Florida calving and breeding may peak in the spring when the energetic demands of winter are finished. This reproductive strategy helps the female through the energetic stress of pregnancy or nursing when there is abundant food and warm water during the summer. (Powell, 2002)

2.3.3 Gestation period/ birth rate

Manatees need to maintain a high survival rate because they reproduce so slowly. The gestation period of manatees is at least 12 and possibly 13 months and females usually produce one calf with each pregnancy. Twins are rare but do occur in approximately 2% of the births. The interval between births is generally two to five years, although a female losing her calf soon after birth could have another calf within two years. If the interval between calving is 3 years and continues over a 36-year period, a female manatee could produce approximately 12 calves during her lifespan. (Brook van Meter, 1989).

2.3.4 Parturition

Shelter-seeking behaviour among cows that are nearing their time of delivery is characteristic of female sirenians in general. Parturition in the manatee has never been witnessed (Hartman, 1979). Fluke presentations are preferred, since the presence of the fluke first allows it to unfold and solidify, making swimming much more efficient after birth. Headfirst presentations can still result in a live calf, but it will initially be more prone to complications, such as difficulty in swimming and surfacing. (Dierauf and Gulland, 2001)

2.3.5 Development

Newborns of the West-Indian manatee have an average length of 1.2 – 1.4 m and weigh 30 kg (Smithsonian Marine Station at Fort Pierce, 1969). Newborns swim using only their flippers, and learn to use their tail several days later. Suckling occurs underwater when the female is suspended at the surface or lying on the bottom. Calves grasp the teat (located under each flipper) and suckle for up to 2 minutes. Calves begin grazing a few weeks after birth and, as they mature, alternate grazing and suckling, becoming less and less dependent on milk. Calves remain dependent on their mothers for up to two years, although they may remain in contact for long periods beyond weaning. This may enhance survival rates by allowing them to learn migration routes, the locations of feeding grounds and winter refuges. (Powell, 2002; Nabor and Patton, 1989) It is seen that orphans are raised by another female (Hartman, 1979).

2.4 Behaviour

The behaviour of manatees can be divided in three time periods, namely Active, Inactive and Eat. Active contains behaviour like aggression, breathing, contact touching, diving, flipper and head splashing, swimming and nostril movement and many others. Inactive contains behaviour like floating and resting. Eat contains the behaviour eating. (Horikoshi-Beckett and Schulte, 2006) In figure 15 is shown how manatees spend their daytime.

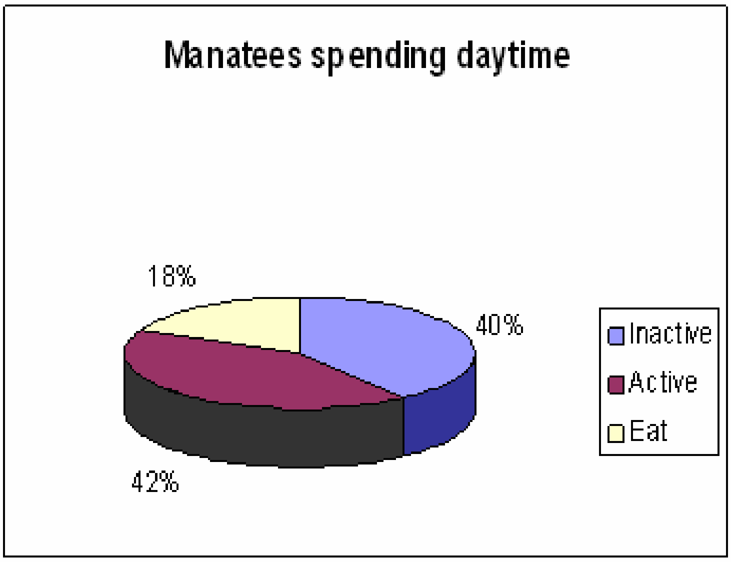


Figure 15 The three main behaviours (inactive, eat and active) by nine captive manatees (Horikoshi-Beckett and Schulte, 2006)

2.4.1 Activity

Manatees are generally arrhythmic (Hartman, 1979), with feeding, resting, travelling, socializing, and other activities showing no consistent differences between day and evening hours. Feeding occurs over 1 – 2 hour periods and totals 6 – 8 hours daily. Socialization among manatees is highly variable, but is apparently higher in winter when animals aggregate in warm-water refuges. Though manatees spend much of their days seeking out food; sleeping, or travelling, they also spend approximately 6 – 10 hours at rest with no apparent pattern in their activities. (Smithsonian Marine Station at Fort Pierce, 1969)

Sleeping
Two basic resting postures are employed by manatees: hanging suspended near the surface and lying prostrate on the bottom. In both positions, the animals lapsed into a somnolent state with their eyes closed and their bodies motionless. The eyes are only opened at the surface. Manatees allow the tail and flippers to dangle in typical suspended resting. When resting suspended, manatees are liable to drift and must regularly compensate for their displacement by making minor corrective movements with tail and flippers. When water is turbulent manatees discourage suspended resting, in stead of they move to the bottom where conditions are more placid. Suspended resting was frequently interspersed with idling and was a more transitory activity than bottom-resting.
When resting on the bottom, manatees are supported on the muzzle, stomach and tail. The flippers are held along the sides or flexed at the wrists and adducted under the chest. The tail usually rests flat on the bottom. It is common for bottom-resting manatees to roll on their sides or backs momentarily.
Bottom-resting manatees have no preference where to sleep. They rest on a variety of substrates, including sand, mud, limestone, oyster bars and beds of aquatic plants.
Manatees generally choose shoals of 1 to 3 meters for bottom-resting, but occasionally rest at greater depths. (Hartman, 1979)

Breathing
Manatees open their nostrils for air at the exact moment of reaching the surface and close them on submersion with equally precise timing. Exhalation is followed instantaneously by inhalation. When disturbed in the act of breathing, manatees interrupted the respiratory sequence and dive, apparently without filling the air in its lungs. In such situations animals resurface immediately. (Hartman, 1979)
An adult manatee exchanges about 90 percent of the air in its lungs by each breath. (Powell, 2002; Waller *et al.,* 1996)
Larger animals take a larger breath then smaller animals, but also the state of activity has direct bearing on the duration of breathing. For adult manatees the length of respiration has an average of 3.8 seconds, while calves have a length with an average of 2.5 seconds. Manatees can hold their breath for 16 minutes and even 20 minutes is recorded. Calves can be submerged for approximately 4.5 minutes. (Hartman, 1979)
Manatees tend to stay submerged for two to three minutes, but longer when resting and more frequently when active (Powell, 2002).

2.4.2 Locomotion
Stabilization in the water arises from the elongate body shape of the manatee, dorsal position of the lungs, and the heavy bones of the manatee, which contribute to the animal's neutral buoyancy by acting as ballast. Most swimming activity in manatees is accomplished solely by dorsoventral undulations of the wide, rounded tail, which also serves as a rudder. Manatees are able to steer, bank, and roll simply by adjusting tail position. Newborns tend to use the flippers exclusively while swimming. (Hartman, 1979; Smithsonian Marine Station at Fort Pierce, 1969)
Flippers are used primarily for turning, but also for precise movements, course corrections, stabilizing position, and for orientation while feeding, idling or socializing. When idling on the bottom while resting or feeding, the flippers provide the sole source of movement, with many manatees using the tips of their flippers to balance upon while resting. Manatees have often been observed "walking" along the bottom using alternating flipper movements (Hartman, 1979, Smithsonian Marine Station at Fort Pierce, 1969).

Swimming speed

The main thrust in swimming is produced by the tail which is moved up and down with powerful strokes. When changing direction of movement, the tail acts as a horizontal rudder by twisting on its axis whilst stroking is stopped. The paddles are used for guiding turns and deceleration; in fast swimming the paddles are raised and held against the sides. (Waller *et al.*, 1996)

Depending on activity, swimming velocities range from 2 to 25 km/hr. Idling manatees swim at 2 to 3 km/hr, cruising animals at 4 to 10 km/hr and animal in flight at 18 to 25 km/hr. The sprints of animals of fleeing animals are short, mostly 20 to 30 meters and never more than 100 meters. (Hartman, 1979; Reeves *et al.*, 1992)

Diving

Manatees dive with their lungs full of air and are similar to whales in this respect but differ from seals (Waller *et al.*, 1996). Diving times varies with activity, but 4 minutes has been suggested as an average in Florida waters (Reeves *et al.*, 1992).

2.4.3 Predation

Manatees have no natural predators (Reep and Bonde, 2006), but all members of the Sirenia have been hunted for food with some populations, especially in the southern hemisphere, still noted to be at risk from poaching and subsistence hunting. (IUCN, 1986; Smithsonian Marine Station at Fort Pierce, 1969)

In Florida, evidence shows manatees were hunted by pre-Columbian societies. After Spanish occupation of Florida, the increase in human population increased hunting pressures on the manatee, heavily impacting population levels. Commercial and subsistence hunting during the 1800s also significantly reduced the population in Florida. (U.S. Fish and Wildlife Service, 1999; Smithsonian Marine Station at Fort Pierce, 1969)

2.4.4 Social behaviour

Manatees live in loosely associated social groups (Hartman 1979), with most cohesive social interaction among manatees occurring only in mating herds (Smithsonian Marine Station at Fort Pierce, 1969).

Though they can form large aggregations, for example at warm water sites, most frequently they are sighted alone or in small ephemeral groups. Manatees are attracted to fresh water, abundant sources of food, good resting places and places where there is minimal human disturbance; consequently manatees are found at higher densities around these shared resources. The strongest social bond is between a mother and its calf. Males sometimes accompany females or females with calves for days at a time in anticipation of the female entering oestrus. There are no family units of mother, father and calf. (Powell, 2002)

Communication

Manatees communicate through sound, sight, taste, and touch. Despite the absence of external ear lobes and the small size of the auditory openings, manatees hear very well. Some studies suggest that manatees may even be able to hear sounds that are too low for humans to detect. Manatee cows respond to the squeals of their calves from almost 200 feet away, and adult manatees have been reported to respond to sounds 160 feet away. (Hartman 1979; Brook van Meter, 1989)

Manatees emit a range of sounds underwater that are within human auditory range. These sounds are believed to be used for communication and not for echolocation or navigation. Indeed, manatees may bump into objects in murky water. Manatees make sounds when they are frightened, sexually aroused or playing. (Brook van Meter, 1989)

Vocalisations

Vocalization plays a role in maintaining contact between adults and between a cow and her calf. Calls are used to maintain contact while feeding and travelling, particularly in turbid water. Rapid calling has been noted when a group is startled and flees an area, or when greeting new arrivals. Information is conveyed by varying the pitch, loudness, and duration of calls. (Brook van Meter, 1989; Smithsonian Marine Station at Fort Pierce, 1969)
A mother and its calf usually stay in close contact with each other and frequently vocalize back and forth using high-pitched squeaks and squeals. If threatened of startled the calf let out a squeal that initiates a rapid duet of squeaks between cow and calf. (Powell, 2002)

2.4.5 Sexual behaviour

The only cohesive association between manatees, besides the cow-calf family unit, is found in the oestrus herd composed of a cow in heat accompanied by courting bulls (Hartman, 1979). These highly active mating herds may retain with the female for more than two weeks and can number 20 or more males. The composition of the herd is dynamic and not all the males will remain with the female the entire time. (Powell, 2002)
Juvenile males join and leave the herd constantly, but a nucleus of mature bulls is always present and in persistent pursuit of the cow (Hartman, 1979).
These mating herds are constantly moving as the females tries to elude her suitors. The males swarm over and under her, jostling to gain a position close and advantageous for the moment she is receptive to mating. Consequently, there is constant and vigorous bumping, rolling, pushing and positioning by the males. The female, after weeks of harassment, will sometimes beach herself to get away from her pursuers' attempts to get underneath her and to have some respite from their constant attentions. Individuals, both males and females and with partners of either sex, engage in sex play. They often grasp each other with flippers, mouthing genitals, rolling and cavorting. (Powell, 2002; Hartman, 1979)
When the herd finally emerged in the water, the cow paused in flight to allow successive copulations. The nearest male rolls on his back, swims up from the rear, mouths the genital area of the cow, then slides fully under her, firmly grasping her from beneath, extruding his penis and effecting intromission. During copulation, the other bulls mouth and embrace the cow from above and from the side. Intervals between matings lasts less than a minute. The copulatory embraces last 15 to 30 seconds. Males can be aggressive during these mating herds. (Hartman, 1979)

Husbandry Guidelines for West-Indian Manatees

Section B: Management in captivity

1 Enclosure

When keeping an animal in captivity it is important that the enclosure is suitable for the species kept in it. Animals should be provided with an environment, space and furniture which is sufficient to allow such exercise as is needed for the welfare of the particular species. (EAZA, 2006)

1.1 Housing facilities

Manatees only require a pool and no dry resting surface (USDA, *2001g*). According to Canadian Association of Zoos and Aquariums (CAZA) the long-term or permanent enclosures for manatees should comply with some standards. It should be of a size that enables the animal to show natural behaviours, should be large enough for the animals to achieve the full range of body motion and physical movements normally performed, and it should contain furniture to physically and psychologically enrich the environment and stimulate normal physical movement and behaviour of the animals. Furthermore it is important that there is a kind of shelter for the animals, this can be natural or man-made. This shelter should provide protection from natural conditions such as sun, rain and snow. (CAZA, 1975) A shelter is especially important when the animal is kept in an outdoor enclosure.

1.1.1 Pool

Circular pools have the advantage over angular pools as they provide better water flow which, in turn, tends to clean the pool. Also active swimmers will tend to sustain fewer surface abrasions when housed in circular pools. (CCAC, 1984)

A wide variety of building materials can be used for the construction of the manatee pool. The principal requirements are that the pool should be water tight, non-abrasive, easily sanitized and resistant to puncture. (CCAC, 1984) Most zoos (6/7) use a concrete pool for housing manatees.

When building a manatee enclosure it is important to consider having different sections in the pool which you can separate from each other. This will make it easier when it is necessary to separate an animal or when an animal has to be examined. (pers. comm. B. Klausen, 2008)

Indoor pool
The air and water temperatures in indoor facilities should be regulated by heating or cooling to protect the manatees from extremes of temperature. Rapid changes in air and water temperatures should be avoided. (USDA, 1998)
The indoor enclosures should be ventilated by natural or artificial means to provide a flow of fresh air for the manatees and to minimize the accumulation of gases and objectionable odours. A vertical air space averaging at least 1,83 meters should be maintained in all primary pools housing manatees. (USDA, 1998)
The indoor housing facilities for manatees should have lighting (USDA, 1998). The size requirements of the pool can be found below in 1.1.2 Dimensions.

Outdoor pool

Manatees should not be housed in an outdoor facility unless the air and water temperature ranges in temperatures which are normally found in the wild. A manatee should not be introduced to an outdoor enclosure when it is not acclimated to the air and water temperature ranges from the outdoor facility. Also it is important when housing a manatee in an outdoor enclosure to be able to change and maintain the water temperature. (USDA, 1999) The size requirements of the pool can be found below in 1.1.2 Dimensions.

1.1.2 Dimensions

Marine mammals must be housed in primary enclosures that comply with the minimum space requirements. (USDA, 2001*c*)

Enclosures smaller than required by the standards of the United States Department of Agriculture (USDA) may be temporarily used for non-medical training, breeding, holding, and transfer purposes. Any enclosure that does not meet the minimum space requirement for primary enclosures (such as medical pools/enclosures, holding pools/enclosures and gate side pools smaller then the minimum space requirements) may not be used for permanent housing purposes. Rotating animals between enclosures that meet the minimum space requirements and enclosures that do not is not an acceptable means of complying with the minimum space requirements for primary enclosures. (USDA, 2001*c*)

The minimum space requirement according to the USDA is based on the minimum horizontal dimension (MHD), depth, volume and the surface area of the primary enclosure (USDA, 2001*c*; USDA, 2001*f*).

The MHD is the diameter of a circular pool of water, the required MHD is two times the average adult length of the longest specimen housed in the pool (USDA, *2001g*).

The minimum depth of the pool must be 1,52 meters (5 feet) or ½ the average adult length of the longest specimen housed therein, whichever is greater. Those parts of the enclosure which do not meet the minimum depth requirements cannot be included when calculation space requirements for sirenians. (USDA, 2001*c*)

If the pool meets the MHD and the depth requirements, then the pool will have sufficient volume to house up to two sirenians (USDA ,*2001g*).

If additional sirenians are to be added to the pool, the volume as well the surface area has to be adjusted to allow for the additional space necessary for such sirenians (USDA, 2001*c*).

According to the USDA the average adult length of a manatee is 3,51 meters. The document Space Requirements of the USDA contains a table in which the volume of water required for an extra manatee is given. The volume of water required for each additional manatee is 35, 50 m^3. (USDA, 2001*c*)

In the same document the required surface areas are given for marine mammals, the required surface area for one manatee is 14,47 m^2. This means that for each manatee added the surface area should be increased with 14,47 m^2. (USDA, 2001*c*)

The opinions of the zoos which answered the questionnaire are divided. According to one expert (1/7) the minimum requirements of the USDA are sufficient, the other experts give different surface areas. The numbers mentioned as surface areas for one manatee are:

114 m^2 (1/7), 153 m^2 (1/7) and 227 m^2 (1/7). Also one expert stated that the minimum surface area for four animals is 150 m^2 (1/7).

1.1.3 Quarantine facility

The Canadian Association of Zoos and Aquariums (CAZA) recommend that all institutions which have marine mammals have a quarantine facility for new arrivals at their institution. This facility should isolate the newly arrived mammal in such a manner that cross-contamination resulting from physical contact, disease transmission, aerosol spread, waste drainage, or the reuse of untreated disease is not possible. (CAZA, 1975)
Ocean pens must be located in a way that prevents the spread of any disease from animal to animal through natural water movement and at a distance from other penned animals as is determined by the supervising veterinarian. If a receiving institution does not have appropriate isolation facilities, the staff should arrange for quarantine at an acceptable alternate site or only receive animals which do not require quarantine. (CAZA, 1975)
The quarantine facility does not need to comply with all the optimal space requirements established for the manatees kept in the primary pool (CCAC, 1984).

1.1.4 Shelter

As said before, manatees kept in an outdoor enclosure should be provided with a shelter to prevent harm to the animal from the sun, wind etc. This shelter must protect the animals from overheating and direct sunlight. When sunlight is likely to cause overheating, sunburn, or discomfort, sufficient shade must be provided to protect the manatees. Also shelter from cold weather should be provided, this to prevent the animals to get cold. This can be done by increasing the water temperature of the pool. (USDA, *2001f*)

1.2 Enclosure boundaries

According to EAZA the enclosure barriers should be designed, constructed and maintained to contain animals within the desired enclosures (EAZA, 2006). Also the in- and outdoor housing facilities should protect the animals from injury and restrict the entrance of unwanted animals and persons (USDA, 2001*b*).

Natural water facilities like a lagoon must have effective barrier fences on all sides of the enclosure that is not contained by dry land. The barrier fence must extend above the high tide water level, so the animals can not leave the enclosure. (USDA, 2001*b*)

Manatees should be protected from abuse and harassment by the viewing public. This can be done by supervising the viewing public or with physical barriers such as fences, walls, glass partitions or creating distance between the animals and the public. (USDA, 2001*b*)

Outdoor enclosures and enclosures not entirely indoors must be enclosed by a perimeter fence that is of sufficient height to keep the animals in and unauthorized persons out. This fence should prevent animals and persons from going through it or under it, and the fence should also be of a sufficient distance from the public to prevent contact between the animals in the enclosures and persons or animals outside the fence. (USDA, 1999)

Glass (4/7), wooden fences (3/7), concrete walls (2/7), acrylic viewing panels (2/7), bridges (1/7), bamboo fences with steel support (1/7), fences of stainless steel (1/7) and wooden shore fences (1/7) are used to separate the visitors from the animals in zoos.

The heights of the boundaries used in zoos vary, this can depend on the kind of boundary used. The heights used are less than one meter (2/7), between one and two meters (4/7) and higher than 2 meters (1/7).

1.3 Substrate

The floors of the holding facility should be hard and durable, impervious to water, readily sanitized, and resistant to physical and chemical damage. It is important that the floors have a pitch towards the drains. (CCAC, 1984)

The walls and ceilings should be coated with an impervious, easily-cleaned material and should be free of gaps and spaces which could house micro-organisms or vermin. All surfaces must be free of sharp projections i.e., nails or wire, which are a potential cause of injury. Electrical fixtures and outlets should be grounded, easily cleaned, and corrosion-resistant. (CCAC, 1984)

1.4 Furnishing and maintenance

Water and power supply

Reliable and adequate sources of water and electric power must be provided by the facility housing manatees. There must be emergency sources of water and electric power in the event of failure of the primary sources, when such a failure could reasonably be expected to be detrimental to the good health and well-being of the manatees housed in the facility.
There should be a plan regarding the evacuation of the animals in the facility in the event of a disaster. This plan should describe back-up systems and/or arrangements for relocating manatees. (USDA, 2001*b*)

Drainage

Adequate drainage must be provided for all primary enclosure pools and must be located so that all the water contained in such pools may be effectively eliminated when necessary for cleaning the pool or for other purposes (USDA, 2001*b*). The drains should be provided with a suitable trap, a catch basket, and a drain line of not less than 10 cm (4 inch) diameter (CCAC, 1984).

Storage

Supplies of food must be stored in facilities that adequately protect such supplies from deterioration, spoilage (harmful microbial growth), and vermin or other contamination. Refrigerators and freezers must be used for perishable food, such as fruits and vegetables. No substances that are known to or may be toxic or harmful to manatees should be stored or maintained in the food storage or preparation areas. Exceptions are cleaning agents; these may be kept in secured cabinets designed and located to prevent food contamination. Food, supplements, and medications may not be used beyond commonly accepted shelf life or the date listed on the label. (USDA, 2001*b*)

Waste disposal

Provision must be made for the removal and disposal of animal and food wastes, trash, and debris. Disposal facilities must be provided and operated in a manner that will minimize odours and the risk of vermin infestation and disease hazards. All waste disposal procedures must comply with all applicable laws pertaining to pollution control, protection of the environment and public health. (USDA, 2001*b*)

Washroom facilities
Washroom facilities containing basins, sinks, and, as appropriate, showers, must be provided and conveniently located to maintain cleanliness among employees. These facilities must be cleaned and sanitized daily. (USDA, 2001*b*)

1.5 Environment

1.5.1 Temperature

The minimum temperature used in manatee pools differs from 20ºC (1/7), 24ºC (3/7) and 28ºC (2/7). The maximum temperature in manatees' pools differs from 23ºC (1/7), 27 ºC (3/7) and 31ºC (2/7). Because manatees cannot deal with cold water temperatures for long periods it is important that the minimum temperature of the pool is above a particular temperature. The opinions about the minimum temperature vary between 20ºC (2/7), 22ºC (4/7) and 25 ºC (1/7). Also manatees have to seek cooler places when the water gets to warm to regulate body heats so the maximum temperature of the water should also be regulated. The opinions of the experts about the maximum water temperature vary between 29ºC (2/7), 30ºC (3/7) and 33ºC (2/7). Extremes of both heat (>33 ºC) and cold (<20 ºC) should be prevented.

The experts opinions are divided about whether manatees need a minimum and maximum air temperature. Four out of seven zoos do not think manatees need a minimum or maximum air temperature, one of these zoos states that they do not need it if the water temperature remains warm or cool enough (1/7). Three zoos (3/7) do think they might need a minimum or maximum temperature but their opinions are divided. The minimum air temperatures mentioned by the experts vary between 18 ºC (2/7) and 22 ºC (1/7). The maximum air temperature temperatures vary between 32 ºC (1/7) and 40 ºC (1/7).

1.5.2 Ventilation

Enclosed facilities should be designed to provide a relatively uniform distribution of clean air, and to avoid strong draughts onto any pen enclosure. The incoming air should be fresh, not recirculated. The volume must be such as to assure an adequate supply of oxygen, reduce noxious or unpleasant odours, dilute airborne pathogens, and reduce saltwater humidity which tends to be corrosive. In accordance with generally-accepted standards for laboratory animal enclosures, a minimum of 10 air changes per hour is recommended for air conditioned rooms, and up to 20 air changes per hour otherwise. (CCAC, 1984)

1.5.3 Lighting

As said before indoor housing facilities for manatees should have lighting, This can be done by natural or artificial means, or both, and should be of a quality, distribution, and duration which is appropriate for the species involved, in this case manatees. Sufficient lighting must be available to provide uniformly distributed illumination which is adequate to permit routine inspections, observations, and cleaning of all parts of the enclosure. The lighting shall be designed so as to prevent overexposure of the manatees contained therein to excessive illumination. (USDA, 1998)
Specific data for what kind of lighting manatees should get is not available at this time.

1.5.4 Noise

The effect of noise on marine mammals is largely speculative. Circulating water systems cannot help but produce background levels of noise from pumps and motors. Animals seem to adjust to these droning sounds without difficulty or overt ill effect. Some species respond adversely to loud piercing sounds which are all too frequent during renovations. Noise from even distant construction activities are transmitted with little attenuation through the ground and into a pool enclosure. Under such conditions, the behaviour of some animals may be noticeably affected and they may go off feed. (CCAC, 1984)

1.6 Water treatment

For aquatic animals the water quality is an essential component of their environment.
The entire life-support system must be carefully designed and monitored. In general the primary enclosure shall not contain water which would be detrimental to the health of the manatees contained therein (USDA, 2001*d*).

Open or closed water systems
In an open system, the water supply is continuous and enters from a natural source, flows through the pool, and exits with no intentional recirculation (Dierauf and Gulland, 2001).
The waste water in an open water system is replaced by fresh water from a natural water flow. One of the simplest solutions is pumping the water from a natural source (e.g. the sea) through the exhibit and the waste out or constructing the exhibit as a sea-pen or fenced off lagoon. Unfortunately, such facilities have to be located in coastal areas and, moreover, such locations have to be free of pollution. "Semi-open" and "closed" systems are in more general use. These respectively provide for continuous partial replacement, or for occasional replacement of water lost through evaporation or waste. In a closed water system, waste water is circulated. (Joustra, 2003)
Closed systems require the most-intensive water treatment, since all the water is reused (Dierauf and Gulland, 2001).
Zoos mainly use a closed water (6/7) in stead of an open water system (1/7). The zoo that uses an open water system (1/7) is based in a natural spring and does not use any kind of filtration.

Fresh water or sea water
Manatees in the wild are found in fresh, brackish and salt water. If manatees are housed in a salt water pool, a source of fresh water should be provided this can be done by a tap, hose or another pool. Manatees should have access to fresh water because they are observed drinking fresh water in the wild. (Brook van Meter, 1989)
Captive manatees have been held in either fresh water or seawater (with freshwater drinking sources) without consequences. Increased skin sloughing has been observed in animals reared in fresh water when moved between seawater and fresh water. (Dierauf and Gulland, 2001)
Zoos mainly keep manatees in fresh water pools (6/7), the other zoo (1/7) provides the animals with two pools of which one contains fresh water and the other salt water. In the pool with salt water fresh water is provided with hoses.

Turnover rates
A water turnover rate of two hours or less is considered desirable for a closed or semi-closed system (CCAC, 1984).
The turnover rate is the amount of time it takes for a treatment system to pass the volume of a pool through its system once. For example a system that services a pool of 100 m^3 of water at the flow rate of 50 m^3 per hour is maintaining a turnover rate of two hours. The water turnover rates used in the zoos are 1 hours (4/7), 2 hours (1/7) and 4 hours (1/7).

Parameters

Zoos use the following parameters to determine water quality:

- Temperature (6/7)
- Bacteria (e.g. coliforms) (5/7)
- pH (5/7)
- Nitrate (NO_3^-) (4/7)
- Nitrite (NO_2^-) (4/7)
- Transparency of water (4/7)
- Ammonia (NH_3) (3/7)
- Salinity (3/7)
- Phosphate (1/7)
- Dissolved oxygen (1/7)
- Ozone (1/7)
- Conductivity (1/7)

The coliform bacteria count of the primary enclosure pool shall not exceed 1000 MPN (most probable number) per 100 ml of water. Should a coliform bacterial count exceed 1000 MPN, then there are the following options:

- The pool may be drained, refilled and tested
- The pool may be treated with a sterilizing agent, such as chlorine, bromine or ozone
- The following procedure may be conducted

  two subsequent bacteria counts must be taken at 48-hour intervals

  the results of these two counts are averaged with first count

  if the averaged count does not fall below 1000 MPN per 100 ml, then the water in the pool shall be deemed unsatisfactory, and the pool must be emptied or treated. (USDA, 2001d; USDA, 2001*a*)

Two tests are generally accepted by the USDA for testing for coliforms and are roughly equivalent; the multiple tube fermentation test and the membrane filter test (USDA, 2001*a*).

Water samples should be taken at least 60-90 cm below the surface of the water near the middle of the pool or the drain. The water samples should be taken from the same place and at the same time of the day, also prior to emptying the pool rather than just after filling the pool. (USDA, 2001*a*)

Records of coliform counts, pH and chemical additive concentrations must be kept, document the time when all samples were taken, record the results of the sampling and should be held for a one year period. (USDA, 2001*a*; USDA, 2001*d*)

The specific water quality tolerances and requirements for marine mammals vary considerably, but typical maintenance ranges for water in the tank are shown in table 2. These are general numbers are for marine mammals, specific maintenance ranges for manatees are not known for all parameters.

Table 2 Maintenance ranges for water in the tank (DEFRA, 2004)

	Marine	**Freshwater**
NH_3	<0,05 ppm	<0,1 ppm
Carbonate hardness	6500 mg/L most	<200 mg/L
Nitrite	<0,1 ppm	
Nitrate	<20 ppm	
0^2	>6 mg/L	>6 mg/L
pH	7,9-8,4	6,5-9
Redox	340+/-20 mV	
Salinity	27,5-32 ppt	5-9 sometimes used in therapy
Specific gravity	1.022-1.025	
Temperature – temperate	<15 ºC	<15 ºC
Temperature – tropical	23- 26 ºC	23-26ºC

It is important that the parameters are checked on a regular basis. In table 3 it is said if water samples should be taken and tested for the parameters on a weekly or daily basis.

Table 3 Schedule check parameters (USDA, 2001*a*)

Parameter:	**Check:**
Coliform	Weekly
Salinity	Weekly
pH	Daily
Chemical additives	Daily
Nitrate (NO_3^-)	Daily
Nitrite (NO_2^-)	Daily
Ammonia (NH_3)	Daily
Temperature	Daily
Transparency of water	Daily

1.6.1 Filtration

To be acceptable, the water filtration and purification system must effectively:

a Remove animal wastes
b Prevent the growth of harmful micro organisms
c Provide an environment that is relatively free of toxic chemicals
d Maintain a reasonable level of clarity (CCAC, 1984)

Mechanical filtration in a water treatment system is primarily designed to remove particulate matter; the most common type is a sand pressure filter. It consists of closed vessels containing a body of sand held above a supporting under drain assembly. Normally water is pumped into the vessel and passes the sand which strains and retains particulate matter. As soon as the sand gets clogged the pressure across the filters increases. When the prescribed level for cleaning the filter bed is reached, the filter flow is reversed and the dirt and water used for the cleaning operation is diverted to waste; sometimes, compressed air is used to "fluidise" the filter bed during the back wash cycle to ensure total cleaning. When the water washing the filter bed appears run clean, the system flow is returned to normal direction and continues to filter the pool's water. (Joustra, 2003)
Sometimes, filtration aids are added to sand filters, like for example the chemical aluminium sulphate. This chemical aids the precipitating (coagulating) of small particles within the water making it easier for them to be trapped by the filter bed. However, as with any chemical addition, testing and control is import as its accumulation within the pool water can cause eye

and mucus membrane irritation. Levels of chemical filtration aids should not exceed 0.3 mg/l. (Joustra, 2003)

Filtration alone is not sufficient to keep the water clean and clear. In paragraph 1.7 Cleaning additional methods to clean the water in the pools are given.

1.6.2 Biological treatment

The use of some form of biological control of organic pollution within aquatic mammal pools is a relatively new development which in part has grown from the popularity of large public aquariums and mixed mammal/fish exhibits. The biological treatments that are mostly used in zoos are: Nitrification (4/7), "protein" skimmer or foam fractionation (1/7) and UV light disinfection (2/7) and are discussed below.

Nitrification

The principles involved in nitrification are the deliberate culturing of species of aerobic bacteria known for their ability to convert ammonia to nitrite (*Nitrosomonas sp.*) and nitrite to the nitrate (*Nitrobacter sp.*). The process is normally achieved in chambers sited after particulate filtration which containing media such as stones or plastic shapes that offer a large surface area for the bacteria to grow on. (Dineley, 1998)

Nitrification is a microbial process by which reduced nitrogen compounds (primarily ammonia) are sequentially oxidized to nitrite and nitrate. The nitrification process is primarily accomplished by two groups of autotrophic nitrifying bacteria that can build organic molecules using energy obtained from inorganic sources, in this case ammonia or nitrite.
In the first step of nitrification, ammonia-oxidizing bacteria oxidize ammonia to nitrite according to the equation: $NH_3 + O_2 \rightarrow NO_2^- + 3H^+ + 2e^-$
Nitrosomonas is the most frequently identified genus associated with this step, although other genera, including *Nitrosococcus,* and *Nitrosospira.* Some subgenera, *Nitrosolobus* and *Nitrosovibrio,* can also autotrophically oxidize ammonia.
In the second step of the process, nitrite-oxidizing bacteria oxidize nitrite to nitrate according to the equation: $NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$
Nitrobacter is the most frequently identified genus associated with this second step, although other genera, including *Nitrospina*, *Nitrococcus*, and *Nitrospira* can also autotrophically oxidize nitrite. (U.S. Environmental Protection Agency, 2002)

For the removal of nitrate there are two possible pathways. There are several large algae that can use nitrate- N for the formation of organic nitrogen compounds, provided there is plenty of light available. If there is not enough light they will start metabolising organic nitrogen and in that way increase the nitrate concentration. Under anaerobic conditions, several bacteria species can use nitrate in respiration instead of oxygen. In the process elementary nitrogen, N_2, is formed, which will escape as a gas. (Joustra, 2003)

"Protein" skimmer or foam fractionation

Complementary to other biological treatments is the use of the "protein" skimmer or foam fractionation. Foam fractionation usually takes place prior to the biological system and is designed to generate foam that can be removed from the system as waste; the bubbles within the generated foam contain dissolved organics that are attracted by the air/water interface of the bubbles. The foam generators on these systems can also incorporate ozone that increases the oxidation of organic population. The effect in fresh water is minimal compared to the effect in salt water. (Dineley, 1998)

UV light disinfection
UV light disinfection is also incorporated into biological treatments: UV-C lamps produce radiation that is damaging to all forms of life by the disruption of a cell DNA bonding. The process takes place in sealed chamber containing an array of UV light tubes that the treated water passes around. The UV tubes are protected by quartz sleeves as glass is opaque to UV-C radiation. An important point to note of UV-C tubes is that they only have an effective operating life of six-months. (Dineley, 1998)

Also one zoo adds beneficial bacteria to the water of the pool. In most zoos combinations of biological treatments and chemical treatments are used (4/7).

1.6.3 Chemical treatment

Various chemical treatments, used in combination with filters, are designed to eliminate micro-organisms, algae, noxious (NOx^-) metabolites, and to prepare the wastes for filtration (CCAC, 1984). Chemicals that may be used in water treatment include Ozone treatment (5/7) and Copper salts treatment (1/7).

Ozone treatment
Ozone is a form of oxygen (O^3) which is very reactive in its ability to oxidise organic material. It is very unstable and has to be produced on site; it is generally mixed with treated water in a special reaction chamber before the water is returned to the pool. It is important that all ozone is removed before the treated water returns to the pool as it can be very noxious and dangerous. (Dineley, 1998)

Ozone has many advantages, including a high potential to kill both bacteria and virus, and has the bonus of producing water with a high clarity that may exclude the need to use coagulation chemicals such as aluminium sulphate. (Dineley, 1998)

However ozone has two disadvantages as well, because of its volatile nature, it does not leave any form of disinfectant residual in the pool water. This can lead to uncontrolled algae and bacteria growth on pool surfaces. Also it is unable to convert organic matter from the ammonia stage in the water conditions (pH levels) found in marine animal pools. (Dineley, 1998)

Nevertheless, these problems can be resolved by using ozone in tandem with chlorination as ozone will breakdown ammonia once the chlorination process has combined with this chemical to formed chloramines. Chlorination will also allow the benefit of in pool residuals to control bacteria and algae growths. Ozone's high effectiveness at oxidising organic matter also allows a lower concentration of total chlorine residuals. (Dineley, 1998)

It is suggested that in totally closed circuit water systems the use of ozone sterilisation is in favour, rather than exclusive reliance on chlorination. Although more expensive, it is more effective and safer for the animals. (Joustra, 2003)

Copper salts treatment
An electrical current can be used to suspend copper and silver ions in water returning to the exhibit from the filtration plant. A periodically alternating current is passed through a pair of electrodes consisting of a copper-silver alloy, resulting in the depleting of metal ions into water flowing through electrode cells. Copper ions act as an algaecide. Greatly decreased growth has been noted in cetacean exhibits, and the little algae that do grow is apparently not healthy since it is more easily removed than untreated algae. Silver ions act as both a bactericide and a flocculating agent. (Joustra, 2003)
Also one zoo uses locron (1/7) as a chemical treatment of the water.

1.7 Cleaning

All facilities must implement a written protocol on cleaning so that surfaces do not constitute a health hazard to animals (USDA, 2001*b*).

Animal and food waste areas other than the pool of water must be removed from the primary enclosures at least daily, and more often when necessary, in order to provide a clean environment, and minimize health and disease hazards.
Particulate animal and food waste, trash, or debris that enters the primary enclosure pools of water must be removed at least daily, or as often as necessary, to maintain the required water quality and to minimize health and disease hazards to the marine mammals. (USDA, 2001*e*)

The wall and bottom surfaces of the primary enclosure pools of water must be cleaned as often as necessary to maintain proper water quality. Natural organisms (such as algae, coelenterates, or molluscs, for example) that do not degrade water quality, prevent proper maintenance, or pose a health or disease hazard to the animals are not considered contaminants. (USDA, 2001*e*)

Equipment and utensils used in food preparation must be cleaned and sanitized after each use. Kitchens and other food handling areas where animal food is prepared must be cleaned at least once daily and sanitized at least once every week. Sanitizing must be accomplished by washing with hot water (82ºC, 180 F, or higher) and soap or detergent in a mechanical dishwasher, or by washing all soiled surfaces with a detergent solution followed by a safe and effective disinfectant, or by cleaning all soiled surfaces with live steam. Substances such as cleansing and sanitizing agents, pesticides, and other potentially toxic agents must be stored in properly labelled containers in secured cabinets designed and located to prevent contamination of food storage preparation surfaces. (USDA, 2001*e*)

Buildings and grounds, as well as exhibit areas, must be kept clean and in good repair. Fences must be maintained in good repair. Primary enclosures housing marine mammals must not have any loose objects or sharp projections and/or edges which may cause injury or trauma to the marine mammals contained therein. (USDA, 2001*e*)

A safe and effective program for the control of insects, ectoparasites, and avian and mammalian pests must be established and maintained. Insecticides or other such chemical agents must not be applied in primary enclosures housing marine mammals except when deemed essential by an attending veterinarian. (USDA, 2001*e*)

Most zoos clean the water in the pool with help of the filtration system (6/7). Many zoos vacuum the bottom of the pool (4/7), this can happen on a daily basis (2/7), 2-3 times a week (1/7) or once a week (1/7). This is done to remove the dirt which is still left by the filtration system on the bottom of the pool. The bottom of the exhibit and holdings of one zoo are siphoned as needed (1/7). And some zoos use manual scrubs or diving scrubs of the pool to remove algae (2/7). The removal of leftover foods and leaves with nets on a daily basis is also used by some zoos (2/7). One zoo uses about 10.000 guppies in the pool that will clean out a lot of the leftover food items (1/7). One institution that keeps manatees does not clean the pool at all, but relies on the natural tides of the natural spring (1/7).

2 Feeding

Providing a good diet, which fulfils as many aspects of natural feeding ecology as possible, is an essential consideration in the welfare of zoo animals. As a basic foundation of animal management, nutrition is also integral to longevity, disease prevention, growth and reproduction. (EAZA, 2006)

2.1 Diet

Manatees are obligate aquatic herbivores, but occasionally ingest numerous invertebrates (Reep and Bonde, 2006; Reeves *et al.,* 1992).
In the United States, captive manatees are fed on a wide variety of foods, including fresh, handcollected manatee- and turtle-seagrasses, lettuce (romaine and iceberg and other), cabbage, kale, carrots, sweet potatoes, bananas, apples, timothy and alfalfa hays, hydroponically grown wheat and oats, various commercially available monkey chow biscuits and other feeds designed for terrestrial species. The exact nutritional requirements of the manatee are unknown. However, many of the above food items have provided nourishment for animals from weaning to reproductive adulthood with concomitant successful rearing of healthy calves. (Dierauf and Gulland, 2001)

The main food items of the diet of a manatee kept in captivity are:

- romaine or iceberg lettuce (7/7)
- fruits like apples, pears and bananas (5/7).

Other items of the diet are:

- sweet potatoes and carrots (4/7).
- broccoli, dandelion greens, cucumber, celery, endive (2/7)
- brussel sprouts, hydrilla, hyacinth, alfalfa and bran with soaked dogfood (1/7).

2.1.1 Food quantity

Marine mammals should be fed on the basis of caloric requirements; small, young, actively growing animals require relatively more energy (food) than older or larger animals. Changes of this amount will occur as the animal goes through physiological and motivational changes, and during different seasons of the year. When preparing to feed marine mammals, one should first determine the caloric value of each food type. Then calculate the amount of food that provides the quantity of calories necessary to keep the animal in good health, making allowances for growth, activity etc. (Perrin, *et al.,* 2002)

The food requirements of a manatee are 5-20 Kcal/kg/day (CCAC, 1984). Adult males and nonlactating females consume about 7-9% of their body weight/day; calves consume about 15% of body weight/day; and lactating females eat about 10-13% of their body weight/day (Best, 1981).
To encounter these requirements, a manatee in captivity should eat on average 4% of their body weight. For an adult manatee in captivity this would be 18-25 kg per animal (5/7).

A general guideline is to provide a diet that incorporates 70–85% leafy green vegetables (e.g. romaine and iceberg lettuce, cabbage, kale) or hydroponic wheat, oats, and/or sprouts; 10 to 20% dried forage such as timothy or alfalfa hay (filtration must be upgraded to handle dried matter), about 5% of vegetables and fruits (carrots, yams, apples, bananas, etc.), and various commercially available dry pelleted feeds (monkey chow, elephant flakes, etc.). The specific vitamin and mineral requirements for manatees are unknown. (Dierauf and Gulland, 2001)

2.1.2 Varied diet

In the wild manatees feed on a wide variety of plant materials of submerged vegetation, but they also eat floating and emergent plants (Hartman, 1979). It is the responsibility of the keeper to monitor the manatee's appetite and feeding behaviour. The daily food intake needs to be adjusted so that the keeper can maintain behavioural motivation for husbandry and training purposes, while at the same time satisfying the animal's appetite.

2.1.3 Seasonal change in diet

Half of the zoos (4/7) have no seasonal changes in the diet of the manatee. These manatees eat the same food types the whole year round, although they have many food types, not a few and those are described in section 2.1 Diet. The other half of the zoos (3/7) do have seasonal changes in the diet, because some food items of the diet are not available throughout the year.

2.2 Nutritional values

A diet of good quality and mixed vegetables should provide a balanced source of fats, proteins, vitamins and minerals. A diet is considered to be "nutritionally balanced" when it provides appropriate levels of known dietary essential nutrients based on current knowledge and information. (American Society for Nutrition, 2007) The essential nutrients are not known for manatees.

2.2.1 Food components

The nutritional status of captive zoo and aquarium animals which rely on vegetables as food is dependent on the quality and composition of the vegetables they consume. See table 4 for the nutrient values of the main food items given to manatees.

Table 4 Nutrient values for the main food items (per 100 grams) given to manatees (Technical University of Denmark, 2007)

Food Item	Energy (cal)	Dry matter	Crude Protein (gr)	Crude Fat (gr)	Moisture (gr)	Sugar (g/100g)	Calcium (mg)	Phosphorus (mg)	Ash (gr)
Lettuce	69	5.1	1.6	0.2	94.9	-	36	45	0.9
Broccoli	187	11.1	5.3	0.6	88.9	2.5	44	87	1.1
Endive	78	6.2	1.3	0.2	93.8	-	52	28	1.4
Carrots	109	8	0.9	0.2	92	-	32	23	0.5
Sweet potatoes	342	19.4	1.9	0.3	80.6	1.03	7	55	0.9
Brussels sprouts	218	15.8	4.1	0.6	84.2	2.12	23	84	1.1
Apples	215	4.7	0.3	0.3	85.3	8.11	4	17	0.3
Bananas	342	5.6	1.3	0.3	74.4	15.90	4	27	0.8
Pears	227	14.6	0.3	0.3	85.4	9.53	10	19	0.3

2.2.2 Water intake

The freshwater requirements of manatees are not well known, but in the wild they are frequently spotted drinking freshwater from outfalls and culverts in salt and brackish water areas (Brook van Meter, 1989). In freshwater pools no extra fresh water is provided (6/7). In one zoo, where one of the pools is outdoors and salt, freshwater is provided from hoses (1/7).

2.3 Nutritional disorders

There are no nutritional disorders known by manatees in captivity (7/7).

2.4 Monitoring food quality

2.4.1 Food storage

Table 5 shows the time food can be stored.

Table 5 How to storage food (Virginia Polytechnic Institute and State University, 2001)

Food	Pantry (room temperature)	Refrigerator	Freezer
Broccoli		3-5 days	
Brussels sprouts		3-5 days	
Cabbage		1 week	
Carrots		2 weeks	
Cauliflower		1 week	
Celery		1 week	
Cucumbers		1 week	
Lettuce		1 week	
Sweet potatoes	2-3 weeks		
Dried vegetables	6 months		
Frozen vegetables			8 months
Apples	Until ripe	1 month	
Bananas	Until ripe	5 days (fully ripe)	
Dried fruit	6 months	2-4 days	
Frozen fruits			1 year
Pears	Until ripe	5 days	1 year

To ensure food stored in the refrigerator, freezer, or pantry is consumed within the expiration dates, practice FIFO (First-In-First-Out). When stocking food storage areas, place recently purchased items behind the existing food items. (Virginia Polytechnic Institute and State University, 2001) For more information about food storage see section B1.4.

All the zoos (7/7) store their food for manatees cooled.

2.4.2 Parameters of food quality

Food quality can be looked from different points of view; safety, nutritive, contributing to health, taste full, appearances, easy to process. The selection of parameters depends on the definition of food quality. Looks, firmness and shelf life are valuable "outside" parameters. "Inside" parameters are taste and analytical measurements. When food safety is of major concern, pesticide residues, mycotoxins levels, amount of heavy metals, dioxins, micro-organism can be measured. Often food quality is also related to specific health promoting substances. Specific foods are expected to have health promoting effects through the presence of these specific nutrients. (Van de Vijver, 2007)

2.5 Method of feeding

It is the responsibility of the keeper to monitor the manatees' appetite and feeding behaviour. The daily food intake needs to be adjusted so that the keeper can maintain behavioural motivation for husbandry and training purposes, while at the same time satisfying the animal's appetite. (CCAC, 1984)

2.5.1 Adults

Free-ranging manatees graze 5 or more hours/day, so captive daily feeding should be dispersed over the day to allow this behaviour. Smaller quantities of food given more frequently during the day will permit normal grazing behaviour and help prevent filtration systems from being overwhelmed. (Dierauf and Gulland, 2001)

Half of the zoos (4/7) feed their manatees 2 to 3 times a day, the other half (3/7) feed their manatees more than 3 times a day. Food items given to the manatees are fed in pieces (6/7), fed in whole (5/7), fed on a fixed feeding spot (3/7) and scatter feed (5/7).

2.5.2 Calves

Manatee calves can be reared in captivity. Please refer to section B5.4 for information about hand-rearing and weaning.

2.5.3 Sick animals

See paragraph 7.5.3 Tube feeding.

3 Behavioural and social structure

3.1 Behaviour

Welfare concerns are associated with captivity, including capture and transport, sources of stress such as restricted space and poor water quality which can lead to reduced longevity (UNEP, 2005). It is very difficult to see what manatees are feeling, because manatees have no noticeable facial expressions. Therefore there are only a few indications of stress. (pers. comm. K. Andersen Hansen, 2008)

Manatees spend many hours of the days seeking food, sleeping, or travelling, they also spend approximately 6-10 hours at rest with no apparent pattern in their activities. (Smithsonian Marine Station at Fort Pierce, 1969) Manatees normally swim a slow 2-3 km/hour propelled by the vertical movements of the flattened tail but they are capable of short bursts of speed up to 25 km/h. (Food and Agriculture Organization of the United Nations, 1996)
They rest intermittently on the bottom or float suspended in the water. They come up for air 20 or 30 times an hour but can remain submerged for 15 to 20 minutes. (Food and Agriculture Organization of the United Nations, 1996) Two basic resting postures that are employed by manatees are hanging suspended near the surface and lying prostrate on the bottom. In both positions, the animals lapsed into a somnolent state with their eyes closed and their bodies motionless. The eyes are only opened at the surface. Manatees allow the tail and flippers to dangle in typical suspended resting. (Hartman, 1979)
In the wild, manatees frequently feed over shallow grass beds in close proximity to deeper water, to which they flee when startled by approaching watercraft or other disturbances. (Nowacek *et al.*, 2004) More information about their behaviour see section A2.4.

3.1.1 Behavioural problems

Part of the zoos does not have behavioural problems (3/7). Only male-male aggression is seen is zoos. (1/7)

3.2 Basic social structure

Behaviour problems can be prevented by a good social structure. Captive marine mammals must be given access to other animals except when they are temporarily maintained in isolation for such purposes as medical treatment or training and given special attention. Attention should be paid to the species involved, their natural habitat and the available space in the zoo. (Ridgway, 1972)

Group size
Manatees live in loosely associated social groups (Hartman 1979), with most cohesive social interaction among manatees occurring only in mating herds (Smithsonian Marine Station at Fort Pierce, 1969).Though they can form large aggregations, for example at warm water sites, but most frequently they are sighted alone or in small ephemeral groups. (Powell, 2002)

A minimum group size of 2 manatees is mentioned by the experts (5/7). Also groups sizes of 1 (1/7) and 3 (1/7) are mentioned. An average group size of 6 manatees is kept in zoos (ISIS, 1973)

Composition of group

Though males and females form mating herds during the breeding period, they can be kept together without problems. Half of the zoos keep both sexes and all have different male/female ratios (4/7) and some zoos only have females (3/7). Manatees in the contacted zoos are kept all in one group (5/7), kept in several groups (1/7) or kept alone (1/7).

3.3 Changing group structure

When introducing of a manatee to a new exhibit environment and to other animals, they must be given time to feel comfortable with the exhibit and with the keepers before the introduction of other animals. It may assist if the initial place of introduction to other animals is a neutral territory. A slow introduction is desirable. (pers. comm. K. Andersen Hansen, 2008)

3.3.1 Minimising procedures for aggression

Compatibility problems can, to some extent, be minimized by adherence to the following procedures:

- Removing either the offending or the victimized animal from the pool.
- Modifying the aggressive behaviour by training techniques
- Either not introducing new animals into an established colony at all or, if this cannot be avoided, bringing them in as pairs or as a group.
- Removing the dominant animal and reintroducing it only after the new ones have become established.

These precautions notwithstanding, it is difficult to solve dominance or other incompatibility problems, once they arise, except by permanent separation. (Verbruggen and Vergeer, 2002)

3.4 Sharing enclosure with other species

In nature manatees share their habitat with all kinds of animals and in most zoos (6/7) manatees share their enclosure with fish (6/7), like guppy's, arapaimas, catfish and pacu's; turtles (4/7); rays (2/7), like freshwater stingrays; capybaras (1/7) and birds (1/7).

3.5 Stress

Temporary stress to some degree is a natural part of life and involves numerous uncertainties in the environmental conditions to which all organisms are exposed. Stress is a condition of the animal as a consequence of a threatening event that causes insecurity in the animal. Being in an artificial environment can induce stress and poor health in an animal especially if the animal is in the initial introduction period. Social stress can include isolation and too few social interactions, and low social status. Animals should be monitored for signs of stress. (Verbruggen and Vergeer, 2002)

The following behavioural indicators can be considered to be negative or undesirable reactions to a social situation:

- Spinning (turning around own axis) (3/7)
- Pacing (2/7)
- Swim same route all day (1/7)

Coldstress is one of the main stressors by manatees, but is less important by captive manatees, because pool temperature is regulated. For more information about the cold stress syndrome see section 7.6.2.

Constant exposure to high levels of noise can potentially cause behavioural disruptions and physiological stress that may adversely affect the manatee. Specific sound sources have been shown to cause profound behavioural disruption in many marine mammal species. For example, the animal could be tolerating the noise, unable to hear the noise, or physiologically reacting with an internal stress response not indicated by physical movement. Stress can result in delayed growth, decreased reproduction, and decreased food intake indicative of a stress response in elevated noise conditions. (Miksis-Olds, 2006; National Academies' Ocean Studies Board, 2003) Stress can be prevented by behavioural enrichment (Ministry of agriculture, nature and fishery, 2002). See section B4 for enrichment.

4 Training and Enrichment

4.1 Training

Training animals for husbandry and medical procedures eliminates stress once associated with shifting and medical exams. Training animals to participate in medical exams has, in many cases, already eliminated the need to dart an animal with a potentially dangerous tranquilizer just to give it an injection or take its temperature. (Martin, 1999)

The main purposes of given training to manatees are:

- Health check (3/7)
- Blood and urine sampling (3/7)
- Body weight (2/7)
- Behaviour (2/7)
- Training on target, which would make it easier to separate the manatees (1/7)

4.1.1 General learning principles

There are several general learning principles, namely positive reinforcement, negative reinforcement, (positive) punishment and negative punishment. For more information about these principles we refer to Gatz, 1998.

4.1.2 Training and welfare

In a human-animal relation, like training manatees, it is important to make sure the animal is in a good state of wellbeing. Important for the animal are the values of predictability and controllability. These ideas can be connected with the learning-principles of conditioning. By classic conditioning an animal learns to predict events in its environment and by operant conditioning an animal learns to influence (control) its environment by the effect of its behaviour. If these conditions are not in balance with the animal, the animal suffers from stress. Stress means the animal is in a bad state, physically and/or mentally. Research on welfare concludes that possible causes of stress (and so a possibility to affecting welfare) can be:

- unpredictability (e.g. the moment of getting punishment)
- uncontrollability (e.g. inconsistent use of commands)
- injury or pain
- excessive fear
- overburdening

Higher chance for illness and untimely mortality are likely effects. (Gatz, 1998)

4.1.3 Training manatees

Marine mammal training programs must rely heavily on positive reinforcement. Because the field of marine mammals minimises the use of punishment techniques, it is highly productive and continues to stand as a model for ethical training of animals. Empirical evidence suggests that positive reinforcement has a much greater influence in motivating animals to voluntarily engage in behaviours, whereas punishment suppresses the expression of behaviour. Additionally, negative reinforcement serves to increase the development of undesired behaviours, such as anxiety, escape, active and passive avoidance, frustration, aggression and learning helplessness (a state of ‘giving up’). (Gatz, 1998)

The first step in training manatees with targets is to determine which food items they prefer. Various fruits, vegetables and biscuits can be offered. The vegetables can be cut into little pieces and used as rewards. At a correct choice, the trainer blows a whistle, the targets are removed and the animal receives a piece of vegetable as a reward. During the training period, in the case of a wrong choice, the animal receives no food reward, the targets are removed and, after an intertrial interval of 30 seconds, the next trial starts. During the tests, the animals are always rewarded with food, but in the case of a wrong choice do not blow the whistle. (Griebel and Schmid, 1997; Young Harper, 2004)
In literature not much is known about training manatees. A few articles cover only a part of the topic training manatees.

More than half of the experts (4/7) are training with their manatees. How many times training is given differs from expert to expert. Answers vary from training once a day (1/7), twice a day (1/7) and three times a week (2/7).
Training sessions length vary between 10 (1/7), 15 (2/7) and 20 minutes (1/7).

Food items that are used during training sessions are items like carrots (2/7), apples (2/7), bananas (1/7), boiled potatoes (1/7), corn on the cob (1/7), mussels (1/7), and vegetables that are preferred by the animal (1/7).

4.1.4 Husbandry training

Basic husbandry procedures and physiological assessment of manatees typically have been limited by the difficulty of restraining these large animals and the risk of injury to subjects and personnel. To address this problem, manatees can be trained for seven tasks: swimming onto a stretcher; stationing for body measurements; voluntary presentation of flippers for venipunctures (medical and lateral); providing urine samples; submitting to rectal measurement of core temperatures; positioning for ultrasound assessment of blubber thickness; and measuring respiration volume and flow rate. Manatees can be trained to perform these tasks using standard conditioning procedures, which eliminated the need to drop water levels or to restrain subjects. The ability to meet these training objectives increased health assessment opportunities, improved the level of health care, furthered collection of baseline biological data, and facilitated physiological research. (Colbert *et al*., 2001)

The foundation for successful husbandry training is desensitisation. Desensitisation is a continuous process by which new and potentially frightening (and often uncomfortable) conditions are slowly introduced to an animal. Such introductions are paired with positive reinforcement. (Gatz, 1998)

4.2 Enrichment

The term enrichment will be defined very broadly, encompassing the animal's entire captive environment, its physical, social and cognitive environment, the role of human caretakers (feeding, cleaning, training, other interactions), diet (type, presentation, and variety), etc. Providing behavioural and environmental enrichment is important for the psychological and physiological wellbeing of any captive animal. (Weiner *et al*., 1999)
Environmental enrichment is anything that changes the animal's normal circumstances. It can be a matter of altering their environment and/or behaviour - for example, by changing a stereotypic swim pattern. It is necessary to provide behavioural enrichment in order to help avoid behavioural problems and reduce other inappropriate behaviour like aggression, stereotypic patterns and stress-related problems, since these are more likely to arise if the animal

is not mentally stimulated. Environmental enrichment programs can increase the activity of the animals. Animals spend more time foraging, interacting and exploring the environment for toys if providing the animals opportunities to engage with different objects or toys. (Weiner *et al*.,1999)

Environment enrichment can provide opportunities and motivation for animals to practice species-appropriate behaviour, can reduce stress and undesirable behaviours, encourage breeding and positive social interaction, and generally improve animal welfare. Enrichment also influences the physical, mental, and social well-being of animals, which often results in the overall health of the animal and may therefore be considered an integral component of a preventative veterinary medicine program. (Martin, 1999)

4.2.1 The exhibit

Naturalistic exhibits are being built at many zoos and often provide great enrichment value for animals. Many exhibits are designed with the species' natural history in mind and target the expression of species-typical behaviour as a goal for the exhibit. Although these exhibits are aesthetically pleasing, they do not always live up to their expectations. (Martin, 1999)
These exhibits may offer the opportunity for animals to practice species-typical behaviour but sometimes the animals lack motivation to use the exhibit to its fullest potential. The lack of activity, overweight animals and aberrant behaviour are reminders at some exhibits that there is something lacking in the lives of the animals that inhabit them. In many cases, it is a lack of motivation. (Martin, 1999)

4.2.2 Social interaction

To make the animals more at ease it is important for animals to be in contact with other animals. Social interaction on its own can be a powerful, inexpensive, enrichment tool affecting positive behavioural changes in an animal which inevitably results in a healthier lifestyle for the animal. (Warrick, 2000)

4.2.3 Food items

There are several food items used in zoos as enrichment for manatees. Half of the experts use food items as enrichment (4/7). The most food items that are used are fresh branches with leaves and bark (2/7), romaine lettuce and dandelions tied onto a hoop and floated in the exhibit (1/7), Whole lettuce (1/7) and in high tide, manatees can eat vegetation from the shore line (1/7)
These food items are fed in pieces (6/7), fed in whole (5/7), fed on a fixed feeding spot (3/7) or scatter fed (5/7).

Some food enrichment devices are:

The ice float
The ice float (see figure 16) remains at the surface of the water and the manatees bob for it and grasp it with their pectoral flippers. Manatees like to roll over onto their backs and rest the float on their stomachs while they munch on it.

Materials:
1 Rubbermaid tub, filled with water, vegetables and fruit. Then freeze.

Figure 16 The ice float (Mote Marine Laboratory, 2005)

The kale feeder

The kale feeder (see figure 17) is designed to float freely throughout the tank suspended just below the surface of the water. The manatees chase it and graze on the kale, much like bobbing for apples.

Materials:
1 90 centimetres long PVC pipe with a 15 centimetres diameter (with holes drilled in it)
2 15 centimetres PVC end caps
Kale

Figure 11 The kale feeder (Mote Marine Laboratory, 2005)

4.2.4 Toys

Because captive manatees do not live in a natural marine environment, several environmental enrichment devices (EED's) have been developed to keep them mentally stimulated. Making EED's for manatees is very challenging because of the manatee's strength and size and the use of their flexible pectoral flippers and lips to manipulate objects. Environmental enrichment devices for these animals must be designed with the following criteria in mind:

- Can not be swallowed
- Can not be torn or ripped (and then swallowed)
- Can not be crushed or broken
- Can not trap or entangle the animal
- Can not cut, poke, or scrape the animal

Most of the zoos (5/7) do not use toys for their manatees. The two zoos that do use toys use floating toys, like balls, and scratch posts and scratch mats.

Enrichment with natural products is not practised in most zoos (5/7), two zoos use natural products, which are live natural plants as water hyacinths (1/7) and plants, which manatees can eat, that naturally grow in the exhibit and manatees that can eat vegetation with the changing tide (1/7).

There are several enrichment toys developed for manatees, like:

The back scratcher
The back scratcher, (see figure 18) is designed to sink to the bottom so that the manatees can rub various parts of their body on the bristles. They have also been seen carrying the entire device around the tank with their pectoral flippers.

Materials:
1 245 centimetres long PVC pipe with a 20 centimetres diameter
2 20 centimetres PVC "T's"
4 20 centimetres PVC end caps
4 industrial strength shop brooms

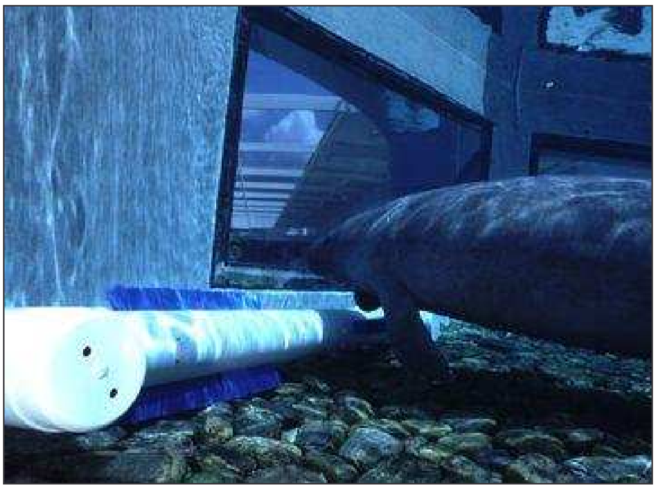
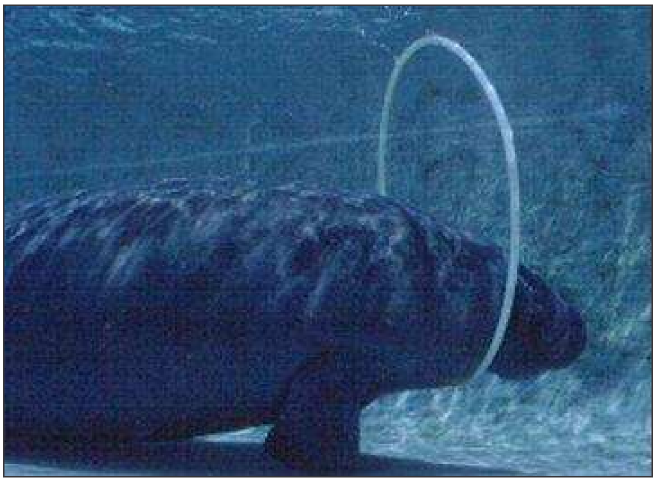


Figure 28 The back scratcher (Mote Marine Laboratory, 2005)

The hulahoop

The hula hoop (see figure 19) hangs vertically in the water and the manatees are often seen wearing it around their necks and paddles.

Materials:
1 extra large hula hoop with holes drilled in half of it



Figue 19 The hulahoop (Mote Marine Laboratory, 2005)

The brush gate

The brush gate (see figure 20) fits into the gate area between a shallow portion of the tank and the medical pool or another part of the pool. The manatees rub their vibrissae on it as well as all other parts of their bodies. This helps remove dead skin as it is sloughed.

Materials:
1 square of 5 centimetres PVC pipe built to the dimensions of the gate
15 industrial scrub brushes
Stainless steel nuts and bolts

Figure 20 The brushgate (Mote Marine Laboratory, 2005)

The texture mat

The texture mat (see figure 21) rests in the shallow area of the manatee exhibit, however it is only presented for short periods of supervised time. The manatees rub their vibrissae back and forth across each of the textures.

Materials:
1 180 centimetres by 90 centimetres industrial non-slip rubber floormat
1 rubber-coated sink drying rack
1 rubber sink mat
1 straw welcome doormat
1 artificial turf doormat
1 textured moulded rubber doormat
1 carpet sample

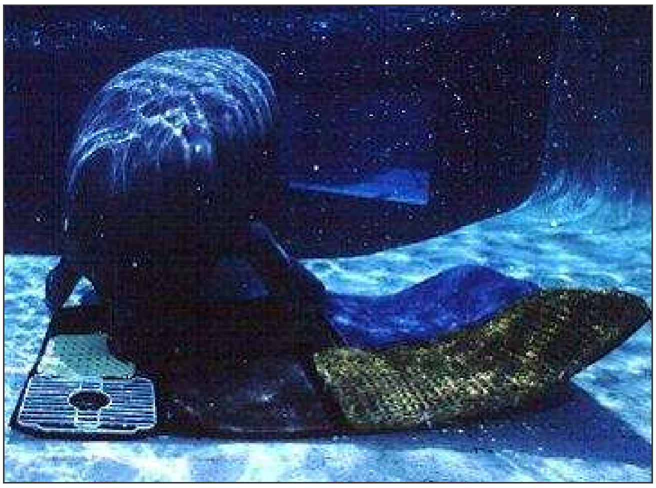


Figure 21 The texture mat (Mote Marine Laboratory, 2005)

The fibre grating square

The fibre grating square (see figure 22) is secured to the outside of the tank and suspended just below the water's surface. The manatees like to rub their vibrissae and pectoral flippers over it.

Materials:
1 45 centimetres by 45 centimetres square of fibre grating
1 nylon rope

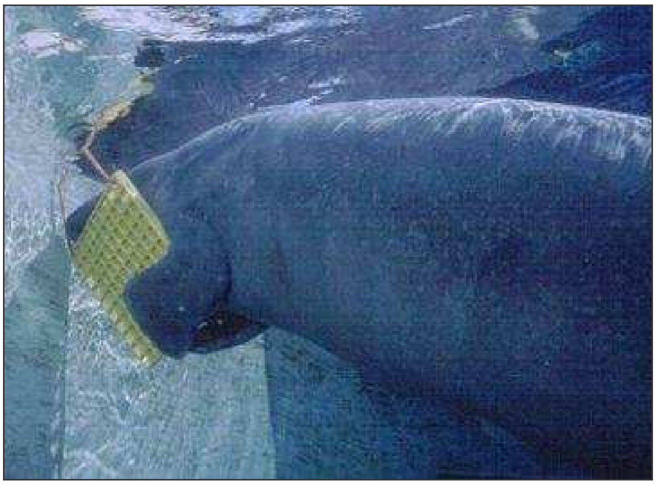


Figure 22 The fibre grating square (Mote Marine Laboratory, 2005)

Vacuum hose
Similar to the hula hoop, the manatees wear the vacuum hose (see figure 23) around their bodies while swimming.

Materials:
1 300 centimetres length of pool vacuum hose, sealed into a loop and partially filled with water

Figure 23 The vacuum hose (Mote Marine Laboratory, 2005)

5. Breeding

5.1 Mating

Behaviour interpret to be mating has been observed in all seasons and there is no clear peak season for calving (Reeves *et al.,* 1992).
Reproductively active adults often form mating herds, which consist of one focal female pursued by several males. Individual male members participating in the herd are transitory, try relentlessly to hold on to the female, and roll over in attempts to gain access to her ventrum for mating. (Bengtson, 1981)
Males can pursue a focal female for two to four weeks; however, physiological oestrus may not necessarily occur during the entire period of pursuit but, instead, could last for only a brief period during the whole mating herd scenario. Observations of captive breeding suggest mating behaviour is not an accurate indicator of female reproductive status (Odell *et al*., 1995), but unpublished hormone data indicate physiological oestrus may range from one to six days (Larkin, 2000; Odell *et al.,* 1995). It is possible that the majority of the two to four weeks of male pursuit comprises the establishment of dominance among males or relates to a strategy of sperm competition, with males breeding as frequently as possible while the female is receptive. Female manatees exhibit promiscuous breeding behaviour, mating with several males in the herd. As a breeding strategy, this may be more specifically described as "scramble competition polygyny". (Larkin *et al*., 2005)

There are several breeding problems observed in the zoos (4/7). Stillbirths, no mating at all and a female who developed uterine infectious during late pregnancy. In half of the zoos (4/7) there is no aggression during the breeding period or no mating has occurred. In 2 of the 3 zoos where mating has occurred there was aggression between male/male or the female was aggressive.

5.2 Pregnancy

The pregnancy process in captivity is similar to the situation in the wild. For information about gestation period and parturition, etc. please refer to section A2.3.

5.2.1 Contraception

The most common methods of reducing fertility have been physical separation, castration of males and contraception of female animals. (Dierauf and Gulland, 2001) There are no contraceptions known for manatees and all zoos (7/7) do not use contraception.

5.3 Birth

In the wild female manatees usually produce one calf with each pregnancy. Twins are rare but do occur in approximately 2% of the births. (Brook van Meter, 1989)
At birth, the fetus emerges from the bathing fluid of the sac surrounding it, and it loses its mother's circulation via the umbilical cord. The difficulty and the duration of the birth are highly variable. If the young mammal goes without oxygen (remains asphyxic) for too long, the oxygen content of the blood becomes inadequate to satisfy the demands of the brain, and permanent cerebral damage may then occur. (Gage, 2002)
Beginning immediately after birth, the mother repeatedly assists her calf to the surface with her flippers or back, allows it to breathe, and then lowers it until a rhythm is established. Newborns

swim using only their flippers, and learn to use their tail several days later. (Nabor and Patton, 1989)

Newborns of the West-Indian manatee in the wild have an average length of 1.2 – 1.4 m and weigh 30 kg (Smithsonian Marine Station at Fort Pierce, 1969). Suckling occurs underwater when the female is suspended at the surface or lying on the bottom. Calves grasp the teat (located under each flipper) and suckle for up to 2 minutes. Calves begin grazing a few weeks after birth and, as they mature, alternate grazing and suckling, becoming less and less dependent on milk. (Powell, 2002; Nabor and Patton, 1989)

In almost half of the zoos (3/7) mating has occurred and calves are born. In one zoo there were 4 youngs born, whereof 2 were stillborns. In one zoo there were 3 youngs born, whereof 1 died within 4 days. In one zoo there were two youngs born.
In 4/7 zoos there is no mating between females and males. In one zoo one calf was born which was fathered in the wild, before the female was captured. Ever since this calf was born the female shows strange behaviour and is sometimes very aggressive to the male manatee in the pool and sometimes to the keepers. There after no other mating has occurred. (pers. comm. A. Mæland, 2008)

5.4 Development and care of young

Development in young has been handled in section 2.3.5. There are no differences known between the development op young in the wild and in captivity situation.

5.4.1 Handrearing

Record keeping
By handrearing, keep a daily log of food intake, faecal production, consistency and appearance, body weight and behavioural notes, including activity, attitude and appetite. Keep careful records on formula adjustments. (Gage, 2002)

Equipment
The following items should be available for handrearing:
- Blender: Heavy duty commercial blender
- Large calf bottles/ baby bottles and a variety of calf and lamb nipples
- Microwave or other method to heating formula
- 12 to 16 French stomach tube may (Gage, 2002; Dierauf and Gulland, 2001)

Criteria for intervention
Wild manatee calves may be abandoned or orphaned. Captive manatee calves that are rejected by the cow, or are unable to nurse either due to poor milk production in the cow or because the calf is weak, by necessity will need to be hand reared. (Gage, 2002)

Manatees can be reared in captivity and the formulas (see table 6 and 7) need to be based on the nutrients in the mother's milk. Analysis of manatee milk has revealed that taurine is a major amino acid and that short- and medium chained fatty acids are abundant. With this information it was determined that the original Esbilac (also known as Milk Matrix 33/40 milk replacer) with 16 ml of either soybean or canola oil was comparable with manatee milk. The most common complication with this formula is usually related to constipation. The constipation is most likely due to starting a dehydrated calf on the formula before correcting and maintaining hydration during the adjustment to the bottle. (Dierauf and Gulland, 2001)

Table 6 Miami Seaquarium Formula for handrearing calves (Dierauf and Gulland, 2001)

Zoologic (Milk matrix 30/55)	400 gr
Goats milk	210 ml
Isomil	120 lm
Water	400 ml
Lactinex	1 tablet/ 100 ml formula
Children's chewable vitamins	1 tablet/ 300 ml formula

Table 7 Seaworld Formula for handrearing calves (Dierauf and Gulland, 2001)

Zoologic (Milk matrix 33/40)	500 gr
Water	1000 ml
Taurine	250 mg/l formula
B-complex	1 tablet/ l formula
Canola oil	4 to 16 ml/100 g formula

The canola oil has to be gradually increased; at 4 ml/100gr formula, the caloric density is approximately 112 Kcal/100gr. These formulas are recommended when the ingredients are available. A number of other formulas have been utilized using goat milk alone or soy-based products. Analysis of manatee milk reveals the presence of a number of sugars, including small amounts of lactose. Manatee calves can tolerate up to 5% of the diet as a carbohydrate. (Dierauf and Gulland, 2001)
None of the zoos had experience with handrearing of manatee calves.

Delivery methods and techniques
Often manatee calves will not nurse from a bottle and it may be necessary to utilize a stomach tube to deliver the formula and prevent dehydration. A 12 to 16 French stomach tube may be used for oral of nasogastric intubation. Calves will often take a few days to accept a nipple when bottle feeding. Formula may be fed with a Johnson and Johnson 'health flow' baby bottle, using a lamb or calf nipple. Initially, introduce the bottle hourly. Some calves prefer nurse on their backs. It is important that all caregivers use similar nursing techniques to encourage the calf to accept a bottle more rapidly. (Dierauf and Gulland, 2001)

Feeding frequency and daily requirements
Once the calf begins suckling, the bottle should be offered every 2 to 3 hours. The maximum formula allowed per feeding should be 300 ml (see table 8). If dehydrated, the calf may need to be tube-fed between bottle-feeding attempts. Calves nursing well on a bottle may gain 0.3 to 0.5 kg of weight per day. A 20 kg calf can be safely given 125 to 150 ml of formula with a stomach tube every 3 hours. If the calf is very thin or hypoglycemic, add 1 ml of 50% dextrose/kg body weight to the formula. If glucose is added, a balanced electrolyte solution is used in place of water. The stool and blood should be monitored for excessive glucose concentrations. The amount of glucose may be reduced as other nutrients are utilized as an energy source. The utilation of fat may reduce the additional glucose requirements.

Table 8 Feeding frequency and daily requirements of formula for manatee calves (Dierauf and Gulland, 2001)

Day 1	Glucose and Electrolyses
Day 2	25% formula, 75% water
Day 3 to 4	50% formula, 50% water
Day 5	75% formula, 25% water
Day 6	100% formula

If there is an intestinal disease and the formula is not appropriate, the calves should be given an elemental formula (see table 9) comprised of:

Table 9 Elemental formula for calves with a disease (Dierauf and Gulland, 2001)

Criticare HM (Mead Jonhson, Evansville, IL)	35 ml
MCT oil (medium-chain triglycerides)	28 ml
Nutramigen (baby formula)	103 ml

This formula contains 200 kcal/100gr and is recommended with thin, hypoglycaemic calves during the initial adjustment period or for severe colitis or pneumototis intestinalis. (Dierauf and Gulland, 2001)

Weaning procedures

The calves may need to be fed for longer periods when they are maintained with adults, and access to solid food is limited to lettuce. When competition is a factor, the calf may need supplementation for 18 to 24 months. Access to green vegetation may be initiated as early as 1 month of age. During the weaning process, the calves should be weighed every week, more or less, depending on the progression of weight gain (or loss). (Dierauf and Gulland, 2001)

Other practical information

Orphaned manatee calves often present with a number of medical problems, including emaciation, hypoglycemia, dehydration, hypothermia, septicemia, entercolitis and constipation. The presence of an umbilical cord can help estimate age, but newborns typically have weight ranges from 17 to 50 kg (average 20-30 kg). Calves should have blood sampled for complete blood counts and serum chemistries. They are sampled once or twice a week to monitor blood glucose levels. With severe hypoglycemia, intravenous 5% dextrose in water is recommended, but intravenous infusion is difficult. Glucose supplementation is designed as a preventive measure until the calf is able to utilize other sources of energy. Underweight neonates that are initially hypoglycemic and respond well to glucose, but are not adequately supplemented, will reach metabolic exhaustion within a week. Therefore, supplemental nutrition must include proteins and fats within the first few days of hand rearing. Manatee colostrum contains 46 Kcal/100gr; milk during midlactation has 189 Kcal/100gr; and 130 Kcal/100gr in the late lactation. Some formulas make up for the lack of caloric density by increasing the daily volume. Manatee colostrum and serum immunoglobulins have been utilized with these neonates. Manatee IgG is given intravenously and/or orally to very young calves. Neonatal calves are often maintained in freshwater pools between 30 and 32˚C with 1 part per million chlorine. (Dierauf and Gulland, 2001)

6. Handling

6.1 Catching/restraining

6.1.1 Capture

When building a facility for manatees it is important to think about how you want to catch or examine these animals when this is necessary.
The easiest way to capture a manatee is to have a separate pool or section of the pool which can be closed off from the other manatees kept in the enclosure (pers. comm. B. Klausen, 2008). Manatees can be trained to follow a target and go into a section of the pool, also these animals can be trained to swim onto a stretcher (Colbert *et al*., 2001). When a facility has a crane installed near the enclosure of the manatees, the manatees can also be trained to get used to being lifted by a crane (Colbert *et al*., 2001).
If the animal is trained to go into that section it will go there when given the sign to go there, when the animal is not trained it can be chased or lured into that pool or section. To lure the animal to that section food items can be used, but if there are more manatees kept in that pool this can cause a problem because more manatees will go to that section. To chase the animal to the section or pool multiple persons have to enter the pool and chase the animal to the area where it will be caught. The persons entering the pool have to take care for the strength of the manatee, especially the fluke of this animal can cause serious injuries. A way to protect the persons entering the pool is to keep enough distance from the animal so they won't get hit and to wear a protective headpiece. (pers. comm. B. Klausen, 2008)
When the animal is in the separate pool or closed-off section the animal can be put in a stretcher or a nylon net. The animal can be trained to swim onto the stretcher but if the animal is not trained, persons have to be in the pool with the animals and put the stretcher or net around the body of the animal so it can be lifted out of the pool. When the animal is in the stretcher or net it can be lifted out of the pool with a crane. The animal can then be placed in a transport box or on a foam pad if it has to be examined. When the animal is in a transport box this box can be placed in a truck. (pers. comm. B. Klausen, 2008)

Manners used to move manatees from the pool to another location (transport box or other pool) are cranes (5/7), slings or stretchers (2/7).

6.1.2 Restraint

Manatees are extremely powerful and will thrash dorsoventrally, laterally, and roll violently during restraint procedures. The fluke is especially dangerous during struggling. These movements can easily cause serious injury to handlers and animal caregivers.
In water rescue situations, handlers must use extreme caution to avoid being entangled in the net and pulled into the water. Manatee calves are also strong and should be restraint with caution. Injury to the manatee or handlers can be minimized by removing the manatee completely from the water. This can be done by draining the water from the pool and stranding the manatee on the pool bottom, of by placing the manatee in a stretcher in low water and moving it to a dry work area. While restrained, the manatee should be placed on thick, closed-cell foam that is a least the length and about twice the width of the animal. At least four or five experienced people are required to restrain an adult manatee during this procedure. Manatee calves can be restrained and supported in the water by a single person. However, calves must be placed in a stretcher before removal from the water, because one person cannot safely lift a manatee calf. Most diagnostic and therapeutic procedures can be accomplished using these stranding methods. Additionally, when an animal is in a stretcher,

its body weight can be determined using a mechanically assisted hoist, crane, or block-and-tackle system. If a manatee becomes too fractious during restraint, a restraint board and/or sedation should be considered. While out of the water, manatees should be sprayed with water to prevent skin desiccation and overheating. (Dierauf and Gulland, 2001)

Chemical restraint can also be safely used in manatees. Generally, chemical restraint is used to calm fractious, healthy, adult manatees. See table10 for the sedative drugs that can be used and in which doses. With these drugs, movement and respiration are decreased, and the manatee may appear asleep, although it will respond to stimuli and have a palpebral reflex. (Dierauf and Gulland, 2001)

Table 10 Commonly used sedative drugs in manatees (Dierauf and Gulland, 2001)

Sedative drugs	Dose	Frequency	Route
Midazolam hydrochloride	0,045 mg/kg[a]		IM
	0,080 mg/kg[b]		IM
Diazepam hydrochloride	0,066 mg/kg[a]		IM
Meperidine hydrochloride	0,5-1,0 mg/kg[c]		IM

[a] For tranquilization with effects lasting 60 to 90 minutes; may be reversed with flumazenil (IM) on an equal-volume basis.
[b] To facilitate intubation for general anaesthesia; may be reversed with flumazenil (IM) on an equal-volume basis.
[c] To provide sedation/analgesia for minor surgical procedures.
IM = Intramuscularly

6.1.4 Stress

Not much is known about stress in manatees, but animals can become agitated when lifted in the air by a crane (Colbert *et al.*, 2001). See B3.5. for more information about stress in manatees.

6.2 Transportation

6.2.1 Transport

The primary enclosure used to transport manatees should comply with the following requirements:
- Straps, slings, harnesses, or other devices used for body support and restraint, must not prevent access to the manatees by attendants, must be equipped with special padding to prevent trauma and injury, must be capable of keeping the animals from thrashing about and causing injury to themselves and the attendants.
- The enclosures used to transport manatees must be large enough to assure that each animal has sufficient space for support of its body in slings, harnesses, or other supporting devices if used, without causing injury due to contact with the primary transport enclosure. (USDA, 2005*a*)

Marine mammals transported in the same primary enclosure must be of the same species and maintained in compatible groups. Marine mammals that have not yet reached puberty may not be transported in the same primary enclosure with adult marine mammals other than their own dams. Socially dependent animals must be allowed visual and olfactory contact whenever possible. Female marine mammals may not be transported in the same primary enclosure with a mature male marine mammal. (USDA, 2005*a*)

Marine mammals must only be placed in animal cargo spaces that have a supply of air sufficient for each live animal contained within. Primary transport enclosures must be positioned in the animal cargo spaces of primary conveyances in such a manner that each marine mammal contained within will have access to sufficient air. (USDA, 2005*b*)

The transport of manatees is less complex than that of cetaceans, pinnipeds or sea otters. This because manatees require and are tolerant of warmer temperatures. The transport appears to be best accomplished using temperature-controlled truck or airplane. (Dierauf and Gulland, 2001)

Airplane
The International Air Transport Association (IATA) made the IATA Live Animals Regulations (LAR). These regulations are a Worldwide Standard for transporting live animals by commercial airlines. Whether it is a pet, an animal transported for zoological or agricultural purposes or for any other reason, the objective of the IATA Live Animals Regulations is to ensure that all animals are transported safely and humanely by air. (IATA, 2007)

The IATA Live Animal Regulations are applicable to Members of the International Air Transport Association and to airlines being parties to the IATA Multilateral Interline Traffic Agreement – Cargo. (IATA, 2006)

The IATA Live Animals Regulations are accepted by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and the Office International des Epizooties (OIE) as guidelines in respect of transportation of animals by air. These Regulations have been used by the Council of Europe as a basis for its code of conduct for the international transport of farm animals. The European Union (former European Community) has adopted the IATA Live Animals Regulations as the minimum standard for transporting animals in containers, pens and stalls. As an increasing number of countries have adopted or accepted these Regulations as a part of their national legislation, shippers are warned that shipping live animals in violation of the Regulations may constitute a breach of the applicable law and may be subject to legal penalties. (IATA, 2006)

The IATA Live Animal Regulations container requirement 55 concerns the manatee species and the dugong.

Truck
Manatees are generally secured in a stretcher and transported in an enclosed truck on thick, 15-20 cm open- or closed-cell foam pads or in a specially designed and padded transport boxes. Cranes and forklifts are generally needed to lift adults. During transport, manatees should be kept moist and shaded. Ambient air temperatures should be kept at 22 to 26ºC. Manatees have been transported for 16 hours in this manner with no apparent ill effects beyond transitory increases in serum lactate dehydrogenase and creatine phosphokinase. (Dierauf and Gulland, 2001)

6.2.2 Transport boxes

The IATA Live Animal Regulations container requirement 55 states the requirements of the transport container of the manatee species and the dugong. The container requirement 55 is also applicable to the following species: beluga, narwhal, killer whale, whale species, dolphin species and porpoise species. (IATA, 2006)

According to the container requirement 55, the materials which can be used to make a transport container are aluminium, canvas, fibreglass, foam rubber, plastics, PVC and wood. The transport box should be waterproof, made from wood with plastic liner, or wood and fibreglass, or moulded fibreglass, or a tubular aluminium frame with waterproof liner. (IATA, 2006)

The size of the box should permit one animal to be suspended in a stretcher of canvas or other suitable material supported on a foam rubber pad. Slits must be made in the stretcher to allow the flippers to protrude outside the stretcher. The marine mammal is accustomed to weightless environment in water and therefore when out of water may be unduly affected by gravity. (IATA, 2006)

The box must be long enough to prevent the animal injuring itself when moving the sling. A clearance of 20 cm must be present at the front and rear of the animal and between the sides of the stretcher bars and sides of the container. (IATA, 2006)

Examples of containers which can be used for manatees are shown in figure 24.

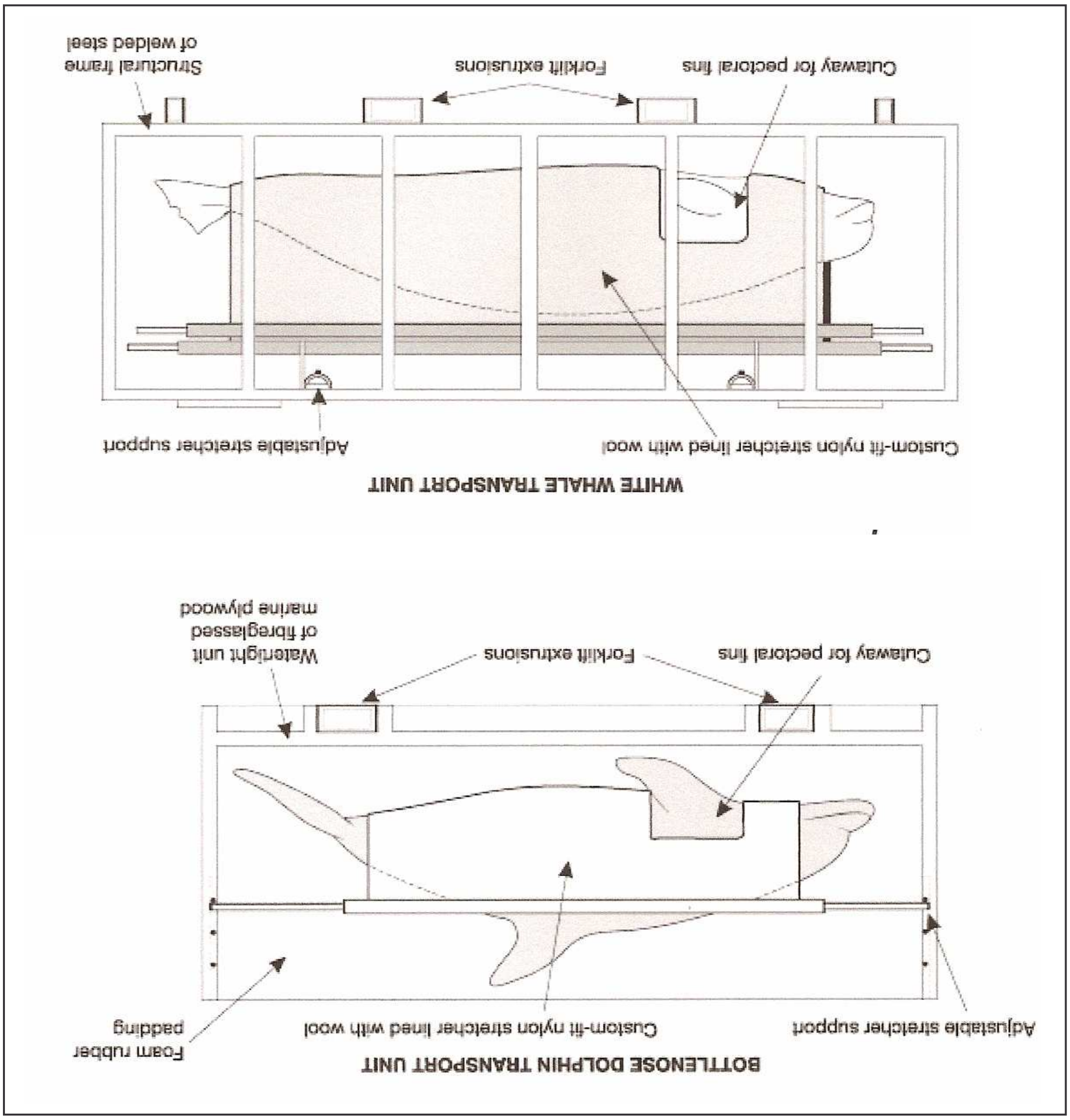


Figure 24 Examples transport unit manatees (IATA, 2006)

According to the experts the best way to transport manatees is:
In a whale/dolphin box misted with water (1/7), on a stretcher (3/7), in a box on soft foam (1/7), placed dry on foam, emerged in water (for very long distances water is best) (1/7).

The critical areas of the body, e.g. under flippers, dorsal fin, tail fluke and head must be thoroughly covered with lanolin/petroleum jelly compound ointment, zinc oxide (or combination of each) to protect the animal against over-heating by retaining moisture, if moisturising by the attendant during transport is not possible. (IATA, 2006)

Padded restraining belts should be used and must be firmly but not forcibly fastened over the animal to prevent violent jumps. When transporting manatees special restraining straps must be used to prevent rolling. (IATA, 2006)

Freshwater spray can be applied throughout the transport process to aid in cooling. Manatees do not require feeding during transport because of their slow metabolic rates. Fasting prior to transport produces a negligible change in waste output. Any solid stool passed during transport must be removed. (Dierauf and Gulland, 2001)

Marking on transport boxes
The markings on the transport boxes must be durable and printed or otherwise marked on or affixed to the external surface of the live animal container. English must be used in addition to the language which may be required by the state of origin. (IATA, 2006)

Each live animal container must be marked, durably and legibly on the outside of the container, with each of the following:
(a) The full name and address and contact number of the shipper, consignee and a 24-hour contact (if it is not one of the aforementioned persons responsible for the shipment).
(b) The scientific and common name of the animal(s) and quantity of each animal contained in the container, as shown on the shipper's certification.
(c) Containers carrying animals which can inflict poisonous bites or stings must be boldly marked "POISONOUS". Aggressive animals or birds that can possibly inflict injury through the bars or ventilation openings of the container must have an additional warning label "This Animal Bites".
(d) Affix special feeding and watering instructions to the container.
(e) In general, tranquillisation is not advocated for the transportation of live animals. However, certain wild species require the use of such medication. Whenever used, they must be administered under competent supervision and the name of the sedative, time of administration and the route of administration must be clearly marked on the container and a copy of the record must be attached to the documents relating to that shipment. Any further medication administered must be recorded and accompany the shipment with the name of the sedative, time of administration and the route of administration. (IATA, 2006)

Labelling
It is mandatory to attach at least one IATA "Live animal" label or tag to each live animal container. Animal containers may have the appropriate labelling imprinted.

The label for live animals should state "Live Animals", the colour should be bright green on a light background, the minimum dimensions for the label are 10 x 15 cm and for the lettering 2,5 cm. (IATA, 2006) In figure 25 a label for live animals is given.

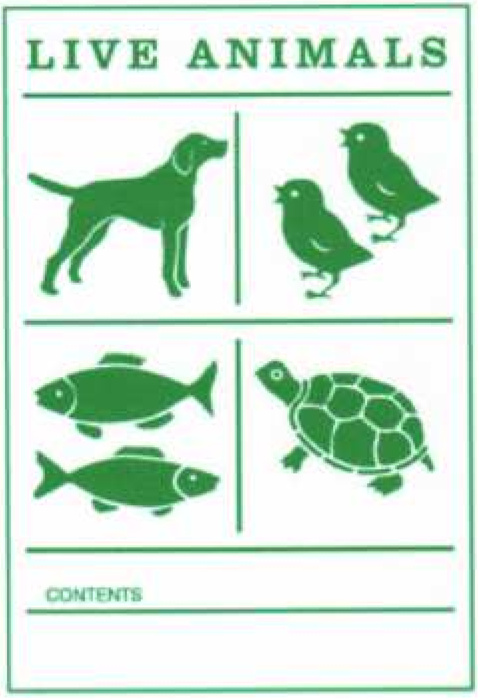


Figure 25 Label "Live animals"

In addition to the live animals label, it is mandatory that the "This way up" labels or markings are placed on at least two opposite sides, these labels may be imprinted on the container. The label for "This way up" should be red or black on a contrasting background and the minimum dimensions are 74 × 105 mm. (IATA, 2006) In figure 26 two labels are given.

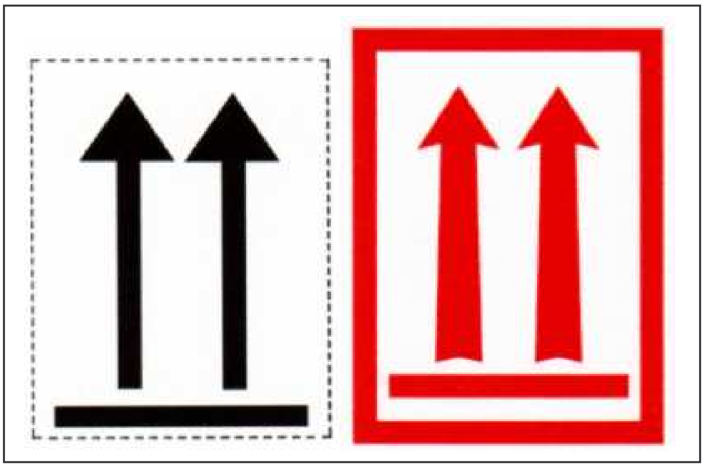


Figure 26 Label "This way up"

6.3 Safety

As said before manatees are extremely powerful and especially the fluke is dangerous. The movements of the fluke during struggles of the animal can cause serious injury to the handlers and animal caretakers (Dierauf and Gulland, 2001). When catching a manatee the safety of the handler and caretakers has to be taking into account. A way to minimise risk of the handlers is to keep enough distance from the animals' fluke so they cannot get hit and to wear protective headpieces. So that if a person gets hit and falls the head is protected. (pers. comm. B. Klaussen, 2008)

7 Health and Welfare

This chapter is a compilation of relevant information of health and welfare from the CRC Handbook of Marine mammal medicine written by L.A. Dierauf and M.D. Gulland.

7.1 General sanitation

Procedures that provide for the cleanliness of exhibits, food preparation and storage areas, and the animal holding areas are important for the control of infectious agents, including parasites, viruses and bacteria. Although routine sanitary procedures are the duty of keepers staff, the veterinarian should oversee and review procedures to be sure that cleaning agents are appropriately used. Effective bactericidal agents are quaternary ammonium compounds, phenolics, chlorhexadine solutions and iodophores. In some cases specific compounds should be used, for instance phenolics in areas contaminated with tuberculosis or dilute (3%) sodium hypochlorite solution in outbreaks of resistant viruses. In the case of manatee enclosures the water quality must be monitored. Also food wastes and excrement should be removed at least daily and disposed in such a way as not to attract insects or rodents. (AAZV, 1998)

7.2 Quarantine

As mentioned in chapter 1: Enclosure, it is necessary that there is a quarantine facility.

The isolation of an animal should be based on the prior medical history of the newly arrived animal. Situations where isolation is recommended are:

- If an animal is recently collected from the wild (less than 30 days prior to arrival)
- If an animal is recently exposed to a new arrival for which an adequate medical history is not available (less than 30 days prior to arrival)
- If there is a lack of documented medical history
- If there are apparent medical problems at time of arrival
- At the direction of the supervising veterinarian (CAZA,1975)

The animal in the quarantine should be under de supervision of a veterinarian and should stay there for a minimum of 30 days. If during the 30-day quarantine additional marine mammals are introduced into the isolation facility, the 30-day period must begin again for all animals already in quarantine and exposed to the new arrivals. (CAZA, 1975)

During the quarantine period individual faecal samples should be collected, if required, at least twice and examined for gastrointestinal parasites. Whenever possible, blood should be collected from the animal and sera banked. When the animal is in the quarantine facility it is also a good opportunity to permanently identify the unmarked animals. A complete physical examination should be performed at the start of the quarantine period and prior to the date that the animal leaves the quarantine facility. During the quarantine period complete medical records should be kept and these should be available for all animals. (CAZA, 1975)

The recommendations and suggestions of the Canadian Association of zoos and Aquariums (CAZA) for the medical procedures which should be performed during the quarantine period for manatees are:

Required	CBC/serum chemistry panel
	Physical examination
Recommended	Direct and flotation faecal exam
	Stool culture and cytology (CAZA, 1975)

7.3 Physical examination

When performing a visual examination of a manatee, it should be visualized in the water to determine position, attitude, and swimming and diving capabilities. Also the breathing rate and breathing excursion characteristics should be determined and an overall subjective assessment of nutritional status should be made. The normal respiratory rate is variable, but three to four breaths per 5 minutes period is typical. The breath excursion should be forceful, constant, and slightly crisp sounding, with no rasping sounds. The breath in a healthy manatee should have a rumen-like “sweet” odour. Malnourished manatees typically have a distinct neck and caudal peduncle with a “sunken-in” appearance of the ventral abdomen. (Dierauf and Gulland, 2001)

The hands-on physical examination should include body weight determination and thorough inspection of the oral cavity for foreign bodies and digital evaluation of the molars. Oral mucous membrane colour and capillary refill time are similar to that in domestic mammals. Auscultation and percussion can be utilized to evaluate the lungs, heart, and gastrointestinal tract. The hindgut fermentation process produces abundant colonic gas, this gas and peristaltic gut sounds are normal findings on abdominal auscultation and percussion in a healthy manatee. (Dierauf and Gulland, 2001)

Traumatic injuries should be characterized regarding the extend of the injury and the presence of musculoskeletal or neurological compromise. The physical examination should also include digital rectal palpation to determine the presence of constipation, diarrhea, or parasites. (Dierauf and Gulland, 2001)

In figure 27 illustrations showing the external features, the skeletal muscles, internal structure and the skeleton of a healthy manatee can be found.

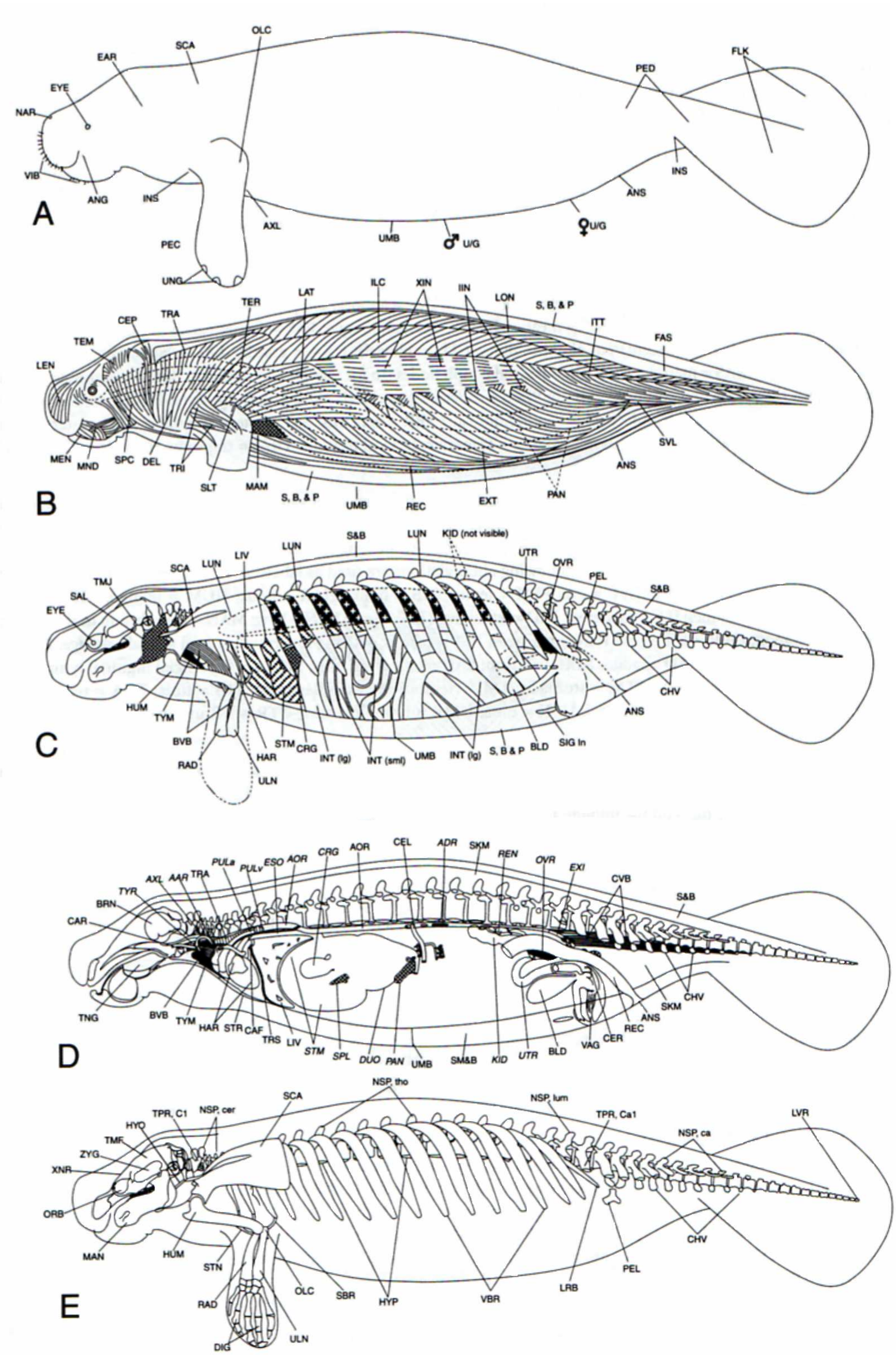


Figure 27 Left lateral illustrations of a healthy manatee (Dierauf and Gulland, 2001) Explanation labels see following page.

Explanation Labels figure 27,
(Layer A) External Features
The following abbreviations are used as labels: **ANG** = angle of mouth; **ANS** = anus; **AXL** = axilla; **EAR** = external auditory opening, ear; **EYE** = eye; **FLK** = fluke entire caudal extremity in manatees; **INS** = cranial insertion of the extremity, flipper and/or fluke; **NAR** = naris; **OLC** = olecranon, palpable bony feature; **PEC** = pectoral flipper, limb; **PED** = peduncle, base of tail, between anus and fluke; **SCA** = dorsal border of the scapula, palpable bony feature in emaciated individuals; **UMB** = umbilicus; **UNG** = unguis, fingernails; **U/G** = urogenital opening; **VIB** = vibrissae.

(Layer B) The superficial skeletal muscles
The layer of skeletal muscles just deep to the blubber and panniculus muscles. The following abbreviations are used as labels: **ANS** = anus; **CEP** = cephalohumeralis; **DEL** = deltoid; **EXT** = external oblique; **FAS** = fascia; **S,B&P** = skin, blubber and panniculus muscle (where present) cut along midline; **IIN** = internal intercostals; **ILC** = iliocostalis; **ITT** = intertransversarius; **LAT** = latissimus dorsi; **LEN** = levator nasolabialis; **LON** = longissimus; **MAM** = mammary gland, in axillary region, thus partly hidden under the flipper; **MEN** = mentalis; **MND** = mandibularis; **PAN** = panniculus, illustrated using dotted lines, is a robust and dominant superficial muscle; a layer of blubber is found on both the medial and lateral aspects of this muscle; **REC** = rectus abdominis; **SLT** = mammary slit, nipple; **SPC** = sphincter colli; **SVL** = sarcoccygeus ventralis lateralis; **TER** = teres major; **TMP** = temporalis; **TRA** = trapezius; **TRI** = triceps branchii; **UMB** = umbilicus; **XIN** = external intercostals.

(Layer C) The superficial internal structures with 'anatomical landmarks' This perspective focuses on relatively superficial internal structures. Skeletal elements are included for reference but are not labelled. The left kidney (not visible from this vantage of the manatee) is illustrated. The relative size of the lung represents partial inflation. The following abbreviations are used as labels: **ANS** = anus; **BLD** = urinary bladder (dotted, not really visible in this view); **BVB** = branchial vascular bundle; **CHV** = chevrons, chevron bone; **EYE** = the eye (note how small it is); **HAR** = heart; **HUM** = humerus; **INT** = intestines, note the large diameter of the large intestines; **KID** = left kidney, not visible from this vantage in the manatee; **LIV** = liver; **LUN** = lung (note the extends under scapula, and over heart); **OVR** = left ovary; **PEL** = pelvic vestige; **RAD** = radius; **SAL** = salivary gland; **S&B** = skin and blubber; **SCA** = scapula; **SIG In** = superficial inguinal lymph node; **S,B&G** = skin, blubber and panniculus muscle, cut at midline; **STM** = stomach; **TMJ** = temporomandibular joint; **TYM** = thymus gland; **ULN** = ulna; **UMB** = umbilical scar; **UTR** = uterine horn; **VAG** = vagina.

(Layer D) A view slightly to the left of the midsagittal plane illustrates the circulation, body cavities, and selected organs. Note that the diaphragm of the manatee is unique and that the distribution of organs and the separation of thoracic structures form abdominal structures requires special consideration. The following abbreviations are used as labels (structures on the midline are in normal type, those off-midline are in italics): ***AAR*** = aortic arch; ***ADR*** = left adrenal gland; **ANS** = anus; **AOR** = aorta; ***AXL*** = axillary artery; **BLD** = urinary bladder; **BRN** = brain; ***BVB*** = brachial vascular bundle (cut); **CAF** = caval foramen; **CAR** = carotid artery; **CDG** = cardiac gland; **CEL** = celiac artery; **CER** = cervix; **CHV** = chevron bones; ***CRG*** = cardiac gland; **CVB** = caudal vascular bundle; ***DUO*** = duodenum; ***ESO*** = esophagus (to the left of the midline cranially, on the midline caudally); ***EXI*** = external iliac artery; **HAR** = heart; ***KID*** = right kidney; **LIV** = liver, cut at midline; ***OVR*** = right ovary; ***PAN*** = pancreas; ***PULa*** = pulmonary artery, cut at hilus of lung; ***PULv*** = pulmonary vein, cut at hilus of lung; **REC** = rectum; ***REN*** = renal artery; **S&B** = skin and blubber; **SKM** = skeletal muscle; **SM&B** = skin, muscle and blubber (cut at midline); ***SPL*** = spleen; ***STM*** = stomach; **STR** = sternum; **TNG** = tongue; **TRA** = tranchea; **TRS** = transverse septum; **TYM** = thymus gland; ***TYR*** = thyroid gland; **UMB** = umbilical scar; **UTR** = uterus; **VAG** = vagina.

(Layer E) The Skeleton Regions of the vertebral column (cervical, thoracic, lumbar, sacral, and caudal), are abbreviated (in lowercase) as **cer**, **tho**, **lum**, **sa** and **cau**, respectively, and are used as modifiers after an abbreviation in caps and a comma. If a specific vertebra is labelled, it will be represented by a capitalized first letter (for caudal, **Ca** will be used) and the vertebral number, i.e., first cervical = **C1**. The following abbreviations are used as labels: **CHV** = chevrons, chevron bones; **DIG** = digits, columns of finger bones; **HUM** = humerus; **HYO** = hyoid apparatus; **HYP** = hypapophysis, ventral midline vertebral process; **LRB** = last, or caudalmost, rib; **LVR** = last, or caudalmost, vertebra; **MAN** = mandible; **NSP** = neural spine (spinous process), e.g. thoracic neural spines = **NSP, tho**; **OLC** = olecranon; **ORB** = orbit; **PEL** = pelvic bone; **RAD** = radius; **SCA** = scapula; **STN** = sternum, if sternabrae are commonly fused; **SBR** = sternal ribs, costal cartilages; **TMF** = temporal fossa; **TPR** = transverse process, **C1**; **ULN** = ulna; **VBR** = vertebral ribs; **XNR** = external (bony) nares; **XIP** = xyphoid process, cartilaginous caudal extension of the sternum; **ZYG** = zygomatic process of the squamosal.

7.4 Diagnostic techniques

7.4.1 Blood collection

Blood samples are taken from the medial interosseous space of the radius and ulna. This site constitutes the brachial vascular bundle. Blood vessels cannot be visualized; hence, practice is required to become proficient at venipuncture. The middle portion of the medial surface of the pectoral flipper is surgically scrubbed with a commercially available iodine surgical solution for at least three minutes prior to venipuncture. The flipper is firmly restrained while the manatee is stranded, and an 18- to 20- gauge, 1-1,5 inch needle (with attached syringe or butterfly set) is inserted between the palpable medial edges of the radius and ulna. (Dierauf and Gulland, 2001)

Blood should be collected from the brachial vascular bundle or the caudal vascular bundle (or fluke), the blood collected from the brachial vascular bundle will be mixed arteriovenous blood. The vessels of the brachial vascular bundle are arranged in a small plexus, and are difficult to isolate for catherization. (Dierauf and Gulland, 2001) For the location of the blood vessels used for blood collection see figure 28.

Six out of seven zoos take blood samples from the pectoral flipper (6/7).

Laboratory tests performed immediately should include a spun hematocrit, plasma protein (via refractometer), and blood glucose (via a reagent test strip or point-of-care glucose analyzer) determinations. The last is especially important for orphaned manatee calves and cold stress syndrome cases. Blood testing should include a complete blood count (CBC), a standard panel of serum analytis, and serum protein electrophoresis. (Dierauf and Gulland, 2001) For the ranges of hematology and biochemistry values of free ranging manatees see table 11.

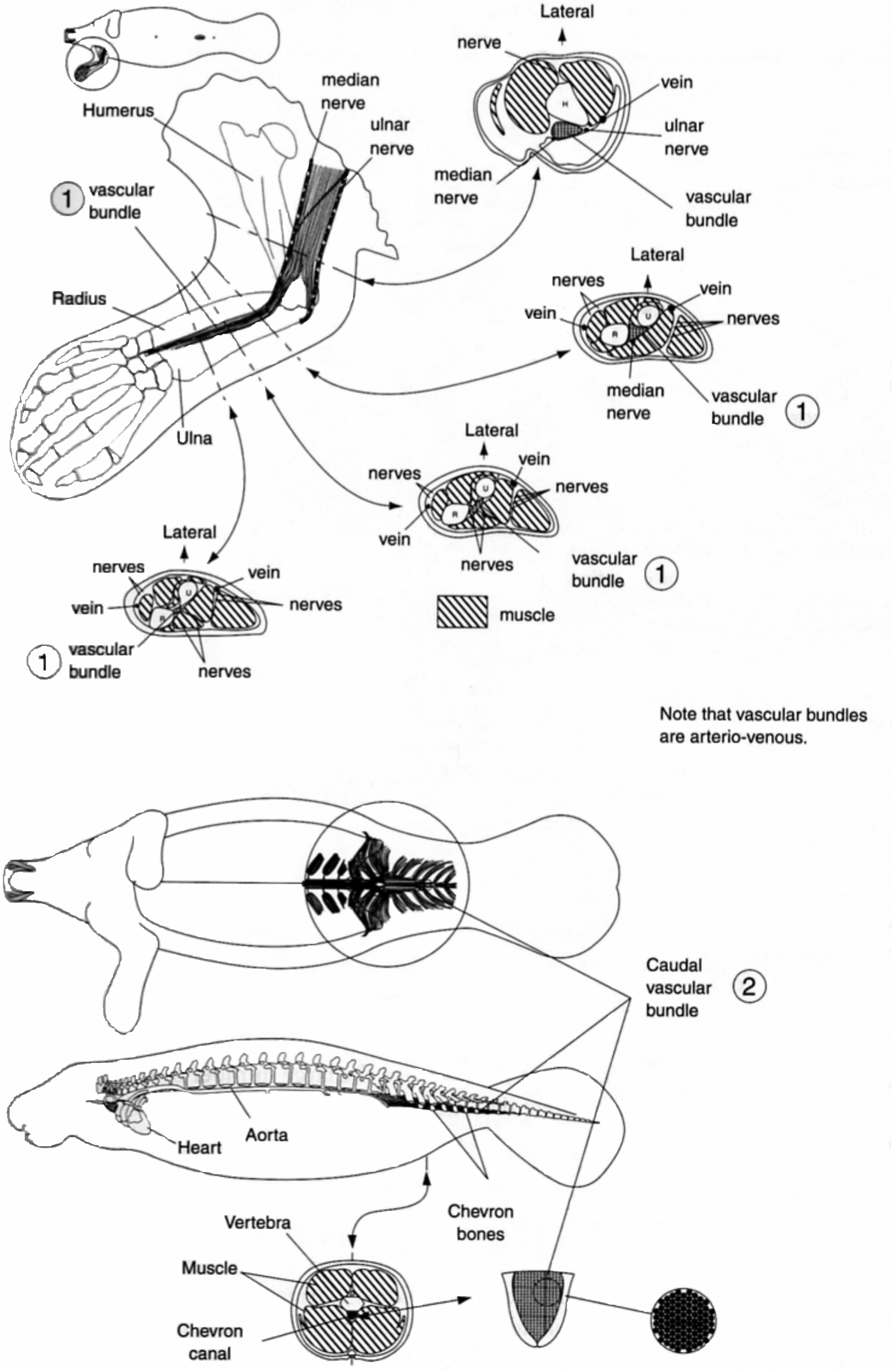


Figure 28 Veins used for blood collection in the manatee (Dierauf and Gulland, 2001)

Table 11 Ranges of Hematology and Biochemistry values in Free-ranging manatees (Dierauf and Gulland, 2001)

Parameter	Manatee
RBC (10^6/mm^3)	2,4-3,4
Hb (g/dl)	9,8- 13,2
HCT (%)	30-40
MCV (fl)	122- 149
MCH (pg)	38-46
MCHC (g/dl)	30-33
Platelets (10^6/mm^3)	195- 412
Reticulocytes (%)	0- 4
nRBC	0
ESR (at 60 min)	7,0- 8,0
Leukocytes/µl	4000- 11800
Neutrophil (band)	0
Neutrophil (mature)	N.D.[d]
Heterophil	960- 8590
Lymphocyte	960- 8590
Monocyte	0 -1020
Eosinophil	0
Basophil	0
Serum proteins (g/d)	6,2- 8,6
Albumin (g/dl)	3,6- 5,9
Globulin (g/dl)	2,6- 2,7
Glucose (mg/dl)	56- 117
BUN (mg/dl)	6,4- 16,0
Creatinine (mg/dl)	0,4-2,1
Bilirubin T/D (mg/dl)	0-0,1/N.D.
Cholesterol (mg/dl)	107- 328
Alkaline phos (U/l)	64-183
ALT (U/l)	6-30
AST (U/l)	5-28
GGT (U/l)	39-64
CK (U/l)	79-302
LDH (U/l)	94-372
Calcium (mg/dl)	10,1-12,2
Phosphorus (mg/dl)	3,0-8,0
Sodium (mEq/l)	142-157
Potassium (mEq/l)	4,2-6,6
Chloride (mEq/l)	90-103
Iron (mcg/dl)	50-199
Fibrinogen (mg/dl)	N.D.

[d] N.D. = not determined

7.4.2 Urine

Urine collection by catherization is difficult because of the small, tight urogenital opening, the distance of the urinary opening from the surface, and animal resistance to the procedure. One can have limited success by manually stimulation urine flow; this is done by applying pressure on the abdomen anterior to the vulva in females or posterior to the genital opening in

males. Patience is required. Urine collection from captive manatees can be successfully accomplished using animals trained to urinate on command. (Dierauf and Gulland, 2001)
The article “Husbandry and research training of two Florida manatees (*Trichechus manatus latirostris*)” written by Colbert *et al.,* 2001 describes how manatees can be trained to urinate on command.

7.5 Therapeutic techniques

7.5.1 Topical

Topical treatment is rarely used in manatees for the obvious reason that the medication usually washes away very rapidly (Dierauf and Gulland, 2001). One zoo has administered ointment to the eyes of a manatee (1/7).

7.5.2 Oral

Oral medication is the best administered by stomach tube. Some manatees may take oral medication concealed in food such as bananas or monkey chow; however, the natural crushing action of the molars usually results in drug loss. In most instances, oral antibiotic therapy is not recommended, because it can result in loss of normal enterocolic flora, diarrhea, and hypermotility. (Dierauf and Gulland, 2001) Three out of seven zoos have given the manatees medicines orally (3/7).

7.5.3 Intubation

Stomach intubation is recommended for fluid therapy and nutritional supplementation, using a foal or small equine size soft plastic stomach tube by either the nasal or oral route. The length of the inserted is predetermined by measuring from the outer upper lip to the level of the caudal tip of a ‘tucked’ pectoral flipper. At this point, the tube is externally marked with tape or a felt-tip pen, which then designates the point of maximum tube insertion. The tube should then be thoroughly lubricated with petroleum jelly. A bite block can be used when using the oral route. Intubation should be done by patient and experienced personnel to avoid inadvertent airway intubation and damage to the easily traumatized caudal oropharynx. Nasogastric intubation is well tolerated by most manatees and prevents the tube from being chewed, potential tube leakage, and/or tube fractures. (Dierauf and Gulland, 2001) One out of seven zoos has used nasal tubing (1/7) and three out of seven zoos have used oral intubation (3/7).

Rehydration

Rehydration is best achieved by gastric intubation. Precise fluid therapy formulas have not been determined for this species, but domestic animal fluid therapy guidelines can be used with a few notes of caution. First of all, extremely ill or dehydrated manatees typically have alimentary tract stasis. Therefore, these manatees should be given only water initially, and only about 50 to 75 % of the calculated total fluid volume should be given to minimize the chance of fluid reflux. A 350 kg manatee may require up to 2,5 litres of water twice a day to improve its hydration status. However, for the first two or three treatments, only 1,0 to 1,5 litres of water should be given and then this volume should be slowly increased. Severely dehydrated or malnourished manatees may be tube-fed three times a day. As with fluid therapy in domestic animals, hematocrit and electrolytes should be closely monitored, and the fluids should be administrated by gravity feed only. (Dierauf and Gulland, 2001)

Tube feeding

Inappetent or malnourished manatees can also be given nutritional supplementation by gastric tube. Manatees that have sustained human-related traumatic injury and cold stress syndrome often develop life-threatening dehydration and gastrointestinal stasis, which, if prolonged, results in cachexia. Reestablishment of gut function and motility is critical for a successful outcome. This can be accomplished by tube feeding the manatee a gruel composed of commercial primate pellets, lettuce, spinach, and water two to three times a day in volumes similar of those for rehydration. The gruel should be thin initially to allow the gastrointestinal tract to adapt to the presence and type of digesta. The gruel should be gradually thickened when flatulence and faecal production are noted. The presence of flatulence is generally a favourable prognostic indicator. If there is excessive reflux of gruel into the tube, the gruel volume should be reduced. However, a small amount of gruel reflux from the previous feeding is normal. As gut function improves, manatees will start to graze on offered vegetation, but tube feedings should not be discontinued until the appetite and daily food intake are normal. Cessation of gruel feedings during the recovery process is a common mistake. (Dierauf and Gulland, 2001)

7.5.4 Intramuscular

Intramuscular drug administration is the preferred route in manatees. Intramuscular injection sites include the caudal epaxial muscles and shoulder muscles. The injection site is disinfected with a commercially available iodine surgical solution for at least three minutes to minimize iatrogenic contamination of internal tissues. Needles recommended for intramuscular injection in adults are 2 to 3,5 inch and 18 to 20 gauge. Needles for calves are 1 to 1,5 inch and 20 to 22 gauge. Common antibiotics and other medications are listed in table 12.

Table 12 Commonly used drugs in manatees (Dierauf and Gulland, 2001)

Drug	Dose	Frequency	Route
Antibiotics			
Ceftriaxone	22 mg/kg	SID	IM
Amikacin	7 mg/kg	BID	IM/PO
Penicillin (G and benzathine)	22000 U/kg	SID	IM
Metronidazole	7 mg/kg	BID	PO
Tetracycline	55 mg/kg	BID	IM
Sulfasalazine[a]	10 mg/kg	BID	PO
Gentamicin[a]	2,5 mg/kg	TID	PO
Antifungal drugs			
Itraconazole	2,5 mg/kg	BID	PO
Gastrointestinal drugs			
Pepto-bismol®	30 ml/animal	TID	PO
Parasiticidal drugs[b]			
Fenbendazole	10 mg/kg	Once	PO
Ivermectin	200 µg/kg	Once	PO
Praziquantel	8-16 mg/kg	Once	PO

[a] For calf enterocolitis only

[b] Manatees that are destined for release to a free-ranging state are not routinely deparasitized unless complications associated with parasitism (e.g. diarrhea, etc) are present.

Key: SID = once a day; BID = twice a day; TID = three times a day; IM = Intramuscularly;
PO = per os; orally.

Combinations of antibiotic use such a penicillin or ceftriaxone combined with amikacin sulphate, is frequently used with life-threatening bacterial infections.
There are no studies performed to determine the drugs dosage for manatees so the amounts are generally based on doses recommended for domestic mammals or humans. (Dierauf and Gulland, 2001) Four out of seven zoos have used injections when giving medicines to manatees (4/7).

7.5.5 Intravenous

Intravenous drugs can be administered via the branchial vascular bundle. However this site is difficult to catherize for long-term intravenous drug therapy, and care must be taken to avoid inadvertent arteriolar injection. (Dierauf and Gulland, 2001)

7.6 Diseases

7.6.1 Brevetoxicosis

Although marine toxins are most frequently a threat to free-living marine organisms, the potential for contamination of natural water used in exhibits or enclosures and food items fed to aquatic animals managed in captivity is real (Fowler and Miller, 2008).
Brevetoxicosis occurs after red tides. Red tides are composed of dinoflagellates that produce neurotoxins. The dinoflagellate *Gymnodinium breve* produces potent neurotoxins known as brevetoxins. (Dierauf and Gulland, 2001)

Brevetoxins act on both the voluntary and the autonomic nervous system. Hemolytic toxins are also produced. Exposure of manatees happens through ingestion of marine vegetation (sea grass and tunicates found on sea grass) and inhalation. Sea grass may remain toxin after the bloom is over. Manatees that strand alive have a variety of neurological signs, including disorientation, incoordination, inability to maintain proper position in the water, hyperflexion, muscle fasciculations, seizures, flaccid paralysis, and dyspnea. (Fowler and Miller, 2008)

Manatees with clinical signs of brevetoxicosis are treated symptomatically with steroids and nonsteroidal anti-inflammatory drugs. Supportive care includes providing fluids, nutritional supplementation, and water buoyancy devices to prevent drowning. In many instances, the manatees survive following this treatment regimen. (Dierauf and Gulland, 2001)

7.6.2 Cold stress syndrome

Water temperatures below 20ºC for extended periods initiate a cascade of clinical signs and disease processes that constitute the manatee cold stress syndrome. Adult manatees appear to handle the effects of cold temperatures better than juveniles or calves. This may be due to surface-to-volume body relationships and a nutritional plane resulting in lowered capacity for heat production. (Dierauf and Gulland, 2001)
The more acute lethal effects of cold temperature probably involve lethargy, anorexia, and terminal hypothermia through metabolic drains to the environment. The chronic lethal effects of cold likely trigger a cascade of physiological changes that predispose these animals to various opportunistic pathogens involving multiple organ systems. Infectious bronchopneumonia, generalized infectious dermatitis, and enterocolitis are common sequelae of the chronic cold stress syndrome. These conditions may require months of pathogen-specific and supportive therapy for favourable outcomes. Bacteria that have been isolated from these disease conditions include *Staphylococcus aureus*, *Morganella morganii*, *Edwardsiella tarda*, *Aeromonas hydrophila*, and various species of *Pseudomonas*, *Vibrio* and

Clostridium. Additionally, secondary fungal infections of the skin (especially *Mucor spp*.) and lungs can occur as this syndrome progresses. Skin lesions may become severe and generalized with ulceration resembling a toxic contact dermatitis. Treatment is based on culture and sensitivity results, and appropriate parenteral antibiotic therapy. Skin lesions should be treated with daily povidone-iodine scrubs, and special attention should be directed to maintaining a clean and bacteria-free water environment. Supportive therapy is of paramount importance. Non-specific clinical signs include shivering, cachexia, anorexia, constipation, and absence of gut sounds and flatulence. Hematological and serum analyte findings may include dehydration, leukocytosis, and elevated lactate dehydrogenase (LDH), creatine kinase (SPK), and creatinine concentrations. Regaining normal gut function and treating initial dehydration involves gastric intubation with water only. This is followed by nutritional support via gastric intubation of a gruel mixture that is gradually increased in consistency and volume. Gruel feeding may be done twice daily as long as faeces are produced. Constipation, characterized by absent or hard, firm faeces, can be treated with oral mineral oil and warm-water enemas. (Dierauf and Gulland, 2001)

7.6.3 Infectious diseases

Abscesses
Juvenile and adult manatees may be unusually prone to chronic abscessation, which is apparently not secondary to trauma. Abscesses may be dermal to subcutaneous, and relatively superficial, or lying deep within skeletal muscle fascial planes. *Staphylococcus aureus* is usually isolated from these lesions. Treatment typically involves surgical drainage and daily flushes with a dilute hydrogen peroxide and povidone-iodine solution, often followed by an antibiotic and/or proteolytic enzyme solution. Surgical drains or Foley catheters can be used to prevent premature closure of the skin. (Dierauf and Gulland, 2001)

Papillomavirus
The clinical signs of papillomavirus are areas of localized epithelial hyperplasia with a defined boundary and an intact basement membrane (Dierauf and Gulland, 2001). These growths, sometimes referred to as being tumours, are limited to the lips, nostrils, and leading edges of the pectoral flippers, reinforcing the belief that the virus is readily transmitted through contact between infected and non-infected manatees (Harbor Branch Oceanographic, 2006).
In general, lesions are focal and randomly distributed. The cutaneous lesions appear as raised, often smooth, plaques that are the colour of the underlying skin. The mucosal lesions vary in colour and often have an irregular surface. The size of the lesions is quite variable, ranging from a few millimetres to in excess of 20 centimetres. (Dierauf and Gulland, 2001)
The papillomas are benign, but they have the potential to become life threatening if they grow to the point where they begin to interfere with feeding, sight, or breathing. Furthermore, similar tumours caused by papillomavirus in other mammalian species (over a 100 types have been reported in humans, including one known to be a cause of cervical cancer in women) have been proven capable of undergoing spontaneous malignant transformations. It is feared that the manatee virus might be capable of doing the same. (Harbor Branch Oceanographic, 2006)

In figure 29 two examples of clinical signs of the papillomavirus are shown.

Figure 29 Manatees with papilloma virus (Harbor Branch Oceanographic, 2006)

There is no therapy available. The lesions are generally self-limiting and will regress. In most domestic animal species, complete immunity follows regression of lesions, but not enough is known to make this statement about manatee papillomas. (Dierauf and Gulland, 2001)

Papillomavirus do not usually grow in tissue cultures and cannot always be detected microscopically. When detected by electron microscopy, papilloma viral particles are naked icosahedrons about 55 nm in diameter. (Dierauf and Gulland, 2001)
Three out of seven zoos have had manatees with the papillomavirus (3/7).

Morbillivirus
Serological evidence of the morbillivirus has been demonstrated in manatees, however signs of clinical disease or active infection due to this virus have not been described. Therefore, these data likely reflect exposure and an immunological response to these pathogens without accompanying clinical disease. Additional investigations are needed to associate these test results with natural history and clinical and pathological data. (Dierauf and Gulland, 2001)

Other infectious diseases reported in adult manatees include systemic mycobacteriosis due to *Mycobacterium marinum* and *M. chelonei*, mycotic dermatitis, hemorrhagic enteritis, pleuritis, lung abscessation, septic metritis secondary to dystochia, and various non-specific dermatopathies.

7.6.4 Parasites

A few species of endoparasites are commonly found in manatees; however, pathological signs or clinical disease are rarely associated with these parasites.
The nasopharyngeal trematode *Cochleotrema cochleotrema* can cause signs of chronic rhinitis and make nasogastric or endotracheal intubation difficult. A trematode (like *Nudacotyle undicola*) has been associated with a severe enterocolitis. Toxoplasmosis due to *Toxoplasma gondii* has been reported to cause fatal encephalitis in West Indian manatees. Dermal parasites described in manatees include copepoda (*Harpacticus pulex*), Cirripedia (*Chelonibia manatii*), and some nematode species. These parasites likely cause opportunistic infestations rather than being primary pathogens. (Dierauf and Gulland, 2001)

Toxoplasma gondii
Toxoplasma gondii is a single-celled protozoan parasite with a complex life cycle involving both definitive hosts and intermediate hosts that support the tissue cyst stage of the parasite. Virtually any warm-blooded vertebrate, including humans, may serve as intermediate hosts. Thus far, only domestic and wild felids (cats) are known to serve as definitive hosts and support the sexual phase of the parasite's life cycle. (Fowler and Miller, 2008)

New hosts are infected through consumption of tissue cysts in muscle, brain, or other organs of intermediate hosts; by consumption of oocyst-contaminated paratenic hosts; or by accidental consumption of sporulated oocysts derived from the faeces of infected cats in soil, in water, or on vegetation, or transplacentally. (Fowler and Miller, 2008)

The clinical signs may be absent or may consists of anorexia, depression, fever, central nervous system (CNS) disease, lymphadenopathy, icterus, abortion, stillbirth, and neonatal mortality.
Neurological deficits are often the most prominent clinical abnormality and may include blindness, papillary mydriasis, decreased mentation, sudden and episodic cessation of activity (e.g. eating, grooming), lack of aggression or increased aggression, unusual or repetitive stereotypical behaviour, ambulatory and proprioceptive deficits, ataxia, paresis, paralysis, loss of bladder tone, obtundation, tremors, seizures, and coma. (Fowler and Miller, 2008)

For animals with *T. gondii* infection, diagnostic blood testing other than protozoal serology is rarely informative. Serologic assays for apicomplexan parasites such as *T. gondii* include three main types; agglutination tests, fluorescence-based tests, and enzyme-linked immunosorbent assay (ELISA). Commonly used agglutination tests for diagnosis of protozoal infection include the direct agglutination (DAT), modified agglutination (MAT), and latex agglutination (LAT) tests. These tests share three excellent attributes: (1) availability on a collaborative or commercial basis, (2) ease of use, and (3) ability to screen animals for which no species-specific antibodies are available. Agglutination tests are widely used to detect antibodies of T. gondii in marine mammals but have not been validated for these animals. (Fowler and Miller, 2008)

<u>Clinical therapy</u>
A variety of antiprotozoal medications have been used in *T. gondii*-infected humans, including atovaquone, sulfonamides (e.g., trimethoprim-sulfamethoxazole (TMS), azithromycin plus pyrimethamine, clindamycin with pyrimethamine and folinic acid, co-trimoxazole, doxycycline, minocycline, and minocycline plus pyrimethamine. Potential side effects of these medications include liver and skin disease, seizures, and leukopenia.
Pyrimethamine, 0.5 mg/kg every 24 hours for two days (q24h), then 0,25 mg/kg q24h for 30 days; TMS, 15 mg/kg orally (PO) q12h for 30 days; and clindamycin, 12,5 mg/kg intramuscularly (IM) q12h, have been used on a limited basis in otters and seals with suspected or confirmed protozoal disease but were thought to be unsuccessful in restoring normal clinical function. (Fowler and Miller, 2008)
Oral anticoccidial medications such as ponazuril (e.g., Marquis, Bayer Animal Health Corporation; 5 mg/kg PO q24h for 30-60 days) and diclazuril (1-10 mg/kg q24-48h) have been more widely used for antiprotozoal therapy in sea otters and harbour seals. (Fowler and Miller, 2008)
There is no specific clinical therapy for manatees.

<u>Prevention</u>
For captive marine mammals, prevention of exposure to *T. gondii* should include efforts to exclude the definitive and intermediate hosts from enclosures, food preparation areas, and water sources. (Fowler and Miller, 2008)

Helminths (Nematodes, Trematodes)
The predominant parasites of sirenians are monostome trematodes (except for one species, *Nudacotyle undicola*), and they are exclusive to sirenians. Nine genera (*Chiorchis*,

Indosolenorchis, *Lankatrema*, *Lankatremoides*, *Rhabdiopoeus*, *Schizamphistoma*, *Solenorchis*, *Taprobonella*, and *Zygocotyle*) may inhabit the stomach, pyloric cecum, and intestine in massive numbers. Some (*Lankatrema*) produce lesions in the stomach and form cystic cavities in the mucosa. Three additional genera of trematodes (*Opisthotrema*, *Chocleotrema*, *Pulmonicola*) inhabit the nasal passages, Eustachian tubes, airways, and lungs, and a fourth genus (*Labicola*) occurs in the upper lip. Diagnosis is by detection of eggs with polar filaments in faeces or sputum. Two genera of nematodes reported from sirenians are *Heterocheilus* and *Paradujardina*. Prophylatic anthelmintic treatment is routinely performed on captive manatees. Both copepods (*Harpacticus*) and barnacles (*Chelonibia*, *Platylepas*, and *Balanus*) have been reported from sirenia, without any associated disease. (Dierauf and Gulland, 2001)

7.6.5 Neonatal diseases

Orphaned neonatal manatees comprise a large percentage of cases presented to manatee rehabilitation facilities in the United States, Mexico, Belize, Colombia and Brazil. In the United States, there are a number of possible causes of premature maternal separation, including death of the cow and/or recruitment of inexperienced cows into the breeding population, because of a loss of experience breeders. Compared with adult manatees, neonates can develop severe medical problems that are labour-intensive and expensive to treat.
Congenital disease is rarely reported, although congenital malformations of the pectoral flipper and umbilical hernias have been reported in manatee neonates. (Dierauf and Gulland, 2001)

Orphaned neonates are usually presented to rehabilitation facilities in a critical medical state. Orphans typically are cachectic, hypothermic and in a state of metabolic exhaustion. Blood studies can indicate severe life-threatening hypoglycaemia, hypernatremia, hypoproteinemia, hypoalbuminemia, and hypogammaglobulinemia. Orphaned manatees have also been described with Gram-negative bacterial omphalitis and secondary peritonitis.
Orphaned manatees can develop enterocolitis, which is also life threatening. Pneumotosis intestinalis has been reported as a sequela to enterocolitis. The entiology of the enterocolonic inflammation is likely multifactorial involving dietary (e.g., artificial formulas), infectious (e.g. *Pseudomonas aeroginosa, Salmonella spp., Clostridium difficile*), and immunological factors. (Dierauf and Gulland, 2001)

Critical care treatment is based on clinicopathological findings. Environment treatment should involve providing an ultraclean water habitat with water temperature maintained around 29ºC, Elemental tube-fed diets consisting of Criticare HN, Nutramigen, and medium-chain triglycerides has been used successfully. One notable exception to oral antibiotics use occurs with calf enterocolitis. In this case, the recommended treatment typically involves combination oral antibiotic therapy in an attempt to ‘sterilize’ the alimentary tract. Combination therapy includes oral sulfasalazine, gentamycin, or amikacin, and metronidazole with bismuth salicylate (see table 12) given by stomach tube for 10 days. This treatment is combined with oral fluid and nutritional therapy as needed and followed by ‘reseeding’ the alimentary tract with Lactobacillus and faecal material from a healthy manatee. (Dierauf and Gulland, 2001)

Also 2/7 zoos have had eye problems with some manatees, and 1/7 zoos have had a case of cancer in one manatee.

7.7 Anaesthesia

Relatively little is known about the anaesthesia of manatees. Midazolam (0,045 to 0,08 mg/kg Intramuscularly) and diazepam (0,066 mg/kg) have been used to facilitate restraint in manatees. Meperidine has been used at up to 1 mg/kg along with midazolam and diazepam to increase sedation. Intubation has been accomplished transnasally by visualizing the larynx endoscopically through one nares while introducing the endotracheal tube through the other nasal opening. Isoflurane has been used to maintain anaesthesia. Mechanical ventilation and the use of a large reservoir system are recommended. Antagonism of meperidine with naloxone and diazepam or midazolam with flumazenil (intramuscularly) has been performed. Doxapram has been used to stimulate respiration in manatees. (Dierauf and Gulland, 2001)

7.8 Euthanasia

Euthanasia may be achieved by one of three basic physiological mechanisms: (1) depression of neurons vital for life (e.g., typically by overdose of chemical anaesthetics), (2) hypoxia, by either direct physical means (e.g., decapitation) or indirect means (e.g., paralytics), and (3) physical disruption of brain activity and destruction of neurons vital for life (e.g., captive bolt). Although many methods will accomplish death, only a few are considered acceptable by published guidelines. (Dierauf and Gulland, 2001)

Acceptable methods of euthanasia by marine mammals are the use of barbiturates and etorphine hydrochloride. The use of barbiturates causes a direct depression of the cerebral cortex, sub cortical structures, and vital centres; and a direct depression of the heart muscle. When using barbiturates the animal must be restrained; also the personnel must be skilled to perform IV injection. (American Veterinary Medical Association, 2007)
There are more acceptable methods for the euthanasia of zoo animals these are inhalant aesthetics, CO_2, CO, potassium chloride in conjunction with general anaesthesia (American Veterinary Medical Association, 2007).

None of the zoos which responded to the questionnaire have euthanized a manatee.

8 Population management

The organisations and programs involved with the management of manatees in captivity are briefly discussed below.

- The International Union for Conservation of Nature (IUCN) is the umbrella organisation for the preservation of nature.
- The European Association of Zoos and Aquaria (EAZA) is a collaboration between the zoos in Europe.
- European Endangered Species Programs (EEP) are set up by EAZA, for the regulation of endangered species in captivity.
- Taxon Advisory Groups (TAG's) are founded for all main groups of vertebrates kept in captivity and investigate the requirements of these main groups. The Marine Mammal TAG is the advisory group where the Trichedae are included.

8.1 Population status

There are 305 European zoos member of EAZA (EAZA, 1988). Of these members, 7 zoos keep West-Indian manatees. These zoos can be found in Denmark, Germany, the Netherlands, France and Portugal. According to the information on the ISIS website on May 2008 the total amount of animals kept in these zoos are 12.11.0 (ISIS, 1973).

8.2 Species management programmes

There is a European endangered species program for the West-Indian manatee (*Trichechus manatus*). The coordinator of this EEP is Bernard Neurohr of Nurnberg zoo. Manatees are included in the EAZA Marine Mammal TAG.

8.3 Individual identification and sexing

8.3.1 Individual identification

The identification of animals is important so that it can be checked if all the animals are still in the enclosure and in good health.
Methods used to identify manatees in captivity are:

- Noting individual scar patterns
- Noting individual characteristics
- Tagging
- Branding
- Microchip (questionnaire)

Manatees can have a lot of scars, these scars form a good method to identify the individual manatees. Also individuals can have individual characteristics which can be used to identify individuals. Examples of characteristics used are differences in nostrils, muzzles, flipper, tail etc. Branding is the most permanent manner of identification.
The use of microchips with manatees is not always possible, because of the thick skin of these animals it is hard to insert the chip. And often when it is inserted the chip is not readable when holding the receiver close to the skin, because it went in to far. (pers.comm. B. Klausen, 2008) Still it is recommended to bring a chip under the skin. Because of the fact that the chips are not always readable also a visual system should be used to identify individuals.

8.3.2 Sexing

The difference between males and females is visible by the different location of the urogenital opening (See figure 30).The urogenital opening of a male is located just below the umbilicus (or navel), while the female urogenital opening is located just above the anus (Xavier university, 2001).
Also a female has mammary glands located at each armpit, though not readily apparent under all viewing circumstances (Bush entertainment Cooperation, 1994*b*).

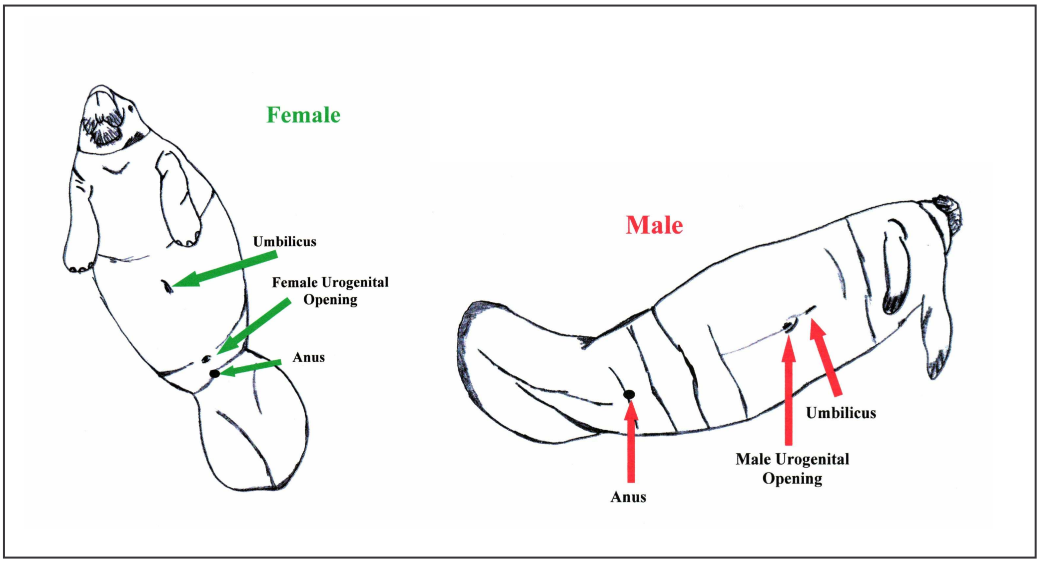


Figure 30 Visible differences in manatee gender (Xavier university, 2001)

8.3.3 Record keeping

The EU Zoos' Directive requires zoos in Europe to keep up-to-date records of the zoo's collection appropriate to the species recorded. Also EAZA recognises the need for proper record keeping in zoos and aquaria. (EAZA, 2006)

Animals should have individual records, except for groups of fish and invertebrates. These records should provide the following information:

- Correct identification and scientific name;
- Origin;
- The dates of entry into, and disposal from the collection and to whom;
- The date, or estimated date, of birth;
- The sex of the animal (where known);
- Any distinctive markings, including tattoo or freeze brands etc.;
- Clinical data, including details of and dates when drugs, injections and any other forms of treatment were given, and details of the health of the animal;
- The date of death and the result of any post-mortem examination;
- The reason, where an escape has taken place, or damage or injury has been caused to, or by, an animal to persons or property, for such escape, damage or injury and a summary of remedial measures taken to prevent recurrence of such incidents. (EAZA, 2006)

These records can be kept in either a card index system, on a computer or other type of retrieval system by means of which information can be quickly examined. EAZA prefers the

use of the ARKS software program and supports the International Species Information System (ISIS). ISIS and its software are currently the global standards for animal record keeping. (EAZA, 2006)
ISIS is currently working to build an online database system called ZIMS (Zoological Information Management System). ZIMS will replace the current ISIS software applications to provide a more accurate, comprehensive database. The existing studbook and ARKS data from the current ISIS system will be moved to the new Zoological Information Management System (ZIMS). (Versleijen and Henselmans, 2006) When ZIMS is finished this will form the global record keeping system for zoos and aquariums.

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9 Legislation

9.1 International

CITES

CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) is an international agreement between governments. Its aim is to ensure that international trade in specimens of wild animals and plants does not threaten their survival.
Of the manatee species the *Trichechus manatus* and *Trichechus inunguis* are listed on Appendix I and the species *Trichechus senegalensis* on Appendix II. This means that the trade of these species is strictly regulated. (CITES, 1963)

9.2 European

The Zoos Directive

The Zoos Directive is the Council Directive 1999/22/EC of 29 March 1999 relating to the keeping of wild animals in zoos. The objectives of the European zoos Directive are to protect wild fauna and to conserve biodiversity by providing for the adaptation of measures by Member States for the licensing and inspection of zoos in the Community, and thereby strengthening the role of zoos in the conservation of biodiversity. This directive has to be implemented by all European member states. (Müntefering, 1999)

The Council Directive 92/65/EEC (Balai Directive)

The Council Directive 92/65/EEC of 13 July 1992, lays down the animal health requirements governing trade in and imports into the Community of animals, semen, ova and embryos not subject to animal health requirements laid down in specific Community rules referred to in Annex A (I) to Directive 90/425/EEC. (Müntefering, 1999)

The International Air Transport Association (IATA)

The IATA Live Animals Regulations is the worldwide standard for transporting live animals by commercial airlines. Whether it is a pet, an animal transported for zoological or agricultural purposes or for any other reason, the objective of the IATA Live Animals Regulations is to ensure all animals are transported safely and humanely by air. (IATA, 2007)

Animal Transport Association (AATA)

The Animal Transport Association is a non-profit organisation dedicated to the safe and humane transportation of animals worldwide by sea, air and land. (AATA, 2005)

9.3 National

All countries in Europe have their own legislation for keeping animals in captivity. In table13 an overview of the zoo legislation in European countries is given.

Table 13 Zoo legislation in EU countries (Eurogroup for animals, 2006)

Country	Name	Date into force	Competent authority
Austria	- Bundesgesetz über den Schutz der Tiere, BGBI. 1 Nr. 118/2004 (includes zoos)	2005	Bundesministerium für Gesundheit und Frauen
Belgium	- Arrêté royal 98-3037 (zoos) - Arrêté ministerial: 3/05/99 (mammals in zoos) - Arrêté ministerial: 5/08/02 (nomination of zoo experts committee)	1999 2002	Service public federal Santé publique, sécurité de la chaîne alimentaire et environment, Inspection générale des services vétérinaires
Cyprus	- 81/2002 (zoos)	2004	Ministry of Agriculture
Czech Rep.	- 162 Act 18/04/03 (zoos) - CITES legislation	2003	Ministry of environment
Denmark	- Order Nº 1023 (zoos) - Order 1021, 1022, 1024 (private keeping, animal trade, live animals in shops)	2003	Civil law division, Ministry of Justice
Estonia	- Animal protection Act - Regulation for the planning and keeping of zoos animals (N. 245) - Regulation for the licensing of zoos	2000 2004 2003	Ministries of Agriculture and environment: Animal welfare inspectors and Environmental inspectors.
Finland	- Act on Animal Welfare 247/1996, amend. 220/2003. - 396/1996, Statute on Animal Welfare - Decisions 2/FHD/2003, welfare requirements for zoo animals	1996 amend 2003	Provincial State Offices (12) Ministries of Agriculture and Forestry
France	- Arrêté 25/03/04 (JO 78, pg 6401) - Code de l'environment - Code rural - Arrêté 25/10/82 (keeping wild animals) - Arrêté 25/10/95 (establishments' control)	2004	Direction de la Nature et des Paysages, Bureau de la faune et de la flore sauvages – Ministries de l'Ecologie et du Développement Durable
Germany	- BGBI I 2002, 1193 Nature Conservation and Landscape Management	2002	Federal Ministry of the Environment, Nature Conservation and Nuclear Safety
Greece	- PD 98/2004 (zoos)	2004	Forestry Department, Ministry of Agriculture
Hungary	- 3/2001. (II. 23.) KöM – FVM-NKÖM - BM (establishment, functioning and maintenance of zoos), amended by 13/2003. (IX. 9.) KvVM-FVM-NKÖM-BM. - 8/1998. (I. 23.), regulation on the protection, keeping, exhibition and use	2001 amend 2003	Ministry of Environment and Water Management

	of protected species of animals - 8/1999. (VIII. 13.) KöM-FVM-NKÖM-BM, regulation on the licensing an keeping of dangerous animals		
Ireland	- S.I. No 440 of 2003, European Communities (licensing and inspection of zoos) Regulations, 2003.	2003	Ministry of Environment, Heritage and Local Government
Italy	- Decreto Legislativo n°73, 21/03/05 (zoos)	2005	Ministry of Environment
Latvia	- Regulation of the cabinet of ministers On the Requirements for Keeping Wild Animals in Captivity and for Establishment of the Collections of Wild Animals (No 185/08.05.2001), amended on 5.04.2005 but not Nr. 232	2005	Ministry of Environment
Lithuania	- Order No. 298, 04/06/02 (amended in 25/09/02 and 25/03/03) (licensing and inspections of zoos). - Order No. 346, 27/06/02 (Keeping Wild Animals in Zoos) - Order No. 308, 27/06/03 (Commission for zoo inspections) - Order No. 250/224, 16/05/02 (Taking of Wild Animals from the Wild to Form Zoological Collections and on Registration of Zoological Collections)	2002	Ministry of the Environment
Luxembourg	- Règlement grand-ducal, 10/02/2003 (zoos) - Loi 15/03/83 (Animal Welfare Act)	2003	Ministére de l'agriculture, de la viticulture et du développment rural, et Ministère de la justice, Administration des Services Vétérinaires
Malta	- Number XXV, 2001 (Animal Welfare Act) - Legal Notice 265, 2003 (zoos)	2001 2003	Ministry of Environment and Rural Affairs
Netherlands	- Dierentuinbesluit (zoos)	2002	Ministry of Agriculture, Nature and Food Quality
Poland	- Act on nature protection of 16 April 2004 (Dz.U.04.92.880) – articles 65-72, 74, 77	2004	Ministry of the Environment, Department of Nature Protection
Portugal	- Decreto-Lei 59/2003 (zoos) - Despacho 7203/2004 (2ªsérie), 12/04 (nomination of the ethical committee) - Portaria 961/2005 (2ªsérie), 22/09 - CITES, native fauna and hunting species	2003	General Direction of Veterinary, Ministery of Agriculture (with cooperation of Ministry of Environment for CITES animals, and conservation evaluations of zoos)
Slovakia	- Act 543/2002 (Protection nature and	2003	Ministry of Environment of

	country, paragraph 44)		Slovak Republic
Slovenia	- Official Gazette of RS, No., 37/2003, 26/04 (Decree on zoos and similar facilities) - Official Gazette of RS, No., 90/2001, (Order on the living conditions for and care of wild animals kept in captivity)	2003	Ministry for Environment and Spatial Planning
Spain	- Ley 31/2003, 27/10/03 (zoos) - Ley 8/2003, 24/04/03 (health and welfare)	2003	Ministry of Environment
Sweden	- Species protection Ordinance (1998:179) (2002 amendment) - DFS 2004:19 Regulations for keeping Zoo animals	2002 2003 (amend. 2004)	Swedish Environmental Protection Agency, Swedish Animal Welfare Agency
UK	- ALL UK except Northern Ireland: Zoo Licensing Act 1981, amended by The Zoo Licensing Act 1981 Regulations 2002	1984 (Amended) 2003 (amended)	Department for Environment, Food and Rural Affairs.

Besides the specific zoo legislation zoos have to comply with general legislation. These are transport laws, environmental laws, veterinary laws and Occupational Health and Safety acts. All countries have there own laws concerning transport, environment, veterinary and health and safety.

Discussion

The data of the captive situation was gathered with the help of literature and a questionnaire.
A lot of information was found during the literature study, but only a few scientific sources are used in this draft of husbandry guidelines. The main reason for this is that most topics are not covered by more than one source. Disadvantage of this is that information is not confirmed by other sources.
Sometimes only general information can be used from sources like USDA, because no species specific information was available. We tried with the help of experts to gather species specific information but this was not possible for all topics.

The questionnaire was send to 13 experts from 13 different zoos worldwide. Of these 13 experts 7 responded and actively contributed to this research by filling in the questionnaire. Two of the respondents were from European zoos, the others were from United States zoos. Six of the zoos are general zoos. This means they do not have only marine mammals, but also other terrestrial animals. Only one zoo of the respondents from the United States keeps specifically marine mammals.

Not all information needed for the topics was asked to the experts, mostly because the questionnaire would become too long and it would take too long for the experts to answer it. Already the some questions of the questionnaire were not all answered and with less explanation than the other questions of the questionnaire. The reason of this can be that some questions were not applicable for the zoos of the experts.

Therefore our priority of questions should have been different. Important questions, about how the situation should be, should have been the first questions on the questionnaire and less important, like number of animals, should have come last. This because in the husbandry guidelines the optimal situations are described and not how the zoos keep this species.

Half of the questions in the questionnaire were open questions. Disadvantage of open questions is that there is no unity in the answers. For a more structured questionnaire it would have been better to use mostly closed questions. (Baarda and de Goede, 2001) So the respondents can choose between categories in stead of all possible answers.

Also the way how questions were asked should have been different. We mainly asked how the situation was in the zoos, in stead of how it should be. Therefore we did not receive all the information that we wanted and what was necessary for the husbandry guidelines.

The experts did not have experiences with all the topics covered by the husbandry guidelines, for example the euthanasia of animals and the transportation of manatees. This means that these topics had to be based on available literature. Main reason for this is that the experiences of the zoos that filled in the questionnaire are based on just a few animals. These zoos together only have 14 males and 24 females.

A problem with the experts could be that their point of view is only focussed on their own manatee enclosure, in stead of a broader view, where more situations of keeping manatees are compared.

www.thesistools.com was used to make and send the questionnaire to the zoos. A disadvantage of using this site was that the respondents were anonymous, so it could not be determined which experts had responded.

A lot is not yet known about keeping manatees in captivity and can only be based on experiences. Because of this it is important that more research is performed about keeping manatees, so that the information is also based on scientific research.

Because of the length of four months to perform this research this was the best methodology possible to gather the information about the captive situation. If there was more time available it would be better to perform interviews with the experts. More questions can be asked during an interview and explanations about answers can be given by the experts. Also in an oral interview persons will give more detailed information than on paper, and the experts can also ask how a question is meant so that the answer is relevant and clearer.

There were some differences in opinions between the experts contacted, these differences concerned the enclosure size, minimum and maximum water temperature, minimum and maximum air temperature, group size and how a manatee should be transported. The differences in numbers can be due to the fact that these were open questions, so every answer could be given.

Some topics were not covered by relevant researches, so no scientific data were available at the time that this draft version was written.
Topics of which no or a little information could be found were:

- Anatomy of manatees; central nervous system, smell, taste and urine system
- Parturition; information about how a manatee is born differs between two literature sources: Hartman, 1979 and Dierauf and Gulland, 2001. Therefore we used the most recent source.
- Enclosure; no species specific information was found, with the exception of enclosure size and depth.
- Feeding; information about nutritional disorders was not found
- Behaviour and social structure; Information about group structure, group size and introduction of animals in a group was not found in literature.
- Breeding; Nothing was found about contraception in female manatees
- Facility design and capture; no species specific information available, most information comes from pers. comm. expert Odense Zoo
- Transport; there was no information found about safety
- Health and welfare; no information was found about surgery
- Population management; no information about identification was found in literature
- Legislation; the laws of each country were hard to find. It is possible that some laws are missing. Language is a problem when searching for national laws.

Enclosure has the highest priority of additional research. Also stress indicators are important, but not found in literature. When keeping an animal it is important to be able to see when an animal is in stress, pain etc.

Because a lot of information in part B is based on literature, this is general information and not always specifically for manatees. In the final husbandry guidelines the general information has to be changed into species specific information, based on the opinions and experiences of the experts contacted.

The opinions of the experts are given under the general information when they were available. So it can be decided relatively easily which opinions form the husbandry guidelines. For the topics on which no opinions are available more discussion is needed between the experts to form an opinion.

Although many information comes from sources with only general information of marine mammals, this draft of husbandry guidelines are still useful for zoos. Now they have some information to hang on to. Also we gathered and compiled relevant information so that it is in one document, so zoos do not have to gather the information themselves.

Glossary

AATA	Animal Transportation Association
CAZA	Canadian Association of Zoos and Aquariums
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
Dam	Term used in animal registration to indicate the mother of the young
EAZA	European Association of Zoos and Aquaria
EEP	European Endangered Species Programme
IATA	International Air Transport Association
LAR	Live Animals Regulations
Manatee/ Manatees	With manatees the West-Indian Manatee (*Trichechus manatus*) is meant
Responsible management	In this case responsible management means the best way of keeping the species with the information available at that moment.
TAG	Taxon Advisory Group
West-Indian Manatee(s)	With West-Indian Manatee(s) is meant the species (*Trichechus manatus*) and both subspecies (*Trichechus manatus manatus*) and (*Trichechus manatus latirostris*)

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