4. METHODS

4.1 Collection of specimens

With good local and regional publicity, many people are aware of the research into the problem of jellyfish envenomation and offer jellyfish specimens they may have caught, or advise of any unusual stings or jellyfish sightings - such offers are always accepted. Even if the jellyfish are not kept a number of interesting specimens have been collected this way, including `Morbakka' and *Physalia* (see Appendix C), Irukandji (*Carukia*), and several other specimens, several of which still cannot be identified.

Some specimens have also been specifically sought, although with *Carukia barnesi* (the Irukandji) which make only brief, erratic appearances at tourist beaches, it is difficult for the author in full-time medical practice to be able to net the waters himself. For this reason surf lifeguards and local fishermen are requested to try to catch them during the course of their work. This has proved successful on just a few occasions (see below). Opportunities to catch jellyfish are always attempted when they appear in waters where the author may be. This has resulted in the description of a Pacific *Physalia physalis* in Australia, for the first time (see below).

Through contacts in the International Consortium of Jellyfish Stings and requests to colleagues a number of specimens of jellyfish have been obtained from areas round the world. These include *Chironex fleckeri* from Borneo (Major Dr J Hooper), *Chiropsalmus quadrumanus* from Puerto Rico (Bertha Cuttress), *Chiropsalmus quadrigatus* from Okinawa, Japan (Dr Araki) and the Philippines (caught by author), *Chirodropus gorilla* from the west African coast (Dr J Gili, Spain), *Chiropsoides buitendijke* from Sri Lanka (Dr M Fernando) *Carybdea* spp. (? *C. marsupialis* from California (S Anderson), Morbakka from Cairns (R Hoare) and Western Australia (L Marsh), and *Physalia physalis* from Pakistan (J Alam)

Irukandji specimens

Three days after a sting at Shute Harbour (Fenner *et al* 1988), the author went to the area near where his case was stung, at the same state of tide, and fortunately similar weather conditions, to try to catch a specimen of the offending jellyfish. A fine net with floats to hold the top on the surface, and weighted on the bottom to hold it as vertical as possible in the water, was secured to the shore on the islet near the area

our case was stung. The other end was attached to a boat anchored 15 meters offshore in 12 meters of water. As the tide started to flood the net billowed out in the current and by swimming behind it (fully protected with a lycra `stinger-suit', hood, gloves, boots and flippers) it was possible to watch the net.

During the next 4 hours two small jellyfish were trapped in the net and transferred to specimen bottles. Both jellyfish specimens were caught near the top of the net, one in only 5 cm of water and the other in 30 cm of water, as suggested by Barnes (Kinsey 1988), but not since corroborated by the author in his findings (see 5.7) that as many such human stings occur at or near the surface of the sea, Irukandji are likely to swim close to and just below it.

The specimens were `box' shaped with one tentacle in each corner. They had a bell diameter of only 1.5cms. and the tentacle length was only 5 cms. They looked macroscopically similar to *Carukia barnesi*. The tentacles were removed whilst still fresh, and stored in liquid nitrogen until they were lyophilised two weeks later. They were then sent to Baltimore for venom analysis and relevant experiments. One tentacle was retained for experiments with the nematocysts (see 5.2.4)

Irukandji antivenom research

The Irukandji is a very fast-swimming, small and transparent jellyfish. As they are also an open-water jellyfish, appearing just sporadically on certain beaches in tropical waters, it is impossible to predict when and where they may be found.

At the author's suggestion, choosing an area near Cairns, north Queensland where Irukandji stings were more common, when there were a spate of Irukandji stings, surf lifeguards were employed to paddle through the water regularly sweeping the water with a fine-mesh net. The contents of the net were then placed into a small container of water, also on the board. Looking at this water, using a black backdrop, they looked for fast-moving jellyfish. This was very slow, quite difficult, and impossible in anything but flat calm weather and sea conditions.

Another method used was for one lifeguard (wearing protective clothing) to take one end of a fine-mesh net out into deep water whilst his companion held on to the other end back on the beach. The deep-water lifeguard then swept round in an arc towards the beach. This method was not effective, as it really needed two people and most lifeguards work by themselves. Also, the net is extremely difficult to pull through the water because of the resistance through the water of the fine mesh. It was thus

possible for the fast-moving Irukandji to escape, as well as being exhausting work for the lifeguard.

In September 1996 a special net was designed after discussions by the author and an engineer, Kim Moss, designer of the stinger-resistant nets used in north Queensland to prevent stings to swimmers. It was a large pyramid-shaped, fine net, attached to a lightweight frame 2.5 meters by 3 meters. Attached to the frame was a rope bridle, which was then attached to some 50 meters of rope. When pulled behind an IRB (Inshore Rescue Boat – a twin-hulled inflatable boat used for surf rescues) the net became vertical and it dropped completely into the water until it was just supported at water level by floats on the aluminium frame. The net, by means of the specially-made rope bridle, then floated on the surface, just off the vertical. Here it could then be slowly towed through the water for as long as necessary, or thought to be necessary, thus covering large amounts of water with relatively little effort.

When the boat stopped, the net immediately floated to the surface, thus trapping any jellyfish in the fine, pyramid-shaped net. The frame was lifted into the IRB and any jelly material shaken down into the tip of the pyramid. This tip was then put in a large bowl of sea water stored in the boat. Using transparent plastic drinking cups this water was then scooped out, inspecting each cup visually, for fast-moving jellyfish. Whenever one was seen it were placed in a separate seawater container until they could be returned to shore. Here the water was drained off and they were placed in small, sealable plastic bags and placed in a freezer to freeze them as quickly as possible.

4.2 Serology investigations

Tentacles

The author obtained tentacles of *Chironex fleckeri* locally, and *Morbakka* from various areas of the Queensland coast (see Appendix C); a tiny amount of tentacle was also collected from two Irukandji specimens collected from Shute Harbour, north Queensland in 1988.

Other tentacles tested by Burnett (Burnett *et al* 1988) were from *Chrysaora quinquecirrha*, *Cyanea capillata, Pelagia noctiluca, Cassiopea xamachana, Physalia physalis, Stomolophus meleagris and Aurelia aurita.*

Serum collection and dilution for testing

Serum from the Australian patients was either collected personally by the author, centrifuged immediately and frozen at –70^{o}c, or transported to the author, some at ambient temperature and others in dry ice to keep it frozen. It was then taken to the local Sugar Research Laboratory, the only laboratory within 400 kilometres where it could be lyophilised, and then it was mailed to Baltimore. Here it was reconstituted and frozen for use. The sera were diluted serially to 1: 5, 25, 50, 100, 150, 200, 300, 450, 900, 1800 and 3600.

Serum from all United States patients was shipped to Baltimore by mail at ambient temperature and frozen at -70'C until use. Sera from 30 healthy patients not on medication were analysed as controls.

4.3 Identification of the specimens

Identification was often very difficult. Some species were identified by using the main taxonomical works of Haeckel (1880), Mayer (1910) and Kramp (1961). However, the more detail that was sought, the more it was realised that each of these books had considerable limitations. In some instances the specific, original articles describing the new species or genus had to be obtained and are referred to in the appropriate place in this thesis. In the case of the Morbakka all the literature proved of little worth and so theories suggested in this thesis have yet to be biologically and taxonomically confirmed.

The same problem seems to be current with the cubozoan from the western coast of California (see 5.7.2).

4.4 Examination of museum specimens

Cubozoan specimens were examined from the Smithsonian Institute in Washington DC, the British Museum of Natural History in London and the South Australian Museum in Adelaide. Other specimens such as *Physalia* spp. were examined in these museums as well as the Australian Museum in Sydney, the Queensland Museum in Brisbane and the Philippines Museum in Manila. Some specimens were in very good condition, but others were very poorly preserved and difficult to identify.

Others were also found that were labelled incorrectly, even in these prestigious museums.

4.5 Experiments with inhibition of nematocysts

Whenever the opportunity arose with live, fresh jellyfish specimens, experiments were conducted using various chemicals to see if they caused inhibition of nematocyst firing. Chemicals included vinegar, magnesium sulphate (epsom salts), 20% aluminium sulphate (called `Stingose' in Australia), formic acid, boracic acid and even insect repellent. The jellyfish tested were *Cyanea capillata* (the “hair jellyfish” or “lion’s mane”)*, Carybdea rastoni* (the “Jimble”), *Carukia rastoni* (the Irukandji), *Tamoya haplonema fenneri* (the “Morbakka”), *Physalia utriculus* (the “bluebottle”) and *Physalia physalis* (the “Pacific man-o’-war”). *Chironex fleckeri* was also tested to recheck Hartwick’s results (Hartwick *et al* 1980).

4.6 Observations of conditions for human jellyfish stings

Over the last thirteen years of surf patrol at Mackay, Queensland, notes have been made for Queensland, of the conditions when stings are most likely to occur. Notes were also made during the frequent travels over the whole Queensland State, tropical Australia and other coastlines of the world. Some of this information was also available from the forms sent in for the database, especially from the 55 Queensland Surf Life Saving Clubs on the east coast of Queensland. One of the greatest observers of conditions and of jellyfish stings, was Dr J Barnes of Cairns, whose extensive works have been mentioned in this thesis.

4.7 Clinical observations of envenomed victims

Over the past thirteen years the author has had the opportunity to both see and treat many patients who have been stung by jellyfish. Case histories of relevant stings are reported (below). It is from the treatment of these victims, together with known pharmacological benefits of some drugs that first aid and medical treatments summarised below were developed. They were done after discussion and collaboration with colleagues and co-authors, mentioned in this thesis.

Several cases of Irukandji envenomation had been investigated and the findings published (Fenner *et al* 1986b; 1988). In the past season 1995-6, and current season 1996-7, there have been a number of serious stings in the Mackay region. This has given the opportunity for the author to collaborate with a local Physician to investigate most of these cases using echocardiography. The results have proved interesting and are reproduced below, and are also to be published (Carney & Fenner 1997).

4.8 Developing policies for first aid

Many years of watching and discussing sting treatments whilst on surf patrol have confirmed the author's view that first aid treatment had to be simple, and easy to remember. Initially treatments were designed around each different jellyfish species - thus needing positive identification first. Most people, even the trained lifesavers who have access to the charts, videos and publications prepared for them, find it practically impossible to positively identify different jellyfish. The policies suggested (see first aid section below) have been simplified as much as possible for treatment `groups' of jellyfish (and other marine animals causing envenomation) so that they are easily remembered.

Mass stings

At times there are so many stings on the beach at once that life guards (professional) or lifesavers (volunteers) may not have enough ice. Policies to overcome this and use alternative treatments that were more readily available were developed, using the principle of "first do no harm" and secondly to use the power of suggestion to overcome any discomfort caused by jellyfish sting. Such treatments were obviously ineffective for more serious stings and all first aid policies call for trained help if simple treatments do not help, or the patient's condition is one to cause concern.

4.9 Personal travel and research

A number of trips to other countries have been planned so that searching and netting for jellyfish was also possible. The opportunity was also taken to speak to locals to try to discover information on local jellyfish, occurrence of stings and their treatments.

4.10 The data base and its sources

This is dealt with under its own `Methods' section under 5.6 below for easier reference.