Tropical box jellyfish: the world's deadliest animals

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[3] Arising from a public lecture delivered by TC in December 2002

Introduction

The opportunity arose, because of a ***sciart*** award (sponsored by the Wellcome Trust and British Arts Council and other bodies, and designed to allow scientists and artists to work together) to brother and sister, scientist Tom Cross and artist Dorothy Cross, to work on the tropical box jellyfish in North East Australia. The data collected in the form of digital video "footage" of swimming, provided the materials used by Marc Shorten in his MSc project. In a talk forming part of the 2002/2003 UCC public lecture series, Professor Tom Cross described the features of these animals and then recounted the work undertaken on swimming biomechanics. A similar format is used in this chapter.

Chironex fleckeri in mid water

General characteristics

Box jellyfish, also known as "marine stingers" or "sea wasps", are of great interest because the group, called Cubomedusa by zoologists, contain some of the most venomous animals on earth, but appear to have been the object of very little scientific study. Cubomedusa are very different from medusae of other classes of the Phylum Cnidaria ("true jellyfish") being far more substantial than animals of Classes Syphozoa or Hydrozoa. Whereas the bell of these other two classes are extremely jelly-like in their consistency, cubozoans are more akin to polyurethane than jelly. Cubozoans are roughly cubical, and this is where they get their vernacular name "box jellyfish". Accentuating the box-like appearance and making them immediately distinguishable from the rest of the medusoid Cnidaria, are four tentacles or tentacle groups, each borne on a blade- or spatula-like pedalium. The pedalia arise from just above the bell-margin and are relatively tough compared with the rest of the bell. The form of the pedalia and the number of tentacles per pedalium are diagnostic of the groupings within the Cubozoa. The pedalia are thought by some to steer the medusae while swimming and are known to function in feeding.

There are two main groupings of the Cubomedusae, the Carybdeidae and Chirodropidae, distinguishable by the fact that in the Chirodropidae there is more than one tentacle per pedalium, in contrast to the Carybdeidae where there is only one tentacle per pedalium. The Carybdeidae range widely over temperate and tropical oceans whereas the Chirodropidae are confined to tropical waters. *Chironex fleckeri* and *Chiropsalmus quadrigatus*, the two species which we studied, are chirodropids. Mature cubozoan medusae vary between two and twenty-five centimetres in bell diameter. *Chironex fleckeri* is the largest species of the class (i.e. averaging 25cm diameter when mature) and *Chiropsalmus quadrigatus* is about 4.5cm in bell height, being slightly smaller in diameter than in height.

Rear view of the bell of *Chironex* showing prominent features

Life cycles of *Chironex fleckeri* and *Chiropsalmus quadrigatus*

In tropical North Queensland, Australia, where our studies were undertaken, *C. fleckeri* and *Chiropsalmus quadrigatus* are annual species. At least in *C. fleckeri (*the life history of *C. quadrigatus* is less well known), the tiny fixed polyp phase occurs in estuaries during the austral winter (May to October). Planula larvae are budded off which give rise to the free living medusa stage. These feed and grow in coastal waters inside the Great Barrier Reef from November to April, with the average size at sexual maturity being 20 to 30 cm in bell diameter. They then return to spawn in estuaries, re-establishing the planula stage. The larger medusae feed on prawns and small fish. The highly toxic venom may have evolved to capture these fast moving prey species in open water. They do not, of course, actively hunt humans but seek their natural prey in shallow waters (often less than a metre deep) over a sandy bottom. Thus, they are abundant off beaches during the Austral summer, where they are accidentally encountered by humans, particularly children, running into the water.

Feeding

Once a prey item is caught in the tentacles, they are retracted to bring the item towards the velarium, before flexing one or more pedalia to feed the item to the manubrium (the animal's mouth). In *C. quadrigatus* there are usually five to nine tentacles per pedalium. In *C. fleckeri* there are up to fifteen tentacles per pedalium in a mature animal, and rarely fewer than nine.

Venom delivery

Chironex fleckeri, which occurs in northern Australia, Papua New Guinea and Indonesia, is the world's most venomous animal, and has resulted in at least 80 fatalities in Australia during the twentieth century. The venom is contained in millions of stinging cells, called nematocysts, spread along the length of the highly muscular and contractile tentacles. These stinging cells contain a coiled and barbed microtubule and aliquot of venom. On physical stimulation or activation by certain chemicals, stinging cells are activated and microtubules are fired into the victim releasing venom.

Pedalium and tentacles of Chironex (photo by Paul Sutherland)

Photomicrograph of discharged stinging cell (nematocyst) of *Chironex* (photo by Jamie Seymour)

Human stinging incidents

A single mature *C. fleckeri* has, in its tentacles, enough venom to kill 60 adult humans. In the Southern Hemisphere, chirodropid stings occur mainly in the austral summer (December-May), but over a longer season in areas closer to the Equator. The database of the International Consortium of Jellyfish Stings has records of over 1100 individual cases of stings (some severe and fatal) from surf life saving clubs in Queensland between 1990 and 1996. In a human stinging incident many thousands of nematocysts are activated. Vinegar is available on North Queensland beaches as a first aid measure against stings. The acidity of vinegar prevents additional stinging cells from firing but does nothing to alleviate the effects of those which have already fired. Where stinging does occur, the most appropriate medical response, after flooding the wound with vinegar, is rapid transport to a properly equipped intensive care unit, where the patient is treated to reduce shock and where necessary, artificially assisted in breathing. An anti-venom agent against *C. fleckeri* stings has been developed by the Australian Government Laboratories and is issued to the emergency services in the danger area.

Stinger suits and exclosures

It was discovered in Australia that a thin layer of synthetic material such as polyester is largely protective, and thus the "stinger suit" has evolved. Women and children are more at risk because of lack of body hair, so nearly all children on beaches are now equipped with these suits during the stinger season. During the Austral summer areas of about 30 metre squared, enclosed by nets, are set up on popular beaches to allow swimming. These stinger nets have a mesh size designed to keep out all but the smallest box jellyfish (though broken off tentacles, that are still capable of delivering a nasty sting, can penetrate the nets).

Chiropsalmus quadrigatus

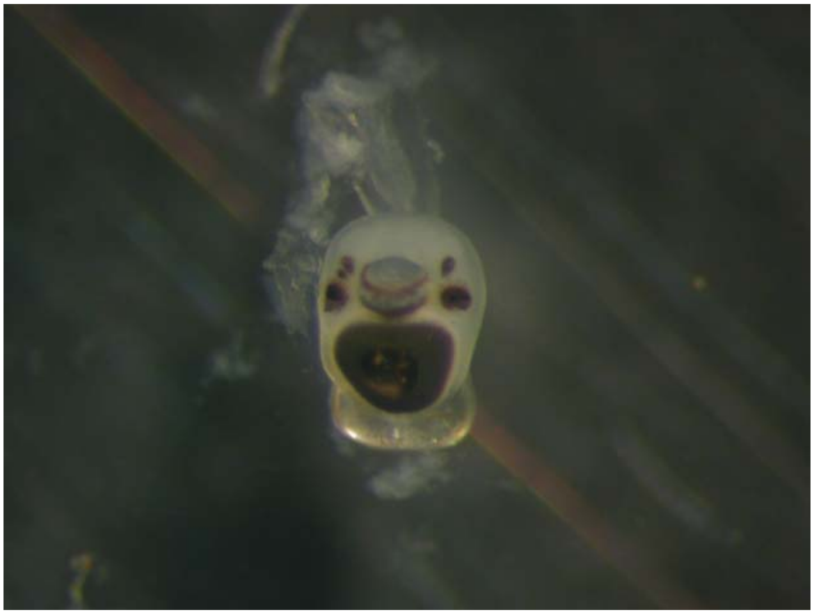
The quoddie, *Chiropsalmus quadrigatus,* is a smaller and less venomous species than *C. fleckeri* but is very similar in appearance and occurs within the same area (but with a much more restricted distribution). We reasoned that it might initially be easier to get suitable video sequences from this species, because of its smaller size and less venomous nature. We were also interested in comparing the biomechanics of swimming in these two species.

Swimming speed

Box jellyfish are active swimmers, whereas jellyfish of the class Scyphozoa, the common medusae in Irish waters, are feeble swimmers, largely at the mercy of the prevailing currents. There is a record from the Cairns area in North Queensland of *C. fleckeri* swimming at 3 knots (i.e. about 1.5 meters per second, very little slower than an Olympic 1500m swimmer) for 30 minutes. It has been questioned whether this was an accurate still water speed, since simultaneous current measurements were not taken. For example, in a later publication it has been reported that large *C. fleckeri* were observed swimming at 10-20 cm per second in still water, and doubt has been expressed that animals could attain ten times that speed. Cubomedusans actively hunt, and exhibit a respiratory rate, on average, an order of magnitude greater than Scyphozoan medusae.

Balance and vision

Between pairs of pedalia, Cubomedusans have organs called rhopalia on which the balance organs (statoliths) and light sensitive organs occur. In *C. fleckeri* there are three light sensitive organs on each rhopalium and at least one of these is a well developed eye with a lens and image gathering neural retina. How the animal processes the images from these eyes when it possesses only a simple nerve net instead of a brain has been the topic of much debate.



Photomicrograph of a rhopalium showing showing balance organ or statolith (top centre) and complex eye (bottom centre)-(photograph by Jamie Seymour)

Swimming action in *C. fleckeri* and *Chiropsalmus quadrigatus*
Like other cubomedusan species, swimming in *C. fleckeri* and *Chiropsalmus quadrigatus* is by jet propulsion. These jellyfish have provided the focus of aesthetic and scientific interest for well over a century due to their speedy and graceful locomotion. At the end of the 19th century, one author described the swimming of the species *Charybdea xaymacana* in rather poetic terms:

> "Charybdea *is a strong and active swimmer, and presents a very beautiful appearance in its movements through the water, the quick, vigorous pulsations contrasting sharply with the sluggish contractions seen in most Scyphomedusae".*

Having described the characteristics features of the Cubomedusae we now describe our field work in Australia and subsequent analyses of the video "footage" in Cork.

Preliminary Australian work-Summer 2000
Considerable work on *C. fleckeri* has been carried out in Cairns, North Queensland and specifically at the James Cook University (JCU) campus, over the last 25 years. Dr Carl Flecker, who first described the species, worked there, as did Dr Jack Barnes, who continued the work. These investigations are being continued by Dr Jamie Seymour of the Department of Zoology, with whom we have been collaborating. Jamie Seymour is an entomologist by training, specialising in butterflies in his early career. He is now working as an ecologist, on several aspects of the box jellyfish and is co-operating closely with Medics on venom research. He

assisted us in every way on the project. He knows where animals are at different times of the day and year. He also knows the best place to capture them and how to handle them.

Jamie Seymour holding large *Chironex fleckeri* and being careful to avoid contact with the tentacles (photograph by Paul Sutherland)

All of the video material available on swimming in *C. fleckeri* and related species was viewed. In viewing these videos, it became obvious that VHS video was not of sufficient quality for detailed analysis. It was, however, possible to get a general impression of swimming movements in *C. fleckeri*, e.g.
1. Jet thrust by contraction of the circular muscles inside the bell (reducing the volume of the bell and causing deformation of the jelly-like mesoglea). This causes outpushing of the velarium (the "skirt" around the aperture) and maximum trust, which in turn leads to maximum velocity.
2. This contraction phase is followed by relaxation of circular muscles and increase of bell volume by springing back of elastic mesoglea or "jelly". This causes refilling of the bell and cessation of forward trust, and forward velocity declines.

It became apparent in this preliminary work that only certain available video sequences were suitable, i.e. those taken in high quality DV, having a background scale and ideally a fixed camera position. It was not possible during the stay in N. E. Queensland in 2000 to do any additional filming, because the stinger "season" does not begin until the austral summer and no suitable animals existed in captivity.

The only suitable section was a 16 second high quality DV sequence from a Digital Dimensions Films video, taken the previous summer in their outdoor sea water tank (8m long X 4m wide X 2m deep) in Townsville, south of Cairns. In this sequence a single animal was swimming against a wall of the tank over which plastic mesh of know dimensions has been draped (supplying background scale). While the camera was moved to a certain extent, it was still possible to record the movements against a fixed background. Using a video analysis software package, it was possible to analyse the sequences in a frame by frame fashion, with each frame representing 1/25 second. This method was also used in subsequent years.

In the 16 second sequence, the animal (bell height 29.9 cm) travelled 310 cm. Average speed was 19 cm sec^{-1} (about 0.6 bell lengths per second). It went through 20 pulse cycles, i.e. 1.25 pulses s^{-1}, travelling on average 15.5 cm per pulse. Concentrating on one of the later pulses in the sequence, it appeared that bell volume decreased by approximately 30% due to circular muscle contraction (calculated by measuring the change in internal bell diameter assuming cuboidal shape). Learning from our experiences in 2000, in the latter two years we based all observations on digital video.

2001 Australian collecting trip

We next travelled to Australia in February 2001. Working again with Jamie Seymour, we began by rising at dawn every morning to seine net on the beaches just north of Cairns. Jamie and his assistant Teresa Carette used a net approximately 4 feet tall and 40 feet long stretched between two poles, which they 'walked' along a beach where it is known box jelly fish feed. We netted every morning for a week without success. The weather had turned unseasonably cold and the storms had begun earlier than usual so Jamie feared that perhaps the animals had disappeared for the season. We travelled south to Townsville to see if we could capture some box jellyfish there, but again failed. The nets were pulled in catching other organisms which we immediately released: barramundi, garfish , baby squid , mangrove seed pods and a heavy, surprised , loggerhead turtle that fled at great speed.

The beaches of North Queensland are stunningly beautiful. Palm trees and wilderness run right down to the shoreline of turquoise water. The presence of *Chironex fleckeri,* such a deadly invisible threat, in the shallow waters of what appears as paradise, is strange.

Dorothy and Tom Cross enroute from Cairns to Townsville, North Queensland

Jamie has jellyfish watchers all along the coast and, towards the end of our stay, we heard there was a large number of *Chiropsalmus quadrigatus* had arrived in Port Douglas, north of Cairns. We drove there and captured many animals to bring back to laboratory for filming. We realised we would have to return the following year when the *Chironex fleckeri* returned again and in the meantime set up some filming tanks for *Chiropsalmus*.

As said before, *Chiropsalmus* is a smaller animal so we could film them in a small tank in the laboratory. Not finding *Chironex* that season may in fact have been fortuitous, since it meant we could experiment with the logistics of netting, transport, grids, camera angles etc. before moving onto the larger and deadlier *Chironex fleckeri*. It was during this period that all the *C. quadrigatus* animals subsequently analysed were collected.

Netting for box jellyfish on Townsville beach using a seine net. Note the protective stinger suits

Videoing in 2001 – *C. quadrigatus*

In this sampling and the next year's sampling periods a digital video camera was used running at 25 frames per second. Videoing was carried out the Cairns campus of James Cook University. The camera was placed 60cm from a glass aquarium. A plastic mesh grid covered the back of the aquarium, to provide scale. The colour of the background behind the mesh was black in order to contrast with the translucent bell and tentacles of the animals. To obtain video of linear locomotion, the camera was placed on its side and the animals brought to the bottom of the tank, before releasing them to swim vertically. This allowed locomotion to take place along the camera's longer axis of view. Measurements of bell diameter were made for each individual prior to videoing by measuring the inter-pedalial distance.

Tank set-up used to film *Chiropsalmus* in James Cook University in Cairns in 2001

2002 Australian collecting trip

We returned to Australia several weeks earlier in 2002 (in late January). The same process of netting began, first in Cairns and then in Townsville, 400 km to the south. In Cairns we caught many *Chiropsalmus* but no *Chironex*. The first day in Townsville we caught no *Chironex*. But the second day we were successful!

However we discovered that when *Chironex* were netted the majority of their tentacles were being torn off. So we changed the capture system. Since the animals swim very close to the surface, with the assistance of Polaroid glasses, Jamie Seymour was extremely successful in catching them by hand, holding them by their bell, which does not have stinging cells. (The animals are so transparent one often sees their shadow on the sands before you see them.) He then placed them in buckets without tentacle damage.

Having gathered several animals in each of four days, we brought them to the same large custom built tank, as mentioned previously, at Digital Dimensions Studio in Townsville. A digital video camera was placed in an underwater housing mounted on a tripod. A perspex sheet was placed between the camera and the background. This helped to constrain the animals' direction of travel. The camera was set up 70cm and at 90 degrees to the perspex sheet, which was 70cm from the wall of the tank, on which a green plastic mesh grid was fixed over a dark blue background.

Set-up used to film *Chironex* in Digital Dimensions Studios in Townsville in 2002

As with *Chiropsalmus*, measurements of bell diameter were made. In contrast to 2001 however, these measurements were generally achieved by bringing the animal alongside Vernier callipers placed against the perspex, while holding the animal by the bell safely on the other side (this trade-off with accuracy was justified considering the potential envenomation that a *C. fleckeri* individual could inflict).

Reviewing video

All footage was reviewed a number of times to assess its suitability for kinematic analysis. Following this, selected sequences were downloaded to computer. In the most suitable footage the line between any two neighbouring pedalia was either parallel or at ninety degrees to the camera lens.

Video frames from two locomotor cycles of *Chiropsalmus quadrigatus* selected at the points of maximum circular muscle contraction and relaxation, with position of landmarks used in subsequent analysis indicated

Editing

Sequences were saved as AVI files before once more being reviewed. For example, after editing the 2002 footage to one hundred-and-thirty-two sequences of 16 *C. fleckeri* individuals, only seven animals were used in the final quantitative analysis. All footage that made it to the final cut was of high quality and clearly illustrative of the locomotion of these species. Each sequence used contained no less than two and a half cycles (pulses) of movement.

Footage from both 2001 and 2002 sampling periods were run through graphics packages allowing the placement of opaque co-ordinate nodes on seven readily-apparent body locations.

The use of "nodes" placed on body of the animal, allowed mathematical interpretation of the position a given body part at any given time.

The positions of the nodes were as follows:

Nodes 1 and 7 fall on the points where the outside of the bell, the base of the pedalia and the velarium meet. These two nodes describe inter-pedalial diameter.

Nodes 2 and 6 describe the midpoints between nodes 1 and 3, and 5 and 7 respectively.

Nodes 3 and 5 are located on vertically opposite corners of the gastric pouches.

Node 4 gauges the distance covered in each frame, and thus characterises gait in terms of speed.

Following the placement of the reference nodes, x and y co-ordinates could be determined in each frame of footage, by moving the computer cursor until it coincided with the centre of the node. Co-ordinates could then be transferred to a spreadsheet for geometrical interpretation of the data.

The data within the spreadsheet for a given sequence of locomotion allowed the determination of several parameters of gait. The distances obtained were recorded in pixels. As a result of making size measurements, a known lateral inter-pedalial distance was available, so that measurements could be converted to millimetres.

Both species were seen to execute a 'pulse-and-coast' style of jet propulsion. One cycle (or pulse) of movement was seen to consist of contraction of the bell causing ejection of water through the velarium and eversion of the velarium. The contraction seen in all animals resulted in a reduced volume in the bell. The contraction stage was followed by a relaxation phase when animals coasted, refilling the bell as the mesoglea returned to its original shape and the velarium was once more inverted. A visual appraisal of the type of movement described above is possible through examination of frames from a sequence of a *C. quadrigatus* individual, as it moves through one cycle.

Video frames from two locomotor cycles of *Chironex fleckeri* selected at the points of maximum circular muscle contraction and relaxation, with position of landmarks used in subsequent analysis indicated

Graphs produced based on the co-ordinates of the nodes during locomotion allowed numerical description of gait. These graphs, having the same horizontal scale, display various parameters that change in the course of movement.

Graphical representation of node movements of *C. quadrigatus* over time

Dye shedding

To visualise the water currents produced during movement, the bright green dye fluorescein was injected into the bell, using a hypodermic syringe. At each contraction (jetting), a spurt of dye was observed moving quickly out through the velarium. When this aliquot of dye reached a certain distance behind the animal it was observed to slow down and roll outwards into a ring structure orientated at right angles to the direction of movement. Hydro-dynamics specialists refer to such ring structures as vortices and their formation during motion as vortex shedding.

Video frames of dye shedding and vortex formation in *Chiropsalmus*

Statistical Analyses

Despite the general similarities of the gaits of the two species, the possibility that there were underlying differences between species, and even size classes within species, became apparent. Statistical testing demonstrated that *C. quadrigatus* ejected a greater volume of water relative to their bell size than *C fleckeri*. There was also a significant within species correlation between relative body size and pulsation frequency, with smaller animals pulsing faster. The computer-based approach to statistical analyses of gait parameters used here has not, to our knowledge, been used previously in this biomechanical context. Therefore this investigation sets a framework for other researchers studying medusan locomotion and animal gaits in general.

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(This lecture was delivered at the UCC Science Faculty Public Lecture Series 1992-93 on December 11, 2002.)

Figure Legends

***Chironex fleckeri* in mid water**

Rear view of the bell of *Chironex* showing prominent features

Pedalium and tentacles of Chironex (photo by Paul Sutherland)

Photomicrograph of discharged stinging cell (nematocyst) of *Chironex* (photo by Jamie Seymour)

Photomicrograph of a rhopalium showing showing balance organ or statolith (top centre) and complex eye (bottom centre)-(photograph by Jamie Seymour)

Jamie Seymour holding large *Chironex fleckeri* and being careful to avoid contact with the tentacles (photograph by Paul Sutherland)

Dorothy and Tom Cross enroute from Cairns to Townsville, North Queensland

Netting for box jellyfish on Townsville beach using a seine net. Note the protective stinger suits

Tank set-up used to film *Chiropsalmus* in James Cook University in Cairns in 2001

Set-up used to film *Chironex* in Digital Dimensions Studios in Townsville in 2002

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