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**DOES TAGGING FOR RADIO ACOUSTIC POSITIONING TELEMTRY
CHANGE SPAWNING BEHAVIOUR OF CHOKKA SQUID,
LOLIGO VULGARIS REYNAUDII?**

BY

P. ROBIN RIGBY

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF SCIENCE

AT

DALHOUSIE UNIVERSITY
HALIFAX, NOVA SCOTIA
AUGUST, 2000

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August, 2000

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TITLE: "DOES TAGGING FOR RADIO ACOUSTIC POSITIONING TELEMTRY
CHANGE SPAWNING BEHAVIOUR OF CHOKKA SQUID, *LOLIGO*
VULGARIS REYNAUDII?"

DEPARTMENT OF BIOLOGY

DEGREE: M.Sc

CONVOCAION: OCTOBER

YEAR: 2000

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DEDICATED TO

All who are willing to go beyond calamari.

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ABSTRACT

Although centuries of work illustrate man's desire to discover loliginid behaviour only recently has the ability to detail spawning behaviour been obtained. Radio Acoustic Positioning Telemetry (RAPT) analysis allows researchers to observe *in situ* behaviour with more accuracy and greater precision and continuity than ever before. However, the transmitters effect on the animals and analytical limitations are unknown. Video analysis combined with data from the literature and general observations allowed 'Normal' behaviour to be represented by sequential dots making it comparable to readouts derived from data collected by RAPT triangulation algorithms. Qualitative comparisons between normal behaviours and behaviours seen by RAPT allowed for biological interpretations of the patterns seen and hence an understanding of movements and *in situ* arrangements over the egg beds along the South African shore. These comparisons and an analysis of change over time of the RAPT data allowed for behavioural change caused by RAPT tags to be assessed. Behavioural change was seen to occur over the first 3 days of experimentation after which they stabilized and observations were made. Notable observations included zone differentiation, approach patterns where female Chokka laid egg strings independently while escort males hovered above, the distinguishing of sneaker males by movement patterns alone and the observation of temporal division of beds by males.

List of abbreviations and symbols used

2D	Two dimensional
cont.	Continued
cm	Centimeters
±	Within the range of
%	Percentage
~	Approximately
0''	Degree, minute, second. Latitude and longitude.
#	Number
#F	Categorical number and sex. F = female
#M	Categorical number and sex. M = male
#S	Categorical number and sex. S = sneaker male
#Fn	Categorical number, sex and test indicator. F = Female, n = NGDR
#Fs	Categorical number, sex and test indicator. F = Female, s = speed
°C	Degrees Celsius
°F	Degrees Fahrenheit
3D	Three dimensional
cms ¹	Centimeters per second
ms ¹	Meters per second
e.g.	<i>Exempli gratia.</i> For example
Esp.	Especially
et al.	<i>Et alia.</i> And others
etc.	<i>Et cetera.</i> And so on
Ex.	Example
FAO	Food and Agriculture Organization (A department of the United Nations)
g	Grams
G.P.S.	Global Positioning System

LIST OF ABBREVIATIONS AND SYMBOLS USED, CONTINUED....

<i>in situ</i>	In the wild
MatLab	Mathematic Laboratory. Created by the Math Works Inc.
MHz	Megahertz
N	North
E	East
S	South
W	West
NGDR	Net to Gross Displacement Ratio
P-value	The difference found by a t test
pers. com.	Personal communication
post. - [prefix]	After in time or order
pre. - [prefix]	Before in time or order
RAPT	Radio Acoustic Positioning and Telemetry
scoping	Surveying a selected area for a specific object or objects
trans.	Translated
TV	Television
VEMCO	Voegeli Engineering & Manufacturing Company
VRAP	Vemco's Radio Acoustic Positioning program
X,Y,Z coordinates	Depth (z) and Distance (x and y) given in a graph

ACKNOWLEDGMENTS

If life were simple and fast solutions worked I would say merely:

Sincere thanks to all of those that helped.

All Praise (Thanks) be to Allah.

However, there is one thing this thesis has taught me and that is that feats such as this are never as simple or quick as one would like! So in acknowledgment of the fact that this like everything else in this world was not a single person heist but that there is blame to go around I would like to extend my thanks to the following people who made this possible. To all those missing from the list I appreciated your love and support, your courage and your wisdom even if you didn't know it then and can't see it here!

Much thanks to:

- A brilliant and patient supervisor - Dr. Ron O'Dor
- My committee, readers and examiners - Dr. Shelly Adamo, Dr. Ram Myers,
Dr. Hal Whitehead and Richard Starr
- All the collaborating scientists - Dr. Malcolm Smale, Dr. Warwick Sauer,
Dr Michael Roberts, Dr. Rodger Hanlon,
and Dr. Marek Lipinski
- The whole crew - out in the water - Dr. Dale Webber especially
- and in the lab - Dr. Yanko Andrade, Frank Ham,
and Catriona Day
- My colleagues - around the office - Dr. Dan Jackson,
Dr. James Wood, Jill Aitken
Alison King, and Marianne Gilbert
- and around the (coffee) pot - Alison Cole
- The HOME front.

This wouldn't have been DONE without you, Thank you.

CHAPTER 1 - INTRODUCTION

Southern African water is a kaleidoscope of life and it is here that species of the Atlantic, Indian and Southern Oceans mingle. 20-30% of the worlds known cephalopod species live in this confluence zone. The Agulhas bank, at the tip of the South African Cape is both warm (13 -18 ° C) and protected, which makes it a prime choice for loliginid breeding (Augustyn, 1990). There are a number of species that take advantage of the Agulhas bank location and environment but the most commonly found loliginid is *Loligo vulgaris reynaudii*. There have been close to forty *Loligo vulgaris reynaudii* spawning sites found along the inshore areas of the Eastern Cape coastline between Algoa Bay and Plettenberg Bay, (Sauer *et al.* 1992). For this reason the area has become known as a Chokka squid breeding ground

Named in 1839 *Loligo vulgaris reynaudii* d'Orbigny was established as a subspecies of the North Atlantic *Loligo vulgaris (sensu stricto)* on the basis of morphological and electrophoretic evidence (Augustyn and Grant, 1988). Found along the continental shelf of southern Namibia and the Cape Province (Augustyn 1989) it is locally known by its Africans nickname Chokka. Chokka squid are more than a common sight off the coast, they are a keystone species in the Agulhas Bank ecosystem and as such knowledge of their life cycle and predator-prey relationships within the region is particularly valuable for the management of both the squid and

(Augustyn, 1990) an industry based on the exploitation of the inshore spawning grounds of a species whose recruitment is highly dependent on complex biological and physiological interactions. (Augustyn *et al.* 1994)

1995 FAO statistics placed cephalopods as the sixth most valuable fishery resource worldwide (Roeleveld, 1998). However squid fisheries are notoriously difficult to manage, as population levels and availability can change drastically and without warning. In reality, the necessary level of management has not been agreed on, and few loliginid fisheries are intensively managed. Past mismanagement or the absence of management has led to a number of serious population crashes and stock losses. Examples include areas such as Northern Japan's 1960's loss of the Pacific Population of *Todarodes pacificus*. Eastern Canada's 1980's loss of *Illex illecebrosus*.

The absence of a management strategy for the South African Chokka fishery is an undesirable situation, from both a resource and economic stand point. The relatively new industry which has grown rapidly since 1984 when Chokka were mainly caught as a by-catch from demersal trawlers accounts for over 90% of the annual Chokka catch which although varies considerably can reach 10 000 tons (Roberts and Sauer 1994). The industry provides employment for 2 500 fishermen and over 1 000 land-based workers and other personnel, it acts as an important source of hard currency from world (esp. European) markets (Augustyn *et al.* 1994). The

economic importance of the fishery aroused government attention in ongoing scientific pursuits, leading to an overall increase in the interest in the welfare and management of the species.

An international workshop held in Cape Town, South Africa in mid-1997 identified a number of features of cephalopod biology in need of further research (Payne *et al.* 1998). Interest stemmed from both fisheries management and general science. Among the identified features, the understanding of life cycles and spatial distribution were perceived as key priorities for research. Physiological and genetic approaches to understanding basic aspects of the life cycle, and their importance for understanding population dynamics, were stressed. Environmental studies were also mentioned as being important in the possible prediction of population trends though links that operate at the level of spawning biology (Lipinski *et al.* 1998).

To some extent these needs had been anticipated beforehand. The primary purpose of the *November 1994 Acoustic Tagging and Environmental Study* in South Africa was to look for environmental influences on the Chokka squid life cycle and fishing (jigging) industry in South African waters (Roberts *et al.* 1994). The project was spearheaded by Dr. Michael Roberts, Dr. Ron O'Dor, Dr. Malcolm Smale, and Dr. Warwick Sauer and was the origin of the umbrella study on which the research for this thesis is based.

In 1994, it was understood that progress was being made in relating 'inter-annual oceanographic variability with global climatic change', but that it was still unclear 'why and how temperature and turbidity affect catches and alter the life cycle' (Roberts *et al.* 1994). The main questions of the study were set out to be:

- 1) To investigate the effects of temperature, turbidity and currents on the spawning process and associated catches.
- 2) To investigate physiology concerning Chokka swimming mechanics/physics and related energetics.

(Roberts *et al.*, 1994)

The fourth experiment as detailed in the original coordination plan for the 'Acoustic Tagging and Environmental study' was the acoustic tagging experiment. Its objectives were:

- 1) To determine the effect of temperature (water column structure), turbidity and currents on spatial behaviour of individual Chokka.
- 2) To undertake a physiological study of swimming behaviour on a spawning ground i.e. swim speeds and emigration-immigration between different aggregations.

3. To undertake a physiological and spatial behaviour study on Chokka predators within the survey area.

All acoustic data were stored for later analysis to be processed by the Dalhousie group headed by Dr. Ron O'Dor.

Radio-Acoustic Positioning and Telemetry (RAPT) of sonically tagged animals provides 'continuous monitoring'. There is no doubt that this results in an increase in the analytical power of studies as tagging removes much of the ambiguity found in hand/eye observation. However, no method should be used without understanding its limitations. In this case, drawbacks include the possible effects of tagging on the animals undergoing observation and the unknowns of the system itself.

The intention of this thesis was originally to detect behaviour from RAPT system read outs. In essence to clarify the limits and strengths of data created by RAPT systems. The purpose of the qualitative comparisons¹ between 'Normal' behaviours and 'RAPT' behaviours was to allow the interpretation of the data collected by the triangulation algorithms. "Normal" behaviour was defined from sequences analyzed from videos taken of Chokka Squid on the Agulhas bank and as described in previous work on untagged animals. This information was collected with the intention of

¹ Largely qualitative comparisons which emphasize methods of presentation of comparative data and the appropriate use of significance testing (Johnson, 1999).

making it as similar to the RAPT data form as possible RAPT behaviour was defined by squid behaviour as it was determined by Mat Lab graphs created from the data collected during the 1994 and 1997 Agulhas bank tagging experiments.

Success in attempting to see similarities between two types of observational methods depends on the behaviours observed being similar. If the observational methods interfere with the behaviours then the behaviours become abnormal and incomparable. The video recordings were checked against previous knowledge, hence the name 'Normal'. As this was not an option with the RAPT behaviours they were checked against themselves in the light of previous knowledge and video recordings that had been transformed into dot sequences. The question is whether the tags altered the behaviours that were being studied? If so, how much and for how long?

There is considerable experimentation that has used different forms of tagging on aquatic animals. However, short of initial queries, there has been little examination of the tag's affect on the experiment or experimental results. Past studies have highlighted the need to reduce tag weight and compensate for buoyancy. However, positive short-term tests on apparent reactions of laboratory animals have silenced the debate over immune reaction, life expectancy, discomfort and behavioural change. The query that becomes apparent in behaviour studies like the one attempted in

South Africa is: How can behavioural studies be done using RAPT system readouts if the system or procedure itself changes the behaviour? The only way to answer these questions was to ask:

How (or how much) does tagging affect behaviour as defined by the video experiments.

In order to answer this an initial detailing of behaviour that can be defined, as 'Normal' or untagged behaviour is essential. The comparison of this 'Normal' behaviour to consistently seen RAPT behaviour is mandatory. The answer will influence the interpretation of RAPT studies.

Analysis of behaviour was made by considering changes over time in: speed, time spent at spawning site, utilization of space, mating behaviours, interactions, and temperature selection. Further comparison to other tagged species gave insights to the kinds of changes to explore. Through these comparisons a conceptualization of the strengths and usefulness of the system was revealed and detailed.

The objectives were to:

1)	Briefly review the spawning behaviour of <i>Loligo vulgaris reynaudii</i> and related squid with the focus on pre-copulatory activity.	Chapter 2- Literature Review
2)	Quantify 'normal' behavior by means of video analysis during: general and interactive utilization of space, mating behaviours, pre- and post- egg attachment interactions, and temperature selection as well as mating and egg laying conditions in and around the egg bed.	Chapter 3- Video Analysis
3)	Quantify RAPT behaviour by means of RAPT tags, during: general and interactive utilization of space, mating behaviours, pre- and post- egg attachment interactions, and temperature selection as well as mating and egg laying conditions in and around the egg bed.	Chapter 4 - RAPT Analysis
4)	Analyze and compare the questions above (detailing difficulties of scale and variable inconsistencies). To look for changes in behaviour with the addition of the tag or the process of tagging the animal, and, if changed, to determine whether the behaviour returns to 'normal' or not, and if it does how long it takes to do so.	Chapter 5 - Comparative Discussion

5)	To give an outline of <i>Loligo</i> behaviour as seen in and around the Agulhas mating grounds.	Chapter 6 – Egg Bed Observations
6)	To give a summary of the strengths and weaknesses of the observation methods (RAPT vs. Video) and suggest the types of analysis that can be performed successfully by each. As well as outline the unique findings of this experiment and suggest where to go to from here.	Chapter 7 - Summary Conclusions

CHAPTER 2 - LITERATURE REVIEW

This chapter compares historical observations of loliginid squid from around the world over centuries, quoting authors directly to reflect both their observations and their changing biases. Thus, this is a chronological review of the development of human understanding of the spawning behaviour of squid over the past two millennia.

Cephalopod reproduction has long been of interest to man. However, the capacity to effectively enter the marine world for the length of time sufficient to observe social interaction of such a small species moving rapidly around in a three dimensional space only recently became a reality. Prior to this century conditions in the natural habitat of marine animals virtually precluded effective analytical observations of the reproductive phases of their life cycles. Visual study of captive specimens maintained in tanks augmented by limited field observations by fishermen and researchers, who saw ongoing activities under conditions of limited time and space, provided the only reference points available. From the earliest writings, the literature has focused on the copulatory phase of the reproductive activities, and tended to refer (by virtue of the capabilities of human eye and eye-hand coordination) to the activities of a few animals or a single individual observed at a particular time.

In 1911 Drew illustrated the long natural history interest man has held for squid and their marine relatives when he noted "Aristotle makes several statements regarding the breeding habits of cephalopods." (Drew, 1911, p341). Whether Aristotle's twenty three hundred year old text was partially based on actual observations of mating animals, or was simply what he understood from tales of fishermen is difficult to know. Drew's choice of sections suggests that Aristotle was aware: of the head to head form of mating, that while the male and female swam together the male used one of his arms which appeared to be somewhat adapted to place sperm into a specific receptacle in the female's body, and during the spawning time the males appeared to be more aggressive of the two sexes, and they took on specific colouration patterns (Drew, 1911).

One of the first modern observers was Drew himself, whose own work was reported in the above noted 1911 paper. Drew based his observations on captive squid, *Loligo pealei*, maintained in aquaria in the Marine Biological Laboratory, Woods Hole, Massachusetts. Much of what he wrote remains as standard information on *Loligo pealei*. His observations were of sexually mature squid which had been caught in fish traps and confined to relatively small aquaria (Drew, 1911). Thirteen carefully drawn biological figures accompany Drew's paper which focused on mating and egg-laying sequences;

Squid have two methods of copulation. By one method sperm reservoirs are attached in the mantle chamber on or near the oviduct and

immediately begin to discharge their contents freely in the water. By the other method sperm reservoirs are attached to the outer buccal membrane and the sperm become stored in a special receptacle in the membrane....

The left ventral arm of the male is always used in transferring the spermatophores.

(Drew, 1911, p350)

The egg strings are composed of two kinds of jelly. One kind is supplied by the oviductal gland and the other by the nidamental....

From the funnel the egg string is drawn between the cirlet of arms...

Fertilization evidently does not take place inside the oviduct. It doubtless may take place in the mantle chamber when sperm reservoirs are present there, and.... sperm are liberated from the receptacle while the eggs are between the arms.

(Drew, 1911, p351)

In 1954 McGowan published observations on the spawning activity of the loliginid species most common on the west coast of North America, *Loligo opalescens*.

McGowan noted that his direct observations, as undertaken in Monterey Bay, California, would allow him to "corroborate and supplement the observations of Drew and... others" (McGowan, 1954, p47).

McGowan utilized three study methods: divers observed the most important known spawning areas and recovered eggs; plankton hauls for squid larvae were done, near the water surface and at the bottom, to determine when hatching took place and how long the larvae remained in the vicinity; and, eggs were reared in the laboratory to determine length of time required for hatching, and to provide larvae of a known age to assist in the identification of those caught in the plankton.

Unfortunately the field observations were mounted too late in the spawning period to see any significant pre-copulatory behaviour. Divers encountered large egg masses "attached to the sandy bottom along the general trend of the 50-foot contour" and observed mating, but the end of the season was obviously near as "the bottom was littered with dead and dying squid" (McGowan, 1954, p49).

McGowan wrote:

There were great numbers of actively swimming squid in the immediate vicinity of the mass. Many females were seen depositing their egg capsules around the periphery of this mass.... The egg capsules were never attached to protruding objects... but always to the base of some previously laid egg capsule. A short distance above the egg mass copulating pairs were seen.

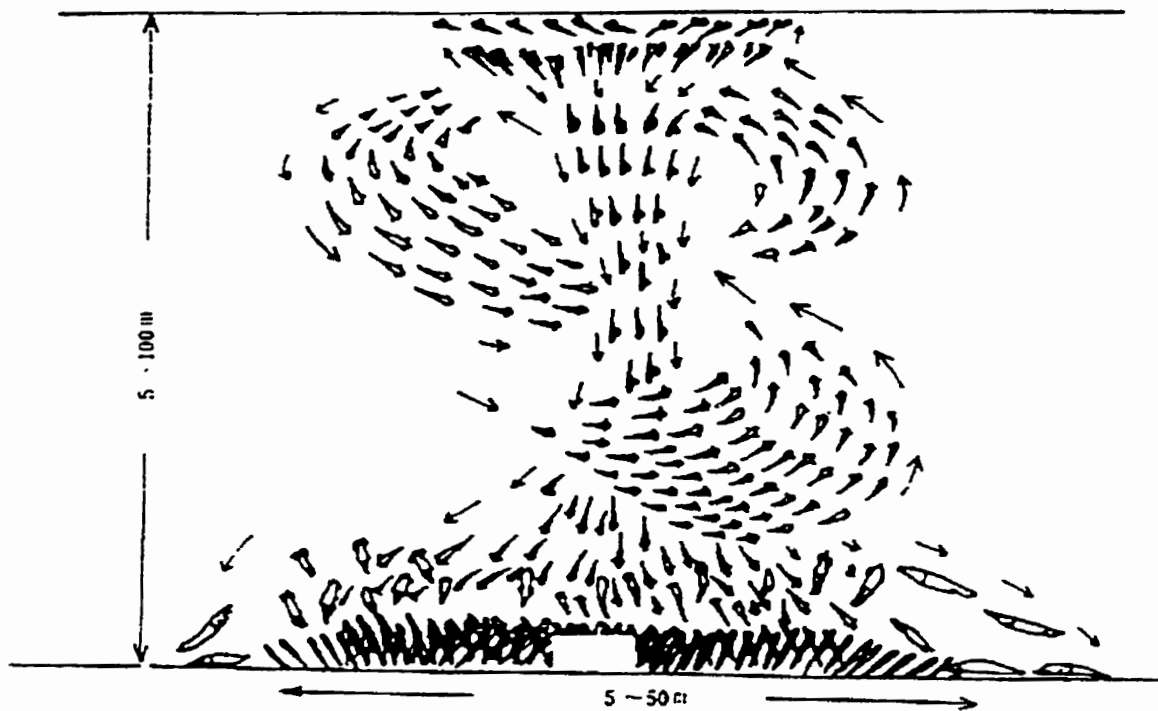
(McGowan, 1954, p50)

McGowan noted that only the first of the two copulation methods described by Drew for *Loligo pealei* were seen in this observation of *Loligo opalescens*. He suggested that since sperm was also found in the buccal seminal receptacles of the females the second method must also be used but "it seems likely that the squid use it some time previous to their arrival on the spawning grounds" (McGowan, 1954, p50).

A 1957 report by Hamabe and Shimizu details observations on squid behaviour during spawning (Hamabe and Shimizu, 1957). The cross sectional diagram drawn as their figure 5 could well be a graphical representation of the spiral swimming pattern of *Loligo vulgaris reynaudii* seen in the video records filmed in the study area which is the focus of this thesis (Figure 1).

FIGURE 1**Mating Groups and Spawning Bed - LOLIGINIDAE**

(Hamabe and Shimizu, 1957)



Going round and round in many small groups, they come near the surface where mating takes place. They descend together to the sea bottom and spawn collectively as though to form a spawning bed

Arnold's 1962 paper noted observations on *Loligo pealei* social behaviour associated with courtship and mate selection seen in the laboratory and "confirmed by watching squid in their natural habitat" (Arnold, 1962, p53). In the introduction the author stated "This paper is an attempt to show that *Loligo pealei* has a social structure concerned with its mating behaviour (Arnold, 1962, p53).

Initial experimentation involved lowering a naturally laid egg mass tied to a cotton cord into one corner of the tank occupied by a small school of male and female squid swimming parallel to each other, "moving back and forth in synchrony" (Arnold, 1962, p54). The animals had been chosen randomly from a larger group, no evidence of any social pattern was observable, and it was assumed no mate selection had occurred. When the egg mass was lowered into the tank the squid immediately "broke formation" and individually investigated it (Arnold, 1962, p54). One after the other, squid, both males and females, swam towards the egg mass with their arms forming a pointed cone, occasionally the egg mass would be flushed with spurts of water from the funnel of an approaching squid. They appeared to feel the egg mass with their arms then dart away and rejoin the group. "The stimulus seemed to be completely visual because of the speed with which the squid responded" (Arnold, 1962, p54). During later observations artificial egg masses constructed of water filled tubing elicited similar responses in captives, while dangling an egg mass from a string in front of a school of squid

in shallow water off a dock or from a anchored boat accomplished the same result.

The investigative behaviour noted above was immediately followed by what Arnold called "dominance determination behavior" (Arnold, 1962, p55). It began with the males raising one median arm above the rest and waving it, sham battles followed wherein the males rushed at each other but rarely touched. Males developed dark brown lateral areas at the base of the arms, a colour pattern characteristic of sexually aroused males. Any given male could be seen trying to place himself between the female of his choice and the rest of the group. Any approaching male was threatened by a waving of the median arm. If intruders persisted the conflicting animals bumped tails, displayed dark colour spots along the lateral margins of the fins and even bit each other. Arnold states:

This behavior continued between all males in the tank until one male was established as a dominant male...The subordinate males each selected a female and would undergo similar combat among themselves. An individual's position in the social structure seemed roughly correlated with its size....Only rarely did one of the mateless males succeed in displacing a mated male. If a new male was introduced to a group that had established a social structure he would immediately be challenged by the other males until he was integrated into the social structure. This would often result in a displacement of an established male and the displaced male would then displace his subordinate....Only rarely did any male attempt to change its mate spontaneously.

(Arnold, 1962, p55-56)

The behaviour of the females was less active. Normally a female would show a slight avoidance of a male but seemed to have no mate preference. While mate selection and challenging went on between the

males, females paid no apparent attention. Once the social structure among the males was established the males attempted copulation with the females.

(Arnold, 1962, p56)

Arnold suggests that the visual stimulus of an egg mass "elicits sexual behaviour" and goes on to state:

This would explain the fact that egg masses are often found attached to *Fucus* or similar sea weeds. Several females deposit their egg strings on a common egg mass which agrees with this hypothesis.... Undoubtedly, there must be another stimulus that elicits the original mating that results in the formation of the original egg mass... it is possible that the original mating occurs spontaneously.

(Arnold, 1962, p57)

For work on his dissertation, which was later published as Fish Bulletin 131 by the Department of Fish and Game in California, Fields studied motion picture film sequences of captive squid taken in 1946 for the purpose of analyzing mating/spawning behaviour. "When transferred alive from fisherman's nets into suitable aquaria, groups of *L. opalescens* continue to mate and spawn" (Fields, 1965, p39).

Fields' subjects were *Loligo opalescens* from the Monterey Bay region:

Female squid seem to differ in their ability to attract males, for several males may single out one or two females from a group and mate several times with them, ignoring the other females present. Later, as mating activity increases, there is general participation, but again certain females are in greater demand than others....These observations suggest that

females ready to mate produce a defusible material that attracts the males.

(Fields, 1965, p41-42)

Fields noted the progressive aspects of colouration (red chromatophores) in male squid as they become "excited" and during the mating activity. (Fields, 1965, p42) He observed the second type of copulation (the head to head activity as discussed by Drew, McGowan and others) only once, and suggests that it may occur primarily during early sexual maturity out in the open ocean (Drew, 1911; McGowan, 1954).

Fishery management was a prime consideration in Fields' research. He looked at the environment of spawning grounds and the timing of the spawning season as well as the activities of the animals themselves.

L. opalescens is known to spawn... in depths of 10 to more that 115 feet, frequently in sheltered bays, where the sea floor is of mud or sand... Various types of evidence were useful in defining the squid's spawning season. The best evidence is the presence of egg masses: these show not only that spawning has taken place but, by the degree of embryo development, tell when it occurred.... Another evidence is the readiness of the animals to spawn when they come into this vicinity. The commercial catch consists entirely of mature squid, most of them gravid.... These evidences strongly suggest that the squid fishery is based entirely upon schools of spawning animals, and that the seasons of availability are, therefore spawning times.

(Field, 1965, p44)

In the final section of the report Fields notes "Indirect evidence derived from several sources confirms the observations that squid die after spawning once" (Field, 1965, p95). In discussion about the changing size (smaller size) of squid in the California squid fishery he discusses both the loss and the seasonality of available food supplies within the region, and the relationship the two distinct squid populations which utilize the same spawning grounds during two times of the year (one from January to June and the other July to December) may have with specific oceanographic conditions and food sources.

In June 1967 two night dives in 30 feet of water off great Stirrup Cay, Bahamas, provided observers in a submersible an unique opportunity to observe sexually mature squid (*Doryteuthis plei* [now reclassified as *Loligo plei*]) within their natural habitat. Unfortunately, the observers note "We saw no courtship or mate selection on either night... the mated pairs were already established on the onset of our observations" (Waller and Wicklund, 1968, p110).

However, their mode of observation was a milestone in squid research. Two 1000 watt undersea lights light up the immediate vicinity and through the window of the submersible researchers saw spawning squid within their natural (except for the light) habitat.

..thousands of squid, in great activity, appeared in the area illuminated by the lights. The cephalopods were densely concentrated near the

bottom... we soon realized that it was not an aimless performance but a mass mating phenomenon. With few exceptions, the squid were paired off and, at a given time several dozen pairs could be seen mating.

(Waller and Wicklund, 1968, p110)

Waller and Wicklund state that the mating of *Loligo (Doryteuthis, to them) plei* was similar to that other authors such as Drew, Arnold and McGowan described for *Loligo plei* and *Loligo opalescens* (*L. plei* Drew, 1911; Arnold, 1962 and *L. opalescens* McGowan, 1954) The authors noted males underwent a colour change when they were challenged by other males and that sometimes two males "performed elaborate maneuvers in unison around a single female before one of them released ink and darted away" (Waller and Wicklund, 1968, p110). The observers commented that they did not see the "sexual avoidance responses" of the females which were noted in the above three references (Waller and Wicklund, 1968, p110). Nor did they see an evidence of mortality at the spawning site.

Occasionally, a second male moved swiftly in on a mated pair and attempted to grasp the female. Inevitably, a struggle followed and sometimes one of the three released ink before contact was broken. The original pair often reunited and mated after the encounter... The females, always smaller than males, were acquiescent to copulation except when a stray male intruded.

(Waller and Wicklund, 1968, p110)

Both forms of mating were seen, but the head-on position was noted only a few times. Immediately after mating the females darted down and implanted an egg strand in the calcareous sand bottom. The single strand egg capsules were implanted very close together, and as spawning progressed they began to form a mop-like cluster. The cluster formed under the floodlight during the initial night of observations was 3-4 feet in diameter.

In their 1981 paper Griswold and Prezioso compare their 1979 observations of *Loligo plei* spawning in nature in Narragansett Bay, Rhode Island to those of Drew and other above noted authors (Griswold and Prezioso, 1981). Their description differs from that of the other authors referenced thus far, and they begin their discussion by stating "The social hierarchy involving egg deposition differs from species to species" (Griswold and Prezioso, 1981, p946).

During a midday dive the authors noted a squid egg mass (50-60 cm in diameter) attached to one side of a small boulder resting on a sand/mud bottom. As they observed the site:

Squid began to appear... and moved toward the egg mass in a semicircle. They stopped about 2.5-3.0 m from the mass and remained stationary approximately 1m off the bottom. The squid were well-defined pairs with the smaller female (mantle length 16-18 cm) parallel and on the left of each male (20-22 cm)... The animals had moderate pigmentation over the mantles, but we did not observe distinctive spots...

nor did we observe color changes... all the animals appeared to be in good condition.

(Griswold and Prezioso, 1981, p945)

Griswold and Prezioso did not see mating activity as documented by earlier references, but observed an egg-laying sequence:

One pair of squid at a time approached the egg mass with their arms held forward and tentacles extended.... They intertwined arms as they extended them into the egg mass. The arms of the female appeared to move delicately over and among the existing fingers of eggs. Each pair that approached the egg mass stayed 2-4 s then moved backward into the same position it had previously occupied in the semicircle. At that time another pair moved forward. There did not appear to be any order in which pairs approached the egg mass, however, no more than one pair approached at any given time. The same pair approached more than once.

(Griswold and Prezioso, 1981, p946)

In the first volume of the series *Cephalopod Life Cycles, Species Accounts* edited by Boyle and published in 1983, spawning activity is an integral part of each of three chapters focused on loliginids: Hixon reviewed *Loligo opalescens* of the northeastern Pacific, Summers wrote on *Loligo plei* in the northwest Atlantic and Worms discuss *Loligo vulgaris* from the northeastern Atlantic and Mediterranean (Hixon, 1983; Summers, 1983; Worms, 1983). In 1984 Arnold drew on his earlier work and that of many others when reviewing available

knowledge on squid mating and courtship in a section he compiled for *The Mollusca: Reproduction* (Arnold, 1984). Both these volumes represent compilations of knowledge circa early 1980's. They are useful as reviews, but neither contains previously unpublished information.

The 1980s saw significant development in collaborative programs which brought South African fisheries and academic based researchers together. Publications based on biological research, fishery productivity and management focused on *Loligo vulgaris reynaudii* began to attract attention near the end of the decade which increased during the first half of the 1990s. Among these papers were several which provided invaluable background information to this study, including: Augustyn, 1990,1991; Augustyn and Grant, 1988; Augustyn *et al.* 1993, 1994; Sauer, *et al.* 1991, 1992, 1993.

The March 1994 issue of the *South African Journal of Science* was a special issue focusing on "Science on the Agulhas Bank". One particular paper "Chokka squid on the Agulhas Bank: life history and ecology" authored by Augustyn, Lipinski, Sauer, Roberts and Mitchell-Innes further 'set the stage' for the commencement of the Acoustic Tagging and Environmental Study, the umbrella research which provided the data analyzed for this study (Augustyn, *et al.* 1994) *Loligo vulgaris reynaudii* was the focus of the lead author's 1989 dissertation, and in the 1994 paper he and his colleagues provide an exceptional summary of

available biological knowledge of this particularly important South African fishery resources. In an abstract of a more recent paper Augustyn notes:

This subspecies is the basis of the only commercial fishery for cephalopods in southern Africa. It is also the most valuable South African fisheries, currently earning more than R100 million (US \$23 Million in 1997) in exports.

(Augustyn, 1996)

CHAPTER 3 - VIDEO ANALYSIS

ABSTRACT

Underwater video recording was done directly over an active egg bed in South Africa's South-East Cape region. The video clips were analyzed for behaviour patterns using medians of speed, Net to Gross Displacement Ratios (NGDR) of 0.96s time clips, and spatial utilization as well as observation of interactions, environment, and direction. The visual behaviour patterns thus created were combined with literature accounts and first hand observation to determine normal (untagged) behaviour around the spawning site. The 'normal' behaviour presented by this video analysis will provide a representation comparable to those found in the radio acoustic telemetry section.

INTRODUCTION

The traditional method of recording behaviour is to observe by eye and record by hand. There are numerous studies that have used this method to record squid behaviour, dating back to Aristotle's studies of habits and anatomy (Drew, 1911). Written recordings are of limited value, however. They lack precision, are prone to bias and often miss key elements of mannerisms or even entire behaviours. Due to its imprecision, data from (human) observational records are difficult to analyze for exact times or locations. Thus, neither speeds nor relative spacing can be determined. Also, many animals in the same vicinity cannot be observed simultaneously; so that, for many observational experiments hand/eye recording does not provide adequate accuracy.

A much more precise method in terms of temporal and spatial detail is video recording, although resolution of image detail may be less than direct observation. Over the years, video recording has supplied researchers with valuable information on animal behaviour. In addition to precision, video has the advantage of allowing the review of behaviours that may otherwise be missed or mistakenly recorded by hand/eye recording.

Video recording allows greater precision in the observation of subjects in their natural habitat. This grants researchers the ability to 'stand back' from the subjects and observe behaviour on a larger scale. Improving the

possibility of clear observation of behaviours in the wild allows subjects to be better understood (Hoar, 1994; Griswold & Prezioso, 1981).

Unique body and chromatophore patterns have been widely used to recognize and characterize specific cephalopod behaviours (Hanlon, 1982). However there are a number of elements of behaviour, the categorization of behaviours using patterns of body movement can provide similar information, with the advantage of spatial significance. These patterns of movement can be recognized on video or readouts created by Radio Acoustic Positioning Telemetry (RAPT) systems. The two recording methods, video and acoustic, provide reliable data.

The purpose of this experiment was to record the behaviour of *Loligo vulgaris reynaudii* over the inshore egg bed in the South African South-Eastern Cape region (Figure 1a) with an underwater video camera. This allowed description and classification of untagged squid behaviour. The three main elements of the animal's behaviour observed are: (1) Patterns, (2) Records of speeds, and (3) Net to Gross Displacement Ratios (NGDR) readings from the lines created from frame by frame analysis. All these initially entail the sequencing and categorizing of events. In combination they allow behaviours to be clearly defined in terms comparable to the output obtained from RAPT system analysis.

FIGURE 2A
The South African Shore Line

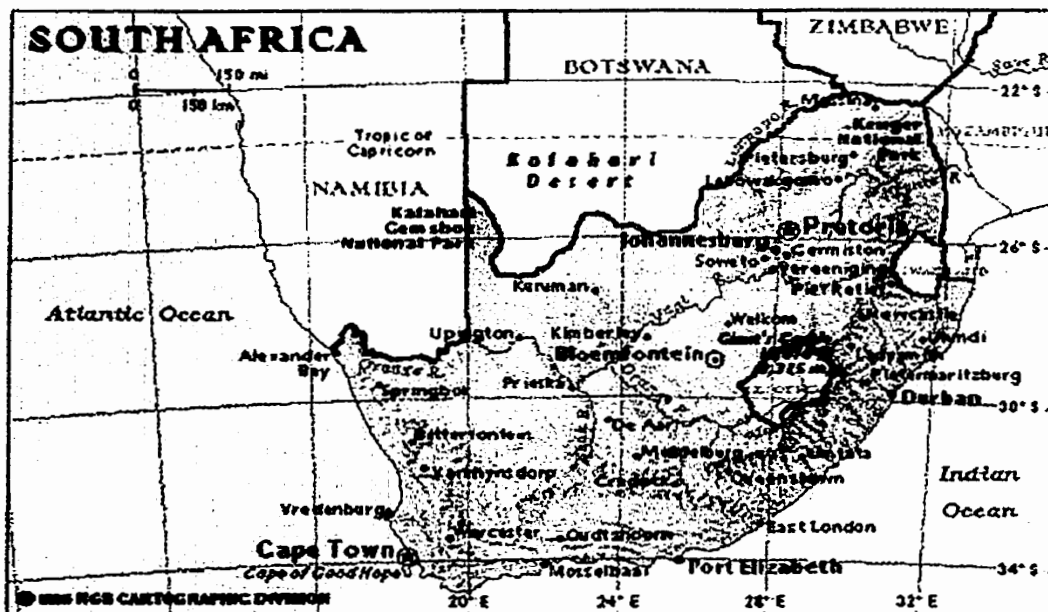
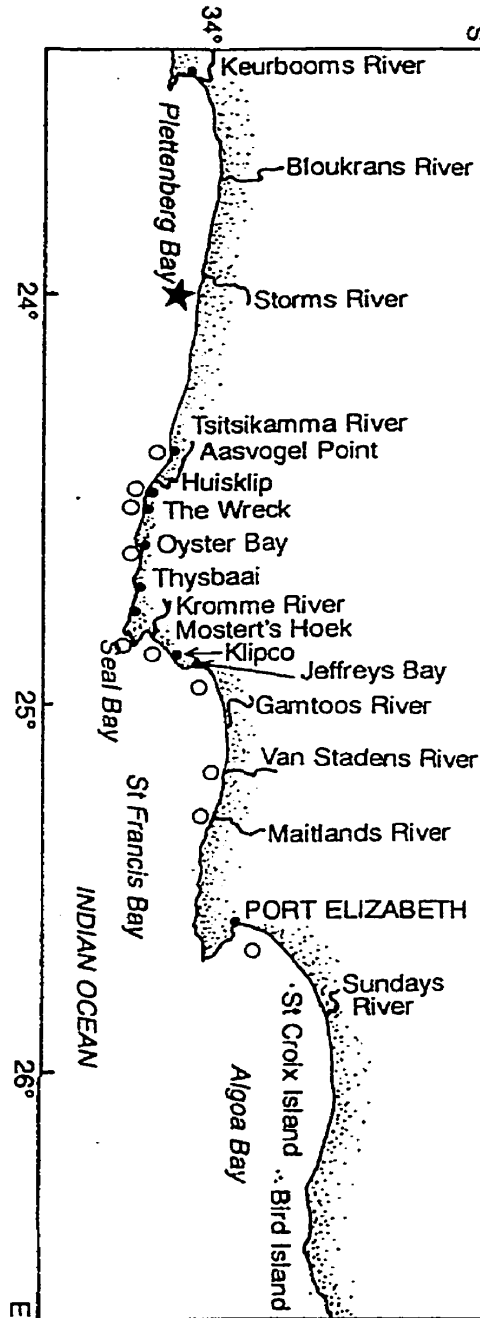


FIGURE 2B**Details of The South-East Coastal Region**

EXPERIMENTAL DESIGN

The video recordings were originally meant to document reproductive behaviour of *Loligo vulgaris reynaudii*. However, they are used here to analyze the specific actions encountered in the movements related to spawning behaviour, in a manner that can be compared to the RAPT system outputs.

In November 1993, the South Eastern Cape coastline between Port Alfred (33° 36' 87" S, 26° 55' 51" E) and the Tsitsikamma Coastal National Park (34° 01' 81" S, 23° 56' 43" E) was the target of 33 videoed dives (Figure 1b). St. Francis acted as the base station for the launching of the research vessel 'Langusta'.

A support crew captained by Peers Pittard, included Dr. Malcom Smale and Dr. Warwick Sauer accompanied Dr. Roger Hanlon to previously selected areas.

Dives were conducted throughout the last three weeks of November 1993 off of the 'Langusta'. Although surface weather conditions were favorable throughout the allocated time, deep-water dives (35-40m) were often cut short due to rough seas and difficult diving conditions (Hanlon, 1999). Underwater visibility was fair (4-6m) on most days chosen for the dives. Water temperature stayed at approximately 50°F. The length of the dives varied between twenty (20) and forty (40) minutes due to the water depths that often reached 40m. In total, the dives yielded a series of twenty-four (24) hours of discontinuous underwater

video-graphy. Recording was done with a Sony HI-8MM camera in a 'Quest' housing, using HI-8 tape.

Editing of the series of video recordings was performed in April 1999, in the Marine Biological Laboratory, Woods Hole, Massachusetts. The selection of video sequences prepared during the editing process included a range of squid behaviours recorded near an egg bed and in the surrounding area. Choices were made based on the clarity of sequence appearance, usefulness for analyzing patterns based on discernable features, and observation of interactions.

Basic behaviour sequences were broken down into actions, which were split into categories based on the subject's gender. The categories were charted and followed through frames of video. Analysis was done to define distinct patterns of movement in reference of circuitousness (NGDR), speed (cm s^{-1}), direction and environment, and the results are shown in categorical relation to each other (e.g.: male approach compared to female approach).

The recorded behaviours are discussed in terms of turning frequency allowing the directness of the various paths to be assessed. This was done by noting the changes in turning behaviour indicated by the net to gross displacement ratios (NGDR) of 0.96s segments of the paths traveled. An increase in the NGDR indicates a straighter swimming path, while a decrease in the NGDR indicates a more circuitous path (Buskey,

1989). Similar NGDR's recorded here imply congruency within the repeated actions.

Movement patterns derived for the categories provide time series of positions. These recorded positions allowed the calculation of speed as distance over time. The speed at which each action occurred was calculated from the distance between positions over the time in which the action took place. Acceleration was ascertained from the change in distance between sequential positions. The comparison of speeds further documents the relative consistency within the categories. Direction and environment are continually noted and referred to as they provide patterning knowledge and reference points, as well as aiding in establishing categories.

The recorded approaches retreats and behaviors around the egg bed can be discussed in terms of turning frequency thus allowing the directness of the various paths to be assessed. This is done by noting the changes in turning behavior indicated by the mean to gross displacement ratios (NGDR) for the 96S segments of the paths of travel.

METHODS

The video-tapes were taken to Dalhousie University, Halifax Nova, Scotia for frame by frame analysis. Examination of the images on the video-tapes were undertaken at the Dalhousie Biology Department with a time lapse video cassette recorder (Panasonic model# AG 6750A). After description of the entire taped sequence, segments were categorized and labeled as identifiable actions. Observed behaviours were subdivided into those that directly related to a) the laying or attempted laying of eggs, or b) those which took place in the surrounding area. The paramount division being: noticeable changes in velocity (directional speed). The behaviours were then broken down into thirteen (13) distinct actions: eight (8) related directly to the laying of eggs, five (5) that relate to activity in the surrounding area. Allowing for variation in activity between males and females within the observed behaviours, a total of nineteen (19) categories were identified and labeled (Table 1- Behaviour Categories).

TABLE 1
Behaviour Categories

1	The approach (within 2m of the egg mass): males (1M), females (1F)
2	The attempted approach (within 2m of the egg mass): males (2M), females (2F)
3	The lay/attachment (within 0 - 0.5m of the egg mass): females (3F)
4	The hover during a lay (within 0 - 0.5m of the egg mass): males (4M)
5	The attempted lay (within 0 - 0.5m of the egg mass): females (5F)
6	The hover during an attempted lay (within 0 - 0.5m of the egg mass): males (6M)
7	The retreat after a lay (within 2m of the egg mass): males (7M), females (7F).
8	The retreat after an attempt (within 2m of the egg mass): males (8M), females (8F).

9	Circling clockwise or anti-clockwise or general swimming ($\geq 2m$ from the egg mass): males (9M), females (9F)
10	Swimming ($\geq 2m$ from the egg mass): males (10M), females (10F)
11	Hovering ($\geq 2m$ from the egg mass): males and females together (11B)
12	Mating ($\geq 2m$ from the egg mass): males and females together (12B)
13	Agonistic behaviour ($\geq 2m$ from the egg mass): males (13M)

The documented occurrences, all with corresponding time frames, were assessed for five (5) clear examples of the categories selected above. In this instance, clear is defined by four (4) aspects: video quality, angle of camera, centralized focus of both subject and reference, and the ability to conclusively label the action in only one of the categories given.

Video quality. Image clarity had to be optimized by increasing the contrast with respect to colour proportions (chrome). This produced relatively sharp lines that allowed distinctions between actions and hence categories to be made and positions drawn. Underwater videography is difficult enough without worrying about picture sharpness while taping. In general, the video was all useable, even though VHS does not allow for overly high resolution (240 lines of horizontal TV resolution). There were only a few sections that had to be disregarded due to lack of picture clarity.

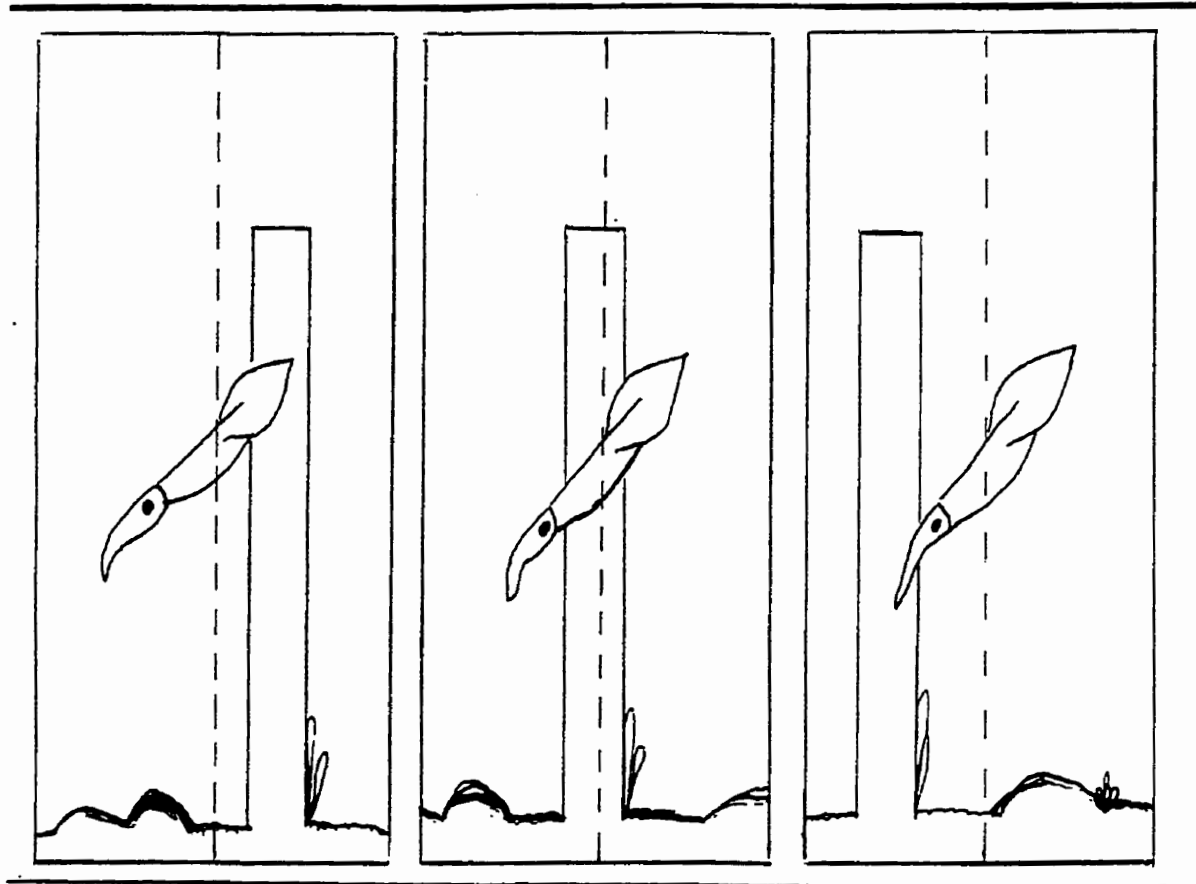
Camera angle. Camera angle involves two considerations, the horizontal and vertical planes. First, the predicament caused by changes in the vertical plane. The shots were often taken while following the subject in order to film an entire action. Therefore, the camera angle was constantly being adjusted. To correct for this in each sequence, a line on the ocean floor or some other reference was used to establish a reference distance. This reference allowed for the triangulation necessary to calculate the changing measurements proportionally. Changes in the horizontal plane result in a parallax. To avoid this the squid had to be captured during an action, which occurred within 30° of parallel to the camera lens. This

allowed the measurements to be taken without the interference of distortions caused by movement in the direction towards or away from the camera. This degrees from parallel limit is suggested by numerous workers in the field of video measurements (Waters and Whitehead, 1990). The 30° limit was confirmed by tests conducted on the ratio of change between cross-sectional radius width and length as the subject moved from parallel to directly facing the camera lens. The matter was simplified in the case of squid as their internalized pen and method of propulsion (jetting) creates a relatively straight action so the angle of motion considered here does not shift significantly.

Subject 'centralization'. The centering of the subject in the field of view is important, unless the camera's distortions have been graphed and taken into consideration, as any individual camera and additional set up has it's own particular distortions of space which are greatest near the edges of the lens. Following the subject through the actions actually reduced this effect on the subject, which remained in the central zone of the camera lens (Figure 3). Edge effect may have altered the reference distances (although most references were relatively central), however, reference points are less sensitive (because total distances are greater).

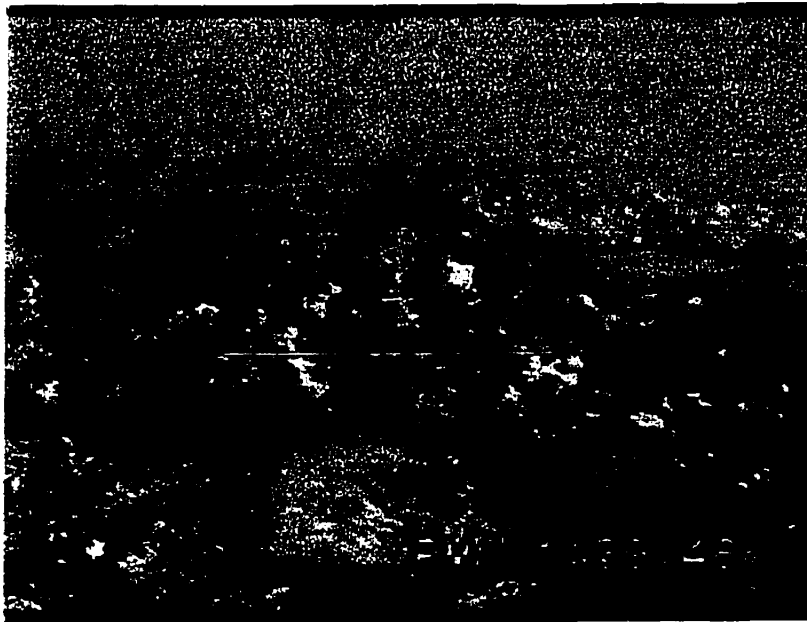
FIGURE 3**Centralization**

Following the subject through the actions, keeping the subject in the central zone of the camera lens.



Categorization. Placing an action into a category meant that the entire sequence of events had to be assessed and understood. Any action that was not seen from its obvious initialization, its visual conclusion, or led to an undistinguishable result (i.e. laying of eggs or not) was not used.

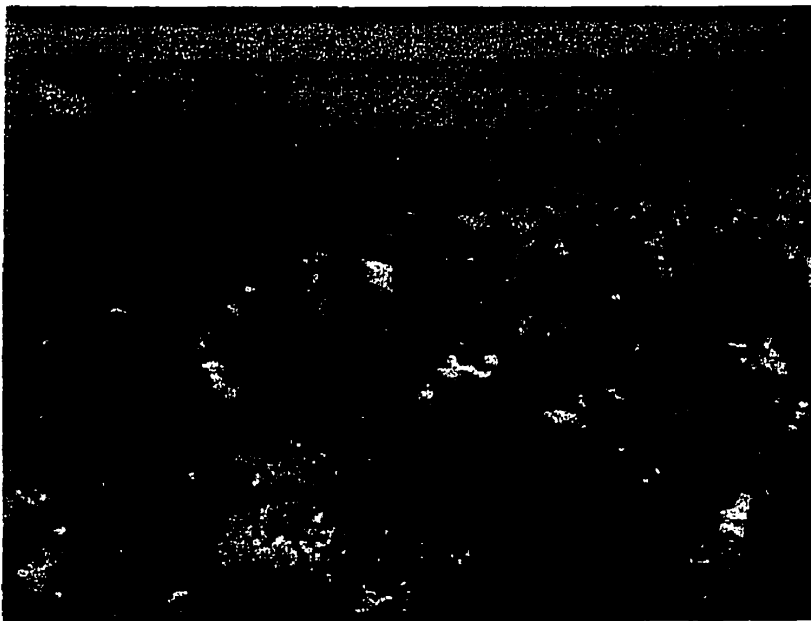
Once a clear example was located it was broken into frames, each representing 0.32 seconds (Figures 4a- 4i) and copied on to acetate paper (which had been taped to the video screen).



4a) Pair Approach egg mass (time 0s)



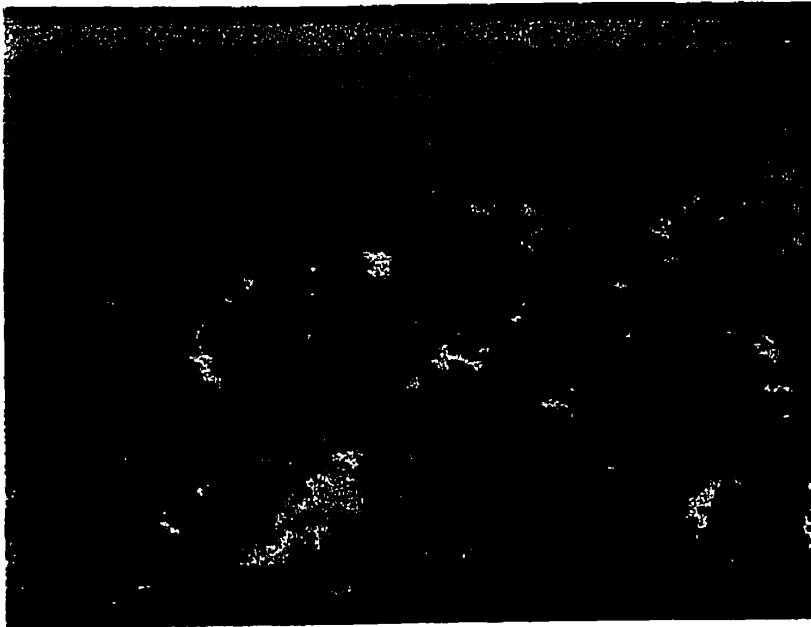
4b) Pair continue to Approach egg mass (time 32s)



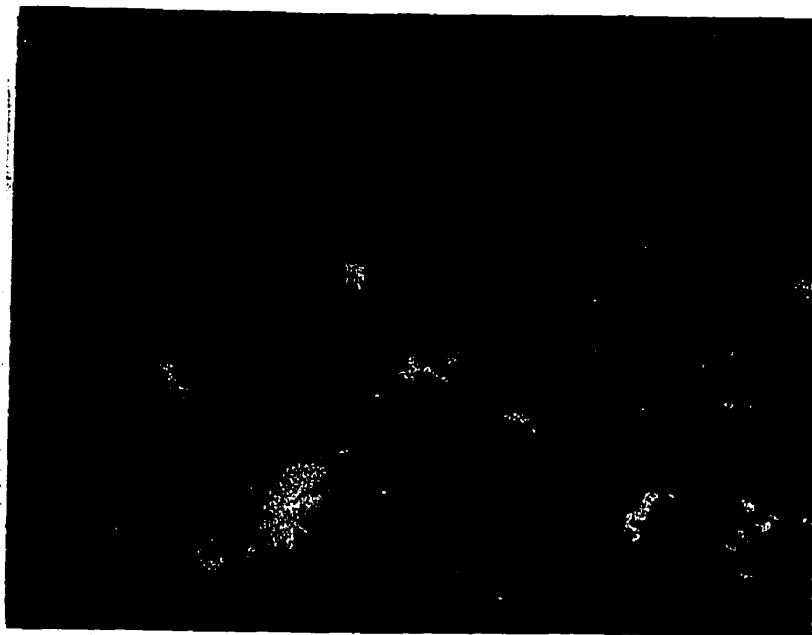
4c) Female descends towards egg mass (time 64s)



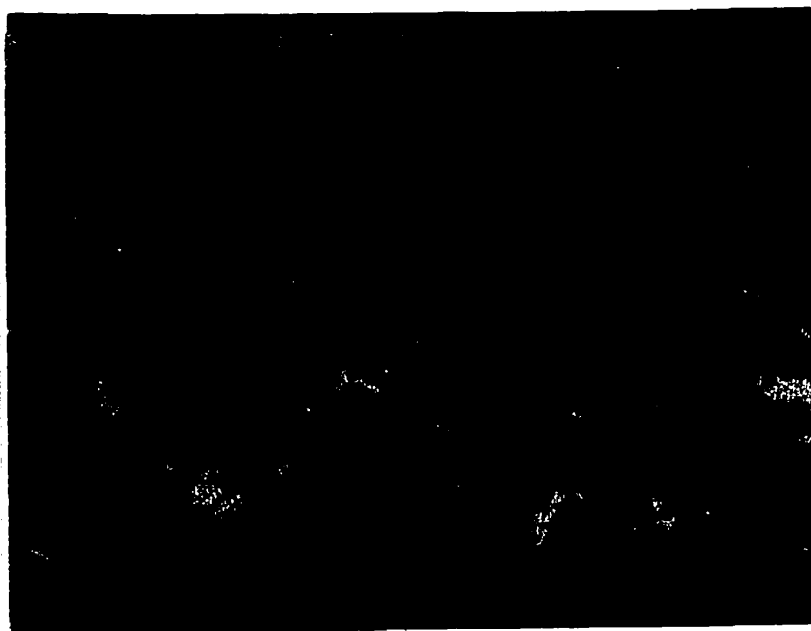
4d) Male hovers while female prepares to attach egg string to the egg mass (time 96s)



4e) Male hovers while female attaches egg string to the egg mass (time 128s)



4f) Male hovers while female secures egg string to the egg mass (time 160s)



4g) Female begins to retreat (time 192s)



4h) Male follows female in retreat (time 224s)



4i) Pair retreats after successful lay (time 256s)

The initial frame copied included 1) the subject, both size and initial eye location and, 2) the baseline which included reference to the egg bed and the size and orientation of the reference point which was usually a large brain coral next to the egg mass. The frames were advanced in 0.32 s intervals and the same information was copied at each point (Figure 5a). The information was then transferred onto graph paper. The transferring process had two goals: one to free up the acetate paper for the next sequence and the other to realign the dots (eye positions) that had been created in a representative pattern, thereby depicting the actual path of action (Figure 5b).

FIGURE 5A
Recording frame by frame sequences

Recording from Video screen to Acetate


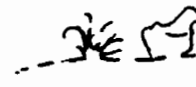


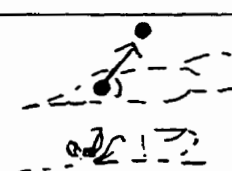

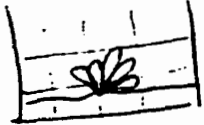


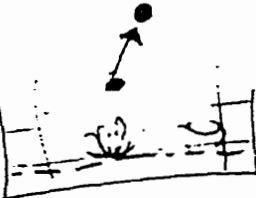
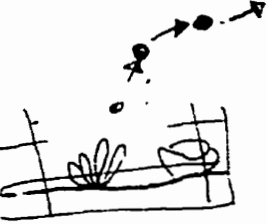
1	The baseline (ocean floor) is drawn, including the egg mass under observation	
2	The reference point is drawn	
3	The subject is drawn (mantle length and eye position recorded precisely)	
4	The frame is then forwarded (0.32s ahead)	
5	The reference point and eye spot are marked and egg mass movement is noted	
6	5 is repeated until the action ceases at which point the acetate is removed from the video screen	

FIGURE 5B**Recording frame by frame sequences****Recording from Acetate to Graph Paper**

1	The baseline (ocean floor) and the egg mass under observation, are drawn near the bottom of a horizontal piece of 8.5X11in, 1cm cubed graph paper	
2	The reference point is drawn	
3	The subject is drawn (mantle length and eye position recorded precisely)	
4	The reference point is corrected to match its next point and the eye-spot copied to its new location. A directional line is drawn between the two eye spots	
5	This sequence is repeated until every eye spot made during the action has been copied with change of orientation, reference and baseline position observed and corrected for each time	

The Net to Gross Displacement Ratio (NGDR), a ratio of the linear distance covered by the path (net displacement) and the total distance covered by the path (gross displacement) over a period of 0.96s was calculated. 0.96s was chosen as the time frame for NGDR as it was the 3rd multiple of 0.32s (10 frames), giving a 'four dot' sequence from which to calculate turning frequency.

The speed of each action was calculated by dividing the distance given by the dot series by the time in which the action took place. The acceleration of the subject was ascertained from the spacing between the representative dots. The time was found from the multiplication of the number of frames by the time between frame intervals. The actual distance was found by correcting the distance that was measurable between the dots on the graph paper by the average size of the mantle length of that gender. The average mantle length of *Loligo vulgaris reynaudii* males is 32 (± 0.4) cm and females is 21 (± 0.3) cm.

The NGDR and the speeds were tabulated under each categorical label and descriptive statistics were calculated (n=5). The median, standard error and standard deviation, at a confidence level of 95% were sought for each category. P- values were calculated for the comparison of attempted actions versus competed actions and males versus female equivalent actions (NGDR-Table 2; Speed-Table 4).

RESULTS

The results are divided into three sections. Section One: Visual displays accompanied by textual and statistical descriptions of selected data showing the movement patterns of the categories given in the methods section. Section Two: Tables of statistical averages used to create the graphs and corresponding text given in section one. Section Three: General Observations.

Clarification of terms used:

#	Corresponds to the behavioural category
F	The following number is for females of that category
M	The following number is for males of that category
n	Indicates the following number relates to the NGDR
s	Indicates the following number relates to the speed
X	Indicates the median of the group
±	Identifies the standard deviation and hence the margin of error found while performing the descriptive statistics on that group

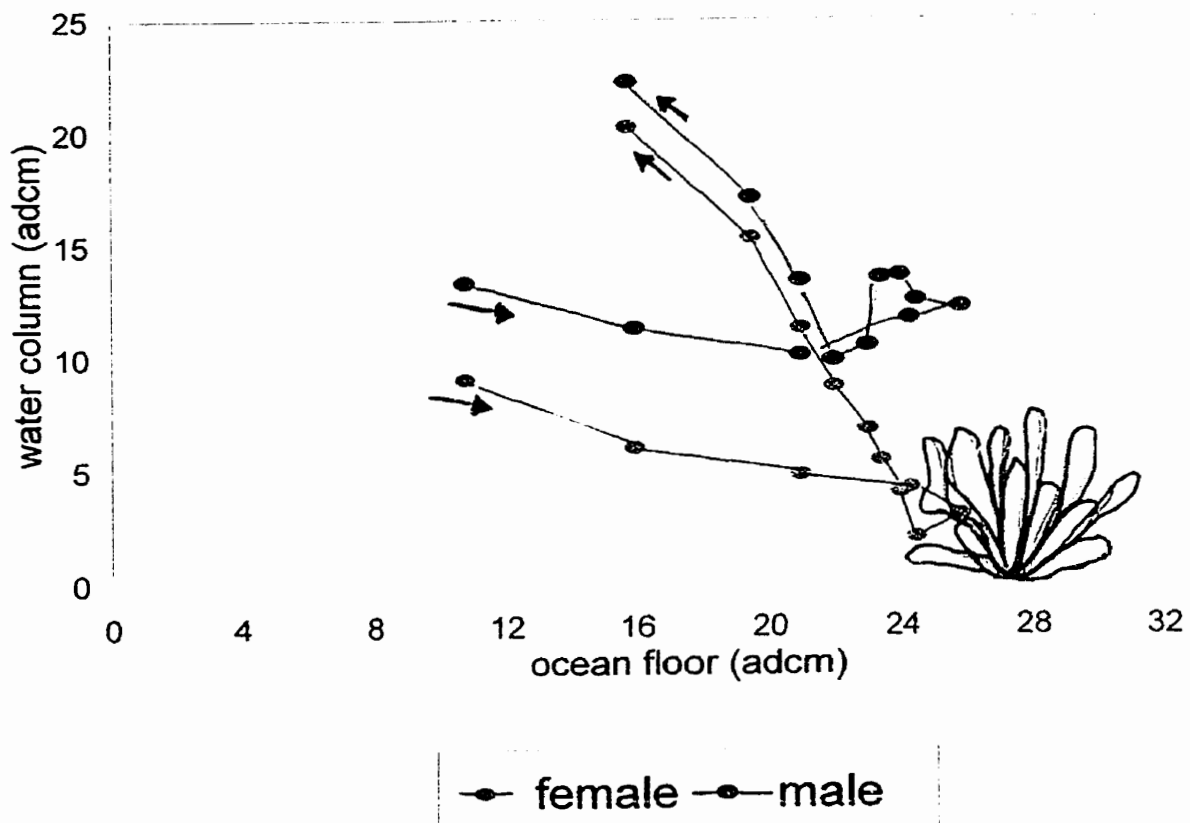
SECTION ONE

The examples shown in the action graphs below have been constructed from actual sequences found in the data collected to produce examples of visual displays of movement patterns created by the subjects. There is one (1) second between each dot, which represents the subject's eye position at that video frame. The graphs are in 'adjustable meters' (adm) as, although they are meter distances, the zero point does not represent an actual zero. In order to display the action in the center of the graph, the zero points have been randomly selected; wherever possible a reference to actual depth is given and/or the egg mass position is shown.

FIGURE 6**An Approach, Lay and Retreat**

The categories included in this display are: 1M - Male Approach, 4M Male Hover, 7M Male Retreat, 1F Female Approach, 3F Female Lay, and 7F Female Retreat.

(Eyespot Positions every second)



The approach (1F and 1M) (within 2m of the egg mass)

An approach to the egg bed is made as a pair; an escorted female with fertilized eggs and an accompanying/guarding male. The pair jets toward a particular communal egg mass (How it is chosen is not clear.) for the specific purpose of attaching eggs to it. The pattern followed is relatively consistent (1Fn, $X= 0.858 (\pm 0.02)$), (1Mn, $X= 0.916 (\pm 0.04)$). It is also similar between males and females. The female descends further as it reaches the egg mass and this translates in to the slightly lower NGDR for the female than the male. The speeds of approach are relatively slower than those of general swimming (1Ms, $X=18.1\text{cm}\cdot\text{s}^{-1} (\pm 4.2)$), (1Ms $X= 16\text{cm}\cdot\text{s}^{-1} (\pm 1.3)$). The male has a relatively constant deceleration as it approaches, leveling off to the hover (4M). The female approaches in a position below and slightly behind the male with a slightly lower speed.

The lay/attachment (3F) (within 0 - 0.5m of the egg mass)

The successful attachment of a string of fertilized egg to the communal egg mass by the laying female. The female attempts to hold position as it attaches the egg string, hence the low NGDR (3Fn, $X= 0.431 (\pm 0.02)$) and speed (3Fs, $X= 7.0\text{cm}\cdot\text{s}^{-1} (\pm 6.2)$). It is not zero due the movement caused by the current and vertical motion in the water column that affects positioning.

The hover during the lay (4M) (within 0 - 0.5m of the egg mass)

The male attempts to stay close to the female as she lays, this requires an almost looped hover allowing a relatively stable position above the

zero, as water currents and general movements in the water column affect the male.

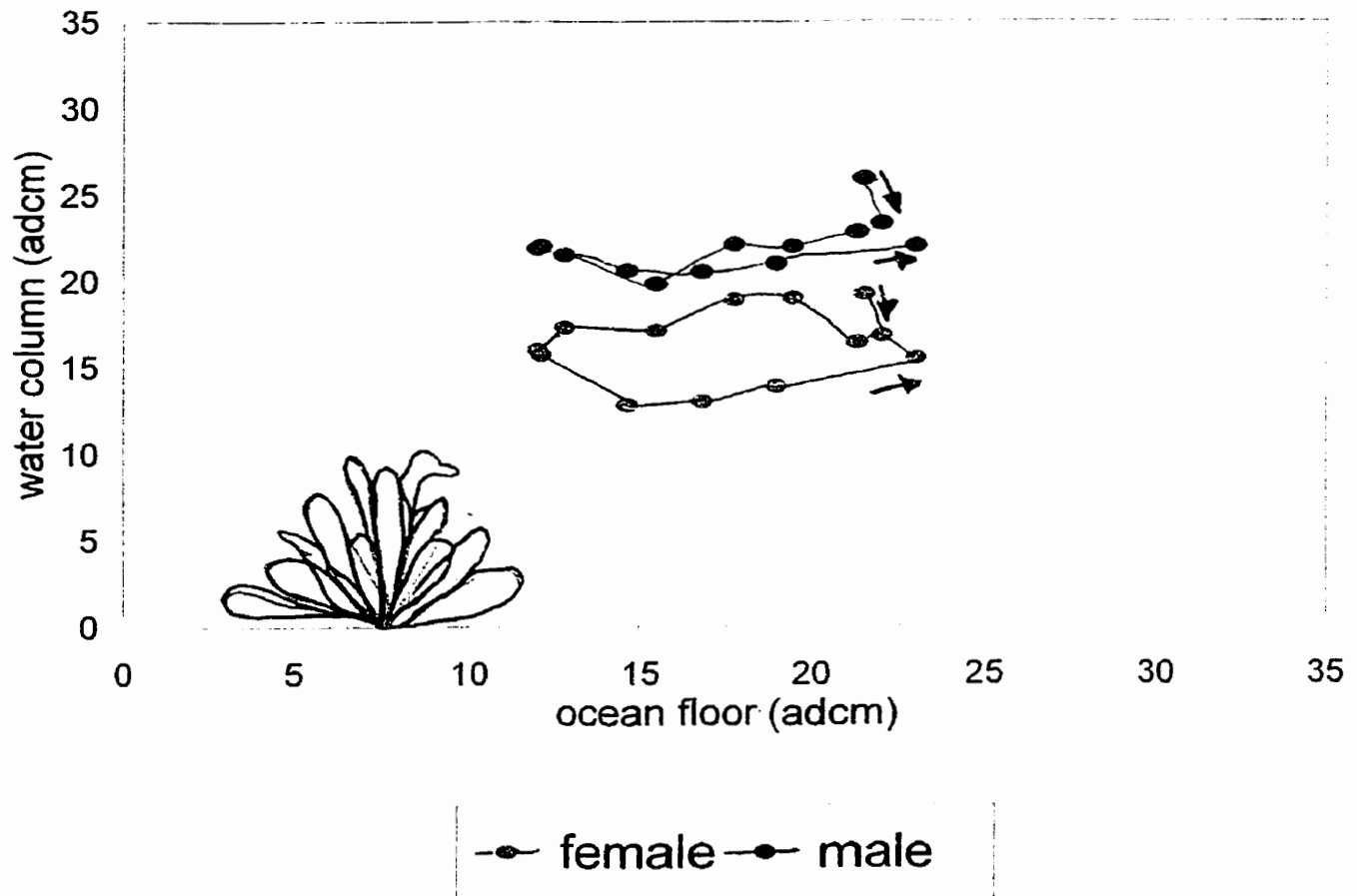
Retreat after a lay (7F 7M) (within 2m of the egg mass)

Departure from the egg bed after the egg string is attached. The subject's body line does not change direction from the approach, therefore there is no looping or turning in the pattern line. Hence the high (straight) NGDR's (7Mn, $X = 0.924 \pm 0.02$), (7Fn, $X = 0.787 \pm 0.18$). The speeds (7Ms, $X = 17.70 \pm 9.56 \text{cm}\cdot\text{s}^{-1}$), (7Fs, $X = 18.10 \pm 5.82 \text{cm}\cdot\text{s}^{-1}$) are similar to the approach but acceleration is greater.

FIGURE 7**An unsuccessful Approach, Attempt (at Laying) and Retreat**

The categories included in this display are: 2M - Male Attempted Approach, 6M Male Attempted Hover, 8M Male Attempted Retreat, 2F Female Attempted Approach, 5F Female Attempted Lay, and 8F Female Attempted Retreat.

(Eyespot Positions every second)



The attempted approach (2F AND 2M) (within 2m of the egg mass)

The approach is "attempted" when the above process of approaching is for some reason disrupted. Hence it has a pattern very similar to the approach 1F versus 2F p -value = 0.93. M&F2 appears to be more 'cautious' hence the lower NGDR's, as the route is usually less direct. As an attempt is terminated before the lay sequence, there is often a rerouted pattern at its end, another explanation for the just slightly lower NGDR's (2Fn, $X = 0.742 \pm 14$), (2Mn, $X = 0.898 \pm 0.03$). The p -value between 1Fn and 2Fn is 0.13. The p -value between 1Mn and 2Mn is 0.51. The speed is slower for the males (2Ms, $X = 13.4 \pm 2.36 \text{cm} \cdot \text{s}^{-1}$). The p -value between 1Ms and 2Ms is 0.07. The deceleration is not as apparent as it is in the approach. The females attempted approach is just slightly faster than one resulting in a successful lay.

The attempted lay (5F) (within 0 - 0.5m of the egg mass)

The lay is "attempted" when the process of laying is for some reason disrupted. The attempted lay therefore has a slightly higher averaged NGDR than the successful lay (between 3Fn and 5Fn $p = 0.37$); this is expected as the line becomes straighter as it jets off due to the disruption (e.g. fright). This reaction is

High-lighted by the slight increase in speed (5Fs, $X = 12.34 \pm 4.5 \text{cm} \cdot \text{s}^{-1}$, between 3Fs and 5Fs $p = 0.20$).

The hover during the attempted lay (6M)(within 0 - 0.5m of the egg mass)

The male reacts to the female's sudden retreat and accompanies her out

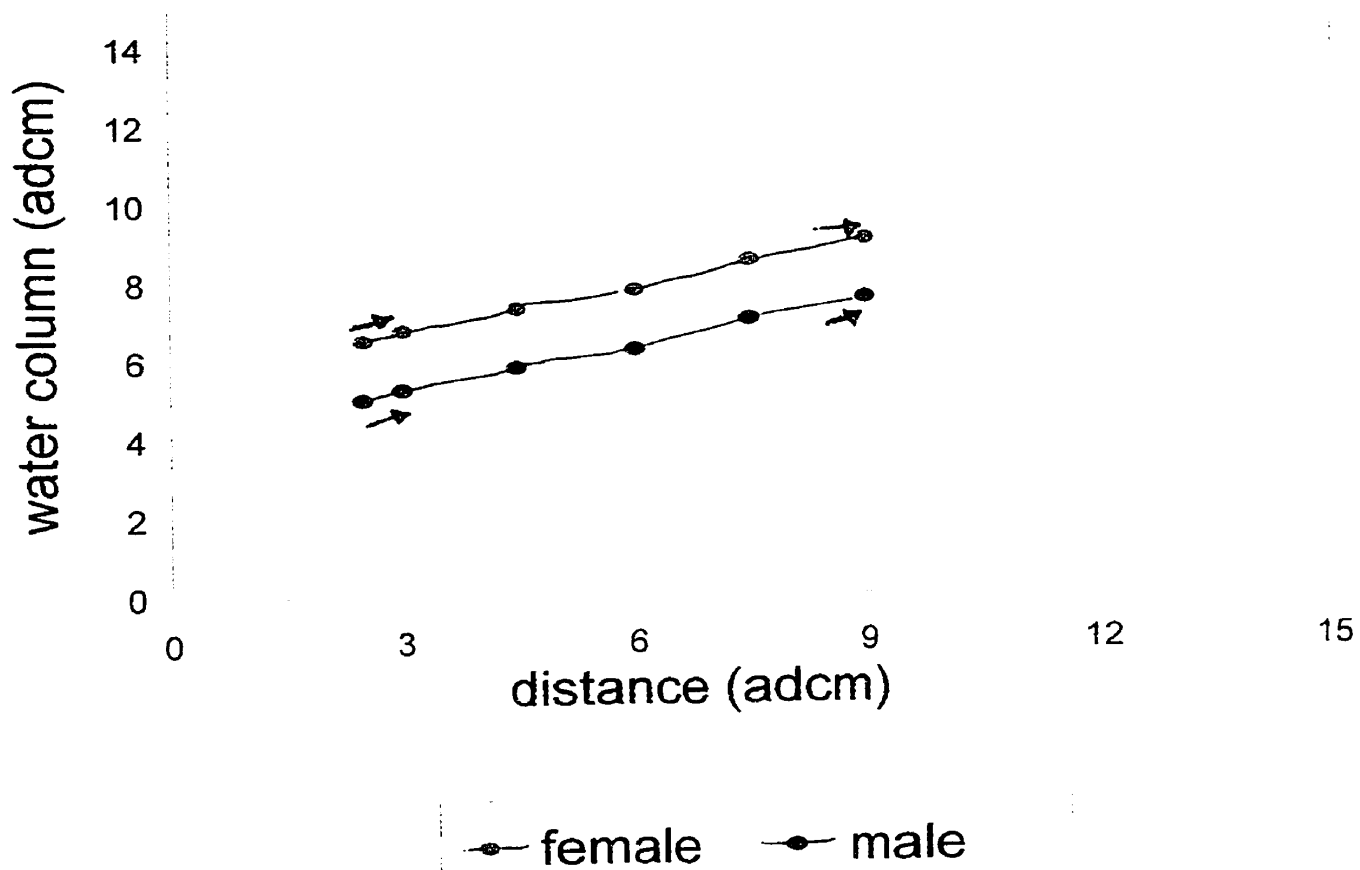
to the periphery of the laying zone. Hence the straightening of the line and the slightly higher NGDR's than occur in a hover during a successful lay (between 4Mn and 6Mn $p = 0.41$). The speed (6M, $X = 11.04 \pm 3.7\text{cm}\cdot\text{s}^{-1}$) increases slowly as the reaction to the female's retreat is often quite delayed.

The departure after an attempt (8F and 8M) (within 2m of the egg mass)

Premature departure i.e. before the egg string is attached to the egg mass. There is no looping or turning in the pattern line. Hence the very straight NGDR (8Fn, $X = 0.94 \pm 0.04$), (8Mn, $X = 0.92 \pm 0.1$). The lower NGDR of the male can be attributed to the fact that male reacts after the female (p -value = 0.6). The mean NGDR for females found in the departure after an attempt is higher than after a successful lay (p -value = 0.14) as they are reacting to a disturbance (example the passage of a potential predator) they clear the area as quickly as possible (a straight line). This 'clearing' is reflected in the faster speeds (8Ms, $X = 25.4\text{cm}\cdot\text{s}^{-1} \pm 17.5$), (8F, $X = 33.4 \pm 26.42\text{cm}\cdot\text{s}^{-1}$). The large variations found in the standard deviations can be reconciled by the fact that the reactions are based on the degree of the disturbance that causes the lay to be aborted.

FIGURE 8**Parallel Mating at ~10m**

The categories included in this display are: 12B Both Male and Female Mating
(Eyespot Positions every second)

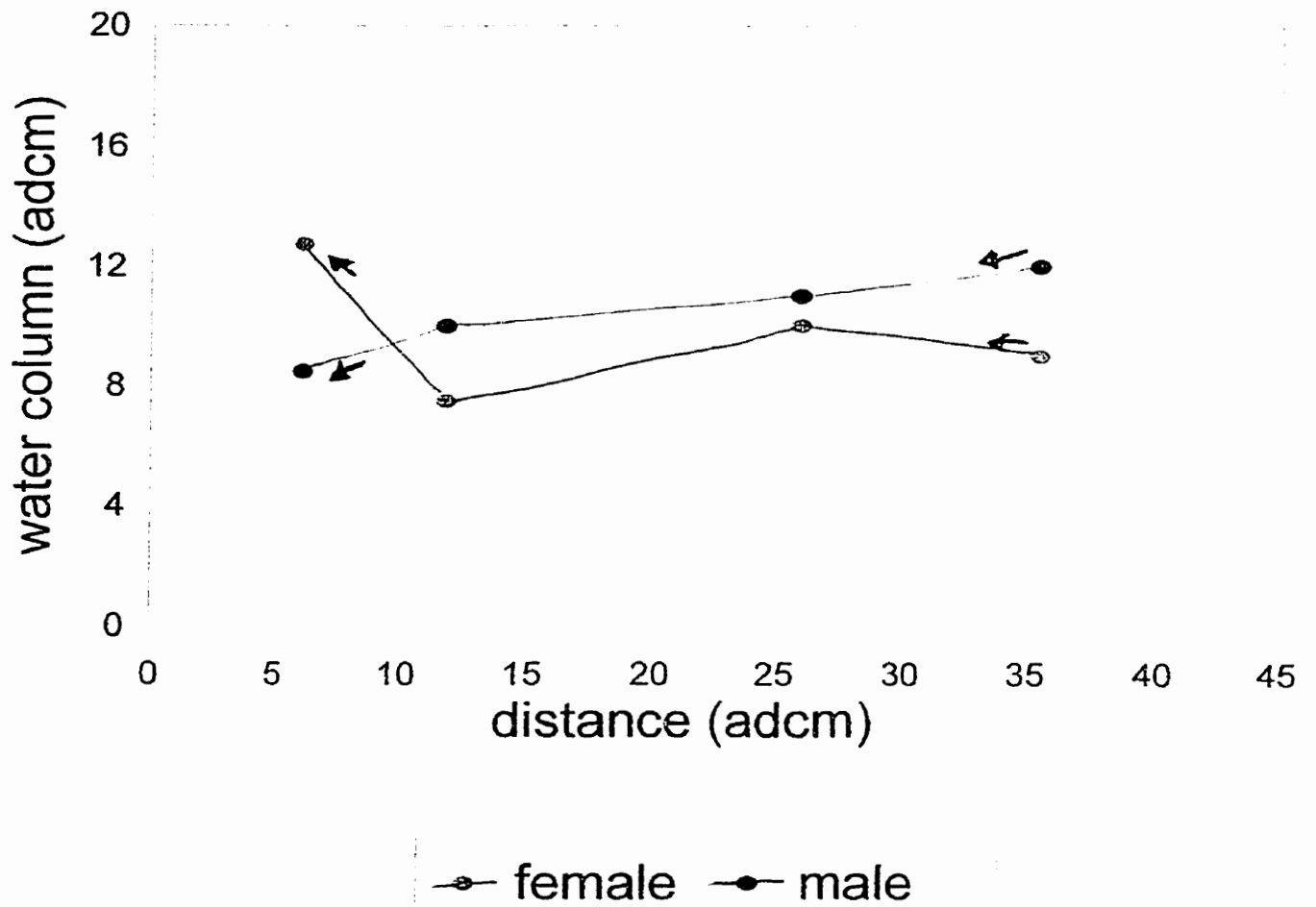


Mating (12F and 12M) ($\geq 2\text{m}$ from the egg mass)

'Parallel' or 'head to head' mating positions are used by the subjects interrupting their circling of the egg bed. Speed slows down slightly as the pair joins. (12Bs, $X = 15.31 \pm 0.66\text{cm}\cdot\text{s}^{-1}$) The NGDR remains quite similar to the general circling (12Bn, $X = 0.800 \pm 0.05$).

FIGURE 9**Agnostic Behaviour**

The categories included in this display are: 13M Male Agnostic Behaviour
(Eyespot positions every second)

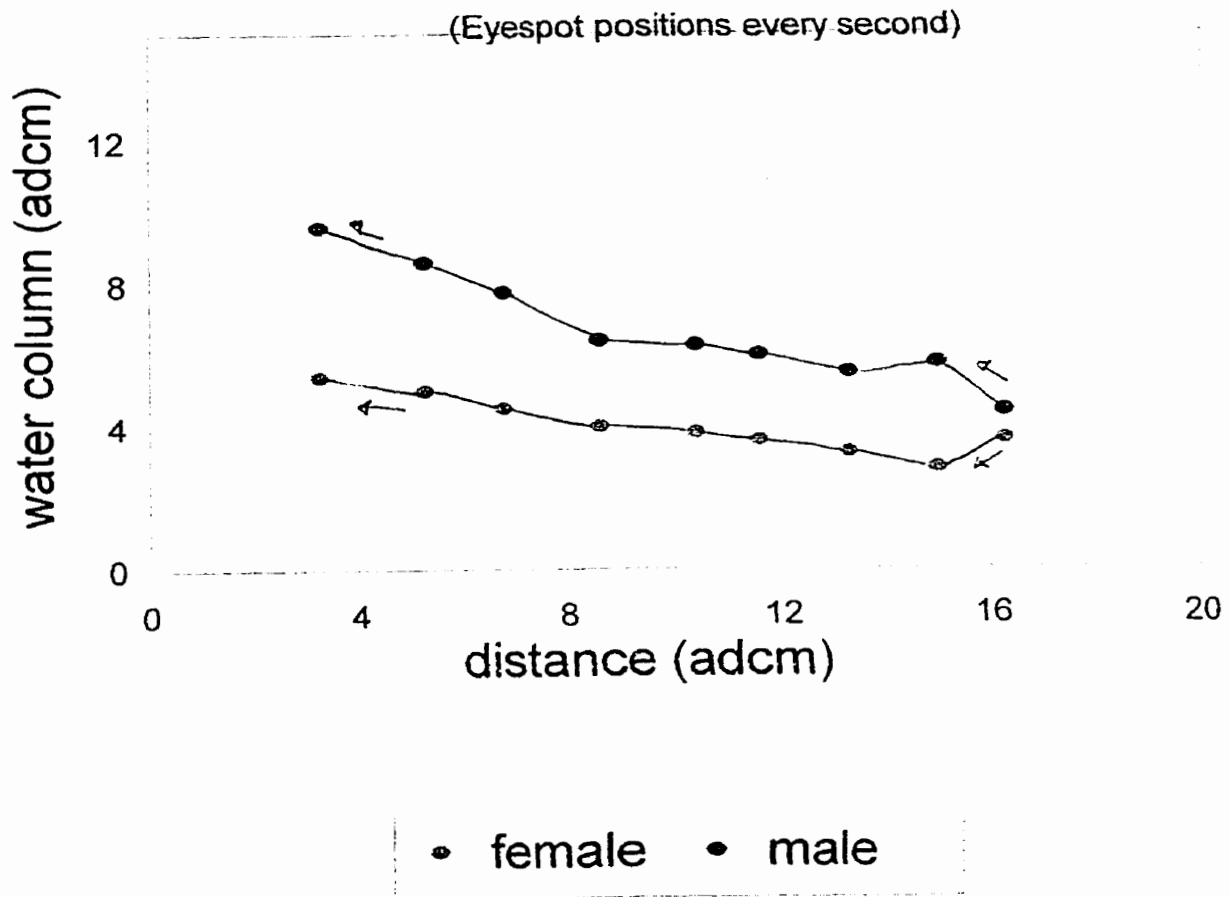


Agonistic Behaviour (13M) ($\geq 2\text{m}$ from the egg mass)

Males compete for mating privileges and often battles erupt. Fighting occurs anywhere and often there are numerous turning points; however, in between these points are surges that are often very fast and very straight having resulting high NGDR (13Mn, $X = 0.932 \pm 0.04$), and fast speeds (13Ms, $X = 67.7 \pm 5.60\text{cm}\cdot\text{s}^{-1}$).

FIGURE 10
Circling, at ~10m

The categories included in this display are: 9M Male Circling, 9F Female Circling however notes on swimming M10 and F10 and hovering M11 and F11 are included as the 3 behaviours often occur together



Circling (9F and 9M) ($\geq 2m$ from the egg mass)

The subjects circle (usually in a clockwise direction) the egg bed from early morning to late afternoon. Although the end product movement is circular the 0.96 s interval over which the NGDR was calculated results in a very straight line (9Mn, $X = 0.966 \pm 0.02$), (9Fn, $X = 0.945 \pm 0.05$). This implies a general smooth circle possessing a large radius. The speeds are comparable to general swimming speeds (9Ms, $X = 45.5 \pm 11.6 \text{cm}\cdot\text{s}^{-1}$), (9F, $X = 43.2 \pm 13.5 \text{cm}\cdot\text{s}^{-1}$).

Swimming (12F and 12M) ($\geq 2m$ from the egg mass)

Not all the swimming is in 'the circle', there is time spent simply in the area, especially in late afternoon that seems to be restricted to general swimming approximately 5 - 20m from the general egg bed. This NGDR result shows (10Mn, $X = 0.67 \pm 0.23$), (10Fn, $X = 0.67 \pm 0.18$) the long slow turns made by both males and females while swimming. The speeds are comparable to those of the circling (10Ms, $X = 48.4 \pm 13.5 \text{cm}\cdot\text{s}^{-1}$), (10Fs, $X = 41.3 \pm 12.7 \text{cm}\cdot\text{s}^{-1}$). Between 9Ms & 10Ms, $p = 0.73$, and between 9Fs and 10Fs, $p = 0.83$).

Hovering (11F and 11M) ($\geq 2m$ from the egg mass)

Hovering in the general vicinity is very similar to hovering above the egg bed. Hence the NGDR's are similar. The NGDR and speed medians are slightly higher for general hovering than hovering above the egg bed, this could be explained by the squid not having a specific area (e.g. the egg mass) over which it is attempting to stay but is simply attempting to stay in the same general area.

SECTION TWO

Median and t-test comparison tables for NGDR & Speed

Table 2: NGDR - net to gross displacement ratio

Categories	#	NGDR Median	Standard Deviation
Approach	1M	0.916	0.049
	1F	0.858	0.020
Attempted Approach	2M	0.898	0.034
	2F	0.742	0.135
Lay/Attachment	3F	0.431	0.025
Hover during Lay	4M	0.392	0.173
Attempted Lay	5F	0.448	0.030
Hover during Attempted Lay	6M	0.502	0.230
Retreat from Lay	7M	0.924	0.022
	7F	0.787	0.179
Retreat from Attempted Lay	8M	0.908	0.105
	8F	0.936	0.043
Circling	9M	0.966	0.020
	9F	0.945	0.047
Swimming	10M	0.668	0.23
	10F	0.671	0.18
Hovering	11B*	0.468	0.108
Parallel Mating	12B*	0.801	0.005
Agonistic Behaviour	13M	0.932	0.040

M - Male

F- Female

B* - Both males and females featured in the same category.

Table 3: T-tests for NGDR comparisons

	1F	2F	3F	5F	7F	8F	9F	10F	1M	2M	4M	6M	7M	8M	9M	10M
1F		.13							.37							
2F	.13									.05						
3F				.37							.64					
5F			.37									.63				
7F						.14							.17			
8F					.14									.59		
9F															.39	
10F																.98
1M	.37									.51						
2M		.05							.51							
4M			.64									.42				
6M				.64							.42					
7M					.17									.75		
8M						.59							.75			
9M							.39									
10M								.98								

*Female versus male mating is omitted as it is one (1).

Table 4: Speed Table

Categories	#	Speed (cm/s ⁻¹) median	Standard Deviation
Approach	1M	18.1	4.21
	1F	15.9	1.30
Attempted Approach	2M	13.4	2.36
	2F	16.3	8.27
Lay/Attachment	3F	5.6	6.15
Hover during Lay	4M	7.9	2.27
Attempted Lay	5F	12.3	4.57
Hover during Attempted Lay	6M	11.0	3.7
Retreat from Lay	7M	17.7	9.6
	7F	18.2	5.8
Retreat from Attempted Lay	8M	25.3	17.5
	8F	33.4	26.4
Circling	9M	45.5	11.6
	9F	43.2	13.5
Swimming	10M	48.4	13.5
	10F	41.3	12.7
Hovering	11B*	11.5	3.5
Parallel Mating	12B*	15.3	0.7
Agonistic Behaviour	13M	67.7	5.6

M - Male

F - Female

B* - Both males and females featured in the same category.

SECTION THREE : General Observations, Spatial Orientation

Frame by frame video analysis allowed the observation of consistently distinctive behavioural patterns. Within the surrounding area behavioural patterns were identified and detailed on their own, the results were used to describe the categories in Section 1. However, it was the observation of distance from the egg mass that created a perspective within which to work. The spatial orientation of the behaviours could be documented and mapped. Areas where particular actions or behaviours consistently took place were identified and named. The zones created by this method are entirely subject to the categorization of behaviours supplied above. Their limits, names and appearance are established only by the acts performed within them.

The egg mass has been placed in the center of this portrayal and it is considered (in Figure 11 and 12) here to be undisturbed by the proximity of others (suggested influence, Figure 13). The laying zone immediately surrounds the egg mass. Only entered by the female, its area is defined by the size of the egg mass. The 'approach' zone is a domed circle with a radius of ≤ 2 m, only well defined pairs attempting to lay or in the process of laying are found here. The approach zone is edged by the 'swim' zone, where antagonistic behaviours, general (afternoon) swimming and hovering are found. Pairs did not remain well defined although they were generally distinguishable. Mating and large scale circling of the area was conducted ~10 m above the egg mass in a 5-7 m 'circling' zone ~10m below the water surface.

FIGURE 10
General Spatial Orientation with in the 'Approach' Zone

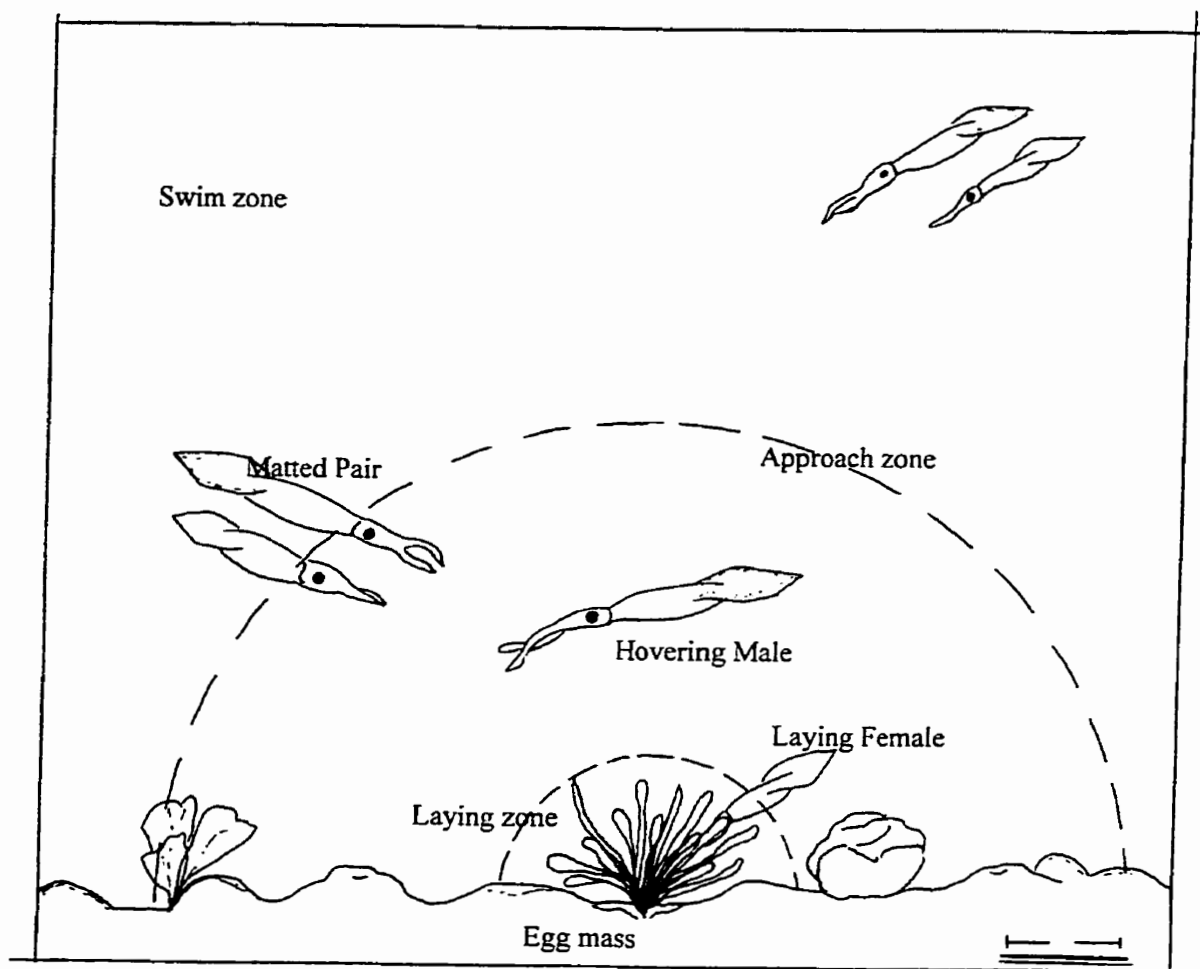


FIGURE 11
Zoning Around an Egg Mass

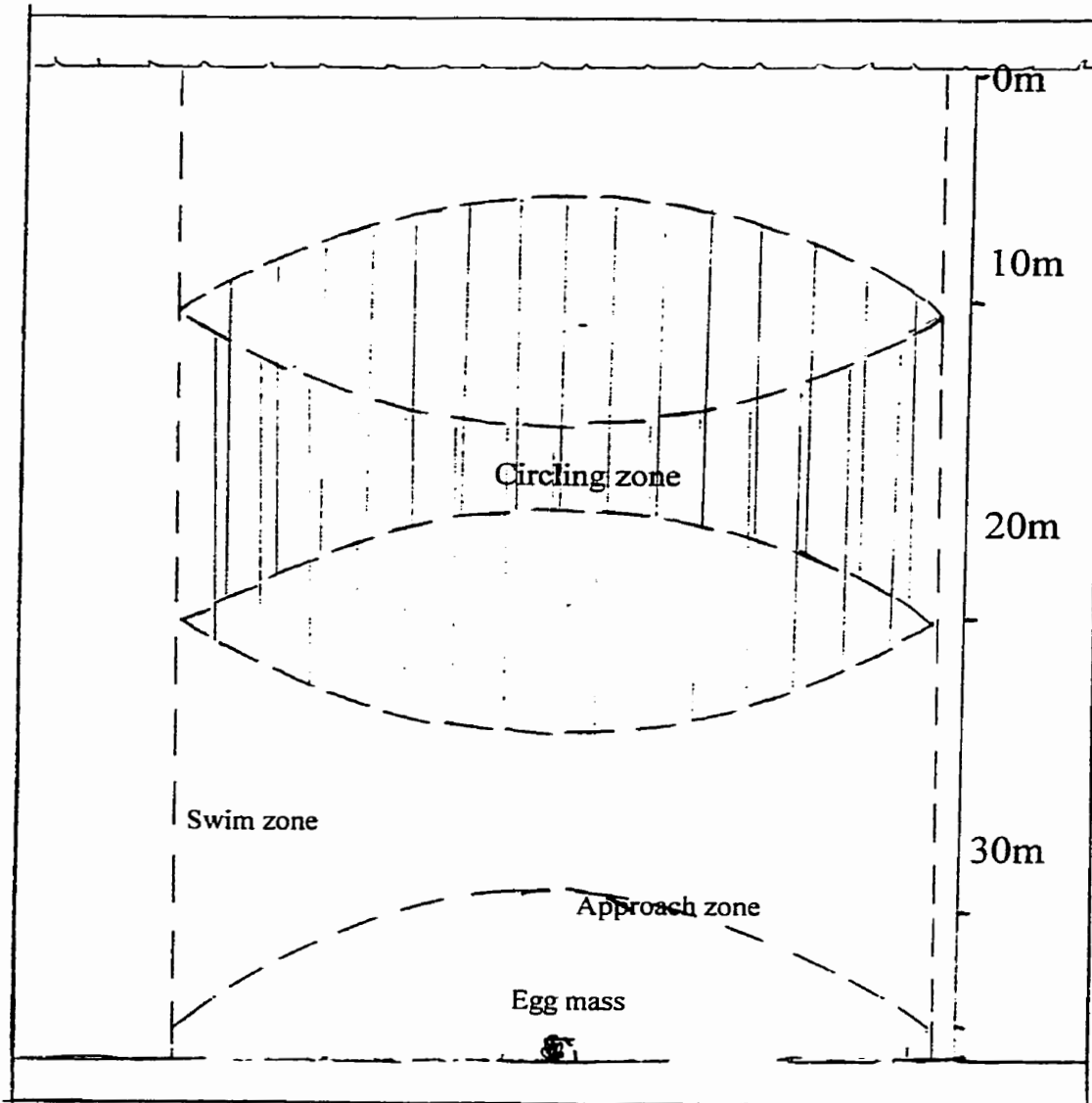
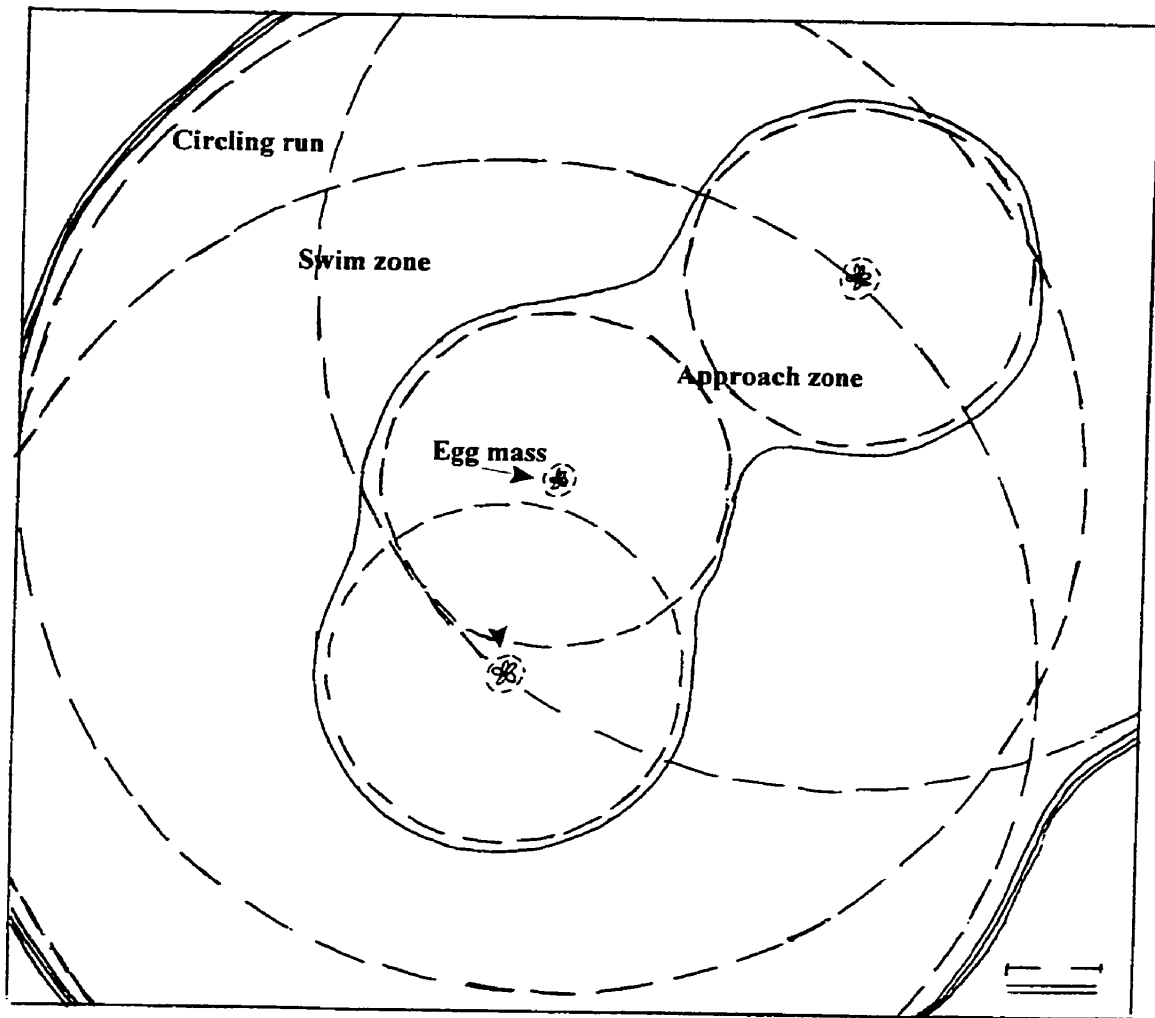


FIGURE 12
Interference -Zoning Around the Egg Masses



DISCUSSION

The purpose of this study was to document the gross body movements and speed of the patterns during reproductive behaviours over the egg bed and in the surrounding area using frame-by-frame video analysis, Thus enabling biological understanding of the RAPT analysis readouts (Chapter 4: "RAPT analysis of spawning behaviour of *Loligo vulgaris reynaudii*").

The behaviours presented in the Results section of this chapter are very similar to the accounts in the literature (Chapter 2: Literature review). The differences show up in the detail given and the change of perspective taken. Unlike laboratory experiments (Drew, 1911; Arnold, 1962) the subjects were surveyed in their natural habitat and so were able to be considered without the physical stresses caused by the disruption of moving them into aquaria. These physical changes may have caused some of the more aberrant behaviours seen in the laboratory experiments (necrophilia etc. observed by Arnold, 1962). The concentration on and quantification of gross body movements is not covered extensively in the older literature (hence it's detail in the previous results section) as the specific acts of copulation and chromatophoric changes were of greater interest to the researchers (Drew, 1911. Arnold, 1965),

It was assumed that Griswold and Prezioso (1981) were correct in saying that the "social hierarchy involving egg deposition differs from species to species". However, it is apparent that the general observations that they

made in 1979 of *Loligo plei* off the coast of Rhode Island are very close to the observations made of *Loligo vulgaris reynaudii* off the southern cape of South Africa. Equivalent 'well defined [mated] pairs' were grouped in the same manner, making several cautious approaches from an almost similar semi-circle waiting area. Although *Loligo vulgaris reynaudii* are slightly larger than *Loligo plei* the female is significantly smaller than the male and very noticeable as she swims under and behind the male. The absence of significant changes in pigmentation and the good condition of the sexually mature individuals around the egg bed was akin to what had been viewed by Griswold and Prezioso (1981).

The notable differences between the 1979 and 1993 observations were that:

- I. Unlike *Loligo plei*, *Loligo vulgaris reynaudii* females approach the egg mass independently. The male hovers 0.5m above the laying female.
- II. Pairs do not wait for other pairs to finish depositing eggs before approaching. Hence several females may 'lay' at the same time.
- III. The waiting area is not a defined semi-circle, but rather a loosely observable one.

Pairs do not wait in attendance as *Loligo plei* was observed to do in 1979. The waiting instead is done indifferently within swim zone or not at all.

The general spatial orientation and relation of activities to time periods could be done only for the immediate vicinity of the egg bed. As the

camera was not in place during long periods of time, and the area that can be seen in the field of view is relatively small, too small to certain of the grand scale spatial utilization. It should be noted, however, that the sexually mature pairs about to lay eggs stayed within or very near the edge of the 'approach zone' until a successful lay was accomplished unless severely disturbed and even then returned quite soon (within 1m - 4m) before continuing, until successful in laying.

Visual stimuli have been recorded as being very important as a source of sexual arousal and egg laying activities (Drew, 1911; Arnold, 1962; McGowan, 1953). Attention is drawn here to the fact that the (human eye's) water visibility has been ~4 -6m on the days experimentation has been done (4m -Griswold and Prezioso, 1981; 4-6m reported by Rodger Hanlon, 1993). This is very close to the observable diameter of the approach zone. Suggesting again the communal egg mass must be seen, sensed and approached. The approaches described here may be linked to the act of flushing introduced egg masses in aquaria (Drew, 1911; Arnold, 1962).

It is of interest that squid are able to hover despite the active swell, although maintaining a stable position even in static water is not easy or 'cheap' as shown by Hoar and colleagues (Hoar *et al.* 1996). Hovering above the egg bed by the male and elsewhere by both genders does take place despite this high cost. The obvious pairing and the discernable high cost hovering over the egg bed prompt a questioning of genetic interest when considered together. The need to physically stand guard at such a

high price seems to demand a reason. Is the male standing guard over his genetic interest? Does the presence of the male at the lay affect the genetic outcome of the spawn? There is potential for spermatophores from different sources: off-shore mating (McGowan, 1953), sneaker males (Hanlon, 1996), other large males (Sauer *et al.*, 1997), and water transmitted spermatophores (Drew, 1911). These questions will only be answered by genetic testing of generational populations and so can only be wondered about at present.

The lack of significant differences shown by the t-tests conducted may be explained by the low power of the tests done. With an n as small as 5 it is very difficult to demonstrate significance. Particularly when the swell is moving the squid during the behaviors under observation. Although the visual differences between the attempts and successful movements have been made in the results section they are hard to corroborate with significance testing due to the small amount of data collected.

The actual purpose of the video analysis was to be able to gather knowledge of the potential importance of specific movements that can be related to spawning behaviours. The next step is to pair the gross body movements displayed by action graphs, thus distinguishing patterns seen in the matrixes produced by the positioning software.

CHAPTER 4 - RAPT ANALYSIS

ABSTRACT

In October/November 1994 and again in November 1997, a four buoy Radio-Acoustic Positioning and Telemetry (RAPT) system was put in place above spawning sites off of the South African shore. By solving 3D hyperbolic equations with data received from the buoys, VEMCO™ software allowed the base station to view and record real-time tracks. Statistically significant tracks were produced back in the lab after various smoothing techniques dealt with the on site noise and distortions. Tracks showed spawning behaviour of large males (32cm mantle length) females (21cm mantle length) and sneaker males (15cm mantle length), all of which concentrated diurnally on the inshore egg beds.

INTRODUCTION

Tracking of marine animals is not new. Passive tagging has been in use for centuries. The information gleaned from passive tagging experiments, planned or unplanned (O'Dor and Webber, 1998) has furthered our understanding of ecology, behaviour and movement patterns. Active tagging, the use of signaling or responding tags has been a more recent development in the field (O'Dor and Webber, 1998).

There are various active tagging techniques that have been developed, each to answer specific questions (Kimley *et al.*, 1999). Electronic tagging allowed large animals to be followed by ships. Providing tracks with spatial errors of <100m, during time periods of <10days, over distances of <100km. This technique provides short-term movements often related to migration or habitat range. Examples of electronic tag use include the tracking of blue shark (Saiarotta and Nelson, 1977; Carey and Scharold, 1990), white shark (Carey *et al.*, 1981, Goldman *et al.*, 1995), marlin (Holland *et al.* 1990; Brill *et al.*, 1993), and yellow-fin tuna (Block *et al.*, 1998).

Archival tagging determines geographic positions from a property of the environment unique to the subject's present location, storing this information in memory for later retrieval. Providing a track with a spatial error of <100km, during time periods of 1-3 years, over distances of <10,000 km. This technique provides large-scale migration and area location for fish with a high recapture

rate (Klimely *et al.*, 1999) or pinipeds that return to a land based colonies (DeLong *et al.*, 1992). Examples of properties used for geo-locating include: Latitudinal distribution of surface temperature at the animals longitude based upon light measurements (Smith and Goodman, 1986), and increase in subsurface irradiance at dawn and its decrease at dusk (Hill 1994).

Localized ultrasonic tags transmit pulses to stationary hydrophones that act as transfer and/or storage sites for location data. The earliest ultrasonic tracking systems for fish appeared in the literature in the mid-fifties (Trefethen *et al.*, 1957). For the first decade or so research focused on monitoring fish moments on a small scale, examples include such work as Carey's 1971 experiments with warm-bodied fish, Kuroki's 1971 work with location and water temperature, Watkins's 1972 research on sound source and 3D hydrophones and Luke's 1973 examinations of pressure and ultrasonic tracking of marine animals (Stasko, 1975). Early versions of localized tagging efforts involved transducers mounted on the sea floor and the time delay between arriving pulses determined by an oscillator to be included later in hand calculated hyperbolic equations in order to determine location (Hawkins *et al.*, 1974). With the advance of computer technology and battery capabilities the hydrophones developed in to independent ultrasonic radio receiver – transmitters (O'Dor *et al.*, 1998). Marine animals can be tracked with a spatial accuracy of 2-10m within an area of 1 km² for indefinite time periods determined by species movement away from the localized area or hydrophone removal.

The intended use of the RAPT system in the South African Environmental Study:

1	Determine the effect of temperature, turbidity and currents on spatial behaviour of individual Chokka.
2	Undertake a physiological study of swimming behaviour on a spawning ground.
3	Undertake a physiological and spatial behaviour study on Chokka predators within the survey area (O'Dor <i>et al.</i> 1994).

The purpose of this study was to describe and classify tagged squid behaviour recorded for *Loligo vulgaris reynaudii* over the inshore egg bed in the South African South-Eastern Cape region (Figure 1) with a Radio Acoustic Positioning and Telemetry (RAPT) system. The two greatest differences between earlier tracking studies and this one:

1	The provision of real time tracking, allowing the operator to focus on individuals that are present rather than just monitoring for presence
2	The accuracy of the positions given. RAPT provides positional accuracy on the order of meters (rather than hundreds of meters). Thus allowing details of gross body movements to be seen and recorded.

Acoustic triangulation is currently the only way of tracking with high-resolution (meters) at intermediate ranges (10's -1000's of meters) in seawater and is the most accurate in freshwater (O'Dor *et al.*, 1998). Large-scale gross body

movement details of spawning behaviour have been beyond research capability until RAPT technology became available. RAPT allows continuous monitoring of real-time behaviours while at the same time accumulating a wealth of data for post-hoc analysis.

The aim of this RAPT analysis:

1	To identify the abilities and limitations of the RAPT system
2	To make biological interpretations from the RAPT readouts i.e. to identify categorizable behaviours
3	To present <i>Loligo vulgaris reynaudii</i> organization over space and time, with reference to speed.

The ability to decipher *Loligo vulgaris reynaudii* movement patterns will clarify the processes that occur on or near the spawning bed. Clear categorization of these processes is necessary before any real attempt can be made to quantify reproductive effort and manage this 'natural resource' sustainably.

No method should be used without understanding its limitations. In this case, drawbacks include the possible effects of tagging on the animals undergoing observation, and on the system's analytical accuracy. The act of tagging involved in this type of behavioral study is theoretically rather invasive. The method of capture, the physical tag and the release, all have potential effects on the animals being observed. Thus questions extend beyond the examination of the

systems accuracy to include the procedure affecting the animals responses and hence the conclusions. A question that became apparent during the data analysis was whether the tags in fact altered the behaviours that were being studied? If so, how much and for how long?

RAPT involves using acoustic transmitters attached to several underwater subjects. The transmitters feed information into semi-autonomous buoys set up over the study area. The buoys package the information, which contains position (in terms of signal arrival times) and performance data (encoded in the pulse intervals) and sends it to the base station over radio frequencies. The receiving computer at the base station is then able to triangulate the individual subject's position in three dimensions (3D) in relation to the buoys and the general underwater topography, as well as decode telemetric information such as performance data or water temperature (O'Dor *et al.*, 1995).

Tagging experiments were conducted during the closed seasons of November, 1994 and 1997. Oyster Bay, an inlet just west of Cape St. Francis (Figure 14) acted as the base for the deployment of the four-buoy RAPT system which was set up using VEMCO software, according to system procedures provided by VEMCO (latest copy: VEMCO manual 1998). In 1994 the experimental group consisting of three (3) large males (~32cm mantle length), four (4) females (~21cm mantle length) and two (2) small 'sneaker' males (~15cm mantle length) were caught, tagged and released over an egg bed which was approximately 1.5km offshore.

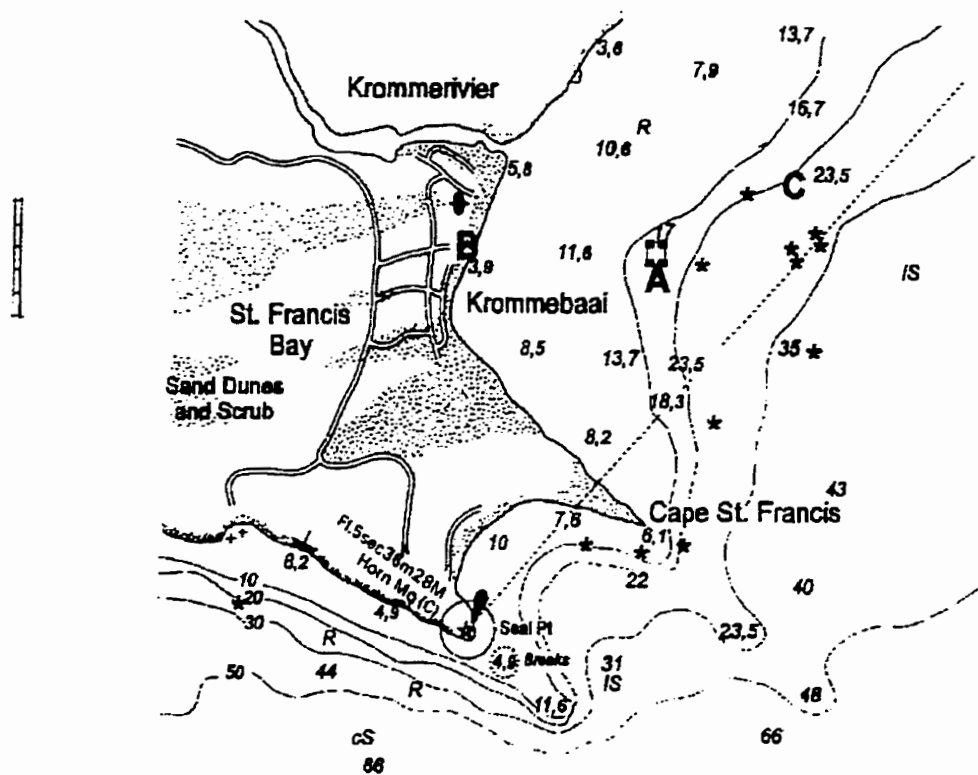
The squid were caught and remained with the larger mating group concentrated near the beds during the day, swimming beyond transmitter range at night and returning for up to one week. Fourteen (14) animal days of tracking were gathered. During this time two minor upwelling events in which colder, clearer water flushed over the egg beds occurred.

Data collection in 1997 was interrupted a number of times by heavy local storms. The buoy array was originally deployed in Oyster Bay for the first two weeks of November and was redeployed during the third week of November in St. Francis Bay (Figure 15). Although the buoys had to be collected and moved due to the severe weather, tracking was possible over short time periods. The 1997 experimental group consisted of three (3) large males (~32cm mantle length), and three (3) females (~21cm mantle length). No sneaker males were caught and tagged successfully. Once again the squid caught remained with the larger mating group concentrated near the beds during the day, swimming beyond transmitter capabilities at night and returning for the period until the buoys had to be removed. Ten (10) animal days of tracking were gathered. The storms interrupted continuous monitoring, however, individual squid were followed before and after storms.

FIGURE 14
Map of Oyster Bay



FIGURE 15
Map of St. Francis Bay



EXPERIMENTAL DESIGN

The primary interest of the hardware design involved was to optimize data collection from simultaneously tagged animals. This meant real time analysis was essential as it was important to follow detailed movements and interactions to understand the mating process. As *Loligo vulgaris reynaudii* females and sneaker males weigh as little as 70g, it was not possible to use large depth modulated transmitters to supply the z coordinate in the hyperbolic positioning algorithm. The only solution to this was to add a fourth buoy to the standard three-buoy system with both surface and bottom receivers arranged in a tetrahedral array.

Accordingly, software had to be developed to solve for an additional hyperbola to provide the best estimate of continuous 3D positions. Further requirements of the program were that it allow for the collection of the maximum amount of data for post-analysis, enabling the production of detailed tracks of multiple fast-moving individuals.

Earlier VRAP (Vemco Radio Acoustic Positioning) software was used primarily for slow-moving benthic species and included averaging and data rejection algorithms to improve position accuracy and eliminate noise. As even small squid can move at $>1\text{ms}^{-1}$ a 'Fast track' feature, that recorded a position for every ping was added for this project. In addition to this the "debug" option was created (and used) to record all the data, so that corrections to trajectories could be made in post-analysis.

Specific design considerations for the system included:

1	A pinger small enough to be attached to a 70g squid without impeding its swimming/jetting.
2	A frequency for the ping high enough that it was well above the hearing threshold frequency of the squid, which also passed through the water with relatively high efficiency.
3	Pings strong enough to be picked up at a range of a 150m from each hydrophone.
4	A system bandwidth sufficient to detect the Doppler shifted ping from a jetting squid.
5	A tracking algorithm fast enough to allow real time tracking of a squid with position updates as often as once per second.

Calibration tests using divers carrying depth transducer/transmitters were conducted in St. Francis Bay approximately 4km offshore with the tetrahedral buoy and hydrophone configuration. Analysis of the equation solutions with small errors in arrival times randomly introduced, indicated that this tetrahedral configuration provided the minimum position error in the zone inside the array (Tim Stone, per. com.). Testing also indicated that positioning for maximum accuracy within a maximum volume requires that all array dimensions be similar, so horizontal array dimensions should not exceed depth by more than about 50%. Thus, placing the buoys in shallow depths limits the range of accurate positioning. If there are no obstructions even the small transmitters can be received from

distances of several hundred meters. Testing of the set up used showed that monitoring a 1s pinger 1m off bottom of the center of the array for 1h using 30s averaging gave standard deviations of 0.3m in x and y and 1.0m in z in 1-2m swells (O'Dor *et al.*, 1997). Accuracies were 1-2m inside the array and 3-5m in a 100m radius, but beyond 200m only the signal strength at individual buoys indicated the general direction. In a calibration test, a diver swam back and forth through the array with a transmitter held in each hand, it was clear which hand held which frequency transmitter (O'Dor *et al.*, 1998). This was possible even while fast-tracking inside the array, but fast tracks outside the array (where squid spent most of their time circling) required post-collection analysis due to random errors. However, 10-20s averaging gave reasonable tracks for squid swimming at up to 0.5ms^{-1} (O'Dor *et al.*, 1997).

METHODS

The experiment ran for fourteen (14) days during the November 1994, closed seasons instituted for conservation and research. The buoy array was mounted around a large egg patch in a cluster of egg patches that formed a bed 1.5km offshore from Oyster Bay, just west of Cape St. Francis (Figure 14), which had been actively fished for several weeks. The buoys were arranged in an approximate 45m square in 30m of water with hydrophones in a tetrahedral array. Two were located at the surface diagonally opposite each other, and the other two were on the mooring ropes at 20m depth (Figure 16).

Mooring ropes were attached to 5m lengths of heavy chain, anchored at one end, to minimize horizontal movement of the buoys while allowing more than 3m of vertical movement for swells. Although each hydrophone includes a pinger that can be activated from the base station on shore as part of a self-calibration position test, the stability of these moorings meant that this feature was rarely required after the initial set up. Setting up included testing and calibrating the system with the help of divers and hand-held transmitters.

After ensuring the accuracy of the system the subjects were tagged. Squid were caught using the traditional hand jig method (Appendix A) and rapidly attachment. No anesthetics were used during the tagging process as previous tests showed that available anesthetics cause trauma and created more problems than they solved (O'Dor, per. com.). The acoustic transmitters (Figure 17) were fitted inside the squid's mantle cavity and attached by securing a plastic washer to the

hypodermic needle that pierced the outer muscle (described in more detail by Webber and O'Dor, 1986). The squid were washed down with salt water before being released. This was all done as quickly as possible, usually less than one minute.

The experimental group consisted of two large males (32cm mantle length, 430g) that were tagged with VEMCO V16T-3H temperature telemetry tags and one large male, two small males (15cm mantle length, 70g) and two females (21cm mantle length, 180g) tagged with V8-2L pingers with intervals of 1.5s.

Once the squid were released the base station initiated acoustic sampling of the buoys polling them via a 458.5375MHz radio link. The base station: a Toshiba 4800CT computer calculated and plotted the positions of the buoys and the transmitters on squid. The actual positions were calculated from the differences in arrival times of the pulse at different elements in the hydrophone array; it was this information that was picked up from the buoys in the 10s polling sessions. Each hydrophone listened and recorded the time of the ping of each transmitter (pinger). 10s 'packages' of this information were transmitted every time the buoy was polled.

In addition to observations of the animals in the array under natural conditions, an attempt was made to monitor behaviours during manipulations including intense night-lighting and experimental fishing. Manual tracking with a directional hydrophone from small boats equipped with G.P.S. provided ground truth and additional position information outside the range of the array.

Post-hoc analysis was done over a number of years at the Dalhousie Department of Biology. The difficulty was in the distinction between noise and usable data.

Anisotropic errors in data positions, irregular time series and the contamination of the data with biologically impossible outliers and the decision to use as much of the original data as possible resulted in the creation of a unique filtering strategy.

The original arrival times for each of the transmitters were tabulated and organized into separate transmitter named files. These files could be split according to any preference that might be required for various observations. The data was then filtered in order to remove unrealistic data (e.g. above the water surface). The filtered data was graphed using an iterative procedure to create an approximating cubic spline (See appendix for details). The trajectory line was then sectioned in to 1s intervals, presenting the sampled data as it appeared after frame-by-frame video analysis. These graphs were created and displayed using MatLab 3D graphs (examples can be seen in the results section) for ease of calculation and orientation. Speeds were calculated from these points and graphed in MatLab (examples can be seen in the result section).

FIGURE 16
Tetrahedral Buoy Array

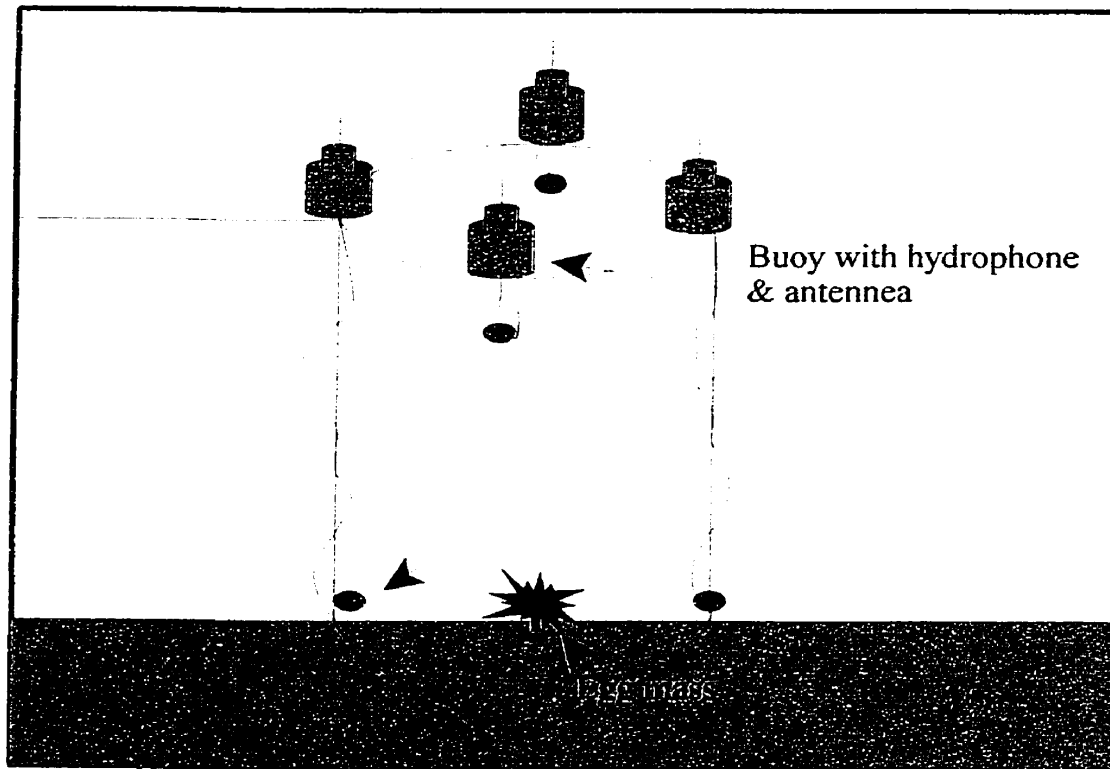
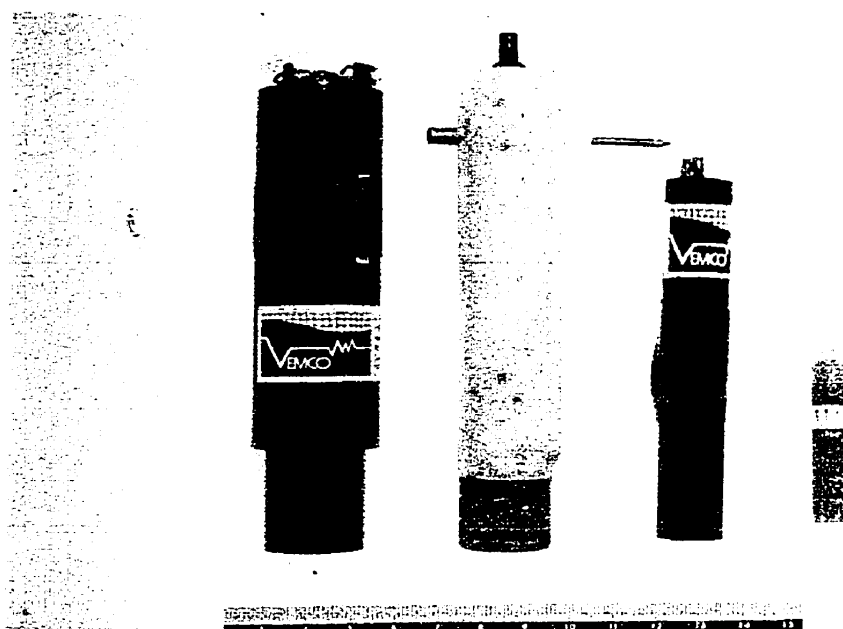


FIGURE 17
Transmitters



RESULTS

The results are divided into five sections. Section 1) MatLab graphs, displaying general examples of the spawning behaviour categories. Section 2) Speeds, median speeds of each group. Section 3) Typical days, for each group. Section 4) Change over time seen by the comparison of the days of RAPT analysis. Section 5) General spatial orientation above and around the egg bed.

Section 1: Actions as displayed by MatLab

Actions that were categorized according to the categorization created during the video analysis are displayed here under the same heading that they were described under in Chapter 3.

Figure 18: Possible Approaches, Lays and Retreats, female

Actual lay sequences could not be determined however various slow loops over the bottom of areas that had been suggested by the video tows to be viable egg mass areas were considered as possible lays.

Figure 19: Circling ~10m, sneaker male

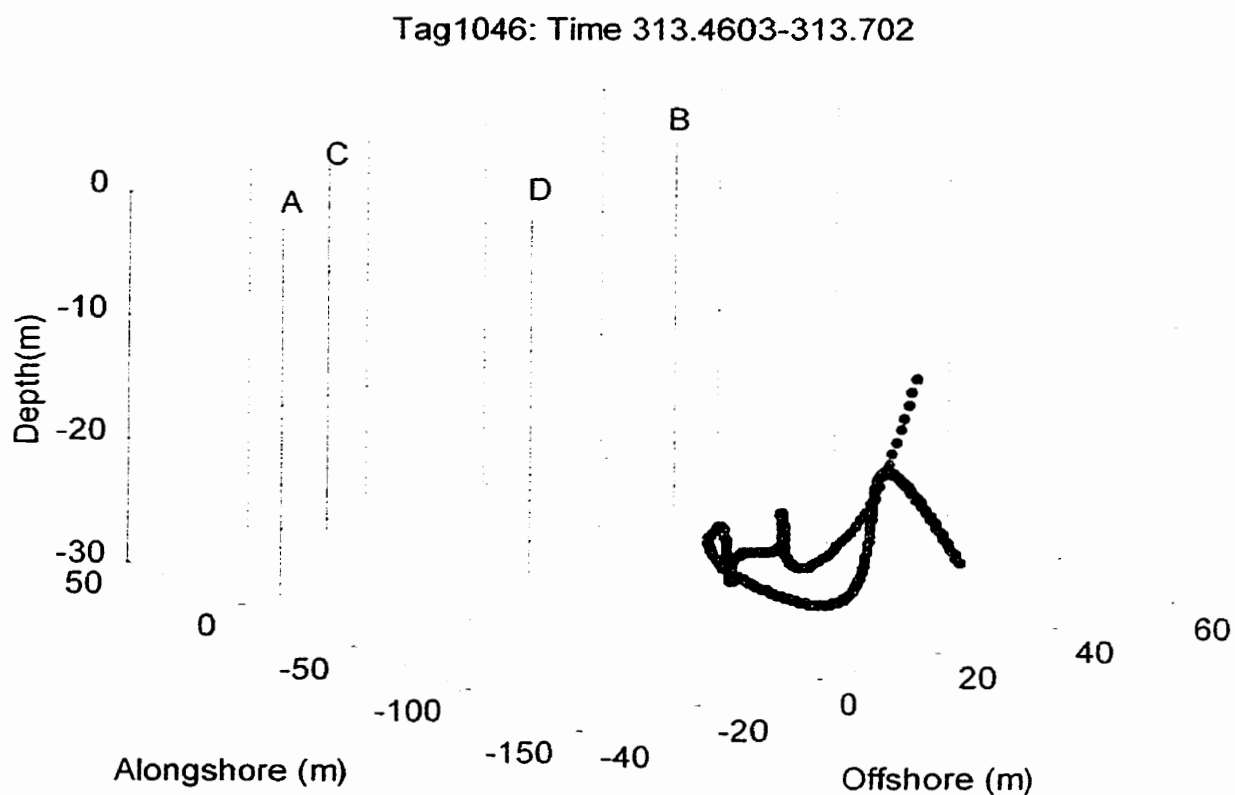
Circling really doesn't occur the way it had appeared to in the video clips. Rather forays within the array were what was seen when the z coordinates were correctly displayed.

Figure 20: Swimming, male

Various general swimming patterns were seen. This one shows a male early in the morning swimming over an area outside the array that was determined to be an egg mass.

Figure 21: Swimming, female

Females swam in large swooping circles, possibly one of the reasons that circling seem to be so prominent in the video sequences (see above for correction). This shows a female circling about 10m off the bottom and finally ending in a possible lay attempt.

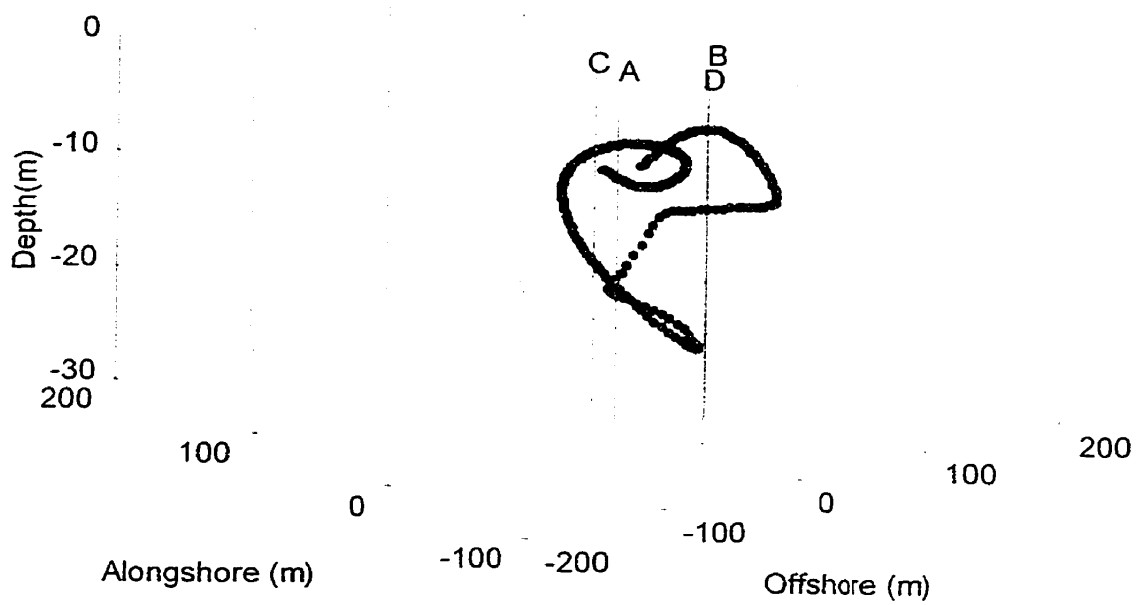
FIGURE 18**Possible Approaches, Lays and Retreats, female**

Categories; 1F Female Approach, 3F Female Lay, and 7F Female Retreat. And/or
 2F Female Attempted Approach, 5F Female Attempted Lay, and 8F Female
 Attempted Retreat.

(Dot interval, 1 min; total time elapsed, 350 min)

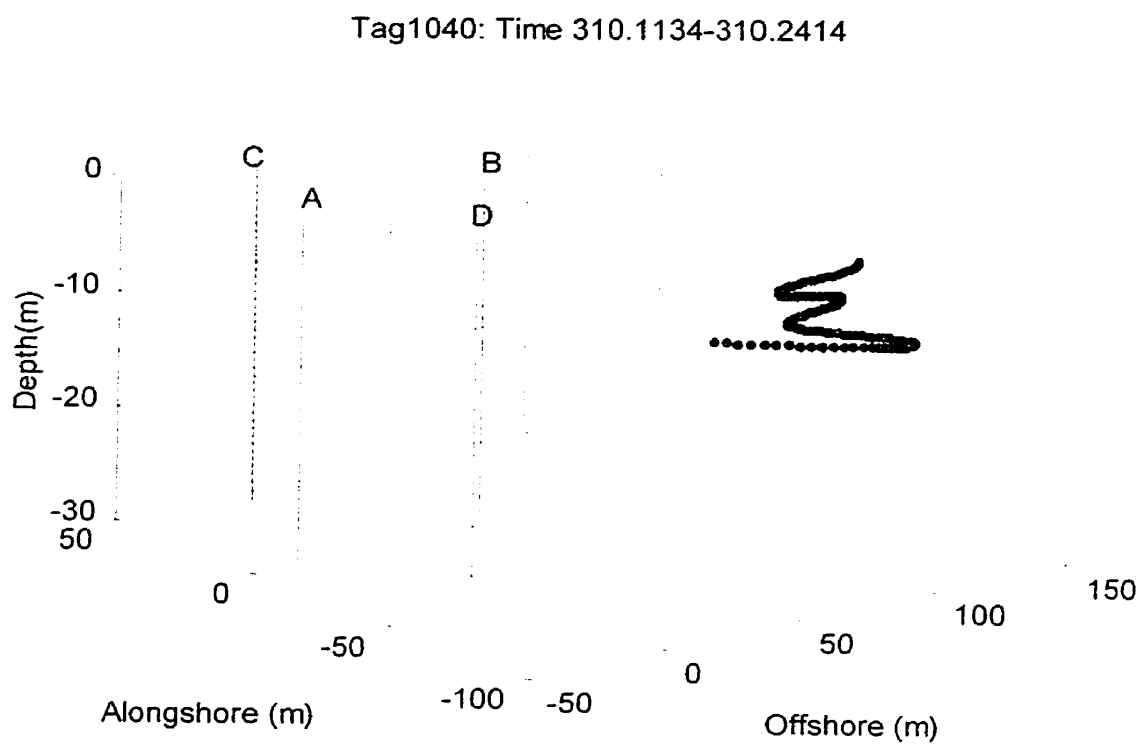
FIGURE 19**Circling ~10m, sneaker male**

Tag1150: Time 310.7052-310.9875



(Dot interval, 1 min; total time elapsed, 405 min)

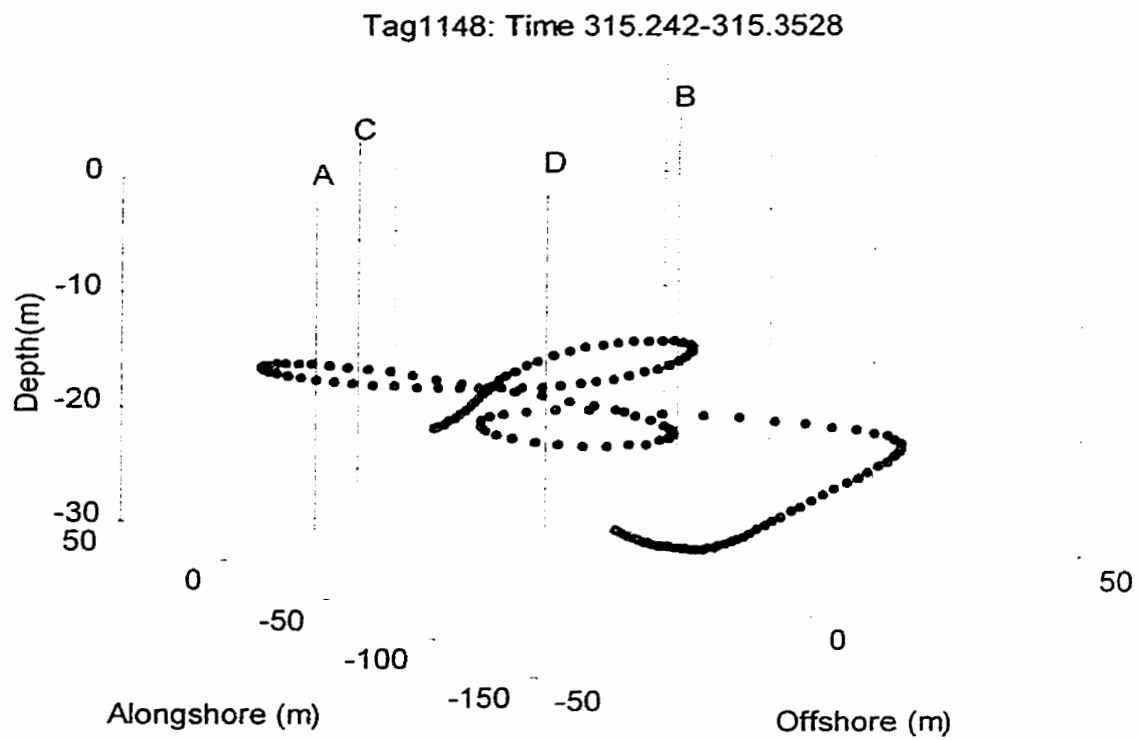
FIGURE 20
Swimming, male



Categories; 10M Male Swimming,

(Dot interval, 1 min; total time elapsed, 180 min)

FIGURE 21
Swimming, female



Categories; 10F Female Swimming,

(Dot interval, 1 min; total time elapsed, 160 min)

Section 2: Speeds of categorized behaviours

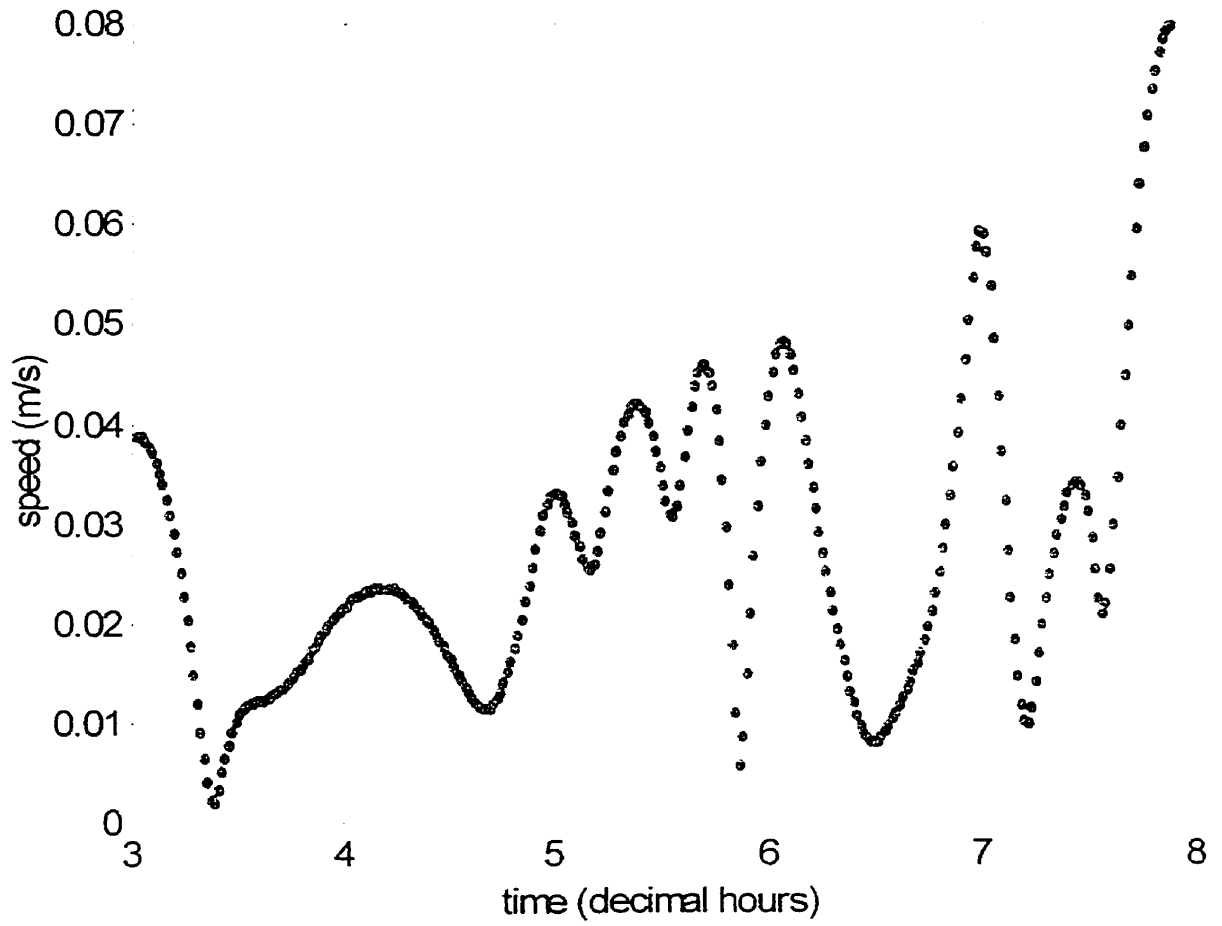
The speeds at which each behaviour was conducted were found after the tracks had been splined hence the results are slower than those for the video analysis as the general movements have been considerably smoothed. The speeds were calculated as distance covered over time taken (see Appendix B for 'speed' program). For each of the points in the splined 3D position graphs a speed was calculated (Figure 22, an example of a speed graph).

Slight changes to the categories were made. Differentiating between attempted versus successful lays was not possible, actions either occurred inside or outside of the array. Specific egg masses had not been located, the array encircled a number of them. This meant that the precise distances from the egg mass were unknown. Actions could only be defined by their patterns. The addition of S for sneaker males was made wherever relevant.

Speeds of selected Behaviours

		Males	Females
1	The approach	2 cm·s ⁻¹	1 cm·s ⁻¹
2	The lay/attachment	n/a	0.5 cm·s ⁻¹
3	The hover during a lay	0.5 cm·s ⁻¹	n/a
4	The retreat after a lay	3 cm·s ⁻¹	3 cm·s ⁻¹
5	Circling clockwise or anticlockwise	10 cm·s ⁻¹ sneakers: 8 cm·s ⁻¹	7 cm·s ⁻¹
6	Fastest Swimming	130 cm·s ⁻¹ sneaker s: 23cm·s ⁻¹	20 cm·s ⁻¹

FIGURE 22
Speed Graph



Section 3: Typical patterns

The continuous monitoring provided by RAPT tags enables the recreation of typical patterns over the entire area for a given time series. A 'general day' for each of the groups (large males, females and sneaker males) was compiled by cross-checking between the different individuals, days, years and beds to get a picture of the entire spawning process. The information from the various subject animals has been pooled together in to their corresponding groups but, each group is considered separately. The day is broken up into two-hour periods, allowing details of the differences between times of day to be portrayed.

- Males often were the first ones there in the morning *or* the last ones there in the evening. If they came in very early they did not stay very late. The males consistently came in early to certain beds *or* stayed late, at others.
- The females did not show up very early nor stay very late, but rather stuck to the sun schedule. Coming in at dawn and leaving around dusk.
- Sneaker males covered more 'ground' than either females or males and made loops that covered more area in their swimming patterns.

NOTE TO USERS

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Microfilmed as received.**

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UMI

A typical day - female

4am - 6am	5:45 females tend to be in range of the hydrophone array.
6am - 8am	Females swim through the array. Detailed loops covering large areas often below the midpoint of the water column (Figure 23).
8am - 10am	The loops get smaller and higher in the water column.
10am - noon	Loops tend to weigh 'heavier' to one side of the array however they are not limited to this area as yet.
noon- 2pm	Females stay on one side of the array or within a particular area, looping within this chosen area (Figure 24).
2pm - 4pm	The now clearly defined one-sided area falls in the water column; it is possible that this time is used to make lays. Figure 25 shows a female's patterns as she makes possible attempts to lay on an egg bed on the edge of the array.
4pm - 6pm	After slow, low swims with possible laying activities the females tend to leave, around dusk.
6pm - 8pm	Most often the females have already departed.
8pm 10pm	No longer at the egg bed except when lights are focused on the spawning bed.
10pm -4am	Gone.

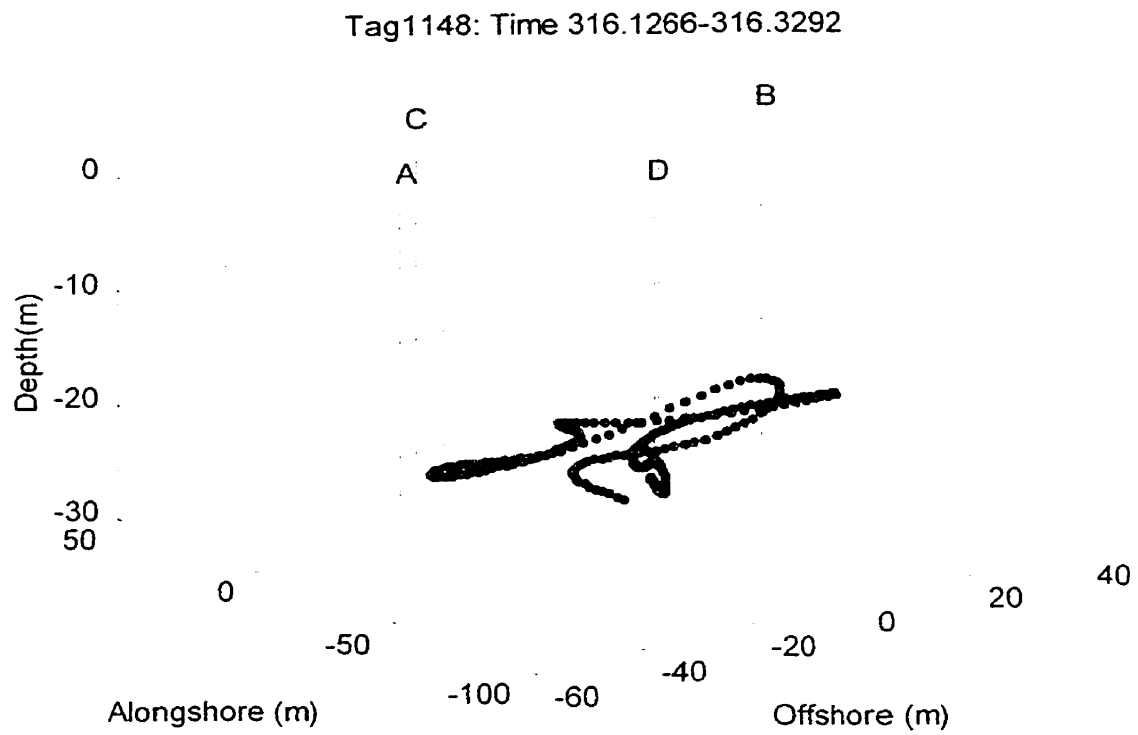
FIGURE 23**A 'swim' through by a female**

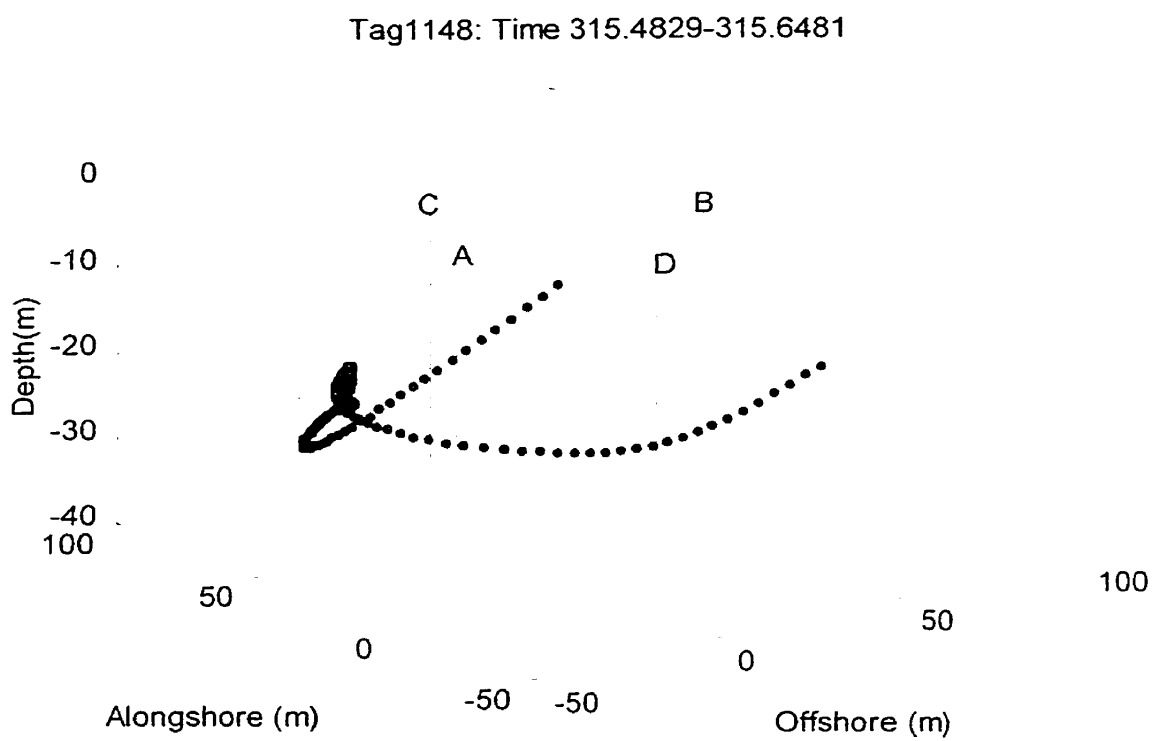
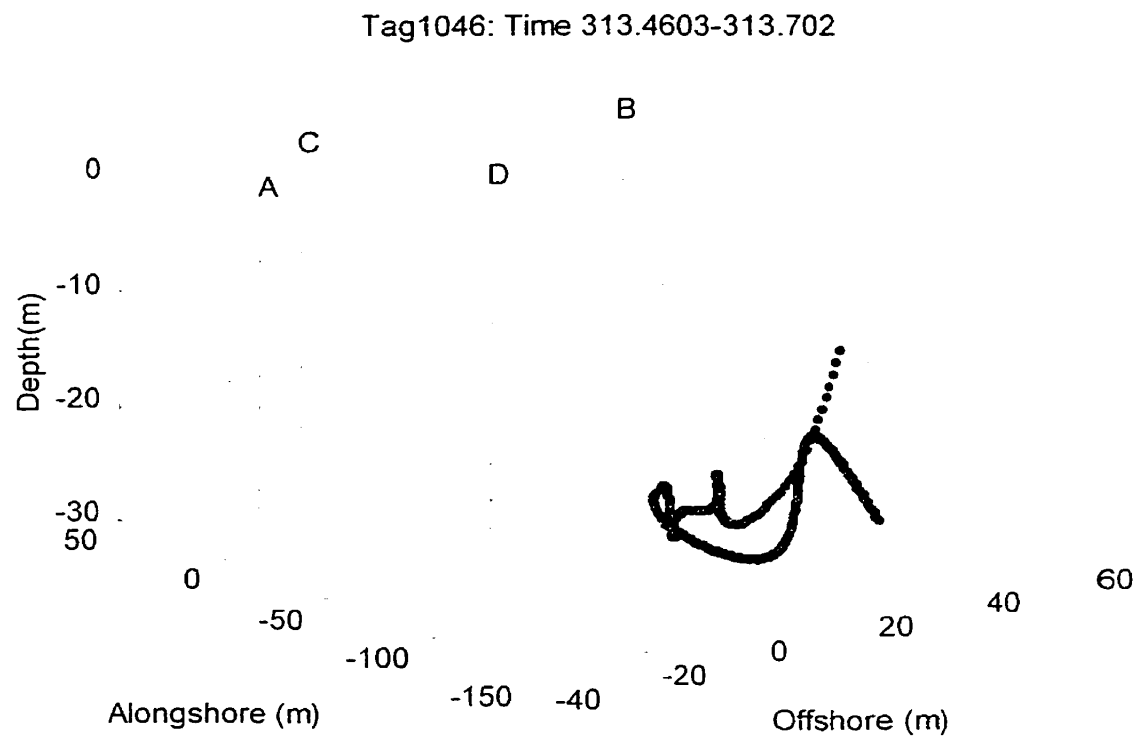
FIGURE 24**One side stationing by a female**

FIGURE 25**Possible 'lays' by a female**

A typical day - sneaker male

4am - 6am	Sneaker males tend to arrive fairly early about 5am. Brief, fast swims over the bed, are the first activity conducted.
6am - 8am	Diagonal passes that go either up or down in the water column as they pass through the array (Figure 26).
8am - 10am	Very intricate patterns, taking up lots of area.
10am - noon	Intricate loops centered within the array covering the entire water column continue (Figure 27).
noon- 2pm	Loops continue.
2pm - 4pm	Loops get lower in the water column.
4pm - 6pm	Circling around the same area as where the females were (often the edge of the array).
6pm - 8pm	Sneaker males stay longer than females but are usually not on the egg bed after dusk, unless the lights are on the array.
8pm 10pm	Gone
10pm -4am	Gone

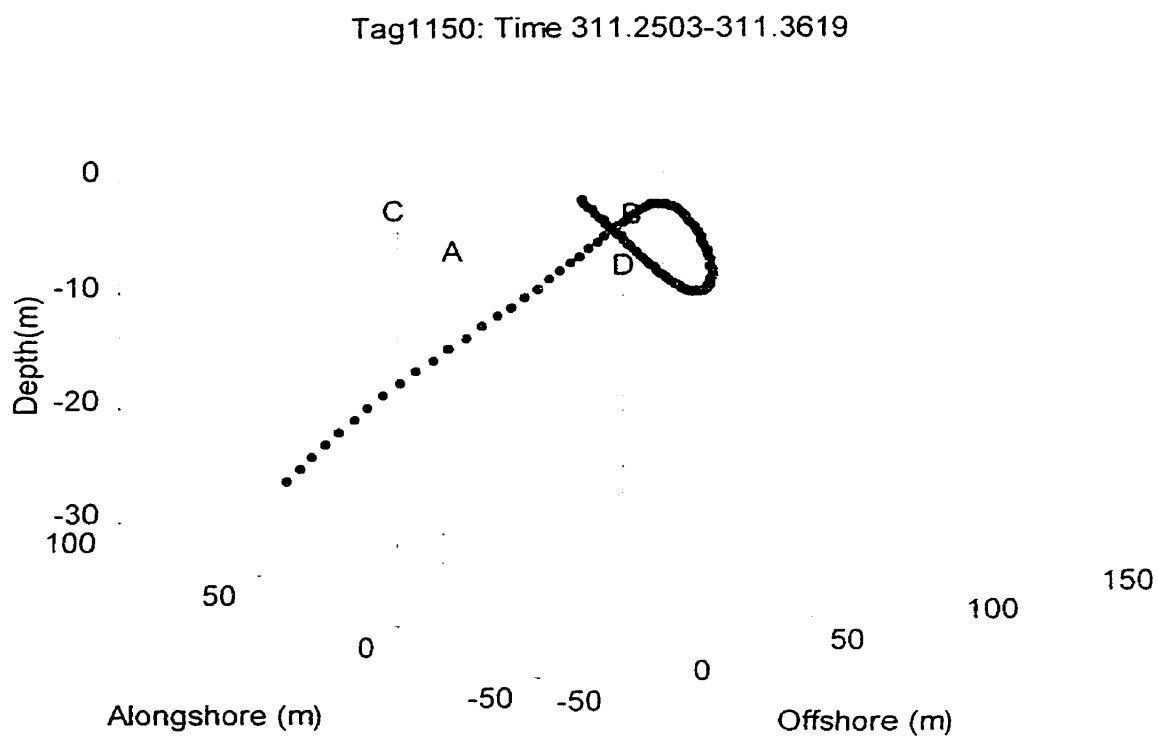
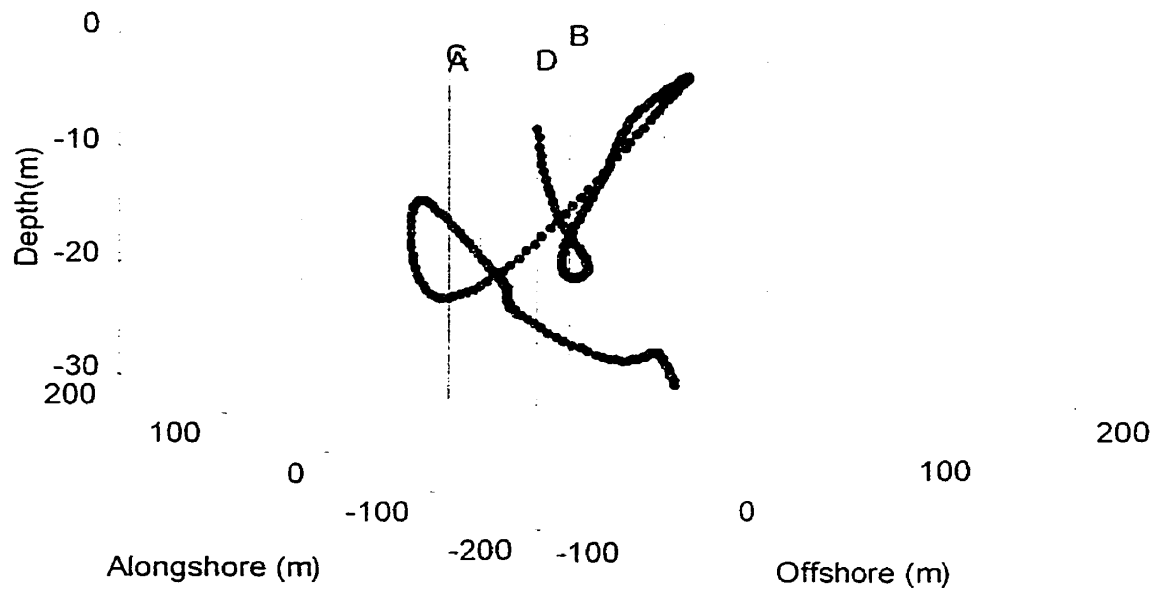
FIGURE 26**A diagonal swim by a sneaker male**

FIGURE 27**Intricate loops by a sneaker male**

Tag1150: Time 310.5256-310.6943



A typical day - large male

4am - 6am	The male often comes in to the morning beds before 4am.
6am - 8am	They usually stay outside of the array until 6/7 am
8am - 10am	About 8/9 the male swims through the array but doesn't actually stay within the array very long. It basically swims though, out and around, not necessarily always in a circle.
10am - noon	Often quite high in the water column the male begins to focus more toward the array for longer periods of time.
Noon- 2pm	Goes down lower in the water column during the afternoon. And begins to loop around a particular area, possibly escorting females.
2pm - 4pm	The afternoon beds are sometimes accessed by 2pm. In this case the male swims through the array but the loops fall in the water column quite quickly.
4pm - 6pm	Low in the water column the male loops around one edge of the array (each male seems to have its own area /edge that it comes back to day after day. But the plan continues to be that they loop and leave (Figure 28). The morning beds are often abandoned by 3pm.
6pm - 8pm	The loops get more intricate as the evening wears on. They occur close to the bottom but often remain on the edge of the array (Figure 29).

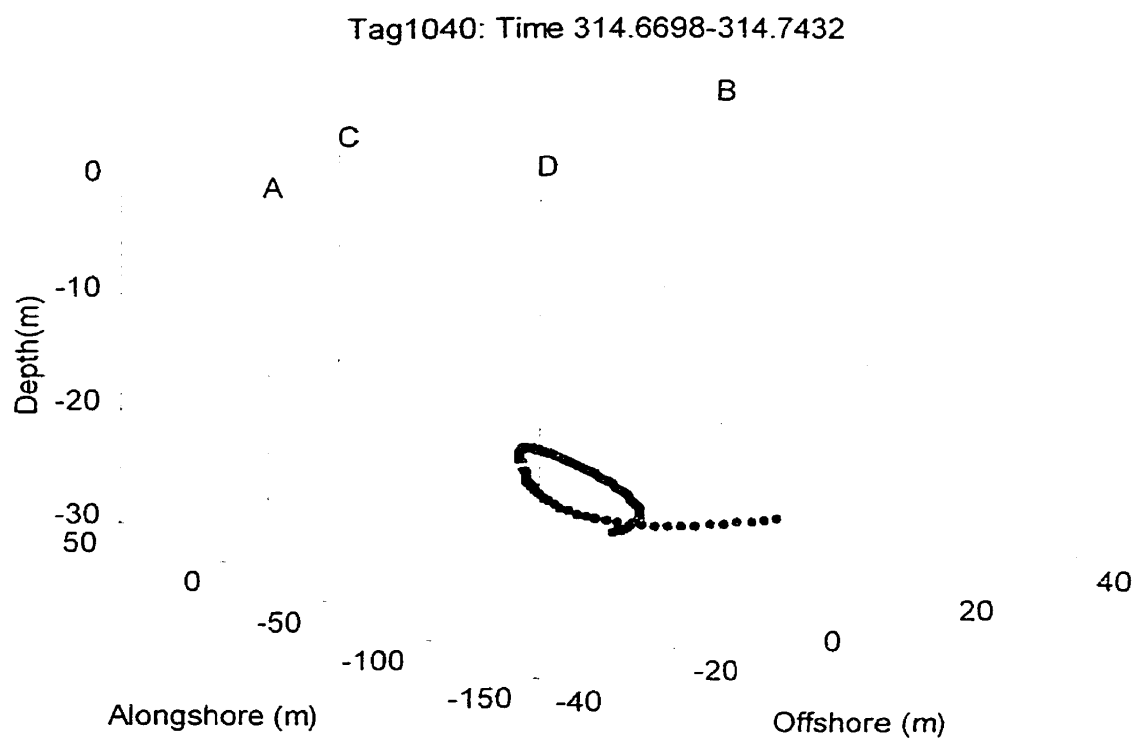
8pm -10pm	Males are often still at the afternoon beds even after dark. Males did not leave the afternoon beds until 10pm.
10pm -4am	Gone

Comments on the Day in the life of a large male

Males do not often stay at the same egg bed all day. They either come in early or stay late. Because of this phenomenon a 'leaving' male was followed with a hand-tracking device. The male swam to another egg bed about 1km away that had also been jigged during the season.

During the comparison between the different egg beds examined over the different years it became apparent that there seem to be some beds that were morning beds and some that were afternoon beds for individual males. Females and sneaker males appeared to remain on the same egg bed all day, but large males visited more than one (egg bed). This behaviour of large males may contribute to the success of the sneakers.

FIGURE 28
A loop by a large male



Section 4: Change over time

Comparisons between the days of monitoring allowed differences to be seen. Hence, the change over time can be recognized.

Females

All the females disappeared right after being tagged, and a minimum of 35 hours passed before they reappeared. The all but one female returned with in hydrophone range with in a 43hour time span. The first day back for every female was extremely shortened; time spent at the egg bed was reduced and some actives were restricted. Loops were not as big and circling was not as fast. Huge circling (and hence pairing?) did not occur. By day three (after tagging) there was no obvious effect in the behaviour patterns.

Sneaker Males

All sneaker males disappeared right after tagging, and a minumum of 25 hours passed before they reappeared. All returned with in 36 hours. Time was not significantly shortened (the average decrease was 2 hours) the first day with in hydrophone range. However, the actions of all of them were generally restricted; loops were not as intricate or as broad or the area covered not as large as they would be later in the experiment.

Large Males

All males disappeared soon after they were tagged (within 2 hours). All returned with in 24 hours. The first day with in hydrophone range their actions paralleled what would later be seen at the bed.

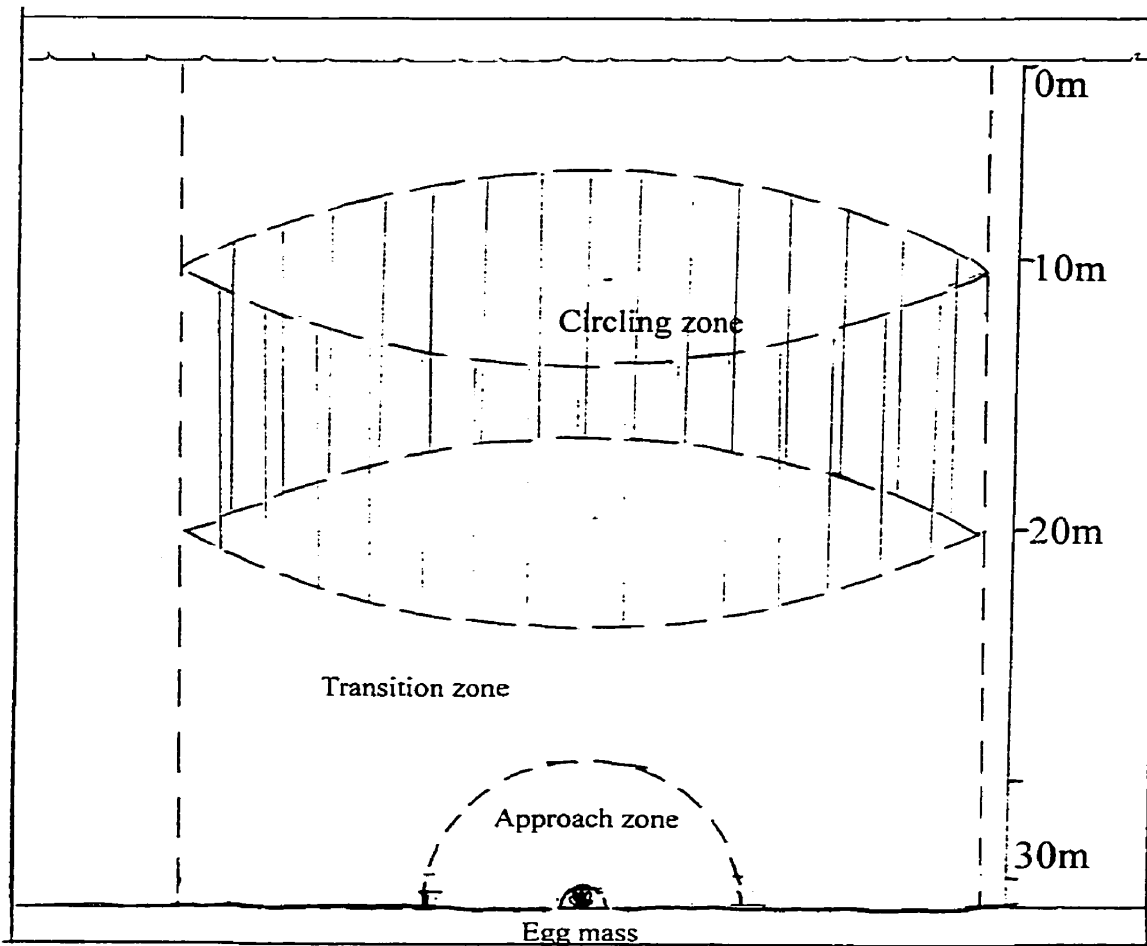
Section Five: General spatial orientation

The goal of this section is to join action over time within space. Displayed below are the figures expressing the conclusion from this perspective.

The graphs produced in MatLab by the data supplied by the RAPT analysis allowed the observation of consistently distinctive behavioural patterns. These patterns were identified and detailed in consideration of the ones found in the Video experiment. However, the biggest improvement offered by RAPT analysis was a quantifying of the spatial orientations. The zones created by this method are entirely subject to the categorization of behaviours. Their limits, names and appearance are established only by the acts performed within them.

On the basis of the portal given in the video section it can be understood that the egg mass after being placed in the center is surrounded by the laying zone. The 'approach' zone continues to appear to be a domed circle with a radius of ~2m. The approach zone is edged by the transition zone, where squid will spend time after dropping down from the height of the circling zone and before attempting to lay. Long looping forays were made through out the immediate vicinity and although not circular the 'circling' area could still be seen ~10 m above the egg mass.

FIGURE 30
General Spatial Orientation with in the array



DISCUSSION

A colloquy on the RAPT results alone is impossible without comparison and relation to the video analysis, for this see Chapter Five. Hence here I find it appropriate to discuss the limitations of this experiment with a RAPT system; its quality and its resolution.

Limitations of this RAPT analysis:

- 1) The lack of data which can be related to:
 - a The continuous time spent at the local.
 - b The low number of tagged subjects.
- 2) The imprecision, caused by topography studies of low resolution

The greatest argument that can be used against this system is the low power of the resulting findings. The fact is that few subjects were tagged for a short period of time over a poorly defined (in terms of egg mass precision) area. The number of subjects was limited by the cost of the individual tags, the number of frequencies that can be tracked and the need to 'time share' between them. During high speed maneuvers only one individual can be effectively tracked. The cost of the transmitters is a factor and will inevitably decrease with greater demand in the market (the general supply and demand concept). However, these studies have contributed to the demand for frequencies.

The short time span that was available for the project's actual physical operation increased the problem of 'low power'. Deployment time also depends on money

available as the skilled personnel used to run the project are not cheap. This could change with long term projects, as continuation time does not require as much manpower as original assembly, although maintaining a charge on the automated buoys is an ongoing problem that will get worse with 'power hungry' digital technology. The date of actual deployment of the buoys was a difficulty here, as continuous monitoring was severely impacted by the time available and the severe weather conditions. This project was also constricted by the period of the closed (conservation) season, which had been set up by the South African government. It thus is simply unfortunate that November brings rather rough seas to the South African coastline.

The limited topography data is less easily excused. Although differential G.P.S., ground surveys and videoing of the area was done, relation to the exact area where the buoys were deployed was very difficult. Relating the video maps and the area of buoy system deployment would have been greatly improved had a video of known coordinates (a video with a transmitter on it) been employed for a few hours on the second and third days after the tag release. Without direct experience (as it has yet to be done) it is hard to appreciate the accuracy required to provide topographic data with resolution equivalent to RAPT.

Noise created both underwater and on the RAPT records was a difficult issue to deal with. Underwater noise created by the mooring chains may well have been the reason that the 'centered' egg mass was passed up in the preference for the one toward the edge of the array. This meant that the clarity or precision available for reconstruction was lessened. This could be improved in future

experiments by decreasing the size of the transmitters further. This decrease in transmitter size would enable the smaller subjects to carry depth recorders. If subjects supply depth, it would remove the need to set the buoys as close to each other as this is only done to provide 3D coordinates from the ping arrival times. If the need for the equal tetrahedral array was removed then the full range capacity of hydrophones could be used, hence they could be set up 300m away instead of 45m!

Noise or errors recorded by the RAPT system itself were due to various issues:

1	Air bubbles in the water.
2	The use of transmitters with close wavelengths.
3	'No solution' spots in the array.
4	Unknown (as yet) factors.

Air bubbles absorb sound, can never be completely removed from the water and increase with rough waters. However, the precaution of minimizing propeller-driven boats is wise. The extra churning increases the insulating effect of the water. 'Bubble walls' are virtually impossible to pass pings through.

Restrictions placed on wavelengths used, creates the problem of having to release transmitters with very similar wavelengths. The problem with this is that they can be mistaken during recording. Hydrophones will independently increase their 'listening' gain when no signal is heard, making it possible to mistake one transmitter for another. Even if this is done briefly it causes flaws in the record.

The tetrahedral array is not perfect and there are errors in recording due to the capabilities of the hydrophones. The blind spots behind each buoy are difficult to resolve and have anisotropic error characteristics. However, the use of more buoys and switching between hydrophone triangles may reduce this error, even if the smaller depth transducers are not forthcoming.

Issues that arise beyond the planned or foreseen will always affect *in situ* behaviour studies. Scientists must simply continue to forge new paths and learn to work with ever improving technologies.

Post-hoc analysis inevitably depends on the ability to gather as much clear data as possible. Great amounts of data were gathered throughout this experiment. The post-hoc analysis benefited by this greatly as questions could be brought back to the original arrival times. The choice to spline the data resulted in smooth curves that represented general patterns. This averaging meant that typical gross body movements could be seen and interpreted. However, this also meant that representative speed records were decreased. After the small side movements are removed and smoothed representations are left, speeds appear to be much lower than before. Before video analysis, it was unclear whether high average speeds were real or a product of accumulating errors. This is unfortunate but unavoidable in this analysis. Future thought on the use of more of the original data will improve this problem.

CHAPTER 5 – DISCUSSION

The intention of this thesis was to detect behaviour from RAPT system read outs, and in doing so clarify the limits and strengths of data created by RAPT systems. A summarized plan would have been:

- I. Resolve gross body movement patterns
- II. Suggest biological interpretations of them
- III. Present their organization over space and time, with relation to speed
- IV. Discover truths about RAPT systems and the South African coastal egg beds

The first was accomplished despite the difficulties found with each method, as comparisons and relations between the two enabled a clearer picture to be drawn. The concern with the video analysis was that the area the video was able to cover clearly was rather small. Thus the impression given when analyzing the film was often skewed. What seemed in the video to be 'proper circles' (circling) ended up to be looping forays, and where the squid were thought to be in the water column was made clear by the z coordinates given by the RAPT analysis. The change in perspective can be seen in the comparison of the general orientation figures of each method.

The RAPT allowed a clearer picture to be drawn as a larger zone was seen. Hence, a less biased reading of the general occurrences could be made. However, the distinctions that had been made in the video analysis of exact

movement, biological interpretation, were lost due to the imprecision's caused by the inability to specify the locations of the egg beds when using the RAPT system. It was the detailing of the gross body movements by the 2D graphs of the video analysis that enabled the clarification of this on the 3D graphs of the RAPT analysis.

Grouping the two methods together enabled the presentation of the patterns organization over space and time. The relations in speed support the idea that squid motion includes irregular reversals that yield higher average speeds than are seen in highly smoothed trajectories.

When normal behaviour is related to the behaviour found by the RAPT system it is clear that the tag disrupts the subjects for a limited amount of time. The disruptions pass; for the large males within a day, the sneakers within two days and the females within three days. Decline of activity over a longer period of time was not detected.

The various reasons for the difference in reaction to tagging and the tag's can be understood by the difference in the size and physical states of the groups. The large males quick recovery from the tagging experience implies that the act of tagging is not a contentious issue. The ease with which the large males return to their normal behavior is most likely due to the fact the tag weighs so little compared to the total body weight. This low percentage of body weight is something to be aimed for in future studies. The sneaker males have more to contend with, as the body weight percentage of the tag

is higher than it is for the large males. This furthers the assumption that the tag weight should be kept down as the sneakers take longer to return to normal behavior after tagging. The females do not only have the extra weight to contend with (similar to the sneaker males), but the presence of the tag amongst eggs in a gravid mantle. The thought that the tag may be more a problem for a female with a mantle full of eggs is not difficult to conceptualize.

The tag effect can be dealt with by simply not using the first few days of data. However the possible affect of the RAPT system must be dealt with more directly. Noise and disruption, primary possibilities to the behaviour seen consistently to the side of the array should be decreased by any means possible. Changes in anchoring material, for example bungee cord anchors such as O'Dor and Jackson are currently testing in Australia, and increasing the distance between buoys by using depth tags. Buoy distance is limited by the need to record the z coordinate. Until the large depth tag design allows a decrease in size, they can only be attached to large squid.

The last point of discussion needs to be about the unique findings of this experiment, which include female's independence when 'laying' and the different allocations of time spent by each group inshore. Gross body patterns have illuminated the mistakes of previous researchers that assumed *Loligo vulgaris reynaudii* to be like other spawning squid. The truth of the matter can be seen in the visual construction of the organization within the approach zone (Chapter 6).

The time spent at the egg bed is significantly different between the three groups. It is notable that the males spend longer than the females in general and that the larger males separate their time between beds. The males extended length of time may be due the increase in possible mating opportunities. Something that the females do not have to be as concerned with as each of them will find some method (see discussion of video analysis for spermatophore sources) to fertilize their eggs. The sneaker males' persistence at one bed may be due to their small size and hence their energy gain relation; This may imply that they get a greater benefit by not wasting time traveling, as cost of transport decreases with animal size (O'Dor and Webber, 1980). The "either/or" situation with the morning/afternoon split by large males may imply just that: morning and afternoon beds for large males. As males are 'only' escorts they have more energy to expend trying harder and diversifying their possibilities. As the large males are able to swim fast and without as great an energy loss as the sneaker males this diversification could outweigh the time lost during travel. Females may have no need to diversify between beds as eggs can be laid anywhere, although some beds may have higher probabilities of hatching. Tag and release studies have shown long-range movements by spawning females (Sauer *et al.* 1998).

CHAPTER 6 - EGG BED OBSERVATIONS

The 1957 figure by Hamabe and Shimizu entitled *Mating Groups and Spawning Bed - Loliginidae* (Figure 1, page 13) shows squid circling a suggested egg mass. The visual interpretation of general spatial orientation observed on and around the *Loligo vulgaris reynaudii* egg beds off the South Eastern Cape of South Africa, given below is quite similar but includes the improved detail confirmed by RAPT.

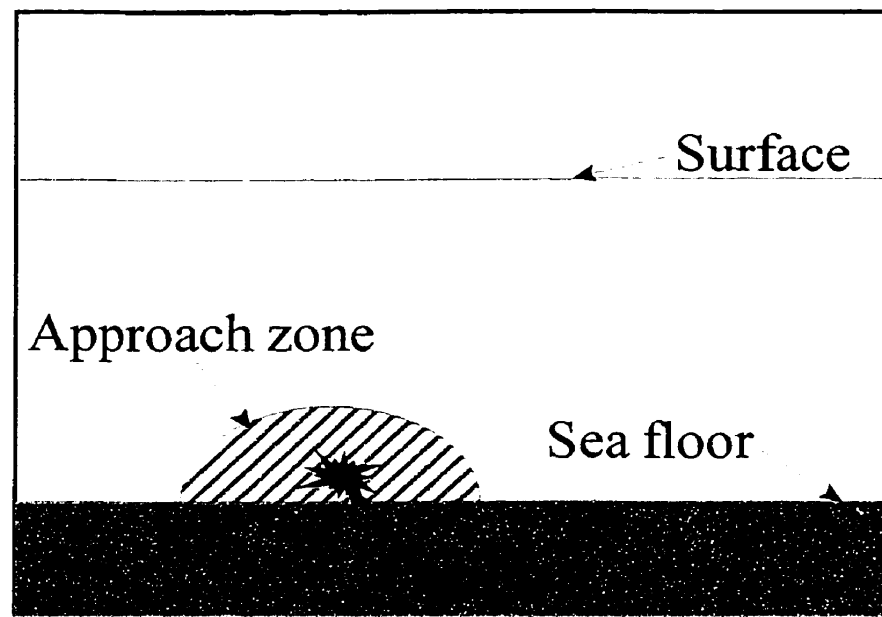
Tagged squid consistently appeared near the array at dawn, arriving from offshore where they spend the night (One large male was tracked manually 2km offshore where it remained immobile until dawn). After arriving inshore all squid 'run through' the egg bed area. Sneaker males made very distinctive diagonal passes. Females spent a bit more time doing these initial runs than the males did, but all three groups participated in these 'scouting' actions. Swimming outside the array did not commonly include entire circles of the array, (although sections seen by video made it appear so). The area covered by the scouting actions begins to narrow as the day progresses. All squid seem to concentrate within the area directly above their chosen egg masses. They cover less area and occur lower in the water column.

Females come in at sunrise and stay until dusk, the small slow movements made within the array were presumed to be egg depositions, higher forays

may well have been mating events. Sneakers also stayed near egg patches all day and sometimes into the evening, maximizing mating opportunities. They tended to cover the array's interior quite well with long sloping transects. Large males either came in very early and left by 1400h or came in late and stayed until much after dark. Different beds seemed to elicit different patterns.

The following clips follow the basic outline:

Figure 31
The Basic Outline

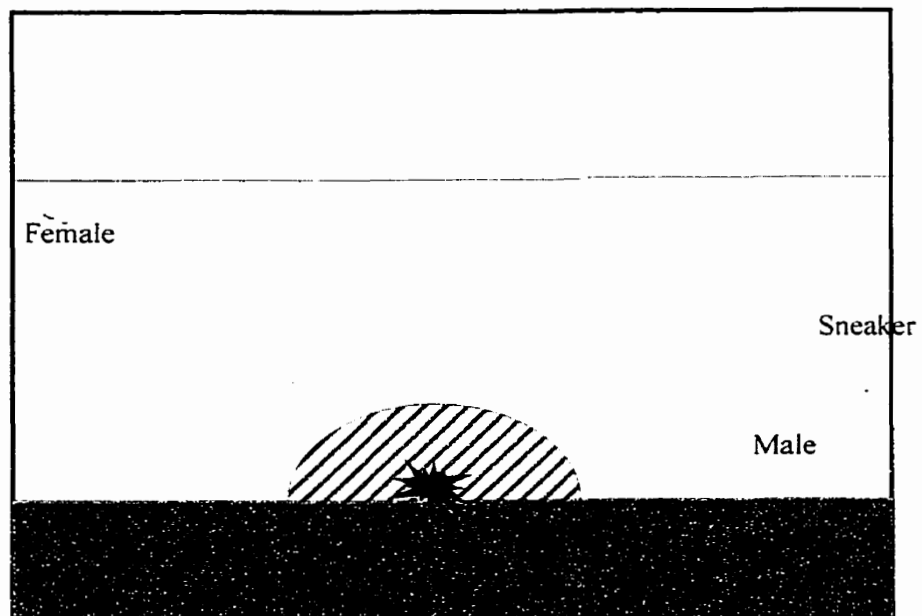


TIME: 2 hours

Scale 1--1m--1

Figure 32
4-6am

Sneaker males arrive and run diagonally through the array
Males appear at morning bed but don't travel through
Female are with in hydrophone range

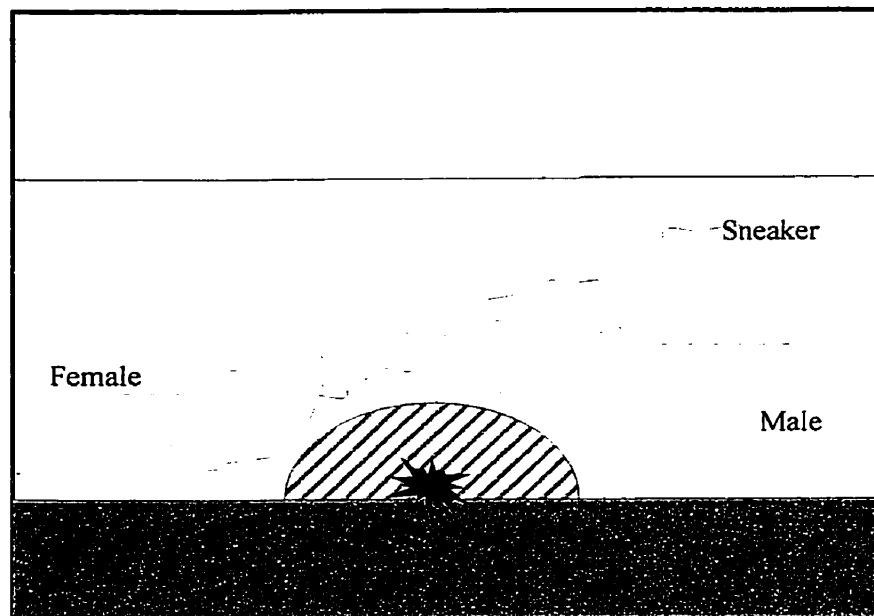


TIME:4-6

Scale 1-- -1

Figure 33
6-8am

Sneaker males continue to run diagonally through the array
Males begin to travel through the area
Female start to scout out the area

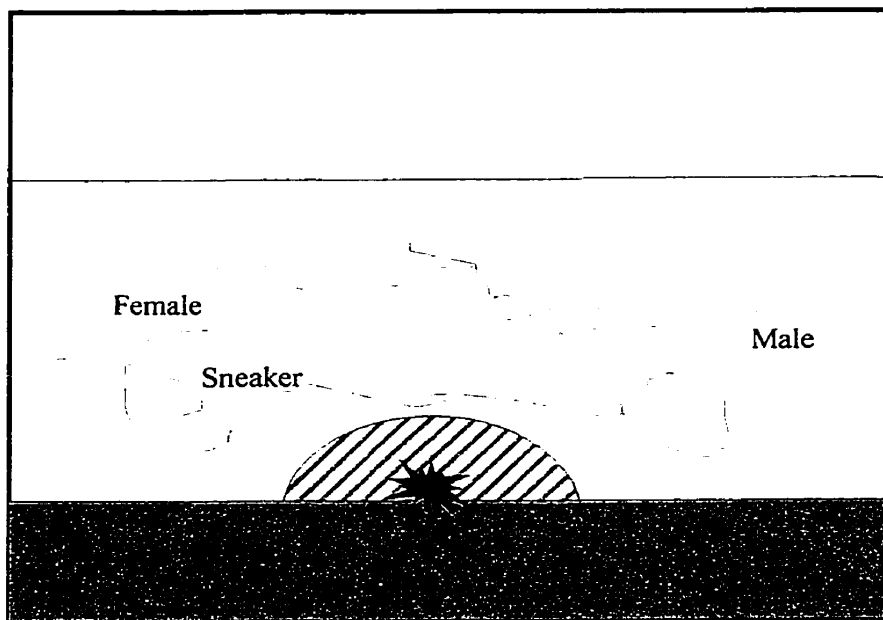


TIME:6 - 8

Scale I— —I

Figure 34
8 -10am

Sneaker males add more loops to their runs
Males travel through the entire area
Female continue to scout out the area

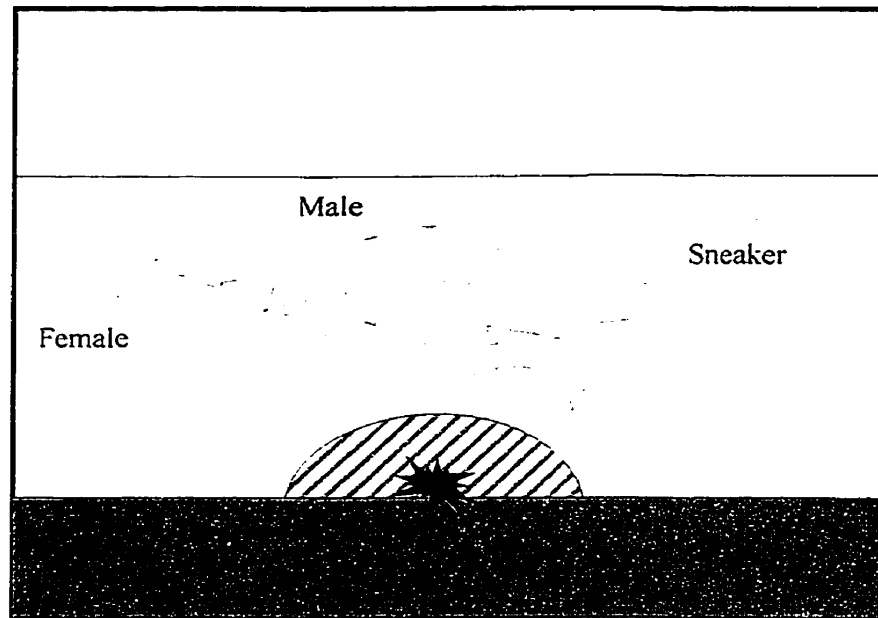


TIME:8 -10

Scale I-- --I

Figure 35
10-12am

Sneaker males concentrate near the females water column mark
Males start to circle in the same area as the females
Female make large circular runs high up in the water column

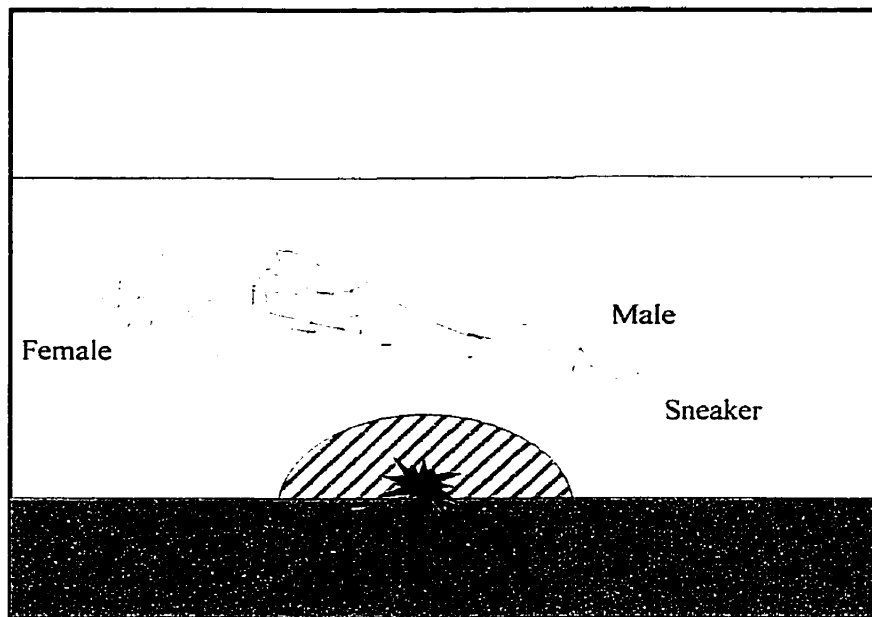


TIME:10-12

Scale I-- --I

Figure 36
12-2pm

Ranges of all groups begin to decrease
Males arrive at afternoon beds

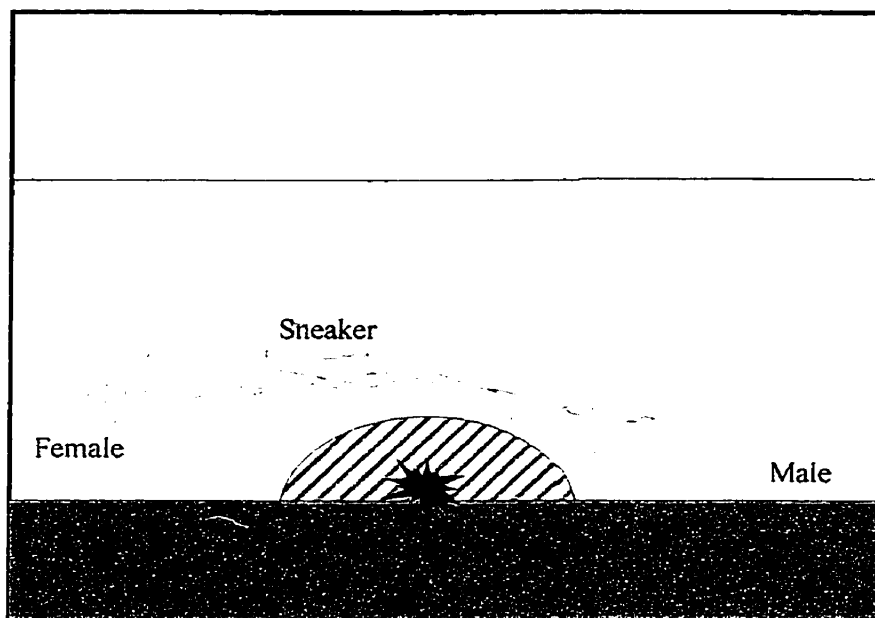


TIME:12-2

Scale I-- -I

Figure 37
2-4pm

The patterns fall in the water column, entering the transition zone
Males leave morning beds

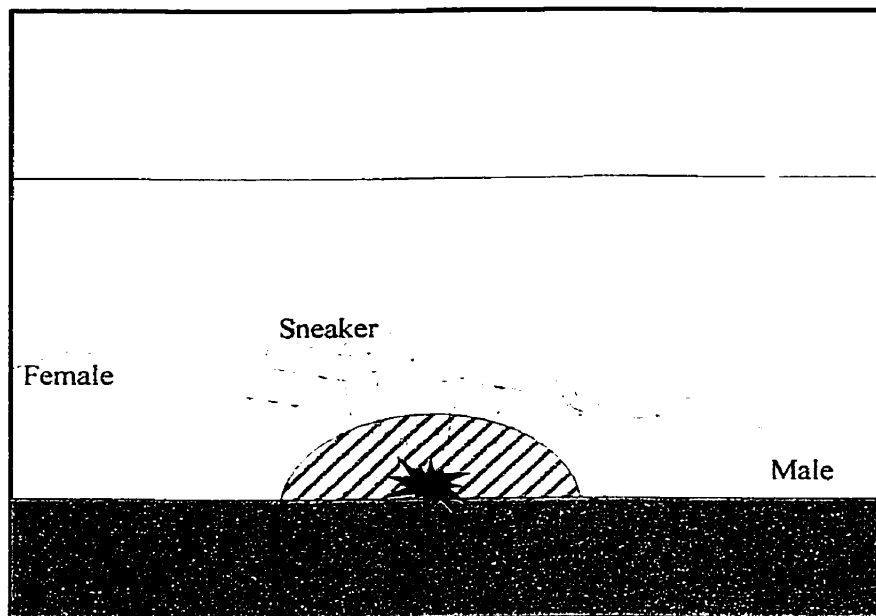


TIME:2-4

Scale I- -I

Figure 38
4-6pm

Concentration around the egg masses by all three groups
Females leave

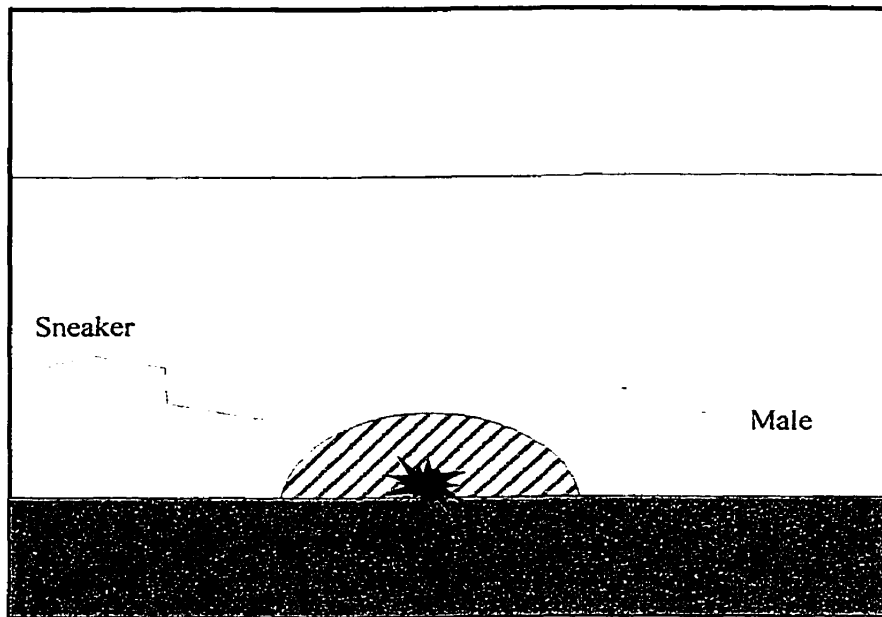


TIME:4-6

Scale I-- -I

Figure 39
6-8pm

Sneakers leave

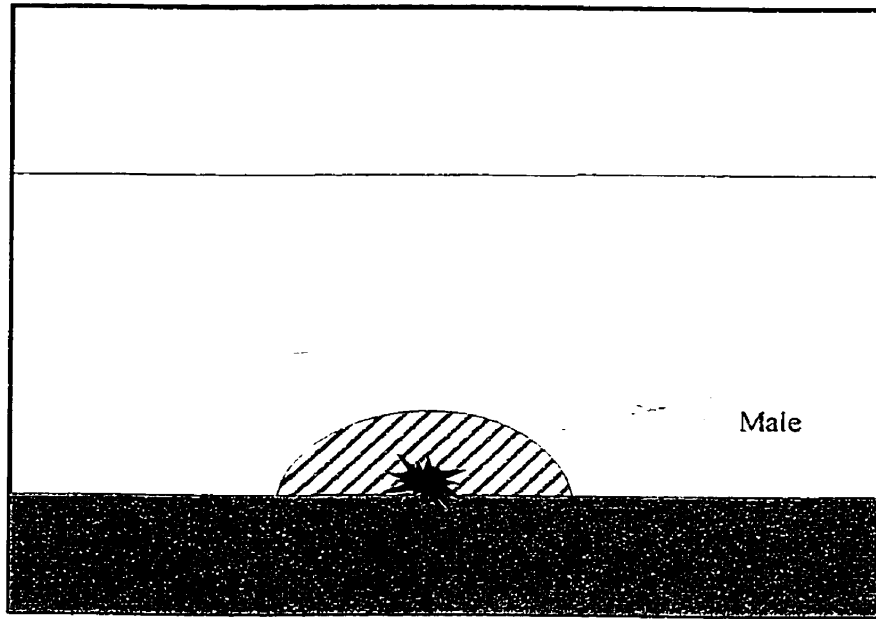


TIME:6-8

Scale I-- --I

Figure 40
8-10pm

Males leave afternoon beds

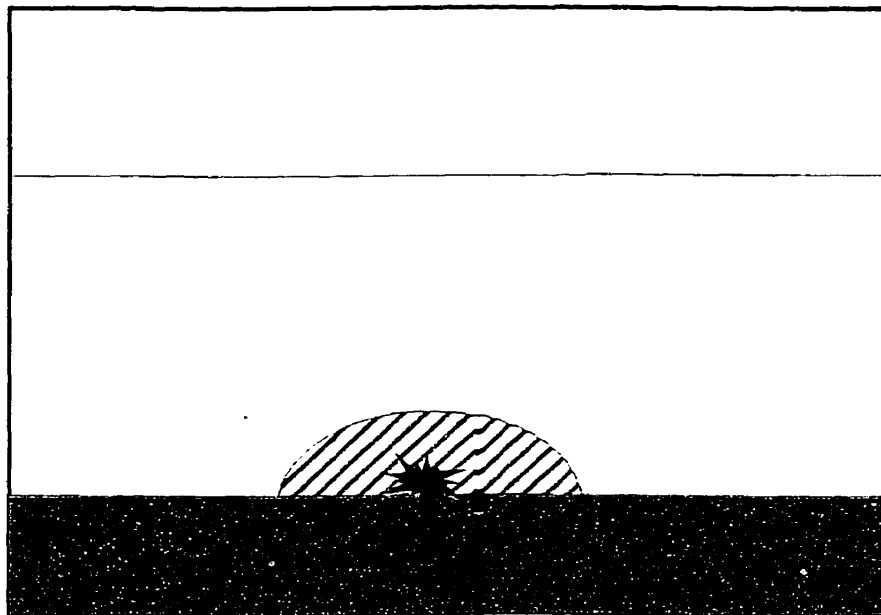


TIME:8-10

Scale I— —I

Figure 41
10pm-4am

No activity



TIME:10-4

Scale I- -I

CHAPTER 7 - SUMMARY OF CONCLUSIONS

The thesis began with two main questions:

QUESTION 1: Does Tagging for Radio Acoustic Positioning Telemetry Change Spawning Behaviour of Chokka Squid, *Loligo Vulgaris Reynaudii*?

ANSWER: Yes.

QUESTION 2: How (how much, how long) does tagging affect behaviour?

ANSWER: Males - One day, Sneakers - Two days, Females - Three days; essentially in proportion to squid/tag weight ratio. However, after a few days delay the tagged subjects behaviour stabilized.

Answering the above questions the thesis is complete, but there were also exiting new and unexpected findings beyond those previously reported (Sauer *et al.* 1997; Hanlon 1996), including the following clarifications:

1. Both video and RAPT show that, unlike the females, the males do not enter the laying zone. (Fig. 4a-i & 11)
2. Analysis of multiple egg beds in 1997 clearly showed that male visits to different egg beds showed similar behaviours but occurred with different temporal patterns.
3. RAPT confirmation that small sexually mature males exhibit distinctive behaviours, consistent with earlier video observations (Hanlon, 1996).

Thus data from RAPT readouts can be used to construct identifiable behaviours, although improvements can still be made. The categorization of gross body movements, both the method and its results will be available for use in future studies, thereby saving time and enabling the biology of the subjects to be understood more clearly and on a new scale. The approach of validating RAPT results with gross body movement analysis of video is a powerful one, although in this first application there was only limited overlap between the scales of RAPT and video. Future validation studies should use wider-angle video from a fixed platform and, if possible include two views that could be used to reconstruct movements in 3D.

The clarification provided by this study of behaviour change is the first based on behavioural tracking studies. The conclusion that after a few days delay behaviour can in fact be considered normal with confidence from the RAPT system readouts allows both researchers and government officials who have born the cost of experiments like this one to breathe a sigh of relief. The suggestions made for circumventing the more obvious limitations of the RAPT system (increased distance of buoys, decreased size of tags) along with the obvious benefits of using combinations of methods and their resultant comparisons will contribute to ongoing and future research. Benefits can already be seen in modeling experiments in: South Africa (Roberts, 1998), currently using a combination of *in situ* video assessments with tracking by RAPT and Australia (O'Dor and Jackson, unpublished) using new buoy anchors and larger arrays.

Appendix- Filtering Strategy

There is considerable distortion of the data as they originally appear in real time caused by small errors in arrival times resulting from echoes and channeling of sound through water layers of different temperature. These can still give solutions to the system of quadratic equations solved to calculate positions (see Figure 42 for a graphic example). When tracking slow moving animals such data can be eliminated by standard statistical approaches with out significant loss of information. When tracking fast moving animals, such as squid it is important to avoid throwing out large movements that may be real. So new approaches were needed. The data from RAPT is both non-periodic and anisotropic, so no standard approach could filter errors from the noisy data to create a sampled trajectory in space (3D). Although the data was contaminated with biologically impossible outliers techniques were required that used as much of the original data as possible. This resulted in the creation of a unique filtering strategy. Each of the hurdles was taken as follows:

Non-periodic sampling rates

The difficulty was that continuous monitoring did not result in continuous data points. The hydrophones can only listen to one frequency at time and must take time out from listening to transmit the radio signals to the base station. This creates gaps and sets up a rotation. The hydrophones cycle through the transmitters frequencies. As a further complication, the option of

which transmitters to listen for is under the control of the land based operator. Selection of only a few available transmitters decreases the gathering of presence and time of day data; however, the more transmitters being listened for the larger the blanks in each pinger's record. These blanks with no data must be connected in order to present a continuous graph. This aperiodic sampling rate precluded the use of standard time series analysis techniques to process the data.

Unrealistic data

Transmitters emit pings, although not in the range to bother squid statolith hearing mechanism. It can be hidden behind large objects, lost or muffed by numerous obstructions on its way to the hydrophones. Or it can be cut off from a particular hydrophone by insulating bubbles either in its path or surrounding the hydrophone. These underwater noises (echoing and ricocheting pings) meant that some data points appeared above the water line or below the ocean floor. These were clearly unrealistic and were excluded by defining the water surface and bottom as parameters.

Unrecoverable data

The tight (45m) tetrahedral array allowed the resolving of depth (z positions), which was essential to movement through space analysis, as the small size of *Loligo vulgaris renaudii* females and sneaker males precluded the use of depth tags. However, the tetrahedral array is still limited. No

solutions were found behind or just in front of the buoys. In 3D space this meant that a cone-shaped funnel behind the hydrophones was a zone of unresolvable data and another source of missing data.

Errors in precision and accuracy

The points in the array were resolvable with a certain error factor, as measurement errors were unavoidable. The problem came with the inconsistency of these errors. The tetrahedral arrangement meant that the errors were anisotropic, different in different directions and at different distances. In the center on the tetrahedral array the error is relatively spherical with an ~ 1 m diameter. As the squid moved from the center point the error extends ovally growing to include errors with the long diameter of half the distance away from buoy set up (e.g. the error has a 20 m main axis forty meters away from the array).

The lack of precision of each point meant that an interpolated graph did not work well; the data points were not perfect, so drawing lines straight through them was not an ideal solution. (See Figure 43 for the difference between interpolation and approximation).

The degree of approximation becomes a factor of biological interpretation. Giving an approximation parameter P , when $p=1$ there is a straight line drawn through the medians given by the data points, when $p=0$ an

interpolating curve that goes through the center of every point in drawn.

Biologically feasible parameters for squid found from working with numerous data sources are usually in the order of $p=1-10^{-8}$.

The smoothing option of the approximating cubic spline was used so that the overshooting of the approximation polynomial could be avoided (see Figure 44 polynomials vs. splines).

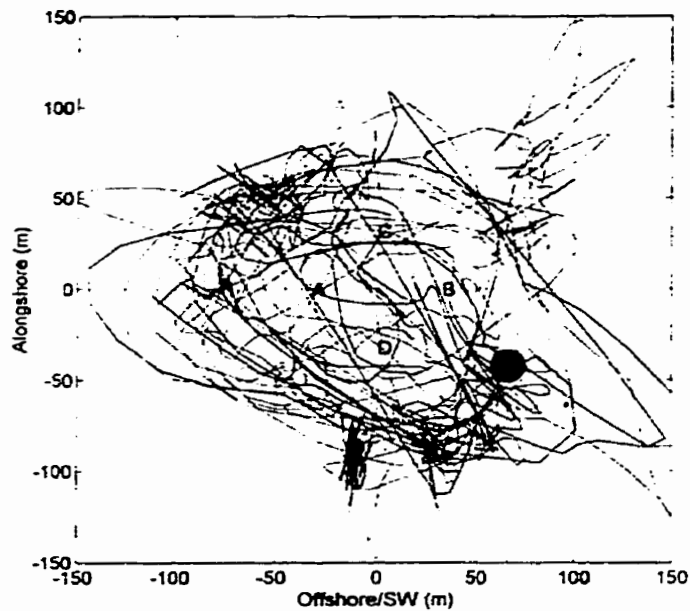
Method of arriving at the final spline however had to take in to consideration the anisotropic error points, which was accomplished by an iterative (repetitive) process of assigning weights to the data points to confirm importance. Distant data points or biologically impossible data points (that would have required speeds unattainable by squid) were eliminated in this manner. The data points were all assigned weights of 1, an approximating cubic spline was then calculated and drawn (by MatLab). The distance between the spline and each point was calculated and its inverse was assigned as a weight, hence the farther away the point from the possible line the less weight it was given. The curve was then re-drawn with these newly assigned weights and the distance between the new curve and the points was drawn. This process was repeated until the curve didn't move (rather its movements were within a given parameter). See Figure 45 the iterative process.

It was this splined trajectory that was reassigned time (t) values that were separated by 1 sec intervals to provide the dotted graphs seen in the results section.

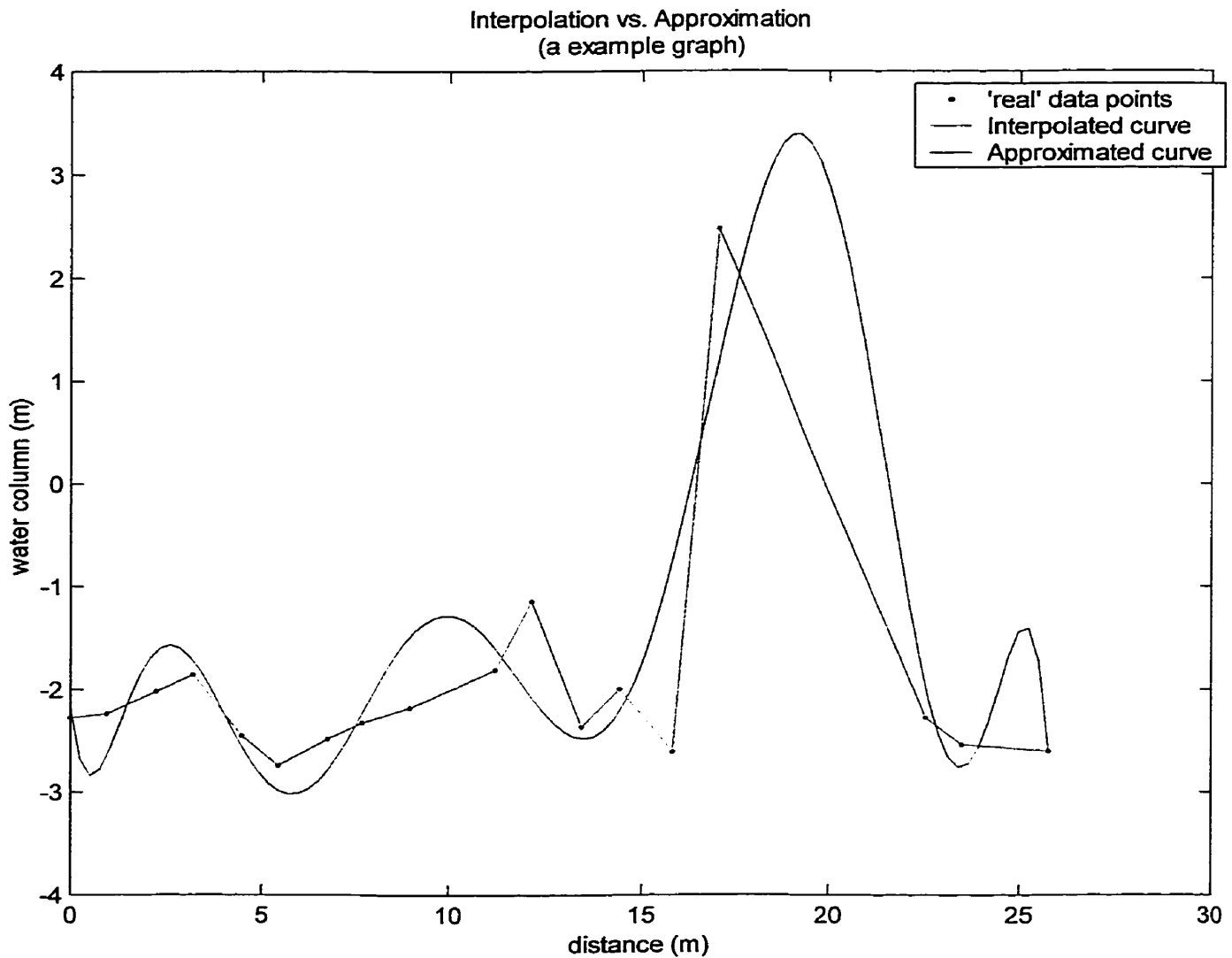
It was recognized that this process left a considerable degree of interpretation of what was a reasonable trajectory for a squid to the operator, and the approach of comparing RAPT and video was developed to show that splined tracks were biologically meaningful.

Figure 42**A 'real-time' graph**

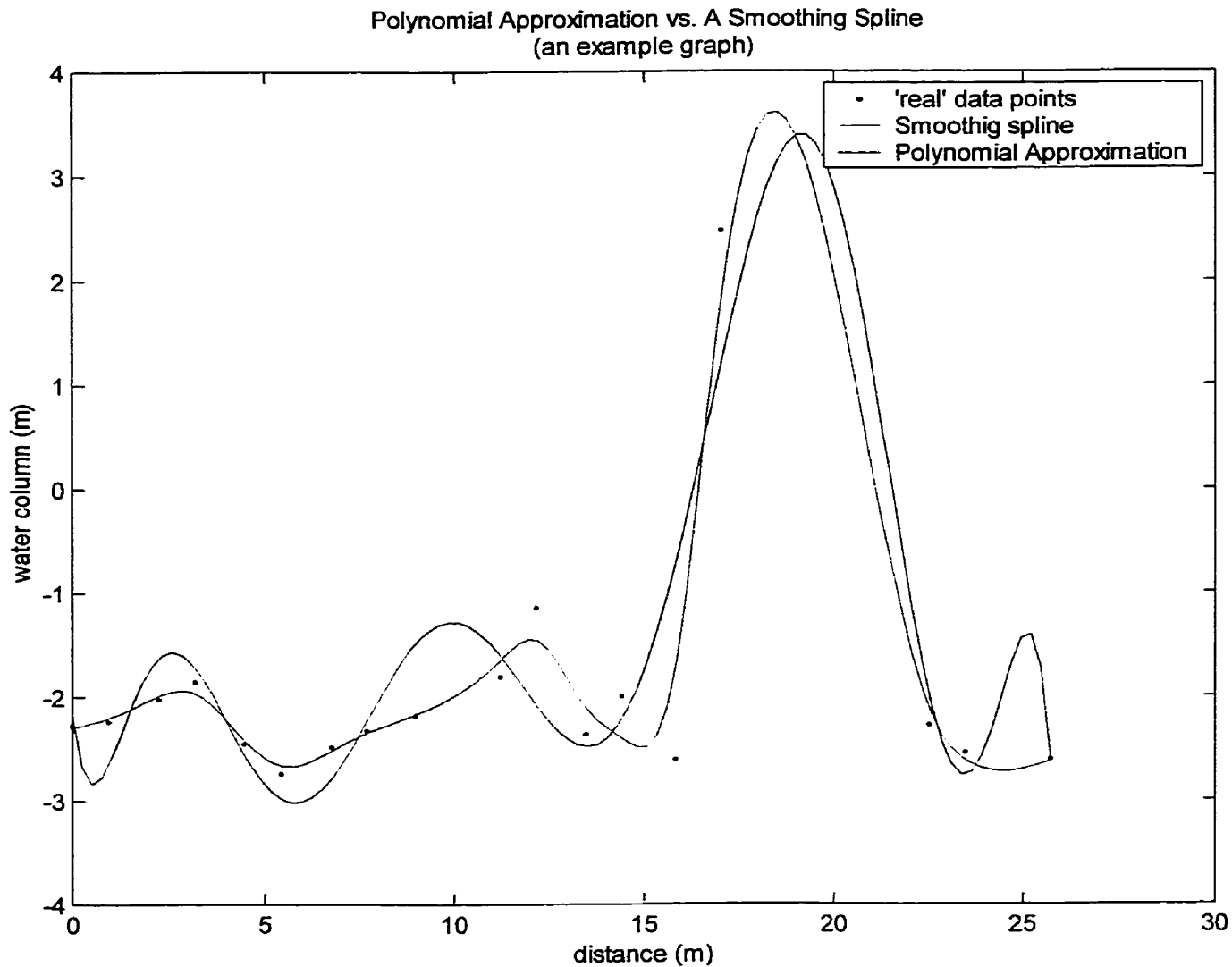
Taken from the 1994 data set



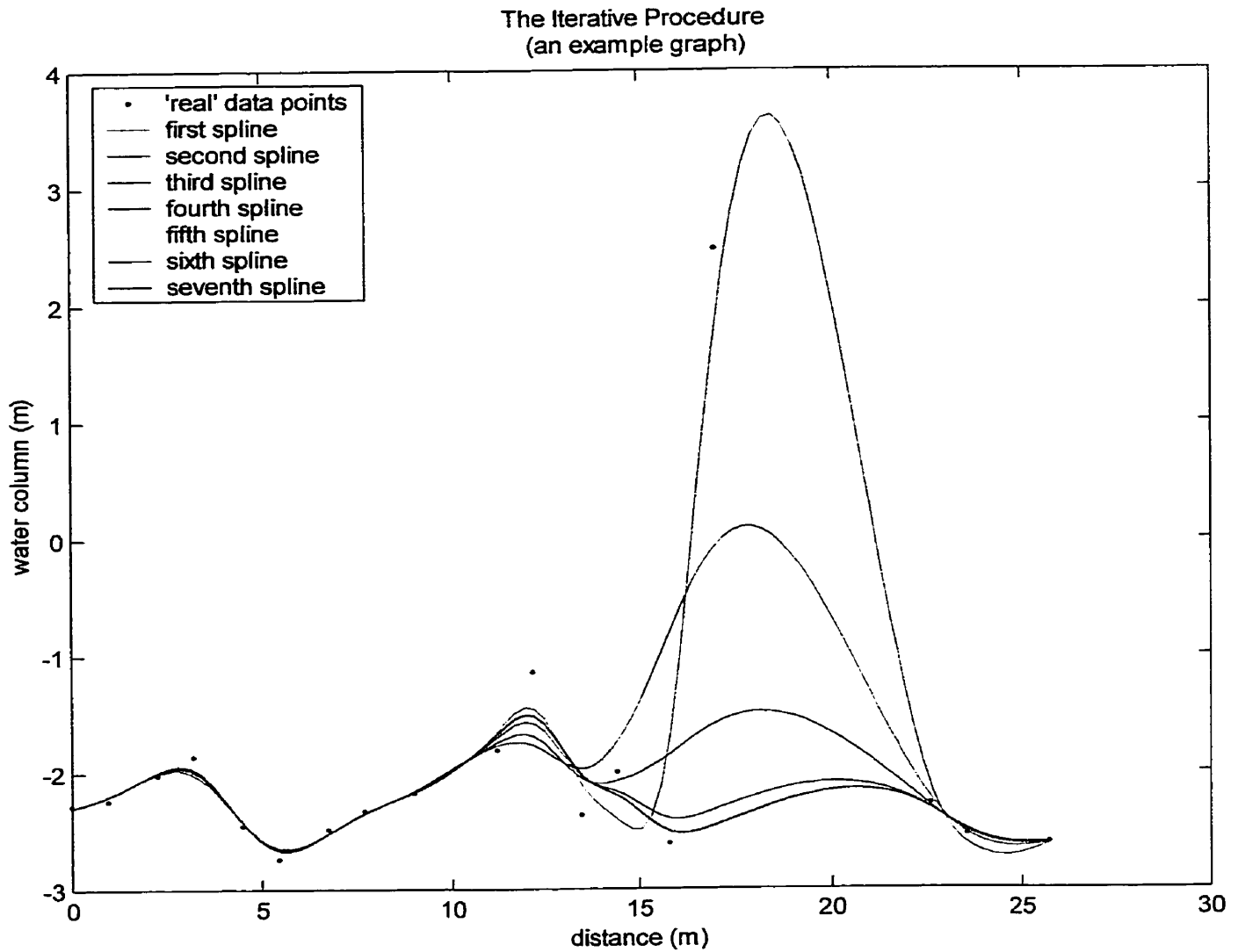
There is considerable distortion of the data as they originally appear in real time caused by small errors in arrival times resulting from echoes and channeling of sound through water layers of different temperature. These can still give solutions to the system of quadratic equations solved to calculate positions.

Figure 43**The difference between interpolation and approximation**

The lack of precision of each point meant that an interpolated graph did not work well; the data points were not perfect, so drawing lines straight through them was not an ideal solution.

Figure 44**The difference between Polynomials and Splines**

The smoothing option of the approximating cubic spline was used so that the overshooting of the approximation polynomial could be avoided (see Figure 44 polynomials vs. splines).

Figure 45**The Iterative process**

inverse was assigned as a weight, hence the farther away the point from the possible line the less weight it was given. The curve was then re-drawn with these newly assigned weights and the distance between the new curve and the points was drawn.

REFERENCES

- Arnold J.M. 1962. Mating behaviour and social structure in *Loligo pealii*. Biol. Bull. (Woods Hole) 123: 53-57.
- Arnold J.M. 1984. Cephalopods (Chapter 6). In: The Mollusca (Vol 7) Reproduction. Tompa A.S., Verdonk N.H., van den Biggelaar J.A.M., Editors. New York: Academic Press. p419-454.
- Augustyn C.J. 1990. Biological studies on the chokker squid *Loligo vulgaris reynaudii* (Cephalopoda: Myopsida) on spawning grounds off the south-east coast of South Africa. S. Afr. J. Mar. Sci. 9: 11-26.
- Augustyn C.J. 1991. The biomass and ecology of chokka squid *Loligo vulgaris reynaudii* off the west coast of South Africa. S. Afr. J. Zool. 26: 164-181.
- Augustyn C.J. 1996. Options for management In: Programme Abstracts and Directory, Benguela Dynamics Symposium: Impacts of variability on shelf-sea environments and their living resources, Cape Town, South Africa, November 1996: p24, abstract only.
- Augustyn C.J., Grant W.S. 1988. Biochemical and morphological systematics of *Loligo vulgaris vulgaris* Lamarck and *Loligo vulgaris reynaudii* D'Orbigny nov.comb. (Cephalopoda: Myopsida). Malacologia 29: 215-233.
- Augustyn C.J., Lipinski M.R., Sauer W.H.H., Roberts M.J., Mitchell-Innes B.A. 1994. Chokka squid on the Agulhas Bank: life history and ecology. S. Afr. J. Sci. 90: 143-154.
- Chotiyaputta C., Yamrungreung A. 1998. Trap fisheries for squid and their impact on spawning. In: Cephalopod biodiversity, ecology and evolution. Payne, A.I.L., Lipinski M.R., Clarke M.R., Roeleveld M.A.C., Editors. Cape Town: Sea Fisheries. S. Afr. J. Mar. Sci. 20: 285-291.

- DiMarco F.P., Hanlon R.T. 1997. Agonistic behavior in the squid *Loligo plei* (Loliginidae, Teuthoidea): fighting tactics and the effects of size and resource value. *Ethology* 103: 89-108.
- Drew G.A. 1911. Sexual activities of the squid, *Loligo pealii* (Les.). I. Copulation, egg-laying and fertilization. *J. Morph.* 22: 327-359.
- Fields W.G. 1965. "The structure, development, food relations, reproduction, and life history of the squid *Loligo opalescens* Berry". Sacramento: California. Dept. Fish and Game. *Fish Bull.* 131: 1-108
- Griswold C.A., Prezioso J. 1981. En situ observations on reproductive behavior of the long-finned squid, *Loligo pealei*. *Fishery Bull.* 78: 945-947.
- Hamabe M., Shimizu T. 1957. Loliginidae spawning. *Rept. Nat. Fishery Inst. Japan-Sea Reg.* 13-18
- Hamabe M., Shimizu T. 1957. The copulation behavior of *Yariika*, *Loligo bleekeri* K. *Rept. Jpn. Sea Reg. Fish Res. Lab.* 3: 131-136
- Hanlon R.T. 1982. The functional organization of chromatophores and iridescent cells in the body patterning of *Loligo plei* (Cephalopoda: Myopsida). *Malacologia* 23: 89-119.
- Hanlon R.T. 1996. Evolutionary games that squids play: fighting, courting, sneaking, and mating behavior used for sexual selection in *Loligo pealei*. *Biol. Bull.* 191: 309-310.
- Hixon R.F. 1983. *Loligo opalescens*. In: *Cephalopod Life Cycles (Vol I) Species Accounts*. Boyle P.R., Editor. New York: Academic Press. p95-114.
- Hoar J.A., Sim E., Webber D.M., O'Dor R.K. 1994. The role of fins in the competition between squid and fish (Chapter 3). In: *Mechanics and physiology of animals swimming*. Maddock L., Bone Q., Rayner J.M.V., Editors. Cambridge: Cambridge University Press. p27-43.

- Johnson, D.H. 1999. The insignificance of statistical significance testing. *J. Wildl. Management* 63: 763-772.
- Kuroki T., Kawaguchi W., Satamoto W., Watanabe H. 1971. A new telemetric apparatus to detect fish location and its surrounding water temperature. *Bull. Jap. Soc. Sci. Fish.* 37: 369-374.
- Lipinski M.R., Butterworth D.S., Augustyn C.J., Brodziak J.K.T., Christy G., Des Clers S., Jackson G.D., O'Dor R.K., Pauly D., Purchase L.V., et al. 1998. Cephalopod fisheries: a future global upside to past overexploitation of living marine resources? Results of an International Workshop, 31 August-2 September 1997, Cape Town, South Africa. In: *Cephalopod biodiversity, ecology and evolution*. Payne A.I.L., Lipinski M.R., Clarke M.R., Roeleveld M.A.C., Editors. Cape Town: Sea Fisheries. *S. Afr. J. Mar. Sci.* 20: 463-469.
- Luke D.McG, Pincock D.G., Stasko A.B. 1973. Pressure-sensing ultrasonic transmitter for tracking aquatic animals. *J. Fish Res. Board Can.* 30: 1402-1404.
- McGowan J.A. 1954. Observations on the sexual behavior and spawning of the squid, *Loligo opalescens*, at La Jolla, California. *California Fish and Game* 40: 47-54.
- Murlis J., Bettany B.W., Kelley J., Martin, L. 1982. The analysis of flight paths of male Egyptian cotton leafworm moths, *Spodoptera littoralis*, to a sex pheromone source in the field. *Physiol. Entomology* 7: 435-441.
- O'Dor R.K. 1998. Can understanding squid life-history strategies and recruitment improve management? *Cephalopod biodiversity, ecology and evolution*. Payne A.I.L., Lipinski M.R., Clarke M.R., Roeleveld M.A.C., Editors. Cape Town: Sea Fisheries. *S. Afr. J. Mar. Sci.* 20: 193-206.
- O'Dor R.K., Andrade Y., Webber D.M., Sauer W.H.H., Roberts M.J., Smale M.J., Voegeli F.M. 1998. Applications and performance of Radio-Acoustic Positioning and Telemetry (RAPT) systems. *Hydrobiologia* 371/372: 1-8.

- O'Dor R.K., Hoar J.A., Webber D.M., Carey F.G., Tanaka S., Martins H.R., Porteiro F.M. 1994. Squid (*Loligo forbesi*) performance and metabolic rates in nature. *Mar. Fresh. Behav. Physiol.* 25: 163-177.
- O'Dor R.K., Webber D.M. 1998. A brief history of marine fish and invertebrate tagging. Stone G.S., Tausig H., Schubel J.R., Principal Editors. "Marine animal telemetry tags". Boston: New England Aquarium. Aquatic Forum Series; Report 98-3: 17-27.
- O'Dor R.K., Webber D.M., Sauer W.H.H., Roberts M.J., Smale M.J. 1995. High resolution, 3-D tracking of squid on spawning grounds with radio-acoustic positioning. In: Proceedings of the 13th International Symposium of Biotelemetry, Williamsburg, Virginia, March 1995. Cristalli C., Amlaner C.J., Newmann M.R., Editors. Fayetteville: University of Arkansas Press. p193-198.
- Payne A.I.L., Lipinski M.R., Clarke M.R., Roeleveld M.A.C., Editors. 1998. Cephalopod biodiversity, ecology and evolution. Cape Town: Sea Fisheries. *S. Afr. J. Mar. Sci* 20 469p.
- Porebski, J. 1970. Observations on the occurrence of Cephalopoda in the waters of the NW African shelf, with particular regard to *Loligo vulgaris* (Lamarck). In: Reports et Proces-verbaux des Reunions Conseil International pour l'Exploration de la Mer. 159: 142-145.
- Roberts M.J. 1998. The influence of the environment of chokka squid *Loligo reynaudii* spawning aggregations: steps towards a unified model. In: Cephalopod biodiversity, ecology and evolution. Payne A.I.L., Lipinski M.R., Clarke M.R., Roeleveld M.A.C., Editors. Cape Town: Sea Fisheries. *S. Afr. J. Mar. Sci.* 20: 267-284.
- Roberts M.J., O'Dor R.K., Smale M.J., Sauer W.H.H. Researchers and coordinators. 1994. Coordination plan for November 1994 acoustic tagging and environmental study. (Squid Research) unpagged

- Roberts M.R., Sauer W.H.H. 1994. Environment: the key to understanding the South African chokka squid (*Loligo vulgaris reynaudii*) life cycle and fishery? *Antarctic Sci.* 6: 249-258.
- Roeleveld M.A.C. 1998. The status and importance of cephalopod systematics in southern Africa. In: *Cephalopod Biodiversity, Ecology and Evolution*. Payne A.I.L., Lipinski M.R., Clarke M.R., Roeleveld M.A.C., Editors. Cape Town: Sea Fisheries. *S. Afr. J. Mar. Sci.* 20: 1-16.
- Sauer W.H.H. 1995. South Africa's Tsitsikamma National Park as a protected breeding area for the commercially exploited squid *Loligo vulgaris reynaudii*. *S. Afr. J. Mar. Sci.* 16: 365-371.
- Sauer W.H.H., Goschen W.S., Koorts A.S. 1991. A preliminary investigation of the effects of sea temperature fluctuation and wind direction on catches of chokka squid *Loligo vulgaris reynaudii* off the Eastern Cape, South Africa. *S. Afr. J. Mar. Sci.* 11: 467-473.
- Sauer W.H.H., Roberts M.J., Lipinski M.R., Smale M.J., Hanlon R.T., Webber D. M., O'Dor R.K. 1997. Choreography of the squids's "Nuptial Dance". *Biol. Bull.* 192: 203-207.
- Sauer W.H.H., Smale M.J. 1993. Spawning behaviour of *Loligo vulgaris reynaudii* in shallow waters of the south-eastern Cape, South Africa. In: *Recent Advances in Cephalopod Fisheries Biology*. Okutani T., Hatanaka H., O'Dor R.K., Editors. Tokyo: Tokai Univ. Press. p489-498.
- Sauer W.H.H., Smale M.J., Lipinski M.R. 1992. The location of spawning grounds, spawning and schooling behaviour of the squid *Loligo vulgaris reynaudii* (Cephalopoda: Myopsida) off the Eastern Cape Coast, South Africa. *Marine Biology* 114: 97-107.
- Stasko A B. 1975. "Underwater biotelemetry, an annotated bibliography". *Fish. Mar. Serv. Res. Dev. Tech. Rpt.* No. 534 31p.

- Summers W C. 1983. *Loligo pealei*. In: Cephalopod Life Cycles (Vol I) Species Accounts. Boyle P.R., Editor. New York: Academic Press. p115-142.
- Swanberg T.R., Geist D.R. 1997. Effects of intraperitoneal transmitters on the social interaction of rainbow trout. *N. Amer. J. Fish. Management* 17: 178-181.
- Trefethen P.S., Dudley J.W., Smith M.R. 1957. Ultrasonic tracer follows tagged fish. *Electronics* 30: 156-160.
- Waller R.A., Wicklund R.I. 1968. Observations from a research submersible - mating and spawning of squid, *Doryteuthis plei*. *BioScience* 18: 110-111.
- Waters S., Whitehead H. 1990. Population and growth parameters of Galapagos sperm whales estimated from length distributions. *Rept. Int. Whal. Comm.* 40: p225-235.
- Watkins W.A., Daher M.A., Fristrup K.M., Howald T.J., 1993 sperm whales tagged with transponders and tracked underwater by sonar. *Mar. Mammal Sci.* 9:55-67.
- Watkins W.A., Schevill W.E. 1972. Sound source location in a three-dimensional hydrophone array. *Deep-Sea Res.* 19: 691-706.
- Webber D.M., O'Dor R.K. 1986. Monitoring the metabolic rate and activity of free-swimming squid with telemetered jet pressure. *J. exp. Biol.* 126: 205-224.
- Worms J. 1983. *Loligo vulgaris*. In: Cephalopod Life Cycles (Vol I) Species Accounts. Boyle P.R., Editor. New York: Academic Press. p143-157.