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DETERMINATION OF NATURAL VARIATION IN BIOLOGICAL AND ECOLOGICAL FACTORS OF *AMERIANNA CUMINGII* (GASTROPODA, PULMONATA) WITH A VIEW TO ITS USE AS A POLLUTION MONITOR FOR THE RANGER URANIUM MINE IN KAKADU NATIONAL PARK

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The Supervising Scientist for the Alligator Rivers Region has research, supervisory and co-ordination responsibilities related to effects on the environment of uranium mining in the Alligator Rivers Region and research and supervisory responsibilities related to effects on the environment of non-uranium mining in a conservation zone declared within the Region.

DETERMINATION OF NATURAL VARIATION IN BIOLOGICAL AND ECOLOGICAL FACTORS OF *AMERIANNA CUMINGII* (GASTROPODA, PULMONATA) WITH A VIEW TO ITS USE AS A POLLUTION MONITOR FOR THE RANGER URANIUM MINE IN KAKADU NATIONAL PARK.

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A thesis submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Honours), University of New England, Armidale, New South Wales, Australia.

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ABSTRACT

It was the central aim of this study to determine the natural variation of the biological and ecological features of *Amerianna cumingii* to assess its use as a biological monitor for the waste water releases of the Ranger Uranium Mine. Four areas were chosen for investigation due to their importance as biological indicators of disturbance and in maintenance of laboratory cultures: reproduction; early development and growth; mortality; and food and feeding.

When a fecundity-size relationship was determined for *Amerianna cumingii* at three different times, (January, March and May) both age and size were important in determining the fecundity of an individual snail. Size at first laying was approximately 6 mm in length. With experiments being carried out at laboratory and field sites it was found that a greater amount of eggs is generally laid in the field than in the laboratory. Also, there was a general decrease in the number of eggs being laid as the monsoonal wet season drew to an end with the greatest number of eggs being laid at the beginning. *A.cumingii* laid egg cases most frequently during the period 0200 to 0800 hours than in any other period of the day. Density (within the range tested) did not significantly effect the egg production of *A.cumingii.*

In an experiment concerning the effect of temperature on early development *A.cumingii* on average takes nine days to develop and hatch at 25.5°C. While at higher temperatures the processes is accelerated with a shorter larval stage and hatching occurs within eight and seven days for temperatures of 30.0°C and 33.0°C respectively. Of the densities tested, growth was effected significantly in three of the five age groups and expected patterns produced in only two age groups due to the high mortality in all vials.

In recently hatched juveniles mortality was widely varied with no difference between sites. The mortality of five age groups of juvenile snails at four densities was high in all cases with no significant difference with age or density. Six survival curves for adult snails were significantly different showing effects of three densities and two sites.

Through a series of food and feeding experiments with various types of energy sources (macrophytes and lettce; epiphytes and settled detritus; and water) it was determined that no food source had a significantly different effect on growth of snails but there were different effects on reproduction. Of the macrophytes and lettuce tested in the first experiment, lettuce was the only food source that increased the egg production over the three weeks of experimentation. In the second experiment, significant results were discovered for the correlation between amount of epiphyte and settled detritus and the number of eggs laid. Finally, it was discovered that there was no significant difference on egg production when *A.cumingii* was offered different water types as food sources.

CHAPTER ONE

1. INTRODUCTION

1.1. USE OF BIOLOGICAL MONITORING

Biological techniques are an important means of evaluation of water quality (Cairns *et al.* 1976). The employment of living organisms as indicators of environmental conditions has potential applications for many forms of resource management but its most effective development has been in relation to water resources (Jones 1979). Aquatic biomonitors provide a better assessment of the environmental damage than do chemical or physical parameters (eg. pH, salinity and temperature) as aquatic organisms respond to their total surroundings and continuously monitor water quality effectively (Cairns *et al.* 1973) without the use of very expensive equipment. Biological monitoring does not replace the need for other forms of monitoring but it is essential in determining the synergistic or antagonistic interactions of waste water releases and the receiving system (Cairns *et al.* 1973).

Classical approaches to biomonitoring have included the acute bioassay which takes death as the end-point of the test (Biesinger and Christensen 1972; Borgmann *et al.* 1978) while modern versions rely on less final results. Such sub-lethal effects are those that disrupt physiological and behavioural activities (Chapman *et al.* 1985) which do not cause immediate mortality but may be significant to the survival of a species (Waldichuk 1979). For example, reproductive failure in Baltic Sea seals has been reported to be associated with high concentrations of DDT and polychlorinated biphenyl in their blubber (Waldichuk 1979). With a lowering of reproductive rate, numbers are reduced and species are in danger of becoming extinct.

Another physiological activity affected by pollution is growth. Cocker (1921; quoted in Imlay 1982) found that freshwater mussels stopped growing when disturbed. With the use of special techniques, annual shell growth can be determined, thus revealing annual amounts of heavy metal present in the water. The use of growth parameters has also long been used to monitor environmental factors affecting particular plant species (Bellamy *et al.* 1972).

The use of aquatic organisms as biological indicators of the presence or absence of pollutants in their surroundings have widely been selected as a means of monitoring

aquatic ecosystems throughout the world. Bayly and Lake (1979; quoted in Connell 1981 p20) describe many Australian species employed in this way, with the list ranging from fish species, rainbow trout (*Salmo gairdneri*) and common carp (*Cyprinus carpio*), to the freshwater amphipod (Genus *Austrochiltonia*). A wide variety of molluscs have also been used throughout Australia including three species of freshwater mussels, *Velesumo ambiguus, Alathyria jacksoni* and *Hyridella australis* to monitor heavy metal pollution (Bayly and Lake 1979; quoted in Connell 1981 p20). Bioaccumulators such as bivalve molluscs, particularly oysters and mussels, are valuable indicators of pollution as they are able to accumulate contaminants in their tissues to levels considerably higher than in sea water (Connell 1981 p19). This accumulation of pollutant in the tissues of these organisms enables easy detection of the pollutant in the surrounding waters.

Freshwater snails have also been valuable monitors of pollution. Arthur and Leonard (1970) used two species of snails *Physa integra* and *Campeloma decisum* to test the effects of copper in soft water, with survival, growth, reproduction and feeding as indicators of the pollutants. Wurtz (1962) described molluscs as slow to repopulate areas from which they are eliminated from and become re-established only through downstream transport. Molluscs (*Physa heterostropha* and *Helisoma companulata*) were, therefore, chosen by Wurtz (1962) to test the effects of zinc.

Borgmann *et al.* (1978) found the rates of mortality, growth and biomass production of *Lymnaea palustris* a means of detecting the effects of lead at concentrations as low as 19 µg/ L. While Flannagan (1974) found concentrations of trisodium nitrilotriacetate above 25 mg/ L imposed an effect on the growth and fecundity of *Helisoma trivolvis*.

Patrick *et al.* (1968) found the freshwater snail *Physa heterostropha* was the most sensitive to potassium chloride when compared to a species of diatom (Nitzschia linearis) and fish (*Lepomis macrochirus*), although, it was not the most sensitive organism to other pollutants tested including phenol, potassium dichromate and naphthenic acids. The conclusions of Patrick *et al.* (1968) therefore, is to include at least three components of the food web in any biological monitoring programme so that complementary information can be provided.

1.2. AIM OF STUDY

The Alligator Rivers Research Institute has carried out a series of biological tests on the waste water releases of the Ranger Uranium Mine into the Magela Creek System. Studies have also been carried out using biological monitors, such as fish, macroinvertebrates and mussels, to determine any effect the discharge is having on the environment *in situ*. To extend the biological monitoring of the mine waste waters, the Institute decided to include freshwater snails in their program. This type of organism was selected for use due to their sensitivity to pollutants, their ability to reproduce many times in the one season, and their short life cycle. Of several species tested for suitability two cogenera species, *Amerianna cumingii* and *A.carinata*, were shown to be acceptable due to their sensitivity to the pollutant, consistant egg laying and their ability to be handled easily.

When using sub-lethal indicators to determine the effect of pollution, knowledge of the natural variation in the biology, ecology and physiology of the species chosen is essential. It is necessary to distinguish between the natural variation and the extra responses of the organism to contamination. In the case of *A.cumingii* nothing is known about its biology, ecology or physiology.

It was the central aim of this study to provide such knowledge to assess the suitability of the snail, *A.cumingii*, as part of the programme for the biological monitoring (by the Alligator Rivers Region Research Institute) of the waste water releases from the Ranger Uranium Mine in Kakadu National Park.

When studying the biology of a species for the first time there is an endless choice of areas to investigate and explore. In describing *A.cumingii*, investigation was limited to areas of natural variation in factors important to biological monitoring. The areas of study were broken up into four main sections: reproduction; early development and growth; mortality; and food and feeding. Reproduction, growth and mortality were selected because previous biological monitoring programmes (for example Biesinger and Christensen 1972; Borgmann *et al.* 1978; Cairns *et al.* 1976) have shown the suitability of these features as a means of determining the effects of pollution in the freshwater system. Knowledge of the best possible food source for *A.cumingii* is essential in sustaining snails in good conditon in laboratory cultures, information about reproduction, growth and mortality also aid in maintenance of snail cultures.

1.3. GENERAL MATERIALS, METHODS AND LOCATION

1.3.1. THE ALLIGATOR RIVERS REGION

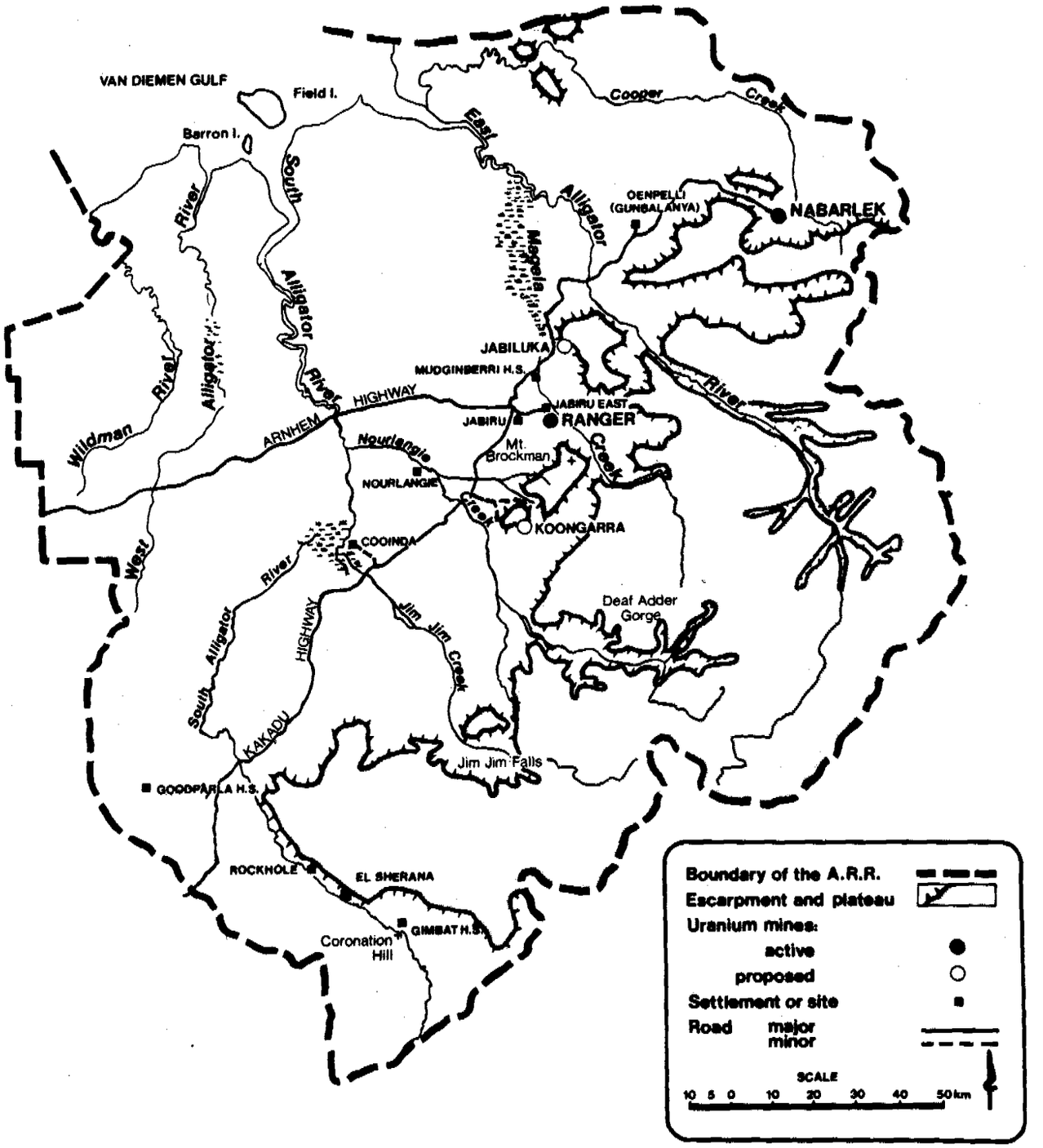
The Alligator Rivers Region is located in the far north of the Northern Territory of Australia and is so named as it is the catchment area of the East Alligator, South Alligator and West Alligator Rivers. In Figure 1.1. the Alligator Rivers Region is indicated by the shaded area and its location is shown in relation to Darwin and Australia.

1.3.2. THE RANGER URANIUM MINE

Airborne radiometric surveys carried out during October 1969 throughout areas of northern Australia indicated uranium orebodies which were confirmed by drilling in mid-1970. The Ranger uranium (Plate 1.1.A.) deposits are located approximately 250 km east of Darwin within the Alligator Rivers Region. Mining commenced at the Ranger site in 1980 and as of mid-1989 there has been 24 591 tonnes of U O recovered by open cut methods.

The Ranger project area is surrounded by Kakadu National Park, an area of World Heritage status, whose waterways support one of the richest biological ecosystems in Australia. Kakadu boasts a diversity of mammals, birds, reptiles, frogs, fish and plants seldom seen anywhere else in Australia. The mine site at Ranger receives, on average, approximately 1 500 mm of precipitation per annum, the majority of which falls within a three to four month period. The runoff from this rainfall is inevitably affected by the chemical composition of its catchment. Thus, the means of containing and disposing of the water that accumulates on the mine site is of great importance as it is this runoff that is the pollutant which is in great danger of damaging the environment. The major water retaining structures (Plate1.1.B.) within the mine site are the tailings dam, the mine pit and four retention ponds.

An important means of regulating any possible effect of the runoff from the mine site is by not permitting release of any stored water into the surrounding ecosystem. Exception would be made under specified conditions and with written approval of the Northern Territory supervising authority.



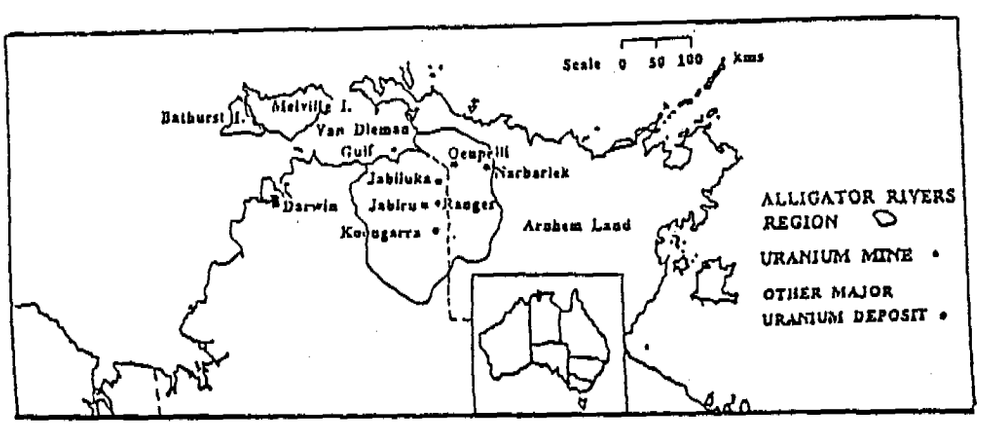


Figure 1.1. The Alligator Rivers Region.

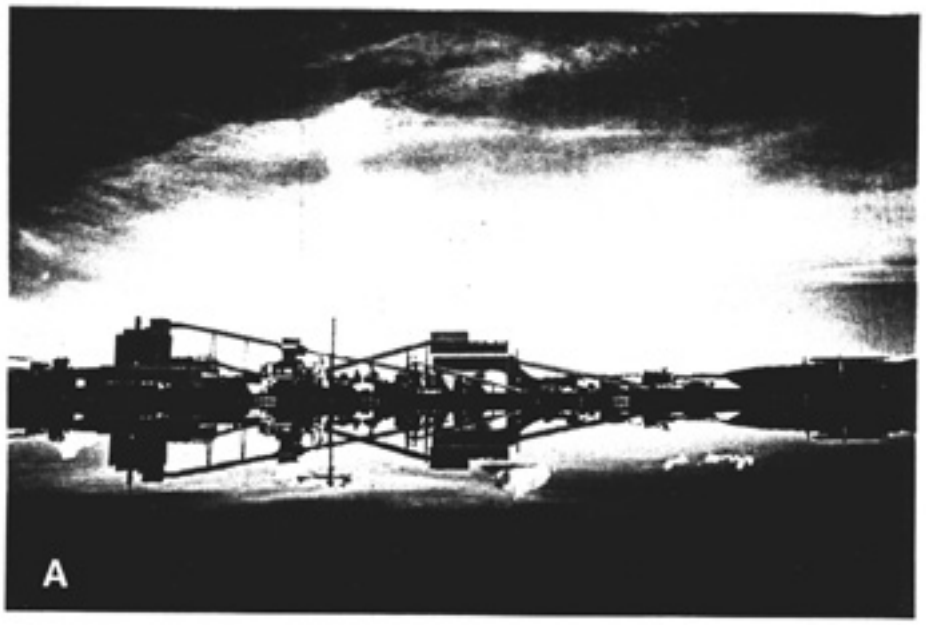
Plate 1.1.

A. Ranger Uranium Mine, processing unit.

B. Aerial shot of Ranger Uranium Mine showing major water retaining structures.

1. Mine pit.
2. Tailings dam.
3. Retention pond two.
4. Retention pond three.
5. Retention pond four.

C. Retention pond four.


A


1
2
3
4
5
B


C

1.3.3. THE ALLIGATOR RIVERS RESEARCH INSTITUTE

The Alligator Rivers Region Research Institute (ARRRI) was established under the Environment Protection Act 1978. In recognition of the exceptionally rich environment of this region (which today includes Kakadu National Park) and the interests of the Aboriginal people of the area, the establishment of a complex regime of environmental protection measures were established. A Supervising Scientist, having responsibilities directly to a Commonwealth Minister, was appointed to direct the activities of the Institute. The broad aims of the Institute are to conduct, co-ordinate and integrate research required to ensure the protection of the environment in the region from any harmful consequences resulting from mining and processing of uranium ore.

1.3.4. RETENTION POND FOUR

Retention pond four (Plate 1.1.B. & C.) is a sediment trap which collects runoff from the waste rock dump. As the capacity is limited in this pond, water is usually discharged to the environment each wet season and is, therefore, of special interest when investigating on the surrounding wet lands. Over several years, the Institute has carried out a series of biological tests on this water in an attempt to confirm safe levels for the discharge of retention pond four water into the Magela Creek System. Studies have also been carried out using biological monitors, such as fish, macroinvertebrates and mussels, to determine any effect the discharge is having on the environment *in situ.*

1.3.5. SNAIL DESCRIPTION

Amerianna cumingii (Plate 1.2.A.) is a large freshwater snail which grows up to 16.5 mm in length, is dark brown in colour, and inhabits the waterways of northern Australia. The shell of snails belonging to the genus *Amerianna* is sinistral with a small, elevated spire and a large last whorl with a large aperture that runs almost the entire length of the shell. Rounded shoulders and a trucated spire separate *A.cumingii* from other species within the genus. There have been no previous studies on the biology, ecology or distribution of this species.

It has been observed in this study that, in the case of *A.cumingii*, both snails produce egg cases after mating. Up to four egg cases can be laid per pair of snails every day with each egg case containing up to sixty eggs inside. The egg case is a jelly-like

Plate 1.2.

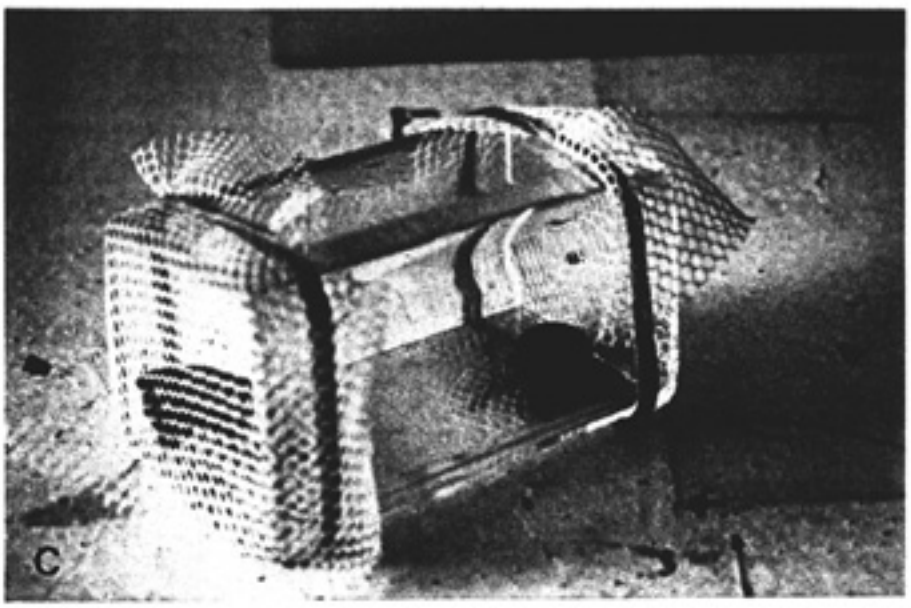
A. *Amerianna cumingii*, showing nail polish used to mark snails.

B. A group of *Amerianna cumingii* of various ages.The snail furtherest to the right showing signs of aging with white markings against the dark of the snail.

C. A pair of *Amerianna cumingii* inside an open-ended, clear perspex vial. Snails were retained within the vial by the mesh held at each end with rubber bands.


A


B


C

substance that protects the developing eggs and provides the prehatchlings with nutrition after they break free from their eggs and before they break out of the egg case.

1.3.6. SNAIL CULTURE

Laboratory stocks of *A.cumingii* have been maintained at the Institute for several years; the original source of snails were derived from floodplain waterbodies of Magela Creek (Humphrey pers. comm.). All snails used in the experiments for this study were derived from laboratory cultures established in September 1990. At the time, sixteen individual snails were placed in a large tank containing aerated tap water. Snails were sustained on a regular suppy of lettuce and the stock was left to reproduce undisturbed until mid-January 1991. At this time, snails greater than 8 mm were collected, measured and snails of each millimetre size class (from 8.0 to 16.5 mm) were placed into a separate tank. Each of these individual tanks was aerated and snails were fed fresh lettuce three times per week.

Snails in the separate tanks, while used in various experiments, were otherwise left free to breed when not required. Their progeny, together with the parental stock were checked and measured regularly for size; with growth, snails were reassigned if necessary to an appropriate holding tank. At approximately three week intervals, tank water with the build-up of nitrogenous wastes was partially or completely replaced with fresh tap water. It was later discovered, during experimentation, that snails reproduced better in creek water than in tap water, therefore, snail cultures were transfered into creek water.

1.3.7. SITE DESCRIPTION

Three different sites were used in the experiments carried out for this study; one laboratory and two field sites. The laboratory site was located at the Institute and two creekside monitoring stations served as the field sites. Both monitoring stations were located on the banks of the Magela Creek; one downstream from George Town Billabong and the other down stream from Djalkmara Billabong (Figure 1.2.).

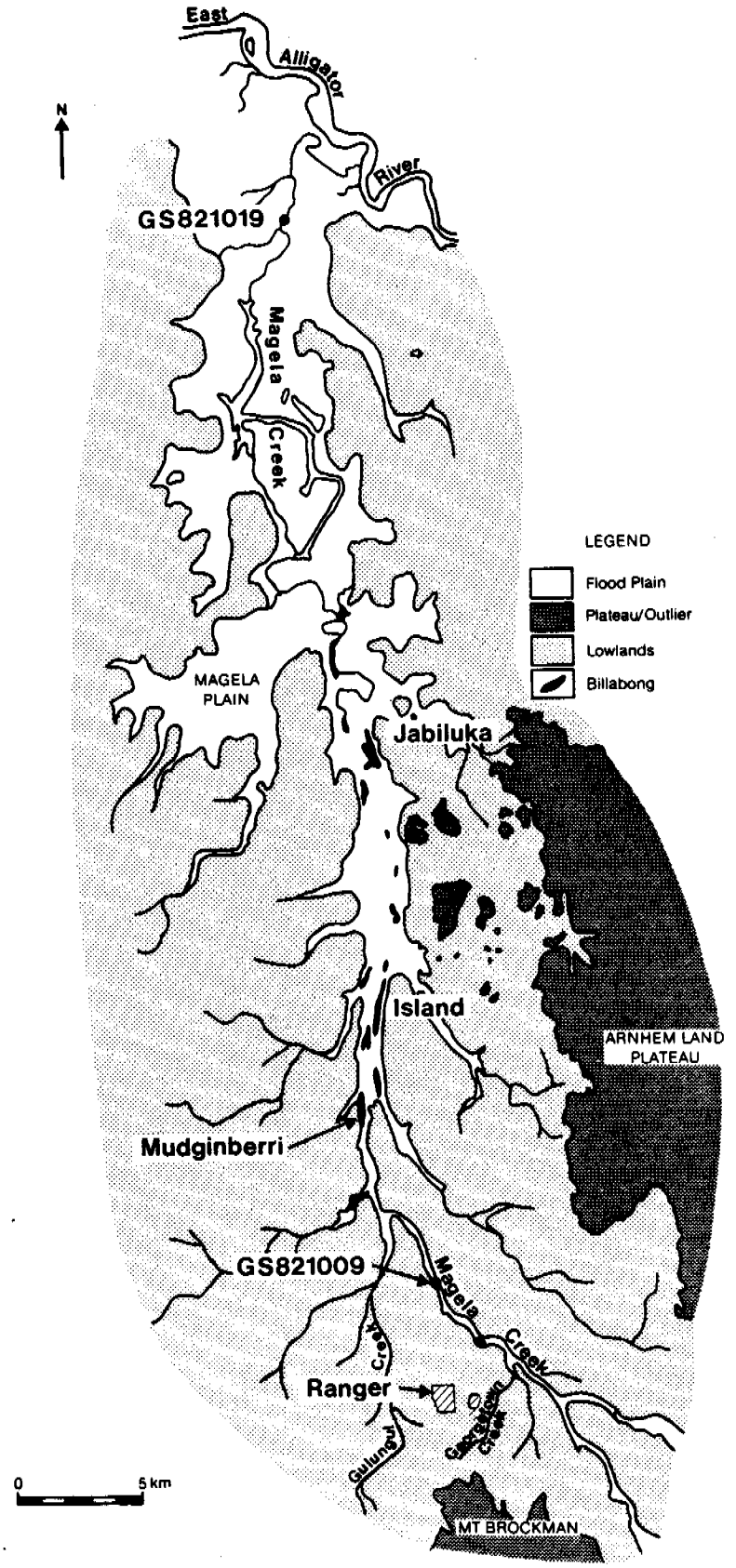


Figure 1.2. Magela Creek System, showing field sites.

Plate 1.3.

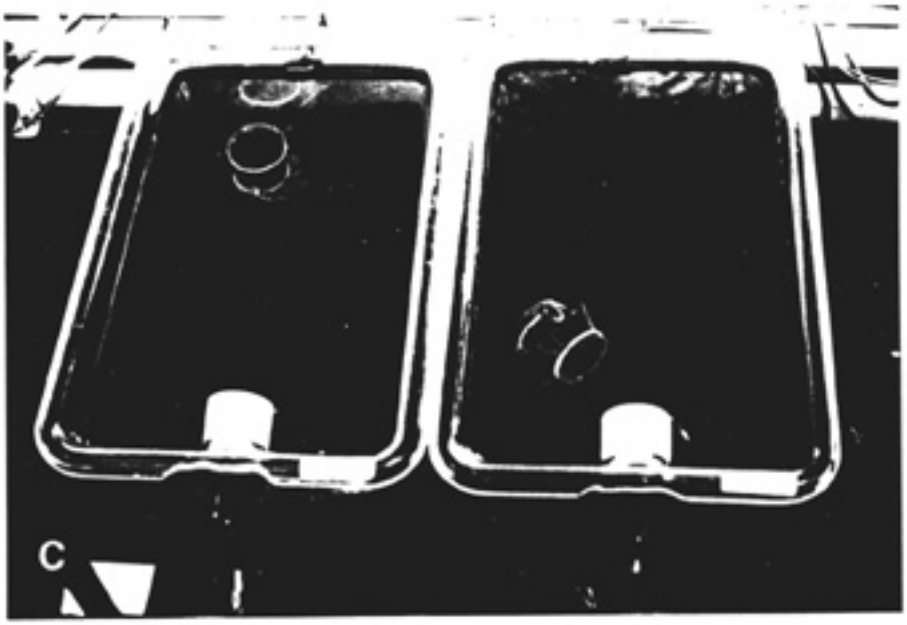
A. George Town creekside monitoring station.

B. Close up of monitoring station showing 9 L tanks positioned on tables with pipes carrying water to the tanks and others taking water away from the tanks so that a continual flow of water is created.

C. Close up of two 9 L tanks each containing one vial. Within each vial two snails are retained for experimental purposes.


A


B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1.3.8. GENERAL EXPERIMENTAL PROCEDURE

In order to avoid repetition, the general experimental procedure used in all experiments will be described first. Any deviation from this procedure will be described in the appropriate sections.

The experimental procedure used at each site was similar in that most experiments utilise 9 L tanks containing Magela Creek water and which are positioned on tables beneath an awning (Plate 1.3.A.). At the laboratory, the creek water in the tanks was aerated and partially replaced each day, from stored Magela Creek water collected prior to the commencement of each experiment, for the duration of all experiments. In contrast, in the field, tanks received a continuous flow of creek water pumped up from the Magela Creek (flow rate through the tanks of approximately 0.15 L / minute) (Plate 1.3.B.).

Certain water quality variables have been measured in the experiments to associate with biological results. The variables measured at the creekside monitoring stations include creek discharge and water temperature (twice daily), conductivity, pH and turbidity (daily) as well as total organic carbon (TOC) and dissolved organic carbon (DOC) (weekly). In laboratory experiments, temperature, conductivity and pH were measured daily with TOC and DOC measurements being made when appropriate.

In all reproduction and food and feeding experiments as well as the experiment involving mortality in recently hatched juveniles, there were similarities in their experimental format. Open ended clear perspex vials (69.1 cm) were used to determine the fecundity of a pair of snails in 9 L tanks (Plate 1.3.C.). For reference, each vial was divided into four quadrants by scratching lines on the outside. The ends of the vials were covered with mesh (2 mm mesh diameter) held in place with rubber bands in order to detain snails without stopping the flow through of water (Plate 1.2.C.). Each pair of snails was fed daily with a disc of lettuce (2 cm diameter). Lettuce remaining from the day before was removed prior to fresh lettuce being added.

When measuring the shell of *A.cumingii* the length is taken to be the distance between the apical tip and the shoulder. This is shown diagramatically in Figure 1.3.

For all statistical analysis the BMDP or the SPSSX statistical package was used within the computer programme minitab. Different tests were selected for individual experiments and are described in the appropriate material and method sections.

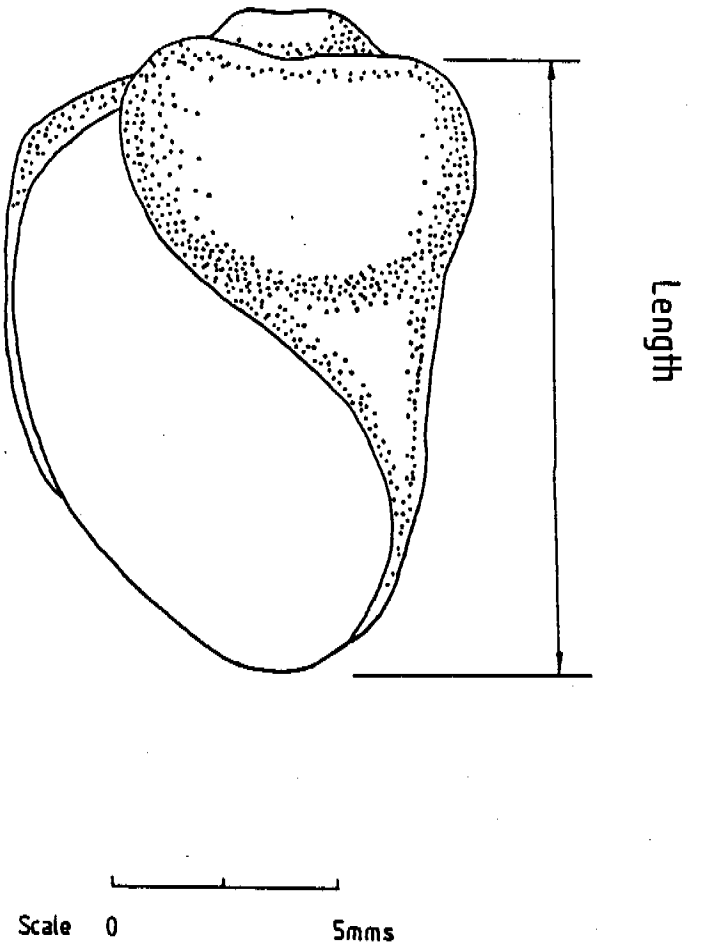

Figure 1.3. Ventral view of the shell of *Amerianna cumingii*.

CHAPTER TWO

2. REPRODUCTION

Different environmental conditions can affect the egg production of freshwater snails, including whether they choose to lay at all. The factors investigated in this study are the size of the snail to determine a fecundity-size relationship, time of day to determine a daily egg laying cycle and density to determine any effect on reproduction.

The fecundity of a snail can be defined as the number of eggs laid by that snail per unit time, typically over a breeding season; due to the limitations of experimentation a time period of one week was used to determine the fecundity. In the case of pulmonate hermaphrodites self-fertilization is possible but since they prefer to cross-fertilize fecundity is determined here, in all but one experiment, for a pair of snails rather than an individual.

2.1. FECUNDITY-SIZE RELATIONSHIP

2.1.1. INTRODUCTION

When determining the relationship between fecundity and size for *A.cumingii* the question of 'what is the best sized snail to use for biological monitoring purposes?' is posed. This investigation was designed to show not only the optimal size when selecting individual snails for biological monitoring purposes but also the fecundity of all other sized snails so that a reference can be made for any snail selected.

2.1.2. MATERIALS AND METHODS

In order to show the widest possible relationship between size and egg production, snails were selected from 4 to 15 mm in size. Different sites were used to show any variability with changing sites and the experiment was carried out three different times during the wet season; late January, mid March and early May, to determine if similar patterns arose at different times of the breeding season.

A. Experiment One

A pair of snails from each size class from 6 to 15 mm were used to determine the fecundity size relationship of *A.cumingii*. Each pair, with individuals of the same size class, were placed into a vial with a disc of lettuce. The ten vials were then placed into a 9 L tank. A duplicate set of vials were also set up in another 9 L tank for both laboratory and field sites (field site being George Town). In all twenty vials were used each containing a pair of snails. At the end of a seven day period the mean cumulative fecundity per coupling of snails was graphed against the mean length of the snail shell with a line of best fit for both laboratory and field data. Differences between laboratory and field sites were statistically tested by a one way analysis of co-variance.

B. Experiment Two

Owing to the determination that snails of 6 mm in length laid eggs the second experiment was extended to include 4 and 5mm snails. Thereby, increasing the number of vials from twenty to twenty eight. Otherwise experiment one and two are identical.

C. Experiment Three

In the third experiment snails were used from 4 to 15 mm in size with fifty seven vials per site being used instead of twenty or twenty eight, as in experiments one and two respectively. This was carried out to reduce errors with a larger sampling number. Djalkmara was the field site used for this experiment, not George Town as in the other experiments. Otherwise, the same procedures used in experiments one and two were followed in experiment three.

2.1.3. RESULTS

Graphs A and B, in Figure 2.1., show similar shaped curves which indicate a low fecundity for small snails followed by a peak size of greatest fecundity and a decrease to a lower fecundity with increased size. In the May experiment the line of best fit for the fecundity-size relationship for both laboratory and field data is a straight line. A possible reason for the dramatic change in shape of the graphs can be explained by the age of the large snails. In the third experiment the large snails appeared to be generally younger than in the other two, as shown by the better condition and hardiness of shell. In the two earlier experiments larger snails showed signs of aging

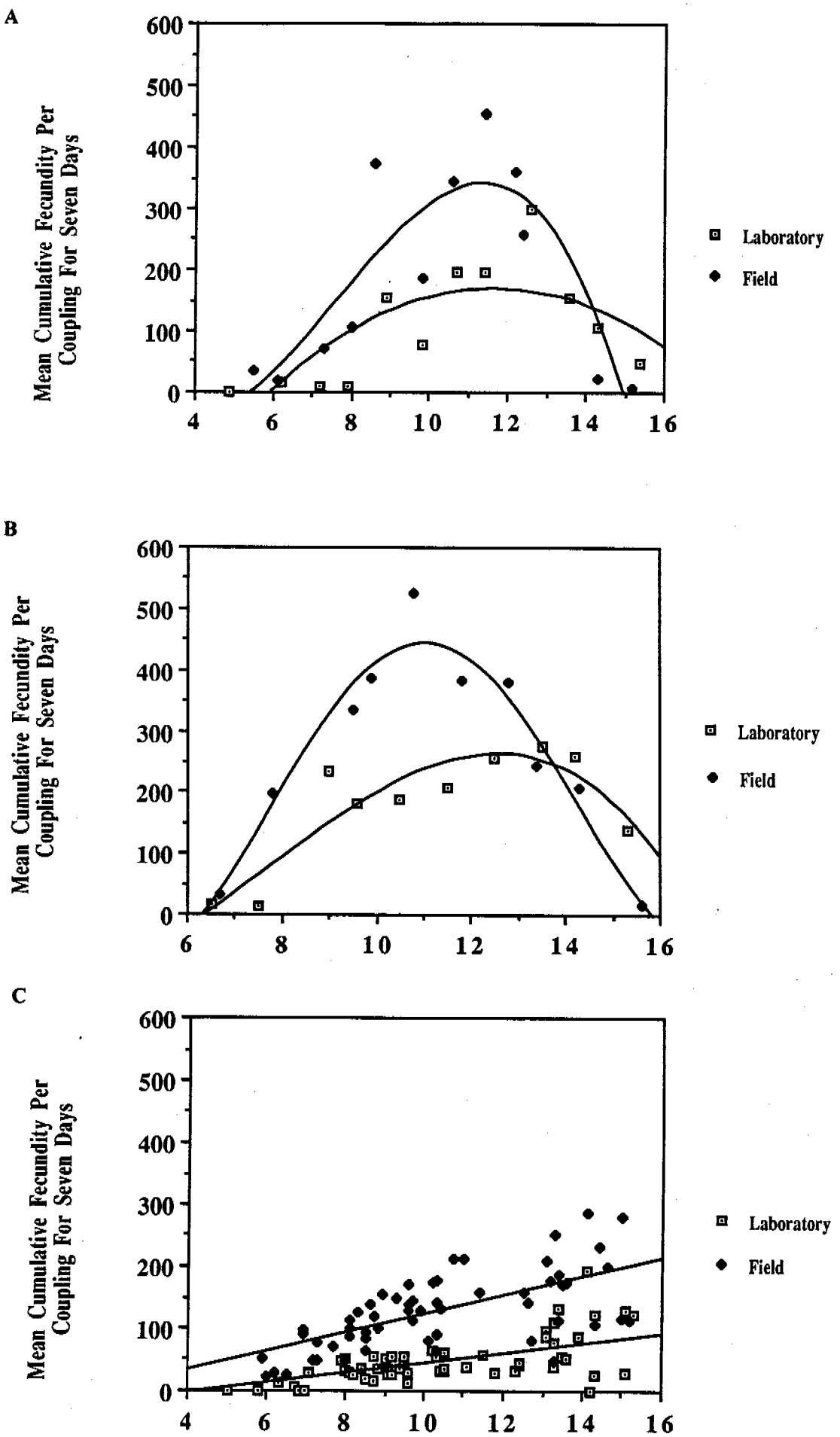
A
Mean Cumulative Fecundity Per Coupling For Seven Days
600
500
400
300
200
100
0
4
6
8
10
12
14
16
Laboratory
Field
B
Mean Cumulative Fecundity Per Coupling For Seven Days
600
500
400
300
200
100
0
6
8
10
12
14
16
Laboratory
Field
C
Mean Cumulative Fecundity Per Coupling For Seven Days
600
500
400
300
200
100
0
4
6
8
10
12
14
16
Laboratory
Field
Mean Shell Length Per Coupling

Figure 2.1

by the lesions and marks that had developed on their shells which appear white against the dark periostracum of the snail.

Laboratory and field sites were found to be significantly different at the 1 % level for the experiments carried out in January (F=13.50, on 3, 14 d.f.) and May (F=63.87, on 2, 110 d.f.) while the March experiment was found to be just not significant at the 5 % level (F=3.32 on 3, 18 d.f.). The reason for the just non-significant finding in March may largely be due to one value from the laboratory (298.5 eggs laid for a one week period) which was much greater than the others. All other values for eggs laid varied from 0 to 198 for the one week period with three quarters of the values being lower than 80. Although, there was a just non-significant difference between sites in March, it was seen in this study that generally more eggs are laid in the field than in the laboratory.

Peak number of eggs being laid in the three experiments varied from approximately 450 in January while in March and May it was approximately 350 and 200 respectively. This reduction in numbers may be related to the reduced water temperature and/ or with the lowering of water levels in the creek both of which were correlates at the time.

When using egg production as an end-point in biological monitoring, it is important to know at what age and size the chosen species begins to reproduce. With this knowledge, appropriately sized specimens can be chosen for monitoring. Similarly, the time required to establish a stock of breeding individuals can be determined. The findings in the fecundity-size relationship experiments showed that *A.cumingii* commence laying eggs at approximately 6 mm in length.

2.2 THE EGG LAYING CYCLE

2.2.1. INTRODUCTION

For any close investigation of the laying of eggs of a particular species, it is important to recognise the period of most frequent egg laying within the day. Also, should egg cases be required for experimental purposes, toxicity testing or monitoring trials for example, then pinpointing the period of laying would expedite procurement of material.

2.2.2. MATERIALS AND METHODS

To investigate the cycle of egg laying in *A.cumingii* each day was broken up into four six hour periods (0200-0800, 0800-1400, 1400-2000, 2000-0200 hours) with egg case counts being made for each period over the twelve days of the experiment. The cumulative number of eggs laid up until the last day of the experiment are presented in a histogram in Figure 2.2.

2.2.3. RESULTS

From Figure 2.2. it is possible to see that a greater number of egg cases were laid in the period from 0200 to 0800 hours than in any other period. Fifteen egg cases were laid in the twelve days of the experiment, while in all three other time periods together only fifteen egg cases were laid. The reason for this is not clear but may coincide with the decrease in water temperature just before dawn from the higher temperatures during the day or just to the affect of first light.

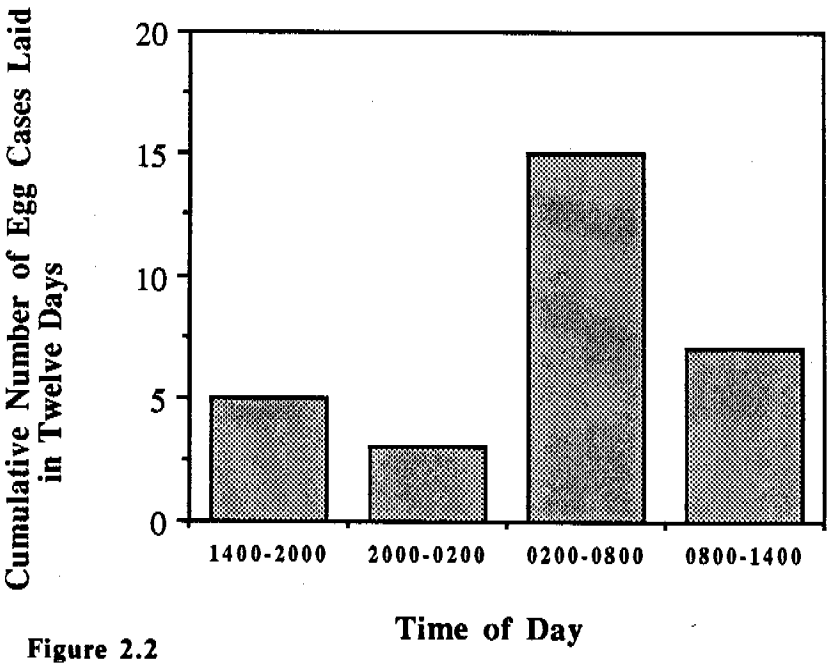

Figure 2.2

2.3. THE EFFECT OF DENSITY ON REPRODUCTION

2.3.1. INTRODUCTION

When maintaining large numbers of any organism in laboratory cultures, density is a critical factor in the health and reproduction of that organism. Therefore, it is important for the maintenance of *A.cumingii* to be aware of the effects of density.

Also, various features of reproduction itself my be affected by the density of the snails.

To enable satisfactory numbers of juveniles for biological monitoring experimentation different densities of adult snails were tested to determine the density which would produce the maximum number of eggs. The effect of density on reproduction during experimentation was investigated in two different ways; by changing the number of snails per vial and by changing the size of the vial while maintaining the same number of snails per vial.

2.3.2. MATERIALS AND METHODS

A. Experiment One

In the first experiment one, two, three or four snails per vial were tested to determine if there was an optimal number of snails per vial to produce the most eggs for a given time period. At the laboratory a duplicate set of vials, each containing a pair of snails either of the 8, 9 or 10 mm size class, were placed into two 9 L tanks. At the end of a seven day period all eggs laid were counted for each vial.

B. Experiment Two

In experiment two, four different sized vials (255.5, 69.1, 43.6, 17.9 cm) were used to change the density experienced by the snails.

A one way analysis of covariance was conducted on each experiment.

2.3.3. RESULTS

The one way analysis of covariance that was conducted on both experiments showed that the densities tested had no significant effect on the egg production over a one week period. In the first experiment the number of snails was found to have no significant effect ($F=0.42$ on 3, 27 d.f.; $P>0.5$) upon egg production, with the covariate of snail length also being not significant ($F=0.32$ on 1, 27 d.f.; $P>0.5$). In the second experiment vial densities between 255.5 and 17.9 cm were found to have no significant effect on egg production ($F=0.06$ on 3, 27 d.f.; $P>0.5$) as was the covariate of snail length ($F=4.90$ on 1, 27 d.f.; $P>0.5$). Therefore, any density of snails, within the range tested, will produce similar numbers of eggs over a one week period and the length of the snails (between 8 and 10.9 mm) will have no effect.

CHAPTER THREE

3. EARLY DEVELOPMENT AND GROWTH

3.1. EARLY DEVELOPMENT

Knowledge of the early (pre-hatching) development, including the timing, of *A.cumingii* may be useful for breeding large numbers of the freshwater snail in laboratory cultures.

3.1.1 INTRODUCTION

The timing of the different stages of early development is known to be affected by the temperature experienced by the organism as it develops. Studies were carried out to investigate the effect of different water temperatures on the development of *A.cumingii* to determine if there is an optimal temperature for raising young in laboratory cultures.

3.1.2. MATERIALS AND METHODS

The first step in describing the early development of any species is in determining the stages it goes through from egg to being born or hatched. The stages of development of *A.cumingii* were determined under a light microscope and were given the same names as described by Morrill (1982, p 403) for the freshwater snail *Lymnaea palustris* : gastrula, veliger, trochophore, hippo and adult-like form.

The temperatures selected were all within the natural variation experienced by *A.cumingii*, (25.5°C, 30.0°C and 33.0°C). Two 9 L tanks were set up at each temperature and five egg cases laid on clear perspex were placed into each tank, with total egg number per tank being approximately equal. Each day all eggs were examined under a light microscope to determine their stage of development until they hatched. A bucket of fresh Magela Creek water was kept at each temperature to enable all tank water to be changed daily with l ittle or no change to the temperature experienced by the developing snails.

3.1.3. RESULTS

At all three temperatures development began slow (Figure 3.1.), with approximately one stage of development occuring each day until the snail reached the adult-like form. At this stage two to three days were needed for the individual snails to grow and to fill their eggs before hatching into the egg cases and often another day to breakout of the egg case. On average it took nine days for snails to develop and hatch at 25.5°C. While for snails raised at 30.0°C or 33.0°C development was accelerated by a shorter veliger or hippo stage and hatching occured within eight and seven days respectively.

The mortality of snails at each temperature is also important in determining the optimal temperature for the early development of *A.cumingii*. The mean mortality for 25.5°C was found to be the highest at 56.3%, the mean mortality at 30.0°C and 33.0°C were found to be very similar at 31.2% and 32.2% respectively. Combining both, time development and mortality the optimal temperature was determined to be between 30.0°C and 33.0°C.

3.2. GROWTH

When maintaining any organism in laboratory cultures, growth rates and eventual size can be important. In biological monitoring experiments, Gray and Ventilla (1971) indicated that growth rates were a more meaningful bioassay technique than mortality tests. Growth represents an 'integration of a wide range of physiological processes' (Stromgren 1982). Therefore, growth may be used as an end-point in biological monitoring, as it indicates an integrated tolerance of an organism to pollution exposure. Examples of previous investigations using growth to indicate the effect of a pollutant include a polychaete worm, *Ophryotrocha labronica*, a brine shrimp, *Artemia salina* (Brown and Ahsanullah 1971) and a mussel, *Mytilus edulis* (Stromgren 1982).

3.2.1. INTRODUCTION

When organisms are kept in cultures at laboratories the density may affect their growth. To determine any effect density has on growth two separate experiments were carried out; one concerning juvenile snails (up to five weeks in age) and the other concerning larger snails (greater than 4 mm).

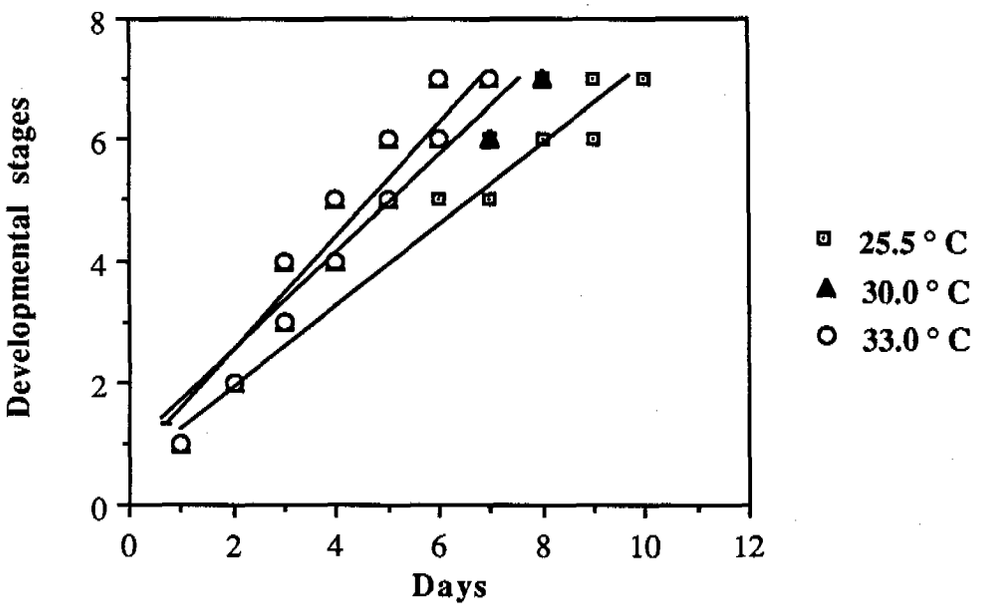

Figure 3.1

3.2.2. MATERIALS AND METHODS

Owing to the fragile nature of the shell of juvenile *A.cumingii*, the experimental techniques used to study the effect of density on growth of juvenile snails differed from that used with the larger snails.

A. Juvenile Snails

Large numbers of hatchlings were procurred after incubation of egg cases laid on the same day. Hatchling snails were placed in large vials at four densities; 10, 20, 40 and 80, with duplicates of each density. As this experiment was to determine the growth of juveniles up to five weeks of age, five replicate sets of vials were set up. The snails in each set of vials were allowed to grow for a different number of weeks, from one to five. Juveniles were removed from their tanks and preserved in 70 % alcohol at which time, length was measured using a compound microscope and a graticule. A separate one way analysis of variance was conducted for each age group.

B. Larger Snails

Different densities of snails were established by the use of a set number of snails in tanks of varying size (2, 4 and 9 L). Thirteen snails ranging in size from 4 mm to 14 mm were randomly selected from the stock, one snail from each millimetre size class except for 6 mm and 7 mm where two specimens were selected, and placed in each tank. Duplicates of each tank size were set up at both the laboratory and one field site, George Town. This experiment was carried out for a period of eight weeks.

Each snail was marked with nail polish, using a coding system whereby no more than two spots were placed on any individual snail. At intervals from six to eight days the shell lengths were measured thus enabling the growth of all individual snails to be followed. Any snail less than 13 mm in length which died in the course of the experiment was replaced with a snail, of the same size or when not possible with a snail from the original millimetre size class of the dead snail, which was appropriately marked. However, snails larger than 13 mm that died were not replaced. Every second day the snails were fed on a diet of lettuce with equal portions of approximately 5 g per tank.

A two way repeated measures analysis of variance was carried out to determine the effects of density, site, time or any interaction on the growth of snails.

3.2.3. RESULTS

A. Juvenile Snails

Of the five different age groups only three showed a significant effect of density on growth. The one week old ($F=10.81$ on 3, 20 d.f.; $P<0.001$), the two week old ($F=13.97$ on 3, 39 d.f.; $P<0.001$), and the four week old groups ($F=24.96$ on 3, 36 d.f.; $P<0.001$) all showed a very highly significant effect of density on growth. The three week old group ($F=2.83$ on 3, 31 d.f.; $P>0.05$) was just not significant at the 5 % level while the five week old group ($F=0.82$ on 2, 49 d.f.; $P>0.1$) showed little or no effect of density on growth.

Greater growth as densities decrease, would be the expected pattern, as it should allow individual snails more room to grow while higher densities crowd individuals, not allowing maximum growth. Figure 3.2. shows this pattern in only two age groups, three and four weeks. The possible reason for the different patterns observed was the very high mortality present in all cases. With high mortality actual numbers per vial change and therefore, densities are not constant throughout the experiment. Also, with high mortality the number of snails measured to determine lengths was very low, thereby, allowing individual snail lengths to have great effect on the mean length of each group.

B. Larger Snails

In the repeated measures analysis of variance carried out to determine the effect of site on the growth of larger snails it was found that there was no significant difference ($F=1.26$ on 1, 77 d.f.; $P<0.2$). With mean growth per week only slightly higher at the field (0.687 mm) than at the laboratory (0.573 mm), growth appears to be very similar at both sites although, it was observed during the experiment that growth was generally greater in the field than in the laboratory. This non significant result may be related to the higher mortality in the field than in the laboratory (see section 4.2.2.B.). With a greater mortality there were a greater number of replacements, thus creating many periods where individual snails were required to become accustomed to experimental conditions. As snails were held in tanks similar to those used in the laboratory experiment, but smaller, the adjustment is not as severe as in the field. The non-significant finding of density on growth ($F=1.94$ on 2, 77 d.f.; $P<0.1$) indicates that *A.cumingii* grows at the same rate, at any of the densities tested.

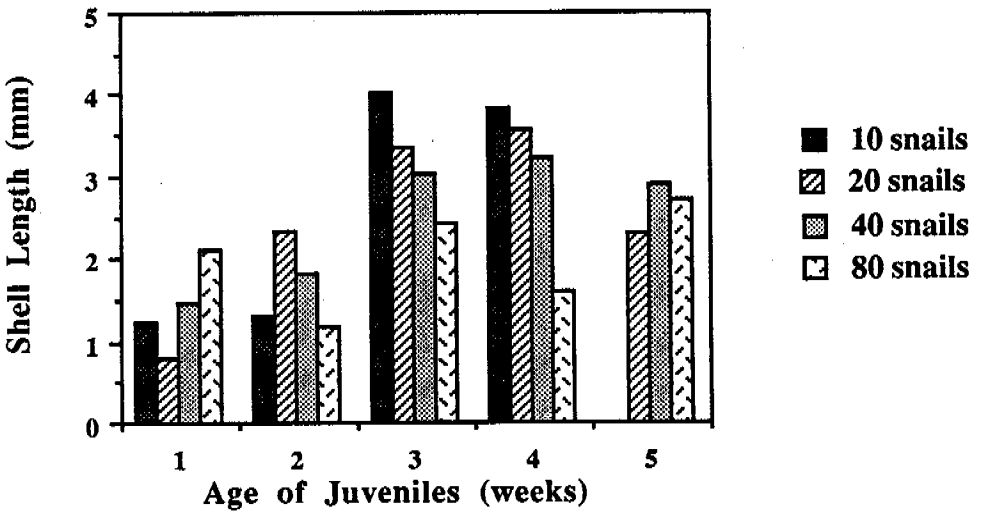

Figure 3.2

A highly significant effect of time was determined (F=6.14 on 7, 539 d.f.; P<0.001) showing periods, throughout the experiment, of varying growth rates. As the experiment continued through the wet season growth was found to be highly significantly (F= 4.82 on 7, 539 d.f.; P<0.001) effected by time. Growth decreased (Table 3.1.) in all but two weeks in the laboratory, while at the field there was only a very general decrease as time passed. The pattern of growth at the field may partially be explained by the combination of pH, conductivity and temperature levels determined throughout the experiment. For example, in week three the mean growth was the highest with 1.467 mm. This period also had high mean pH (5.99) and mean conductivity (18.86us/cm) levels and the highest mean temperature (32.14 C) of the experiment. The order of these values does not parallel the order of growth in the field and therefore, it can only partially contribute to the growth of *A.cumingii* in the field and to the pattern during the eight weeks of this experiment.

Table 3.1.
The mean growth of large (>4 mm) *Amerianna cumingii* over a period of eight weeks at two sites, laboratory and field.

Site / Week	Laboratory	Field (George Town)
1	0.820	0.743
2	0.874	0.715
3	0.797	1.467
4	0.588	0.446
5	0.555	0.638
6	0.121	1.069
7	0.575	0.050
8	0.253	0.371

CHAPTER FOUR

4. MORTALITY

The freshwater snail *Lymnaea stagnalis* was found to be a suitable test organism for pollution monitoring using mortality as a criterion (Canton 1977). This species is sensitive to disturbance, able to reproduce many times in the one season, has short life cycles and are cosmopolitan in distribution so are readily available for experimentation. *A.cumingii* also has these characteristics except for the distribution but is still readily available due to laboratory cultures. Therefore, to allow the assessment of mortality as an end-point in biological monitoring, several experiments were carried out to determine the variation in mortality of snails subjected to experimental conditions.

When Wier and Walter (1976) used mature and immature freshwater snails, *Physa gyrina*, to determine 50 % lethal tolerance levels to cadmium, it was discovered that the immature snail was three times more sensitive to this element. In an attempt to determine if this was also true for *A.cumingii*, snails were divided into groups made up of recently hatched snails, juveniles and adults.

4.1. RECENTLY HATCHED JUVENILES

4.1.1. INTRODUCTION

The investigation of mortality in *A.cumingii* must begin with recently hactched juveniles to thoroughly determine the change in mortality with age.

4.1.2. MATERIALS AND METHODS

Two 9 L tanks were set up at both laboratory and field (George Town) sites, each tank containing five vials with a pair of snails of the same millimetre size class in each. Snails vary in size from 10.0 to 14.9 mm with all size classes being used. On the fourth day of the experiment, eggs were counted and adult snails removed from the vials. The vials were then placed back in the tanks and the egg cases left to incubate for a period of ten days. After the adults were removed the large mesh, used to retain the adult snails,was replaced with smaller mesh (0.5 mm) to prevent the escape of any hatchlings.

After the ten day incubation period vials were placed in 50 % ethanol to preserve all hatchlings. Counts were made of juveniles alive and dead at the time of preservation (as determined by the presence or absence respectively of soft tissue in the shells). Snail shells with no soft tissue parts or with obviously decaying parts were counted and considered dead before preservation. By using this data the variability of mortality of recently hatched juveniles was determined.

4.1.3. RESULTS

From the two sample analysis of variance conducted on data collected there was no significant difference ($t=0.10$ on 36 d.f.; $P>0.5$) between laboratory and field sites in the mortality of recently hatched *A.cumingii*.Table 4.1. shows the mean values of mortality at laboratory (32.28%) and field (31.24%) sites to be similar. Both had large standard deviations (laboratory 29.60%; field 17.93%) which produce wide variation in mortality at this age.

4.2. DENSITY DEPENDENT MORTALITY

4.2.1. INTRODUCTION

The need for considerable numbers of snails for experimental work necessitates efficient management of laboratory cultures. Knowledge of mortality experienced by *A.cumingii* at different densities may enable better maintencance of the snail.

4.2.2. MATERIALS AND METHODS

A. Juveniles

Mortality data was collected during the experiment carried out in section 3.2.2. A two way analysis of variance was calculated to determine the effect of density on mortality.

B. Larger Snails

Mortality data was collected during the experiment carried out in section 3.2.3. Survival curves were graphed for the three densities at both laboratory and field sites. A generalised Wilcoxon (Breslow) statistic and a generalised Savage (Mantel-Cox) statistic were calculated to determine if the survival curves were significantly different.

Table 4.1.
Mortality of recently hatched *Amerianna cumingii*.

Site	Number of Vials	Mean Mortality (%)	Minimum Mortality (%)	Maximum Mortality (%)	Standard Deviation
Laboratory	25	32.28	0.00	98.00	29.60
Field	29	31.24	4.00	78.00	17.93

Table 4.2.
The mean percentage mortality of five age groups of juvenile *Amerianna cumingii* at four different densities.

Age (weeks)	1	2	3	4	5
Density (number per vial)					
10	76.71	67.50	76.71	70.38	90.00
20	77.08	56.10	68.54	67.21	90.00
40	77.08	68.25	69.29	64.45	56.96
80	87.13	85.45	73.32	81.27	68.24

4.2.3. RESULTS AND DISSCUSSION

A. Juveniles

It was discovered that there was no significant effect of density (F=2.22 on 3, 38 d.f.; P<0.1) on the mortality of juvenile snails. The different ages were also found to have no significant effect (F=1.134 on 4, 38 d.f.; P<0.1) on mortality. In all cases mortality was extremely high, with a range of 56.96 to 90.00 % mortality per vial (Table 4.2.).

B. Larger Snails

Figure 4.1. shows the survival curves of the three densities tested (2, 4 and 9 L) at both sites (laboratory and field) it can be seen that mortality at the field site is higher than the mortality at the laboratory site. Variables controlled in the laboratory with the static stored water may contribute to this reduction in mortality at the laboratory but it is not easily explained .

A generalised Wilcoxon (Breslow) statistic was determined and the differences between the survival curves were found to be highly significant (49, 127 on 5 d.f.; P<0.001). In support of this the generalised Savage (Mantel-Cox) statistic (59.801 on 5 d.f.; P<0.001) was found to be highly significant.

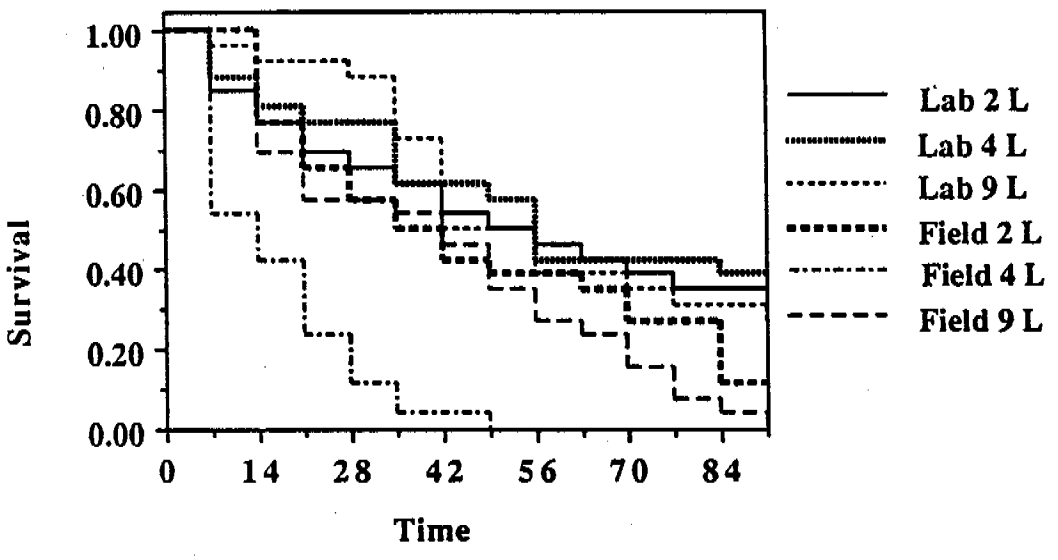

Figure 4.1. The survival curves of large (>4 mm) *Amerianna cumingii* at three densities (2, 4 and 9 L) and two sites (laboratory and field).

CHAPTER FIVE

5. FOOD AND FEEDING

Freshwater pulmonate snails may be termed 'grazing herbivores' with a diet consisting of living and decaying plant material. Thus, food items and energy sources for snails may include and be derived from macrophytes, epiphytes and/or detrital material. The food of natural populations of *A.cumingii* is unknown. While laboratory stocks of snails will readily consume supplies of lettuce leaves, the nutritional value of this food is unknown.

5.1. INTRODUCTION

With regards to food and feeding the aims of this study on feeding were to provide some information upon possible food sources for *A.cumingii* (natural and unnatural) including the palatability of some potential food items in terms of utilisable energy sources. This information is essential for the optimal feeding conditions for snails during experimentation. Possible food sources that were investigated are macrophytes, epiphytes and settled detritus and dissolved organic substances.

5.2. MATERIALS AND METHODS

In all food and feeding experiments, duplicate pairs of snails of 8, 9 and 10 mm size classes were used for each treatment (type and amount of food). Egg numbers were determined and scraped off daily for each vial, which held one pair of snails each. At weekly intervals, for the three weeks of all experiments, shell lengths and snail weights were measured. All experiments were carried out at the laboratory.

5.2.1. Macrophytes and Lettuce

In the initial investigations of the macrophytic food source, palatability testing was carried out on a wide variety of macrophytes found in the Magela Creek waterbodies. On the basis of this broad screening, three palatable macrophytes were selected for further investigation.

For lettuce and one macrophyte, a comparison was also made between two quantities of plant material given to snails, one or two portions per day. The remaining two macrophytes were supplied to the snails at the rate of one portion per day, thus

creating a total of six treatments. Statistical analsis of data was carried out using a repeated measures analysis of covariance with size of snail being the covariant.

5.2.2. Epiphytes and Settled Detritus

Epiphytes were procurred by exposing vials to billabong water stored in a large tank, for one and two week periods. From counts of algal blooms it was determined that there was approximately 65% more epiphytic material accumulated in a two week period than in a one week period. Settled detritus is unavoidably associated with the colonisation of epiphytes but determination of exact amounts accumulated on vials was unknown. Vials with fresh epiphytic material were given to experimental snails daily.

5.2.3. The Water

The contribution of organic material dissolved or suspended in water was tested using different concentrations of billabong (relatively enriched with organic matter) and stored Magela Creek waters (low in organic matter). Snails were exposed to three different concentrations of billabong water (diluted where necessary with stored Magela Creek water) and stored Magela Creek water (control). In addition snails were also exposed to the billabong water after it was passed through a 1-2μm filter to determine whether or not snails utilise the dissolved fraction in water for nutritional requirements.

The water rich in organic matter was collected prior to the commencement of the experiment at Anabangbang Billabong. All tanks used in these experiments were 2 L in size and were totally replenished with fresh water daily. To avoid any accumulation of settled detritus on the vials they were suspended vertically in the water and all vials were cleaned daily.

5.3. RESULTS

5.3.1. Macrophytes and Lettuce

When *A.cumingii* was fed various macroph ytes it was found that the type of macrophyte used as a food source was responsible for significant differences in the number of eggs laid ($F=4.05$ on 6, 34 d.f.; $P<0.01$). Although, the covariate of size of the snail was found to have no significant effect upon egg production ($F=1.62$ on 1, 34 d.f.; $P>0.20$) indicating no difference in egg production from snails 8.0 to 10.9 mm in size. Table 5.1. shows that larger numbers of eggs were laid by snails fed lettuce than

Table 5.1.
The mean number of eggs laid by *Amerianna cumingii* when fed on different macrophtic diets.
1- one portion of lettuce; 2- two portions of lettuce; 3- one portion of *Caldesia oligoeocca;* 4- two portions of *Caldesia oligoeocca;* 5- one portion of *Persicaria attanuata;* 6- one portion of *Hydrilla verticillite;* 7- no food.

Macrophytes	1	2	3	4	5	6	7	Mean
Week one	7.13	9.66	3.19	3.22	4.37	6.33	1.99	4.82
Week two	4.70	43.53	0.00	0.03	0.00	0.17	0.00	1.78
Week three	72.17	162.47	0.00	0.00	0.00	0.00	0.00	9.21
Mean	19.75	56.01	0.35	0.43	0.49	0.95	0.22	4.79

Table 5.2.
The mean growth of *Amerianna cumingii* over a three week period whilst fed on various macrophytic diets.
1 - one portion of lettuce; 2 - two portions of lettuce; 3 - one portion of *Caldesia oligoeocca;* 4 - two portions of *Caldesia oligoeocca;* 5 - one portion of *Persicaria attanuata;* 6 - one portion of *Hydrilla verticillite;* 7 - no food.

Macrophytes	1	2	3	4	5	6	7	Mean
Week one	-0.16	-0.27	-0.28	-0.12	-0.17	0.03	0.03	-0.13
Week two	0.19	0.19	0.07	-0.08	-0.37	-0.22	-0.11	-0.05
Week three	0.30	-0.03	0.23	-0.07	0.23	-0.05	-0.13	0.07
Mean	0.11	-0.04	0.01	-0.09	-0.10	-0.08	0.07	-0.04

by snails fed any other macrophyte, with two portions of lettuce producing almost twice as many eggs (mean eggs laid per week =7.48) as one portion (mean eggs laid per week =4.44).

The type of macrophyte fed to snails was found to have significant effect on the number of eggs laid per week ($F=14.91$ on 2, 69 d.f.; $P<0.001$). There also existed a significant interaction between macrophyte treatment and time ($F= 9.60$ on 12, 69 d.f.; $P<0.01$), with numbers of eggs being laid decreasing with all food types except lettuce, which increases. Energy stored prior to the experiment may explain production of eggs during week one, for snails fed on diets other than lettuce, with little or no egg production in weeks two and three when stored energy was consumed.

Table 5.2. shows the mean growth of snails fed on the various macrophytic diets over a three week period. In many cases negative values were determined indicating shell breakage or chipping. The effect of different macrophytes and lettuce had no significant effect ($F=1.03$ on 6, 34 d.f.; $P>0.4$) on the growth of individual snails with no significant effect of the covariate, shell length ($F=0.47$ on 1, 34 d.f.; $P>0.4$).

5.3.2. Epiphytes and Settled Detritus

It was discovered that the amount of epiphytic material fed to *A.cumingii* had a significant effect on egg production ($F=5.95$ on 2, 15 d.f.; $P>0.01$). Larger amounts of epiphytic material fed to snails enabled them to lay more eggs. Table 5.3. shows the egg production of snails fed on two weeks of epiphytic growth per day (mean 4.92 eggs laid per week) is greater than that for snails fed on one week (mean 3.42 eggs laid per week) or no epiphytic growth (mean 0.36 eggs laid per week).

There was also a significant time effect on egg production ($F=5.36$ on 2, 30 d.f.; $P>0.01$) with a decrease as time progresses (Table 5.3.). This may indicate that the energy derived from the epiphytic material was not sufficient to allow egg production to continue as 'normal' after energy stored prior to the experiment was consumed. With greater amounts of epiphytic material reproduction may have continued for a longer period of time.

The effect of epiphytic material on snail growth was found to be not significant ($F=0.18$ on 2, 15 d.f.; $P>0.5$), indicating that the energy gained from consuming the

Table 5.3.
The mean number of eggs laid by *Amerianna cumingii* when fed on different amounts of epiphytic material.
1 - clean vial; 2 - one week of epiphytic growth; 3 - two weeks of epiphytic growth.

Epiphytes	1	2	3	Mean
Week one	1.11	10.19	13.47	5.70
Week two	0.33	1.14	3.86	1.45
Week three	0.03	1.65	1.04	0.68
Mean	0.36	3.42	4.92	2.42

Table 5.4.
The mean growth of *Amerianna cumingii* when fed on different amounts of epiphytic material.
1 - clean vial; 2 - one week of epiphytic growth; 3 - two weeks of epiphytic growth.

Epiphytes	1	2	3	Mean
Week one	-0.09	-0.43	-0.10	-0.21
Week two	-0.10	0.07	-0.03	-0.02
Week three	-0.03	0.03	0.00	0.00
Mean	-0.08	-0.11	-0.04	-0.08

amount of epiphytic material offered to the snails was not sufficient to allow growth to occur. As seen in Table 5.4. growth in many cases was negative due to chipping of shells during experimentation. The chipping may have occurred due to the poor quality of the diets snails were fed.

5.3.3. The Water

When *A.cumingii* was offered different water types as sources of energy it was discovered that there was no significant effect ($F=0.61$ on 5, 30 d.f.; $P>0.5$) on egg production. Table 5.5. shows the mean egg production of snails supplied with the different water types as energy sources. The effect of time on egg production was also tested and was found to be significant ($F=16.00$ on 2, 60 d.f.; $P<0.001$) with fewer eggs being laid as the experiment continued.

The effect of the different water types was also tested on growth of *A.cumingii* and found to be not significant ($F=0.47$ on 2, 60 d.f.; $P>0.5$). Once again growth values (Table 5.6.) were marginal with negative and positive values indicating breakage or chipping of shells during the experiment.

Table 5.5.
The mean number of eggs laid by *Amerianna cumingii* when fed on enriched water diets.
1 - 100 % billabong water; 2 - 50 % billabong water and 50 % creek water; 3 - 25 % billabong water and 75 % creek water; 4 - 100 % creek water; 5 - filtered billabong water; 6 - unfiltered billabong water.

Water Type	1	2	3	4	5	6	Mean
Week one	3.52	6.56	2.60	8.65	0.11	5.36	3.76
Week two	0.06	0.14	0.06	0.03	0.54	1.79	0.26
Week three	0.19	0.00	0.00	0.00	0.14	0.00	0.02
Mean	0.72	0.96	0.38	1.07	0.23	1.48	0.75

Table 5.6.
The mean growth of *Amerianna cumingii* when fed on enriched water diets.
1 - 100 % billabong water; 2 - 50 % billabong water and 50 % creek water; 3 - 25 % billabong water and 75 % creek water; 4 - 100 % creek water; 5 - filtered billabong water; 6 - unfiltered billabong water.

Water Type	1	2	3	4	5	6	Mean
Week one	-0.15	-0.03	0.00	-0.10	-0.35	0.02	-0.10
Week two	0.03	-0.05	-0.07	-0.10	0.12	-0.22	-0.05
Week three	0.02	-0.15	0.00	0.00	-0.03	-0.07	-0.04
Mean	-0.03	-0.08	-0.02	-0.07	-0.09	-0.09	-0.06

CHAPTER SIX

6. DISCUSSION AND CONCLUSIONS

6.1. DISCUSSION

From the experiments in this study, many discoveries were made about the natural variation in the biological and ecological factors of *A.cumingii*. With a view to the use of this freshwater snail as a biological monitor for the Ranger Uranium Mine all findings in this study are important in establishing base line information about this previously uninvestigated species.

The mortality of juvenile snails was found to be very high in experiments involving different densities at both laboratory and field sites and therefore, may create problems if this parameter was used to determine the effect of a pollutant. At this stage of the life cycle, *A.cumingii* is very fragile and the smallest disturbance may be lethal. For this exact reason, this parameter is used as an indicator of pollution but all care must be taken to prevent accidental death through handling or experimental error so the true effect of the pollutant can be determined. The same care is essential in the natural mortality of hatchlings. If mortality of juveniles is to be used as an indicator of pollution, all caution should be taken to ensure that mortality due to pollution can be identified and separated from the high natural mortality of this species.

Juvenile growth experiments were effected by the high mortality so that very little could be derived from data in a quantitative manner, although a pattern of lower densities giving rise to high growth did appear for two periods tested. As a biological monitoring parameter, juvenile growth appears to be very variable and highly affected by mortality and therefore, further investigation should take place prior to possible use.

Mean growth for snails greater than 4 mm was found to be similar for both laboratory and field sites although, the growth at the field greatly varied over the eight weeks of the experiment it was observed that growth was generally greater than at the laboratory. The lower survival of snails in the field than in the laboratory may have contributed to this finding. In the event of biological monitoring, adult snail growth and mortality findings will be most helpful in maintenance of the snails in the laboratory cultures. Therefore, if laboratory methods are used, greater variability of growth and higher mortality in the field will not be a major concern.

Of importance in maintaining snail cultures is the establishment of the optimal temperature for the early development of *A.cumingii*. At 33.0 C juveniles hatched in a period of seven days while at lower temperatures the development was found to be slightly slower. Knowledge of mortality at the different temperatures was also critical in determination of an optimal temperature for the developing eggs. As snails developing in water at 25.5 C had a mortality of only 56.3% compared with 32.2% and 31.2% at 31.2 C and 30.0 C respectively, it was determined that the optimal temperature for the early development of *A.cumingii* was between 30.0 C and 33.0 C, with juveniles hatching between seven and eight days after being laid.

No significant difference was found in the densities of snails tested to produce the maximum number of eggs per vial. In terms of biological monitoring experiments more juveniles will, therefore be produced by placing fewer snails per vial and having a greater number of vials than having many snails per vial. It is recommended to use one snail per vial for experimentation to reduce the variabilities of egg production. (Cross-ferilisation would have occurred in the snail culture prior to the experiment).

The most prolific time of egg laying for *A.cumingii* was found to be between 0200 and 0800 hours. This information may assist in the refinement of the use of eggs in a biological monitoring protocol. By enabling a shorter period of time to be allocated to the egg laying portion of the experiment, juveniles hatching will be as close as possible to the same age, thereby reducing possible variables.

When selecting individual snails for experiments involving reproduction it is important to take the many variables into consideration. Fecundity was found to vary considerably with size in the series of experiments carried out in this study. In attempting to produce the maximum number of eggs per vial it is also important to select individual snails that are of a size that lay many eggs; small snails tend to lay less eggs than larger snails. Also to be noted is that when two snails are of the same size but different ages the younger snail will produce the most eggs. A further consideration is the time of the year as *A.cumingii* almost exclusively produces during the monsoonal wet season with the greatest number of eggs produced at the start of the wet and fewer as it continues.

In the food and feeding trials it was found that a large quantity of epiphytic material fed to snails enabled a greater number of eggs to be laid than the number laid by snails on little or no epiphytic material. As epiphytes are a food source found in the natural habitat of *A.cumingii* it is feasible to assume that epiphytes contribute to the

natural diet of this species of freshwater snail. Lettuce, although not present in natural surroundings was able to supply snails with energy to produce the greatest number of eggs of all food sources tested. This indicates the possible use of a native macrophyte in the natural diet of *A.cumingii* . If so, it was not one of the macrophytes tested in this study.

6.2. CONCLUSIONS

Results achieved in this series of experiments will be employed by the Alligator Rivers Region Research Institute in designing monitoring methods using *A.cumingii* as a pollution monitor of the waste water releases from the Ranger Uranium Mine in Kakadu National Park. Knowledge gained in this study will assist in biological monitoring in several ways.

FOOD AND FEEDING

- Lettuce was found to be an adequate food source for snails. Although, not a natural food it was found to maintain snails in cultures. The advantage of using lettuce over epiphytic material is in its availability in appropriate quantity and quality for experimentation.

- Snails produce more eggs when they are fed than when they are starved; therefore, in experiments involving production of eggs it is essential to feed snails.

- When maintaining snails in large tanks algae should be allowed to grow on the surface of walls to produce a supplement to the diet of lettuce. However, it is still important to change the water in tanks regularly when it becomes green with too much algae otherwise it may become detrimental to the health of the snails.

LOCATION OF SNAIL CULTURES

- It is recommended that snail cultures are maintained at the laboratory rather than at the field as mortality, although variable at both sites, tends to be lower in general at the laboratory.

- The above was supported by the more stable growth of adult snails in the laboratory than in the field.

EARLY DEVELOPMENT

- While water temperature is between 30.0 C to 33.0 C, there is no need to separate developing eggs into temperature controlled tanks as development time and mortality are relatively low within this range. If water temperature falls close to 26.0 C, and development is still required for experimentation, then eggs need to be placed into tanks with controlled water temperature.

REPRODUCTION

- To obtain hatchlings as close to the same age as possible, snails should be allowed to lay between the period 0200 and 0800 hours as this is the most productive egg laying period. For practical purposes, leaving snails in vials overnight to lay their eggs would be acceptable.

- There are important considerations when selecting and using individual snails for experimentation on reproduction. Egg production was found to vary with age, size, site and time of season. For optimal egg production the largest, healthiest (ie shell without markings of age) snail should be allowed to lay at the field during the early wet season.

- It is also recommended that one snail per vial be used for experiments to reduce the variability of egg production per vial.

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