

**UNIVERSITY OF CAPE COAST**

**EXPLORATION OF THE ECOTYPES OF *Achatina achatina* (L) FOR  
SNAIL FARMING IN GHANA.**

**BY**

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PHILOSOPHY DEGREE IN ZOOLOGY**

**AUGUST, 2011**

**DECLARATION**

CANDIDATE'S DECLARATION

I hereby declare that this thesis is the result of my own original work and that no part of it has been presented for another degree in this University or elsewhere.

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SUPERVISOR'S DECLARATION

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

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## ABSTRACT

Intraspecific variation in *Achatina achatina achatina* Linne (one of the numerous giant African snails) in Ghana was studied using chronological features, body characters and reproductive potential. Snails were collected from June to August, 2006, from four ecological regions namely Ashanti (Amansie East district), Central (Assin South district), Eastern (Suhum- Kraboa- Coaltar district) and Western (Wassa West district).

In this study, the four snail populations showed a broad range of variation in size, colour pattern of shell, aestivation pattern and reproductive potential. However, electrophoresis of the haemocyanin (blood pigment) and DNA of the snails from the four ecological regions did not show any differences between them.

Juvenile snails from the Western ecotype grew larger than those of the Ashanti ecotype. There was a significant difference in the aestivation pattern of the four ecotypes. Populations were characterized as distinct on the basis of shell colour, body colour, banding pattern and distribution of grey speckles on the body.

The results obtained from this research indicate that the Western ecotype was more amenable to easy culture. The basis for selection of the best ecotype were growth rate, life history traits such as clutch size, hatchability of eggs, survival of hatchlings and susceptibility to harsh environmental conditions or aestivation pattern. Therefore, the Western ecotype is recommended for farming in Ghana.

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## **DEDICATION**

This work is dedicated to Mr. Joseph Kwesi Parden, Mrs. Juliana Parden, Miss. Mary Parden and my siblings.

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## **CHAPTER ONE**

### **INTRODUCTION AND LITERATURE REVIEW**

Variation, that is differences in the frequency of genes and/or traits among individual organisms within a population, forms the basis of both evolution and classification. According to Begon *et al.*, (1990), several patterns of variation are found in living things. These have resulted in the differentiation of populations into races, varieties, polymorphic forms, ecotypes and subspecies.

A "geographic race" is an aggregate of phenotypically similar populations of a species inhabiting a geographic sub-division of the range of that species that differs taxonomically from other populations of that species. But although a race is restricted to a geographic sub-division of the range of a species, it is part of a larger species in spite of its certain diagnostic differences (Mayr, 1987). A variety, used more in plant systematics is usually put in a rank below sub-species. Varieties are usually the result of selective breeding and diverge from the parent species or sub-species in relatively minor ways.

A phenomenon similar to variety in animal biology is polymorphism, which is the occurrence together in the same locality at the same time of two or more discontinuous forms of a species in such proportions that the rarest of them cannot be maintained merely by recurrent mutation (Ford, 1975).

For example, the life-cycle of some cnidarians may alternate between two polymorphic forms polyp and medusa. With a few exceptions, a colonial polyp is sedentary attached to the substratum by the end opposite the mouth. Some well known solitary polyps are the sea anemone and the freshwater hydra. Thus, its tentacles are typically considered to point upward and outward (Bridge *et al.*, 1995). In some Orders the polyp stage is strongly or completely reduced. Polyps of some species propagate vegetatively, forming colonies (if the progeny remain attached to one another) or clones (if the progeny separate) of microscopic size. The sizes of individual polyps usually range from 2 to 1000 mm (Collins, 2002).

Polymorphism is exhibited by some land snails and such snails are polymorphic with respect to the body color, banding and shell colour. *Cepaea nemoralis* (grove snail) is one such species which exhibits polymorphism. The grove snail or brown-lipped snail is one of the most common species of land snails in Europe. It is a relatively small snail, growing to a height of about 20 mm and a width of 25 mm. Their semi-glossy shell has 4½ to 5½ whorls (Clarke *et al.*, 1996). Bands may either be present or absent (unbanded dominant to banded) and there is further variation in number, one to five bands in a certain order (but never more than five bands). *Cepaea nemoralis* occurs in three main colours which range from yellow and pink to dark brown, with a range of light and dark bandings and with the order of dominance as : brown > pink > yellow (Cowie and Jones, 1998; Dvořák *et al.*, 2003). Banded yellow snails are known to predominate in grassy habitats where the bands are presumed to provide camouflage amongst the linear or often vertical shadows, whereas unbanded



morphs ( especially unbanded pink and unbanded brown ) show preference for wooded sites (Barker, 2001). These shell colours were thought to act as camouflage to avoid predation from, for example, the song thrush, but also have implications for the body heat of the animal: darker shells heat up more quickly, with consequences for rates of metabolism and loss of moisture crucial in snail locomotion (Cowie and Jones, 1998; Dvořák *et al.*, 2003; Juříčková *et al.*, 2001).

Another dimension of polymorphism is provided by ants which although are basically social insects, but exhibit variation in size. A typical ant colony or nest contains an egg-laying queen and many adult workers together with their brood (eggs, larvae and pupae). Workers, which are by far the most numerous individuals in the nest are responsible for nest construction, nest defence and maintenance, foraging and tending the brood and queen (Shattuck and Barnett, 2001). Workers in a single nest can all be the same size or they can vary greatly in size. In some cases the variation in size can be so extreme that large workers are twice the size of small workers. If variation between small and large workers is discontinuous, with respect to variations in sizes of the heads and mandibles then the workers are described as polymorphic (Shattuck and Barnett, 2001).

Ecological types otherwise known as ecotypes are defined as local populations of a species which become adapted to their local habitat or distinct forms of a species occupying particular habitats (Begon *et al.*, 1990). Such ecotypes which may also be described as ecological races are the first step in species formation. However, while races are phenotypically similar populations of a species inhabiting a geographical sub-division of its range, ecotypes are distinct

forms of a species occupying particular habitats. If populations or groups of populations show ecological differences, regardless of morphological characteristics; they are said to be ecotypes. Therefore the term ecotype is applied to any population differentiated in respect of any characteristic attributed to the selective action of ecological factors.

The different habitats present different environmental conditions to the various ecotypes to which they respond differently. The biotic response of species to their changing environment may involve modifications in their physiology, morphology and distribution (Hughes, 2000). One consequence is that the individuals of a species that occur in one part of its range often look different from those that occur elsewhere (Raven and Johnson, 1996). For example, it should be expected that if two individuals of the same species occur at different areas where one area abounds readily in food and the other area is deficient in food sources, there may be differences in the alimentary tract (Riddihough and Pennisi, 2001).

Patterns of ecotypic variations are exhibited in several species of animals including fishes, amphibians, birds, reptiles and mammals. For example ecotypes of killer whales, termed “resident,” “transient,” and “offshore” have been described in the coastal waters of the Northeast Pacific Ocean. These terms were originally designated by killer whale researchers to describe the whales’ patterns of occurrence, particularly in British Columbia and Washington. “Residents” were generally in inland waters all summer, whereas “transients” only appeared occasionally, and “offshore” whales were seen only in outer coast waters (AFSC

Quarterly Research Reports, 2003). In terms of diet, resident killer whales are known to be primarily fish-eaters; in contrast to transients that feed primarily on marine mammals. Relatively few feeding observations have been made for the offshore type, but initial data suggest they also eat fish (Matkin *et al.*, 2007).

The three recognized Northeast Pacific killer whale ecotypes also exhibit some morphological variations. The resident (fish-eating) type has a more falcate dorsal fin and greater saddle patch variation. In particular, a black cup, finger, swirl or open area may intrude into the top of the white saddle patch (Matkin *et al.*, 2007). However, in the transient (mammal-eating) type the dorsal fin is often more triangular with a broad base and the saddle patch is large and uniform (without black intrusions, although sometimes with a “feathering” pattern along the front edge of the saddle). Then in the offshore-type, the dorsal fin is often rounded at the tip with multiple nicks in the fin (Matkin *et al.*, 2007). The saddle patch can also have black intrusions like resident-type whales (AFSC Quarterly Research Reports, 2003).

In trouts, three ecotypes are found which are the lake-resident, lake-river and riverine ecotype. Trout within the lake-resident ecotype use a combination of one lake (Kukaklek or Nonvianuk) and the respective lake outlet and inlet tributaries. During the spawning (April-June) and post-spawning or feeding season (July-September) the fish remain at the lake outlets or migrate upstream through the lakes to the inlet tributaries, where sockeye salmon begin spawning activity in August and September (Meka *et al.*, 2000).

Fish within the lake-residents ecotype exhibit both migratory and non-migratory behaviour and sockeye salmon influence the movement of trout during the post-spawning and winter seasons. Trout within the lake-river ecotype use the lakes, inlet tributaries and the Alasgnak River main stream. During spawning season they migrate downstream to the braided reaches of the main stream. Fish within this ecotype made longer migrations than any other ecotype and there was no record of movement between lakes indicating lake basin fidelity. Fish within the riverine ecotype exhibit both highly variable seasonal migratory behaviour, as well as non-migratory behaviour (Meka *et al.*, 2000).

Ecotypic variation seems to be even more pronounced in invertebrates. Populations of the shore-dwelling rough periwinkle, *Littorina saxatilis*, in some tidal areas in Galicia, Spain are large and robust ecotype with a ridged and banded shell which occupies the upper intertidal zone of barnacles, whereas a small and fragile ecotype with a smooth unbanded shell is confined to the lower intertidal zone of blue mussels. There are two main differences between the ecotypes. On the average the size of the ridged and banded ecotype is nearly twice the size of smooth and unbanded. Also the relative area of the shell aperture of smooth and unbanded ecotype is greater than that of the ridged and banded (Rolan-Alvarez, 2007). They also differ in behaviour and even physiology. However, there is a metre-wide mid-shore zone in which a small proportion of the snails are hybrids.

Basically, ecotypes had evolved by strong divergent selection favouring morphological differences in shell size, ornamentation and colour. The large foot

and shell aperture, present in the lower-zone ecotype, provide the best holdfast to the rocky surface during wave-splash (Rolán-Alvarez, 2007). The distribution of Galician snails overlap in narrow hybrid zones and also in this case strong divergent selection maintains the morphological differentiation in spite of a gene flow. In some non-tidal areas (e.g. Sweden) different ecotypes occupy adjacent shores of different substrata such as boulders and rocks (Rolan-Alvarez, 2007).

A possible scenario for the *Littorina* snails is related to size differences. In Sweden, for example, small individuals are favoured on cliff substrates probably because they resist wave forces better due to a less pronounced hydrodynamic profile. In contrast, large individuals are favoured among boulders, because these are more robust against crab attacks. Size differences may impose technique problems during mating that prevent mates of unequal sizes to mate at random. However, ecotypes can interbreed (Rolan-Alvarez, 2007).

Studies on the endemic land snail genus *Mandarina* of the oceanic Bonin Islands in Japan have provided evidence that speciation on different islands of the three main archipelagos was such that similar ecotypes evolved independently in different lineages and islands (Davison and Chiba, 2006). As most of the characters involved are inherited, then variation between ecotypes must represent genetic differences between populations. However, while the diversity of ecotypes present at each site is dependent on the regime of natural selection and competition, habitat or ecology still must have an important role (Davison and Chiba, 2006).

In West Africa, a Giant African land snail, *Achatina achatina*, is one such species thought to exhibit ecotypic variation (Cobbinah, 1992). However, this is an allusion that has not been substantiated by any empirical evidence. It is alluded that there are several ecotypes i.e. locally adapted populations of *Achatina achatina*, showing differences in growth rates, size and aestivation patterns (susceptibility to dry environmental conditions), shell and or body colouration and even flavour. The indication now is that *Achatina achatina* exhibits extensive geographical variation and superimposed on this is a pattern of extensive variation within populations.

The most popular terrestrial snails in Africa belong to two main genera: *Achatina* (Lamarck) and *Archachatina* (Albers). Some local names of *Achatina achatina* are; ‘Nwapa’ (Akan), ‘abobo’ (Ewe), ‘kreketete’ (Hausa), ‘elonkoe’ (Nzema) and ‘waa’ (Ga). Species of both genera are common south of the Sahara, *Achatina achatina* being the most common species in West Africa, while *Archachatina marginata* occurs more commonly in Southern Nigeria and in the Congo Basin (Cobbinah, 1992; Hodasi, 1984).

The giant African snails actually belong to the family Achatinidae (Mollusca, Gastropoda, Pulmonata, Stylommatophora) whose size have intrigued scientists and shell collectors (Parkinson *et al.*, 1987). It is the large size to which some of the species can grow that has earned them the description “giant African snails” or “giant West African snails” (Monney, 1992; Mead, 1961; Hodasi, 1986).

There are many genera and species which are geographically irregularly distributed. Some members especially of the genera *Achatina* and *Archachatina* reach their peak of diversity and abundance in the forest zones of West Africa (Parkinson *et al.*, 1987). *Achatina achatina* (L) can measure up to 20 cm in shell length. It was Bruggen (1986) who chose an arbitrary criterion of shell length of 80 mm or more as the qualification of a giant snail and therefore estimated that there were 33 genera of 200 species in the Achatinidae out of which 8 genera with 55 species qualify as giant snails. On the basis of shell features, Bequaert (1950) estimated the possible number of *Achatina* subgenera to be 8 with 65-80 species. Also, 4 subgenera and many species of *Archachatina* were described. Hence, there are overlaps in the many species and sub-species and because no breeding experiments have been carried out, no concrete evidence has been established in there. In all the attempts to unravel the biology of giant snails for farming, this is one area that seems to be overlooked in numerous experiments and therefore the phenomenon of ecotypes needs to be explored for the farming of giant African snails.

In Ghana, four species of the giant African snails are recognized. These are *Achatina achatina*, *Achatina fulica*, *Archachatina marginata* and *Archachatina degneri*. All four species of the Achatinidae are eaten in Ghana. The most popular of these is *A. achatina* whose descriptive name in one of the local languages translates literally as “good snail” (Monney, 1994).

*Archachatina marginata* described as “Tarkwa nwapa” in one of the Ghanaian languages is one species of the Achatinid group of snails. The albinos of *Archachatina marginata* predominate and it is the commonest giant African snail in Nigeria. This species can be recognized in several ways. The most obvious is the tip of the shell is rounded or blunt. Several *Archachatina* species also have a distinctive red, orange or pink tip to the shell. The *Archachatina* species have a dome-shaped shell apex and lay small number of large eggs. *Archachatina marginata* grows large and usually becomes as big as *Achatina achatina*. They have been known to reach 20 cm in shell length although as with all maximum sizes this is unusual. Morphological adaptation such as the presence of the shell makes it tolerant to dry weather (Barnes, 1991).

*Archachatina degneri* is an unpopular species with most tribes in Ghana but, it appears to be cosmopolitan in distribution. This species has become naturalized to human dwellings and can be found in farmlands. It is argued that chewing *Archachatina degneri*, for example, does not crush and render soft the pieces of snail but instead merely breaks them up into smaller, rubbery firm particles. It is felt that too much time is spent cooking *A. degneri* to make it tender. The low popularity of *A. degneri* is also due to the allergic reaction some people get after consumption apart from its characteristic smell which puts off snail consumers. Sometimes snail collectors have to pick *Archachatina degneri* from tree tops which could be as high as 5 m. This has resulted in the perception that *Archachatina degneri* is not tolerant to “fouled” earth. Collectors in some areas in Ghana would not collect *Archachatina degneri*. Even in situations where



*Archachatina degneri* is collected, it is sold at other places out of the collector's area.

*Achatina fulica*, described in literature as “notorious” giant African snail was first observed in Ghana in 1984. Although it originates from East Africa, it illustrates one of the most interesting and best documented examples of the extension of geographic range of a land mollusc by man's activities. It is very prolific and can establish itself in other countries. How it appeared in West Africa is not clear but it was first reported in Cote d'Ivoire in 1982 and in Ghana it was described as “Sefwi nwa”. It can be farmed in Ghana for export but Ghanaians have not yet accepted it although it is edible.

*Achatina achatina* grows to about 200 mm in length and has a much more patterned shell than the other three species. The shell is slightly more yellow with distinct brown jagged bands. The ground colour of the shell of *Achatina achatina* is golden olive brown and may be either dark longitudinally streaked or unstreaked. The body of the snails is either black with numerous grey specks or unpigmented that is creamy white and therefore albinos. The species enjoys the widest patronage on the basis of its delicacy (Monney, 1994). Additionally, the white (albino) forms of *Archachatina marginata* are not eaten by some people on religious grounds. It is also believed that *Archachatina marginata* feeds on human excreta and this has resulted in it being abhorred by many consumers (Monney, 2001). Lastly, Ghanaians have not yet accepted *Achatina fulica* although it is edible, hence marketing it in the country will pose some difficulties. In the light of these reasons, it becomes clear that the best choice of species for research is

*Achatina achatina*. This is because *Achatina achatina* has been accepted and therefore has high patronage compared with the other three species.

### **Economic importance**

Species of giant African snails have been introduced to new countries for use as food (Overton, 2004; Cooling, 2005). Some species of *Achatina* and *Archachatina* form a substantial source of animal protein in the diet of some West Africans. *Achatina fulica* was introduced to Singapore during the Second World War as a potential source of protein and snail meat has been consumed by humans throughout the world since prehistoric times.

It is high in protein (12-16%) dry weight and iron (45-50 mg/kg), low in fat (0.05-0.8%) and contains almost all the amino acids needed by humans (Cobbinah, 1992; Ntiama-Baidu, 2005, in Cooling 2005). Snail meat was thought to contain aphrodisiac properties and was often served to visiting dignitaries in the late evening at the Imperial Court in Rome (Cobbinah, 1992).

In addition to the nutritional value of snail meat, a recent source has shown that the glandular substances from edible snails cause agglutination of certain bacteria which are responsible for various ailments, including whooping cough. Edible snails also play an important role in folk medicine. In Ghana, the extracted blue blood haemocyanin is believed to be good for growing infants (Cobbinah, 1992). The high iron content is considered important in the treatment of anaemia (Cobbinah, 1992).

Snails are important in many food chains and they are frequently used by fish and wildlife as food. Many beetle species consume the Giant African Snail

such as the lampyriid and the coprine beetle. The domesticated duck and a variety of other bird species also eat the Giant African Snail. The importance of thin-shelled snails in animal diets is often underestimated. Snails may serve as pollution and acid rain indicators. They are intermediate hosts for many important parasites (Burch, 1962).

*Achatina fulica* is used for religious purposes in Brazil as deity offering to Obatala as a substitute for the African Giant Snail (*Archachatina marginata*) that is used in Nigeria, because they are known by the same name (*Igbin*, also known as *Ibi* or *Boi-de-Oxalá* in Brazil) in both Brazil and Nigeria (Neto *et al.*, 2009).

Some people also believe that these snails have medicinal properties and are therefore used in native medicine in West Africa (Parkinson *et al.*, 1987; Overton *et al.* 2004; Cooling 2005). For example, it was thought to arrest constipation, restore vitality and stop bleeding when fluid from snail was applied to a fresh cut. Scientists have identified a compound known as acharan sulphate produced by the snail which has anti-tumor properties (Lee *et al.*, 2003; Cooling, 2005).

Shells of giant African snails are rich sources of calcium are ground and added to feeds for layers. Empty shells of large specimens are also used as containers for storing salt and sugar (Parkinson *et al.*, 1987). In a few ethnic groups in Ghana such as Senya / Beraku, meals associated with some festivals at certain times of the year should by all means include snail meat. Figures available indicate that Ghana exported 620 kilogrammes of snails in 1994 to the Netherlands and 1,050 kilogrammes to the USA (Daily Graphic, 2003).

In addition to farming for meat, several species of Achatinidae, including *A. fulica*, are maintained in temperate regions outside Africa as laboratory animals (Nisbet, 1974; Plummer, 1975; Raut and Barker, 2002).

*A. fulica* distributes in its faeces spores of *Phytophthora palmivora* in Ghana; *P. palmivora* is the cause of black pod disease of cacao (*Theobroma cacao*). The fungus also infects black pepper, coconut, papaya and vanilla (Raut and Barker, 2002). *A. fulica* spreads *P. colocasiae* in taro and *P. parasitica* in aubergine (*Solanum melongena*) and tangerine (*Citrus reticulata*) (Mead, 1961).

In many places the snail is seen as a pest. Suggested preventative measures include strict quarantine to prevent introduction and further spread. Many methods, including hand collecting and use of molluscicides and flame-throwers, have been tried to eradicate the giant snail. Generally, none of them has been effective except where implemented at the first sign of infestation. In some regions, an effort has been made to promote use of the Giant East African Snail as a food resource, the collecting of the snails for food being seen as a method of controlling them. However, promoting a pest in this way is a controversial measure, as it may encourage the further deliberate spread of the snails.

One particularly catastrophic attempt to biologically control this species occurred on South Pacific Islands. Colonies of *A. fulica* were introduced as a food reserve for the American military during the Second World War and they escaped.



**Plate 1: Giant African land snail (*Achatina achatina*)**

**Key:**

**S – Shell**

**T – Tentacle**

**H – Head foot**

**Ds – Dark stripe**

A carnivorous species was later introduced, but it instead heavily harvested the native *Partula sp*, causing the loss of several species within a decade

United States of America importations of snails were worth more than \$4.5 million in 1995 and came from 24 countries. This included preserved or prepared snails and snails that were alive, fresh, chilled, or frozen. Major exporters to the United States of America are France, Indonesia, Greece and China. The United States of America exported live, fresh, chilled, or frozen snails worth \$55,000 to 13 countries; most were shipped to Japan, the Netherlands, and the United Kingdom (Raut and Barker, 2002; Cooling, 2005).

Giant African land snails are illegal in the United States and many other countries due to their potential as a devastating invasive species. They are highly invasive and can cause extensive damage to important food crops and other agricultural and natural resources. They have a voracious appetite and are known to eat at least five hundred different types of plants, including peanut, beans, peas, cucumbers and melons (Mead, 1961). If fruits or vegetables are not available, the snails will eat a wide variety of ornamental plants, tree bark, and even paint and stucco on houses. When these snails are introduced into a nonnative environment, they can reach such enormous numbers which can pose a serious conservation problem. They may eat native plants modifying the habitat and may also out-compete native snails (Mead, 1961). While their small size limits the quantity of plant material consumed per animal the aggregated nature of the infestations can lead to severe damage in infested plants (Raut and Barker, 2002). The snail causes

great economic loss for farmers due the large amounts of vegetation it consumes. Not only does it decrease the income for farmers, but it also impacts their living conditions and decreases food resources for humans, animals, and other species.

In the US state of Florida it has been estimated that *A. fulica* would have caused an annual loss of USD 11 million in 1969 if its population had not been controlled (Kliks *et al.*, 1982).

*Archachatina marginata* is potentially an intermediate vector of the rat lungworm *Angiostrongylus cantonensis*, causing eosinophilic meningoencephalitis in humans. This parasite can be contracted by ingesting improperly cooked snail meat or by handling live snails and transferring snail mucus to the human mucus membranes such as those in the eyes, nose and mouth. Giant African land snails are legal pets in many places, however, under no circumstances are they allowed to be kept as pets where illegal to do so. The United States Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) is interested in finding these snails and encourages those using these snails in classrooms, nature facilities or keeping them as pets to turn them in voluntarily without fear of penalty (Raut and Barker, 2002).

In many Asian, Pacific and American societies *A. fulica* may play a role in the transmission of the metastrongylus causative agents of eosinophilic meningoencephalitis (*Angiostrongylus cantonensis* and *A. costaricensis*). *A. fulica* is a vector for the bacterium *Aeromonas hydrophila* (also found in shellfish in New Zealand). The parasites carried by the snail are usually passed to humans

through the consumption of raw or improperly cooked snails (Kliks *et al.*, 1982; Raut, 2002).

In Japan, *A. fulica* are also a general nuisance when found near human habitations and can be hazardous to drivers, causing cars to skid. Their decaying bodies release a bad odor and the calcium carbonate in their shells neutralises acid soils, altering soil properties and the types of plants that can grow in the soil (Mead, 1961). In India *A. fulica* attained serious pest status, particularly in 1946/1947, when it appeared in epidemic proportions in Orissa and caused severe damage to vegetable crops and rice paddies (Raut and Barker, 2002).

### **Justification**

Although some members of the family Achatinidae form a substantial source of animal protein in the diet of some West Africans, the main source of supply of snails for the table is a picking harvest in which the snails, abundant in the rainy season, are collected, sold or eaten. Seasonality of the supply of the giant snails limits their use as source of animal protein on regular basis throughout the year and they are rather expensive during the dry season. It has been observed that abundance of snails depends very much on the rainfall distribution in a particular year. The supply of snails on the Ghanaian market is therefore erratic and a lot of people have been agitating for a means of providing a regular supply and hence the establishment of snail farms has been expected (Elmslie, 1982; Monney, 1992).



Apart from the dangers from snake bites, scorpion sting and snail hunting by collectors, there are other threats to survival of snails in the wild. The greatest threat is that of habitat destruction through indiscriminate forest clearance and the use of poisonous chemicals. Although there is no evidence of the effects of insecticides on the snails, it is possible that those which persist for some time in the environment may affect the snails. It has been noted by Hodasi (1989) that West African agricultural practices alter the environment in ways which do not augur well for the survival of the snails in the wild. For instance, the usual agronomic practice of the West African farmer is the “slash and burn” method, that is after the bush is cleared the vegetation is allowed to dry and then set ablaze. Snails being slow crawlers cannot move fast enough to escape from such fires resulting in accidental burning of not only the breeding stock but young snails. In addition, the farmer moves on to a fresh piece of land each year; that is shifting cultivation. Thus each year, the area of land rendered uninhabitable for snails is increased by some means.

Moreover, often snails which have been collected for sale lay eggs in the market stalls and no attempt is made to incubate them. If no attempt is made to incubate eggs laid by snails during transport or in the course of their being traded, potential addition to the next generation will be drastically reduced. It is such factors that are leading to the decline of snail populations in some countries. Many species of land snails in Australia and throughout the world are considered to be declining and at a risk of extinction (Wells, 1988; Ponder, 1997; IUCN, 2000).

In the past, some districts in Ghana attempted to protect the snails in the wild by enforcing local legislation. In such areas, people were prevented from collecting snails until the chief of the area was satisfied that the rainy season had advanced enough and that the snails had fully recovered from the previous dry season and laid their eggs. The chief's decision is however not based on any empirical evidence. With the foregoing, it becomes clear that farming snails in Ghana will help a great deal to satisfy the increasing demand whilst protecting them in the wild. For inhabitants who have limited space and capital resources, successful farming of snails could help them raise the level of animal protein in their own diets since scarcity of protein rich foods affects many African countries. The shell which is rich in calcium can be used as a supplement in enriching the calcium content in poultry feed (Plummer, 1975). Snails farmed could also be sold to generate income for these farmers.

When reared in captivity, snails could be made available throughout the year without going to search in the wild, thus avoiding the hazardous snail collection expeditions. In captivity, factors affecting growth can also be controlled; thus avoiding natural enemies and aestivation for example, so that they may grow throughout the year. Moreover, properly organized snail farming industry would provide occupation for the unemployed and the importance of an organized snail farming industry for generating foreign exchange cannot be overemphasized. Thus regular mass production of popular endemic snails in Ghana where snail meat is appreciated would provide essential nutrients. Apart from being cheap, snails have the advantage of being easily transported and easy

to store alive for a considerable length of time. The small unit size also means that producers for household consumption can harvest just what is required for a meal (Monney, 1992).

Contrary to popular expectation, the setting up of commercial snail farms is progressing at a very slow pace due mainly to the fact that prospective enthusiastic farmers do not have information about the many species of the giant African land snails and which ones are commercially viable (Monney, 1998). If the issue of species preference is taken into consideration then the choice of species for farming in Ghana is *Achatina achatina*. Research into farming giant African snails with Africa in mind has been sporadic (Barry, 1959; Ajayi *et al.*, 1978; Hodasi, 1982; Olufokunbi *et al.*, 1989; Monney, 1994).

There is an urgent need for researching into the genetics of the various species with a view to selecting the best ecotype(s) for maximum body size, rapid growth rate, elimination of aestivation, high reproductive capacity and resistance to pests and diseases. Therefore adequate information on the best ecotype(s) of *Achatina achatina* viable for commercial snail farming is crucial in helping prospective farmers make well informed choices on which ecotype(s) to farm. It is in the light of these that this research was carried out to obtain information on the pattern of variation in *Achatina achatina*. This, it is hoped will serve as a basis for selection of the best breed for farmers.

### **Statement of the problem**

There is existence of ecotypes of *Achatina achatina* in the four regions. These ecotypes from the four regions have significant differences in their reproductive potential, growth rate and aestivation pattern.

### **Study Objectives**

The present study takes a critical look at the various ecotypes of *Achatina achatina* to obtain information on the following:

- the reproductive potential of the ecotypes
- the differences in the growth rate of juvenile snails of the various ecotypes
- the response to dry environmental conditions among the various ecotypes (aestivation pattern).

This information will help establish a basis for the selection of the best ecotype(s) for commercial snail farming.

## CHAPTER TWO

### MATERIALS AND METHODS

*Achatina achatina* inhabits the forest zones in the southern part of Ghana. Snail density is high in forest reserves where cultivation activities are minimal. However, in areas where cultivation activities are extensive, it has resulted in the dwindling of snail populations which consist of juvenile and medium-sized cohorts. This is as a result of human predation and habitat destruction (Duah and Monney, 1998). Therefore sampling of populations in four different geographical regions provided information on the various ecotypes of *Achatina achatina*. Snails from four localities were examined and used for the investigations. The four districts with their capitals were Amansie East – Bekwai, Assin South – Nyankumase, Suhum-Kraboia-Coaltar – Suhum and Wassa West - Tarkwa (Figure. 1).

Each collection site was visited thrice, that is once each in the months of June, July and August, 2006 respectively. On each visit the investigator established a relationship with the snail collectors, obtained information about the time of hunting and collected samples. Samples of snails collected were used in the indoor experiment which was set up in an Animal House behind the School of Biological Sciences block University of Cape Coast. The outdoor system was set

up in the snail production unit at the Technology Village of the School of Agriculture University of Cape Coast in the Central Region.

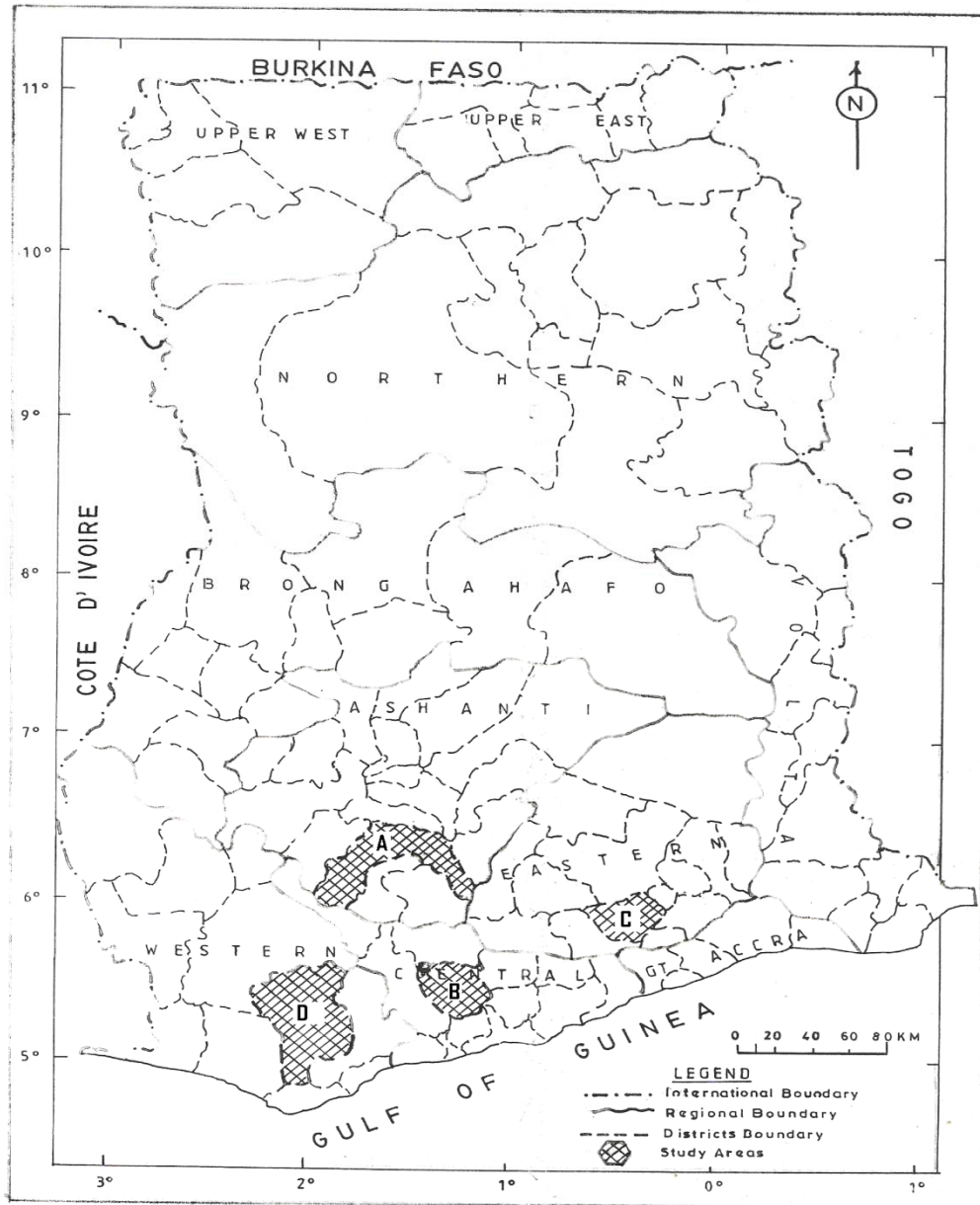
### **The study areas**

#### **Suhum-Krabo-Coaltar district in the Eastern Region**

The Suhum Krabo Coaltar District with district capital at Suhum is in the Eastern Region of Ghana. The region occupies 19,323 km<sup>2</sup> which represents 8.1% of the total land area of Ghana. The population is made up of 49.2% males and 50.8% females, giving a sex ratio of 49:51 males to females (Public-Private Partnership Programme, 2006).

The Suhum Krabo Coaltar District (5° 50'N; 0° 16'W and 6° 08'N; 0° 38'W) is located in the southern part of the Eastern Region. It shares boundaries with the West Akim District to the west, the Akwapim North and New Juabeng Districts to the east, the Akwapim South District to the south and the East Akim District to the north (Public-Private Partnership Programme, 2006). The snails were collected from Tetekasom, Amanse and Nankese with nucleus at Suhum (Fig. 2).

The district falls within the forest-dissected plateau. Most of the land is elevated at an altitude of 500 to 1,000 m above sea level. The terrain is generally undulating. The Atewa range, the highest elevation, rises to about 2,000 m above sea level. The district is well supplied with water by rivers such as Densu, Essiem and Kua. It also forms the catchment area for some streams.

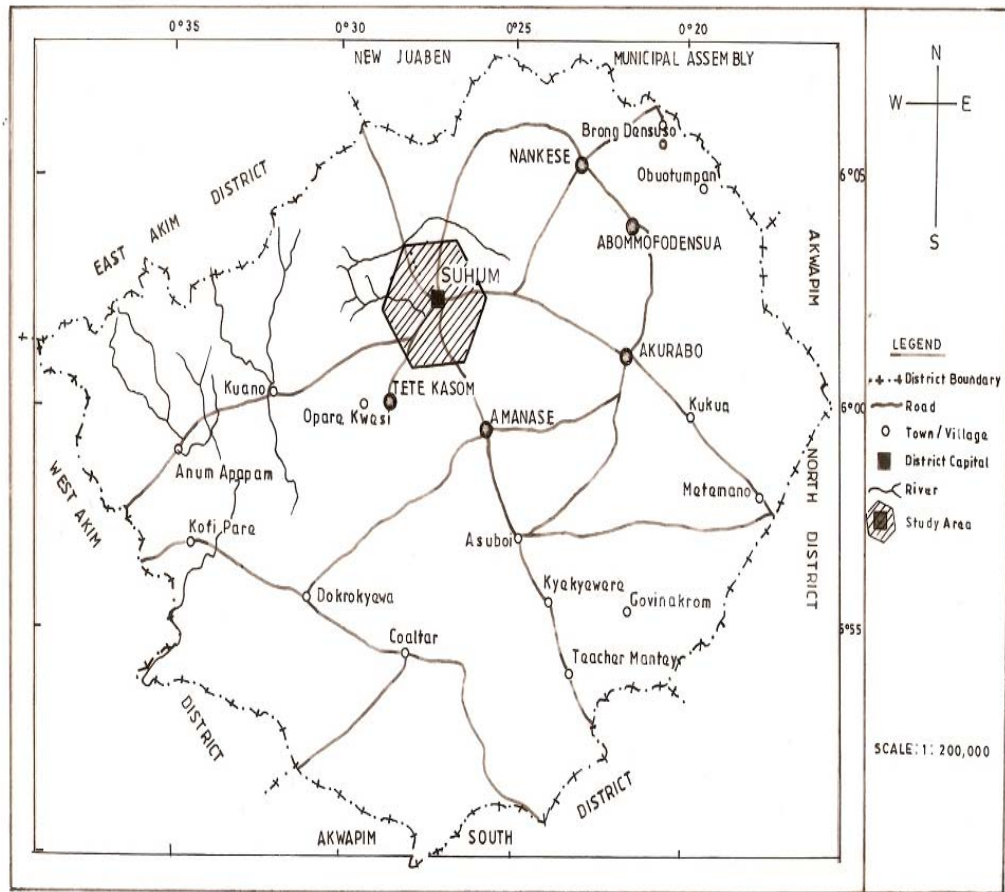


**Fig. 1: Map of Ghana showing the study districts**

Districts – Capital	Latitude	Longitude
A- Amansie East - Bekwai.	06 <sup>0</sup> 37'N	01 <sup>0</sup> 35'W
B- Assin South - Nyankumase.	05 <sup>0</sup> 27'N	01 <sup>0</sup> 09'W
C- Suhum-Kraboia-Coaltar - Suhum.	06 <sup>0</sup> 03'N	00 <sup>0</sup> 27'W
D- Wassa West - Tarkwa.	05 <sup>0</sup> 19'N	01 <sup>0</sup> 59'W

The main vegetation type is forest however, the original forest is giving way to secondary forest in areas such as Asuboi and Kyekyewere, due to logging and farming activities. *Pennisetum purpureum* and other grasses are prevalent in the southern part of the district, a development which favours livestock farming but not the survival of snails in the wild. The snails were collected from secondary forests close to farms. These forests were lands left to fallow after some logging activities had taken place.





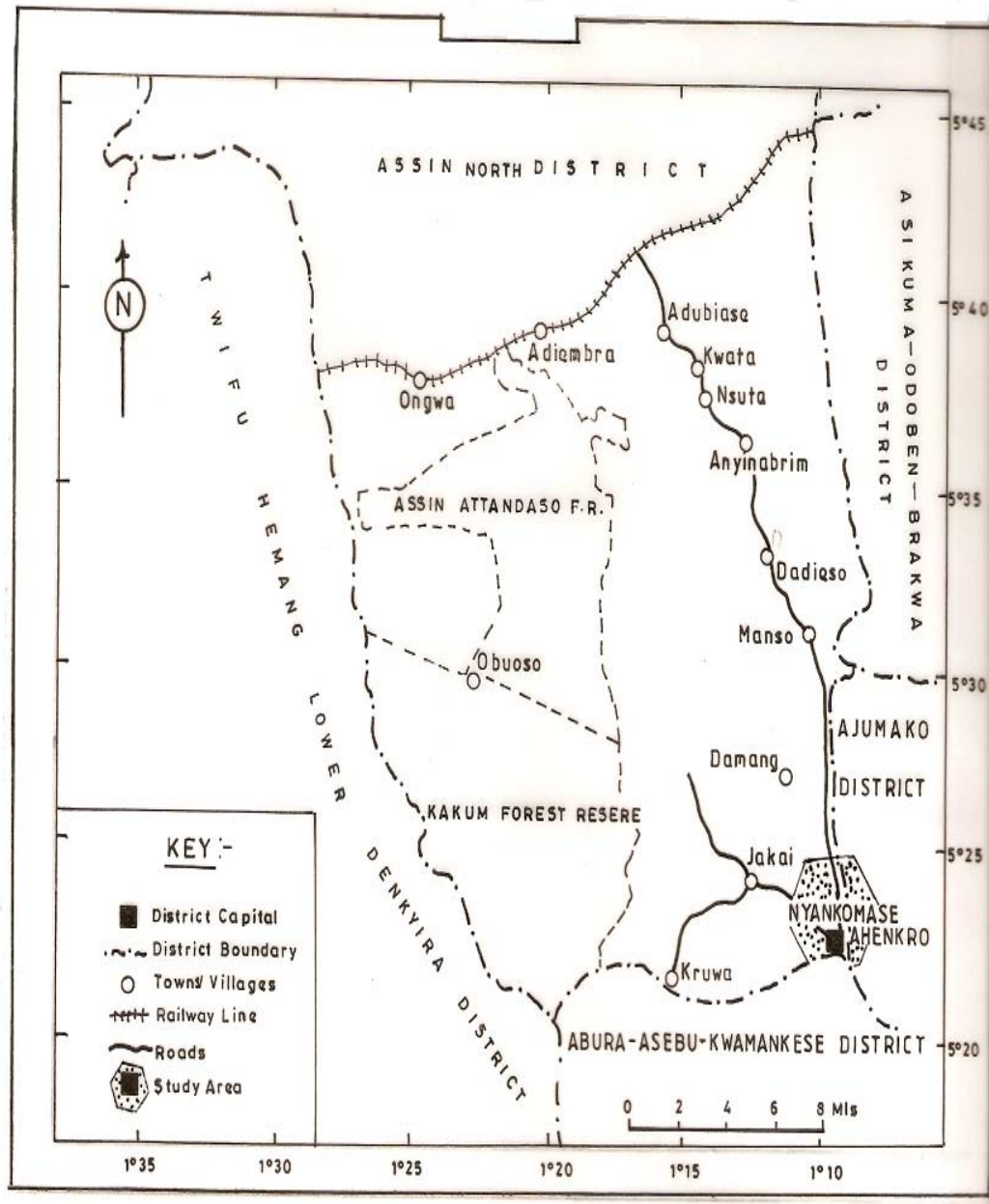
**Fig. 2: A map of Suhum-Kraboa-Coaltar District showing the study areas**

### **Assin South District in the Central Region**

The Assin South District is one of the thirteen (13) districts of the Central Region of Ghana. Nsuaem-Kyekyewere is the district capital. The district shares common boundaries with Twifo - Hemang - Lower - Denkyira District on the West, Abura - Asebu - Kwamankese District on the South, Asikuma - Odoben-Brakwa and Ajumako - Enyan-Esiam to the East and Assin North to the north.

The Assin South District falls within the moist tropical forest mainly deciduous forest. Rainfall pattern is bi-modal. The major rainy season falls within April - July which is the major farming season in the district. The minor rainy season falls within September - November. It is during the major rainy season that members of the genus *Achatina* reach their peak of abundance (Public-Private Partnership Programme, 2006). The area has an annual rainfall of 1500 to 2000 mm. Annual temperatures are high and range between 26 °C – 30 °C. Relative humidity ranges from 60 - 70%. The climate allows cultivation of a variety of food crops, cash and non-traditional export crops. The snails were collected from Kruwa, Damang and Jakai with nucleus at Nyankomase (Fig. 3).

The District comes under relative cool and moist South-West monsoon winds that blow from the Atlantic (November to February). However, the dry harmattan or North-East Trade Winds blow from the North. The snails were collected from parts of the Kakum Forest Reserve close to farms. These parts of the forest form the ecotone or buffer between the forest reserve and the farms of surrounding communities.



**Fig. 3: A map of Assin South District showing the study areas**

## **Wassa West District in the Western Region**

The Western Region covers an area of 23,921 km<sup>2</sup>, which is about 10% of Ghana's total land area. It is located in the south-western part of Ghana, bordered by Ivory Coast on the west, Central Region on the east, Ashanti and Brong-Ahafo Regions on the north and on the south by 192 km coastline of the Atlantic Ocean. The southernmost part of Ghana, Cape Three Points, near Busua, is in the Ahanta West District of the region. Cape Three Points is bounded to the north of the Wassa West District by the Wassa Amenfi District, the south by the Ahanta West District, the West by the Nzema East District and the East by Mpohor Wassa East District. The District has a total land area of 2354 km<sup>2</sup>.

The District lies within the South-Western Equatorial Zone. It therefore has fairly uniform temperature, ranging from 26°C in August to about 30°C in March. Sunshine duration for most part of the year averages 7 hours per day. Relative humidity is generally high; 70 – 80% in the dry season and 75 – 85% in the rainy season. The Wassa West District experiences the highest rainfall in Ghana. It has a mean annual rainfall of 1878.3 mm, with the major rainfall season starting from March and the minor rainfall season starting from September and November to February as the dry season. It is during the major rainy season that *Achatina achatina* reach their peak of abundance (Public-Private Partnership Programme, 2006). The rainy season has an important effect on the environment in creating watersheds, large expanses of stagnant water bodies, deep trenches and

gullies as well as leaching of soil nutrients (Public-Private Partnership Programme, 2006).

The District lies within the rainfall belt with the height of economic trees ranging between 15 – 40 m high. The forest is full of climbers and lianas which are able to reach into the upper tree layer. Economic trees include *Khaya ivorensis*, *Triplochiton scleroxylon*, *Chlorophora excelsa*, *Entandrophragma cylindricum* among others. In recent times, most part of the rich forest has been reduced to secondary forest through increased human activities such as excessive open cast mining, farming activities and indiscriminate logging. These have impacted negatively on the natural vegetation. These anthropogenic activities have contributed greatly to the destruction of the snails' habitat through indiscriminate forest clearance and probably the use of poisonous chemicals from some mining companies. Although there is no evidence of the effects of these poisonous chemicals on snails, it is possible that those such as cyanide which persist for some time in the environment may affect the snails. Thus, each year, the area of land rendered uninhabitable for snails is evidence by some means. The District, however, can still boast of large forest reserves like the Bonsa Reserves (209.79 km<sup>2</sup>) and Neung Reserve (157.84 km<sup>2</sup>).

Some of the snails were collected from parts of the Subri River Forest Reserve close to farms of surrounding villages. These parts of the forest form the ecotone or buffer between the forest reserve and the farms of village folks where the collection of fire wood and other materials are allowed (Fig. 4).

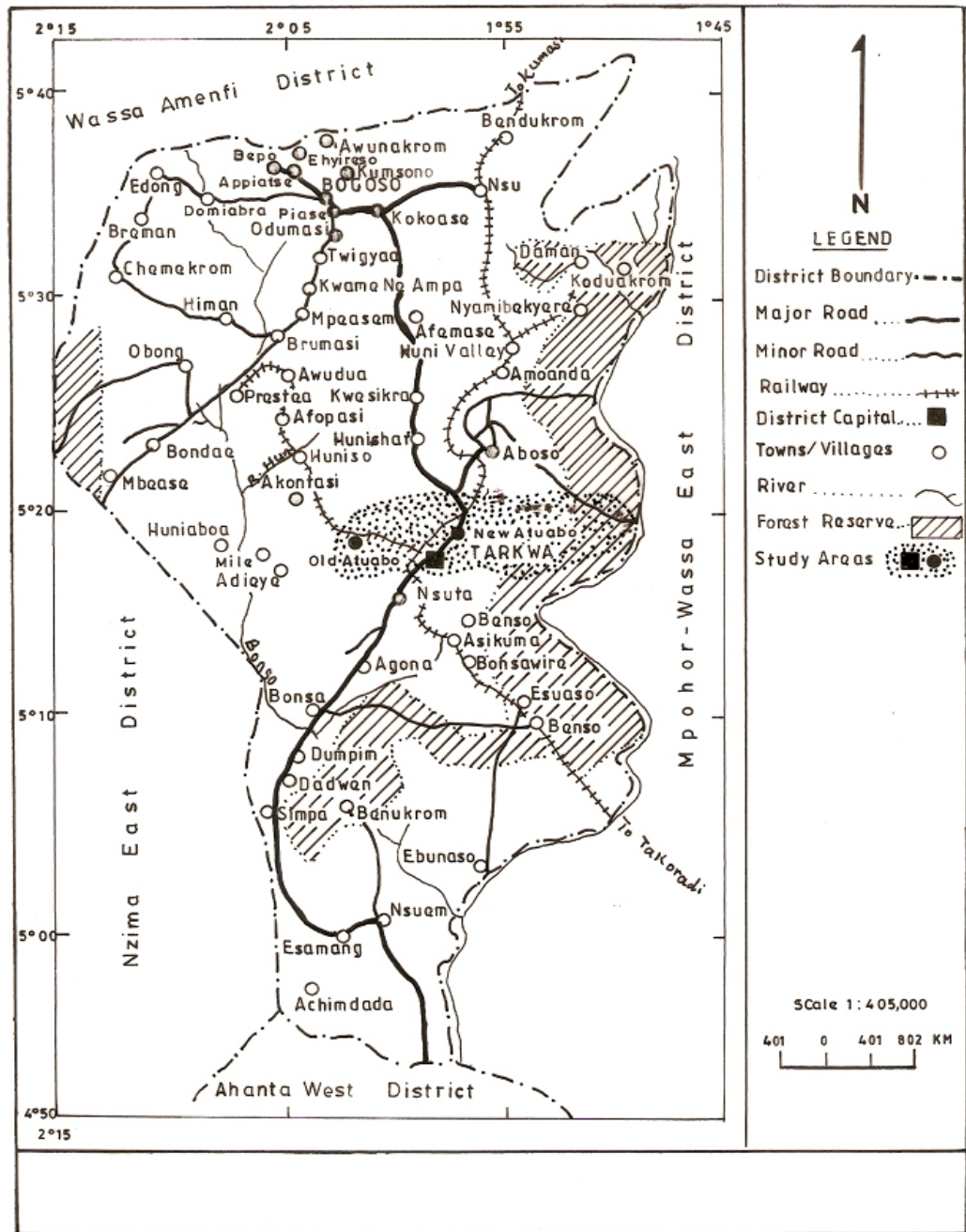


Fig. 4: A map of Wassa West District showing the study areas

### **Amansie East District in the Ashanti Region**

The Ashanti Region is centrally located in Ghana. It lies between longitudes 0.15W and 2.25W and latitudes 5.50N and 7.46N. The region shares boundaries with Brong-Ahafo- region in the north, Eastern region in the east, Central region in the south and Western region in the South West. The region occupies a total land area of 24,389 km<sup>2</sup> representing 10.2% of the total land area of Ghana. It is the third largest region after Northern (70,384 km<sup>2</sup>) and Brong Ahafo (39,557 km<sup>2</sup>) regions (Public-Private Partnership Programme, 2006). More than half of the region lies within the wet, semi-equatorial forest zone. Due to human activities such as logging and bushfires, the forest vegetation of parts of the region, particularly the north-eastern part, has been reduced to savanna woodland. The region has an average annual rainfall of 1270 mm and two rainy seasons. The major rainy season starts in March, with a major peak in May. There is a slight dip in July and in August, tapering off in November. It is during the major rainy season that *Achatina achatina* reach their peak of abundance (Segun, 1975; Public-Private Partnership Programme, 2006). December to February is dry, hot and dusty (Public-Private Partnership Programme, 2006).

The Amansie East district is located in the southern part of the Ashanti region and shares boundaries with Amansie West and Central Districts to the West , Bosomtwe–Atwima– Kwanwoma District to the north, Adansi East, Adansi North and Asante Akim South District to the East.

The climate of the district is the semi-equatorial type. The first major rainfall season starts from March and ends in July. The minor rainy season starts from September and ends in November. The annual rainfall is between 1600 mm – 1800 mm. Relative humidity is fairly moderate but high during the rainy season. It ranges between 70 and 80 percent in the wet season. The temperature regime and rainfall pattern enhance the cultivation of many food crops.

The Amansie East District lies within the moist – semi- deciduous forest zone. Some of the tree species are *Khaya ivorensis*, *Triplochiton scleroxylon* and *Entandrophragma cylindricum*. Parts of the forest have been reserved. The forest reserves include Bosomtwe Range Forest Reserve and Fun Forest Reserve. Outside the forest reserve, human activities, particularly farming and timber extraction have reduced the primary forests to secondary forests. *Chromolaena odorata*, popularly called ‘Acheampong’ shrub seems to be the predominant vegetative cover in many parts of the district. The snails were collected from secondary forests close to farms at Pepeedan, Adumasi and Dunkwa with nucleus at Bekwai. These forests were lands left to fallow after some logging activities had taken place (Fig. 5).



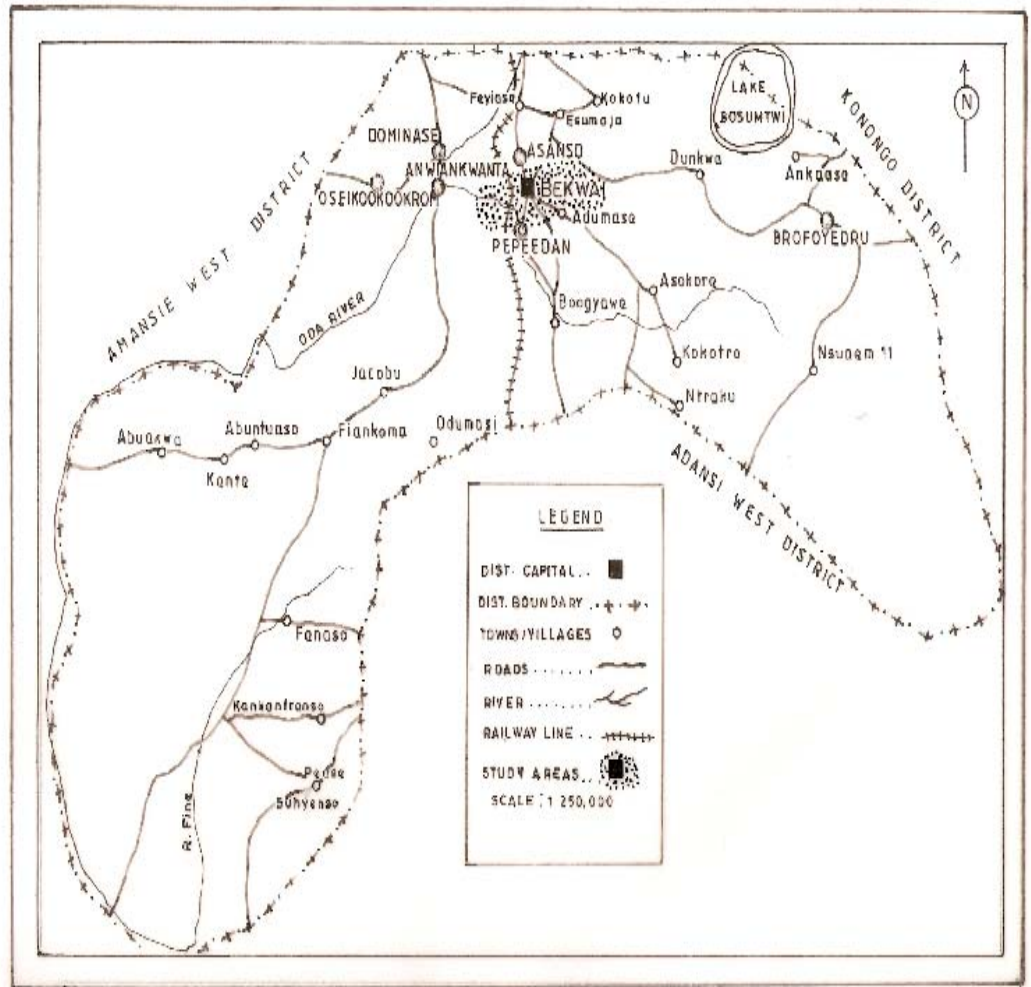


Fig. 5: A map of Amansie East District showing the study areas

### **Collection of snail samples**

The investigator visited these localities and solicited the help of local snail collectors for the snail harvest from their hunting expeditions. In the Wassa West district, snail collection was done mainly in the early hours of the morning that is 5:00 am - 8:00 am during the rainy season. In the Amansie East district, there was a combination of early morning and night snail hunting. However, the Assin south and Suhum-Kraboia-Coaltar districts depended mostly on night snail hunting. Samples were obtained from collectors and data on maximum shell length recorded. A total of two hundred and sixty adult reproductively matured snails of 12- 17cm shell length were obtained from the four different regions. Thirty snails from each of the four different regions were isolated from each other and not mixed for the breeding and rearing experiment.

### **Laboratory work**

#### **Electrophoresis**

Electrophoresis of the haemocyanin (blood pigment) of the various ecotypes of *Achatina achatina* from the four districts was performed to ascertain whether there were variations among the snails. This process was replicated four times with each ecotype in September, 2006.

The shell of one snail from each ecological region was broken and 1ml of haemocyanin was drawn into each of four different test tubes using a syringe and 5 drops of an anticoagulant were immediately added to the haemocyanin in each

of the four different test tubes. The four solutions were then centrifuged for 5 minutes and then decanted. After that, 100ml of 0.65% saline was added to each of the samples, centrifuged for 5 minutes and then decanted. This process of washing was repeated two times. After centrifuging with the 0.65% saline solution, 150ml of distilled water and one part of carbon tetrachloride (CCl<sub>4</sub>) were added to each solution in the test tubes and centrifuged for 5 minutes. The haemocyanin solutions were applied for the run (Brody and Kern, 2004).

A 50:50 mixture of the two buffer solutions: Barbitone, pH 8.6 and Tris, pH 9.2 was prepared. A cellulose acetate plate was pleated on the solution and allowed to impregnate for thirty minutes. The Shandon tank was filled with the two buffers to the same level as follows: Barbitone at cathode and Tris at anode. A Red contact was established between the two chambers by means of wicks prepared from filter papers.

After impregnation, the cellulose acetate plate was dried by pressing it gently between two blotting papers. The four haemocyanin samples were then applied on a line drawn with a pencil at the middle of the cellulose acetate plate using four different applicators (one applicator for each sample). The plate was then placed upside down on the bridge between the two wicks after the anode and cathode ends were indicated. The electrophoresis was run for twenty minutes using the Kayagaki voltage / current regulator power pack. This process was replicated on three more cellulose acetate plates.

After running the electrophoresis, the plates were transferred into 3% aqueous trichloroacetic acid (TCA) for three minutes. The plates were then placed

in the staining solution, poncess solution, for ten minutes. The plates were then washed in three changes of TCA for three minutes each. The strips were kept in methyl alcohol after which the various bands were identified.

### **Analysis of DNA**

Samples of snail foot were obtained and put in four separate conical flasks with hot water at about 70.0 °C. The foot was then macerated with 2% cetyl triethylammonium bromide (CTAB) solution. After that it was vortexed lightly with 500ul of CTAB. The solution was then incubated in 65.0 °C waterbath for thirty minutes with intermittent vortexing. This was followed by addition of phenol and phenol-chloroform. Chloroform : isoamyl (24:1) was added and mixed vigorously by shaking and inverting the tube up and down about 100 times. The mixture was then centrifuged at 14,000 rpm for ten minutes. The aqueous phase which contained the DNA was pipetted off into new labelled Eppendorf tubes. About two times the volume of cold isopropanol was added and the tube inverted several times until the DNA began to precipitate. The samples were put into a refrigerator for thirty minutes and immediately spun at 10,000 rpm for five minutes, after which the isopropanol was poured off. The DNA pellet was then washed with 70% ethanol. The ethanol was poured off leaving the DNA pellet which was then dried by turning it upside down on paper towels for one hour. The DNA was re-suspended in 50µl of tris acetate (TAE) buffer and incubated at 65.0 °C water

bath for fifteen minutes. After the incubation, the DNA solution was allowed to cool at room temperature and then kept in the freezer (Brody and Kern, 2004).

A micropipette was used to measure 4 $\mu$ l of sample DNA into labelled tubes, allowing the DNA to settle at the bottom of the tubes. After that 1 $\mu$ l of the enzyme was added followed by the addition of 1 $\mu$ l of 10x restriction buffer by 6 $\mu$ l of distilled water to each tube and closed. The contents were mixed by gently flicking the tubes with the finger and spun in the microcentrifuge for three seconds. The tubes were then incubated at 37.0 °C for one hour to provide the necessary condition for the reaction. After the incubation, 0.1 $\mu$ l of 0.5M ethylenediaminetetracetic acid (EDTA) was added to stop the reaction. About 5g of agarose was transferred into a conical flask containing 500ml of 1x tris acetate (TAE) buffer. The content was swirled and the flask was placed inside a microwave oven and heated to melt the agarose. The agarose was allowed to cool to about 40.0 °C. A comb of 15 teeth was then inserted 1cm from the sealed end. About 5 $\mu$ l of ethidium bromide was added to the warm agarose solution and swirled to mix uniformly to make 0.001% concentration. The agarose solution was then poured gently onto the mould until the bottom of the tray was covered by the agarose. The gel was allowed to solidify.

After solidifying, the comb and the tape were carefully removed and the gel was carefully mounted into the electrophoresis chamber. 1x tris acetate (TAE) buffer was poured gently into the electrophoresis tank until the gel in the chamber was covered with the running buffer. A volume of 4 $\mu$ l of distilled water was added to 3 $\mu$ l of the DNA preparation and then 5 $\mu$ l of loading dye (bromophenol

blue) added and slowly pipetting the mixture up and down until the contents in the tubes were uniformly coloured. A volume of 10µl of each DNA preparation was loaded into separate wells and the position or order of loading noted. In addition to this, the DNA of *Theobroma sp.* (cocoa) was also loaded in separate wells at the ends in order to make the DNA of the snail ecotypes to stand out. The tank was covered and connected to the power supply of 100V and ran for two hours. The power was switched off to remove the gel from the casting tray. It was rinsed with distilled water and visualized under ultra – violet light. A photograph of the gel was then taken and the movement or differential separation of the DNA compared among the samples to determine DNA polymorphism.

## **Breeding experiments**

### **Project sites**

Breeding experiments were carried out for information on the fecundity, fertility, hatchability of the eggs and aestivation pattern of the snails from the four ecological regions.

The snail production unit at the Technology Village was the site for rearing of ecotypes of *Achatina achatina* for the outdoor set up (Plate 2) while the Animal House was used for the indoor set up. These two sites were chosen for the breeding experiment. The Technology Village is located about 1.2 km from the Science Faculty on the Farm Road. The Technology Village is a specialized branch established by the School of Agriculture of the University of Cape Coast which undertakes research activities on both plants and animals. It

consists of several units including the gari processing, grass cutter rearing, mushroom production and snail production. Samples of snails collected were observed for morphological differences, such as length and weight. This was done to compare which, that is, whether outdoor or indoor would favour snail rearing better.

#### **Random selection of snails for breeding in both indoor and outdoor culture**

Sixty pieces of plane paper were cut and thirty of them were numbered leaving the other thirty blank. These pieces of papers were mixed thoroughly in a bowl for the random sampling of the snails for either outdoor culture or indoor culture. Whenever a numbered piece of paper was selected from the bowl the snail picked was used for outdoor culture whereas selecting a blank paper indicated that the snail picked should be used for indoor culture for each ecotype.



**Plate 2: Snail production unit at the Technology Village**



## **Snail farming systems used**

Three main snail farming systems have been established based on the snail's biology and behaviour. The indoor Systems were started in France (Chevallier, 1979; Daguzan,1983; Gomot and Deray,1987) . The snails are kept in a controlled environment where hibernation is controlled (Runham, 1989 ). In the outdoor systems , the snails are kept in the natural environment. The techniques used are open air or free range (Chevallier, 1986 ; Cobbinah,1992), outdoor fed system ; mini – paddock , trench pen system and movable pens ranchings. The mixed systems involve using indoor breeding pens to provide growing snails which are then fattened outside.

However, in this project, there were two rearing units:

- Indoor using the closed pen system.
- Semi-natural conditions using the mini-paddock system.

## **Culturing of snails under indoor condition**

### **Closed pen culture**

The closed pen is made of wooden boxes. These are provided with decomposed grasscutter droppings mixed with soil and covered with nylon mosquito netting, bordered on its edges by wooden strips to prevent the escape of snails. Cleaning is minimal, as the faeces of the snails become mixed with the soil, which is changed every 4-6 weeks (Monney, 1998). However, in this research, the pens were built of cement blocks and covered with nylon mosquito net bordered on sides by wooden strips. The pens (100 x 100 x 50

cm) were kept in the animal house behind The School of Biological Sciences close to the botanical garden (Plate 3).

### **Reproductive potential**

The heights, width and weights of thirty *Achatina achatina* snails from each of the four ecological regions were measured and recorded. Ten snails from each ecological region were kept separately in four pens. This arrangement was replicated three times for each ecotype (i.e. Ashanti, Central, Eastern and Western) for the breeding experiment.

The snails were reared using the closed pen system. The snails were fed on ripe, pawpaw, *Carica papaya* fruits and wild lettuce, *Lactuca taraxacifolia*. Feed was provided *ad libitum* to provide the best condition for breeding while water was sprinkled on the snails every other day. Data on **the number of clutches**, the clutch size- number of eggs in each clutch, successes in hatchability- percentage of eggs that hatched (number of hatchlings), mortality of hatchlings, juvenile and young ones and growth rate from hatchling through juvenile to young were observed and recorded.



**Plate 3: Closed pen culture for the indoor experiment**

**Key:**

**C – Cement block**

**B – Bordered net cover**

## **Response to dry environmental conditions**

Thirty snails from each ecological region were observed from November, 2006 to the end of March, 2007 during the dry season to establish the susceptibility of the various ecotypes to dry environmental conditions (dry weather). The snails were fed on ripe *Carica papaya* fruits and dandelion leaves *ad libitum*.

Data on the number of snails that went into aestivation and the time (in days) these snails spent in aestivation from November, 2006 to the end of March, 2007 during the dry season were observed and recorded for each ecotype.

After the specified duration of dry condition, water was however sprinkled on the snails every other day to keep the pens moist.

## **Culturing of snails under semi-natural conditions**

### **Mini-paddock system**

The mini – paddocks consisted of rectangular pens (160 x 120 x 40 cm) in a fenced area and kept under shade. The pens were built with cement blocks and covered with nylon mosquito net bordered on sides by wood (Plate 4). The mini – paddock systems were filled with decomposed grasscutter droppings (Monney, 1998). Since the snails also depended on these decomposed droppings as a source of nutrients, the decomposed droppings together with the soil were changed every three months to prevent the deficiency in some particular sources of nutrients which resulted from the selective feeding behaviour of the snails (Cobbinah, 1992).



**Plate 4: The mini-paddock system used for the outdoor experiment**

**Key:**

**C – Cement block**

**B – Bordered net cover**

### **Reproductive potential**

The length, weight and width of thirty *Achatina achatina* snails from each of the four ecological regions were measured and recorded. Ten snails from each ecological region were kept separately in four pens. This arrangement was replicated three times for each ecotype (i.e. Ashanti, Central, Eastern and Western) for the breeding experiment.

The snails were reared using the mini-paddock system. The snails were fed on ripe *Carica papaya* fruits and wild lettuce, *Lactuca taraxacifolia*. Feed was provided *ad libitum* to provide the best condition for breeding whiles water was sprinkled on the snails every other day.

Data on **the number of clutches**, the clutch size- number of eggs in each clutch, successes in hatchability- percentage of eggs that hatched (number of hatchlings), mortality of hatchlings, juvenile and young ones and growth rate from hatchling through juvenile to young were observed and recorded for each ecotype every two weeks for six months.

### **Incubation of eggs**

Whenever eggs were observed, they were removed from the soil, counted and then incubated in earthenware bowls filled with soil at the base, after which the eggs were then covered with soil (Plates 9 and 10). Water was sprinkled on the soil in the earthenware bowl from time to time to keep the eggs from dehydration. The hatchlings (juvenile snails) were transferred into the closed pen

made of wood and kept under the semi- natural condition based on the week of collection for each ecotype (Plates 11 and 12).

### **Specific growth rate (SGR)**

Specific growth rate (SGR) expressed as percentage shell length per day defines the instantaneous rate of length increase over a time period (t) throughout the life of the snail. It reflects the pattern of growth, thus indicating how much length is gained per unit time.

%/day was calculated by the formula:  $SGR = 100 (\ln W_2 - \ln W_1) / t_2 - t_1$   
(Ricker, 1975)

Where,  $\ln W_2$  and  $\ln W_1$  = natural logarithms of the initial and final weights (in grams) of snails.

$t_2 - t_1$  = breeding period (in days) for which snails were reared from  $W_1$  to achieve  $W_2$ .



**Plate 5: *Achatina achatina* from Ashanti ecotype laying eggs**



**Plate 6: *Achatina achatina* from the Ashanti ecotype laying eggs (at close range)**

**Key:**

**E – Eggs,      S – Snail,      H – Head foot**





**Plate 7: *Achatina achatina* eggs laid in the soil (indoor set up)**



**Plate 8: *Achatina achatina* eggs laid in the soil (outdoor set up)**

**Key:**

**E – Eggs,     S - Soil**



**Plate 9: Incubated eggs of *Achatina achatina* in earthenware bowl (indoor set up)**



**Plate 10: Incubated eggs of *Achatina achatina* in earthenware bowl (outdoor set up)**

**Key:**

**S – Soil,      L – Leaf,      E – Earthenware bowl**



**Plate 11: Juvenile snails of *Achatina achatina* in wooden pen**



**Plate 12: Wooden pens containing juvenile snails of *Achatina achatina***

**Key:**

**B – Bordered net cover,    W – Wooden pen,    J – juvenile snails**

## **Response to dry environmental conditions**

One major problem snail farmers face is how to control the rate and period at which the snails aestivate especially during the dry season. Aestivation is the state of dormancy during the dry season (warm season). Long periods of aestivation by the snails result in low yield due to the quiescent state of the snails. At this period, feeding and mating behaviour are affected and hence there is suspension of breeding activities.

Thirty snails from each ecological region were observed from November, 2006 to the end of March, 2007 during the dry season to establish the susceptibility of the various ecotypes to dry environmental conditions (dry weather). The snails were fed on ripe *Carica papaya* fruits and dandelion leaves *ad libitum*.

Data on the number of snails that went into aestivation and the time (in days) these snails spent in aestivation from November, 2006 to the end of March, 2007 during the dry season were observed and recorded for each ecotype. Water was however sprinkled on the snails every other day.

After the specified duration of dry condition, the watering regime was continued to restore the moist condition in order to determine the number of snails that come out of aestivation and the time they take to come out of aestivation.

The information obtained on the pattern of variation in *Achatina achatina* was analysed to come out with deductions about the best ecotype of *Achatina achatina* viable for the establishment of commercial snail farming in Ghana. This

will serve as a basis for selection of the best breed for prospective farmers to make well informed choices on which ecotype(s) to farm.

## **CHAPTER THREE**

### **RESULTS**

#### **Electrophoresis**

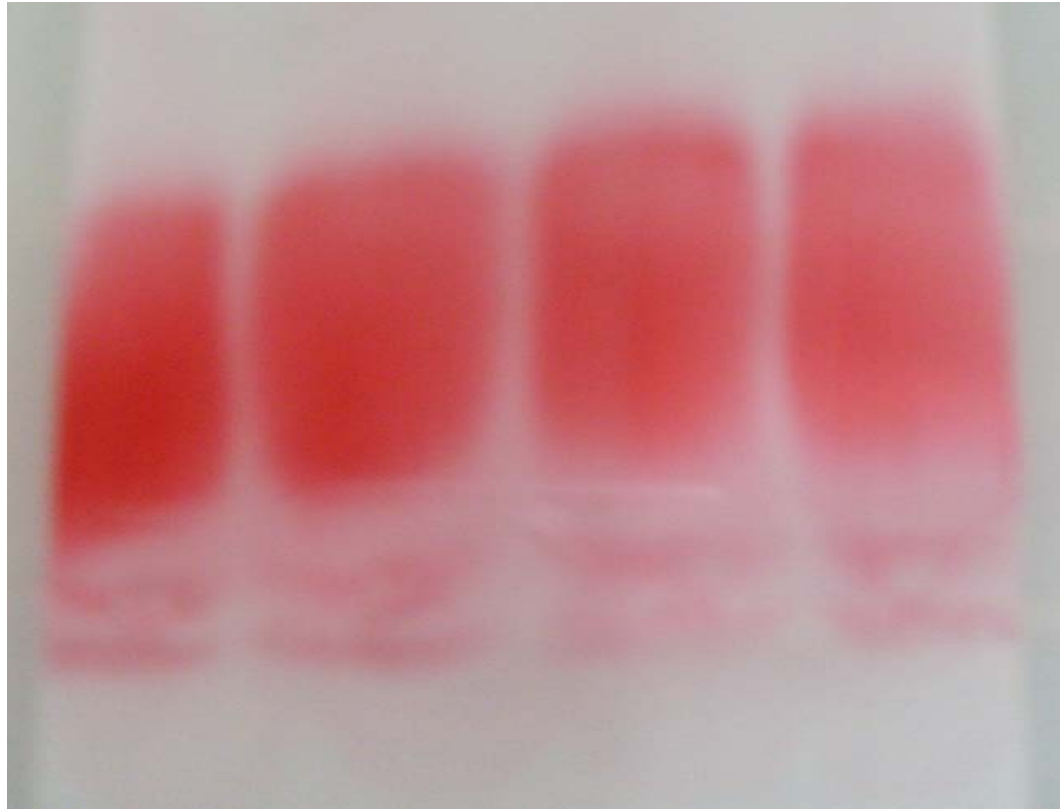
Electrophoresis of the haemocyanin of the four ecotypes of *Achatina achatina* snails from the four districts did not show any differences in the movement of the molecules as shown in Plate 13a. This indicates that there is no significant difference in the molecular composition of the haemocyanin of snails from the four districts.

The analysis of the DNA from the four snail ecotypes did not show any differences in the differential separation as shown in Plate 13c.

#### **Examination of the four snail ecotypes**

Examination of snails sampled from the four districts showed some morphological differences in shell colour, pattern of stripes on shell and body colour. Snails from Bekwai in the Ashanti Region had 11 to 16 thick black stripes which extended from the mouth of the shell to the apex. Each stripe branches into two along its length with zig-zag and continuous from the mouth to the apex of the shell. The black skin of the snail has evenly distributed grey speckles throughout (Plate 14). Snails from Nyankomasi in the Central Region had 10 to 14 thin single black stripes which extend from the mouth of the shell. Each stripe

assumes a zig-zag pattern midway along its length and continuous to the apex of the shell. The black skin of the snail has grey speckles not evenly distributed throughout (Plate 15).



**ASHANTI                  CENTRAL                  EASTERN                  WESTERN**

**Plate 13a: Bands of the haemocyanin from the four ecotypes of *Achatina* snails**



**ASHANTI                  CENTRAL                  EASTERN                  WESTERN**

**Plate 13b: Magnified portion of bands from the electrophoresis of the haemocyanin from the four ecotypes of *Achatina* snails**





T T T A C E W T

**Plate 13c: Analysis of DNA from the four ecotypes of *Achatina* snails and DNA of *Theobroma sp.* (Cocoa)**

**Key:**

- A- Ashanti ecotype**
- C- Central ecotype**
- E- Eastern ecotype**
- W- Western ecotype**
- T- *Theobroma sp.* (Cocoa)**

**Table 1:** Percentage shell fill, number of whorls and mean distance between adjacent stripes of the different ecotypes of *Achatina achatina* (Figures in brackets are standard error)

Ecotypes (Regions)	S F (%)	N W	M DBAS (mm)
Ashanti	85	6.00 (± 0.00)	6.00 (±0.19)
Central	78	6.00 (± 0.00)	11.52 (±0.24)
Eastern	84	6.00 (± 0.00)	14.18 (±12.20)
Western	91	6.00 (± 0.00)	12.20 (±0.43)

**Key:**

% SF- Percentage shell fill

NW- Number of whorls

MDBAAS-Mean distance between adjacent stripes

The Tarkwa specimens from the Western Region had 11 to 18 thin black stripes which extend from the mouth of the shell to the apex. The stripes do not continue smoothly to the tip but some break into spots along the length of the shell. The black skin of the snail has dense evenly distributed grey speckles (Plate 14. A-D).

However, snails from Suhum in the Eastern Region had olive brown shell with 10 to 13 thick and broad single black stripes which extend from the mouth of the shell and continuous to the apex of the shell. There were some stripes which assume zig-zag pattern and the distances between adjacent stripes are wider when compared with the other ecotypes as shown on Table 1. The Eastern ecotype had the widest distance of 79.56 mm between adjacent stripes on the shell while, the Ashanti ecotype had the least distance of 6.00 mm between adjacent stripes on the shell among the four ecotypes (Table 1). The black skin of the snail had evenly distributed grey speckles throughout (Plate 17).

There was overlap in the stripe pattern for twenty snails from the Western ecotype. These snails had a combination of the stripe pattern of the Ashanti and Western ecotypes. The twenty snails had thick black stripes which extended from the mouth of the shell however, the stripes do not continue smoothly to the tip but, some break into spots along the length of the shell. In addition to this, each stripe branches into two along its length (Plate 15).



A



C



E



W

**Plate 14: Four ecotypes showing banding patterns on the shell and body colour**

**Key:** A – Ashanti ecotype  
 C – Central ecotype  
 E – Eastern ecotype  
 W – Western ecotype



**Plate 15: Western ecotypes showing combination of the stripe pattern of the Ashanti and Western ecotypes**

**Table 2:** Some parameters of the different ecotypes of *Achatina achatina* (Figures in brackets are standard error)

Ecotypes (Regions) (mm)	M W (g)	M SL (cm)	M A W
Ashanti	360.91 (±6.63)	14.05 (±0.11)	77.34 (±0.57)
Central	331.30 (±12.00)	13.09 (±0.18)	73.60 (±1.05)
Eastern	359.70 (±9.31)	14.59 (±0.14)	76.12 (±0.77)
Western	382.57 (±9.74)	14.43 (±0.17)	79.56 (±0.79)

**Key:**

M W- Mean weight of snail

MS L- Mean shell length of snail

MAW- Mean aperture width

**Weight of parent stock of snails from the four ecotypes**

Among the four snail ecotypes, those from the Western region were heaviest compared to the other three. Snail specimens from the Central region had the least mean weight. However, the Eastern ecotypes were longest with a mean

length of 14.57, while the Central ecotype had the least mean length of 13.09. The Western ecotype had the highest aperture width while the Central ecotype had the lowest aperture width. All the four ecotypes had the same number of whorls on the shell as shown in table 1.

Out of sixty snails observed from each of the four districts, the Ashanti ecotype had the highest percentage with a heavy shell fill while the Central ecotype was the lowest (Table 1).

**Table 3:** Egg laying in two of the four ecotypes (Figures in brackets are standard error)

Ecotypes (Regions)	Number of clutches		Average clutch size		Mean egg weight/g
	Indoor	Outdoor	Indoor	Outdoor	
Ashanti	5	5	195.0 (±81.2)	264.0 (±75.6)	0.1701 (±0.002)
Western	2	3	154.5 (±10.6)	222.7 (±40.2)	0.1793 (±0.003)

### Egg laying

Snails from the Ashanti ecotypes laid most egg clutches for both indoor and outdoor set up. This was followed by those from the Western region while the Eastern and Central ecotypes did not lay any eggs. However, the Ashanti ecotype laid the higher mean number of eggs compared to those of the Western ecotype.

This was however not significantly different ( $P > 0.05$ ). Eggs laid by snails from the Western Region were heavier than those laid by snails from the Ashanti Region. There was a significant difference ( $X^2= 2.04, 150, P < 0.05$ ) in the weight of eggs laid between the Ashanti and Western ecotypes (Table 2).

**Table 4:** Incubation period, mean humidity and mean temperature of indoor and outdoor experimental areas (Figures in brackets are standard error)

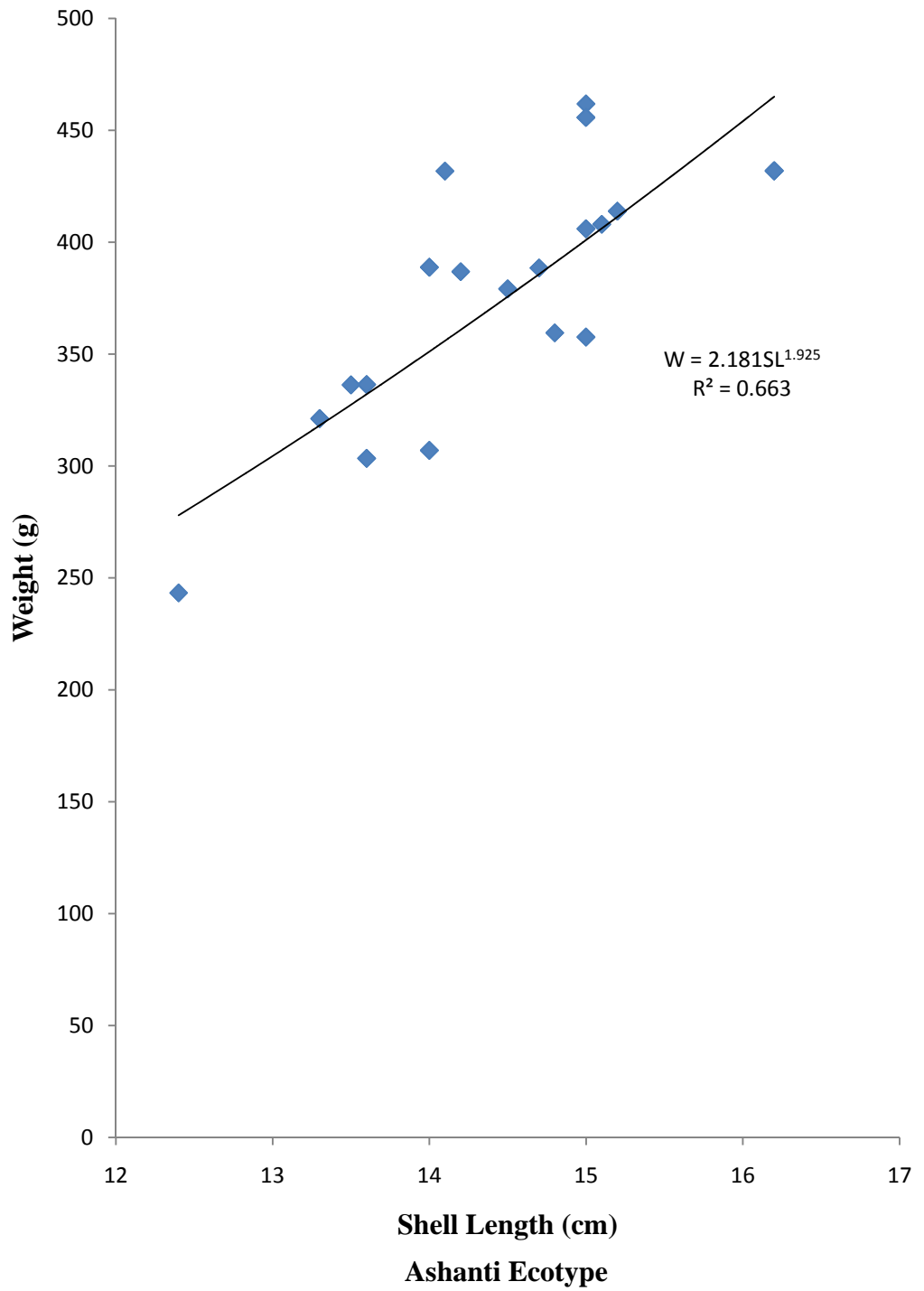
Ecotypes (Regions)	Mean time taken for eggs to hatch		Mean temperature		Mean humidity	
	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
Ashanti	25.50 (±3.54)	20.00 (±2.55)	27.03 (±0.97)	29.59 (±2.01)	76.94 (±8.36)	69.38 (±10.7)
Western	25.0 (±1.41)	19.67 (±.52)	27.03 (±0.97)	29.59 (±2.01)	76.94 (±8.36)	69.38 (±10.7)

Sample size = 60 snails for each ecotype.

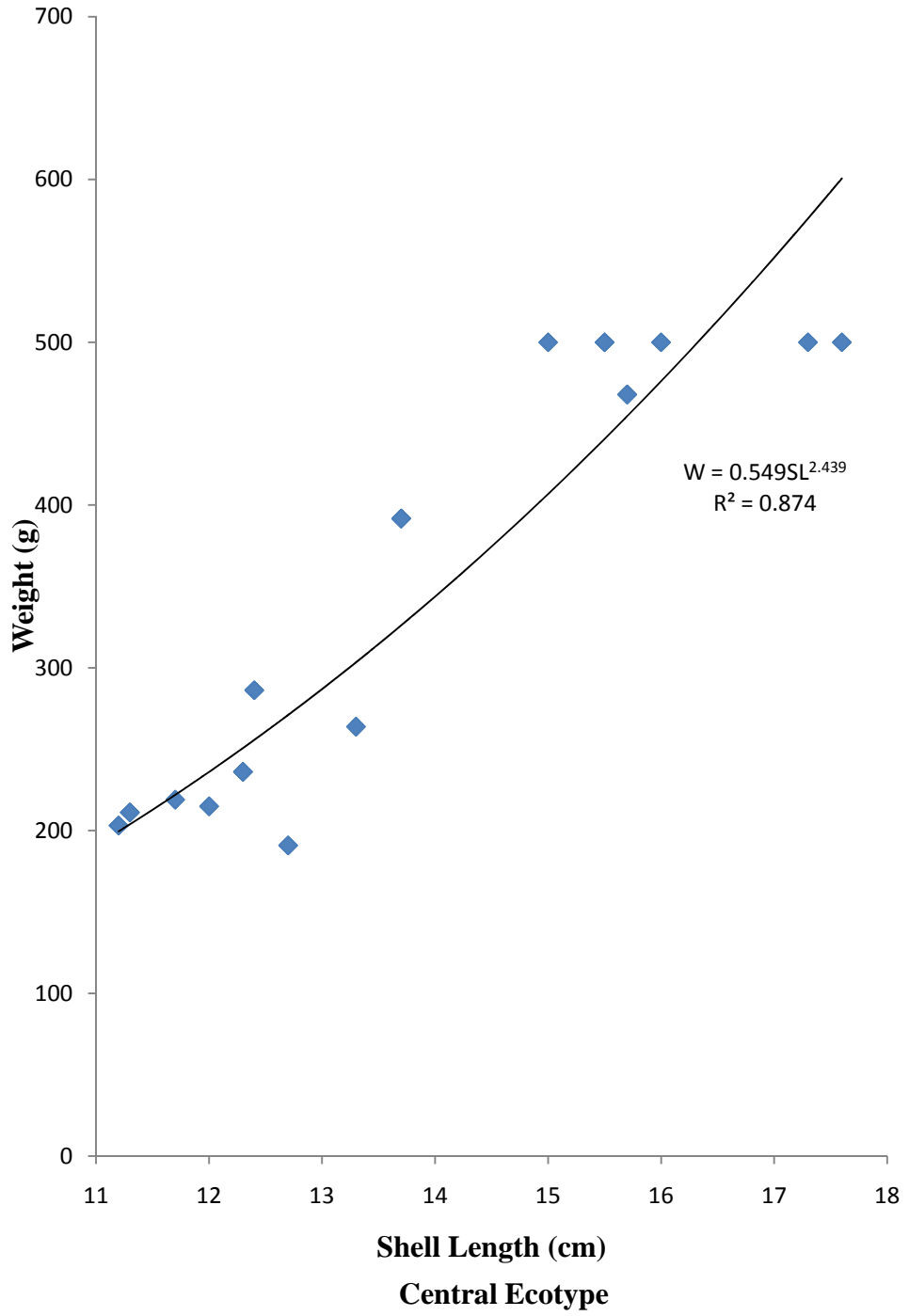
### **Incubation period**

Hatching periods for eggs laid by snails from the Ashanti ecotypes were longer compared with those for the Western ecotype. The outdoor set up recorded shorter incubation period compared with those from the indoor (Table 3). Relative humidity and temperatures were relatively higher for the outdoor experimental area compared with those of the indoor area.

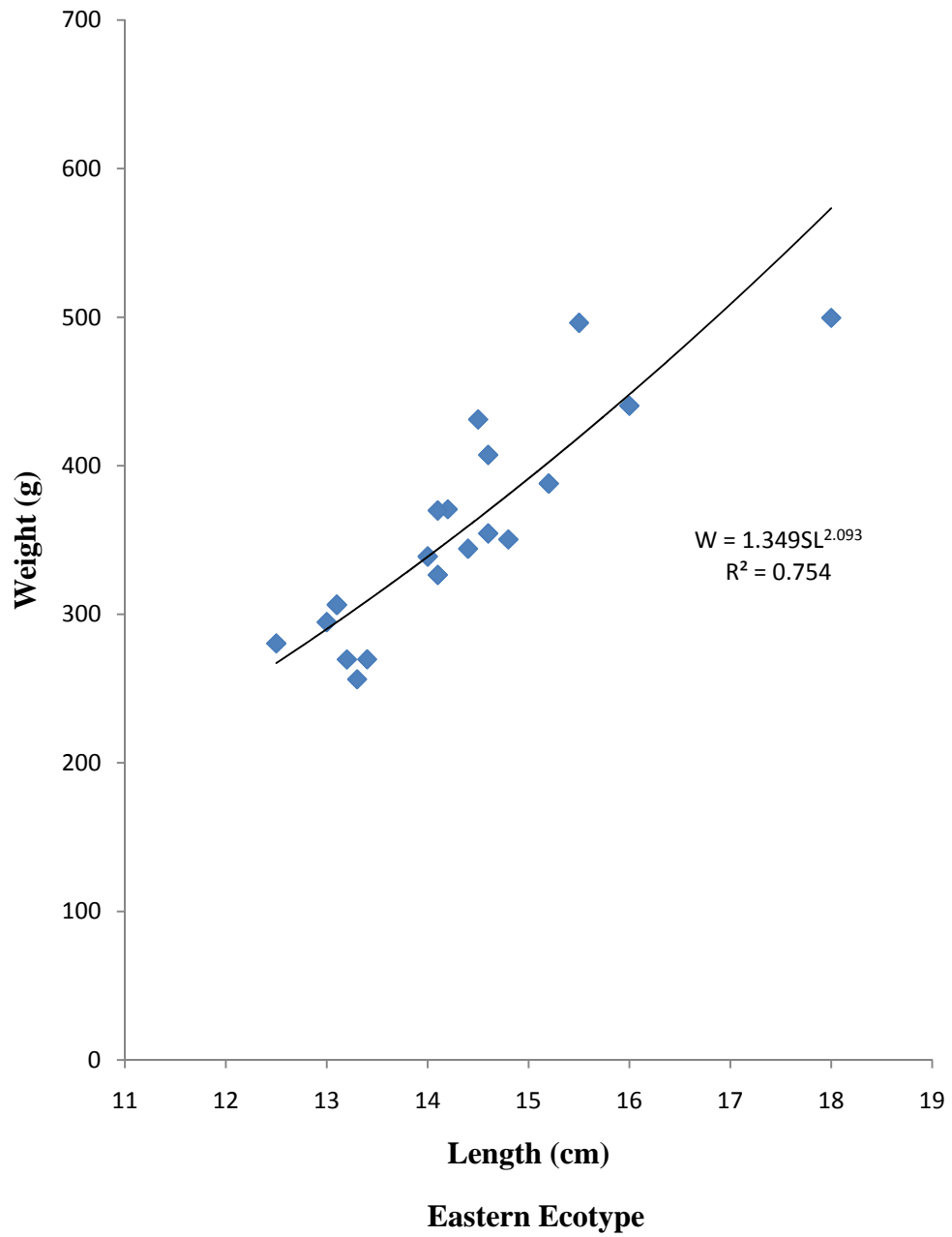




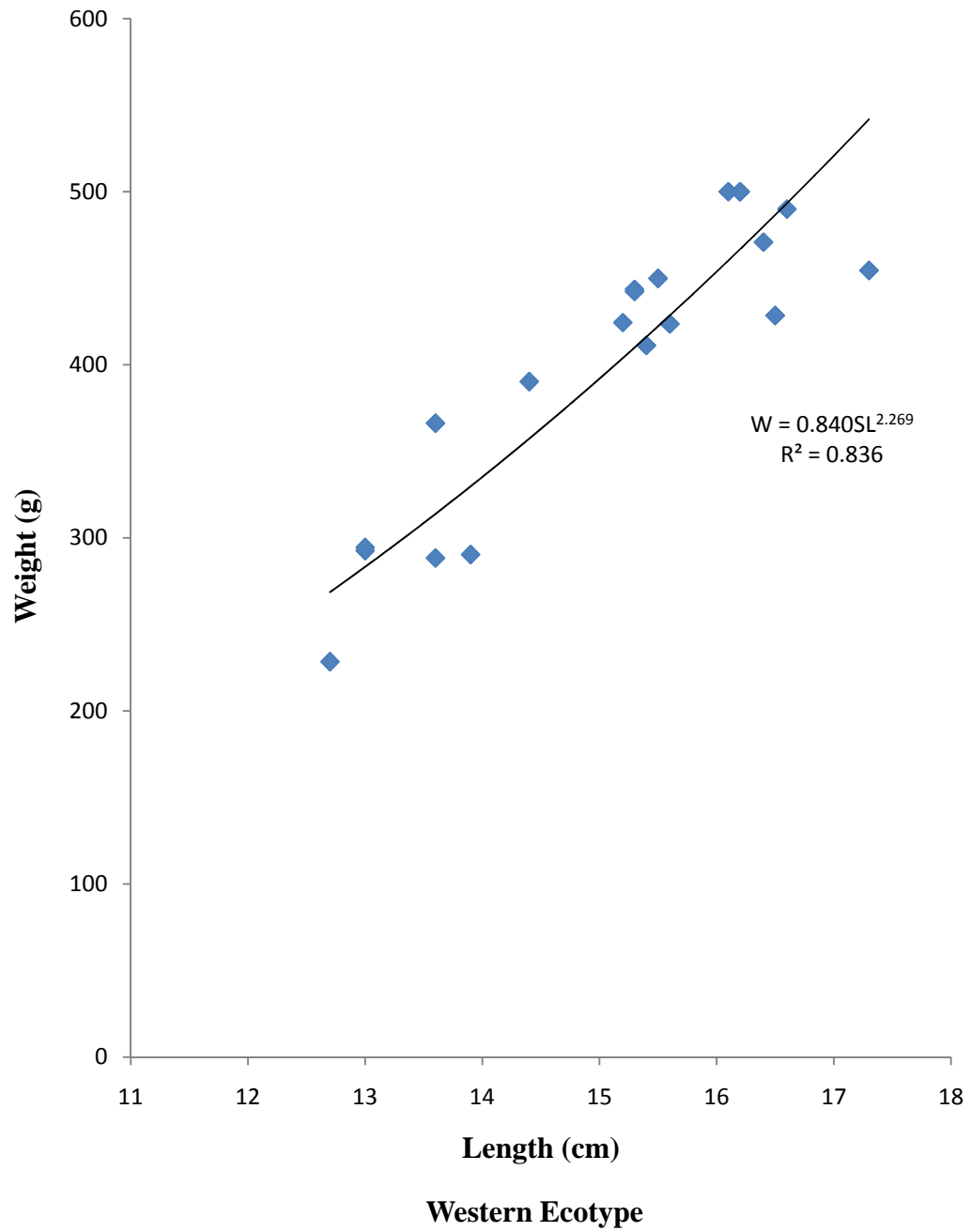
**Fig. 6: The relationship between the weight and shell length of the Ashanti ecotype**



**Fig. 7: The relationship between the weight and shell length of the Central ecotype**



**Fig. 8: The relationship between the weight and shell length of the Eastern ecotype**



**Fig. 9: The relationship between the weight and shell length of the Western ecotype**

### **Weight and shell length relationship**

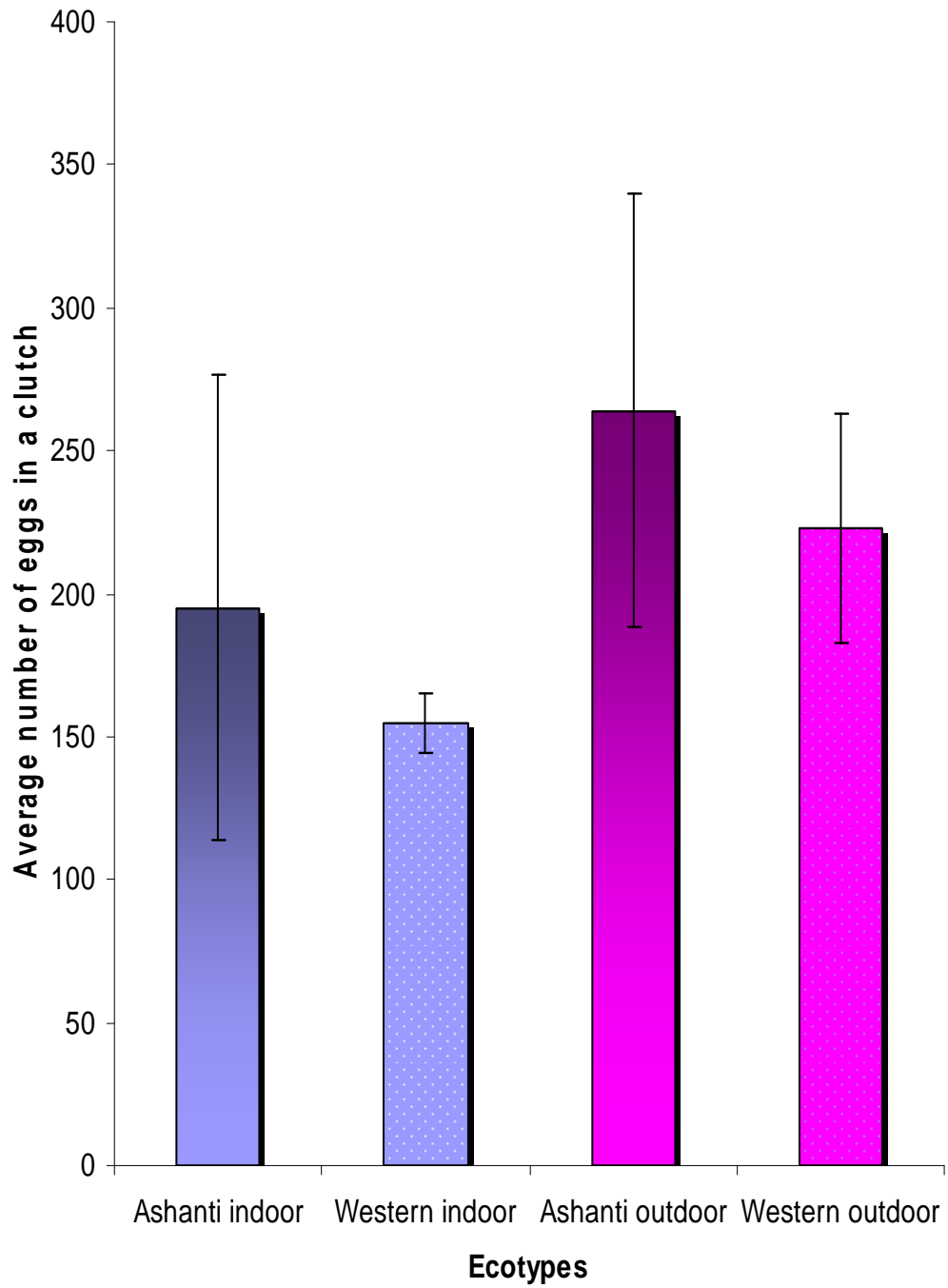
The relationship between the weight and shell length of the snail indicates a negative allometric growth (i.e. snail grows longer than it puts on weight = snail is too light for its shell length). However, this can be justified since the flesh of the snail fills only a small portion of the shell and also since the shell is supposed to be more protective in nature. From the equations above, one cannot use the Weight-Shell length relationship to differentiate between the various ecotypes.

### **Total egg laying**

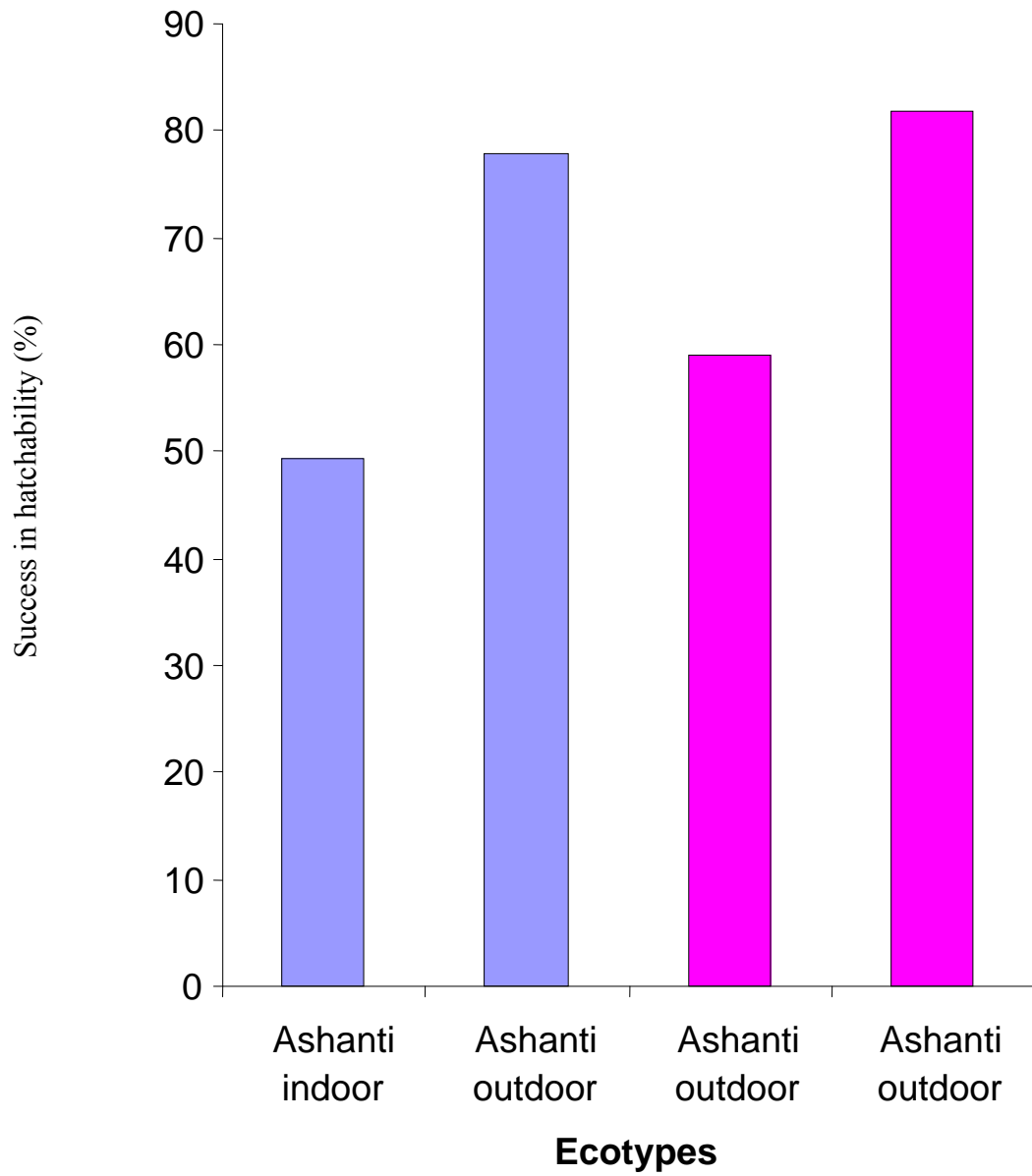
Total number of eggs laid was higher in snails from the Ashanti region compared with those from the Western region in both indoor and outdoor breeding system. The Eastern and Central ecotypes did not lay eggs during the experimental period. Egg laying was higher for the outdoor set up compared with those obtained from the indoor breeding system (Figure 6).

### **Hatchability**

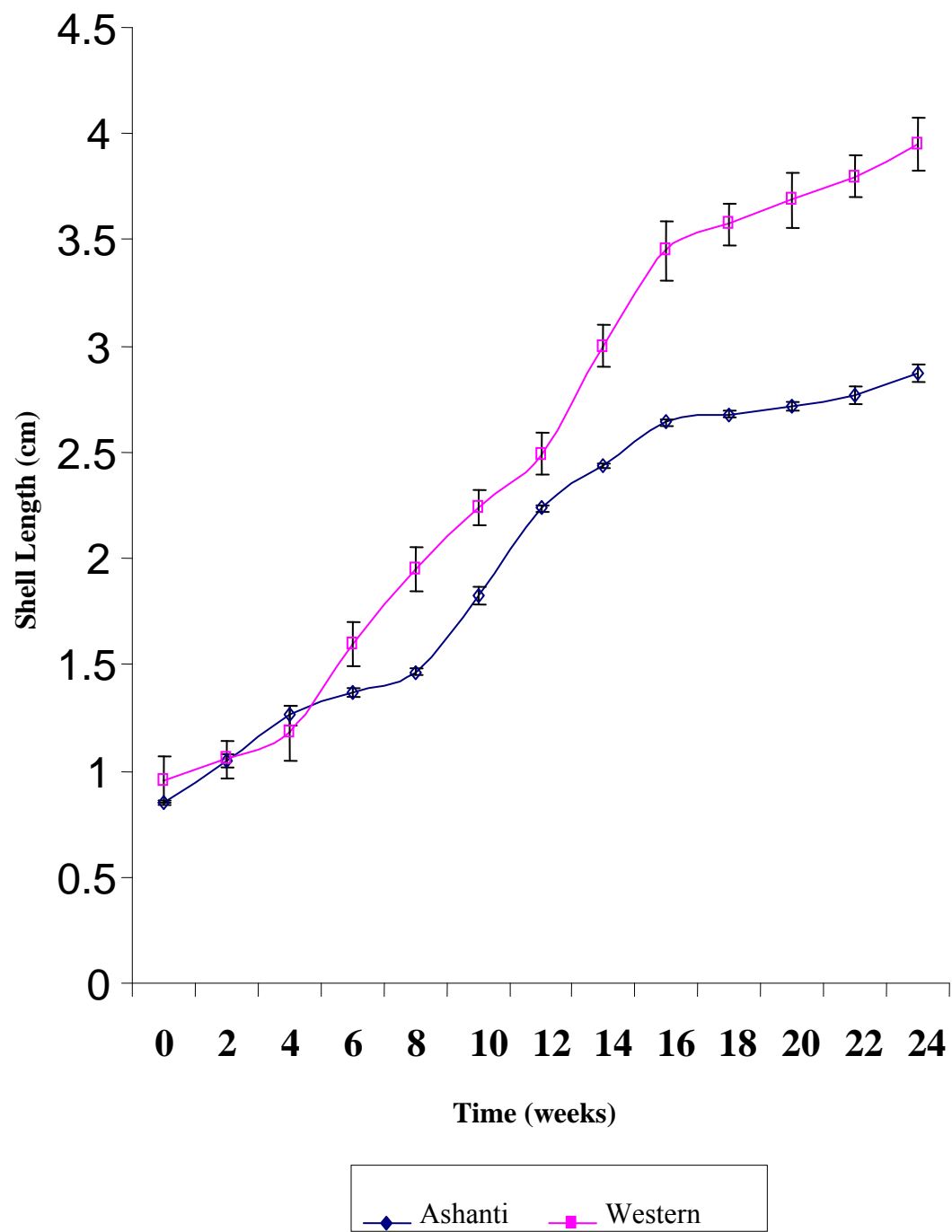
Hatchability, which is the percentage of eggs that hatched or the number of hatchlings produced, were higher for the eggs laid by snails from the Western region than those from the Ashanti region for both indoor and outdoor breeding system. In addition to this, the outdoor set up recorded higher success in hatchability compared with those of the indoor system (Figure 7).



**Fig. 10: The reproductive capacity of the Ashanti and Western ecotypes for the indoor and outdoor breeding system**



**Fig. 11: Percentage of hatched eggs of the Ashanti and Western ecotypes for indoor and outdoor breeding system**



**Fig 12: Life growth trends of juvenile snails from Ashanti and Western ecotypes reared indoors in wooden boxes for 24 weeks**



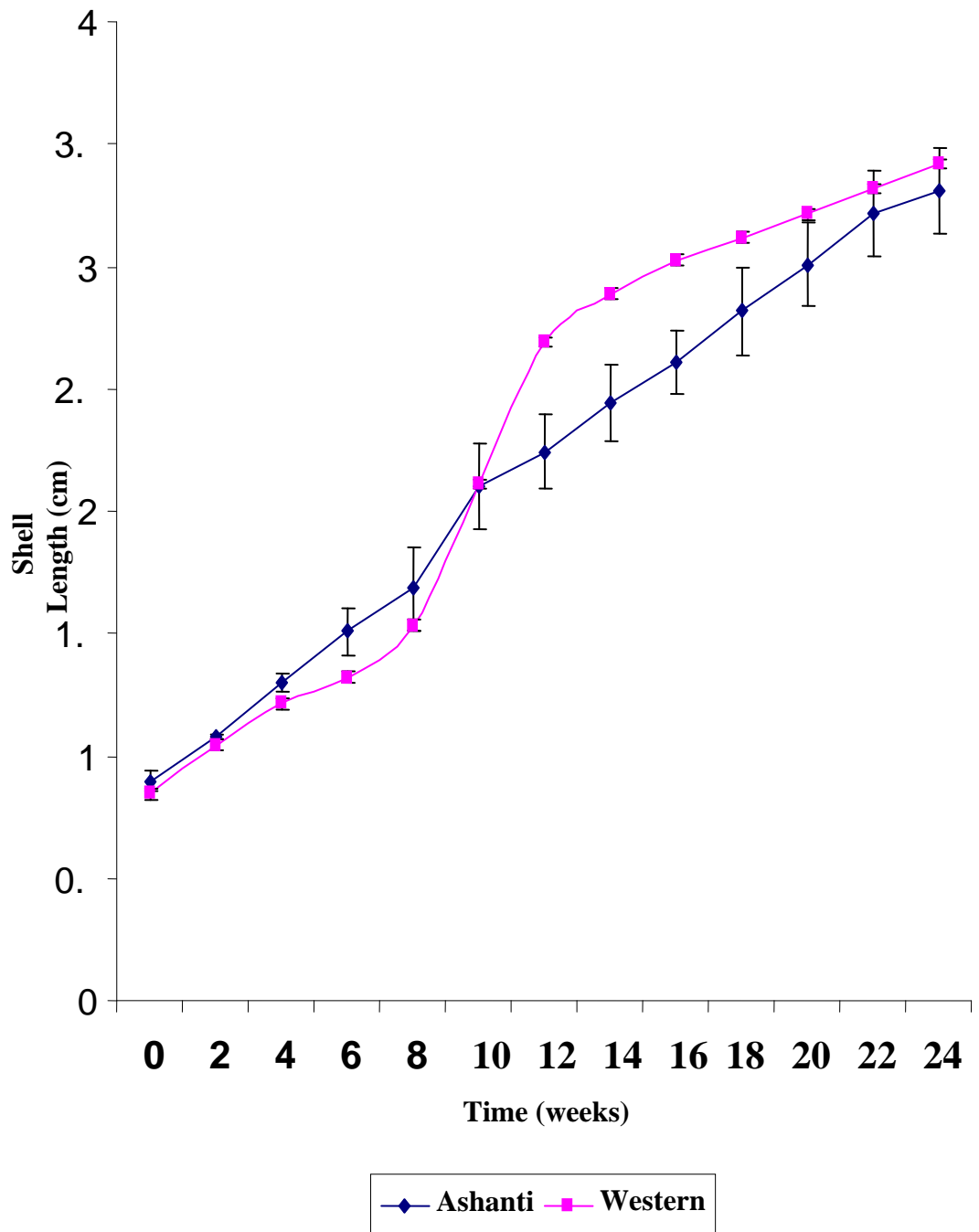
### **Growth pattern of juvenile snails from Ashanti and Western Regions reared indoor**

The growth of the snails is characterized by three distinct phases; an initial slow phase (week 0 – 4), a sharp incremental phase (week 6- 18), followed by another phase of slow growth (week 18-24). The growth of juvenile snails from both Ashanti and Western regions follow a normal sigmoid curve characteristic of many organisms (Figure 8).

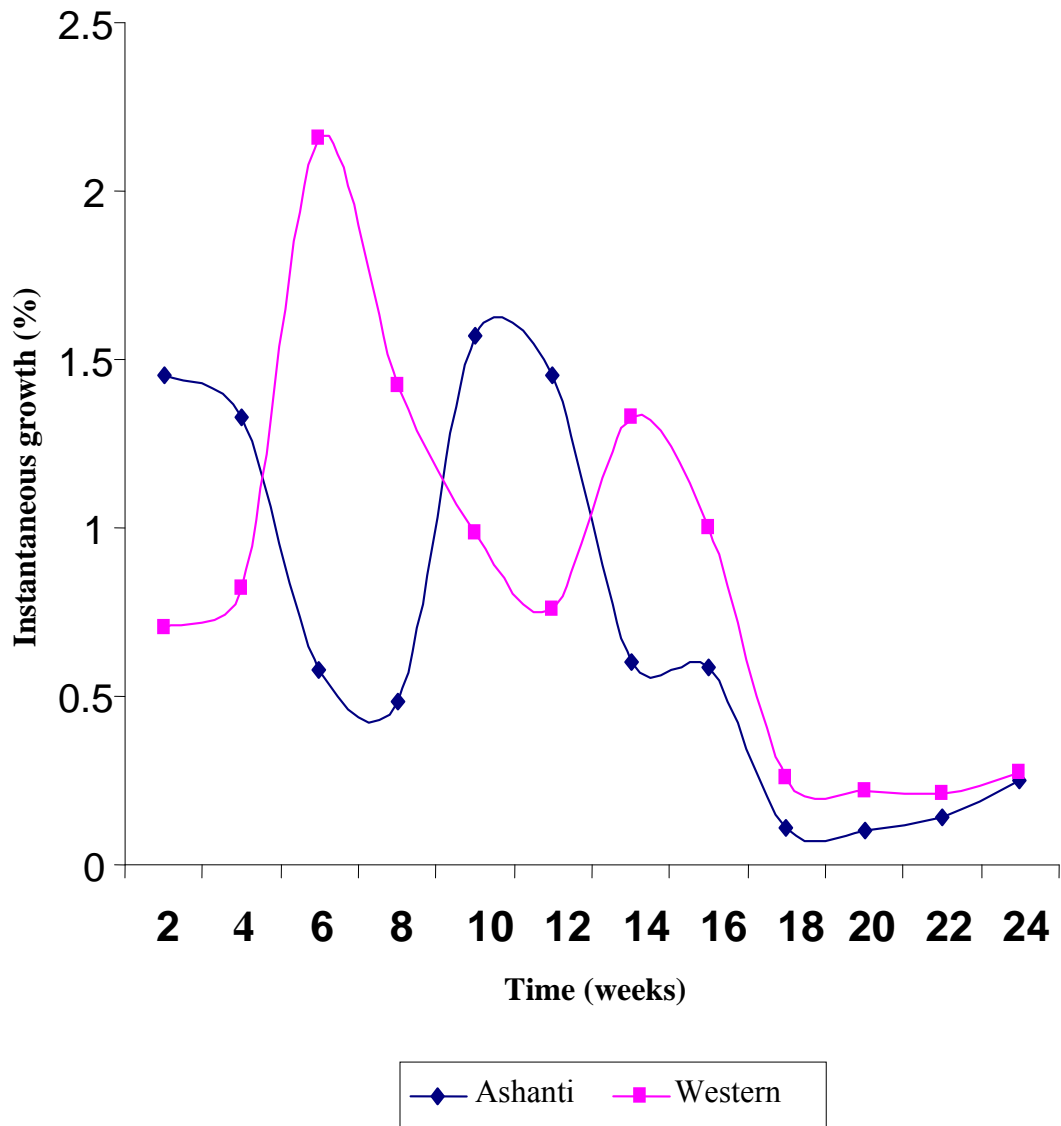
### **Growth pattern of juvenile snails from Ashanti and Western Regions reared outdoor**

The growth of the juvenile snails from the Ashanti Region was characterized by three distinct phases; an initial slow phase (week 0 – 8), a sharp incremental phase (week 8- 10), followed by another phase of slow growth (week 10-24). The growth of the juvenile snails from the Western Region was also characterized by three distinct phases; an initial slow phase (week 0 – 6), a sharp incremental phase (week 6- 12), followed by another phase of slow growth (week 12-24). The growth of juvenile snails from both Ashanti and Western regions follow a normal sigmoid curve characteristic of many organisms (Figure 9).

Generally, the juvenile snails from Western ecotypes grew heavier and larger than those of the Ashanti ecotype for the indoor set up as shown in figure 8 and 9.



**Fig 13: Life growth trends of juvenile snails from Ashanti and Western ecotypes reared outdoor in wooden boxes for 24 weeks**



**Fig. 14: Specific growth rate of juvenile snails from the Ashanti and Western ecotypes reared indoors**

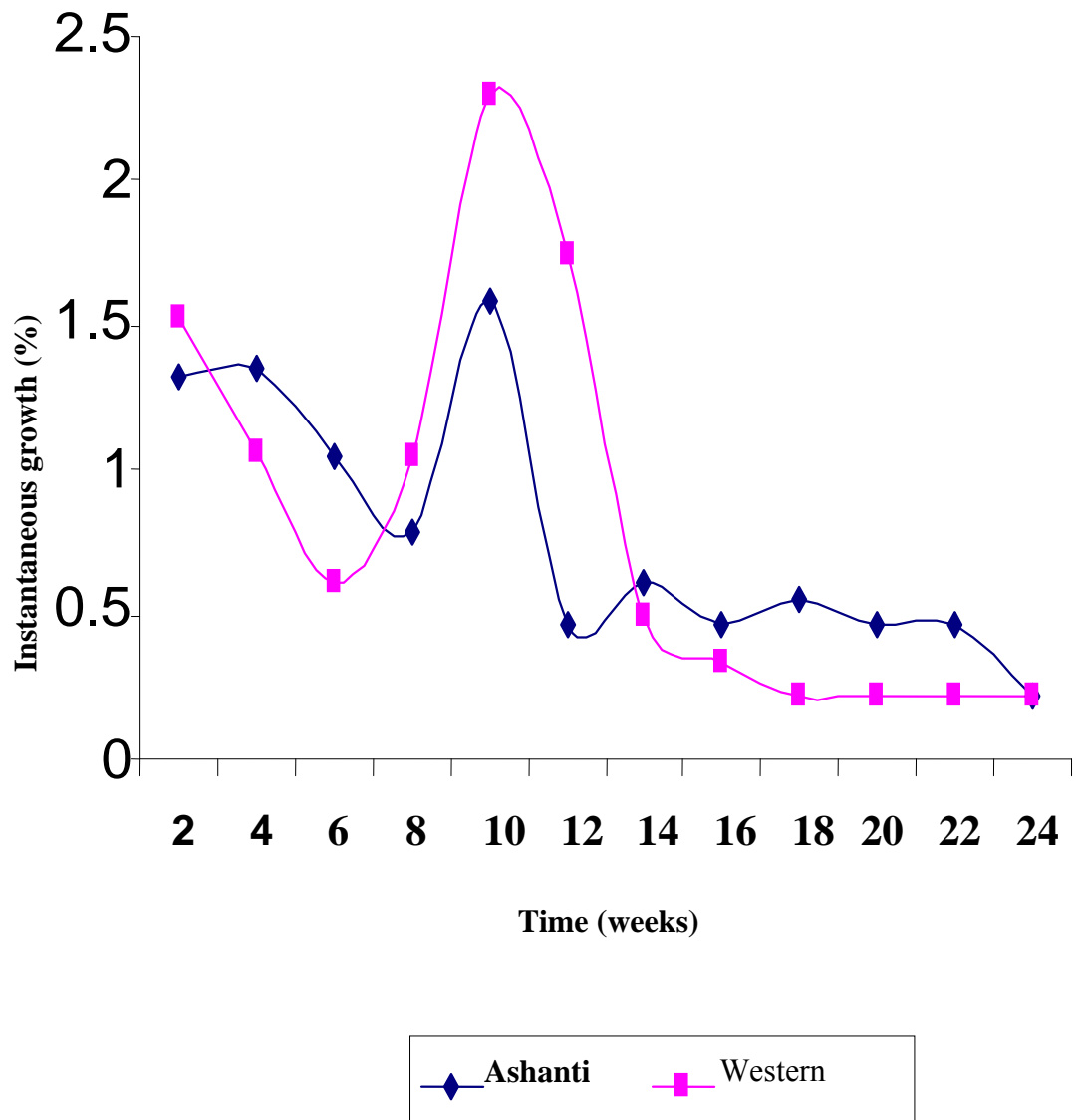
### **Specific growth rate (SGR) of juvenile snails from Ashanti and Western Regions reared indoors**

The instantaneous growth rate provides information on the growth at every stage. It was observed that there were two main peaks in the growth pattern of the two ecotypes; week 6 and 14 for the Western and week 2 and 12 for the Ashanti ecotype. It is also observed that generally, growth was higher for the juvenile stage and slow in the adult stage as shown in figure 10.

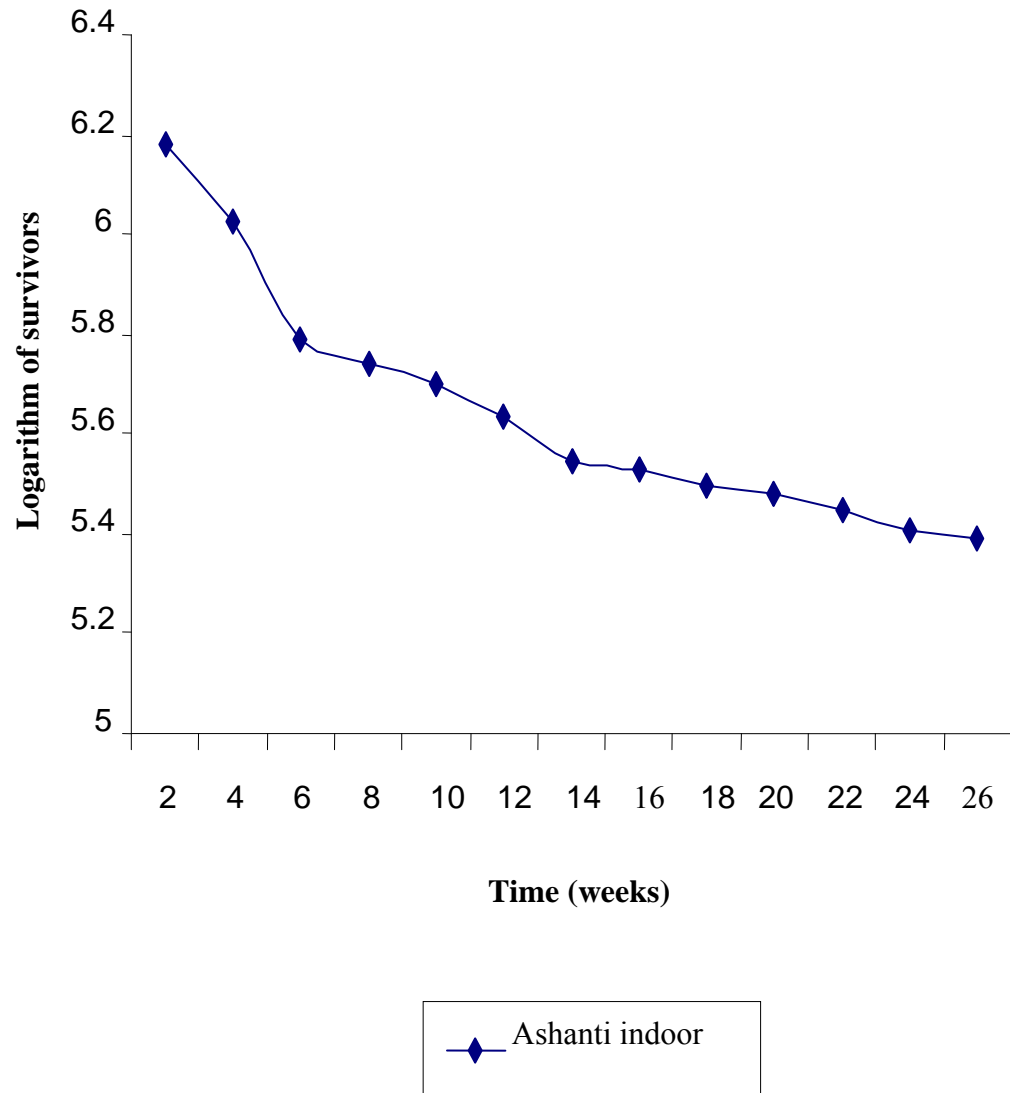
### **Specific growth rate (SGR) of juvenile snails from Ashanti and Western Regions reared outdoor**

It was observed that there was one main peak in week 10 in the growth pattern of the two ecotypes. It was also observed generally that, growth was higher for the juvenile stage and slow in the adult stage.

Generally, the weekly rate of increase in shell length were (respectively) higher in the juvenile snails from the Western ecotype compared to those of the Ashanti ecotype in both the indoor and outdoor experimental designs as shown in figure 11.



**Fig. 15: Specific growth rate of juvenile snails from the Ashanti and Western ecotypes reared outdoor**



**Fig. 16a: Survivorship curve for juvenile snails from the Ashanti Region reared indoors**

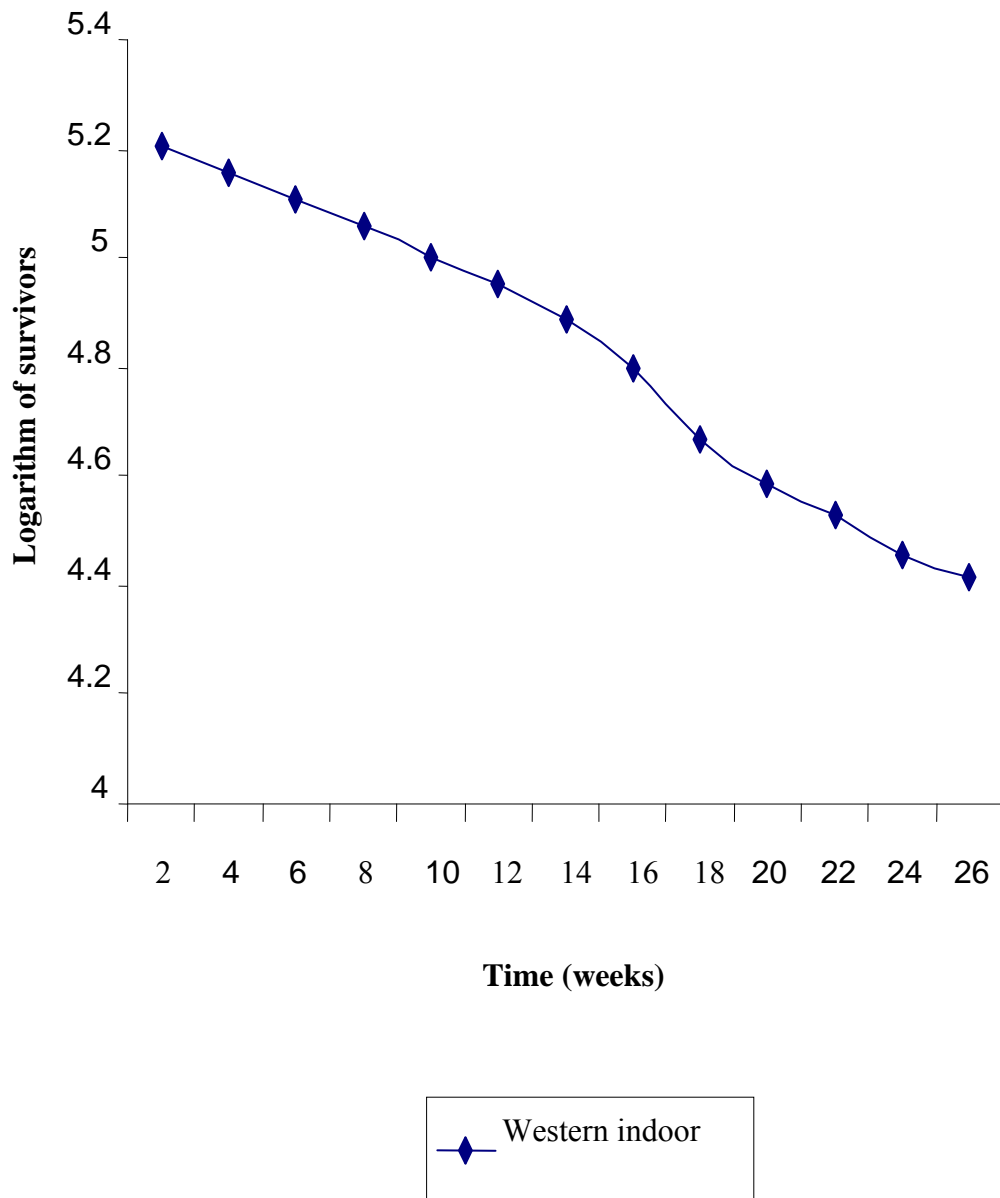


Fig. 16b: Survivorship curve for juvenile snails from the Western Region reared indoors

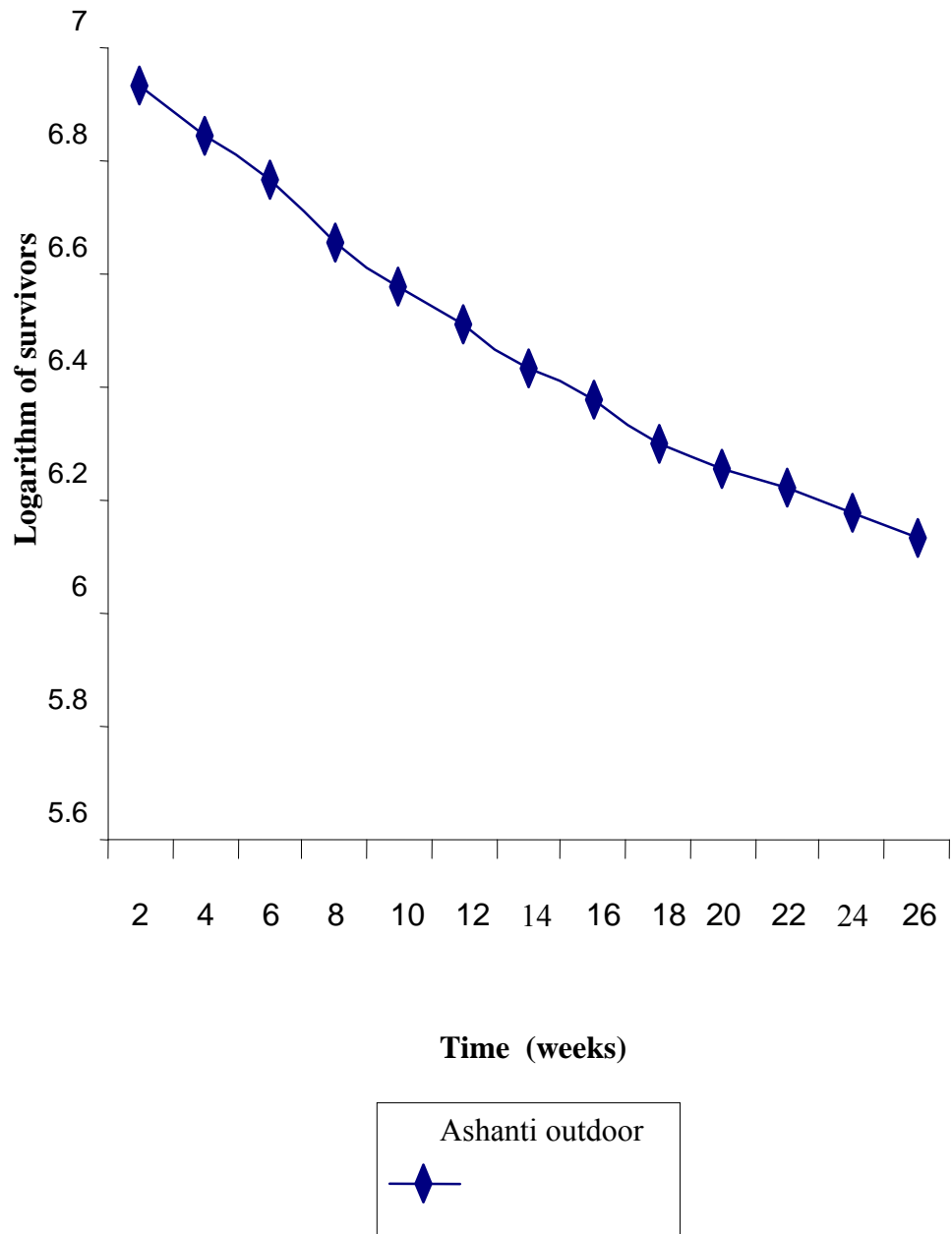


Fig. 16c: Survivorship curve for juvenile snails from the Ashanti Region reared outdoor



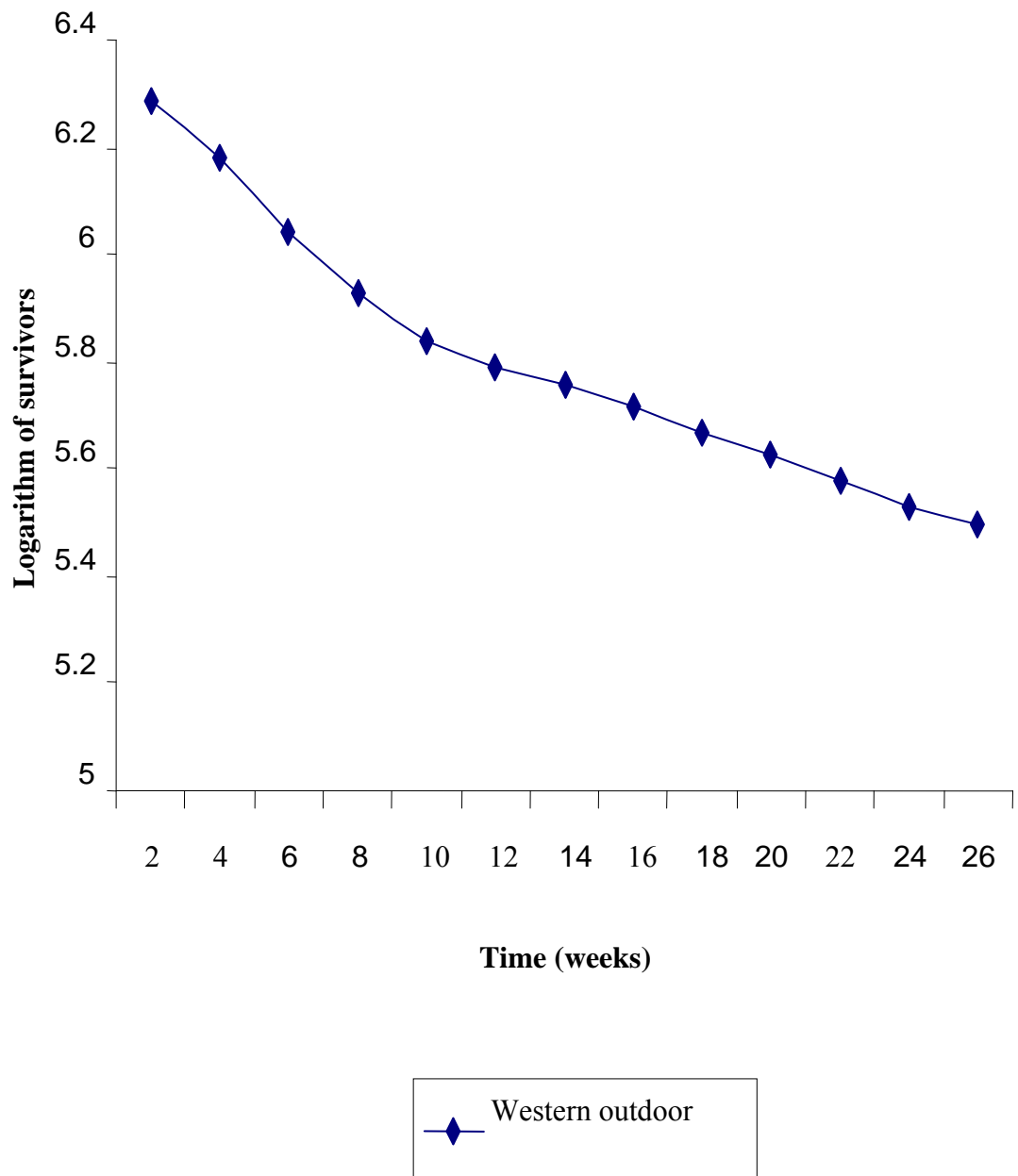


Fig. 16d: Survivorship curve for juvenile snails from the Western Region reared outdoor

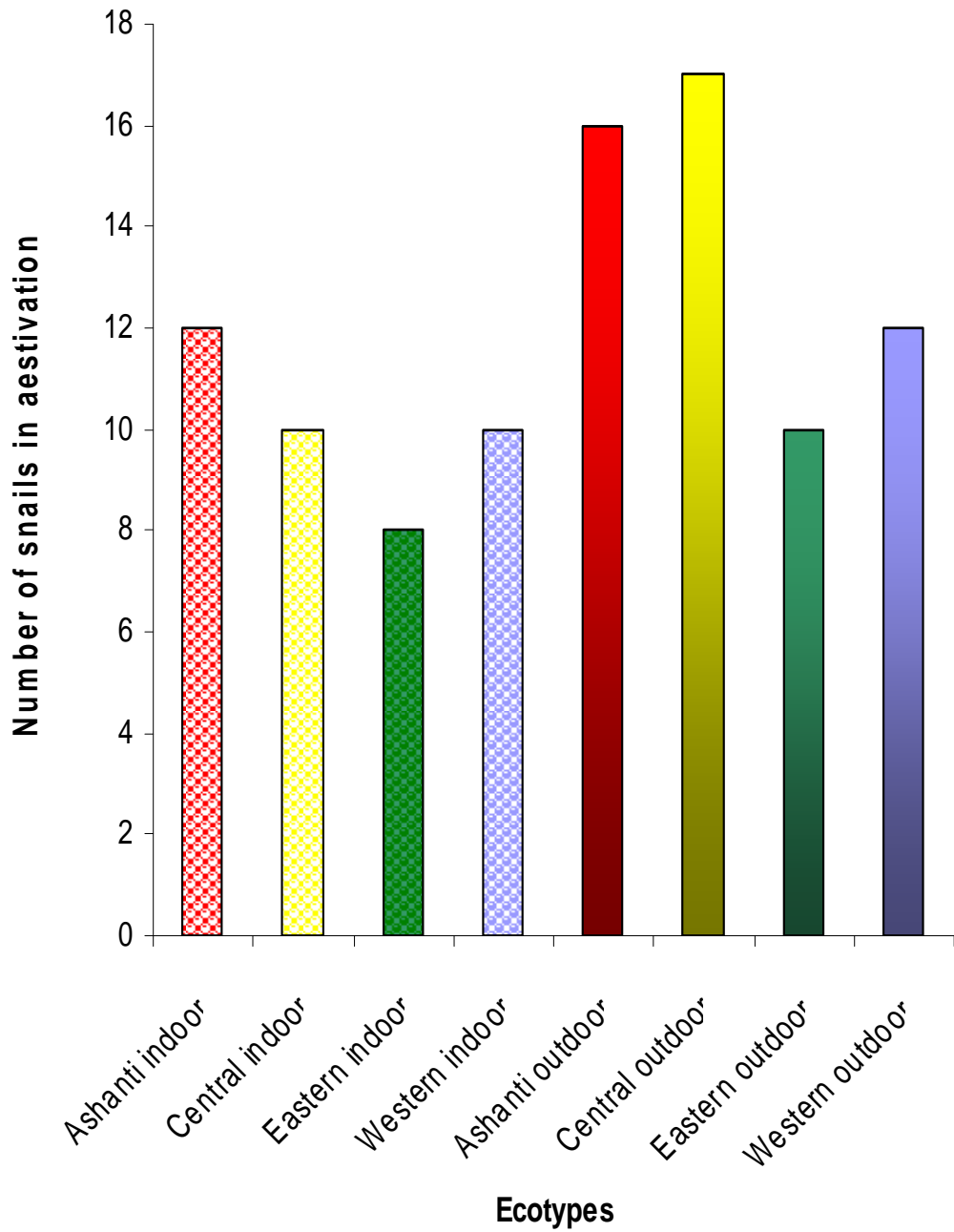
### **Survivorship curve for juvenile snails from the Ashanti and Western Region reared indoor and outdoor**

Generally, mortality was higher in the juvenile snails from the outdoor system than for those in the indoor system as in figure 12 a-d. Mortality in the juvenile snails was high from the first week to the eighth week in both indoor and outdoor experiments.

The percentage of juvenile snails surviving at the end of the research was higher in the Western ecotype compared with those of the Ashanti ecotype for both indoor and outdoor experiment (figure 12a-d).

### **Aestivation pattern of ecotypes of snails for the indoor and outdoor breeding experimental designs.**

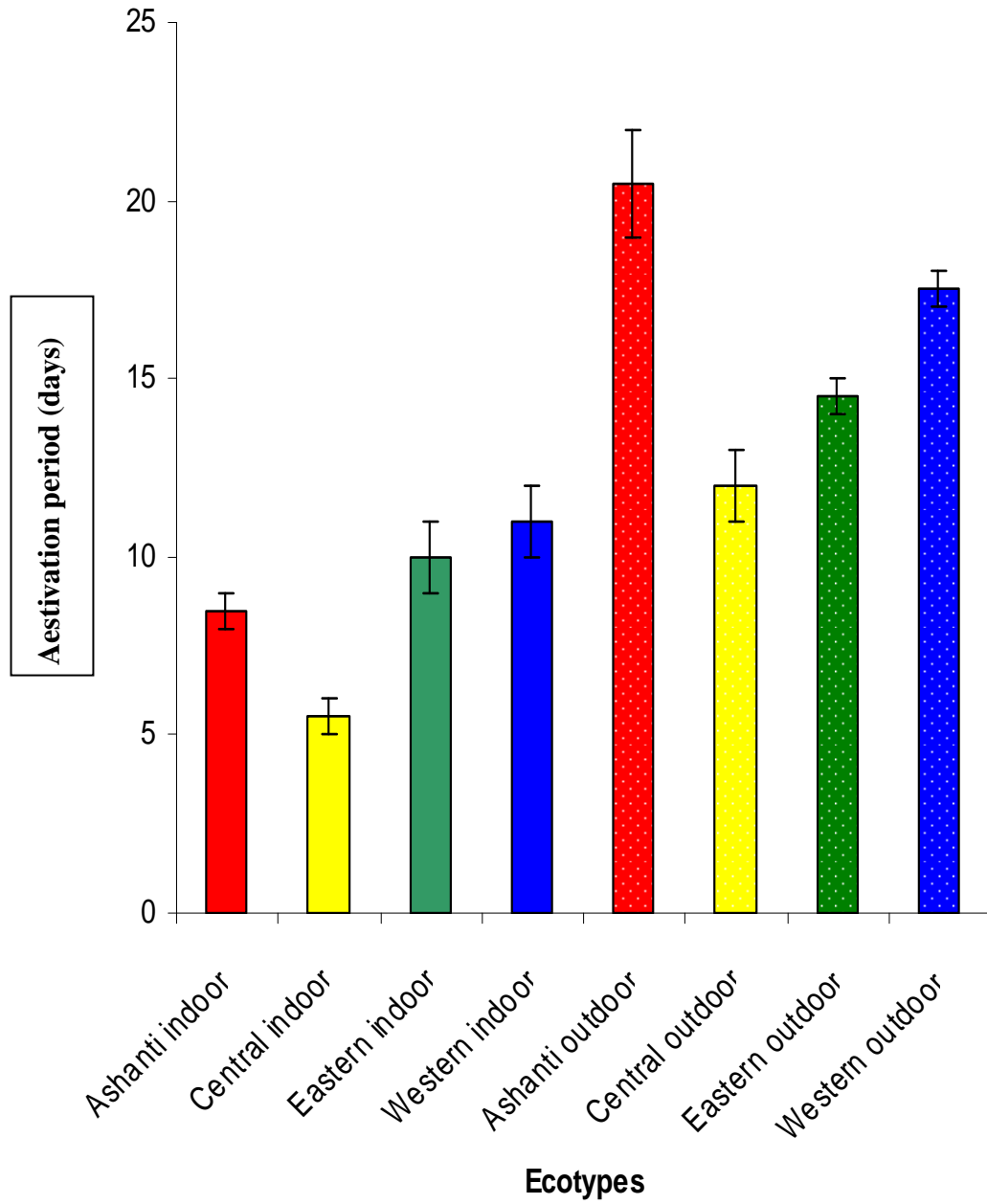
In the indoor experiment, snails from the Ashanti region recorded the highest number of snails aestivating. This could be attributed to differences in weather conditions or rainfall pattern in the region of collection. This was followed by the Western, Central and Eastern ecotypes respectively. However, the Western ecotype spent the longest period of 11 days in aestivation while the Central ecotype spent the shortest period of 6 days in aestivation. This could be attributed to the adaptation of the various ecotypes to the environmental conditions such as humidity, precipitation and temperature existing in their places of collection (figure 13).



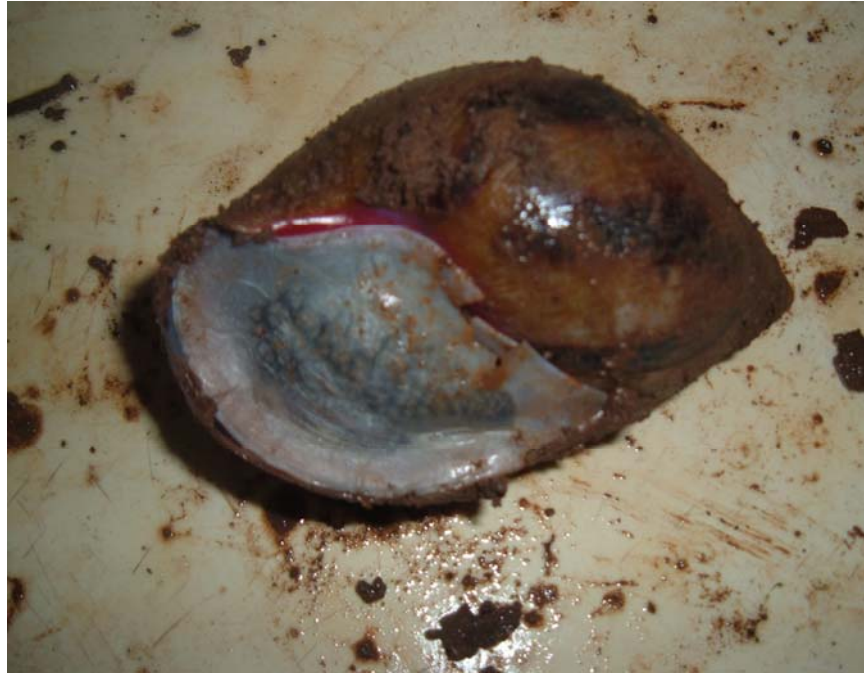
**Fig. 17: The number of snails that aestivated for the four ecotypes of snails for the indoor and outdoor breeding experimental designs**

However, for the outdoor experiment, ecotypes of snails from the Central region recorded the highest number of 17 snails aestivating. This could be attributed to differences in weather conditions or rainfall pattern in the region of collection (Central). This was followed by the Ashanti, Western and Eastern ecotypes respectively. However, the Ashanti ecotype spent the longest period of 21 days in aestivation while the Central ecotype spent the shortest period of 12 days in aestivation for the outdoor experiment (figure 14).

Generally, the number of snails which went into aestivation was higher for the outdoor experiment compared with those from the indoor experiment (figure 14). In addition to this, aestivating snails from the outdoor experiment spent longer period in aestivation compared to those in the indoor experiment.



**Fig. 18: Aestivation pattern of the four ecotypes of snails for the indoor and outdoor breeding experimental designs**



**Plate 16:** *Achatina achatina* snail from the Eastern Region sealed into its shell by a calcareous layer in full aestivation



**Plate 17:** *Achatina achatina* snail from the Eastern Region in aestivation

**Key:**

E - Epiphragm



**Plate 18:** *Achatina achatina* snail from Ashanti Region coming out of aestivation



**Plate 19:** *Achatina achatina* snail from Eastern Region coming out of aestivation

**Key:**

E – Broken epiphragm, B – Exposed body of snail, S - Shell



**Plate 20: *Achatina achatina* snails buried in the soil to avoid aestivation (indoors)**



**Plate 21: *Achatina achatina* snails buried in the soil to avoid aestivation (outdoor)**

**Key:**



S - Snail



**Plates 22: *Alluaudihella flavicornis*, natural enemy of snails**

**Key:**

D – Dorsal portion  
V – Ventral portion

## CHAPTER FOUR

### DISCUSSION

The study of speciation has been one of the most active areas in evolutionary biology, because it is believed to be one of the keys to full understanding of the process of evolution (Rolan-Alvarez, 2007). Species are groups of interbreeding populations that are reproductively isolated from other such groups (Coyne and Orr, 2004). Speciation, therefore, is the process by which the isolation barrier arose in an ancestral population. One of the most important mechanisms contributing to speciation is when the isolation barrier emerges as a by-product of the process of adaptation to a new environment (Rolan-Alvarez, 2007). Initial adaptation to a new environment by many species has often resulted in the establishment of ecological types.

The study was a geographic survey, sampling replicate populations and hypothesized that there may be different forms of *Achatina achatina*. In addition, common breeding experiments to determine whether any morphologically different forms represent genetically inherited traits were performed.

#### **Electrophoresis**

Electrophoresis of the haemocyanin (blood pigment of snails) of the four ecotypes of *Achatina achatina* snails did not give any visible difference in the

movement of the molecules as shown in plate 13. This indicates that there is no distinct difference in the molecular composition of the haemocyanin of snails from the four districts. The implication is that, although there may be the existence of ecotypes of *Achatina achatina*, this has not resulted in the formation of new species.

There were no differences in differential separation of the DNA from the four snail ecotypes as shown in Plate 13c. This indicates that the DNA was from the same species and therefore the four snail ecotypes are not different species.

### **Examination of the four snail ecotypes**

Examination of the snails sampled from the four districts indicated some morphological differences in shell colour and stripe patterns as well as body colouration. There were differences in the nature, pattern, thickness and ranges of black stripes on the shells of the four ecotypes. There was a significant difference ( $P < 0.05$ ) in the distances between adjacent stripes on the shells of the snails from the four ecological regions (Table 1). There was a significant difference ( $P < 0.05$ ) in the number of stripes on the shells of the four snail ecotypes. In addition to this, there were some differences in the distribution of the grey speckles in the black skin of the four snail samples from the four districts studied as shown in plates 14 (A-D). The differences in the distribution of grey speckles in the black skin of *Achatina achatina* ecotypes from the four regions could be partly environmentally or physiologically influenced.

In the slug, *Carinarion* species, there are strong indications that colour of skin is influenced by environmental conditions and physiology of snail. Jordaens *et al.*, (2000), showed that food may influence body pigmentation in *Carinarion* species. In *Arion fasciatus*, one of the three *Carinarion* species, a diet of carrot, paper or lettuce resulted in the loss of the yellow-orange lateral bands (Jordaens *et al.*, 2000). The nature and possible ‘adaptive’ significance of food-induced changes in body pigmentation have also been explored. The few studies that have addressed this issue in slugs suggested that darker animals are better adapted to low temperatures and high altitudes and that pale animals are better adapted to high temperatures and low altitudes (Reise, 1997).

There was overlap in the stripe pattern for twenty snails from the Western ecotype. These snails had a combination of the stripe pattern of the Ashanti and Western ecotypes. This could be due to the fact that the Western region shares boundary with the Ashanti region and hence some snails might have crossed over and bred with the Ashanti ecotypes. Since they are of the same species this could have resulted in the transfer of these traits among the two ecotypes.

### **Length, weight and width of parent stock from the four ecotypes**

Snails from the Western region had the highest mean weight of 382.57g ( $\pm 9.74$ ) with a corresponding mean length of 14.43cm ( $\pm 0.17$ ) and width of 79.56mm ( $\pm 0.792$ ). This was then followed by snails from the Ashanti ecotypes with a mean weight of 360.91g ( $\pm 6.63$ ) with a corresponding mean length of 14.05cm ( $\pm 0.11$ ) and width of 77.34mm ( $\pm 0.573$ ). The Eastern ecotype had a

mean weight of 359.70g ( $\pm 9.31$ ) with a corresponding mean length of 14.59cm ( $\pm 0.14$ ) and width of 76.12mm ( $\pm 0.779$ ). Finally, snails from the Central ecotype recorded the lowest mean weight of 331.30g ( $\pm 12.0$ ) with a corresponding mean length of 13.09cm ( $\pm 0.18$ ) (Table 3) and width of 73.60mm ( $\pm 1.05$ ). However, there was a significant difference ( $P < 0.05$ ) in the lengths, weights and widths of the parent stock of snails from the four ecotypes used for the experiments (Appendix I).

The results in Table 1 indicate that snails from Western region were significantly heavier than those from the other three regions. Snails are cold blooded animals and therefore sensitive to changes in humidity and temperature (Cobbinah, 1992). When humidity falls below 75%, as is the case during the dry season (November to March), *A. achatina* becomes inactive and seals itself in its shell in order to prevent water loss from the body. This reaction is typical of all snail species. In unfavourable conditions, they go into dormancy, feeding activity stops and they rely on stored nutrients in the body until favourable conditions arise when they come out of aestivation and begin to feed.

Cobbinah (1992) showed that there was a correlation between length of aestivation and size of the snail. Therefore, the differences in size of snails may be attributed partly to the differences in the length of aestivation period and weather conditions in the four regions. Thus, the shorter the aestivation period, the longer the feeding period and the heavier and larger the ecotype (Cobbinah, 1992). Hence snails from Wassu West District in the Western region, with a mean annual rainfall of 1878.3 mm, which is the highest and longest in Ghana, might have

shorter aestivation periods and therefore longer feeding period as compared with snails from the other three regions. The rainfall pattern in this region creates favourable conditions (food availability and high humidity) which enable snails to feed for longer periods resulting in the Western ecotype being heavier and larger than the other three ecotypes.

### **Shell fill of snail's body**

There was a noticeable difference between the four ecotypes from the four ecological regions in the extent to which the body of the snails filled the shell. Out of hundred snails observed from each of the four ecological regions, the Western ecotype recorded 91 snails being the highest while the Central ecotype recorded 78 snails being the lowest number of snails which could withdraw completely into the shell without leaving empty space at the mouth (Table 1). The implication is that snails with shorter aestivation period and longer feeding period often have heavy shell fill due to the length of the feeding period. Snails from the four districts however had the same number of whorls on the shell as shown in Table 1.

### **Number of clutches and mean eggs laid**

The results, summarized in Table 2 show that out of the four ecotypes of *Achatina achatina* snails set up for the indoor and outdoor breeding experiments, Ashanti and Western ecotypes laid eggs while Central and Eastern ecotypes did not lay eggs during the experimental period. Some individuals of *Achatina*

*achatina* have been observed not to lay eggs for three successive seasons. Whether this is due to infertility or growth aberrations is not known (Cobbinah, 1992).

Ecotypes from Ashanti Region produced five clutches of eggs (being the higher) with a mean of 195 ( $\pm 81.2$ ) and 264 ( $\pm 75.6$ ) eggs for indoor and outdoor experiments respectively (Table 2). Those from the Western Region produced two and three clutches of eggs each with an average of 154.50 ( $\pm 10.61$ ) and 222.7 ( $\pm 40.2$ ) eggs in a clutch for both indoor and outdoor breeding experiments respectively (Table 2). Although egg laying by snails from Ashanti ecotype was higher, this was not significantly different ( $\chi^2 = 2.92$ ,  $df = 1$ ,  $P > 0.05$ ) from those of the Western ecotype. Hence egg laying in snails from both the Ashanti and Western regions was relatively the similar under the same treatment. This indicates that the two ecotypes have relatively the similar reproductive potential. Generally, snails used for the outdoor breeding experiment produced higher mean number of eggs in a clutch as compared to those of the indoor experiment. However, there was no significant difference ( $\chi^2 = 2.92$ ,  $df = 1$ ,  $P > 0.05$ ) in egg laying by snails from both the indoor and outdoor experiment (Appendix II).

#### **Time of egg hatching and temperature and humidity readings**

At a mean temperature of 27.0 °C ( $\pm 0.97$ ) and a mean relative humidity of 76.94% ( $\pm 8.36$ ), eggs laid by snail ecotype from the Ashanti region took an average of 25.5 ( $\pm 3.54$ ) days to hatch while, eggs laid by snails from the Western region took 25.0 ( $\pm 1.41$ ) days to hatch for the indoor experimental set up (Table

3). However, at a mean temperature of 29 °C ( $\pm 2.01$ ) and humidity of 69 % ( $\pm 10.70$ ), eggs laid by snails from the Ashanti region took an average of 20.0 ( $\pm 2.55$ ) days to hatch while those laid by snails from the Western region took an average of 19.7 ( $\pm 1.52$ ) days to hatch for the outdoor experimental set up (Table 3).

Generally, eggs laid by snails from the outdoor experimental set up hatched earlier than those from the indoor experimental set up. This could be attributed to the differences in temperature and relative humidity in the animal house and the snail production unit of the Technology Village respectively. There was a significant difference ( $P < 0.05$ ) in the temperature and the relative humidity readings from the indoor and the outdoor experimental areas (Appendix II) and this could be the reason. There was no significant difference ( $\chi^2 = 2.92$ ,  $df = 1$ ,  $P > 0.05$ ) in the time of hatching for eggs laid by the Ashanti and Western ecotypes. However, there was a significant ( $\chi^2 = 2.92$ ,  $df = 1$ ,  $P < 0.05$ ) difference between the time taken for the eggs to hatch in both the indoor and outdoor experimental set up (Table 3). Hatchlings emerged out of the soil on the day of hatching.

### **Hatchability**

Hatchability, which is the percentage of eggs that hatched or the number of hatchlings produced, was higher for the outdoor breeding experiment. The outdoor breeding experiment recorded a hatchability of 1029 and 539 hatchlings out of 1320 and 659 eggs laid by the Ashanti and Western ecotypes representing



78 % and 82 % respectively (Figures 6 and 7). The Western ecotype recorded the higher success in hatchability for the outdoor experiment.

The indoor breeding experiment recorded a hatchability of 482 and 182 hatchlings out of 975 and 309 eggs laid by the Ashanti and Western ecotypes representing 49.4 % and 58.9 % respectively as shown in figure 6 and 7. The Western ecotype recorded the highest hatchability for the indoor set up. However, there was a significant difference ( $\chi^2 = 2.92$ ,  $df = 1$ ,  $P < 0.05$ ) in hatchability between the Ashanti and Western ecotypes for both indoor and outdoor set up. Although the number of eggs laid by the Ashanti ecotype was higher than the number laid by the Western ecotype, the Western ecotype recorded a higher hatchability in both the indoor and the outdoor experimental designs. Therefore, under the same breeding conditions the proportions of hatchlings produced by the Western ecotype were relatively higher than those produced by the Ashanti ecotype.

Although, hatchability or the number of hatchlings produced was higher in the outdoor set up, it did not differ significantly ( $\chi^2 = 2.92$ ,  $df = 1$ ,  $P > 0.05$ ) from that of the indoor set up. The Central and Eastern ecotypes did not lay eggs confirming Cobbinah's (1992) findings that not all adult snails breed during each breeding season. Some individuals may not lay eggs for three successive seasons. Whether this is due to infertility or growth aberrations is not known (Cobbinah, 1992).

### **Early life growth pattern**

Growth in terms of mean weekly shell length gain (MWSLG) and specific growth rate (SGR) snails from Ashanti and Western ecotypes monitored in wooden boxes are shown in Figures 12, 13, 14 and 15 respectively. Western juvenile snails showed progressively higher growth trend after the sixth and twelfth week of breeding compared with Ashanti juvenile snails in both indoor and outdoor experimental set up (Figures 12 and 13).

It was observed that there were two main peaks in the growth pattern of the two ecotypes; weeks six and fourteen for the Western ecotype and weeks two and twelve for the Ashanti ecotype (Figure 14). This could be attributed to the differences in the time of egg laying between the two ecotypes. The Western ecotype laid eggs in early November, 2006, while the Ashanti ecotype laid eggs in late November, 2006, resulting in about two weeks difference in the hatching time. Therefore, while eggs laid by snails from the Western region hatched in late November, 2006, those laid by snails from the Ashanti region hatched in December, 2006 and as a result experienced dry weather conditions at the time of hatching. It was also observed that generally, growth was faster at the juvenile stage and slowed in the adult stage. It was observed that in the indoor system there was a significant ( $\chi^2 = 0.53$ ,  $df = 21$ ,  $P < 0.05$ ) difference in growth between juvenile snails of both the Ashanti and Western ecotypes (Appendix II). This observation indicates that under favourable conditions, juvenile snails from the Western ecotype grew larger than those of the Ashanti ecotype.

However, there was only one peak (week 10) in the growth pattern of the two ecotypes for the outdoor set up (Figure 15). This is because snails from Ashanti and Western ecotypes laid eggs in late November, 2006 and hatched in December, 2006. Therefore eggs laid by snails from both regions hatched in December, 2006 and as a result experienced dry weather conditions at the time of hatching. This resulted in reduced feeding activities as hatchlings had to contend with the dry weather conditions which they usually do not experience at normal hatching periods, that is during the rainy season. There was rapid growth from the eighth week to the tenth week after which growth decreased rapidly to the twelfth week. It was also observed generally that, growth was faster at the juvenile stage and slowed in the adult stage. There was no significant ( $\chi^2=0.23$ ,  $df = 1$ ,  $P > 0.05$ ) difference in growth for juvenile snails from both the Ashanti and Western ecotypes.

#### **Growth rates (MWSLG and SGR)**

The weekly rate of increase in shell length was higher for juvenile snails of the Western ecotype than those of the Ashanti ecotype for both indoor and outdoor experiments (Figures 14 and 15). Specific growth rate (SGR) for Western juvenile snails differ significantly ( $P < 0.05$ ) from those of Ashanti juvenile snails for the indoor design. However, specific growth rate (SGR) for Western juvenile snails did not differ significantly ( $P > 0.05$ ) from those of Ashanti juvenile snails for the outdoor experimental design (Figures 14 and 15). Although juvenile snails from the outdoor experimental design (for both Ashanti and Western ecotypes)

recorded higher daily increase in the shell length respectively, this was not significantly different ( $P > 0.05$ ) from the record obtained from those of the indoor experimental design (Figures 14 and 15).

There was a significant difference in SGR and MWLG for juvenile snails from the Ashanti and Western ecotypes for the indoor set up. Although SGR and MWLG were not significantly different among the populations for the Ashanti and Western ecotypes in the outdoor set up, the relatively higher SGR of Western population led to final snail shell lengths. A situation where growth rates between two populations do not differ significantly but results in significant differences in final weights or lengths is attributed to expression of mathematical function. Thus growth rate expresses a constant function with time. Therefore the significant difference in snail shell length between Ashanti and Western populations at the end of the experiment, in spite of insignificant differences in growth rates, was expected.

### **Aestivation pattern of the parent stock of the four ecotypes of snails**

Aestivation is defined as a condition of dormancy in which the snail has formed an epiphragm and remains entirely quiescent (Nisbet, 1974). Dryness inhibits growth and even stops some life processes of snails such as feeding and reproduction. During the aestivation period, the aperture of the mouth of the shell is temporarily closed by a calcified material known as epiphragm which is a whitish, fragile material (Nisbet, 1974). The epiphragm consists of organic matrix

of dry mucus in which are embedded variable amounts of calcareous granules (Machin, 1968).

During stress conditions, snails may bury themselves in the soil or hide beneath stones in order to avoid direct solar radiation (Schmidt-Nielsen *et al.*, 1971). Terrestrial snails also owe their survival in dry environmental conditions to the protection afforded by the shell during periods of inactivity. Although some water passes through the shell, the rate of evaporative loss is minimal (Machin, 1968).

However, in adverse conditions, some snails may aestivate without burrowing. This enables observation of the process of epiphragm formation. The animal retracts within its shell, leaving about 2.5mm of the internal surface of the lip exposed. The collar is extended to close the retracted foot and secretes a yellowish mucous layer which slowly whitens, finally becoming a thin, tough, shiny and slightly flexible structure with a fine slit that filled the line of the pneumostome (Nisbet, 1974; Thompson, 2004). During this period, most of the activities of snails are suspended. When humidity falls below 75%, as is the case during the dry season, *Achatina achatina* becomes inactive, seals itself into its shell with a white, calcareous layer and aestivates in order to prevent loss of water from the body (Cobbinah, 1992; Odaibo, 1997).

The results in Figure 17 indicate that during the period between December and March, a total of 55 snails went into aestivation for all the four ecotypes for the outdoor set up. The breakdown was as follows; 16, 17, 10 and 12 snails for Ashanti, Central, Eastern and Western ecotypes respectively for the outdoor set

up (Figure 13). This trend in aestivation pattern was attributed to low humidity and high temperature conditions during this period, confirming the findings of Ajayi *et al.*, (1980) that from November to March each year, giant African land snails aestivate because of the hot dry weather. These environmental factors affected the behaviour of the snails which resulted in these numbers aestivating. However, those which did not aestivate used behavioural means such as burrowing into the soil to overcome these conditions as in plates 20 and 21. But, some species aestivate on exposed surfaces of rocks, sometimes fully in the blazing sun, and survive (Schmidt-Nielsen *et al.*, 1971).

In the outdoor set up, the Ashanti ecotype recorded 16 snails aestivating between 27<sup>th</sup> of December, 2006 to 26<sup>th</sup> of January, 2007, spending an average period of 20.5 days in aestivation. This ecotype recorded the longest period of aestivation. This could be attributed to their adaptability to the rainfall pattern in the region of collection or ecological area and hence in spite of the watering regime this result was obtained (Figure 18). The annual mean rainfall of the Ashanti region is higher than that of the Central region and as a result, snail ecotype from the Ashanti region had become adapted to that rainfall pattern. Due to this, snails from the Ashanti region were not adapted to the harsh environmental conditions (low relative humidity and high temperature) in the Central region.

The Central ecotype recorded the highest number of 17 snails aestivating. This could be attributed to the nature of their shell fill that resulted in the snails bodies being exposed to these environmental factors (high temperature and low

humidity) of dryness. The shell fill of some of the snails from this ecotype was such that the body of the snail protruded outside the shell therefore exposing the snails to desiccation which resulted in more than half of the snails aestivating. These snails aestivated from the 5<sup>th</sup> to 26<sup>th</sup> of January, 2007 spending an average period of 12 days before coming out of aestivation and this was the shortest period spent in aestivation (Figure 18). This is because the Central ecotype had adapted to the robust weather conditions in the Central Region resulting in their ability to overcome such stress condition over a short period.

Ten snails went into aestivation from 1<sup>st</sup> December, 2006 to 19<sup>th</sup> January, 2007 in the pens housing snails from the Eastern Region. These snails spent an average period of 14.5 days in aestivation. However, snails in this same pen which did not aestivate burrowed into the soil to avoid desiccation (Plates 20 and 21). The Eastern ecotype recorded the least number of snails which went into aestivation. This was as a result of the specialized behaviour of burrowing into the soil which was often observed in this ecotype as shown in Plates 19 and 20. During stress conditions, snails may bury themselves in the soil or hide beneath stones in order to avoid direct solar radiation (Schmidt-Nielsen *et al.*, 1971).

However, pens housing snails from the Western Region recorded 12 snails aestivating from 29<sup>th</sup> December, 2006 to 28<sup>th</sup> January, 2007, spending an average period of 17.5 days in aestivation which was the second longest period of aestivation among the four ecotypes. This was as a result of the dry conditions created by the low humidity of 40% and high temperature of 35<sup>0</sup>C in the environment especially from late December, 2006 to the end of January, 2007.

This observation confirms Cobbinah's (1992) findings that when humidity falls below 75%, as is the case during the dry season, *Achatina achatina* becomes inactive, seals itself into its shell with a white, calcareous layer and aestivates in order to prevent loss of water from the body (Cobbinah, 1992; Odaibo, 1997). Within the outdoor experimental set up, the number of snails that went into aestivation for each ecotype and the days spent in aestivation differed significantly from each other.

However, the indoor experimental set up recorded a total of 40 snails aestivating. The breakdown was as follows: 12, 10, 8 and 10 snails for Ashanti, Central, Eastern and Western ecotypes respectively (Figure 17). Aestivation pattern of snails for the indoor breeding experiment however gave a different trend with the Ashanti ecotype recording 12 snails aestivating from 9<sup>th</sup> December, 2006 to 26<sup>th</sup> December, 2007, spending a mean period of 8.5 days which was the third longest period of aestivation. The Central ecotype recorded 10 snails aestivating from 15<sup>th</sup> January, 2006 to 24<sup>th</sup> January, 2007, spending a period of 5.5 days being the shortest aestivation period. However, the Eastern ecotype recorded 8 snails aestivating from 12<sup>th</sup> January, 2007 to 23<sup>rd</sup> January, 2007, spending an average period of 10 days as shown in figures 17 and 18 respectively. This was attributed to their ability to burrow effectively into the soil to avoid desiccation as shown in Plate 21. Lastly, the Western ecotype recorded 10 snails aestivating from 11<sup>th</sup> December, 2006 to 28<sup>th</sup> January, 2007, spending an average period of 11 days being the longest period in aestivation. Within the indoor experimental set up, the number of snails and the days spent in aestivation did not



differ significantly ( $P > 0.05$ ) from each other. Thus the four ecotypes aestivated at the same rate under the same breeding conditions (Appendix IV).

Generally, the number of snails which went into aestivation was higher for the outdoor experiment compared to those from the indoor experiment (figure 17). This could be attributed to a substantially high humidity and a corresponding low temperature conditions in the animal house which were as a result of the enclosure hence cutting off the dry external conditions from the outside while maintaining relatively high moisture content in the animal house for the indoor experiment. Aestivating snails from the outdoor experiment spent longer period in aestivation compared to those in the indoor experiment. There was a significant difference ( $P < 0.05$ ) in aestivation period by snails from the indoor and outdoor experiment and this could be attributed to the significantly different humidity and temperature conditions at the experimental sites.

These results indicate that in *Achatina achatina* population, morphological, behavioural and physiological adaptations to dry conditions are effective and seem to ensure sufficient water reserve. The observation is typical of snails and most common in the dry season, but snails will also aestivate if dry spells occur during the wet season. For the snail farmer, aestivation means the loss of valuable growing time and hence avoiding or reducing the time spent in aestivation would be an advantage (Cobbinah, 1992). When rain falls the epiphragm breaks and water stored before aestivation pours out of the aperture and the snails emerge to eat the fresh herbage and the soft soil as in plate 18 and 19 (Ajayi *et al.*, 1980; Odaibo, 1997).

### **Mortality of snails**

Generally, mortality was higher in the juvenile snails from the outdoor system than for those in the indoor system (fig. 16a-d). Mortality in the juvenile snails was high from the first week to the eighth week in both systems. This could be attributed to dry weather conditions which prevailed during the period (November –March) of hatching which was not the normal period for breeding. Additionally, those from the outdoor system experienced massive invasion of field mice, lizards and a fly, *Alluaudihella flavicornis*. *Alluaudihella flavicornis* (Plate 22), feeds on the body of the snail and reduces it to a putrefying mass. Invasion of mice was more serious in the outdoor system resulting in higher mortality than in the indoor system. This resulted in mortality being higher in the outdoor system.

### **Percentage of snails surviving**

The percentage of juvenile snails surviving at the end of the research was higher in the Western ecotype than in Ashanti ecotype for both indoor and outdoor experiments. However, there was no significant difference ( $P > 0.05$ ) in the percentage of juvenile snails surviving for both Ashanti and Western ecotypes. In addition to this, the percentage of snails for the indoor experiment that survived did not differ significantly ( $P > 0.05$ ) from those of the outdoor experiment.

Most environmental changes even those caused by human activities will directly affect different behavioural, anatomical, morphological or life-history traits. Artificial habitats, habitat fragmentation and population bottlenecks caused

by human induced activities might affect the extent and distribution of biodiversity at the genetic level. Several sources of evidence provide a link between ecological adaptation, vertical distribution and phenotypic differentiation between ecotypes. The main morphological differences between these ecotypes are size, relative area of shell aperture, morphological, behavioural and even physiological traits.

## **CHAPTER FIVE**

### **CONCLUSION**

In the study of the four ecotypes of *Achatina achatina*, examination of the snails sampled from the four ecological areas indicated some morphological differences in the shell colour and stripe patterns as well as body colouration.

The study of the four ecotypes of *Achatina achatina* snails has shown that Ashanti and Western ecotypes were able to lay eggs while Central and Eastern ecotypes failed to lay eggs when kept under the same conditions for both the indoor and outdoor systems from August 2006 to June, 2007. The Western ecotypes have proved to be more amenable for snail farming compared to those of the Ashanti, Central and Eastern ecotypes for both the indoor and outdoor experimental designs.

There was a significant difference in mean weekly shell length gain (MWSLG) and specific growth rate (SGR) of juvenile snails from Ashanti and Western ecotypes. Juvenile snails from the Western ecotype grew heavier and larger than those from the Ashanti ecotype. However, in the outdoor system, there was no significant difference in mean weekly shell length gain (MWSLG) and specific growth rate (SGR) by shell length of juvenile snails from Ashanti and Western ecotypes. Although, the juvenile snails from the outdoor set up grew

larger than those of the indoor set up, there was no significant difference in growth of juvenile snails in both systems.

The outdoor set up generally had the highest number of snails aestivating and the longest period (the number of days) spent in aestivation while the indoor set up had the least number of snails aestivating and the shortest period (the number of days) spent in aestivation. Although, the Ashanti ecotype spent the longest period in aestivation for the indoor and outdoor experimental set ups, this could be improved by a more efficient watering regime. The indoor experimental set up is a better system in reducing aestivation in the farming of snails.

Although mortality of juvenile snails was higher for the outdoor set up compared with those of the indoor set up, this was due to predation mainly by field mice. However, the number of juvenile snails that survived from the outdoor set up was higher than those from the indoor system.

In the study of the four ecotypes, Ashanti and Western snails had the highest fertility and fecundity rates. There were no significant differences between the growth of the juvenile snails. Ashanti and Western ecotypes appeared to be more amenable to easy culture among the four ecotypes studied for snails farming in Ghana. In Ghana, these two ecotypes would be recommended as the best candidates for snail farming.

The allusion of the existence of ecotypes of *Achatina achatina* was suggested based on morphological, anatomical and behavioural traits. Traits measured were shell length, body weight, aperture width, reproductive capacity and aestivation pattern. Populations were characterized as distinct on the basis of

shell colour, body colour, banding pattern and distribution of grey speckles on the body.

## **Recommendations**

- An efficient watering regime of 10 litres especially during the dry season could possibly eliminate or at least reduce aestivation in *Achatina achatina* snails.
- A more effective covering of mosquito net and wire mesh when provided would prevent predators (especially field mice) from entering the pens housing the snails.
- It is also being recommended that cross breeding these four ecotypes in further experiments would confirm whether or not they are the same species.

## REFERENCES

- AFSC Quarterly Research Reports (Oct-Nov-Dec 2003). Surveying Killer Whale Abundance and Distribution In the Gulf of Alaska and Aleutian Islands.
- Ajayi, S. S., Tewe, O. O., Moriarty, C. and Awesu, M. O. (1978). Observations on the biology and nutritive value of the African giant snail, *Archachatina marginata*. *East Africa Wildlife Journal* **16**: 85-95.
- Ajayi, S.S., Tewe, S.O. and Milligan, J.K. (1980). Influence of seasonality, on aestivation and behaviour of the forest African giant land snail, *Archachatina marginata* (Swaison). *Bulletin Annual Health Proceedings* **28**: 328pp.
- Barker G. M.(ed.) (2001). The biology of terrestrial molluscs, CAB international, *Proceedings of the Royal Society of Queensland* **5**: 150 - 153.
- Barnes R. D. (1991). The Invertebrate Zoology. British Marine Life Study Society Saunders College Publishing, 5<sup>th</sup> Edition. pp 32 - 47.
- Barnes S. K., Callow P. and Olive, P. J. W. (1993). *The Invertebrates: A new synthesis*. Blackwell Science, 2<sup>nd</sup> Edition. pp 130-132.
- Barry, D. H. (1959). Observations on the giant African snail. *West African Pharmacist* **1**: 92-93.
- Begon, M., Harper, J. L. and Townsend, C. R. (1990). *Ecology Individuals, Populations and Communities*. Blackwell Scientific Publications. 2<sup>nd</sup> Edition. pp 497-503.
- Bequaert, J. C. (1950). Studies in the Achatinidae, a group of African land snails. *Bulletin. Museum of Comparative Zoology Harvard* **105**: 1-126.



- Bouillon, J., Medel, M.D., Pagès, F., Gili, J.M., Boero, F. and Gravili, C. (2004).  
Fauna of the Mediterranean Hydrozoa. *Scientia Marina*, **68** (Suppl. 2)  
Hydroids from Reunion Island and Indian Ocean.
- Bridge, D., Cunningham, C. W., De Salle, R. and Buss, L. W. (1995). Class-level  
relationships in the phylum Cnidaria: Molecular and morphological  
evidence. *Molecular Biology Evolution* **12**:679-689.
- Brody, J.R. and Kern, S.E. (2004). History and principles of conductive media for  
standard DNA electrophoresis. *Animal Biochemistry* **333**(1):1-13.
- Bruggen, A. C. (1986). Aspects of the diversity of the land mollusks of the  
Afrotropical Region. *Revue Zoologie Africaine* **100**: 29-45.
- Burch, J. B. (1962). How to Know the Eastern Land Snails. 1<sup>st</sup> edition Wm. C.  
Brown Co., Publishers, Dubuque, Iowa pp 214.
- Clarke, B., Johnson, M.S., and Murray, J. (1996). Clines in the genetic distance  
between two species of island land snails: how "molecular leakage " can  
mislead us about speciation. *Philosophy of Transient Royal Society of  
London Bulletin* **351**: 773-784.
- Chevallier, H. (1979). Les escourgats. Dargand, Paris: Un Elevaged d` Avenir.
- Chevallier, H. (1986). Final fattening and outdoor breeding of the edible snail  
*Helix aspera*. *Snail Farming Research* **1**: 1-8.
- Cobbinah, J. R. (1992). Snail farming in West Africa – a practical guide.  
Wageningen: Technical Centre for Agricultural and Rural Co-operation.  
*British Crop Protection Council Monograph* **11**: 49-55.
- Collins, A. G. (2002). Phylogeny of Medusozoa and the evolution of

cnidarian life cycles. *Journal of Evolutionary Biology* **15**: 418-432.

Cooling, V. (2005). Risk Assessment of the Giant African Snail Bowdich in New Zealand. LPSC 7700 Integrative Report.

Cowie, R. H. and Jones, J. S. (1998). Gene frequency changes in *Cepaea* snails on the Marlborough Downs over twenty-five years. *Biological Journal of the Linnean Society* **65**: 233-255.

Coyne, J, and Orr, H. A. (2004). Speciation in snails, Sinauer: Sunderland, Massachusetts. Oxford Journals Life Sciences Integrative and Comparative Biology **44** (5): 400.

**Daily** Graphic (2003). Snails, another gem in export trade. Business News of Tuesday, 18<sup>th</sup> February, 2003.

Daguzan, J.(1983). Les escargot et l'heliculture. Paris: Information Technique de Services Veterinaires.

Davison, A. and Chiba, S. (2006). Labile ecotypes accompany rapid cladogenesis in an adaptive radiation of *Mandarina* (Bradybaenidae) land snails. The Linnean Society of London, *Biological Journal of the Linnean Society* **88**: 269–282.

Duah, O. A. and Monney, K. A. (1998). Population density estimation and feeding ecology of the giant snail *Achatina achatina* in a forest reserve. East African Wildlife society. *African Journal of Ecology* **37**: 366-368.

Dvořák, L., Honěk, A. and Martínková, Z. (2003). The spread of *Cepaea nemoralis* (L.) populations in the Czech Republic. 2003 BCPC Symposium Proceedings No. 80: *Slugs & snails: agricultural, veterinary and environmental perspectives*, pp. 99–102.

- Elmslie, L.J. (1982). Snails and Snail Farming. *World Animal Review* **41**: 20-26.
- Ford, E. B. (1975). *Ecological Genetics*. The Chaucer Press limited. 4<sup>th</sup> Edition.  
pp. 20-60.
- Gomot, L. and Deray, A. (1987). Les escargots. *La Recherche* **18** : 302-311.
- Hodasi, J. K. M. (1982). The effect of different light regimes on the behaviour and biology of *Achatina achatina*. *Journal of Molluscan Studies* **48**: 283-293.
- Hodasi, J. K. M. (1984). Some observations on the edible giant land snails of West Africa. *World Animal Review* **52**: 24-28.
- Hodasi, J. K. M. (1986). Some observations on the feeding behaviour and food preferences of the giant West African snail *Archachatina marginata* (Swainson). In *Snail Farming Research*: Associazione Nazionale Elicicoltori Cherasco (CN) Italy, (L. J. Elmslie ed) **1**: 54-66.
- Hodasi, J. K. M. (1989). The potential for snail farming in West Africa. In: Slugs and snails in world agriculture: *BCPC Monograph* (I. F. Henderson ed) Number **41**: 27 – 31.
- Hughes, L. (2000). Biological consequences of global warming: is the signal already apparent? *Trends in Ecological Evolution* **15**: 56–61.
- IUCN (World Conservation Union). 2000 IUCN Red List of threatened Species. IUCN Species Survival Commission: Gland.
- Juříčková, L., Horsák, M. and Beran, L. (2001). Check-list of the molluscs (Mollusca) of the Czech Republic. *Acta Societas Zoologicae Bohemicae* **65**: 25-40.

- Jordaens, K., Van Riel, P., Geenen, S., Verhagen, R. and Backeljau, T. (2000). Food-induced body pigmentation questions the taxonomic value of colour in the self fertilizing slug *Carinarion* spp. *Evolutionary Biology Group, Groenenborgerlaan 171, B- 2020. Royal Belgium Institute of Natural Sciences, Vautierstraat 29, B- 1000 Brussels, Belgium.*
- Kliks, M. M., K. Kroenke and J. M. Hardman (1982). *Eosinophilic Radiculomyeloencephalitis: An Angiostrongyliasis Outbreak in American Samoa Related to Ingestion of Achatina fulica Snails, American Journal of Tropical Medicine Hygiene* **31** (6): 1114-1122.
- Lee, W. G., Mark, A. F. and Wilson, J. B. (2003). Ecotypic differentiation in the ultramafic flora of the South Island, New Zealand. *New Zealand Journal of Botany* **21**: 141-156.
- Machin, J. (1968). The Permeability of the Epiphragm of Terrestrial Snails to Water Vapor. *Biological Bulletin*. pp. 87-95.
- Matkin, C. O., Barrett-Lennard, L. G., Yurk, H., Ellifrit, D. and Trites, A. W. (2007). Ecotypic variation and predatory behavior among killer whales off the eastern Aleutian Islands, Alaska. *Fishery Bulletin*. pp. 1- 4
- Mayr, E. (1987). The onotological status of species: Scientific progress and philosophical terminology. *Biology and Philosophy* **2**:145-166.
- Mead, A. R. (1961). *The Giant African Snail: A Problem in Economic Malacology*. The University of Chicago Press, Chicago. pp 257.
- Meka, J., Knudsen, E. E. and Douglas, D. C. (2000). Alagnak watershed rainbow

trout seasonal movement. pp 35-42. *Biotelemetry* 15: Proceedings of the 15th International Symposium on Biotelemetry. Juneau, Alaska, USA.

International Society on Biotelemetry

Monney, K. A. (1992). Observations on the food and feeding habits of

*Archachatina degneri*. *Tropical Ecology* **33**: 148-156.

Monney, K. A. (1994). Effects of different dietary regimes on growth

and reproductive function of farmed *Achatina fulica* Bowdich. *Snail*

*Farming Research* **5**:14-22.

Monney, A.K. (1998). Prospects for Snail farming in West Africa. *Tropical*

*Science* **38**: 238-247.

Monney K. A. (2001). Funny factors in the maintenance of a polymorphism.

*Bulletin of The Malacological Society of London* **36**: 3-6.

Neto, N. A. L., Brooks, S. E. and Alves, R. R. N. (2009). "From Eshu to Obatala:

animals used in sacrificial rituals at Candomblé "terreiros" in Brazil".

[\*Journal of Ethnobiology and Ethnomedicine\*](#) **5**: 23.

Nisbet, R. N. (1974). The life of archatinidae in London. *Proceedings of*

*Malacological Society of London* **41**:1171.

Odaibo, A. B. (1997). *Snail and Snail Farming. Nigeria Edible Land Snails.*

Ibadan:

Stirling-Horden Publishers **1**: 1-11.

Olufokunbi, B., Philips, E. O., Omidiji, J. O., Ogbonna, U. O., Makinde, H. T.

and Apansile, O. J. (1989). The economics of commercial domestication

of the African giant land snail (*Archachatina*) (*Calachatina*) *marginata*

- (Swainson) in Nigeria. In: *Slugs and snails in the world agriculture*: BCPC Monograph. I. F. Henderson (ed) **41**: 41-48.
- Overton, J. M., Barker, G. M. and Price, R. (2004). Estimating and conserving patterns of invertebrate diversity: a test case of New Zealand land snails. *Conservation Science Journal* **15** (5) : 731–741.
- Parkinson, B., Hemmen, J. and Groh, K. (1987). *Tropical land snail shells of the world*. 1<sup>st</sup> ed. Wiesbaden Verlag Christa Hemmen, Western Germany. pp 279.
- Plummer, J. M. (1975). Observations on the reproduction, growth and Longevity of a laboratory colony of *Archachatina (Calachatina) marginata* (Swainson) subspecies ovum. *Proceeding Malacological Society London* **41**: 395- 413.
- Ponder, W. (1997). Conservation status, threats and habitat requirements of Australian freshwater and terrestrial Mollusca *Memoirs of the Museum of Victoria* **56**: 421-430.
- Public - Private Partnership Programme (2006). Report of Ministry of Local Government and Rural Development.
- Raut, S. K. (2002). Present status of the giant African land snail *Achatina fulica* Bowdich in Kolkata (Calcutta), India. pp. 35. *In Proceedings, 7th International Congress on Medical and Applied Malacology. 21-24 Oct 2002, Los Banos, Laguna (Philippines). Malacological Society of the Philippines, Incorporated.*
- Raut, S. K. and Barker, G. M. (2002). *Achatina fulica Bowdich and Other*

- Achatinidae as Pests in Tropical Agriculture*, pp 55-114. In G. M. Barker [ed.], *Molluscs as Crop Pests*. CABI Publishing, Hamilton, New Zealand.
- Raven, H. P. and Johnson, B. G. (1996). *Biology (Student Research Beyond The Classroom)*. McGraw-Hill, 4<sup>th</sup> edition. pp 427 – 428.
- Reise, H. (1997). *Deroceras juramum* – a Mendelian colour morph of *D. rodnae* (Gastropoda: Agriolimacidae). *Journal of Zoology London* **241**: 103-115.
- Ricker, W. E. (1975). Computation and interpretation of the biological statistics of fish population. *Bulletin, Fish Reserve Board Canada* **191**: 1-382.
- Riddihough, G. and Pennisi, E. (2001). The evolution of Epigenetics. *Science* **293**:1063.
- Rolan-Alvarez, E. (2007). Sympatric speciation as a by-product of ecological adaptation in the Glacial *Littorina saxatilis* hybrid zone. *Journal of Molluscan studies*. Advance Access published 9<sup>th</sup> January.
- Runham, N. W. (1989). Snail farming in the United Kingdom. In: *slugs and snails in world agriculture*. British Crop Protection Council Monograph **11**: 49-55.
- Schmidt-Nielsen, K., Taylor, C. R. and Shkolnik, A. (1971). Desert snail: problems of heat, water and food. *Journal of Experimental Biology* **55**: 385–398.
- Segun, A. O. (1975). The giant land snail, *Archachatina (Calachatina) marginata* (Swainson). Benin City Ethiope Publishing House.
- Shattuck, S. O. and Barnett, N. J. (2001). Australian National Insect collection

CSIRO entomology. Australian species of the ant genus *Diacamma*  
(Hymenoptera: Formicidae). *Myrmecologische Nachrichten* **4**: 1-10.

Thompson, R. (2004). Raising snails. The Alternative Farming Systems  
Information Center, National Agricultural Library. Special reference  
briefs. pp. 96-105.

Wells, S. (1988). Snails going extinct at speed. *New Scientist* **117** (11): 46-48.



## APPENDICES

### APPENDIX I

#### **Some parameters of the different ecotypes of *Achatina achatina* parent stocks**

##### **General Linear Model: w versus treatment**

Analysis of Variance for w, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Width	1	1000746	128399	128399	144.28	0.000
Length	1	148959	172186	172186	193.48	0.000
Treatments	3	28578	28578	9526	10.70	0.000
Error	234	208241	208241	890		
Total	239	1386524				

##### **One-way ANOVA: Weighta/g, weightc/g, weighte/g, weightw/g**

Analysis of Variance

Source	DF	SS	MS	F	P
Factor	3	79661	26554	4.80	0.003
Error	236	1306863	5538		
Total	239	1386524			

##### **One-way ANOVA: widtha/mm, widthc/mm, widthe/mm, widthw/mm**

Analysis of Variance

Source	DF	SS	MS	F	P
Factor	3	1113.3	371.1	9.31	0.000
Error	236	9410.5	39.9		
Total	239	10523.9			

**One-way ANOVA: lengtha/cm, lengthc/cm, lengthe/cm, lengthw/cm**

Analysis of Variance

Source	DF	SS	MS	F	P
Factor	3	81.73	27.24	18.12	0.000
Error	236	354.80	1.50		
Total	239	436.53			

**Number of stripes on the shells of the four ecotypes**

**One-way ANOVA: Response versus Subscripts**

Analysis of Variance for Response

Source	DF	SS	MS	F	P
Subscrip	3	1841.01	613.67	97.17	0.000
Error	196	1237.86	6.32		
Total	199	3078.87			

Individual 95% CIs

For Mean

Based on Pooled

StDev

Level	N	Mean	StDev	CI
1	50	6.000	1.370	(-*-)
2	50	11.520	1.681	(-***)
3	50	14.180	3.385	(-***)
4	50	12.200	3.017	(-***)

Pooled StDev = 2.513

## APPENDIX II

### **Egg laying in the four ecotypes**

Two-Sample T-Test and CI: Eggs indoor, Eggs outdoor

Two-sample T for Eggs in vs Eggs out

	N	Mean	StDev	SE Mean
Eggs indoor	2	642	471	333
Eggs outdoor	2	990	467	331

Difference =  $\mu$  Eggs indoor -  $\mu$  Eggs outdoor

Estimate for difference: -348

95% CI for difference: (-6309, 5614)

T-Test of difference = 0 (vs not =): T-Value = -0.74 P-Value = 0.594 DF = 1

### **Weight of eggs**

Two-Sample T-Test and CI: weghtw/g, weighta/g

Two-sample T for weghtw/g vs weighta/g

	N	Mean	StDev	SE Mean
weghtw/g	100	0.1793	0.0398	0.0040
weighta/g	100	0.1701	0.0210	0.0021

Difference =  $\mu$  weghtw/g -  $\mu$  weighta/g

Estimate for difference: 0.00920

95% CI for difference: (0.00031, 0.01809)

T-Test of difference = 0 (vs not =): T-Value = 2.04 P-Value = 0.043 DF = 150

### **Incubation period of eggs**

Two-Sample T-Test and CI: Indoor, outdoor

Two-sample T for Indoor vs outdoor

	N	Mean	StDev	SE Mean
Indoor	2	25.250	0.354	0.25

outdoor 2 19.800 0.283 0.20

Difference = mu Indoor - mu outdoor

Estimate for difference: 5.450

95% CI for difference: (4.072, 6.828)

T-Test of difference = 0 (vs not =): T-Value = 17.02 P-Value = 0.003 DF = 2

Both use Pooled StDev = 0.320

### **Two-Sample T-Test and CI: hatchlings indoor, hatchlings outdoor**

Two-sample T for hatchlings in vs hatchlings out

	N	Mean	StDev	SE Mean
Hatchlings	2	332	212	150
Hatchlings	2	784	346	245

Difference = mu hatchlings in - mu hatchlings out

Estimate for difference: -452

95% CI for difference: (-4102, 3198)

T-Test of difference = 0 (vs not =): T-Value = -1.57 P-Value = 0.360 DF = 1

### **Hatchlings**

One-way ANOVA: Ashanti indoor, Ashanti outdoor, Western indoor, Western outdoor

Analysis of Variance

Source	DF	SS	MS	F	P
Factor	3	457930	152643	1.01	0.476
Error	4	605289	151322		
Total	7	1063219			

### Two-Sample T-Test and CI: Ashanti, Western

Two-sample T for Ashanti vs Western

	N	Mean	StDev	SE Mean
Ashanti	2	1148	244	173
Western	2	484	247	175

Difference = mu Ashanti - mu Western

Estimate for difference: 664

95% CI for difference: (-2459, 3786)

T-Test of difference = 0 (vs not =): T-Value = 2.70 P-Value = 0.226 DF = 1

### Incubation Temperature

Two-Sample T-Test and CI: Ashanti, Western

Two-sample T for Ashanti vs Western

	N	Mean	StDev	SE Mean
Ashanti	2	22.75	3.89	2.7
Western	2	22.33	3.78	2.7

Difference = mu Ashanti - mu Western

Estimate for difference: 0.42

95% CI for difference: (-16.07, 16.91)

T-Test of difference = 0 (vs not =): T-Value = 0.11 P-Value = 0.923 DF = 2

Both use Pooled StDev = 3.83

### Humidity

Two-Sample T-Test and CI: Humidity/o/oo\_1, Humidity/%

Two-sample T for Humidity/o/oo\_1 vs Humidity/%

	N	Mean	StDev	SE Mean
Humidity	16	69.4	10.7	2.7
Humidity	16	76.94	8.36	2.1

Difference =  $\mu$  Humidity/o/oo\_1 -  $\mu$  Humidity/o/oo

Estimate for difference: -7.56

95% CI for difference: (-14.50, -0.63)

T-Test of difference = 0 (vs not =): T-Value = -2.23 P-Value = 0.034 DF = 30

Both use Pooled StDev = 9.60

APPENDIX III

**Growth of juvenile snails**

**Growth of juvenile snails reared indoor**

Two-Sample T-Test and CI: Ashanti, Western

Two-sample T for Ashanti vs Western

	N	Mean	StDev	SE Mean
Ashanti	13	2.014	0.734	0.20
Western	13	2.53	1.11	0.31

Difference =  $\mu$  Ashanti -  $\mu$  Western

Estimate for difference: -0.519

95% CI for difference: (-1.291, 0.253)

T-Test of difference = 0 (vs not =): T-Value = -1.40 P-Value = 0.176 DF = 20

**Growth of juvenile snails reared outdoor**

Two-Sample T-Test and CI: Ashanti, Western

Two-sample T for Ashanti vs Western

	N	Mean	StDev	SE Mean
Ashanti	13	2.171	0.818	0.23
Western	13	2.289	0.969	0.27

Difference =  $\mu$  Ashanti -  $\mu$  Western

Estimate for difference: -0.118

95% CI for difference: (-0.846, 0.610)

T-Test of difference = 0 (vs not =): T-Value = -0.33 P-Value = 0.741 DF = 23

### **Growth of juvenile snails reared in the indoor and outdoor**

One-way ANOVA: Ashanti indoor, Western indoor, Ashanti outdoor, Western outdoor

Analysis of Variance

Source	DF	SS	MS	F	P
Factor	3	1.866	0.622	0.73	0.537
Error	48	40.676	0.847		
Total	51	42.542			

### **Specific growth rate of juvenile snails reared indoor**

Two-Sample T-Test and CI: Ashanti, Western

Two-sample T for Ashanti vs Western

	N	Mean	StDev	SE Mean
Ashanti	12	0.721	0.571	0.16
Western	12	0.846	0.590	0.17

Difference =  $\mu$  Ashanti -  $\mu$  Western

Estimate for difference: -0.125

95% CI for difference: (-0.618, 0.368)

T-Test of difference = 0 (vs not =): T-Value = -0.53 P-Value = 0.604 DF = 21

### **One-way ANOVA: Ashanti indoor, Western indoor, Ashanti outdoor, Western outdoor**

Analysis of Variance

Source	DF	SS	MS	F	P
Factor	3	0.117	0.039	0.11	0.951
Error	44	14.968	0.340		
Total	47	15.085			



### Specific growth rate of juvenile snails reared outdoor

Two-Sample T-Test and CI: Ashanti, Western

Two-sample T for Ashanti vs Western

	N	Mean	StDev	SE Mean
Ashanti	12	0.778	0.439	0.13
Western	12	0.832	0.703	0.20

Difference =  $\mu$  Ashanti -  $\mu$  Western

Estimate for difference: -0.054

95% CI for difference: (-0.557, 0.448)

T-Test of difference = 0 (vs not =): T-Value = -0.23 P-Value = 0.823 DF = 18

### Survival of juvenile snails reared in the indoor and outdoor systems

One-way ANOVA: Ashanti in, Western in, Ashanti out, Western out

Analysis of Variance

Source	DF	SS	MS	F	P
Factor	3	932	311	1.05	0.379
Error	44	12994	295		
Total	47	13926			

Individual 95% CIs For Mean

## APPENDIX IV

### **Aestivation pattern of snails**

#### **Aestivation pattern of snails reared indoor**

One-way ANOVA: Ashanti, Central, Eastern, Western

Analysis of Variance

Source	DF	SS	MS	F	P
Factor	3	2.67	0.89	0.18	0.908
Error	8	40.00	5.00		
Total	11	42.67			

#### **Aestivation pattern of snails reared outdoor**

One-way ANOVA: Ashanti, Central, Eastern, Western

Analysis of Variance

Source	DF	SS	MS	F	P
Factor	3	10.92	3.64	2.08	0.181
Error	8	14.00	1.75		
Total	11	24.92			

#### **Aestivation pattern of snails reared outdoor**

One-way ANOVA: Snails indoor, Snails outdoor

Analysis of Variance

Source	DF	SS	MS	F	P
Factor	1	28.13	28.13	4.14	0.088
Error	6	40.75	6.79		
Total	7	68.88			

**Aestivation periods recorded from the indoor and outdoor systems**

One-way ANOVA: Time/days, Time/days\_1

Analysis of Variance

Source	DF	SS	MS	F	P
Factor	1	108.78	108.78	11.27	0.015
Error	6	57.94	9.66		
Total	7	166.72			

**Aestivation periods recorded from the indoor and outdoor systems**

One-way ANOVA: out Snails, in Snails, out Time/days, in Time/days

Analysis of Variance

Source	DF	SS	MS	F	P
Factor	3	138.17	46.06	5.60	0.012
Error	12	98.69	8.22		
Total	15	236.86			