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**FACTORS INFLUENCING THE
LIFE CYCLE
OF *CTENOCEPHALIDES* SPP.**

by

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for the degree Magister Scientiae in the
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CHAPTER 1

GENERAL INTRODUCTION

The origin of fleas (Siphonaptera) has been the subject of great controversy, but it is considered that fleas evolved from a mecopteran-like ancestor in the late Mesozoic and have evolved with the mammals. There is little doubt that fleas became parasites of mammals comparatively early in the history of their hosts. A small number of species, mainly of the genus *Ceratophyllus*, have become secondarily adapted to birds (mostly Passeriformes and sea birds) (Holland, 1964; Kettle, 1995). A fossil flea, scarcely different from living species and displaying all the specialised features associated with them, have been found in Baltic amber dating from 50 million years ago (Holland, 1964). A number of host associations suggest ancient lineages and the distribution of the fleas, which extends to all continents, including Antarctica and a range of habitats and hosts from equatorial deserts through tropical rain forests to the northernmost Arctic tundra, indicates a long history of dispersal and evolution (Holland, 1964).

Fleas were formerly considered as predators and not as ectoparasites because they did not spend the whole of their lives on their hosts and it was thought that they were only dependent on their hosts for an occasional meal of blood. However, some fleas such as *Tunga penetrans* burrow through the skin of their host and become endoparasitic. Recent researches have demonstrated that fleas are as specialized and as intimately dependent, each on its particular host, as are endoparasites on theirs (Burt, 1970). Fleas probably arose as winged scavenging flies, feeding as larvae on the excrement in the refuges of burrowing mammals. Almost countless generations of such pre-fleas may have eked out a sheltered life in pre-historic burrows before the first pioneer crept into the fur of a passing ratlike occupant (Kim, 1985). Possibly there is an even shorter step between piercing the dried outer layer of excrement to reach the semi-fluid matter below it and piercing a mammal's skin and imbibing the first drink of blood. Blood as food may confer such advantages that the insect is immediately started along the risky road to overdependence and overspecialization and once fleas became parasites, their fate was linked to the fate of their hosts (Kim, 1985).

There is a well-established correlation between the level of evolutionary development of the flea and that of the host. The ancient primitive hosts have the most primitive fleas, and the more recently evolved mammals have the more specialized fleas. In as much as the fleas have adapted to the vestiture of the mammals and to the survival problems posed by the special activities of their hosts, in general, evolution obviously proceeded more slowly in the fleas than in the animals they infest. The same species and even subspecies of fleas often occur in areas separated by barriers such as desert and mountain ranges (Kim, 1985). Selective pressures influencing evolution would include environmental factors associated with the host, environmental factors on the host, physiological adaptability and capacity for dispersal, isolation and eventually speciation (Holland, 1964).

The Siphonaptera are ectoparasites on a wide range of terrestrial mammals and birds. Their life cycle is such that they are particularly associated with mammals that spend part of their life in nests, dens, holes or caves (Kettle, 1995). Fleas that infest mammals can vary in their range of hosts. About 25% occur on only a single mammalian species or genus. Others have wider ranges of hosts (Dryden, 1993). The fact that fleas generally parasitise a range of hosts, and have the ability to transfer from one host species to another, makes them of medical importance in the transmission of disease from animals to humans. While feeding, fleas inject a poisonous saliva which causes the familiar itch and swelling (Smit, 1964). The medically most important families are the Ceratophyllidae, Ctenophthalmidae, Leptopsyllidae, Pulicidae and Tungidae (Kettle, 1995). The medically important species are among those that infest more than one mammalian order, for example *Tunga penetrans* and *Xenopsylla cheopis*, and those with a very broad host range, for example *Echidnophaga gallinacea*, *Ctenocephalides felis* and *Pulex irritans* (Kettle, 1995).

The cat flea, *Ctenocephalides felis* (Bouché), is primarily responsible for flea allergy dermatitis (FAD) in both dogs and cats. FAD is a hypersensitization to antigenic components contained in the saliva of fleas. The clinical disease associated with the development of the hypersensitive state is the most common allergic dermatologic

disease of dogs and cats (Rust & Dryden, 1997). The cat flea can also serve as a vector of typhus-like rickettsia and is the intermediate host for cestodes like the dog tapeworm, *Dipylidium caninum* Railliet 1892. The cysticeroid stages of *Dipylidium caninum* develop in the larvae of the cat flea (Brown, 1975; Noble & Noble, 1982). The cat flea has also recently been implicated in the transmission of *Bartonella henselae*, the etiologic agent of cat scratch disease (Rust & Dryden, 1997).

Without having an understanding and appreciation for the life cycle of the flea it is almost impossible to gain control over a flea infestation (Kuepper, 1999). This is because only about 5% of a potential flea population is in the adult stage at any one time (Figure 1.1). Cat fleas undergo complete metamorphosis. The four developmental stages in the life cycle are the egg, the larva, the pupa and the adult (Kuepper, 1999).

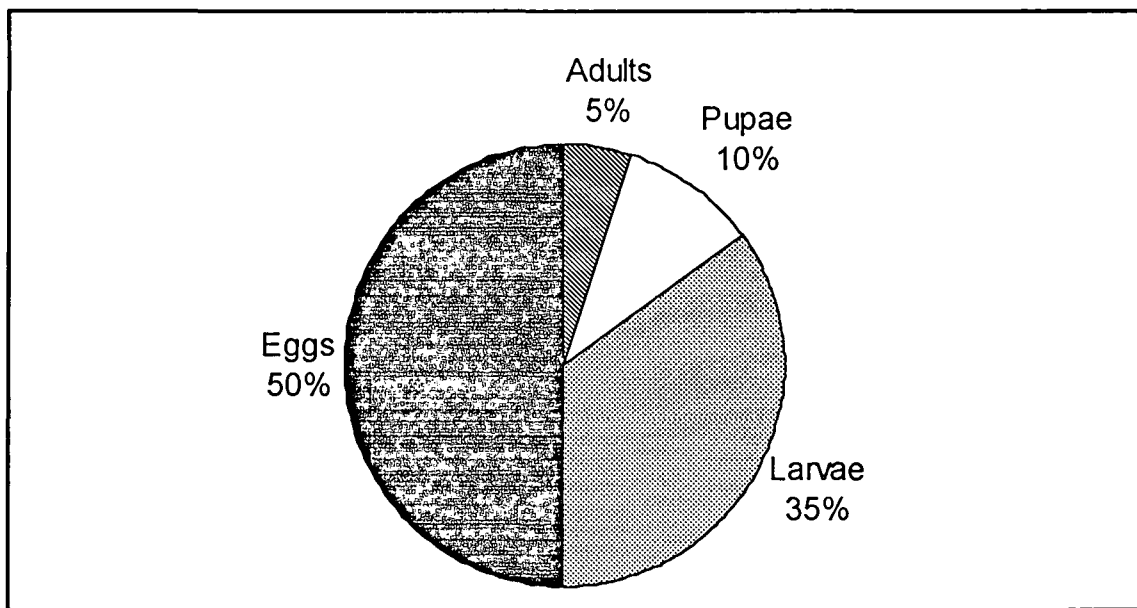


Figure 1.1 The four stages in the life cycle of a flea with approximate percentages of a typical infestation (After Kuepper, 1999).

Because of the small size and nature of the flea in its immature stages, it is only the adult fleas that people come in contact with. This means that 95% of the fleas in a pet's environment are developing in the other three stages of life that are not seen and therefore

are not aware of. A typical flea population consists of 50% eggs, 35% larvae and 10% pupae. These stages of the life cycle allow an ever continuing development of fleas even when all the adult fleas of a current generation are killed. The key, then, to control fleas is to consistently interrupt their life cycle at an immature stage so that they do not develop into adults (Kuepper, 1999). Fleas that bite humans in flea-infested houses and yards are not fleas that have left pets but rather fleas that have recently emerged from cocoons and are seeking the first blood meal (Dryden, 1992).

The ability of the cat flea to sense a host animal as well as the effect of environmental conditions, mainly temperature and relative humidity (RH), are the two most important factors affecting the longevity of the flea. The better conditions are for development, the more active, but shorter the life cycle will be. In general, a moist, warm environment will cause rapid development throughout the flea's life cycle (Kuepper, 1999). The abundance of adult cat fleas fluctuates with seasonal changes. The warm months of spring and summer give rise to the highest numbers, whereas few are found during the cold months of late fall and winter (Metzger & Rust, 1997).

Attempts to control fleas on pets and in the environment can be expensive, time consuming and often frustrating. New information concerning the biology and environmental epidemiology of fleas infesting dogs and cats greatly enhances our understanding of these important ectoparasites. In the past, the primary recommendations for control of cat fleas have focused on applying insecticides to the host and environment. Reports of insecticide resistance to multiple categories have been increasing, but the impact of resistance on cat flea control remains unknown. As our understanding of the life cycle and habits of the cat flea has improved, insect growth regulators as well as physical and cultural mechanisms have been incorporated into our control programs (Rust & Dryden, 1997).

The comparative biology of *C. felis* and *C. canis* have been neglected, perhaps because the physical differences between the two species tend to be small (Baker & Elharam, 1982). The general aim of this study was to determine:

- The influence of certain factors on the development and survival of *C. felis* and *C. canis*.
- The effect of different environmental conditions on the different stages in the life cycle. Every stage was exposed to various conditions, including different combinations of temperature and RH.
- The conditions for optimal development for both *C. felis* and *C. canis* and the influence of *D. caninum* on the life cycle of the two species.

CHAPTER 2

MORPHOLOGICAL CHARACTERISTICS OF *CTENOCEPHALIDES FELIS* AND *C. CANIS*

Introduction

More than 2 000 species and subspecies of fleas are known throughout the world. Pulicoidea, Malacopsylloidea and Ceratophylloidea represent the three superfamilies that occur in South Africa (Segerman, 1995). The family Pulicidae in the superfamily Pulicoidea, contains many medically important species. Pulicinae, Archaeopsyllinae and Xenopsyllinae are the subfamilies in this family which occur in South Africa. The subfamily Pulicinae has five genera, Archaeopsyllinae has one genus and Xenopsyllinae has four genera (Table 2.1) (Segerman, 1995). *Ctenocephalides felis* (the cat flea) and *C. canis* Curtis 1826 (the dog flea) from the subfamily Archaeopsyllinae and *Pulex* species from the subfamily Pulicinae are found in a large enough number and with sufficient regularity to be of medical importance, chiefly in connection with the transmission of diseases. They also may act as intermediate hosts of animal parasites (Brown, 1975).

In morphological taxonomy, three sets of characteristics are generally employed in identifying fleas: chaetotaxy (setae, pseudosetae, combs, or ctenidia), thoracic and leg structures and the structure of male segment IX and female sternite VII and spermatheca (Ménier & Beaucournu, 1998). Accurate identification of fleas can also be based on the presence, size and position of the eyes, the location of the ocular setae, presence, arrangement and setae of combs, structure and appendages of the head and the genitalia (Brown, 1975). Figure 2.1 shows the most important characteristics used in this study, namely the head, combs, and some of the leg structures and cuticular plates.

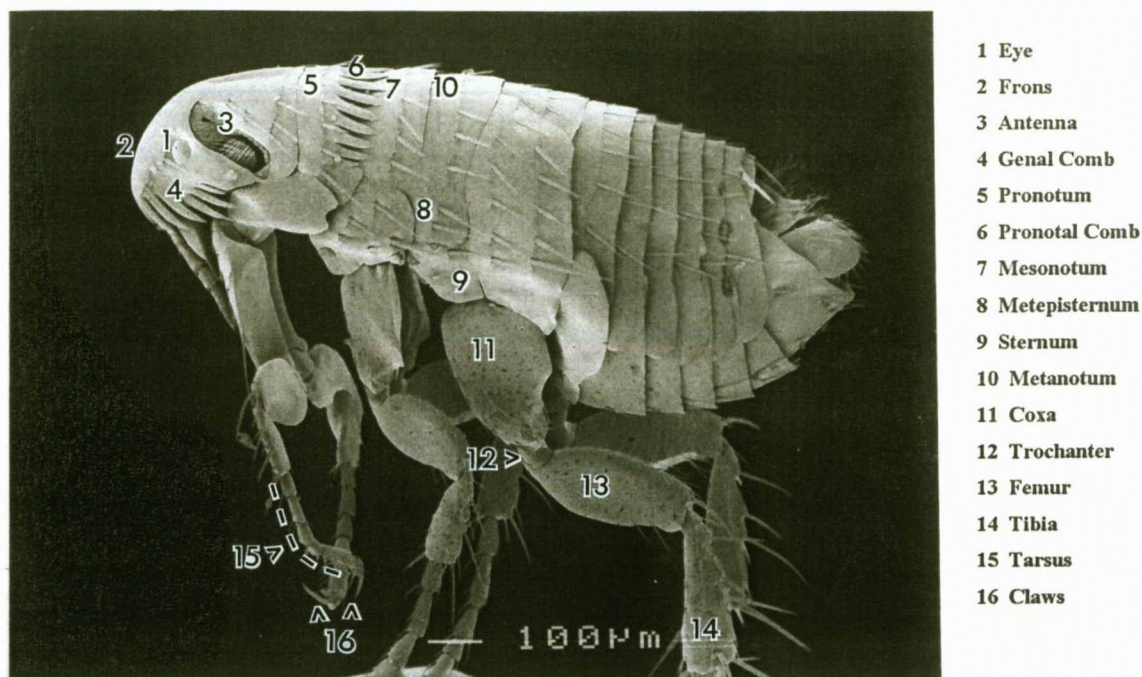


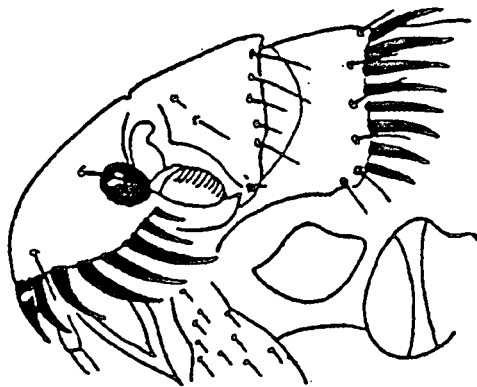
Figure 2.1 Some external structures of a female *C. felis*.

Table 2.1 Superfamilies, families and subfamilies of the order Siphonaptera in Southern Africa. The number of genera within each subfamily is indicated in parenthesis (modified from Segerman, 1995).

PULICOIDEA	TUNGIDAE	Tungidae (1)
	PULICIDAE	Pulicinae (5) Archaeopsyllinae (1) Xenopsyllinae (4)
MALACOPSYLLOIDEA	RHOPALOPSYLLIDAE	Parapsyllinae(1) Ctenophthalminae (1)
	HYSTRICHOPSYLLINAE	Dinopsyllinae (1) Listropsyllinae (1)
CERATOPHYLLOIDEA	ISCHNOPSYLLINAE	Thaumapsyllinae (1) Ischnopsyllinae (6)
	LEPTOPSYLLIDAE	Leptopsyllinae (1)
	CERATOPHYLLIDAE	Ceratopsyllinae (2) Chimaeropsyllinae (1)
	CHIMAEROPSYLLIDAE	Epirimiinae (3) Chiastopsyllinae (3)

In general the species and subspecies determination of fleas is based mainly on the study of male segments corresponding to the external genitalia (Ménier & Beaucournu, 1998). However, the presence or absence of combs and setae and the shape of the head are the most important characters used to distinguish the subfamilies, genera and species of Pulicidae, due to the homogeneity of the external genitalia among the species in this family (Ménier & Beaucournu, 1998). The presence of genal and pronotal combs is a distinctive character of the subfamily Archaeopsyllinae which can thus be easily separated from the other two subfamilies, Pulicinae and Xenopsyllinae, which lack these combs (Figure 2.2) (Howell, Walker & Nevill, 1983). Although only one genus of Archaeopsyllinae occurs in South Africa the genal and pronotal combs can further be used to distinguish between the genera in this subfamily. When both the genal and pronotal combs are present, and the base of the genal comb is parallel to the long axis of the head, the flea belongs to the genus *Ctenocephalides* Stiles & Collins 1930 which includes *C. felis*, *C. canis*, *C. damarensis* and *C. connatus*. However, in the genus *Ctenocephalides* the external genitalia are discouragingly homogeneous (Ménier & Beaucournu, 1998).

PRONOTAL AND GENAL COMBS

*CTENOCEPHALIDES* SP.

WITHOUT COMBS

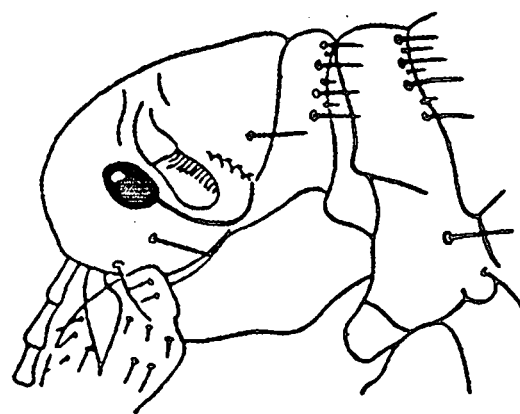
*PULEX* SP.

Figure 2.2: A schematic representation of the heads of a *Ctenocephalides* sp. and a *Pulex* sp. (Brown, 1975).

C. felis and *C. canis* look very much alike but they can be separated taxonomically by a few distinctive chaetotaxic characters which are clearly visible when using the scanning electron microscope. One of these is the presence of the notches with setae on tibia III. *C. canis* has eight setae, an additional two compared to the six of *C. felis*. *C. orientis* has seven of these setae while *C. damarensis* and *C. connatus* both also have six setae on tibia III (Lewis, 1967). The latter two species, however, can be distinguished from *C. felis* by other characteristics (Ménier & Beaucournu, 1998).

When seen from the side, the head of the female *C. canis* is strongly rounded, while the head shape of the female *C. felis* is more sloping. The first tooth of the genal comb of the cat flea is the same length as the others, while the first tooth of the genal comb of the dog flea is half the length of the others (Lewis, 1967; Hinkle, 1996). Another characteristic to distinguish the two species is the number of setae on the metepisternum. Two setae occur on the metepisternum of *C. felis* in contrast with the three setae on the metepisternum of *C. canis* (Oberholzer & Ryke, 1993). Furthermore, *C. felis* has seven to ten setae on the medial side of femur III, in contrast with the 10 to 13 setae on the medial side of femur III of *C. canis* (Hinkle, 1996).

There are two recognized subspecies of *C. felis*, namely, *C. felis strongylus* and *C. felis felis* which are both primarily parasites of carnivores. These subspecies in southern Africa present a challenging taxonomic problem that cannot be solved with certainty (McCrindle, Green & Bryson, 1999). In their taxonomic study on the genus *Ctenocephalides* Ménier and Beaucournu (1998) used aedeagus characters and found it impossible to differentiate these subspecies from each other. Speculation exists that either *C. f. felis* does not exist in southern Africa, or is a synonym of *C. f. strongylus*. Whatever the origin of *C. f. strongylus* and *C. f. felis* may be, they are morphologically only distinguishable by the subjective evaluation of their cephalic curvature and the number of pre-apical plantar spiniforms on the fifth fore-tarsal segment of the male (Segerman, 1995). *C. f. felis* is cosmopolitan and can adapt to diverse hosts in practically all parts of the world. *C. f. strongylus*, which is the primitive form, is known with certainty from the

whole afrotropical region and has also been recorded from Egypt (Lewis, 1967; Segerman, 1995; Menier, 1995-1996).

Due to its homogeneity, *Ctenocephalides* is a genus difficult to analyze. A morphological study by Ménier and Beaucournu (1998), based on new criteria, allows differentiation of most of the members of the genus. These new characters, namely, the description of the phallosome apex, or aedeagus, allow differentiation between taxa, but the relationships of the species are still doubtful because it is impossible to generalize on the phyletic concordances between hosts and fleas.

A morphological differentiation between *C. felis* and *C. canis* was necessary in view of further comparative experimental studies on the two species. Scanning electron microscopy (SEM) provides access to the greatest possible detail of structures that are difficult to study with other methods. Using SEM in addition to other methods, the morphological characteristics of the two species could be established with certainty, making the later differentiation between the two species easier.

The aim of this study was:

- To identify and describe the most useful and reliable characteristics to distinguish between *C. felis* and *C. canis*.

Material & Methods

Fleas

All fleas used for microscopic examination were newly emerged, unfed adults of *C. felis* and/or *C. canis* reared under laboratory conditions. *C. felis*, originally obtained from cats in Botshabelo near Bloemfontein, and *C. canis*, originally obtained from dogs in Grahamstown, were used to start the laboratory colonies. The test animals and artificial rearing of the immature stages are discussed later.

Preparation of material for scanning electron microscopy

Specimens which had been fixed in 70% ethanol had to be dehydrated to 100% ethanol. Some specimens had to be cleaned carefully with a soft sable hair brush under a stereo dissection microscope to remove any attached dust or debris. The specimens were then dehydrated through a series of ethanol concentrations and critical point dried using standard techniques.

Inverted, conical mounting stubs were manufactured (Department of Instrumentation of the Faculty of Natural Sciences) to enable a tilt of the SEM stage of 90°. The stub also ensured a black background on micrographs. The commercial brand Japan Gold Size (Winsor and Newton) was used to mount specimens on the stubs. Specimens were then coated with gold and examined in a *Joel Winsem JSM 6400* scanning electron microscope at 10 kV.

Results

Figure 2.1 shows the general external morphology of a female *C. felis*.

Tibia III

Adult fleas have three pairs of very well developed legs of which the hind legs are enlarged for jumping. The leg of an adult flea consists of a coxa, trochanter, femur, tibia and tarsus. At the posterior border of tibia III is a series of notches, each with one or more setae. On tibia III of *C. felis* there are six notches with setae compared to that of *C. canis* which has an additional two notches with setae (Figure 2.3a & b).

Head and genal comb

C. felis and *C. canis* can easily be separated by the shape of the head of the females. The head of the female *C. felis* is twice as long as it is high when seen from the side, whereas the head of the female *C. canis* is less than twice as long as it is high when seen from the

side (Figure 2.4a & b). In both species the head shape of males is very similar to that of the female *C. canis* and cannot be used as a distinguishing character.

The first tooth of the genal comb of *C. felis* is the same length as the others, whereas the first tooth of the genal comb of *C. canis* is half the length of the others (Figure 2.5).

Metepisternum

The metepisternum is a thickened cuticular plate on the ventral side of the thorax. On the metepisternum of *C. canis* are three setae whereas only two setae occur on the metepisternum of *C. felis* (Figures 2.6a & b). On the opposite metepisternum of the flea illustrated in figure 2.6b, however, only one seta was present (Figure 2.7).

Femur III

The femur is the segment of the leg between the trochanter and tibia. The medial side of femur III of *C. felis* contains nine setae, in contrast with the 14 setae on femur III of *C. canis* (Figure 2.8a & b).

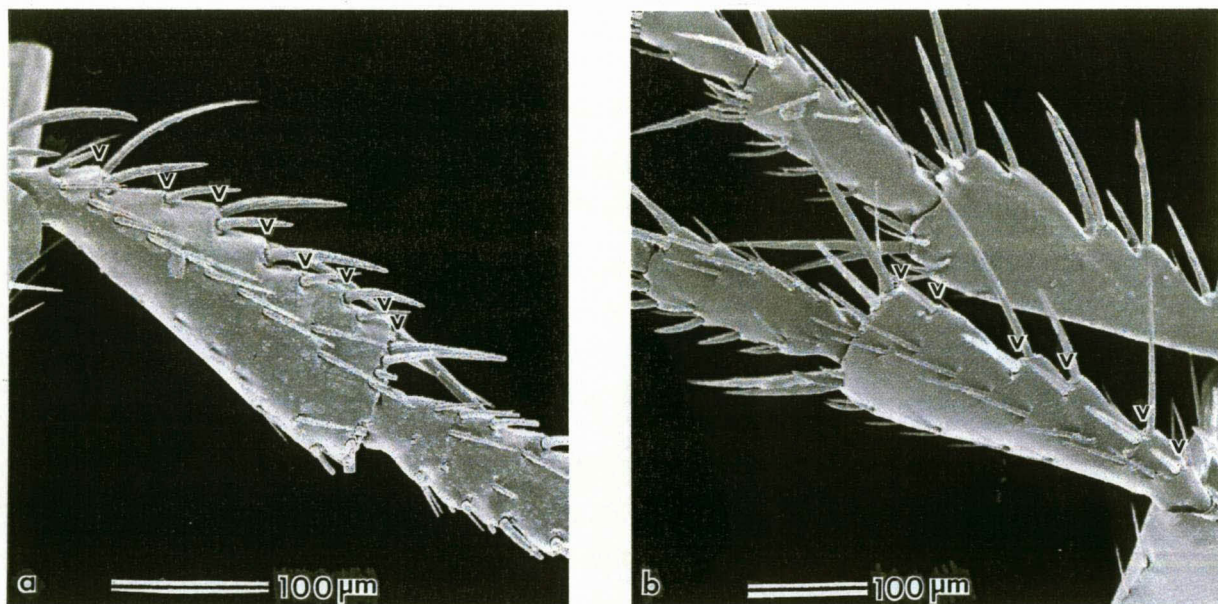


Figure 2.3 Scanning electron micrographs of tibia III of a) *C. canis*, with eight notches with setae and b) *C. felis* with six notches with setae as indicated.

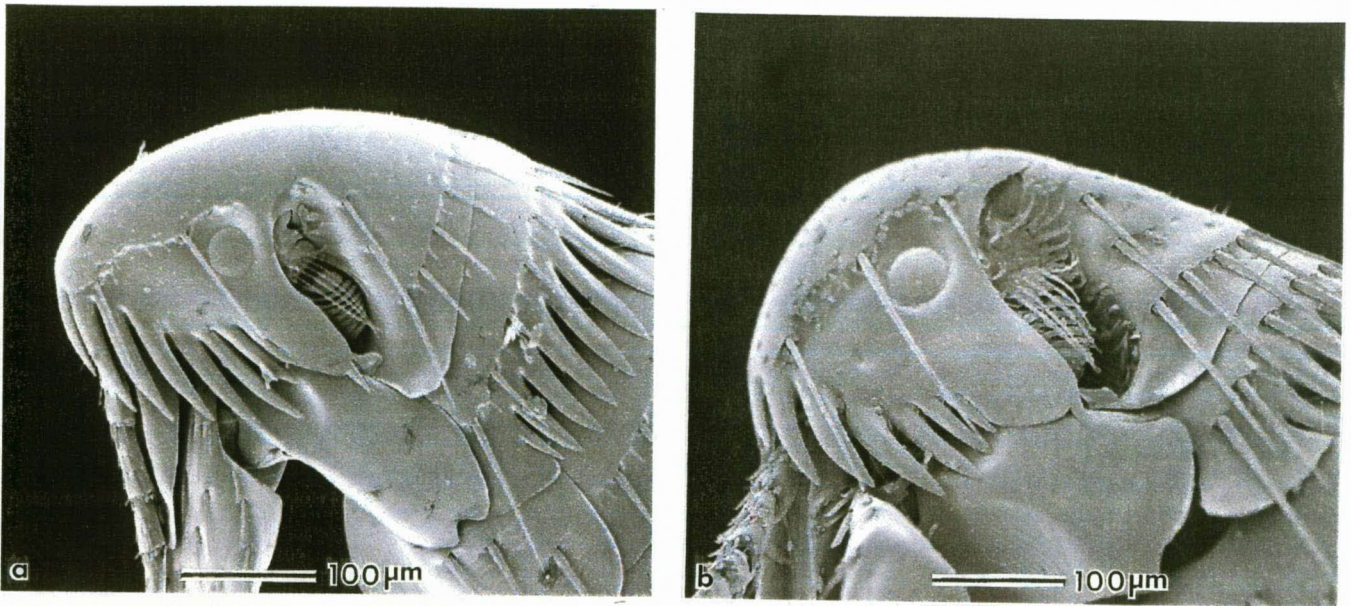


Figure 2.4 Scanning electron micrograph of the head of a) female *C. felis* and b) female *C. canis*.



Figure 2.5 Scanning electron micrograph of the head of a female *C. canis* showing that the first tooth of the genal comb is half the length of the others.

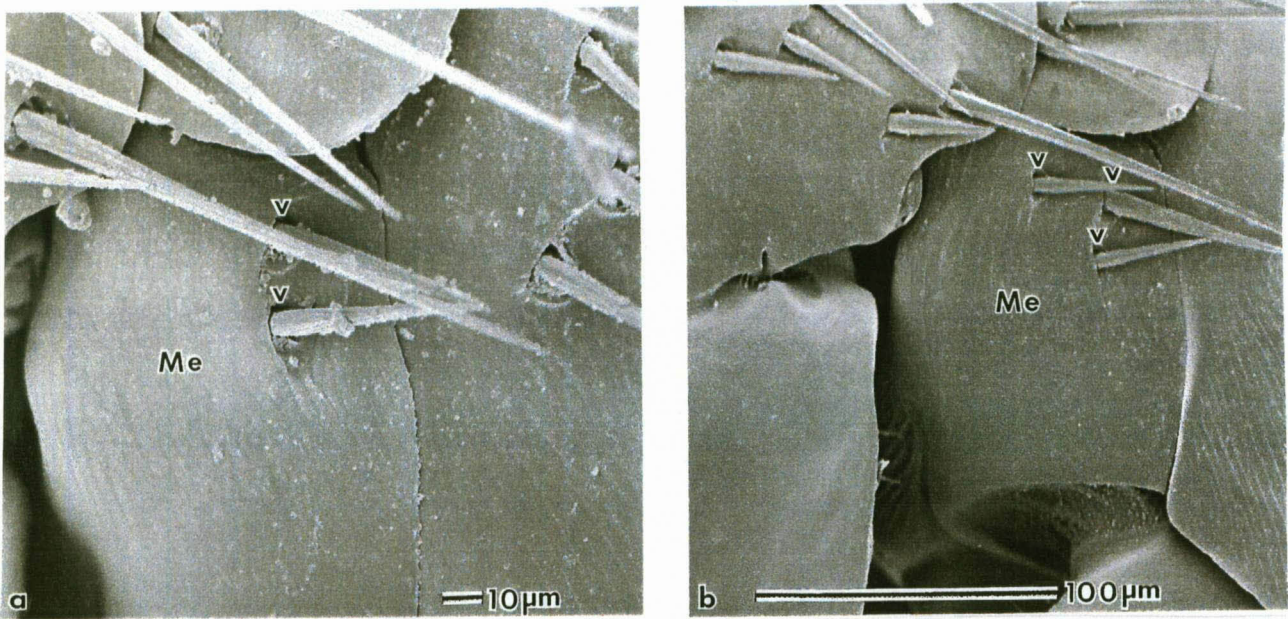


Figure 2.6 Scanning electron micrograph of the metepisternum (Me) of a) *C. felis* with the two setae indicated and b) *C. canis* with three setae as indicated.

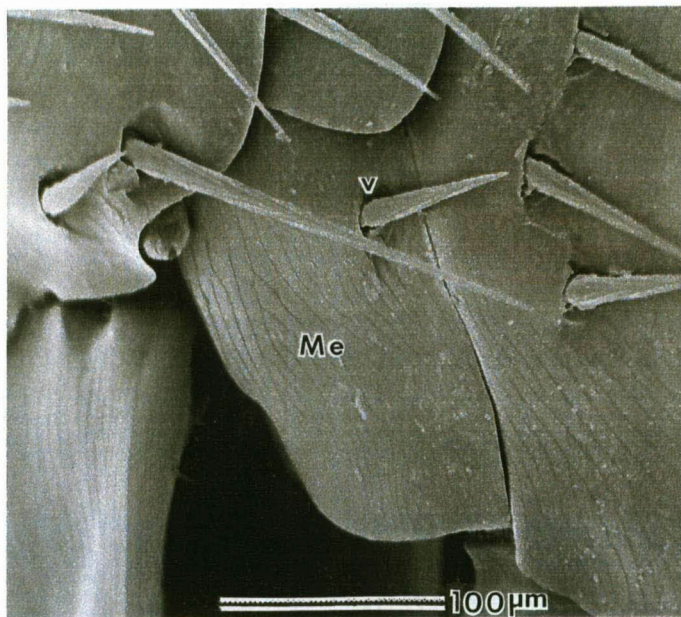


Figure 2.7 Scanning electron micrograph of the opposite side of the specimen in figure 2.6b, showing only one seta on the metepisternum.

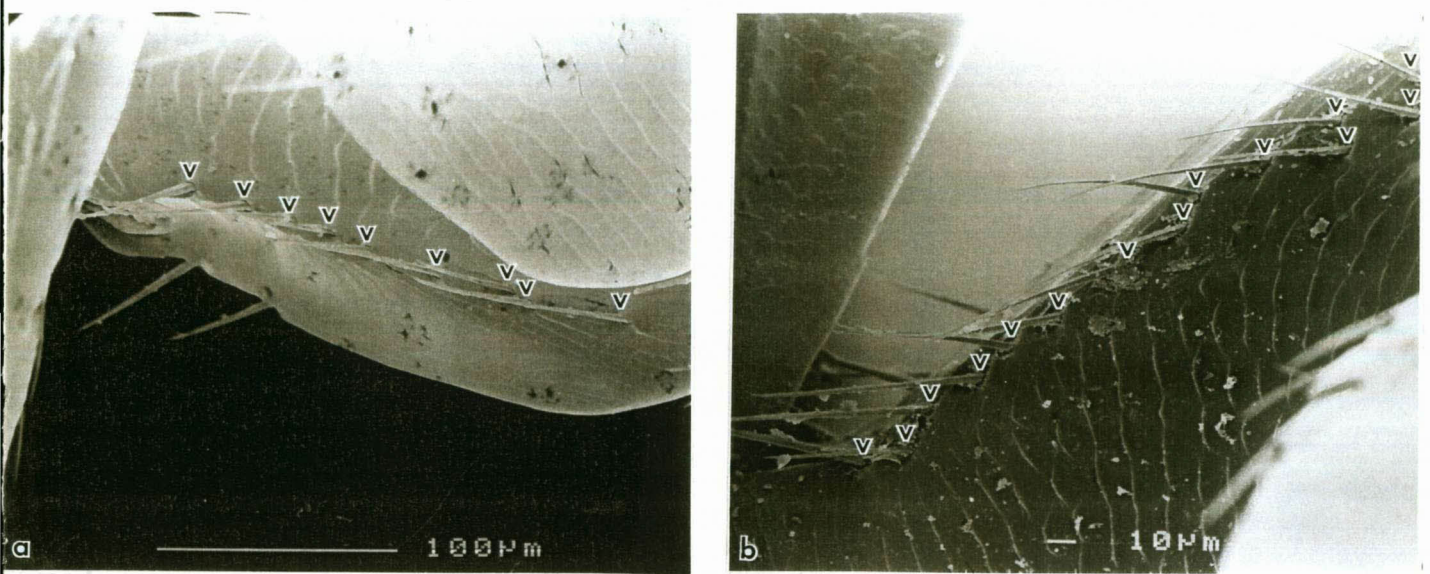


Figure 2.8 Scanning electron micrographs of femur III of a) *C. felis* containing nine setae and b) *C. canis* containing 14 setae as indicated.

Discussion

Characters used for identification must be of such a nature that they are clearly visible when studied under a light microscope. Light microscopy that can be used includes compound microscopy where the specimen is cleared in potassium hydroxide and mounted in Bouin-Glycerine on a slide and stereo microscopy where specimens are studied alive, dead or anaesthetised. Though the first method of microscopy requires that the specimen should be killed, it is useful because light is transmitted through the specimen, which facilitates better observation. The specimen is also permanently preserved for repeated studying. The stereo dissection microscope provides many more options to study a specimen. The specimen can be studied alive or, if necessary, anaesthetised and at the same time stay undamaged. Readily visible characters of a dead specimen that does not need to be cleared can also be studied in this way. Disadvantages

of using the stereo dissection microscope in this study, was that it was difficult to identify fleas accurately according to the different characters besides the head shape. Even when the fleas were anaesthetised proper observations were not possible, because they were still moving to some extent. Some of the fleas were needed for starting a new laboratory colony and could therefore not be killed. Although not without limitations, both the compound and stereo dissection microscopes can be very useful, depending on the nature of the study.

The SEM contributed to represent very clear images of the fine detail of the important differences between *C. felis* and *C. canis*, which were not clearly visible under the light microscope. The only disadvantage is that the specimen has to be killed in order to study it. However, the SEM can be used to confirm uncertain observations made under a light microscope.

Although the head shape cannot be used as distinguishing character among males, it is a prominent characteristic that can be most easily used to distinguish between female *C. felis* and *C. canis* by using a light microscope. The SEM micrographs clearly show the distinctness of the head shape as well as the number of notches with setae on tibia III that can therefore be employed as reliable distinguishing characters. These results correspond with that of Segerman (1995) who found that the shape of the head and the number of notches with setae on tibia III are useful distinguishing characters. The notches with setae on tibia III are fairly clear under the light microscope, but might be difficult to count in anaesthetised fleas under the stereo microscope. The legs of anaesthetised fleas tend to vibrate, which makes it impossible to count the setae. According to Lewis (1967) the number of stout bristles between the long postmedian and apical bristles of the dorsal margin of tibia III is also a characteristic to distinguish between *C. felis* and *C. canis*. *C. felis* has only one stout bristle compared to the two stout bristles of *C. canis* in this position on tibia III. However, this characteristic was not visible in the present study.

Although the teeth of the genal comb is readily visible under the light microscope, the first tooth and the length of it with respect to the rest of the teeth, is not a very practical

character to use in identification. However, this character is ideal to distinguish between the two species when using the SEM. Hinkle (1996) also found that the length of the first tooth is a reliable character to distinguish between *C. felis* and *C. canis*. The ability to rotate the specimen, provides an excellent image of the front view of the flea. The number of setae on the metepisternum is also a very useful character for SEM purposes, but cannot be used to identify live fleas, because the specimen has to be absolutely still to make an accurate identification. The tendency that the number of setae on opposite metepisternums differs had been found several times during the study. Instead of the three and two setae that normally occur on the metepisternum of *C. canis* and *C. felis*, respectively, at least one out of every 50 fleas of both species contained only one seta on the metepisternum. However, these results are not sufficient to state that the characteristic is unreliable.

A characteristic also being investigated in this study is the occurrence and number of setae on the medial side of femur III. To study this character the flea was cleared with potassium hydroxide and the femur had to be removed carefully from the specimen and rotated to different angles in order for the medial side to be visible. Therefore, the appearance of setae on femur III is not a very useful character to use in light microscopy. In the present study nine setae were found on femur III of *C. felis*, that is five less than the 14 setae on femur III of *C. canis*. The number of setae found in *C. canis* differs from previous authors who reported 10 to 13 setae on the medial side of femur III in *C. canis* (Hinkle, 1996). The setae on the medial side of femur III and the length of the genal tooth might have played an important role in identification, but due to the fact that it is difficult, if not impossible, to make accurate observations with respect to live fleas under a light microscope, these characteristics are not applicable in the studies to follow.

CHAPTER 3

THE INFLUENCE OF TEMPERATURE AND RELATIVE HUMIDITY ON THE DEVELOPMENT OF CAT FLEA EGGS

Introduction

Female *Ctenocephalides felis* have six ovarioles in each ovary of which half contain mature oocytes. Egg production begins two days after the first blood meal and reaches a maximum in six to seven days (Linley, Benton & Day, 1994). Flea eggs are small, pearly-white, oval in shape and translucent. They measure about 478 μm in length, 308 μm in width and are just visible to the naked eye (Bacot & Ridewood, 1914; Linley *et al.*, 1994). The eggs are usually laid while the female flea is on the host animal (Dryden, 1992). The majority of the eggs are laid during the last eight hours of the scotophase. The eggs laid by the female are not attached in any way to the skin, fur or feathers of the animal on which the fleas are parasitic. Initially, the chorion of the egg is wet, which tends to prevent immediate drop-off, but it dries rapidly and because they are very smooth, the eggs fall off the host animal easily and, thus, can be spread all over the pet's resting area. The rate at which eggs drop off or are dislodged from the pelage is influenced by grooming, hair coat length and host activity (Bacot & Ridewood, 1914; Rust & Dryden, 1997).

The development and morphology of the cat flea egg reflect specialized adaptations in the life cycle of this ectoparasite, with respect to the host and the environment. The cat flea egg is fragile by comparison to other insect eggs, particularly those oviposited in harsh environments. Also, the cat flea egg is less ornate externally than the eggs of other

siphonapteran species. There is no surface reticulation, but a more or less uniform covering of small, round, nodular tubercles. A slightly raised disk with micropyles are usually visible at the posterior end of the egg and a disk with aeropyles are also present anteriorly, but are usually less conspicuous (Marchiondo, Meola, Palma, Slusser & Meola, 1999).

The chorion of the cat flea egg provides physical protection for the embryo and probably allows some gaseous exchange. However, the rate of diffusion of oxygen through this solid structure would be limited and inadequate to meet the demands of the developing embryo. The respiratory needs of the developing cat flea egg are provided by the presence of external aeropyles connected to the air-filled chamber of the inner chorionic network that create a layer of air in the chorion that largely surrounds the embryo and is connected to the outside air of the microenvironment (Linley *et al.*, 1994).

While some insect eggs develop without any uptake of water, flea egg development is highly sensitive to changes in temperature and RH (Marchiondo *et al.*, 1999). To determine the extent of heat and moisture necessary in an environment where flea egg development would be optimal, flea eggs were exposed to different combinations of temperature and RH. The specific aims were to determine:

- The extent of moisture gain or loss when exposed to different percentages of RH.
- The viability of eggs exposed to different combinations of temperature and RH.

Material & Methods

Rearing of fleas

Cat fleas used in all the studies were obtained from an existing cat flea colony reared at the Department of Zoology and Entomology at the University of the Free State. Flea-infested laboratory cats, used to provide a supply of flea eggs, were housed at the Experimental Animal Unit of the University of the Free State. The cats were kept in a temperature-

regulated room ($25\pm 2^{\circ}\text{C}$) of 4.5 m x 2.5 m. The room contained an aluminium table of 2.5 m x 0.5 m and plastic basins with pet blankets for the cats to sleep in. Pans with commercial cat litter, fresh food and water were provided daily when the room was cleaned. The animals were kept in accordance with guidelines set forth by the National Code for the handling and use of animals for research, education, diagnosis and the test of drugs and substances in South Africa (Erasmus, 1990).

Optimum conditions for the rearing of fleas in the laboratory were obtained as follows: All stages of the life cycle were kept in a large glass container with a saturated sodium chloride solution, which provided a RH (relative humidity) of 75%. The container was kept in a temperature-regulated room at 25°C . The cats were re-infested weekly with unfed laboratory reared fleas not older than three days. Eggs were collected daily, starting two days after infestation, by wiping the bedding and aluminium table, where the cats most often rested, with a paintbrush. Eggs were separated from debris by sifting the debris with a plastic sieve (openings 1 mm x 2 mm). The eggs were then transferred to a standard rearing medium where the larvae hatched and started feeding. The rearing medium was composed of sand, bloodmeal and bonemeal in the ratio 7:2:1. At the end of the larval stage the larvae spinned cocoons and pupated.

Pupae were separated from the larval medium after 10 days by sifting the medium with a plastic sieve (openings 1 mm x 2 mm) and transferred to special jars where the adults could emerge and collect at the bottom of the jar. Adult fleas were used to re-infest cats, in order to maintain the flea colony, and for experiments. Adults were anaesthetized with CO_2 to count out required numbers for specific experiments. When eggs were needed for experiments, the required numbers were collected with an automated aspirator. Where an experiment required it, eggs were weighed on a Mettler Toledo UMT2 Balance that can weigh accurately to four decimals.

Experimental conditions

Glycerol solutions of different concentrations were used to obtain desired RH percentages inside closed containers. Figure 3.1 shows the relationship between glycerol concentrations and percentage RH. Incubators, set and regulated at different temperatures, were used to obtain the different temperatures required for the experiments.

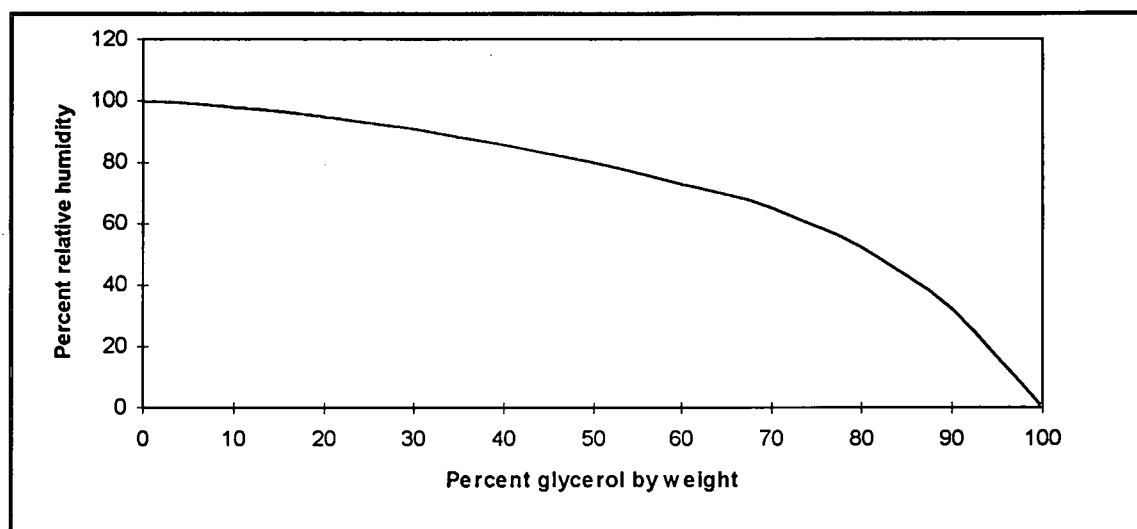


Figure 3.1 Percentage relative humidity over aqueous glycerol (Miner & Dalton, 1953).

Change in egg mass at different percentages of RH

Microtitre Plates, each containing 96 cells, 10 mm deep and 8 mm in diameter, were used to expose eggs to different percentages of RH. Three groups of twenty-five eggs were exposed to 20%, 50% and 85% RH, respectively, at a constant temperature of 25°C. Eggs were weighed individually, daily for three days. Differences between groups exposed to different conditions, were tested for significance ($P < 0.05$) with the use of t-tests and analysis of variance (ANOVA) followed by the Tukey test.

Effect of temperature and RH on egg hatching

To determine the effect of temperature and RH on egg hatching, cat flea eggs were placed in five different petri dishes. Each petri dish contained 25 eggs and was exposed to different combinations of temperature and RH. First the RH was kept constant at 75%

and the temperatures varied from 15°C to 25°C and 35°C. Alternatively the temperature was kept constant at 25°C and RH differed from 50% RH to 85% RH. Eggs were monitored daily for any emerged larvae.

Experiments were done in duplicate and in all cases the similarity of data sets allowed pooling of the data.

Results

Change in mass at different percentages of RH

Three groups of cat flea eggs were exposed to different percentages of RH at a constant temperature of 25°C. In total all three groups lost mass over three days. Table 3.1 shows the summary statistics of the changes in mean mass of the eggs at different conditions. At 20% RH the eggs lost between 16.7% and 78.2% mass (mean 44.6%) and between 0.1% and 35.4% mass (mean 9.5%) at 50% RH. Although the mass of some of the eggs at 85% RH stayed constant throughout the study, the eggs lost between 0% and 50% mass (mean 6%). Statistical analysis showed that all groups of eggs differed significantly ($P < 0.0001$) in the amount of mass lost after exposure to different RH. Mass lost at 20% RH versus 50% RH differ with 10.8 μg ($P < 0.001$), while there was a significant difference of 11.92 μg between the mass lost at 20% RH and that lost at 85% RH ($P < 0.001$). The difference between the mass lost at 50% and 85% RH was not significant ($P > 0.05$).

Table 3.1 Change in mean mass of eggs over three days after being exposed to different percentages of RH at a constant temperature of 25°C.

	20% RH	50% RH	85% RH
Initial mean mass (µg)	31.2	32.5	33.2
Mass loss (%)	44.6	9.5	6.0
Standard deviation	5.4	2.2	3.2
Minimum (%)	16.7	0.1	0
Maximum (%)	78.2	35.4	50

Effect of temperature and RH on egg hatching

Eggs started to hatch from the fourth day of exposure to 15°C and 75% RH. In total only 72% of the eggs hatched. All the eggs exposed to 25°C and 75% RH hatched on the second and third days of exposure and altogether 86% of the eggs exposed to 35°C and 75% RH hatched, all within the first two days. A constant temperature of 25°C and varying RH of 20%, 50% and 85%, respectively, resulted in the following: After three days 12% of the eggs at 20% RH hatched, while the rest of the eggs did not hatch at all. All the eggs exposed to 50% RH hatched, except for two eggs, which hatched on the fourth day. The remainder of the eggs hatched on the third day of exposure. Although most of the eggs (90%) exposed to 85% RH hatched, it occurred over a period of eight days.

At temperature 15°C and 75% RH the majority of the 72% eggs which hatched, hatched on the fifth and seventh days. With a 10°C increase in temperature, 100% of the eggs hatched of which almost all hatched on the third day of exposure. With a further 10°C increase in temperature the percentage of eggs hatched decreased slightly. All of the 86% eggs hatched within the first two days of exposure (Figure 3.2).

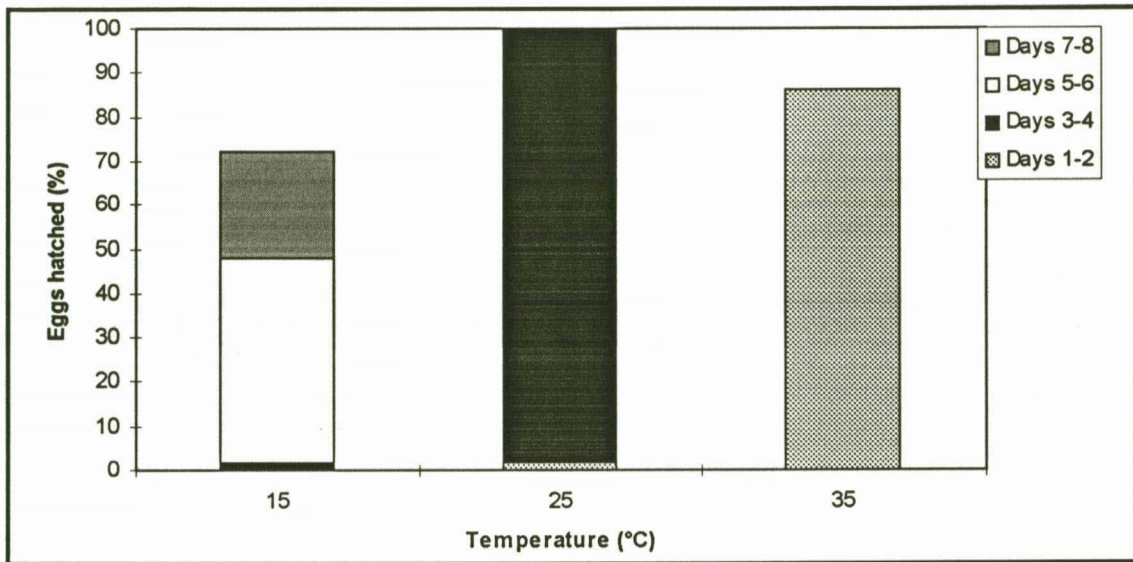


Figure 3.2 Percentage of eggs hatched after being exposed to different temperatures at a constant RH of 75%.

Figure 3.3 shows the percentage of eggs hatched after being exposed to a constant temperature of 25°C and varying percentages of RH. Eggs exposed to 20% RH and 50% RH hatched on the third and fourth days of exposure. At 20% RH only 12% of the eggs hatched, while 100% of the eggs hatched at 50% RH. After exposure to 85% RH, eggs started to hatch from the first day, but the majority of the eggs hatched on the third day of exposure.

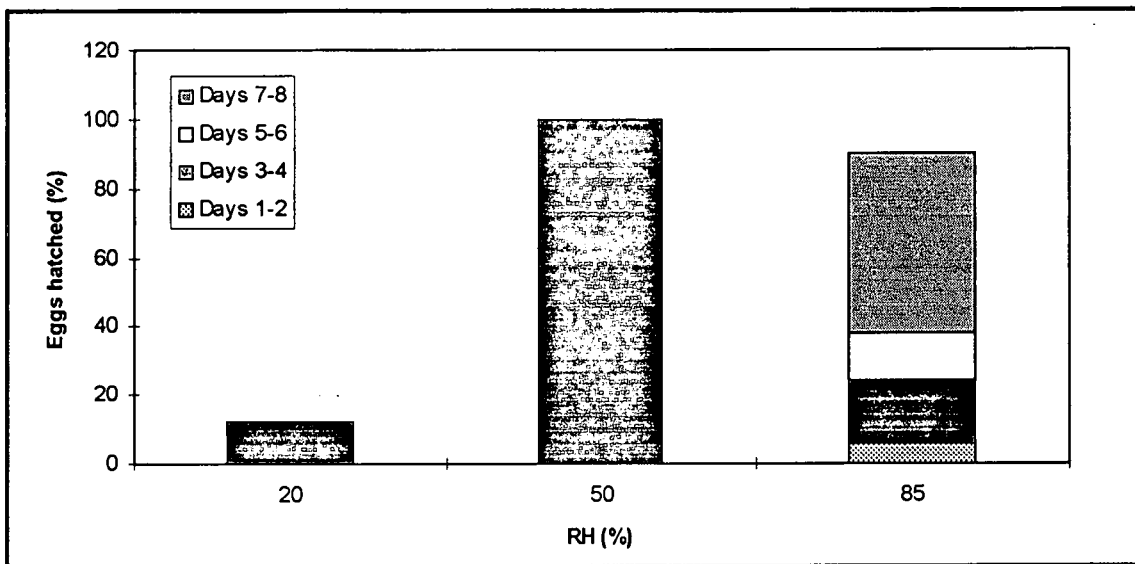


Figure 3.3 Percentage of eggs hatched after being exposed to different percentages of RH at a constant temperature of 25°C.

Discussion

In this study eggs lost the largest amount of mass ($> 13 \mu\text{g}$), and thus the most moisture, after being exposed to an RH of 20% at 25°C. Although the eggs at 50% RH lost more moisture than the eggs exposed to 85% RH, both groups lost $< 4 \mu\text{g}$. Although 12% of the eggs exposed to 20% RH hatched on day three, the remainder seemed to shrink and did not hatch at all. This implies that a RH of this low level is almost lethal to cat flea egg development. Studies by Silverman *et al.*, (1981) similarly showed that RH below 50% will cause desiccation and destruction of eggs. Temperatures above 25°C and RH above 50% resulted in survival rates of at least 86%. Dryden and Rust (1994) found that nearly all eggs hatched when the RH was greater than 50% at 27°C. In this study a combination of 25°C and 75% RH appeared to be optimal for cat flea development since all the eggs, exposed to these conditions, hatched. Optimum RH alone, however, could not achieve the same success in combination with temperatures of 15°C and 35°C. Although a higher temperature increased the rate of development, it did not necessarily result in the

highest percentage survival. This phenomenon is confirmed by Dryden and Rust (1994) who reported that only 40% of eggs hatched at 37°C when they were held at 75% RH and Silverman *et al.* (1981) found that eggs kept at 35°C hatched only in moist air (75-92% RH). Although Silverman *et al.* (1981) who reported that egg hatching will increase with increasing RH, they found that hatching decreased to 70% in saturated air. They explained that the failure of eggs to hatch in warm saturated air may have been due to accumulation of heat within the egg. Eggs kept below 75% RH desiccated.

This study corresponds to the results of Dryden and Rust (1994), since all the eggs hatched on the third and fourth days at 25°C and 50% RH and 90% of the eggs exposed to 85% RH hatched within eight days. All the eggs exposed to different temperature-RH combinations in this study hatched within eight days. According to Dryden & Rust (1994) eggs normally hatch within ten days depending on the environmental conditions in which they dislodge from the host's pelage and drop off. An increase in temperature increased the rate of development as eggs exposed to lower temperatures took longer to hatch. In the present study the time required for eggs to hatch increased from two to seven days when temperature decreased from 35°C to 15°C. The same trend occurred in a study by Dryden (1993) who showed that 50% of eggs deposited under environmental conditions of 70% RH and 35°C hatched within 1.5 days, whereas 50% of the eggs deposited at 13°C hatched within six days.

This study confirmed that the development of cat flea eggs is highly sensitive to changes in temperature and RH. The tendency in this study was that eggs lost moisture even at a very high RH. This is an indication of the high permeability and thus susceptibility of eggs to desiccation. Thus, flea eggs require a warm, moist environment to hatch and will desiccate rapidly in dry conditions. According to Dryden and Rust (1994) only a small percentage of eggs in most households and yards ultimately develop into adults. Although only a fraction of the eggs develop to adulthood in the natural environment, populations survive because of the large reproductive capacity of the female cat flea and due to multiple mating. Multiple mating by females is an advantageous strategy for cat fleas

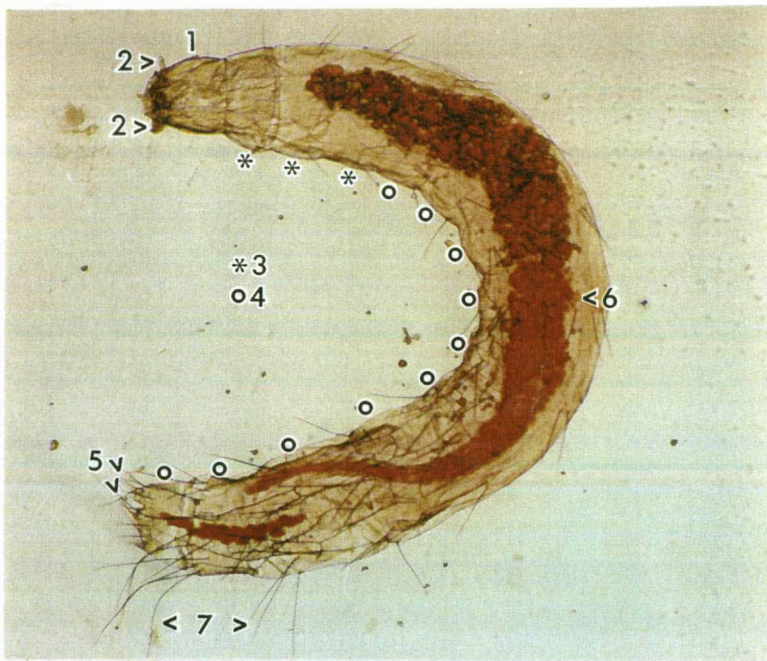
concerning survival (Osbrink & Rust, 1984; Dryden, 1992; Hsu & Wu, 2000). The egg and larval stages of cat fleas are the stages in the life cycle that are most susceptible to heat and desiccation. Since extreme fluctuations in temperature and RH will cause desiccation and destruction of eggs, the micro-environmental conditions in which the eggs are deposited is therefore of prime importance to their survival rate. Although the flea eggs are laid on the pet, they drop off together with adult flea feces in the pet's environment as the pet moves around. Not surprisingly, Rust (1992) found that most of the cat flea eggs and larvae recovered from an infested structure were found in close proximity to the host's resting place which usually provides a protected microhabitat in order to ensure the survival of the immature stages.

CHAPTER 4

THE INFLUENCE OF EXTERNAL FACTORS ON THE DEVELOPMENT OF CAT FLEA LARVAE

Introduction

A *Ctenocephalides felis* egg hatches into an approximately 2 mm long, eyeless, legless, but very active larva. Flea larvae are slender, segmented, sparsely covered with hair and transparent white (Dryden, 1992). The larva consists of a head, three thoracic somites and ten abdominal somites, the last being much smaller than the ninth and provided with a pair of downwardly and backwardly directed processes, the anal struts (Figure 4.1). Anal struts consisting of a pair of blunt, hooked processes distinguish flea larvae from those of dipterous insects. A newly hatched larva is more cylindrical, *i.e.* of more uniform width, than those of later instars. As the larvae grow the fore and hind ends assume a more tapered form. The head does not increase in size at the same rate as the other parts of the body and so an older and consequently larger larva has a relatively smaller head than a small larva and the front portion of the body appears more pointed (Bacot & Ridewood, 1914). Although the first larval instar is only about 2 mm long, fully developed larvae can be 4-5 mm in length (Dryden, 1993). On top of the head of a newly hatched larva is an egg-breaker, by means of which a slit is formed in the egg-shell through which the larva emerges. This egg breaker was first described in the larvae of *Ctenocephalus felis* and *Ctenocephalus fasciatus* (Bacot & Ridewood, 1914). No trace of the egg-breaker is to be seen in the larvae after the first moult (Bacot & Ridewood, 1914).



1. Head
2. Antennae
3. Thoracic somites
4. Abdominal somites
5. Anal struts
6. Alimentary canal
7. Setae

Figure 4.1 A second instar *C. felis* larva as seen under a light microscope (40 x magnification).

During this stage of the life cycle larvae almost constantly look for food. Larvae feed on organic matter, such as skin scales, tiny insect parts and most importantly, adult flea fecal material which consists of dried, but undigested blood (Kuepper, 1999). According to Rust and Dryden (1997) larvae will also consume the egg chorion and are cannibalistic. The nutritional requirements of larvae greatly limit the number of sites in and around structures that are suitable for *C. felis* development (Dryden & Rust, 1994).

The larvae of cat fleas are free-living and very active, moving with remarkable celerity through and upon the debris of the nests and dry rubbish among which they live. When moving quietly, the larvae crawl over an even surface supporting the body on the ventral setae and extending and contracting the segments. The larvae, however, have periods of quiescence, during which they lie coiled up, either for repose or for concealment (Bacot & Ridewood, 1914). Larvae are negatively phototactic and positively geotactic and therefore, avoid direct sunlight in their microhabitat by crawling in under organic debris (grass, branches, leaves or soil). Because of their susceptibility to heat and desiccation, flea larvae are not likely to survive outdoors in shade-free areas. Flea development

outdoors probably occurs only in areas with shaded, moist ground where a flea-infested animal spends a significant amount of time to allow adult flea feces to be deposited into the larval environment (Dryden, 1993). Likewise, in the indoor environment, flea larvae probably only survive in the protected microenvironment under a carpet canopy or in cracks between hardwood floors in humid climates (Dryden, 1993). Since larvae prefer a dark environment and will burrow down into any available materials, with carpeting, furniture fabric, underneath furniture cushions, and cracks in floors being particular favorites inside a home (Kuepper, 1999). Thus, development occurs at the base of carpet fibers and in the outdoor environment most larval activity is likely to be restricted to the upper few millimeters of the soil (Dryden & Rust, 1994). Although larvae will drown in water, moisture in the larval environment is essential for development and exposures to a low RH are lethal. Before pupal formation, the larvae are extremely sensitive to desiccation (Dryden, 1992; Dryden & Rust, 1994). The larvae molt twice before spinning a cocoon and developing into the pupal stage (Dryden, 1993).

At the end of the larval period, just prior to the spinning of the cocoon, the larva become less active, takes on a more opaque white colour than before and the body becomes shorter and fatter (Bacot & Ridewood, 1914). The full-grown larva is soon followed by a pre-pupal period in which the levels of juvenile hormone decrease. During this stage the mature larva adopts a u-shape position during which it expels its intestinal contents and becomes creamy white in appearance. This is followed by a period of quiescence which is ended by spinning a silk cocoon in which the larva soon pupates, but if not ready to spin a cocoon, it stretches out at full length (Chen, 1934).

In view of the requirements for the survival of *C. felis* larvae, some aspects concerning development of the larvae under various conditions were investigated. The specific aims of this part of the study were to determine:

- The influence of temperature and RH on the mass, duration of the larval stage and survival of developing larvae.
- The effect of different rearing media on larval development.

Material & Methods

Mass, duration of the larval stage and survival of larvae developed under different conditions

C. felis eggs, collected from laboratory cats, were individually placed in Microtitre Plates with a little dried blood (see chapter 3). The eggs in each Microtitre Plate were then exposed to different combinations of temperature and RH. Desired temperatures and RH were obtained as described in Chapter 3. First RH was kept constant at 75% and the temperature was varied from 15°C to 25°C and 35°C. Then temperature was kept constant at 25°C and RH was varied from 50% RH to 85% RH. In this way larvae, emerged under different conditions were obtained. As soon as the eggs hatched, the larvae were removed from the Microtitre Plate and individually placed in different petri dishes containing a little standard rearing medium. Each larva was then returned to the temperature and RH combination under which it emerged. The larvae were left to complete the life cycle, but were weighed daily on a Mettler Toledo UMT2 Balance in order to compare the mass, development time and survival of larvae developed under different conditions. One-way analysis of variance (ANOVA) followed by the Tukey test was used to test for the significance ($P < 0.05$) of differences in mass between groups exposed to different conditions.

Different larval rearing mediums

Flea eggs were obtained and placed in Microtitre Plates as described above, but were exposed to optimum conditions (25°C; 75% RH) for flea development as determined in the previous experiments. As the eggs hatched, the larvae were transferred to petri dishes (25 larvae per dish) containing different rearing media, or components of that. The larvae were allowed to feed *ad lib* on the different diets under optimum conditions. The different diets to which the larvae were exposed included the following:

- a) A mixture of blood- and bonemeal in the ratio 2:1 which served as a control (standard rearingmedium)
- b) Adult flea feces

- c) Bloodmeal only
- d) Bonemeal only
- e) A mixture of even parts of blood- and bonemeal (in the ratio 1:1)
- f) Cat food pellets

Apart from the rearing medium, each petri dish contained enough sand to cover the bottom of the petri dish. The larvae were then left to complete the life cycle.

All experiments were done in duplicate and due to the similarity of the data sets, they were pooled.

Results

Mass of larvae that developed under different conditions

As shown by figure 4.2a larvae in all the groups exposed to different conditions, showed a rapid increase in mean mass immediately after emerging from the eggs, except for the group of larvae exposed to 15°C and 75% RH, which gained mass only gradually. This tendency continued for the duration of the first instar. Figure 4.2b shows that during the second instar the rate of mass increase was slightly lower among larvae at 75% RH exposed to 25°C and 35°C, respectively, while the group of larvae at 15°C showed a higher rate of mass increase than in the previous instar. The larvae at 25°C exposed to 50% RH and 85% RH continued to gain mass rapidly. During the third instar the group of larvae exposed to 15°C and 75% RH continued to gain mass until day 32 of larval development. From day 33 onwards the mean mass varied between 144 µg and 187 µg until day 63 when it decreased rapidly before the larvae developed into pre-pupae. At 25°C and 75% RH larvae gained mass for five more days from the beginning of the third instar after which the mean mass varied between 206.3 µg and 258.3 µg before the larvae finally continued to lose mass from day 42 until the end of the larval stage. At 35°C and 75% RH the third instar started on day nine after larvae emerged from the eggs. During the first three days of the instar there was an increase in mean mass after which the mass varied between 165.2 µg and 200.5 µg until day 22, whereafter it drastically decreased.

The mean mass of larvae developed at 25°C and 50% RH, varied between 152.9 µg and 165 µg during the third instar and finally started to decrease on day 28 before development into pre-pupae. At 25°C and 85% RH there was a variation in the mean mass between 155.1 µg and 168.8 µg before the mean mass started to decrease on day 23 until the end of the larval stage (Figure 4.2c).

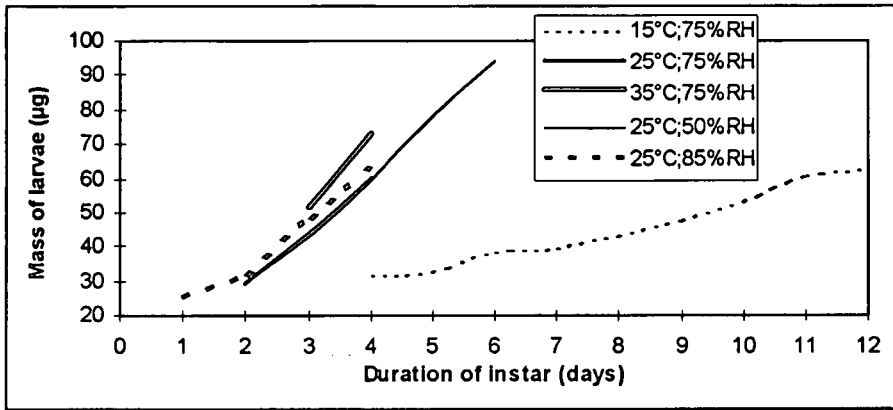


Figure 4.2a Change in mean mass of first instar larvae which developed under different conditions.

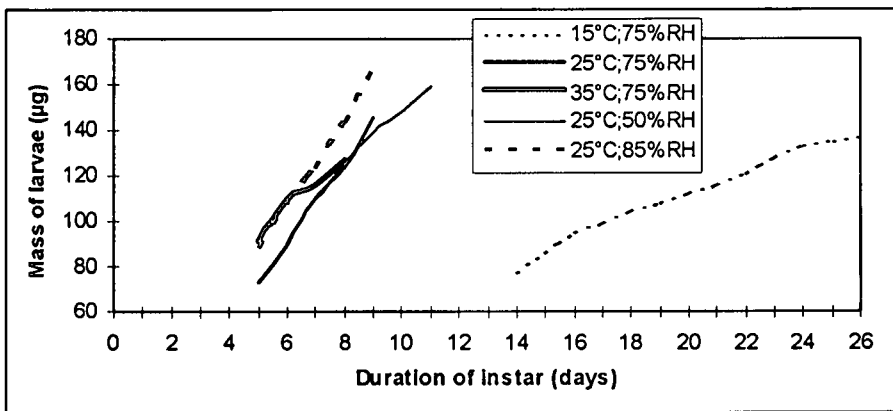


Figure 4.2b Change in mean mass of second instar larvae which developed under different conditions.

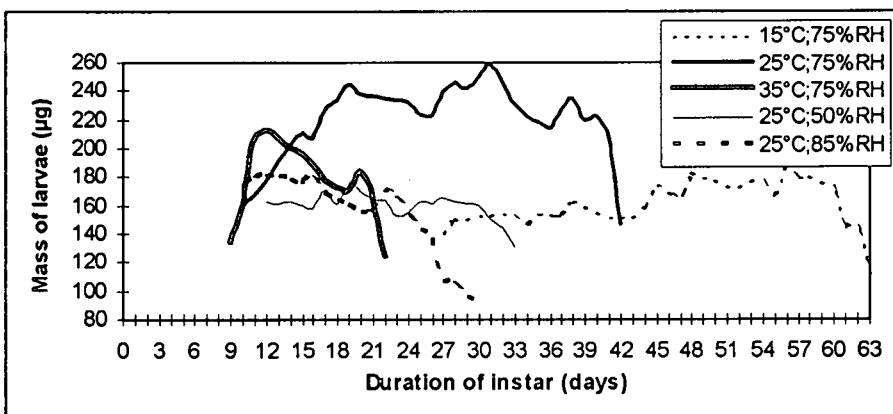


Figure 4.2c Change in mean mass of third instar larvae which developed under different conditions.

Table 4.1 shows the summary statistics of the general mean mass of third instar larvae which developed under different combinations of temperature and RH. Larvae that developed at 75% RH weighed between 31.7 μg and 187 μg at 15°C, between 29.3 μg and 258.3 μg and 51.6 μg and 212.4 μg at 25°C and 35°C, respectively. At 25°C and 50% RH the mass of larvae varied between 29.7 μg and 176 μg and between 25.4 μg and 180.8 μg at 85% RH. Third instar larvae which developed at 25°C and 75% RH had the highest general mean mass of 209.3 μg , followed by third instar larvae which developed at 35°C and 75% RH with a mean mass of 182.8 μg . A temperature of 25°C and 50% RH resulted in third instar larvae with a mean mass of 167.8 μg , while the mean mass of third instar larvae developed at 25°C and 85% RH was 154.4 μg . Third instar larvae with the lowest mean mass (161.6 μg) were those which developed at 15°C and 75% RH (Figure 4.3). Statistical analysis showed that the mean mass of the groups exposed to different conditions, differed significantly, except for the two groups exposed to 15°C; 75% RH and 25°C and 85% RH ($P > 0.05$).

Table 4.1 Summary statistics on the mass (μg) of flea larvae which developed under different conditions.

	Mean	Standard deviation	Minimum	Maximum
15°C;75% RH	161.6	45.2	31.7	187.0
25°C;75% RH	209.3	61.6	29.3	258.3
35°C;75% RH	182.8	47.4	51.6	212.4
25°C;50% RH	167.8	38.4	29.7	176.0
25°C;85% RH	154.4	46.8	25.4	180.8

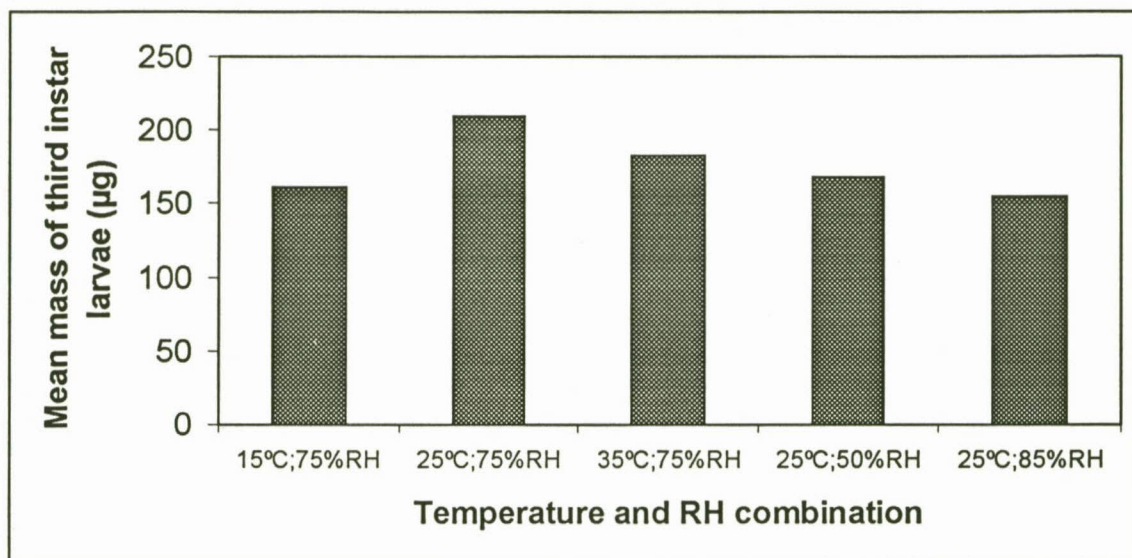


Figure 4.3 Mean mass of third instar larvae developed under different conditions.

Duration of instars and larval developed under different conditions

The duration of the larval stage at a temperature of 15°C and 75% RH was the longest, 41.2 days, which is the time since the larvae emerged from the eggs until the beginning of the pre-pupal stage. The first and second instars were completed in 9.2 days and 11.2 days, respectively, and the third instar lasted 20.8 days. The larvae that emerged and developed at 25°C and 75% RH moulted for the first time on day three and again 5.2 days later before they reached the pre-pupal stage 25.5 days later on day 33.7. At 35°C and 75% RH, the duration of the larval stage was 14.6 days of which 1.6 days were for the first instar, 3.8 days for the second instar and 9.2 days for the third instar. At a temperature of 25°C the duration of the larval stage was 26.9 days and 22.4 days at 50% RH and 85% RH, respectively. The length of the first (5.4 days) and second (5.3 days) instars at 50% RH were almost equal, while the duration of third instar was 16.2 days. At 85% RH, larvae took 3.9 days to complete the first, 4.9 days to complete the second and 13.6 days to complete the third instar (Figure 4.4).

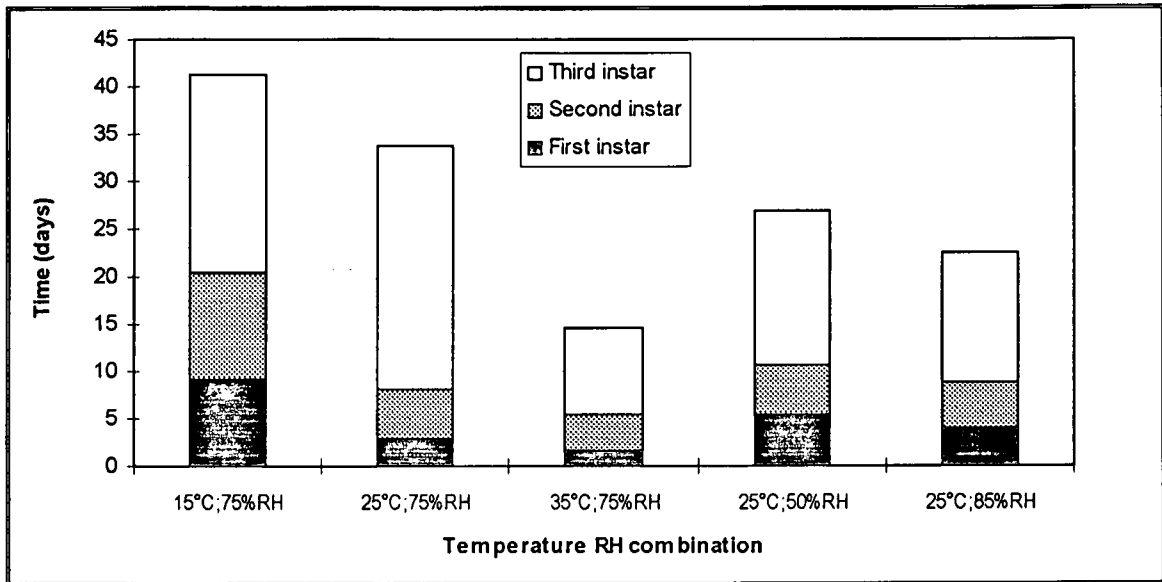


Figure 4.4 Duration (days) of development for flea larvae exposed to different conditions.

Survival of larvae exposed to different conditions

At 75% RH 100% of the larvae that developed at 25°C reached the pre-pupal stage, while only 50% of the larvae at 35°C completed the larval stage. At 15°C 70% of the larvae developed to pre-pupae and at 25°C 45% and 90% successful development occurred at 50% and 85% RH, respectively (Figure 4.5).

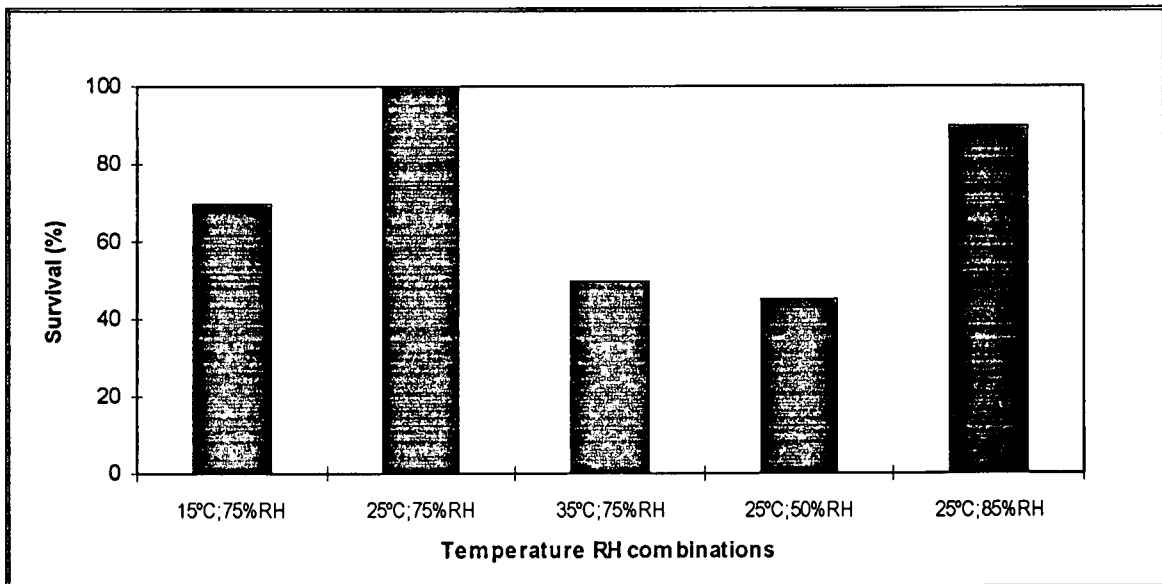


Figure 4.5 Percentage of flea larvae which developed into naked pupae under different conditions.

Development of adult fleas from larvae fed on different rearing mediums

Ninety-eight percent of the larvae fed on standard rearing medium successfully developed into adults, while adult flea feces as rearing medium resulted in the second highest survival of 73%. Sixty-nine percent of the larvae fed on a mixture of even parts of blood- and bonemeal completed the life cycle, while 50% of the larvae fed on bloodmeal alone, reached adulthood. Forty-six percent of larvae which fed on cat food pellets developed into adults. None of the larvae which was fed on bonemeal alone developed into adults (Figure 4.6).

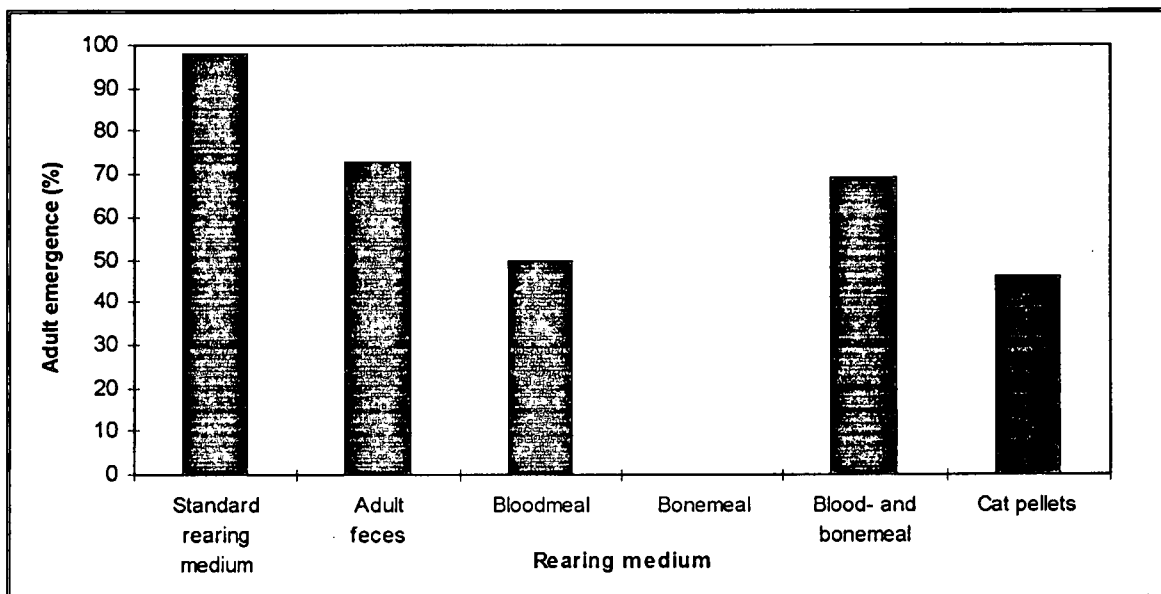


Figure 4.6 Percentage of larvae fed on different rearing mediums which developed into adult fleas.

Discussion

During the first instar the rate of mass increase of larvae decreased with a decrease in temperature. Temperatures above 25°C resulted in rapid increase of the mean larval mass. However, according to Rust and Dryden (1997), larval development is restricted by temperatures above 35°C. At a constant temperature of 25°C, the mass increase remained more or less constant regardless of changes in RH during the first and second instars, except at 85% RH where mass increase seemed slower at first. The same rapid

mass increase was continued throughout the second instar, while the rate of mass increase among larvae reared at a low temperature also accelerated slightly during this time. During the third instar, the changes in mass were very variable. The mean mass of all the groups of larvae reared under different conditions varied over time and eventually decreased, in most groups drastically, towards the end of the larval stage. Larvae reared at 35°C and 75% RH reached the highest mean mass increase, while 15°C and 75% RH resulted in the lowest mean mass increase among larvae.

The period of development for *C. felis* larvae depended on temperature and RH. The larval stage was completed more than twice as fast at 35°C (14.6 days) than at 15°C (41.2 days) at a constant RH of 75%. However, conditions under which the larval stage was completed the fastest, did not necessarily provide the highest survival rate. The opposite was also true for conditions under which the larval stage was much longer. This corresponded with a study by Rust and Dryden (1997) during which they found that at 13°C and 75% RH, about 50% of the larvae reached the pupal stage within 34 days and about 80% of larvae pupated in eight days when reared at 32°C and 75% RH. According to Kern, Richman, Koehler and Brenner (1999), larvae reared at 50% RH, had development times twice as long as larvae reared at 65% to 85% RH. Silverman *et al.*, (1981) found the same tendency namely that larval development requires 10 days at 50% RH, but only five days at 90% RH. They suggested that it is perhaps reflecting a consequence of energy transfer into water conservation mechanisms at the expense of rapid development. In this study, however, it was found that the development time for larvae reared at 25°C and 50% RH was only five days longer than larvae developed at 25°C and 85% RH.

Optimal conditions for larval development were found to be 25°C and 75% RH. According to Rust and Dryden (1997), temperatures ranging from 20°C to 30°C and RH in excess of 70% are required for optimum larval development. In the present study survival rates decreased by half as the temperature increased to 35°C and 75% RH and only 70% of the larvae survived at 15°C. At the optimum temperature (25°C) a high RH of 85% resulted in 90% survival in contrast with the 45% survival at 50% RH. Kern *et*

al. (1999) found that cat flea larval survival was > 90% at temperatures of 21°C to 32°C, but survival dropped to 34% at 38°C. They also found that RH of < 45% or > 95% resulted in no larval survival at optimum temperatures and that an RH of 65% to 85% resulted in > 90% larval survival. Development of larvae is restricted mainly by temperature outside a range of 4°C to 35°C and by RH ≤ 50%. In studies by Silverman *et al.* (1981) and Dryden (1997) no larvae survived where the RH was 100%, as it was found that fungi developed on the rearing medium of larvae exposed to 100% RH.

In this study standard rearing medium, which is currently used to rear the laboratory flea colony, was found to be the most sufficient rearing medium of those tested, since 98% of the larvae fed on the medium eventually developed into adult fleas. Adult feces and an equal part of blood- and bonemeal resulted in almost the same survival rates, but neither could provide survival above 80%. Although feces from adult *C. felis* constitute the natural food for the larvae, some authors have reported that dry blood from different hosts also serves as satisfactory larval nutrition (Linardi, De Maria & Botelho, 1997).

Only 73% of the larvae fed on adult flea feces in the present study developed into adults. However, according to Linardi *et al.* (1997) flea larvae require nutrients contained in the feces of adult fleas for successful development. Adults consume more blood than is necessary for their own use when sucking on their hosts because of this requirement. The excess blood is eliminated as undigested or partially altered host blood, which dries on the fur of the host or goes directly or indirectly into the host nests, burrows and shelters, which constitutes the habitat of flea larvae (Linardi *et al.*, 1997). According to Moser, Koehler and Patterson (1991) adult flea feces are the main natural larval diet of *C. felis*. However, larvae will also eat flea eggs and other injured flea larvae and can be reared exclusively on flea eggs after the first moult. As an alternative to adult feces, larvae can develop on dried blood as food, but are unable to develop on various substances such as feathers and cat excreta.

Although only 50% of the larvae fed on bloodmeal alone in this study survived, some authors reported unsupplemented dried blood as a satisfactory flea larval diet, whereas

other laboratories reported just the opposite (Moser *et al.*, 1991). Moser *et al.* (1991) found that adult emergence was high for fleas reared on heat dried blood. Silverman and Appel (1994), however, found that dried blood needed supplementation with either brewer's yeast or dog chow for it to be a successful larval diet. From a study by Richman, Koehler and Brenner (1999), it appeared that the dried blood may or may not be a satisfactory diet for laboratory-reared cat fleas, depending on the process used to prepare it. The drying temperature of blood determined its quality as a sufficient food source (Moser *et al.*, 1991).

In the present study no larvae fed on bonemeal survived. A study by Silverman and Appel (1994) showed that adult flea feces and dried blood supplemented with bonemeal, provided adequate nutrition for larval fleas to develop and emerge as adults. Although not a high percentage, some larvae fed on cat pellets, developed into adult fleas. This indicated that cat pellets must contain components that were nutritious to flea larvae. As mentioned above Silverman and Appel (1994) reported that dried blood needed supplementation with either brewer's yeast or dog chow for it to be a successful larval diet.

In conclusion, in rearing the larvae of cat fleas it is essential that the environmental conditions should provide a suitable microhabitat and the surroundings in which they are kept should provide the necessary components to provide a sufficient diet.

CHAPTER 5

FACTORS INFLUENCING THE DEVELOPMENT OF THE PUPAL STAGE

Introduction

One factor that commonly results in control failure of *Ctenocephalides felis* is tolerance to insecticides of pupae and pre-emerged adults. Although developing larvae and recently emerged adults often can be controlled effectively with insect growth regulators and insecticides, respectively, adult fleas continue to emerge after premises are treated (Dryden & Smith, 1994). The pupal stage begins when the late third larval instar voids its gut contents in preparation to form a cocoon and pupation. The mature larva spins a small, silken cocoon, about 5 mm long, inside which development into the adult stage will occur, followed by a pre-pupal stage during which the larva becomes inactive and adopts a u-shaped position. After the short pre-pupal period, the mature larva transforms into a pupa. The u-shaped larva begins pupal development about 18 hours after completion of the cocoon (Rust & Dryden, 1997). The process is called metamorphosis and can be affected by temperature. However, if the larva is disturbed shortly after completing the cocoon, it will leave the cocoon and either spin a second cocoon or develop as a naked pupa. About 50% of the larvae will successfully spin another cocoon, about 43% will develop as naked pupae and the rest will die (Rust & Dryden, 1997).

The cocoon is originally white, but the silk fibers secreted by the salivary glands are sticky and a layer of dust and debris quickly adheres to the outside of the cocoon and camouflage it (Rust & Dryden, 1997). Cocoons are typically found in areas that are hidden from sight and protected from environmental dangers, e.g. in soil, under vegetation, in carpets, under furniture, and on animal bedding. The base of carpet fibers is a particularly favourite area for larvae to spin cocoons indoors (Dryden, 1992; Dryden & Rust, 1994). After pupal development is completed, the adult within the cocoon is

known as the pre-emerged adult and will remain quiescent until stimulated to emerge from the cocoon. The pre-emerged adult is thus the stage that can extend the longevity of the flea (Sousa, 1999). Pre-emerged adult cat fleas will emerge from cocoons in response to certain stimuli, but have not been shown to possess specific behavioral or developmental mechanisms for overwintering (Metzger & Rust, 1997). Although the development of pre-pupae and pupae inside cocoons has been studied in several species of fleas, not much is known for *C. felis* in this regard. There is a basic, but incomplete, understanding of pre-emerged adult *C. felis* longevity (Metzger & Rust, 1997).

The aims of the study were to:

- Examine the influence of temperature and RH on pupa-to-adult development.
- Compare the size of pupae developed under different percentages of RH.
- Investigate the necessity of the cocoon with regard to pupal development.

Material & Methods

Pupal development under different conditions

C. felis eggs were collected and incubated at optimum conditions (25°C; 75% RH). The larvae that hatched from the eggs were kept in petri dishes provided with standard larval rearing medium and were allowed to develop under different conditions in order to investigate development, pupal formation and emergence of adults. The larvae were exposed to either a constant RH of 75% and varying temperatures of 15°C, 25°C and 35°C, or a constant temperature of 25°C and varying RH of 50% and 85%. The larvae were left undisturbed under these conditions to develop into pupae. The first 25 cocoons that were completed within a 24 hour period at each temperature-RH combination, were carefully transferred to petri dishes and were left undisturbed to develop at that same temperature-RH combination. The cocoons were monitored for the emergence of adults.

Size of pupae

Flea eggs were incubated under optimum developmental conditions. Fifty of the newly

emerged larvae were transferred to two petri dishes (25 per petri dish) containing standard larval rearing medium. The petri dishes were exposed to a constant temperature of 25°C at either 50% or 85% RH. The larvae were allowed to develop and eventually spin cocoons. The cocoons (< 24 hours old) were then carefully opened by removing the silk strands and the naked pupae were weighed on a Mettler Toledo UMT2 Balance. The unpaired t-test was used to test for the significance ($P < 0.0001$) of differences in mass between the two groups exposed to different conditions of RH.

Naked pupae

Newly emerged larvae, emerged under optimum conditions, were transferred individually to the cells of a Microtitre Plate (see chapter 3). Each of the cells contained rearing medium, equivalent to standard rearing medium but without any sand. When the larvae reached the third instar, ten of them were transferred to an empty petri dish containing only a little rearing medium. The larvae were allowed to develop further and were regularly provided with adequate rearing medium but no excess material (e.g. sand and debris) was available in the petri dish. The larvae were left undisturbed to pupate, but their development was monitored daily.

All experiments were done in duplicate and since the results more or less corresponded with that of the original experiments, the data were handled as one set.

Results

Pupal development under different conditions

Table 5.1 shows the duration of the pupal stage and the number of adult fleas emerged from the cocoons at different conditions. The duration of the pupal stage was considered as the period from when the pupae were collected (< 24 hours after completion of the cocoon) until the adults emerged. With the RH constant at 75%, adult emergence was 68% at 15°C, 86% at 25°C and 4% at 35°C. A temperature of 25°C resulted in 78% emergence at 20% RH, 70% at 50% RH and 82% at 85% RH. Where the RH was kept

constant at 75%, 38% of the pre-emerged adults at 15°C and 6% at 25°C remained quiescent for more than 130 days after cocoon formation before emerging from the cocoons. The same tendency occurred at 25°C and 85% RH where 8% of the pre-emerged adults only emerged after 130 days from cocoon formation (Figure 5.1).

Table 5.1 Duration of the pupal stage and emergence of adult fleas.

Time (days)	RH 75%			Temperature 25°C		
	15°C	25°C	35°C	20% RH	50% RH	85% RH
1	0	0	0	0	0	0
2	0	0	2	0	0	0
3	0	0	-	3	0	0
4	1	0	-	8	2	4
5	3	0	-	6	8	5
6	5	15	-	4	4	2
7	1	8	-	10	2	8
8	1	17	-	4	5	11
9	2	-	-	4	2	7
10	2	-	-	-	7	-
11	-	-	-	-	5	-
> 130	19	3	-	-	-	4
Adults emerged < 12 days	34	43	2	39	35	41

Figure 5.2 shows the duration of the pupal stage under different combinations of temperature and RH. At a constant RH of 75% adults emerged from the cocoons at 25°C between days six and eight after the cocoons were completed, between days four and 10 at 15°C and the duration of the pupal stage was the shortest at 35°C where emergence of adults occurred only on day two. Where the temperature was constant at 25°C, all the adults started to emerge at more or less the same time in spite of varying RH. Adults emerged between days three and nine from the cocoons exposed to 20% RH and all the

viable cocoons exposed to 50% RH and 85% RH started to emerge from day four up to days eleven and nine, respectively.

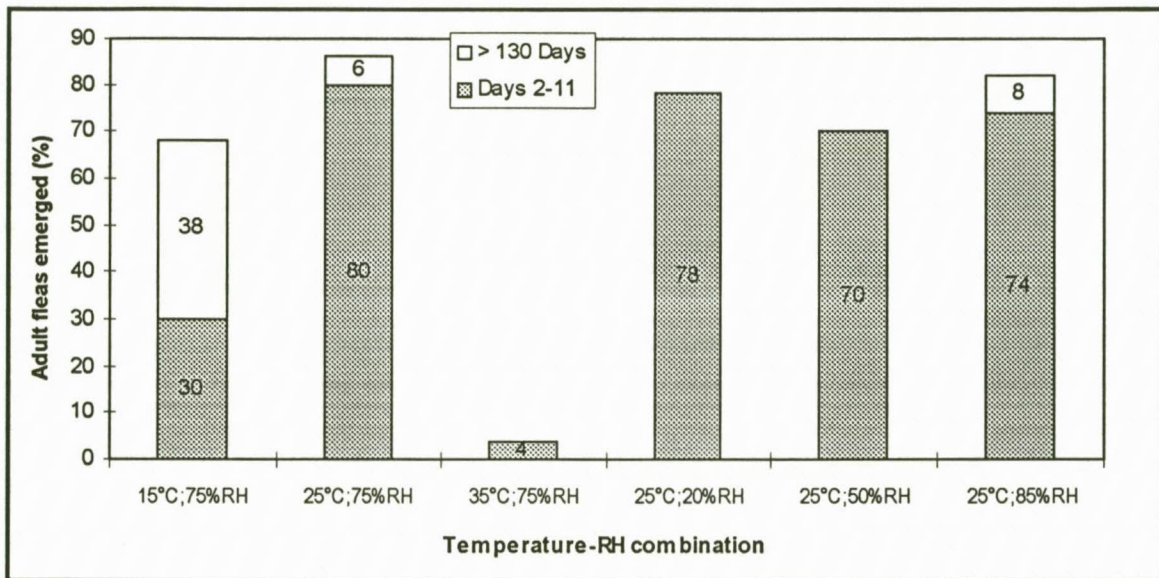


Figure 5.1 Percentage adult flea emergence from pupae kept at different environmental conditions.

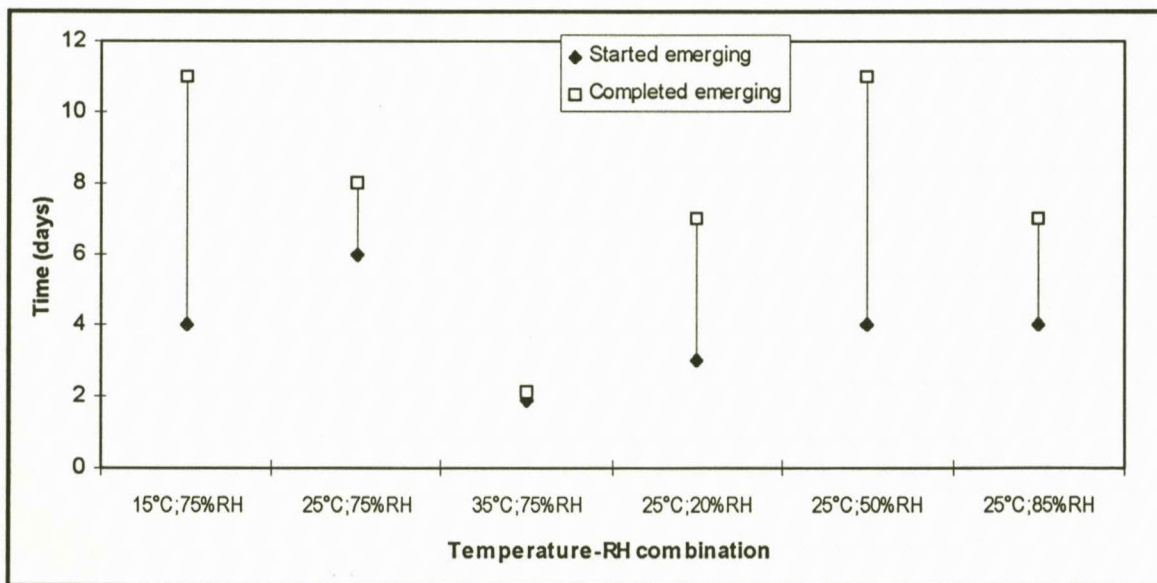


Figure 5.2 Duration of the pupal stage when kept at different environmental conditions.

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Size of pupae

Table 5.2 indicates that pupae weighed between 1 786.5 μg and 7 050.5 μg at 50% RH and between 4 934.7 μg and 10 791.5 μg at 85% RH where the temperature was kept constant at 25°C. At the lower RH the mean mass was 4 140.3 μg and it was 7 183.9 μg at 85% RH. Statistical analysis showed that there was a significant ($P < 0.0001$) difference in mass between the two groups exposed to the different percentages of RH.

Table 5.2 Pupal mass following development under different conditions of RH at a constant temperature of 25°C.

	50% RH	85% RH
Mean mass	4 140.3	7 183.9
Standard deviation	1 184.7	1 518.4
Minimum	1 786.5	4 934.7
Maximum	7 050.5	10 791.5

Naked pupae

The viability of naked pupae was determined by the percentage adult emergence. As figure 5.3 shows, 35% of the third instar larvae which were allowed to complete the life cycle died before they developed into pupae, while 5% successfully developed into naked pupae but died before any further development could occur. The remaining 60% of the larvae all developed into naked pupae, completed the life cycle and eventually emerged as adult fleas. Of the latter larvae, 68% developed into completely naked pupae, while 32% spun only a few silk strands from the side to the bottom of the petri dish, they effectively also developed into naked pupae.

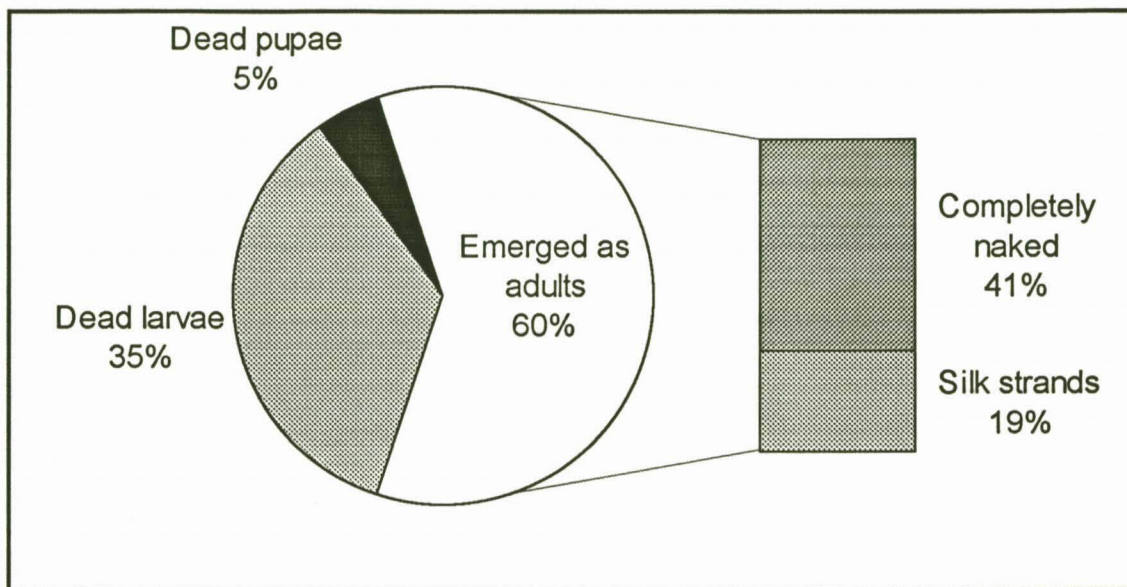


Figure 5.3 Development and survival of naked flea pupae.

Discussion

Since a temperature of 25°C in combination with 75% to 85% RH resulted in the highest adult emergence, this combination of temperature and RH appeared to represent the optimal conditions for pupal development as was also noted for egg and larval development in the previous chapters. At 75% RH and 15°C a moderate percentage of adult emergence occurred although not as high as at 25°C and it seemed that temperatures near 35°C are detrimental to the pupal stage. In their study, Rust and Dryden (1997) found that exposure to temperatures below 3°C for more than 10 days is lethal to the pupal stage, however, they also found the same tendency at temperatures near 35°C. Silverman *et al.*, (1981) and Kern *et al.*, (1999) reported that when temperatures surpass 35°C, 30% of the larvae formed cocoons, but died as pupae and did not survive to adulthood.

Temperature clearly influenced the developmental time and emergence of adult fleas

from cocoons in this study. Under the laboratory conditions in this study, adults emerged from cocoons within three days at higher temperatures, but the duration of the pupal stage increased with a decrease in temperature. Dryden and Rust (1994) also reported that the developmental time of pre-pupae to adults was prolonged when reared at cooler temperatures. As discussed in the next chapter, the gender of the immature fleas also affects the length of time spent in the pre-pupal and pupal stages. According to Dryden & Rust (1994), the pupal stage normally lasts for an average of six to nine days, depending on the environmental conditions (Dryden, 1992; Dryden & Rust, 1994). However, it was also reported that adult fleas emerged from the cocoons in about five to fourteen days at optimum conditions (Sousa, 1999; McGlennon, 2000). These results differ significantly from the present study where, although a small percentage, adults started to emerge within a minimum of 24 hours after completion of the cocoon. The time from when the life cycle is completed until the adult fleas emerge can vary greatly, since pre-emerged adults can remain quiescent for an indefinite period awaiting proper stimuli (Dryden & Rust, 1994).

Although the pupa is the most resistant of the immature stages to desiccation, low RH is harmful to the pre-emerged adult, while an increase in RH decreased the duration of the pupal stage (McGlennon, 2000). Apparently, RH did not have a significant influence on the development of pupae in the present study, since exposure to different RH at a constant temperature resulted in survival rates between 70% and 82%. According to Silverman, *et al.* (1981), the rate of development within the cocoon is the same regardless of RH, tested at 16°C and 27°C. Dryden and Rust (1997) reported the same tendency as their study resulted in about an 80% emergence of adults after exposure to 27°C and 2% RH. At 16°C in saturated air, 92% of the pre-emerged adults survived for 70 days compared with 62% of emerged adults (Rust & Dryden, 1997). Higher temperatures not only decreased the duration of the pupal stage, but higher RH also resulted in larger pupae. Silverman *et al.* (1981) reported the same tendency and found that the larger pupae may have been due to expanded intersegmental membranes caused by the uptake and retention of water by larvae and pupae.

The phenomenon that pre-emerged adult fleas stayed quiescent in the cocoons for over 130 days after cocoon formation, was also reported by Rust and Dryden (1997) who found that about 60% of adult fleas successfully emerged from cocoons held at 13°C by day 140 after eggs were collected. Prolonged quiescence of pre-emerged adults inside cocoons may result from a combination of factors such as the physical barrier of the cocoon to emerge and reduced activity at low temperatures. Inside cocoons, quiescent, pre-emerged adults can survive for extended periods of time before emerging. As temperatures decrease, the time to adult emergence from the cocoon often becomes longer and less predictable (Metzger & Rust, 1997). In addition to favourable environmental conditions, there are other particular activities that will cause a flea to emerge from its cocoon, like host-produced stimuli in the environment. Thus, emergence will be delayed depending not only on temperature but also emergence stimuli, which explains why some of the fleas in the present study remained quiescent in spite of optimum conditions for development. According to Dryden (1993) temperature and physical pressure may be the primary stimuli for emergence, while changes in light (movements), carbon dioxide and noise are also thought to be stimuli for emergence of the adult flea (Kuepper, 1999; Sousa, 1999). Although vibration is commonly reported to cause emergence of fleas, this has not been confirmed through research (Dryden, 1993). These specific stimuli reduce the chance that adults will emerge in the absence of a host, especially in view of the wide range of mobile hosts they may infest (Rust & Dryden, 1997). The ability to survive for extended periods in the cocoon is especially important for species such as *C. felis*, which infest mobile hosts that may not frequently return to a nest or burrow (Dryden & Rust, 1994).

This suggests that the pre-emerged stage is ideal for prolonged survival during the absence of hosts or during unfavourable environmental conditions (Silverman *et al.*, 1981; Metzger & Rust, 1997; Rust & Dryden, 1997). The ability of the fleas to prolong their life span, during the pupal stage as pre-emerged adults, is a major reason why the increase in temperature and humidity towards the end of spring and beginning of summer can cause an explosion of flea activity. It is also the reason why fleas are notorious for attacking people when a long unoccupied house or room is entered (Kuepper, 1999).

According to observations by Dryden and Smith (1994) and Rust and Dryden (1997) on larvae placed in flat-bottom Microtitre Plate wells, larvae successfully completed cocoon formation only against the sides of the wells. Such behaviour may be caused by a need for three-dimensional orientation or a tactile stimulus. Thus larvae need contact with a vertical surface to successfully spin a cocoon. Although the flat-bottom petri dishes used in the experiments of this study provided a three-dimensional orientation, none of the larvae succeeded in spinning complete cocoons.

Although development of naked pupae resulted in adult emergence of 60%, this is lower than the percentage adults that emerged from cocoons under optimal conditions in a previous experiment. However, Rust and Dryden (1997) reported that over 95% of the larvae successfully completed development as naked pupae, demonstrating that the cocoon is not essential for successful pupation and development to the adult stage. The cocoon probably serves as protection against elements and predators such as ants. According to Dryden and Rust (1994), it does not act as a barrier to water vapour, as the humidity inside and outside the cocoon are the same. The cocoon serves as a barrier to emergence, helping to ensure that a host is present when the adult flea emerges, because adult fleas reared from naked pupae emerged sooner from the pupal cuticle than do adults reared in cocoons (Rust & Dryden, 1997).

Failure of many flea control programs often may be related to inadequate control of developing pupae and pre-emerged adults. Cocoons are typically found in areas that are hidden from sight and protected from environmental dangers. The base of carpet fibers is a particularly favourite area for larvae to spin cocoons. It has been documented that cat flea cocoons placed in carpet are highly tolerant to a variety of insecticides (Dryden & Smith, 1994; Metzger & Rust, 1997). Because of this, fleas may continue to emerge from cocoons for as long as four weeks after insecticide application to the environment (Dryden, 1993). Dryden and Rust (1994) reported that cocoons are not a barrier to insecticides. The survival of developing pupae in insecticide-treated households is not due to any protection afforded by cocoons, but due to the lack of penetration of the insecticide into the carpet canopy (Dryden & Rust, 1994).

CHAPTER 6

THE INFLUENCE OF ENVIRONMENTAL CONDITIONS ON ADULT *CTENOCEPHALIDES FELIS*

Introduction

Metzger and Rust (1997) noted that the gender of the immature fleas affected the length of time spent in the pre-pupal and pupal stages. The observation that female fleas developed faster than males has been recorded often, but its biological significance has not been investigated (Dryden & Smith, 1994). Immediately upon emerging from its cocoon, the new adult flea, about 2.5 mm in length, begins to look for a suitable host (Dryden, 1993). A host's body heat and odor, subtle changes in light and shadow and traces of carbon dioxide exhalation can all be cues to the flea's finely tuned sensors. Bristles near the flea's genitalia may serve to detect shifts in air movements, a further clue to the proximity of a potential host (Duplaix, 1988). According to Metzger and Rust (1997) distribution frequencies for males and females on hosts were quite similar. Adult *Ctenocephalides felis* begin feeding almost immediately once they acquire a host. Adult fleas remain in the fur or hair of the host most of the time (Chen, 1934). The cat flea is metabolically dependent on a constant food source. Once feeding is initiated, the flea must have access to a blood source almost continuously (Dryden, 1992). Both male and female fleas bite and suck blood to sustain themselves and provide food for their offspring. The life span of the adult female can be as long as several months during an active "flea season" or longer during a less active time, while the male's life span is thought to be about half that of the female (Kuepper, 1999).

If a newly emerged cat flea does not immediately acquire a host it can survive for from 10 to 60 days before requiring a bloodmeal. The environmental requirements for

development and survival of newly emerged *C. felis* off the host, depend greatly on temperature and RH (Dryden, 1993; Kuepper, 1999). Although males consume less blood than females, they feed more frequently (Sousa, 1999). Once feeding is initiated, size discrepancies between male and female become apparent (Dryden & Rust, 1994).

The aims of this part of the study were to determine:

- The duration of the pupal stage and to what extent this would be influenced by the gender of the emergent adult.
- The ratio of males and females emerged from cocoons.
- The influence that high and low RH would have on the size of emergent adults.
- The duration of the survival period of unfed adults kept under different conditions.
- The success of development after feeding of fleas on dogs instead of cats.

Material & Methods

Duration of the pupal stage

Twenty-five pupae, approximately one day old and reared under optimum conditions (25°C; 75% RH) for flea development, were randomly selected from the laboratory's rearing system. The pupae were placed in a separate container and their development monitored every 12 hours. In this study, the pupal stage was considered to be the time before adult fleas emerged from cocoons which were spinned in the previous 24 hours. The duration of the pupal stage as well as the gender of every newly emerged adult was determined.

Influence of RH on size

Cat flea eggs were incubated under optimum conditions until they hatched. The newly emerged larvae were then placed in petri dishes containing standard larval rearing medium. Twenty larvae were exposed to 50% RH and another twenty larvae to 85% RH at 25°C. The larvae were allowed to complete the life cycle and the emergent adult fleas were weighed while they were anaesthetised with carbon dioxide.

Survival of unfed adults

Fleas were obtained from the laboratory cat flea colony as described in chapter 3. Immediately after they emerged from the cocoons, the fleas were exposed to different environmental conditions which included a constant RH (75%) with varying temperatures (15°C, 25°C and 35°C) and a constant temperature (25°C) with varying RH (50% and 85%). Within each combination of temperature and RH the fleas were allocated to two groups of 25 each and kept in glass vials either with or without cat hair. The live fleas were counted daily.

Development of eggs of *C. felis* fed on dogs

Two dogs were infested with cat fleas from the laboratory colony. Before infestation they were combed thoroughly to make sure that they did not contain any fleas. They were then infested with 100 newly hatched cat fleas each and the fleas were allowed to feed. On the third day after infestation, eggs were collected from the pens. Eggs were also collected from the infested cats according to the procedure described before. The eggs collected from either the cats (n = 50) or the dogs (n = 50) were kept separate, allowed to hatch and reared at 25°C and 75% RH. Emergence and survival of adults from the two groups, fed on dogs, were determined and compared.

Experiments were done in duplicate and in all cases the similarity of data sets allowed that they could be pooled.

Results

Duration of the pupal stage of males and females under optimum conditions

The pupal stage for males was consistently longer than for females. From the 50 adult fleas that emerged from the selected cocoons, 64% were females and 36% males. All the females emerged between days one to three, while the males emerged between days 2.5 and 5 from cocoons spun within the previous 24 hours. Twelve females emerged on day 1.5, while most males, six, emerged from the cocoons on day 3. Females took an average

of 1.9 days and males an average of 3.7 days to emerge from the cocoons (Figure 6.1).

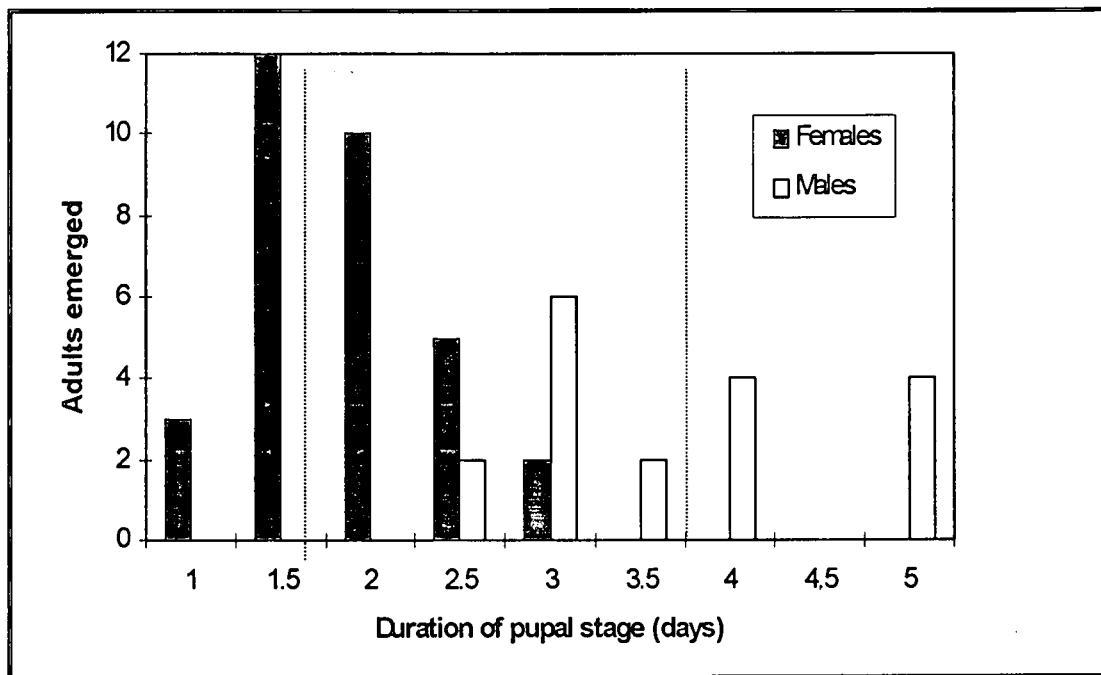


Figure 6.1 Duration of pupal stage for male and female fleas with the mean duration for each gender indicated with dotted-lines.

Influence of RH on size

Since the same ratio of males and females was weighed at both RH's, the mean mass of the two genders were pooled and considered as the mean mass of the two groups of fleas developed under the two different RH's respectively. The mean mass of fleas exposed to 25°C and 50% RH was 4 479.6 µg, compared to the 8 750.5 µg of fleas exposed to 25°C and 85% RH. The lower RH resulted in adult fleas weighing between 2 497.1 µg and 6 873.3 µg and fleas at the higher RH weighing between 6 731.9 µg and 11 983.7 µg. Statistical analysis showed that there was a significant ($P < 0.0001$) difference in mean mass between adult fleas reared at different percentages of RH (Figure 6.2).

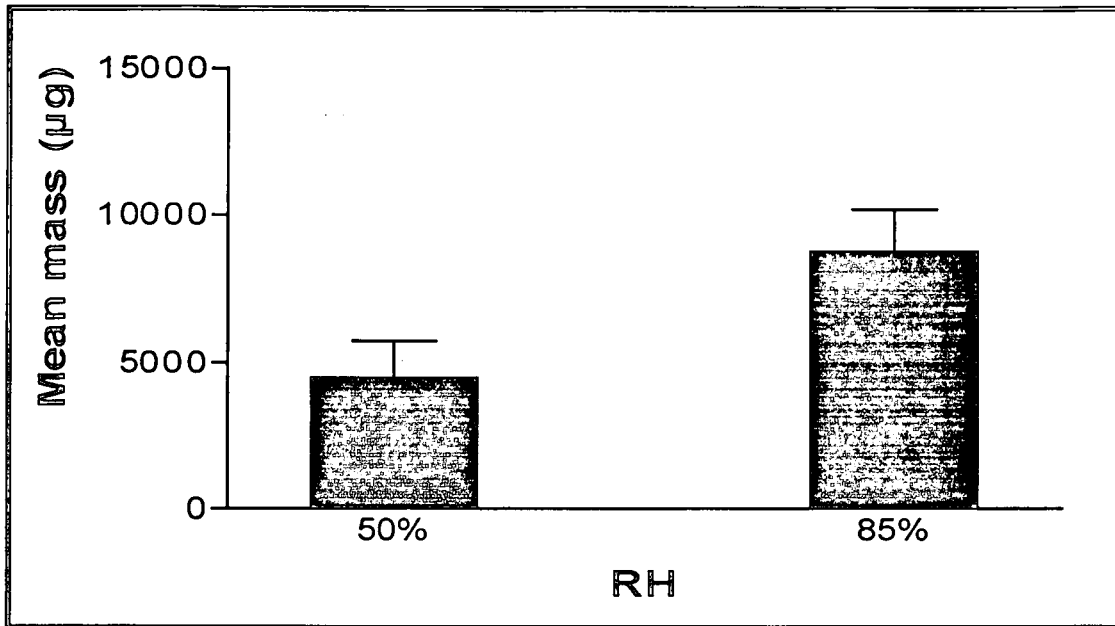


Figure 6.2 The effect of RH on the size of adult fleas.

Survival of unfed adults

Figure 6.3 shows the percentage survival of adult fleas exposed to different conditions. Fleas survived the longest (19 days) at 15°C and 75% RH where their environment was provided with cat hair. At the same conditions, but where no hair was present, all the fleas were dead six days earlier at day 13. A high RH of 85% and 25°C resulted in a survival period of almost two weeks where cat hair was present, but the fleas were all dead five days earlier where no hair was provided. At 35°C and 75% RH the survival period was the shortest, namely six days where hair was present and five days where hair was absent. In general, fleas kept in vials provided with cat hair, lived longer than fleas in empty vials, except at 25°C and 50% RH where the survival periods were equal. Thus, at an RH of 75% fleas survived 31.6% longer at 15°C, 10% longer at 25°C and 16.7% longer at 35°C where cat hair was present. At a constant temperature of 25°C fleas survived 46.2% longer at 85% RH where hair was present in the environment, while no difference in survival occurred between environments with and without cat hair at 50% RH.

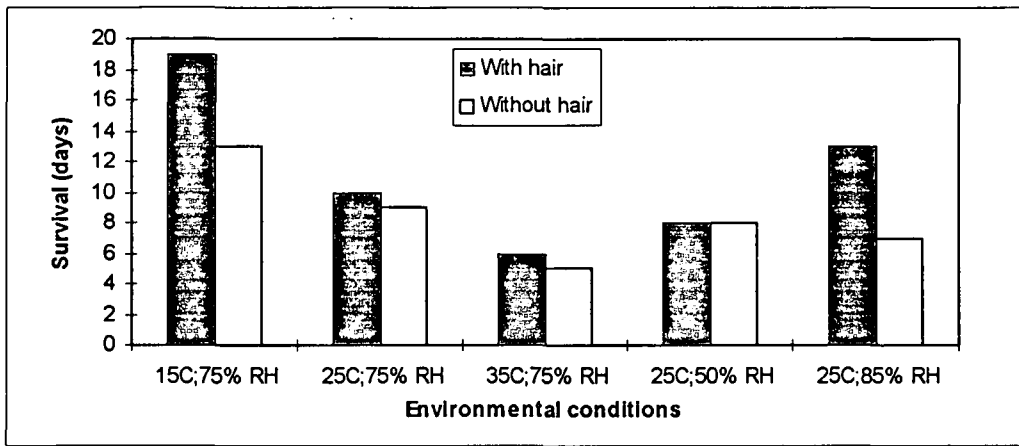


Figure 6.3 Mean survival period (days) of adult fleas exposed to different environmental conditions.

Development from eggs of *C. felis* fed on dogs

The course of the life cycle of fleas fed on cats and dogs were closely similar. Ninety-five percent of the eggs laid by the fleas fed on cats hatched, while 93% of the eggs laid by fleas fed on dogs, hatched. Respectively 92% and 89% of the larvae from the two groups spun cocoons and pupated. From the eggs laid by fleas fed on cats, 88% developed into adults, while 83% of the eggs laid by fleas fed on dogs, reached the adult stage (Figure 6.4).

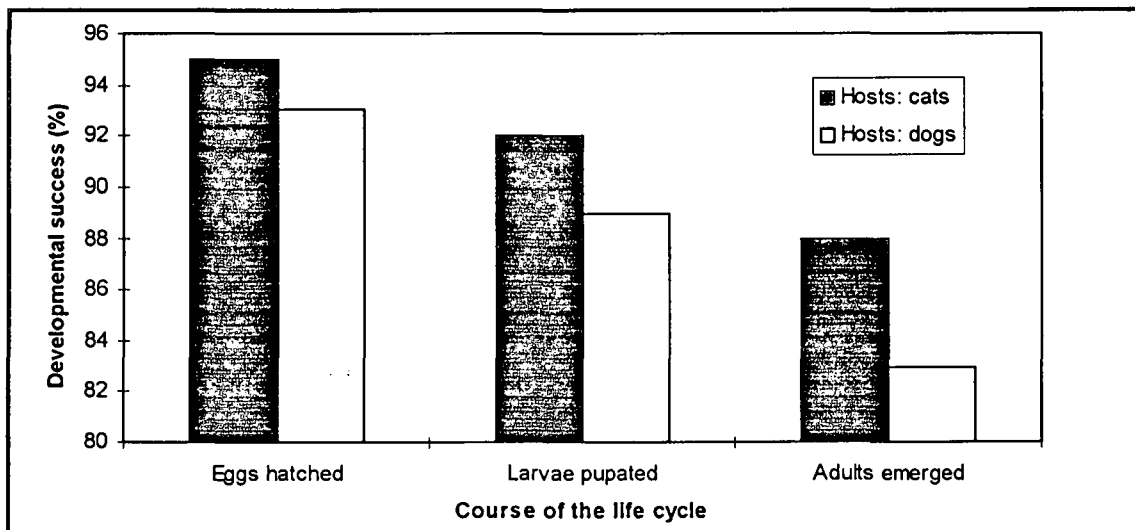


Figure 6.4 Developmental success (%) of *C. felis* fed on cats and dogs.

Discussion

Dryden (1993) has reported that the ratio of female cat fleas on the host varies from 1:1.8 to 1:2.2 and suggested that the greater frequency of females on the host may result from increased longevity or the ability of females to evade host grooming activity more effectively. However, the ratio of unfed male to female cat fleas emerged in the present study was 1:1.8. Thus, the emergence of unfed males and females in the present study and occurrence of males and females on a host in the study conducted by Dryden (1993), occurred in a similar ratio. However, a larger sample size than used in the present study would be necessary to confirm these results. According to Metzger and Rust (1997), the time for pupal maturation and emergence of female cat fleas from cocoons was one to two days shorter than those of males at 26.7°C and 80% RH. The females in this study similarly began emerging from the cocoons 1.8 days earlier than did males at 25°C and 75% RH. This difference in developmental time supports the differential emergence pattern of females and males referred to as protogyny (females emerge first). Although there exist different opinions about the biological significance of this phenomenon, it may occur for some of the following suggest reasons. It may prevent inbreeding of fleas from the same cohort by increasing the possibility that females will mate with males from other cohorts (Metzger & Rust, 1997). Alternatively, it may be associated with sperm displacement of competing males mating with females, where the last male to mate with a female would have the greatest chance of passing its genes on to the next generation (Metzger & Rust, 1997).

It was shown in the present study that higher RH (85%) resulted in larger adults than those that developed at a lower RH of 50%. Silverman *et al.* (1981) found that immature *C. felis* reared at 92% RH, produced larger adults than those reared at 50% RH. According to Silverman *et al.* (1981) this can be due to the uptake and retention of moisture by larvae, pupae or adults that expanded their intersegmental membranes. However, in their study the adult fleas were measured and the rigid structures, such as the head and tibia were also the largest in adults whose immature stages had been exposed to the higher RH. Silverman *et al.* (1981) stated that as in larval development, this might

indicate priority energy utilization in which water was conserved at the expense of growth.

Although newly emerged cat fleas can survive several days before requiring a blood meal, survival in the off-host environment depends greatly on temperature and RH (Dryden, 1993). In the present study longevity increased significantly at optimum RH and a lower temperature compared to combinations of higher temperatures and optimum RH. Where RH varied at optimum temperature the opposite seemed to be true since higher RH increased longevity. These results correspond with what Rust & Dryden (1997) reported namely that the longevity of fleas was more than twice as long at optimum temperature in saturated air than at 50% RH.

Fleas kept in vials containing no hair jumped perceptibly more compared to fleas kept in vials with hair, where they mainly crawled around through the hair. The unfed adults, that were kept with cat hair, lived perceptibly longer than the fleas kept in glass vials without any hair. This can be explained by Dryden's (1993) finding that jumping requires more energy than crawling around through a host's pelage. Expenditure of energy would reduce longevity and thus, decrease the flea's chances of finding a host. Therefore, if not stimulated, newly emerged fleas do not move away from their site of emergence.

Although the survival rate of *C. canis* seemed lower than that of *C. felis*, there was no significant difference between the success with which the two species fed on dogs as hosts and developed afterwards. *C. felis* is less host specific than *C. canis* which is mainly restricted to dogs as hosts (see chapter 7). That explains why *C. felis* fed successfully on dogs and this corresponds with the results of Dryden (1993) who reported that *C. felis* is the most common flea species associated with dogs and cats. *C. felis* and *C. canis* occasionally occurs in mixed infestations on dogs. In such cases, *C. felis* usually predominates (Dryden, 1993).

CHAPTER 7

COMPARISON OF THE BIOLOGY AND ECOLOGY OF *CTENOCEPHALIDES* *CANIS* AND *C. FELIS*

Although *Ctenocephalides felis* is considered to be the most common flea infesting both dogs and cats worldwide, mixed infestations of *C. felis* and *C. canis* also occur. The physical differences between species of *Ctenocephalides* tend to be small and there may be considerable variation between individuals within a species as well as between sexes (Dryden, 1992). Identification, therefore, is often difficult. However, clear differences between the cat and dogs flea do exist as discussed in chapter 2. The most conspicuous of these characters involves the head and tibia III. The head of the dog flea is strongly rounded anteriorly, in contrast with the distinctly elongated head of the cat flea. Further, tibia III of the dog flea contains eight seta-bearing notches, while the cat flea has only six seta-bearing notches (Figure 7.1).

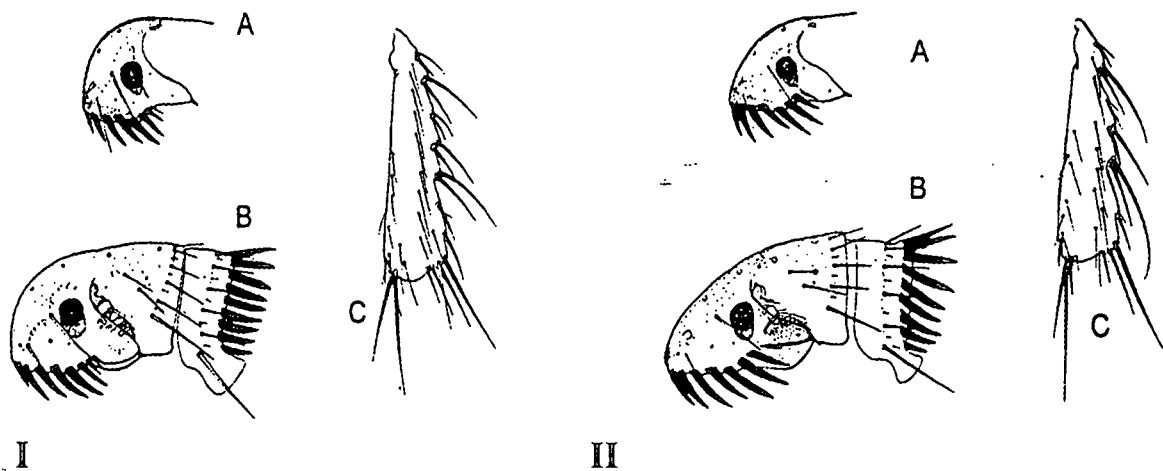


Figure 7.1 *C. canis* (I) and *C. felis* (II); A) front of the male head, B) female head and pronotum and C) tibia III.

The life cycle of *C. canis* is very similar to that of *C. felis*. However, differences in behaviour between the two species occur and seem to be largely dependent of the range of environmental conditions that their larvae are capable of tolerating (Wall & Shearer, 1997). Whilst the optimum temperature and RH for the successful *in vitro* rearing of *C. felis* have been reported, there are no published data available for *C. canis*, apart from incomplete studies (Baker & Elharam, 1992). *C. canis* appears to be practically cosmopolitan but is more abundant in temperate countries than in the tropics (Bedford, 1932). *C. canis* infests dogs and cats and other animals like rats, but may also attack man (Brown, 1975). Together with *C. felis* (cat flea) and *Pulex irritans*, *C. canis* can also act as intermediate host of *Dipylidium caninum*, the double-pored tapeworm (Wall & Shearer, 1997).

During a survey in Queensland cat fleas were found on 38% of the cats examined, being most prevalent on younger cats. Dog fleas were found on only two cats during the survey. According to a survey by Baker and Mulcahy (1986) in the Dublin area *C. felis* was the most common flea found on cats and dogs. Dryden (1993) also stated that the most common flea infesting cats and dogs worldwide is *C. felis*. A survey conducted in northern central Florida indicated that *C. felis* was the flea species found on 92.4% of infested dogs and 99.8% of infested cats (Dryden, 1993). During a survey in Indiana *C. felis* was found on 93% and 97% of flea-infested dogs and cats, respectively. *C. canis* was found on only 18% of the flea-infested dogs (Dryden, 1993). According to Segerman (2000) a survey in southern Africa indicated that *C. canis* is restricted to the central and southern parts of southern Africa, mainly the Western Cape, Eastern Cape, Free State, Northwest Province and Gauteng, while *C. felis* appears throughout the whole of southern Africa including the neighbouring states (Figure 7.2).

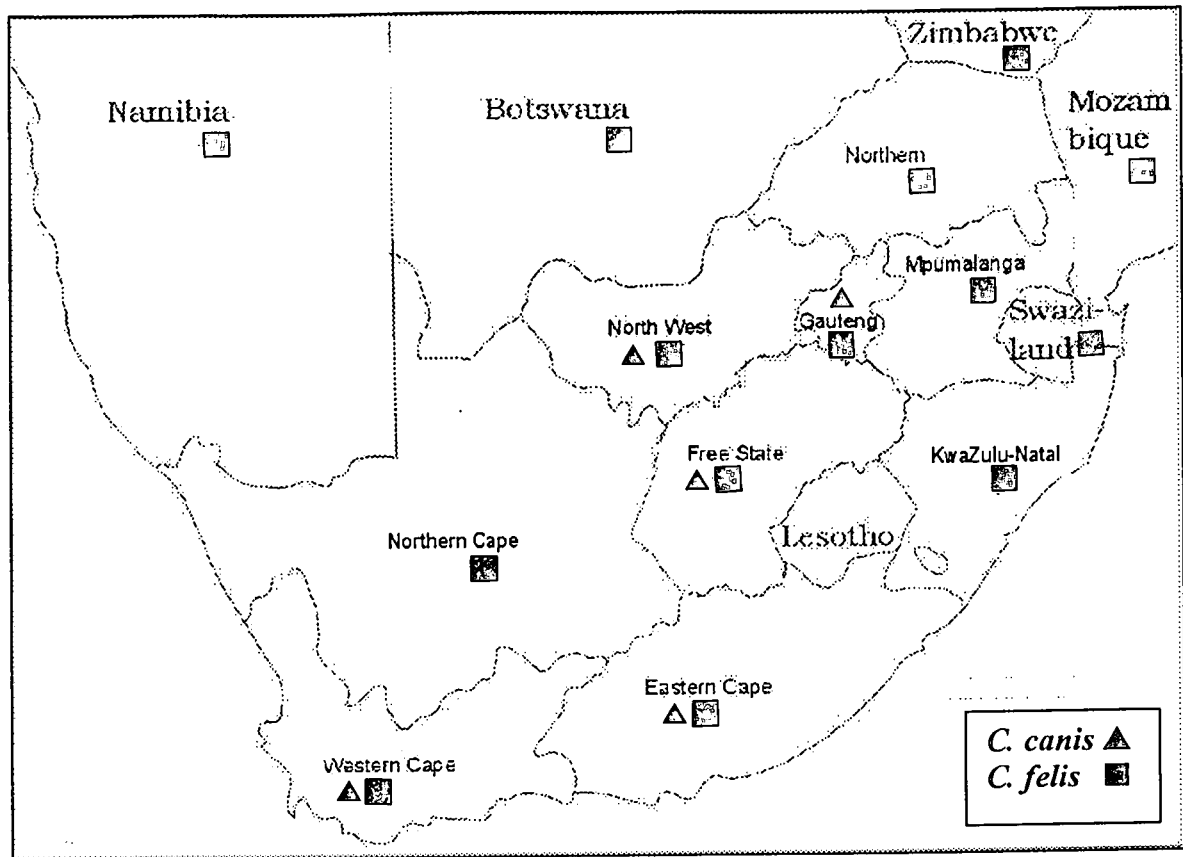


Figure 7.2 The distribution of *C. canis* and *C. felis* in southern Africa (after Segerman, 2000).

Certain questions arose as to the similarity of the biology of the two species and the aims of this part of the study were to investigate and compare certain aspects of this:

- Development and survival of *C. canis* under different conditions.
- Development and survival of the two species under optimal conditions.
- The success of the two species to complete the life cycle on cats as hosts.
- The possibility of interbreeding.
- The susceptibility to infection with *D. caninum*.
- The geographical distribution of the two species in southern Africa.

Material & Methods

Study animals

Dogs, used as study animals were kept at the Experimental Animal Unit at the University of the Free State. The dogs, two adult Beagles, were held in pens (10 m x 2 m) provided with large plastic containers with pet blankets to sleep in. Fresh food and water were provided daily when the pens were cleaned. The animals were kept in accordance with guidelines set forth by the National Code for the handling and use of animals for research, education, diagnosis and the test of drugs and substances in South Africa (Erasmus, 1990).

Flea colony

A laboratory colony of *C. canis* was started by collecting fleas from dogs in Grahamstown in the Eastern Cape province where dogs were known to be infested with *C. canis*. The fleas were combed from the dogs and kept in containers provided with dog hair. Back in the laboratory the fleas were anaesthetized with carbon dioxide, provisionally identified under a light microscope and the selected *C. canis* were released on dogs to continue feeding as soon as possible. After a few days, eggs were collected from the pens of the dogs and the eggs were hatched and the larvae reared in the laboratory in the same way as was described for *C. felis* (see chapter 3). The *C. canis* colony was allowed a few weeks to establish. The fleas were then once again examined under a light microscope to make sure that the colony consisted of pure *C. canis*. After the dogs were thoroughly combed and bathed to make sure that they were free from any fleas, they were re-infested with the identified *C. canis*. The colony was again allowed some time to establish before the fleas were used for experiments.

Exposure to different conditions

C. canis was exposed to different combinations of temperature and RH and was allowed to complete the life cycle as was described for *C. felis* in previous experiments (see chapter 6). Eggs were counted out in petri dishes, 25 per petri dish, with sufficient standard rearing medium. Some of the petri dishes were exposed to a constant RH of

75% with varying temperatures of 15°C, 25°C and 35°C and some to a constant temperature (25°C) and RH of 50% and 85%. All the petri dishes were monitored daily and the number of live individuals were counted.

Comparison between *C. canis* and *C. felis* under optimal conditions

Ten eggs from each of the *C. canis* and *C. felis* colonies were counted out petri dishes that were provided with sufficient standard rearing medium. The fleas were then left under conditions for optimal development (see chapter 6). The petri dishes were examined daily to compare the success of completion of the life cycle.

Host specificity

Two cats, cleaned in the same way as described above for the dogs, were infested with 80 newly emerged specimens of *C. canis* and the fleas were allowed to feed. On the second day after infestation, the room in which the cats was kept, was searched for eggs. After a week the cats were re-infested with *C. canis* and the same procedure was followed. The experiment was done a third time. As a control, the same procedure was followed infesting dogs with *C. canis*.

Interbreeding

Fifty adult fleas from each sex were counted out from the *C. canis* colony and transferred to different containers. The same was done with the *C. felis* colony. The males of each species were then brought together with the females of the opposite species. Two dogs were combed and bathed to get rid of any fleas on them and each was then infested with the 100 fleas of mixed species as described above. The dogs were kept in separate pens far away from each other. Dogs were used as hosts because it was found previously that *C. felis* can complete its life cycle successfully on dogs (see chapter 6). From the second day after infestation, the dog pens were searched daily for flea eggs. After a week the dogs were re-infested in the same way and the same procedure was followed.

Infection with *Dipylidium caninum*

C. canis was obtained from the laboratory colony, while *D. caninum* eggs were obtained when collecting flea eggs from infected laboratory cats. The tapeworm uterine capsules were counted out one by one with a very fine paintbrush from the mixture of flea eggs and debris and stored in a petri dish under conditions for optimal cat flea development until they were required. The flea eggs were placed in a Microtitre Plate as described in chapter 3, one in each cell, at optimal conditions. As soon as they hatched, a small amount of dried blood was added. The larvae were then allowed to develop and were monitored daily. In this way larvae required for the various experiments were available as they reached the desired instars. As previous workers have shown, fleas become infected during the larval period by eating tapeworm ova (Zimmerman, 1937). Larvae were reared to the desired larval instar, removed and put in a clean petri dish free from food. After being deprived from food for 12 hours, 30 larvae, ten from each instar, were transferred to three different petri dishes, with 50 *D. caninum* uterine capsules and a little dried blood in each petri dish. After 24 hours, five larvae from each petri dish were randomly selected, removed from the petri dishes and immediately prepared for microscopy. Thus, after repeating the experiment, altogether 10 larvae from each instar were examined.

For microscopical examination, a larva was transferred to a drop of Bouin-Glycerine on a cavity slide and covered with a coverslip. The slide was then left overnight to allow the Bouin-Glycerine to clear the larva. The larva was then ready for microscopic examination. A Nikon E800 microscope fitted with a camera was used. Larvae were studied segment by segment using the 60x and 100x oil immersion lenses and Differentiating Interference Contrast (DIC). One-way analysis of variance (ANOVA) followed by the Tukey test was used to test for the significance ($P < 0.05$) of differences between groups exposed to different numbers of uterine capsules.

Geographical distribution

A survey was done in southern Africa on cats and dogs (mainly on dogs). Fleas were collected in various towns and cities from cats and dogs by combing them. The collected fleas were preserved in 70% ethanol. Back in the laboratory the fleas were identified.

Although all the experiments were done in duplicate, the data were similar and therefore pooled in one set.

Results

How the exposure to different conditions influences the development of *C. canis*

After being exposed to different environmental conditions, the highest percentage egg hatching of 94% occurred at 25°C and 75% RH. Two percent of the *C. canis* larvae died before they spun cocoons and 4% of the pupae never emerged into adults. Thus, altogether 88% of the eggs developed into adult *C. canis*. Eggs exposed to 85% RH at the same temperature resulted in an 86% hatching, of which 6% of the larvae did not develop into pupae and a further 6% of the pupae did not develop into adults, so that 74% of the eggs developed into adults. At 35°C and 75% RH, 72% of the eggs hatched. The 12% larvae that did not develop into pupae and 8% of the pupae that died before developing into adults, resulted in the emergence of only 53% adults. Although 64% of the eggs exposed to 15°C and 75% RH hatched, only 44% developed into adults as 6% of the larvae and 14% of the pupae died. At 25°C and 50% RH only 42% of the eggs hatched into larvae of which 6% died. Four percent of the pupae did not develop into adult *C. canis*, thus only 32% of the eggs reached adulthood (Figure 7.3).

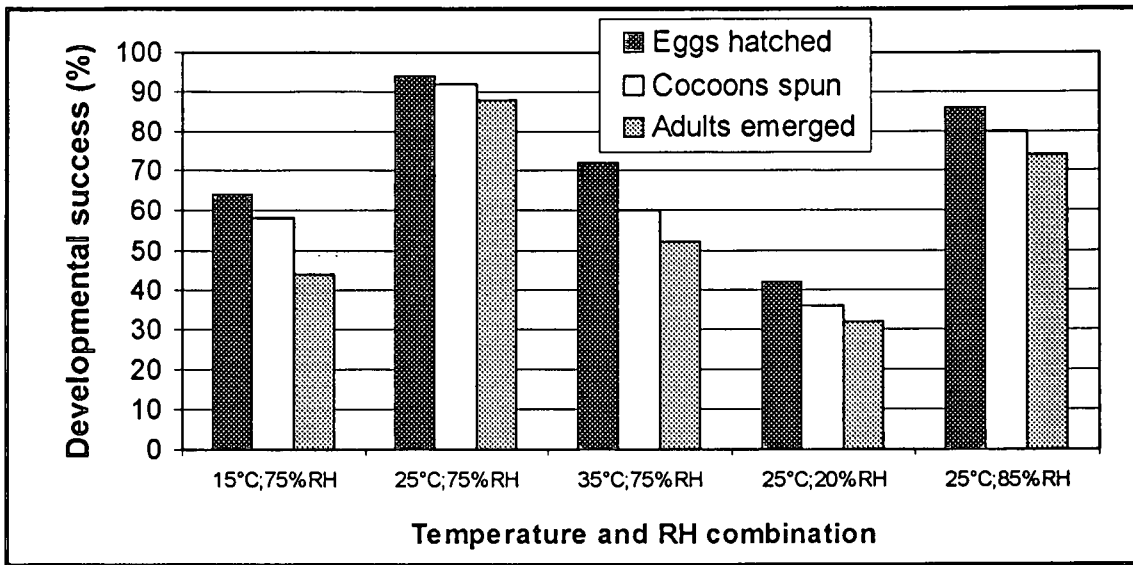


Figure 7.3 Percentage developmental success of different stages of *C. canis* exposed to different conditions.

Comparison between *C. canis* and *C. felis* under optimal conditions

In both species eggs started to hatch from day two after being exposed to optimal conditions. *C. canis* eggs hatched within two to seven days, while the eggs of *C. felis* hatched within two to four days. The *C. canis* larvae spinned cocoons between days 18 and 24 and the *C. felis* larvae spinned cocoons between days 15 and 21. Adult *C. canis* emerged between days 23 and 36, whereas the *C. felis* emerged between days 20 and 30 (Table 7.1).

Table 7.1 Comparison between the developmental times of *C. canis* and *C. felis*.

	<i>C. canis</i>	<i>C. felis</i>
Stage of life cycle	Time to develop (days)	
Eggs	2-7	2-4
Larvae	18-24	15-21
Pupae	23-36	20-30

Figure 7.4 shows 82% of the *C. canis* eggs hatched, but 10% of the larvae died before they spun cocoons. Of the 72% cocoons that were spun, 4% did not develop into adult

fleas. Thus, 68% of the eggs reached the adult stage. Ninety-two percent of the *C. felis* eggs hatched but 8% of the larvae died. The remaining 84% larvae all successfully spun cocoons. Eighty-one percent of the eggs eventually developed into adults as 2% of the pupae died.

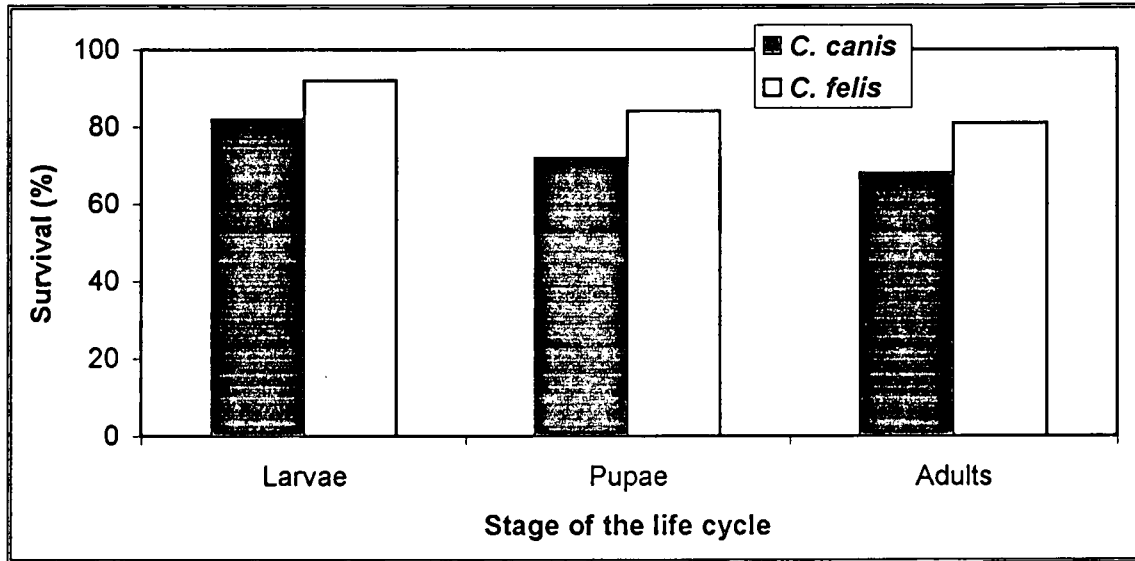


Figure 7.4 Comparison between the developmental success of *C. canis* and *C. felis* under optimal conditions (25°C and 75% RH).

Host specificity

When cats were infested with *C. canis* for the first time, the room was swept and searched for flea eggs on the second day after infestation, but no eggs were found. During the second infestation, three eggs were found during microscopic examination of debris. The eggs were transferred to a petri dish provided with standard rearing medium and kept at optimal conditions for flea development, but the eggs did not hatch. The experiment was done a third time, but again no eggs were found (Table 7.2).

Table 7.2 Success of development of *C. canis* fed on cats and dogs, respectively.

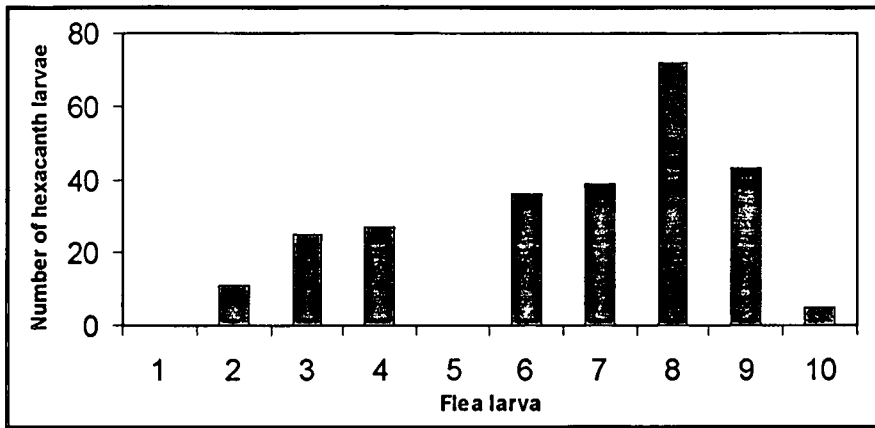
	<i>C. canis</i> on dogs (control)			<i>C. canis</i> on cats		
Eggs	80	80	80	0	3	0
Larvae	72	77	74	0	0	0
Pupae	68	76	74	0	0	0
Adults	68	73	74	0	0	0

Interbreeding

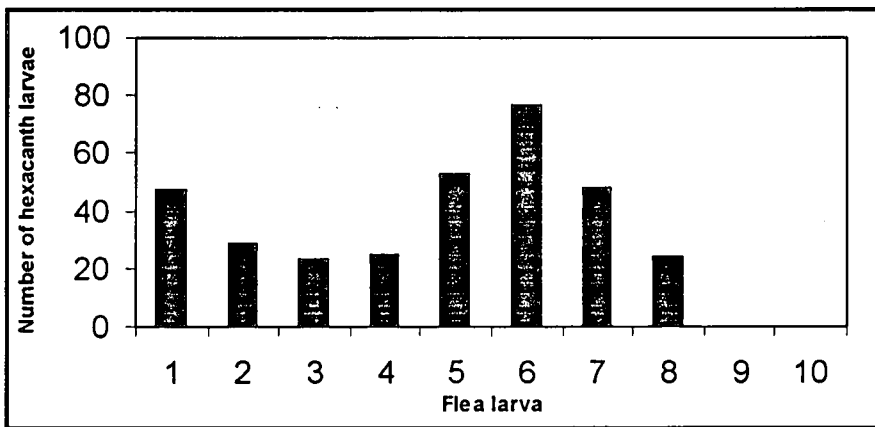
Where females from the one flea species were allowed to feed and mate with males of the opposite species, no eggs were found. The pens of dogs were thoroughly searched, the containers in which the dogs slept were swept with a paintbrush and the collected debris was searched under a microscope, but with no success. Even when the experiment was carefully repeated a week later, no eggs were found.

Infection with *Dipylidium caninum*

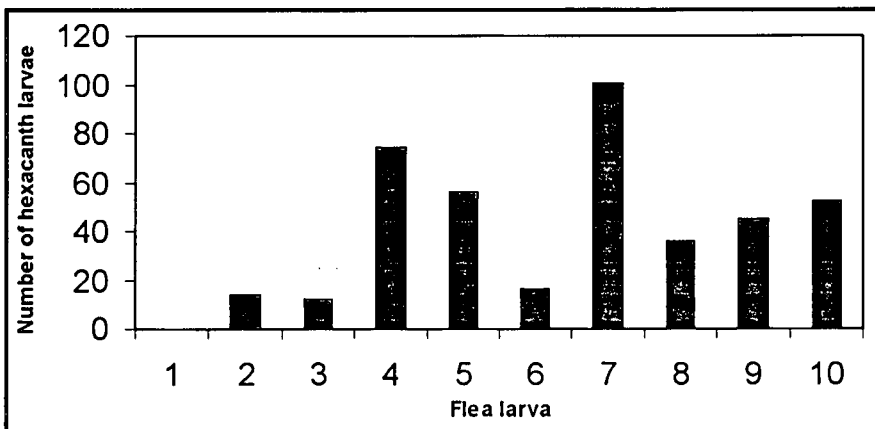
All except two of the first instar *C. canis* larvae, were infected with *D. caninum* after being exposed to uterine capsules. Although only one hexacanth larva was found in one of the flea larva, another two flea larvae contained as many as > 70 and > 40 hexacanth larvae, respectively (Figure 7.5a). All the second instar larvae contained more than 20 hexacanth larvae, except for two flea larvae that were not infected. The highest infection was 77 hexacanth larvae in one of the flea larva (Figure 7.5b). Only one of the third instar flea larva was not infected after being exposed to uterine capsules of *D. caninum*. The rest of the flea larvae contained from 12 to as many as 100 hexacanth larvae per flea larva (Figure 7.5c).



a) First instar



b) Second instar



c) Third instar

Figure 7.5 Number of hexacanth *D. caninum* larvae that infected the different instars of *C. canis* larvae.

Figure 7.6 shows the total number of hexacanth larvae per ten of flea larvae for each instar. A total of 12.9 parasites per 10 flea larvae were found in the first instar larvae, while 32.6 parasites per 10 flea larvae were found in the second instar flea larvae. The most severe infection occurred in the third instar flea larvae that contained 40.5 parasites per 10 flea larvae. Although the intensity of infection increased with the increase in flea larval instars, there were no significant differences in the levels of infection of the different instars.

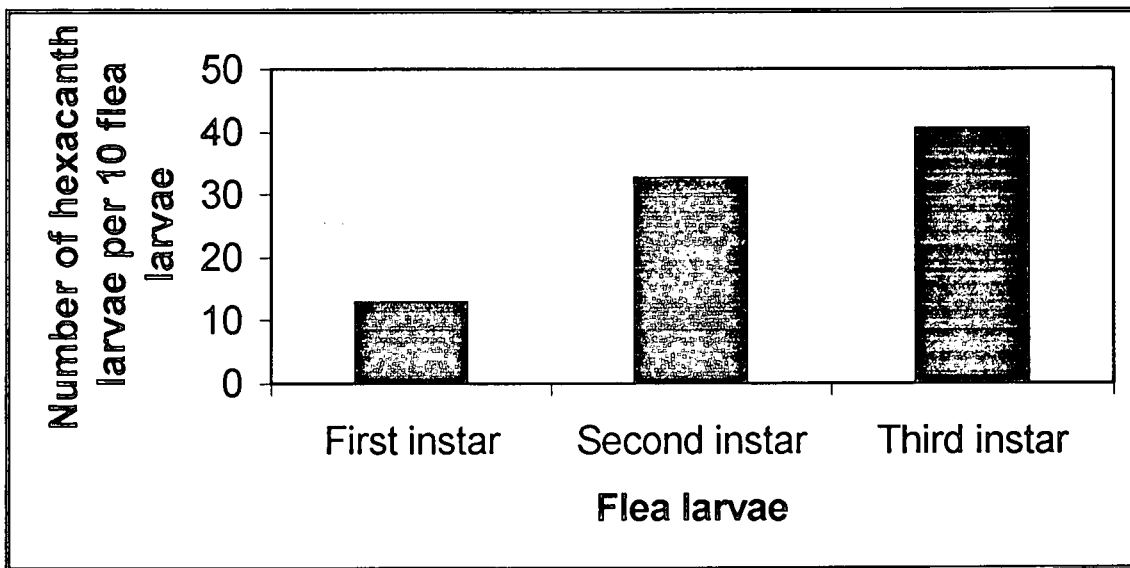
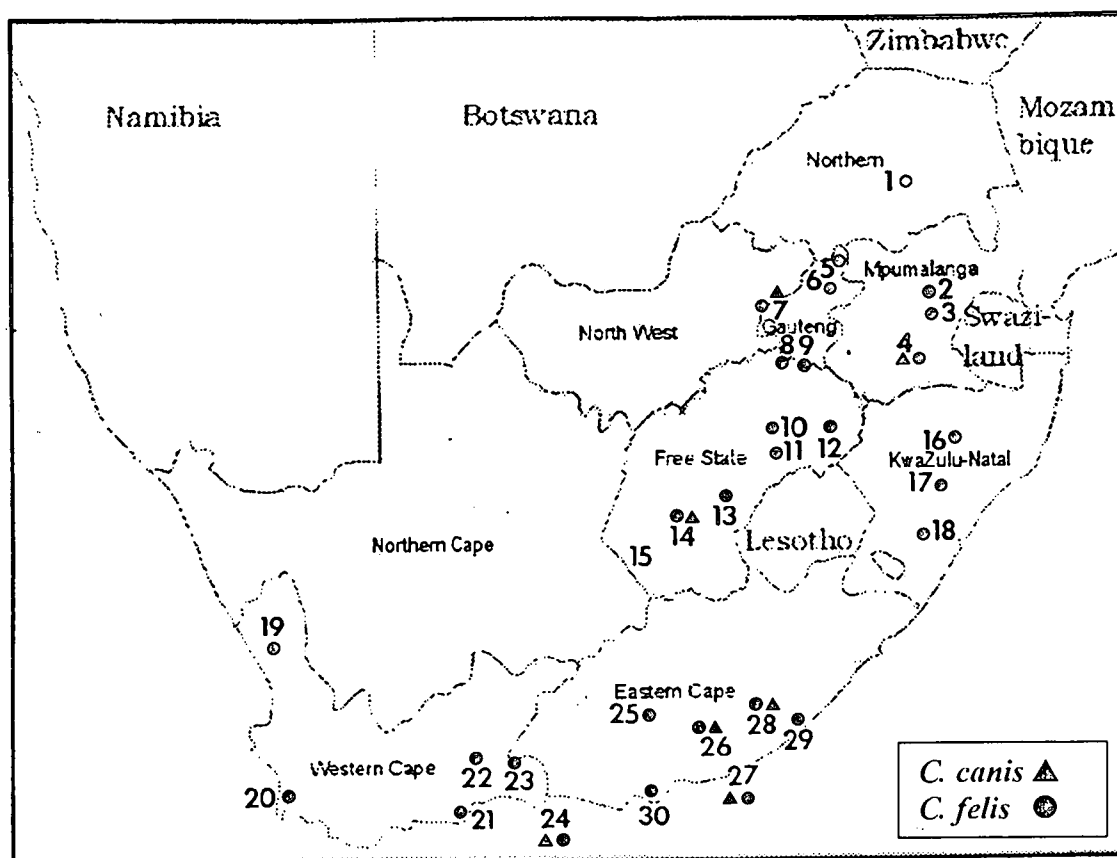


Figure 7.6 Total number of hexacanth larvae that infected the different instars of *C. canis* larvae.

Geographical distribution

The survey was done in all the provinces of South Africa, except Northern Cape and Northwest and a total of 1 693 specimens were collected. Figure 7.7 shows that *C. felis* was found in all 30 towns/cities where fleas were collected, except in Fauresmith in the Free State where no *Ctenocephalides* species were found. Thus, *C. felis* was found in the Northern Province, Mpumalanga, Free State, KwaZulu-Natal, Eastern Cape and Western Cape. *C. canis* was found in seven of the towns where surveys were done, including Eastern Cape, Western Cape, Mpumalanga, Free State and Gauteng. *C. canis* was mainly found in the disadvantaged communities of the townships. The highest frequency of *C.*

canis occurred in Grahamstown in the Eastern Cape and in addition to that reported by Segerman (2000) this species was also found in Ermelo in Mpumalanga. Segerman (2000) described the distribution of *C. canis* as uncommon and restricted for the most part to urban centers of Western Cape, Eastern Cape, Free State, Northwest Province and Gauteng. According to Segerman (2000) *C. felis* was found widely spread in Western Cape, Eastern Cape, Free State, KwaZulu-Natal, Mpumalanga, Gauteng, Northern Province and Northern Cape, Namibia, Botswana, Zimbabwe and Mozambique.



- | | | |
|----------------|---------------------|------------------------|
| 1 Pietersburg | 11 Bethlehem | 21 Mossel Bay |
| 2 Nelspruit | 12 Harrismith | 22 Oudtshoorn |
| 3 Barberton | 13 Excelsior | 23 Uniondale |
| 4 Ermelo | 14 Bloemfontein | 24 Plettenberg Bay |
| 5 Medunsa | 15 Fauresmith | 25 Cradock |
| 6 Pretoria | 16 Dundee | 26 Grahamstown |
| 7 Sandton | 17 Ladysmith | 27 Port Alfred |
| 8 Johannesburg | 18 Pietermaritzburg | 28 King William's Town |
| 9 Germiston | 19 Lutzville | 29 East London |
| 10 Lindley | 20 Cape Town | 30 Port Elizabeth |

Figure 7.7 A map of southern Africa which indicates the distribution of *C. canis* and *C. felis* based on a survey during the present study (1998-2000).

Only 10% of the fleas collected from dogs during the survey were *C. canis*, while 86% were *C. felis* and 4% belonged to either *Pulex irritans* or *Echidnophaga gallinacea*. Based on the findings of a survey carried out during the present study, *C. canis* (1%) and *C. felis* (99%) were the only species found on cats (Figure 7.8).

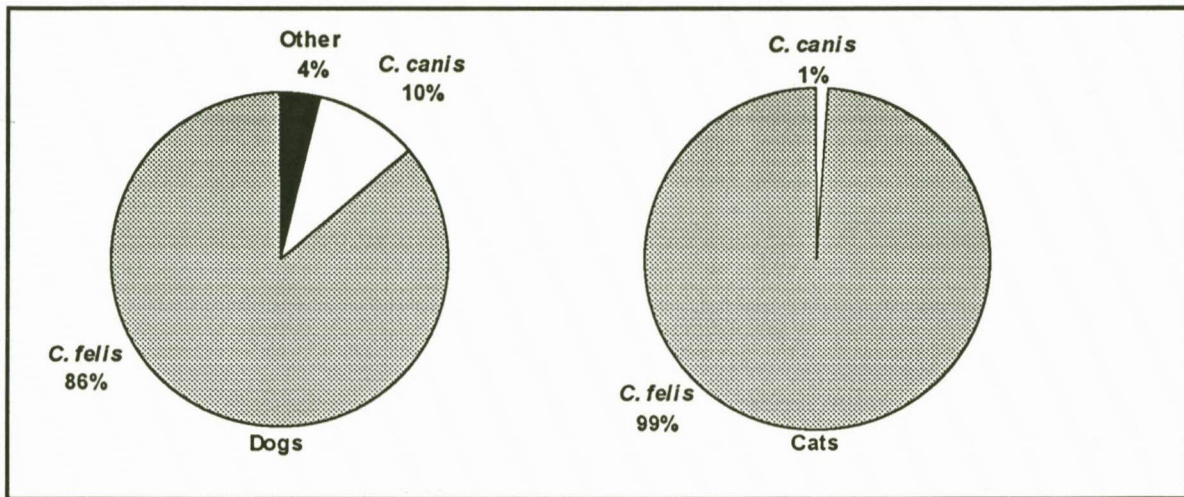


Figure 7.8 Graph showing the prevalence of *C. canis* and *C. felis* found on dogs and cats, respectively.

Discussion

In this study, development of the different stages in the life cycle of *C. canis* occurred at all the combinations of temperature and RH, but a temperature of 25°C and RH above 75%, resulted in the best development and survival. The ideal combination was found to be 25°C and 75% RH. This corresponds with the findings of Baker and Elharam (1992) who described 25°C and 75% RH as optimal for rearing *C. canis*, as was also found for *C. felis* (see chapter 6). In this study exposure to different conditions seemed to have a similar effect on *C. canis* as it had on *C. felis*. Baker and Elharam (1992) also found that the effect of different temperature and RH combinations showed the same tendency concerning the development of the different stages in the life cycle for both *C. canis* and *C. felis*.

Until now studies on the biology of *C. canis* and *C. felis* has been comparatively neglected (Wall & Shearer, 1997). Although it was found in previous experiments that optimal conditions for *C. canis* development correspond with that of *C. felis*, the direct comparison of the development of these two species under the same conditions, showed that *C. felis* was more successful. Ten percent less *C. canis* eggs hatched than those of *C. felis* and the same tendency, namely the higher mortality among larvae than among the pupae occurred in both species. These results confirmed that as in *C. felis*, the egg and larval stages are the less tempered stages of the life cycle. It also appeared that the life cycle of *C. canis* was a few days longer than the life cycle of *C. felis*. However, this study was not comprehensive enough to make such a statement and some more research has to be done on this topic.

Although three eggs were found in the present study no eggs laid by *C. canis* fed on cats, hatched. According to Segerman (1995) no previous study has reported the failure of *C. canis* to complete its life cycle successfully after feeding on cats, but Baker and Elharam (1992) found in their study that no eggs laid by *C. canis* feeding on cats, developed into adults. Bacot and Ridewood (1914) also found that very few eggs (2%) from *C. canis* fed on cats hatched, while all the larvae died within 48 hours of hatching. The failure of *C. canis* to complete its life cycle on cats as hosts indicates a high degree of host specificity for this flea species. According to Segerman (1995) the dog flea's principal hosts are dogs and domestic rats (*Rattus* sp.) Strays have been reported from the suricate and a hare (Segerman, 1995). *C. canis* was previously found in great numbers on dogs, but rarely on cats and is becoming more scarce as dogs are more frequently kept in environments similar to that of pet cats (McGlennon, 2000).

Since no eggs had been found where mixed infestations of males and females of different species were released on a host, it seemed that *C. canis* and *C. felis* are unable to copulate and mate. Wall and Shearer (1997) also found that although *C. canis* and *C. felis* are closely related and morphologically very similar, they cannot interbreed and are therefore truly distinct species. A morphological study by Ménier and Beaucournu (1998) of the aedeagus of *Ctenocephalides* spp. showed almost no differences based on some

phallosome structures (hamulus, lobes and tubus interior) between *C. canis* and *C. felis*, no indication could be found in the literature concerning morphological differences of the aedeagus that would prevent mating between the two species.

As in *C. felis* (see chapter 8), hexacanth larvae were found in all three instars, which indicated that *D. caninum* is able to infect *C. canis* during any stage of the larval development. In all three instars some larvae were heavily infected, while others contained no hexacanth larvae at all. Thus, the different instars of *C. canis* are equally susceptible to infection by *D. caninum*. However, the third instar flea larvae contained 27.6 more parasites per 10 flea larvae than the first instar larvae, while the second instar larvae contained 19.7 more parasites per 10 flea larvae than the first instar larvae. The difference in the number of parasites per 10 flea larvae between the second and third instar flea larvae was 9.7. The intensity of infection differed significantly between first and second and first and third instars, but not between the second and third instars.

C. felis live and multiply extremely well on both dogs and cats and is the most common flea species to attack domestic pets (Dryden, 1993). *C. canis*, on the other hand appears to have experienced some difficulty in getting itself firmly established and is much more host specific to dogs and therefore more restricted in distribution. Where the two species occur sympatrically *C. canis* competes rather unsuccessfully with *C. felis*. Hopkins and Rothschild (1953) concluded that *C. canis* is much less adaptable than *C. felis* and has a much more restricted geographical distribution and host range.

CHAPTER 8

THE ROLE OF *DIPYLIDIUM CANINUM* IN THE LIFE CYCLE OF *CTENOCEPHALIDES FELIS*

Introduction

A cyclophyllidean tapeworm that infects dogs and cats has been known for generations. Linnaeus named it *Taenia osculis marginalibus oppositus* in 1748 and *T. canina* in 1758. Bloch renamed the tapeworm as *T. cucumerina* in 1782 and in 1863 Leuckart named it *Dipylidium cucumerinum*. In 1892 Railliet finally changed the name to *D. caninum* as it is known today. The generic name is derived from a combination of the Greek words “dis” and “pyle” meaning “two” and “gate”, respectively, referring to the two genital openings of the double reproductive system in each proglottid (Grove, 1991).

That arthropods may act as intermediate hosts for worm parasites was first observed by Leuckart in 1858. Later investigations, even to the present day, have been mainly centered on the life cycle of the parasites with reference mainly to infections in definite hosts and very little attention has been given to the infection in the arthropod, or insect in particular, as it was generally believed to be more or less harmless in this host. That a definite defensive reaction might occur in insects infected with worms, was suggested while examining living *Ctenocephalides canis* larvae which had been fed eggs of the dog tapeworm, *D. caninum* (Chen, 1934). The cysticercooids in the coelomic cavity of the flea larvae were surrounded by small individual cells, presumably leucocytes. The reaction was later observed in stained sections of *C. canis* larvae and the phenomenon suggested a pathological condition in the insect host, heretofore unnoticed (Chen, 1934). *C. felis* was shown to be an intermediate host of *D. caninum* and it was found that the parasite developed very slowly until the flea became an adult on a mammal (Pugh, 1987).

The development of the larval form of *D. caninum* was accidentally disclosed by Melnikoff in 1868 while studying the embryology of the dog louse, *Trichodectes canis*. He discovered the cysticercoïd, the larval form of this tapeworm, in the body cavity of this louse. His discovery was confirmed by Leuckart in 1886 and many others. Later in 1888 Grassi found that these cysticercoïds were present in adults of *C. felis*, *C. canis* and *Pulex irritans*. Since adult fleas are not provided with biting mouthparts as are the louse *Trichodectes*, the mode of infection of the fleas long puzzled parasitologists. Several investigators, including Grassi and Rovelle, Sonsino, Railliet, Brumpt and others, conceived the idea that flea larvae might be responsible for the infections, but unfortunately their microscopical examinations of the flea larvae were always negative. In 1920 Joyeux confirmed, however, that the idea of these early investigators was right and experimentally demonstrated that the flea larvae became infected by eating organic debris contaminated with eggs of this tapeworm (Chen, 1934; Soulsby, 1982).

D. caninum occurs throughout South Africa and is worldwide in distribution (Reinecke, 1983). This tapeworm, often called the cucumber tapeworm, occurs in the small intestine of dogs, cats or other small carnivores as the definite host, and arthropods like fleas or lice as the intermediate host. The definite host is infected when it ingests an infected intermediate host. The feces of an infected dog or cat may contain proglottids that are shed from the tapeworm, and these have a characteristic size and shape (Wall & Shearer, 1997). *D. caninum* occasionally infects humans, particularly children (Noble & Noble, 1982). Most infections occur in children under eight years and about one-third are in infants under six months of age (Brown, 1975). Symptoms in children are absent to mild (Noble & Noble, 1982). Gastrointestinal symptoms and eosinophilia are the common findings. Control of this tapeworm involves not only eliminating the adult worm from the dog or cat but also flea control since without the presence of fleas the tapeworm cannot complete its life cycle (Cheng, 1986).

Diagnosis involves finding uterine capsules or entire proglottids in feces, since free eggs are not usually found in the feces of infected animals (Meyer & Olsen, 1971). The proglottids are distinguishable from those of other common species of tapeworms in that

the proglottids have two genital pores, one on each side of the proglottid, while the others have only one. *D. caninum* is therefore also known as the "double-pored tapeworm" (Cheng, 1986).

Following the description given by Leuckart (Cheng, 1964), the typical life cycle may be divided into three stages which involve an intermediate and a definite host:

Stage 1. The eggs develop within the uterus of the adult cestode until they contain larvae infective to the intermediate host. In this first larval stage, the embryonated ovum contains an oncosphere that bears six functional hooks. Such ova are contained in capsules within the gravid proglottid (12 mm x 2.7 mm) of the cestode. The capsules are held together by an outer embryonic membrane (Despommier & Karapelou, 1987). The proglottids separate from the strobila and are voided with feces, or leave the host spontaneously by crawling out of the anus. The uterine capsules each containing 5-20 eggs, are released by the contractions of the proglottids or by their disintegration (Noble & Noble, 1982). Each egg measures approximately 35-60 μm in diameter (Cheng, 1964).

Uterine capsules are usually released when the host is resting or sleeping. The den or bed of the host is where the flea larvae are also found. Adult fleas lay their eggs in the resting place of the host animal and have fine tubular mouthparts adapted for blood sucking so they are incapable of taking in cestode eggs (Baer, 1971; Noble & Noble, 1982). Unlike the adult, the larval flea has simple chewing mouthparts and feed on debris in the host's environment. This would include *D. caninum* eggs deposited there by the disintegration of the proglottids. Flea larvae of *C. felis*, *C. canis* and *P. irritans*, are attracted to the isolated proglottids that they break open, eat the eggs and become infected. After the eggs hatch as hexacanth larvae in the flea larvae, they develop into cysticercoids, which is the infective stage for the definite host (Despommier & Karapelou, 1987) (Figure 8.1). This is an example of hyperparasitism, since the flea is itself a parasite (Baer, 1971; Schmidt & Roberts, 1977).

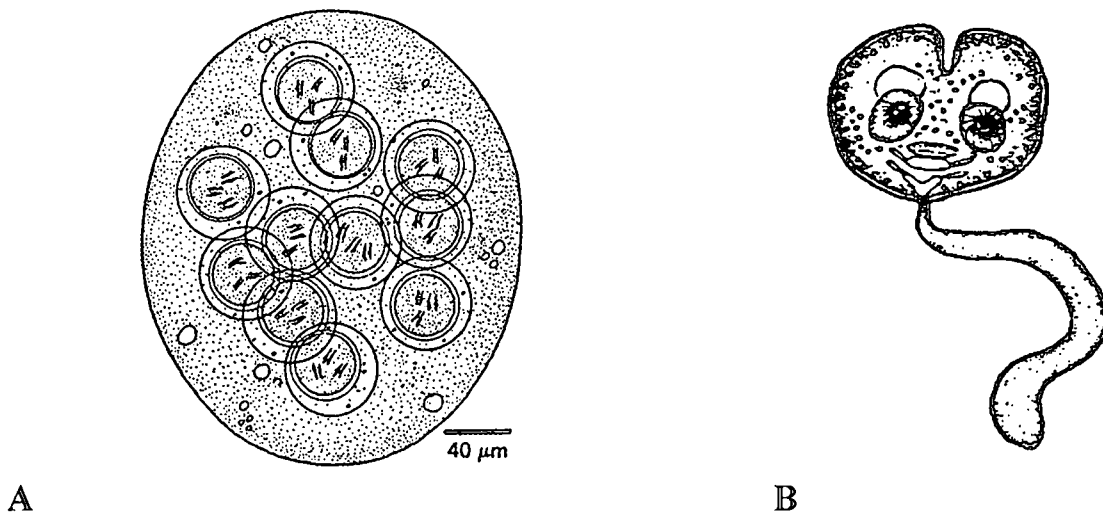


Figure 8.1 (A) An egg enclosing hexacanth larvae and (B) a cysticeroid larva (after Cheng, 1964).

Stage 2. The globular egg contains an oncosphere, measuring 20-23 μm in diameter. The oncosphere contains both germinative and somatic cells, six keratinaceous hooks with their associated muscles and a penetration gland. Growth in fleas, however, is more prolonged and development is associated with the stages of metamorphosis of the insect host. Oncospheres develop very little in the larval flea, considerable growth takes place during the pupal stage and final development is completed in adult fleas when the latter begin to take blood meals (Olsen, 1974). Possibly the nutritional level of the larval body cavity is insufficient to permit growth to the cysticeroid level of organisation (Smyth, 1962). *D. caninum* causes no mortality of flea larvae but significant mortality of infected flea pupae when the parasite is growing most rapidly (Zimmerman, 1937; Kettle, 1990). The oncospheres in the digestive tract of the flea migrate into the haemocoel (Brown, 1975). They immediately start to develop into cysticeroids, but their presence triggers off a host defence reaction and the cestode larvae become surrounded by leucocytes that inhibit further development. Just before the metamorphosis of the flea, however, these leucocytes are mobilised in order to phagocytose and destroy larval tissues preceding formation of the pupa. Freed from restraint the cysticeroids complete their development

so that the adult fleas emerge containing infective cysticercoids. A cysticercoid will consist of a scolex, two small rostellar hook arrangements and a tailing bladder (Baer, 1971; Despommier & Karapelou, 1987).

Stage 3. The tapeworm larva becomes fully developed only some time after the emergence of the adult flea and it is then infective to the appropriate definite host. The accidental ingestion of the cysticercoid larva contained in the intermediate host by the definite host is the beginning of the third stage. The cysticercoid escapes into the small intestine, evaginates its scolex and on reaching its proper location in the intestine of the definitive host, attaches itself and grows into the adult strobilate cestode (Zimmerman, 1937). The worm grows to maturity within the lumen of the small intestine in three to four weeks (Chen, 1934; Despommier & Karapelou, 1987).

The adult tapeworm measuring about 15-70 cm in length, is slightly pink in colour, and consists of 60-175 proglottids. A rhomboidal scolex possesses a retractable rostellum armed with 30-150 rose-thorn-shaped hooks arranged in transverse rows. Below the rostellum are four prominent suckers (Brown, 1975; Reinecke, 1983). The gravid proglottids are characterized by being longer than broad, resembling rice grains and measure 8-23 mm. A mature proglottid contains two sets of male and female reproductive systems and a genital pore on each side of the proglottid. The uterus develops as a network of canals or cavities (Schmidt & Roberts, 1977; Noble & Noble, 1982). Testes are numerous (± 200 testes per proglottid) and fill the medullary parenchyma between the excretory canals. The ovaries and vitelline glands are in separate clusters with the latter posterior (Figure 8.2) (Mönnig, 1938; Cheng, 1964; Olsen, 1974).

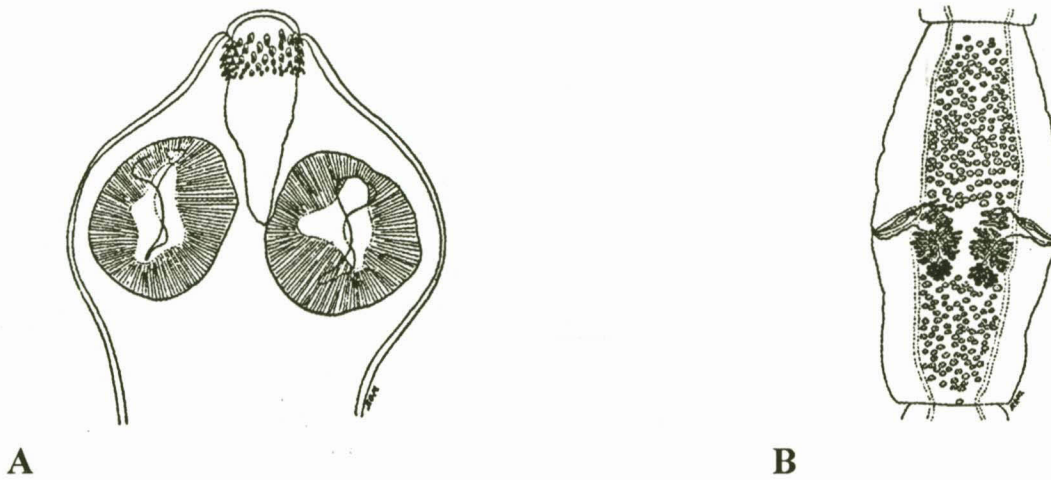


Figure 8.2 (A) A scolex with hooked rostellum and (B) a mature proglottid enclosing two sets of reproductive organs (after Soulsby, 1968).

The objectives of this part of the study were to determine:

- The susceptibility of *C. felis* larvae for infection with *D. caninum*, the instar during which infection occurs and the position of the parasite in infected *C. felis* larvae.
- The survival rate of *C. felis* larvae exposed to different numbers of *D. caninum* eggs, grouped together in uterine capsules.
- The time that *D. caninum* uterine capsules will stay viable.

Material & Methods

Experimental procedure

Fleas used in this study were obtained from the laboratory colonies (see chapter 3), while *D. caninum* eggs were obtained when collecting the flea eggs from tapeworm infected laboratory cats. The tapeworm uterine capsules were counted out one by one with a very fine paintbrush from the mixture of flea eggs and debris and stored in a petri dish under conditions optimal for *C. felis* development (25°C; 75% RH) until they were required.

The effect of *D. caninum* in *C. felis* was investigated under conditions optimal for *C. felis* development.

All experiments in this chapter started with the same basic procedure: Flea eggs were placed in a Microtitre Plate (see chapter 3), one in each cell, at optimum conditions. As soon as they hatched, a small amount of bloodmeal was added. The larvae were then allowed to develop, but were monitored daily. In this way larvae required for the various experiments were available as they reached the desired instars. As other authors have shown, fleas become infected during the larval period by eating the tapeworm eggs (Despommier & Karapelou, 1987). When larvae reached the desired larval instars, they were transferred from the Microtitre Plates to a clean petri dish, without any food. After being deprived from food for 12 hours, the larvae were ready for use in the various experiments.

The experiments implied the exposure of a certain number of *C. felis* larvae to a certain number of *D. caninum* uterine capsules for 12 hours. After 12 hours a little bloodmeal was added to the dish and development was allowed to proceed as usual. When the 12 hours had expired, the larvae were mounted in a drop of Bouin-Glycerine on a cavity slide and covered with a coverslip. The slide was then left overnight to allow the Bouin-Glycerine to clear the larvae after which they were ready for microscopic examination. A Nikon E800 microscope fitted with a camera was used. Larvae were studied segment by segment using the 60x and 100x oil immersion lenses and Differentiating Interference Contrast (DIC).

Infection of *C. felis* by *D. caninum*

Thirty flea larvae, ten from each instar, were placed in three different petri dishes, with 50 *D. caninum* uterine capsules in each petri dish, for a period of 24 hours. After 24 hours five larvae from each petri dish were randomly selected, removed from the petri dish and mounted for microscopy. The number and position of *D. caninum* larvae infecting the *C. felis* larvae were determined under the microscope. One-way analysis of variance (ANOVA), followed by the Tukey test, was used to test for the significance ($P <$

0.05) of differences between mean numbers of hexacanth larvae found in the different larval instars.

Survival of *C. felis* larvae exposed to different numbers of *D. caninum* uterine capsules

Fifty flea larvae, ten per petri dish, were exposed to tapeworm uterine capsules. The petri dishes contained 10, 20, 30, 50 and 100 uterine capsules respectively. Another ten *C. felis* larvae were placed in a petri dish, containing only standard rearing medium and served as a control. After 24 hours all larvae were removed and placed in clean petri dishes with fresh rearing medium and allowed to complete their life cycle. After completion the number of adult fleas or other stages of the life cycle were counted by examining the petri dishes under a light microscope in order to determine survival of fleas.

Survival and intensity of infection of *C. felis* larvae exposed to a high concentration of *D. caninum* uterine capsules

Hundred *C. felis* larvae, a mixture of all the instars, were exposed to more or less 600 *D. caninum* uterine capsules. After 24 hours five flea larvae were removed and prepared for microscopy. The remaining 95 larvae were transferred to a bigger plastic container with fresh rearing medium and left at optimum conditions to complete the life cycle. After completion of the life cycle, the adult fleas were counted and the container was searched under a light microscope for any other stages of the life cycle.

Viability of *D. caninum* uterine capsules

Ten flea larvae (from different instars) were exposed to 80 tapeworm uterine capsules that have been kept at optimum conditions for *C. felis* development for over three months. After 24 hours five larvae were removed from the petri dish and prepared for microscopy. The number and position of the hexacanth larvae of *D. caninum* in the *C. felis* larvae were determined under the microscope.

All experiments were done in duplicate and because the data were similar, that was

pooled in one set.

Results

Infection of *C. felis* by *D. caninum*

After being exposed to 50 *D. caninum* uterine capsules, the 10 larvae from each instar were examined and the number and position of hexacanth larvae were determined. Figure 8.3 shows two hexacanth larvae next to each other with their outer body walls clearly visible. However, the hexacanth larvae were rarely as clearly visible as this (Figure 8.4). In most cases some of the six hooks were fully visible, while only the tips of the hooks of others could be seen (Figure 8.5), and sometimes the six hooks were seen from directly above (Figure 8.6).

Although some flea larvae from each instar were sometimes not infected at all, hexacanth larvae were found in all three the larval instars. The highest infection among first instar flea larvae was 72 hexacanth larvae found in one of the larvae, whilst two second instar larvae contained as many as 78 and 85 hexacanth larvae, respectively. The highest individual infection was among the third instar larvae where 90 and 112 hexacanth larvae, respectively, were found in two of the flea larvae. Nineteen of the 112 hexacanth larvae found in the one flea larva, were located in the head (Figure 8.7). Hexacanth larvae were in general distributed through various segments, although there was a higher frequency of occurrence in some of the segments.



Figure 8.3 Two hexacanth larvae next to each other with their outer body walls clearly visible (60 x magnification).



Figure 8.4 The six hooks of a hexacanth *Dipylidium caninum* larva (100 x magnification).

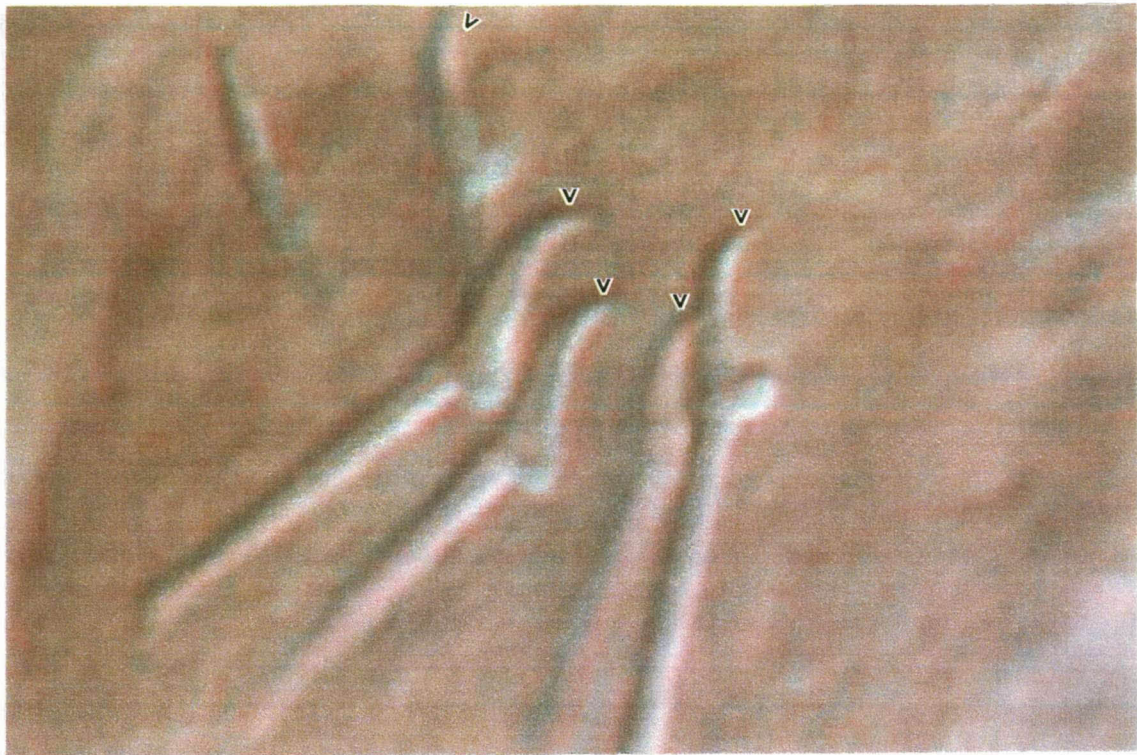


Figure 8.5 Four of the six hooks of a hexacanth larva fully in focus and two of which only the tips are visible (100 x magnification).

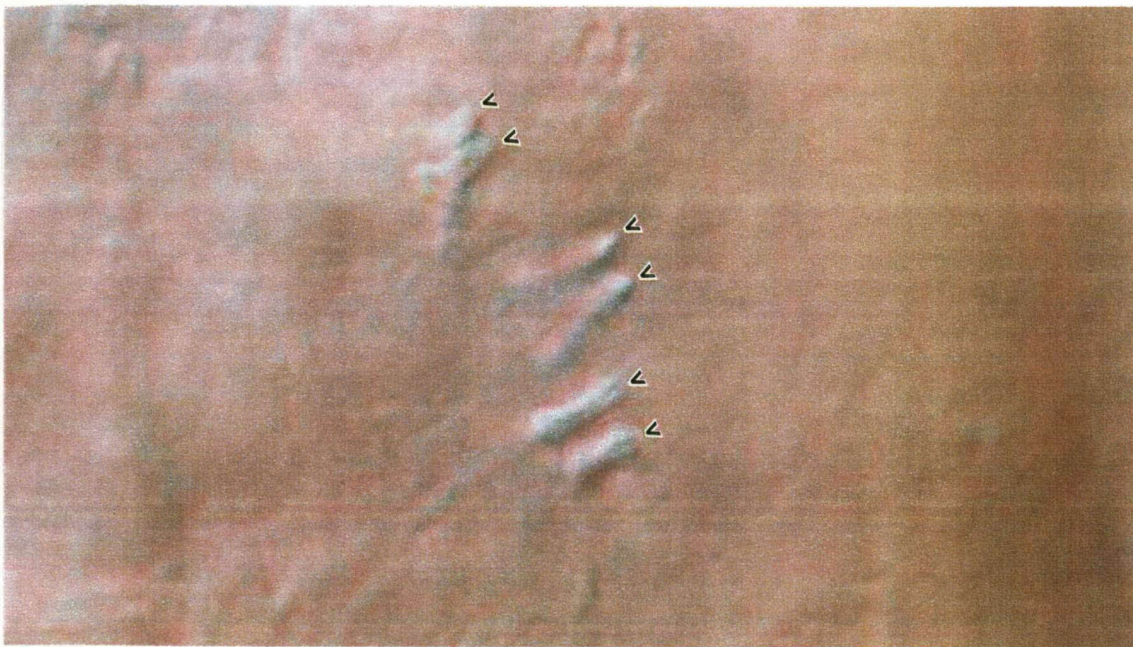


Figure 8.6 The tips of the six hooks of a hexacanth larva as seen from directly above (100 x magnification).

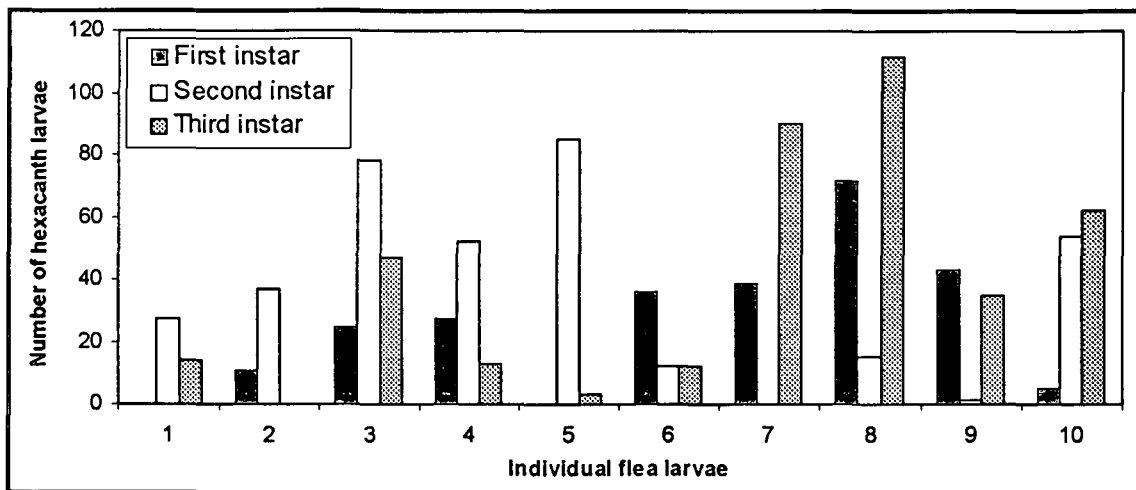


Figure 8.7 Number of hexacanth *D. caninum* larvae infecting individual flea larvae of different instars of *C. felis* larvae.

The intensity of infection was 25.8 hexacanth larvae per flea larva in first instar larvae, while second instar flea larvae contained 36.1 hexacanth larvae per flea larva. Third instar larvae had the highest intensity of infection, namely 38.8 hexacanth larvae per flea larva. Although the intensity of infection found in the third instar *C. felis* larvae was higher than in the two other instars (Figure 8.8), the difference was not significant.

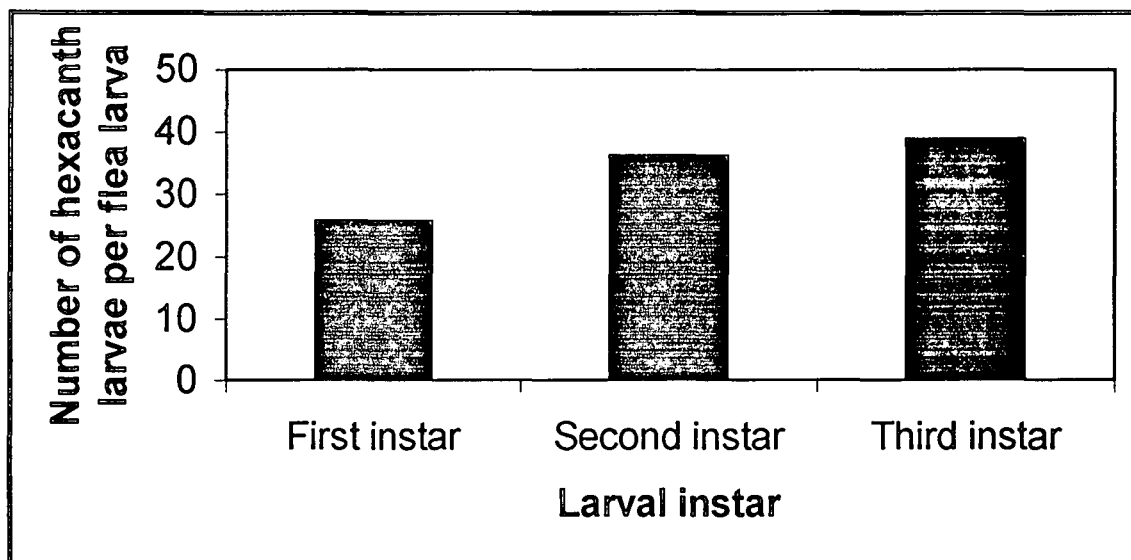


Figure 8.8 Average number of hexacanth *D. caninum* larvae found per flea larvae in the different larval instars of *C. felis*.

Survival of *C. felis* larvae exposed to different concentrations of *D. caninum* uterine capsules

A mean of 95% of the *C. felis* larvae fed on standard rearing medium (control), survived and developed into adults, while 95% of the larvae exposed to 10 uterine capsules, developed to adulthood. Exposure to 20 and 30 uterine capsules per ten flea larvae resulted in 90% and 84% survival of adults, respectively. From the larvae exposed to 50 uterine capsules, 58% developed into adult fleas, while 63% of the larvae exposed to 100 uterine capsules completed the life cycle (Figure 8.9). Where flea larvae were exposed to 30 and 50 uterine capsules, respectively three and two dead pupae were found, while four pupae died after larvae had been exposed to 20 uterine capsules and another four at exposure to 100 uterine capsules. No dead larvae were found.

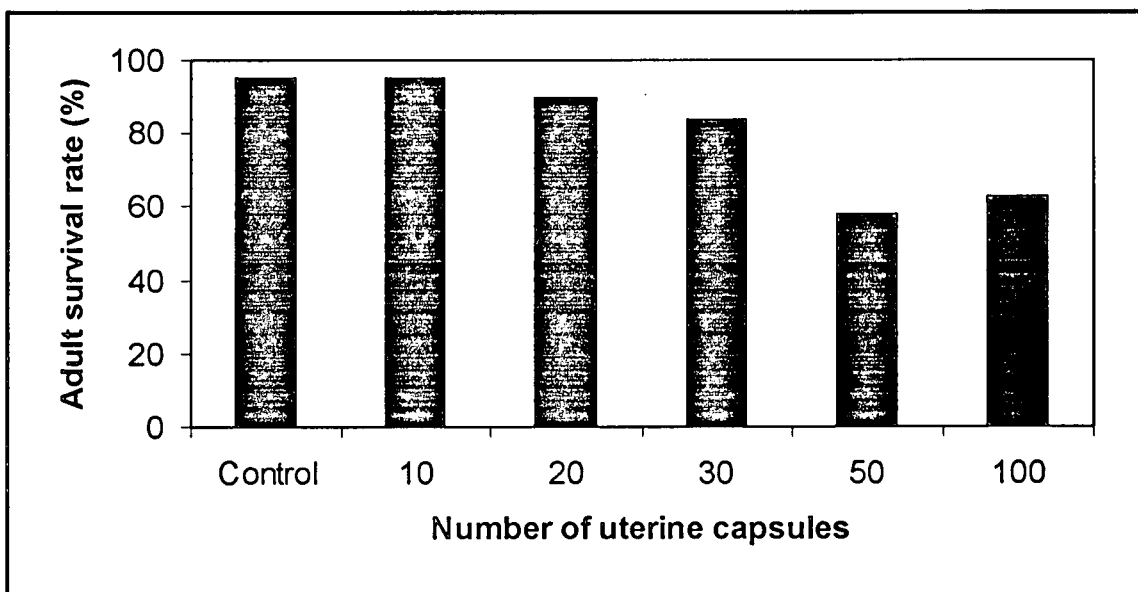


Figure 8.9 Survival rate of adult fleas after exposure of larvae to different concentrations of uterine capsules.

Survival and intensity of infection of *C. felis* larvae exposed to different concentrations of *D. caninum*

Figure 8.10 shows that infection with *D. caninum* occurred in all the *C. felis* larvae examined after exposure to ± 600 uterine capsules. Although one larva contained only a few hexacanth larvae, all ten larvae were found to be infected and the intensity of

infection of hexacanth larvae per flea larva was 67.2. Eighty-four percent of the remaining *C. felis* larvae in the larger container, completed the life cycle and emerged as adult fleas. During the search for other stages of the life cycle 1% dead larvae and 15% dead pupae were found (Figure 8.11).

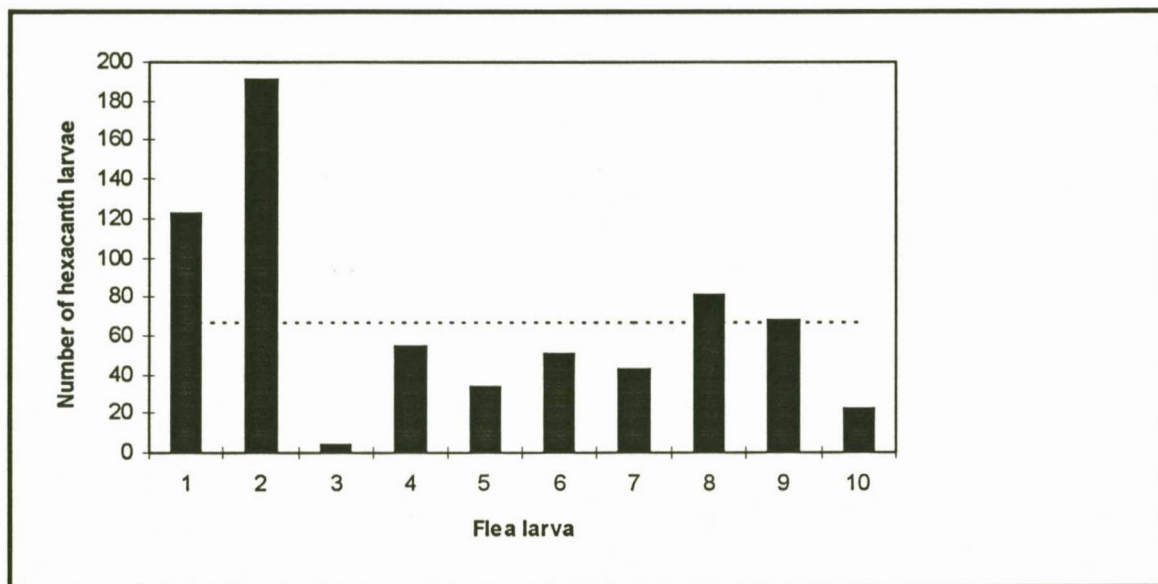


Figure 8.10 Number of hexacanth *D. caninum* larvae infecting individual *C. felis* larvae. The dotted-line indicates the average intensity of infection of hexacanth larvae per flea larva.

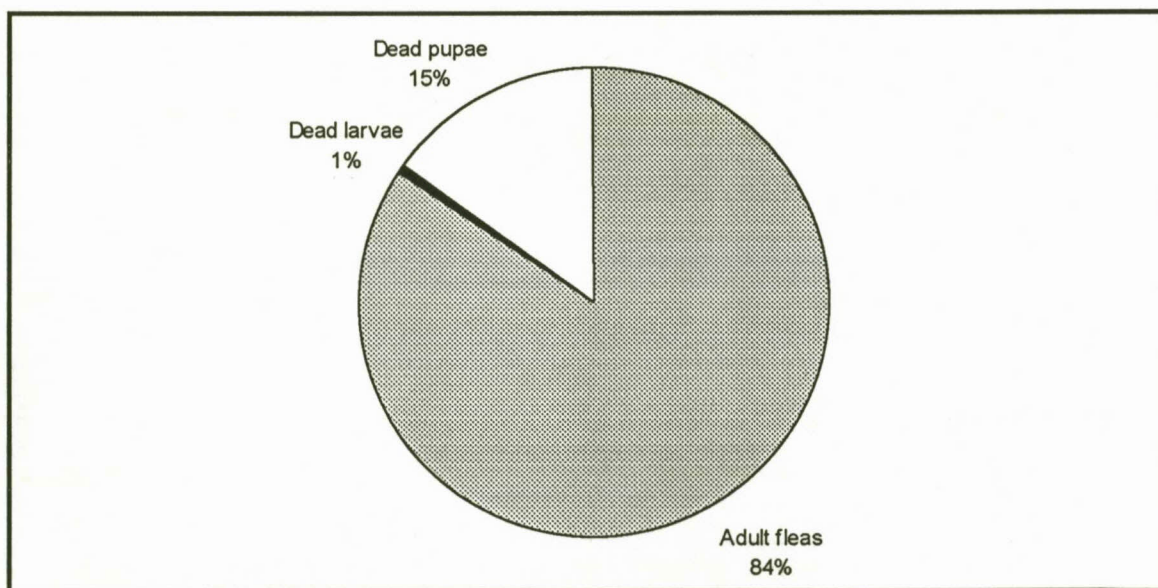


Figure 8.11 Percentage of individuals present after being allowed to complete the life cycle following exposure to high numbers of uterine capsules.

Viability of *D. caninum* uterine capsules

All but two of the *C. felis* larvae exposed to three month old uterine capsules were infected with *D. caninum* and two of the *C. felis* larvae contained as many as 197 and 192 hexacanth larvae respectively (Figure 8.12). To give an indication of the intensity of infection of the flea larva in which 197 hexacanth larvae were found. Figure 8.13 shows the various hooks in only a small part of a segment of a *C. felis* larva.

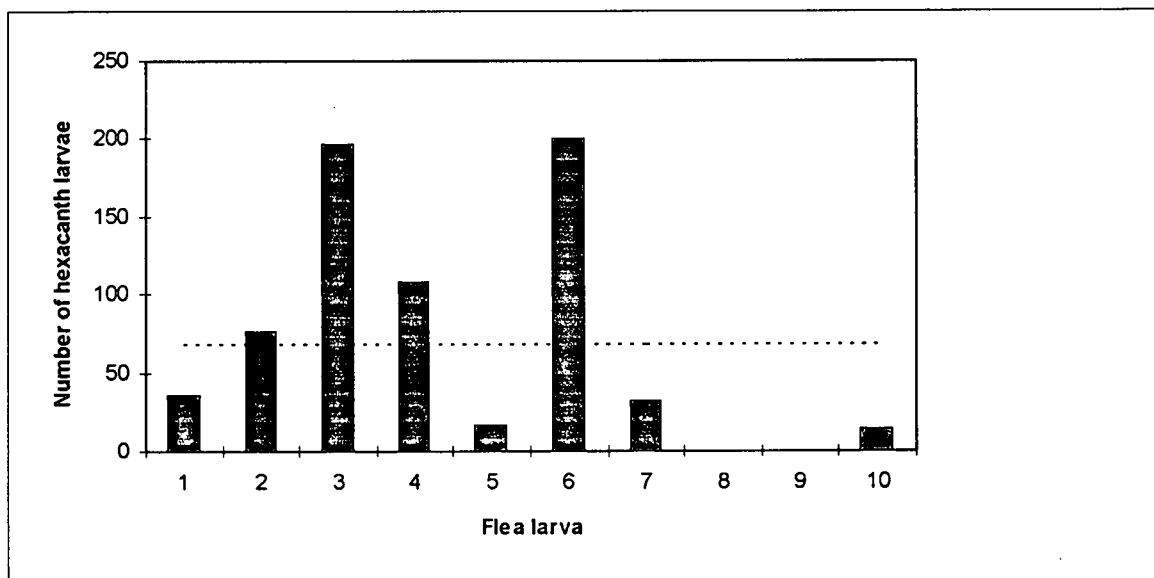


Figure 8.12 Number of hexacanth *D. caninum* larvae infecting individual *C. felis* larvae. The average intensity of infection of hexacanth larvae per *C. felis* larva is indicated by the dotted-line.

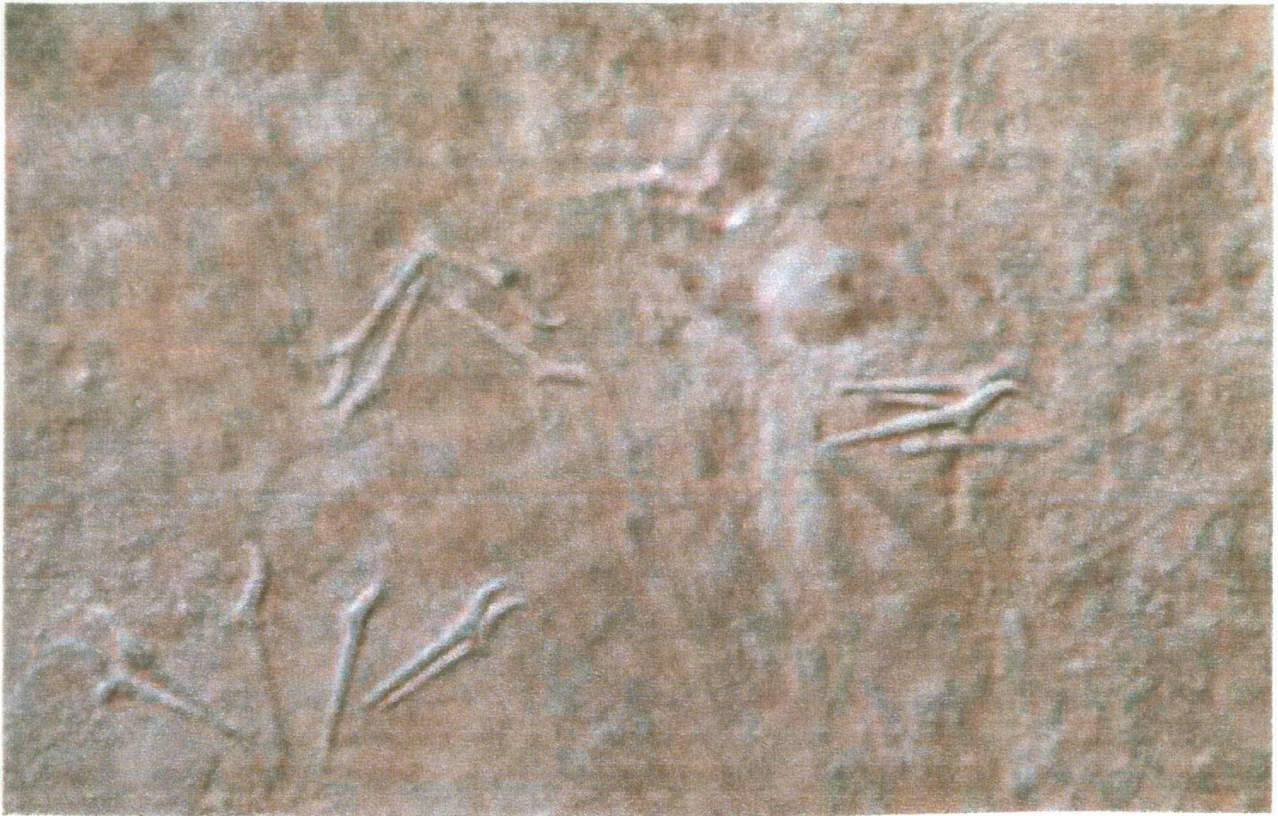


Figure 8.13 The hooks of various hexacanth *D. caninum* larvae in one segment of a *C. felis* larva (60 x magnification).

Figure 8.14 shows the intensity of infection per segment of the flea larvae after ten flea larvae were exposed to 80 uterine capsules. The head, segment seven and segment 10 contained a mean of 104, 99 and 90 hexacanth larvae, respectively, while the intensity of infection in the rest of the segments varied between 57 and 79.

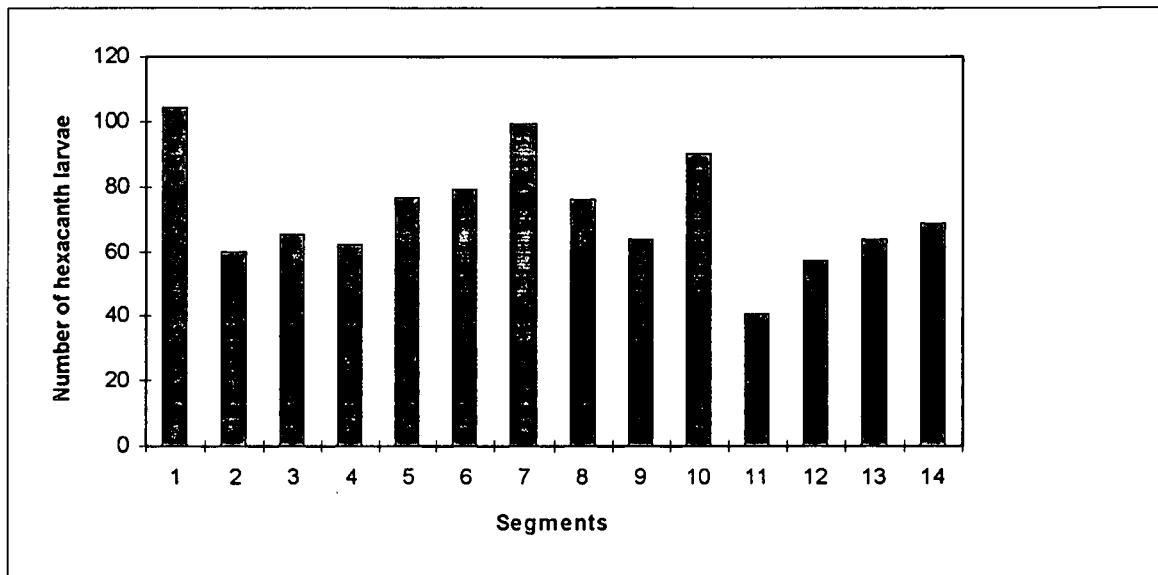


Figure 8.14 Mean number of hexacanth larvae per segment in flea larvae.

Discussion

Although at least two of the hooks from the hexacanth larvae were usually clearly visible, some hooks were barely visible especially when located near the digestive tract. After each moult the larvae became more sclerotised which made the visibility of the hexacanth larvae less clear so that, all hexacanth larvae were not equally well defined. The numbers of hexacanth larvae that infected flea larvae in the different experiments, might thus be more than the actual numbers counted.

Three of the segments contained slightly more hexacanth larvae than the rest of the segments. Furthermore, no connection could be made between the number of hexacanth larvae in a certain segment and the occurrence of hexacanth larvae in the rest of the larva. Nothing could be found in the literature about the distribution of hexacanth larvae in different segments of flea larvae.

When *C. felis* larvae from different instars were exposed to *D. caninum* uterine capsules, hexacanth larvae were found in all three instars, which indicated that *C. felis* can get

infected during any period of the larval stage. The fact that infection intensities varied from high to zero in all the instars is a typical overdispersion that is characteristic of parasites in/on hosts (Baer, 1971).

D. caninum was found to have a negative effect on *C. felis*. No mortality occurred amongst the flea larvae, but some pupae died before they could develop into adults. According to Pugh (1987) an average parasite infestation did not cause mortality to *C. felis* larvae unless they were weak or moribund. The results of this study indicated that *D. caninum* is not very harmful to its larval intermediate host at optimum conditions for *C. felis* development (25°C; 75% RH), although mortality increased at high intensities of infection. From Pugh's (1987) study, it seems that the host-parasite relationship is a dynamic one and the effect of the defence mechanism of *C. felis* can be altered by varying conditions, particularly temperature (Pugh, 1987). Thus, the effect of *D. caninum* on *C. felis* might change with varying temperatures.

Higher mortality occurred among pupae than among larvae throughout the study. The conclusion can be made that *D. caninum* affects *C. felis* in the pupal rather than in the larval stage. Kettle (1990) and Zimmerman (1937) also found that *D. caninum* causes no mortality of flea larvae but rather affected the infected flea pupae in which the parasite is growing most rapidly. As might be predicted, temperature is one of the major factors affecting the rate of development in the intermediate host. Pugh and Moorehouse (1985) studied the infection of *D. caninum* in *C. felis* and found that the parasite's development rate increased with increased temperature. No perceptible growth occurred at 20°C, but development accelerated for every 5°C increment and was also affected to some extent by the RH (Smyth & McManus, 1989).

The study further showed that tapeworm eggs stayed viable for at least three months as tapeworm larvae appeared in flea larvae which had been exposed to eggs which were kept under optimum conditions for over three months.

CHAPTER 9

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Abstract

ABSTRACT

The behaviour and survival of *C. felis* are highly dependent on biotic and abiotic factors in the environment and therefore developmental and nutritional requirements demand that eggs must be laid in an environment which provides the suitable microhabitat for *C. felis* development. Since eggs and larval stages of the cat flea cannot survive major fluctuations in temperature and humidity, sites for development are usually limited. It was found that eggs desiccated at low relative humidities and hatched faster at higher temperatures. The percentage adult emergence also depended on the type of rearing medium on which the larvae fed. The duration of the larval stage increased with a decrease in temperature and eggs exposed to high relative humidity resulted in larger larvae. However, *C. felis* populations survive because of a large reproductive capacity of the female and the ability of the pupal stage to survive environmental fluctuations. The pupa is the immature stage that is most resistant to desiccation and it is during this stage that *C. felis* is able to prolong its life cycle in order to survive unfavourable conditions. The fully developed adult flea can remain quiescent within the cocoon, presumably awaiting proper stimuli for emergence. Optimal conditions for *C. felis* development were found to be $25\pm 2^\circ\text{C}$ and $75\pm 2\%$ relative humidity.

In spite of the many similarities between *C. felis* and *C. canis*, a comparative study showed conspicuous differences in the micro-morphology of these two species. Although only small differences were found in the biology of *C. felis* and *C. canis*, the host specificity and geographical distribution of the two species varied greatly. *C. felis* is the most common flea species to attack domestic pets, *C. canis* is more restricted in distribution.

Apart from its association with various diseases and flea allergy dermatitis (FAD), *C. felis* can also serve as the intermediate host for the dog tapeworm, *D. caninum*. *C. felis* become infected with the parasite during any period of the larval stage when larvae ingest

the eggs of *D. caninum*. Although it was found that *D. caninum* were only slightly harmful to its intermediate host under optimal conditions for *C. felis* development, the negative effect on the intermediate host increased with an increase in the intensity of infection.

Key words: *Ctenocephalides felis*, cat flea, *C. canis*, dog flea, *Dipylidium caninum*, dog tapeworm, temperature, relative humidity, optimal conditions, survival

opsomming

OPSOMMING

Omdat die gedrag en oorlewing van *C. felis* afhanklik is van biotiese en abiotiese faktore in die omgewing, moet eiers in 'n omgewing gelê word wat 'n geskikte mikrohabitat voorsien vir *C. felis* ontwikkeling. Gedurende die studie is gevind dat eiers uitdroog by lae relatiewe humiditeit en vinniger uitbroei onder hoër temperature. Die persentasie volwasse vlooi wat ontpop het, het ook afgehang van die voedingsmedium waarop die larwes gevoed het. Geskikte omgewings vir optimale ontwikkeling is gewoonlik skaars, omdat die eier en larwale stadiums nie groot temperatuur- en humiditeitsfluktuasies kan oorleef nie. Die lengte van die larwale stadium het toeneem met 'n afname in temperatuur en eiers wat aan hoë relatiewe humiditeite blootgestel is, het groter larwes tot gevolg gehad. *C. felis* populasies oorleef egter as gevolg van die wyfie se hoë reprodukerende vermoë en die weerstandbiedendheid van die papie stadium teen fluktuasies in die omgewing. Van die onvolwasse stadiums is die papie die meeste bestand teen uitdroging en verlenging van die lewensiklus kan tydens dié stadium plaasvind ten einde ongunstige toestande te oorleef. 'n Volledig ontwikkelde volwasse vlooi kan rustend in die kokon bly totdat 'n geskikte stimulus die vlooi stimuleer om te ontpop. Ontwikkeling van *C. felis* was optimaal by $25\pm 2^\circ\text{C}$ and $75\pm 2\%$ relatiewe humiditeit.

Ten spyte van heelwat ooreenkomste tussen *C. felis* en *C. canis*, het 'n vergelykende studie ooglopende morfologiese verskille tussen dié twee spesies aan die lig gebring. Hoewel slegs klein verskille in die biologie van *C. felis* en *C. canis* gevind is, is daar groot verskille in die gasheerspesifisiteit en geografiese verspreiding van die twee spesies.

Behalwe dat *C. felis* met verskeie siektes en allergiese dermatitis geassosieer word, kan die katvlooi ook optree as tussengasheer van die hondelintwurm, *D. caninum*. *C. felis* word geïnfecteer met die lintwurm gedurende enige tyd van die larwale stadium wanneer die vlooi larwe die eiers van *D. caninum* insluk. Hoewel daar gevind is dat *D. caninum*

slegs effens skadelik is vir sy tussengasheer onder optimum toestande vir *C. felis* ontwikkeling, het die negatiewe effek toeneem by hoër infestasies van infeksie.

Sleutelwoorde: *Ctenocephalides felis*, katvlooi, *C. canis*, hondevlooi, *Dipylidium caninum*, honde lintwurm, temperatuur, relatiewe humiditeit, optimale toestande, oorlewing