# A Study of the Mayfly Parasite *Spiriopsis adipophila*, Arvy and Peters (1972) (= *Spirinella adipophila*, Arvy and Delage 1966) in *Ephemera danica* from the River Pang, a Tributary of the River Thames

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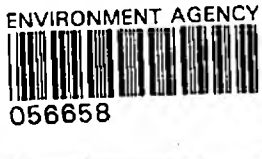

# Preface

**Acknowledgement**

I would like to thank all the staff in the Biology section at Fobney Mead for their help throughout this project. I would especially like to thank Willie Yeomans for both help and advice throughout the work and for proof reading the drafts. I would also like to thank Alan Tubb who encouraged me to complete the work within the required time period, Dr. Whitfield for providing and helping to interpret the transmission electron microscope images, and finally Cyril Bennett for all his help and advice.

**Declaration**

I certify that the work of this report and the views expressed within are my own.

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# Abstract

The mayfly parasite *Spiriopsis adipophila* , Arvy and Peters (1972),(=*Spirinella adipophila* Arvy and Delage 1966) was recorded to be present in *Ephemera danica* larvae taken from the River Pang, a tributary of the River Thames. The geographical distribution of this parasite was found to be uneven along the length of the river such that the intensity of infection was much greater within the *E. danica* larvae sampled from the downstream sites compared to those taken at the upstream sites. The was no apparent reason for this uneven geographical distribution, as it did not seem to be related to either pollution or the absence of another required host or hosts which the parasite may require to complete its life cycle. Examination of the relationship between *E. danica* and *S. adipophila* found that the parasite was overdispersed within the host community and that there was no relationship between the larval length and the intensity of infection. Experimental work with *S. adipophila* specimens revealed that the parasite was stimulated to uncoil at high temperature (40°C) although no response, measured as a change in morphology, was recorded when exposed to internal and external fish secretions. The presence of an additional membrane boundary layer around the parasite and the possible detection of an intracellular developmental stage were observed through the use of a transmission electron microscope.

# Introduction and Aims

## Introduction

An article by Bennett (1994a), reported the first appearance of the mayfly parasite *Spirinella adipophila* (Avry and Delage 1966) in the British Isles. The presence of this parasite, coupled with pollution and low river flows was suggested to be associated with a dramatic decline in the mayfly population of the River Wey, a tributary of the River Thames. This project was initiated as an attempt to gather information about this enigmatic parasite. In 1966, Arvy and Delage proposed the name *Spirinella adipophila* for a protozoan which was found to parasitise the tissues of the mayfly nymph *Ephemera vulgata* L. Discovered in the Ezyies region of France, *S. adipophila* was believed to be endemic to that area. Six years later, Arvy and Peters (1972) proposed that the name be changed to *Spiriopsis adipophila* as Johnston (1941) had used the name *Spirinella* previously to designate a genus of Silurian Brachiopods (cited from Arvy and Peters 1972). Throughout this project, this parasite is therefore referred to as *Spiriopsis adipophila* in conjunction with the new name designated by Arvy and Peters (1972).

## Description of *Spiriopsis adipophila*

*Spiriopsis adipophila* was first described by Arvy and Delage (1966) as a vermiform organism contained within an oval chamber situated within and at the end of a long flat ribbon which is coiled about ten times. The ribbon is believed to be formed by secretion of protein into an envelope which develops to produces this characteristic coiled shape. This coil appears to be present in two distinct forms, here described as 'crinkled' and 'smooth'. The parasite is believed to communicate with the exterior by means of a small canal situated

near the outer end of the ribbon (Arvy and Delage 1966). Arvy and Delage have speculated that the ribbon acts to provide a floating device as means of transport for the parasite. The overall size of *S. adipophila* has been found to be relatively constant. The specimens examined during this work were found to have a mean coil size (taken from the measurement of 20 individuals isolated from *E. vulgata* from the River Kennet) of 69.8$\mu$m x 61.2$\mu$m, with the chamber in which the parasite is located of mean size 15.1$\mu$m x 8.3$\mu$m. These measures correspond closely to those found in the initial work by Arvy and Delage (1966).

**Classification**

The present lack of information about *S. adipophila* has meant that its classification is still debatable. Desportes *et al.*(1975) believe that *S. adipophila* is a protozoan belonging to the phylum 'Sporozoa' (= Apicomplexa). This name is often considered confusing since 'spores' are not present in many members of the group (Lee *et al.*1985). All members of the Apicomplexa are parasitic and some are extremely important disease causing agents. Among them are the malarial parasites of man and animals, the coccidia and piroplasms of animals and the gregarines of invertebrates (Lee *et al.*1985). Soldan (1980) places *S. adipophila* among the subclass Coccidia. Some species in this subclass are homoxenous and others heteroxenous (i.e have more than one type of host at different stages of their life cycle). Coccidians are found in the digestive tract epithelium, blood and other cells, and although found mainly in vertebrates may be present in invertebrates (Lee *et al.*1985).

**Host and Distribution**

Since its initial discovery in *E. vulgata* from the streams and rivers in the Ezyies region of France (Arvy and Delage 1966), *S. adipophila* has been documented in the other mayfly

species belonging to family Ephemeridae, and appears to be widely distributed throughout Europe. *Ephemera lineata* Eaton from Czechoslovakia (Soldan 1980) and *Ephemera danica* Müller from Luxembourg (Delvaux 1975) were found to be infected with *S. adipophila*, as were *Epheremra vulgata* specimens from Poland (Arvy and Sowa 1976). Most recently *S. adipophila* has been found in *E. danica* taken from the Thames catchment in England (Bennett 1994b). During this project *S. adipophila* was also discovered in *E. vulgata* taken from the River Kennet, which is also a tributary of the River Thames. *S. adipophila* has also been recorded in other mayfly families. Delvaux (1975) reported *S. adipophila* in *Ephemerella ignata* Poda and Bennett (1994b) found the same parasite in the tissues of *Heptagenia lateralis* Curtis, although the size of *S. adipophila* found in *H. lateralis* was recorded to be significantly smaller than those found in the Ephemeridae (Bennett 1994b). It can therefore be concluded that *S. adipophila* is far more widespread than originally believed. To date the most extensive survey is that of Bennett (1994b) who has reported the presence of *S. adipophila* in mayfly larvae sampled from many rivers in the Thames catchment.

**Effect of *S. adipophila* on its mayfly host**

It is not known from where, or when *S. adipophila* first appeared in the British Isles, and whether mayflies are the only host or whether other hosts are required for the parasite to complete its life cycle. The methods of transmission and reproduction of *S. adipophila* are also unknown. Added to this the effect, if any, of the parasite on the mayfly hosts have yet to be investigated. It has been speculated that *S. adipophila* is partly responsible for a decline in the mayfly numbers along certain rivers (Arvy and Delage 1966, Bennett 1994a), however as yet there is no direct evidence to support this claim. Soldan (1980) stated that

infections of *S. adipophila* are restricted to the parietal and visceral fat bodies of the mayfly larvae but could detect no histopathological effects on such tissues or increased mortality in heavily infected larvae. Arvy (1979), cited from Bennett(1994b), stated that large infections of *S. adipophila* totally destroy the adipose tissue although again, no increased mortality was observed. Delvaux (1975) documented that in certain rivers in Luxembourg, *E. danica* larvae, which usually has a semivoltine life cycle Elliot *et al.*(1988), were present in three generations and suggested that this was due to the presence of *S. adipophila* in the ovaries leading to a delay in the development of the eggs after laying. There was however no direct evidence that *S. adipophila* caused this delayed development of the mayfly eggs after laying, as such a three generation life cycle has been recorded in *E. danica* larvae in other rivers (Elliot *et al.*1988).

**The River Pang**

The work completed during this project took place within the catchment of the River Pang. (The *S. adipophila* specimens used in section two were also isolated from *E. danica* larvae obtained from the River Pang). The River Pang was chosen as the site of study partly because of its convenient location, but mainly because *E. danica* were found to be both abundant and 'heavily' parasitised by *S. adipophila* (Bennett, personal communication). The River Pang is situated at the eastern end of the Berkshire Downs in southern England, and is a tributary of the River Thames (Fig. 1). The Pang is predominantly groundwater-fed although the flow is supplemented by run off from tributaries draining the relatively impermeable tertiary outcrop to the south (Ruse 1993). The catchment area of approximately 173 $km^2$ is mainly rural with the only significant sewage discharge entering the river at Bradfield. No direct abstraction for drinking water occurs, however groundwater is

abstracted from the chalk catchment throughout the year, Ruse (1993).

**Aims**

To date, very little is known about the biology of *Spiriopsis adipophila* or its association with, and effects on, the mayfly host. The aim of this work was to attempt to provide more information about *S. adipophila* and its association with its mayfly host, which in this case was *Ephemera danica*. It was originally planned to carry out an extensive distribution survey of the Thames catchment area, but this had largely already been completed by Bennett (1994b). The work carried out during this project was split into two main sections. The first section involved an examination of the geographical distribution of *S. adipophila* within the River Pang catchment, which also allowed a more detailed examination of the epidermological relationship between *S. adipophila* and *E.danica*. The second section involved experimenting with isolated *S. adipophila* specimens with the aim of discovering something of its biology and life cycle. All the experimental work was completed in the laboratory at Fobney Mead, Reading. In addition, some ultrastructural work on *S. adipophila* was carried out in conjunction with Dr Whitfield of Kings College, London.

# Methods

## Section 1: General Methodology

### 1:1 Collection of *Ephemera danica* Larvae

*E. danica* larvae were collected using a standard Freshwater Biological Association (FBA) pond net with mesh size 1000 $\mu$m. *E.danica* larvae can be easily collected using the standard kick/sweep sampling technique once the appropriate habitat of a sandy or gravelly substrate has been located within the river.

### 1:2 Dissection: Isolation of *Spiriopsis adipophila* from *Ephemera danica* larvae

The method of dissection was that used by Bennett (1994b), who gave a personal demonstration of his technique. The body length of each *E. danica* specimen was recorded as the measure from the tip of head to the tip of the abdomen, such that the lengths of the antennae and the caudal filaments were not included. The dissection of each *E. danica* larva was carried out on a glass microscope slide. The abdomen was separated from the upper body posterior to the metathorax, laterally bisecting the larva. The upper body (consisting of the prothorax, mesothorax and metathorax) was discarded once the upper part of the gut had been removed. The lower gut and associated fatty tissues were then carefully removed from the abdomen, as this is where the majority of *S. adipophila* are located (Arvy and Delage 1966), after which the remainder of the abdomen was then also discarded leaving only the required material on the slide. The isolated material was macerated in a few drops

of water and examined under a compound microscope. Care was taken to ensure that the mounted needles (used to carry out the work) were thoroughly cleaned between each dissection so that no cross contamination occurred.

## 1:3 Counting *S. adipophila*

Numerous techniques for counting the number of *S. adipophlia* per nymph were experimented with. The method adopted did not record the absolute number of *S. adipophila* present, but was used as a standard technique throughout. All the results were therefore comparable. (Counting absolute numbers is more accurate but is also very time consuming). The slide prepared during the dissection was examined using a compound microscope at x160 magnification. Three horizontal transects were viewed across the slide (effectively examining 90 fields of view) and the number of *S. adipophila* present recorded using a click counter.

## Evaluation of counting procedure

The counting procedure was tested to evaluate whether it could be used as a standard method for counting the number of *S. adipophila* per nymph. It was necessary to determine if the position of the three transects examined on the slide would bias the results. To do this, two randomly chosen slides were examined by the above method five times with the number of *S. adipophila* counted each time being recorded. Using these results, a Chi-squared test was preformed with a null hypothesis that the distribution of *S. adipophila* on the slide was random. The results from the Chi-squared tests are shown overleaf.

Test slide 1. Chi-squared= 1.15679 ( N=5, P>0.05)

Test slide 2. Chi-squared= 1.03008 ( N=5, P>0.05)

In both cases the results accept the null hypothesis meaning that the distribution of *S. adipophila* on the slides was random, implying that the position of the three transects examined on each prepared slide did not bias the results.

## 1:4 Fish Dissection

Each fish was killed by a blow to the head followed by pithing. Using a scalpel blade a sample of mucus was scraped away from the skin and fins. A ventral cut was made from the buccal cavity to the vent, the digestive tract carefully removed in its entirety and the remainder of the fish discarded. The remainder of the dissection was performed using a binocular microscope at x60 magnification. The gall bladder was removed carefully, using watchmakers forceps. The digestive tract was then isolated and dissected open. Macroscopic food debris was removed and a scrape of the stomach and intestinal wall was taken. The dissection equipment was cleaned before each part of the dissection, with each isolated section being used immediately in the experimental work.

## Section 2: Survey of the River Pang

### Site selection

The survey of the River Pang involved examining *E. danica* larvae for the presence of *S. adipophila* from a total of 11 sites on the Pang, and 1 site on the River Bourne (Fig.2). In the initial survey, seven sites were selected to cover the entire length of the river (Fig.2). This was then increased through a more extensive survey which involved five additional sites, four on the Pang, and one on the River Bourne (Fig.2). The sites from Tidmarsh to Folly Bridge are shown in Figures 3,4 and 5.

### 2:1 Site Sampling Procedure

In the initial survey, 20 *E. danica* larvae were collected from each of the 7 selected sites (Fig. 2) unless none could be found after a 20 minute search. The individuals were preserved in 10% Neutral Buffered Formalin on site. In the secondary survey of the additional 5 sites, (Fig.2), 10 individuals were collected and preserved in the same fashion. Finally, 30 additional larvae were taken from the Tidmarsh site, as a more extensive survey of the *E. danica* community was performed. The larvae collected from each site were returned to the laboratory and the number of *S. adipophila* per nymph recorded using the dissection and counting techniques outlined in methods section 1.

### Macroinvertebrate Sampling

The macroinvertebrate communities were examined at two selected sites, Folly Bridge and the site at the confluence of the rivers Pang and Bourne (Fig 2). Comparisons of the macroinvertebrate communities were made between these sites as *S. adipophila* was found

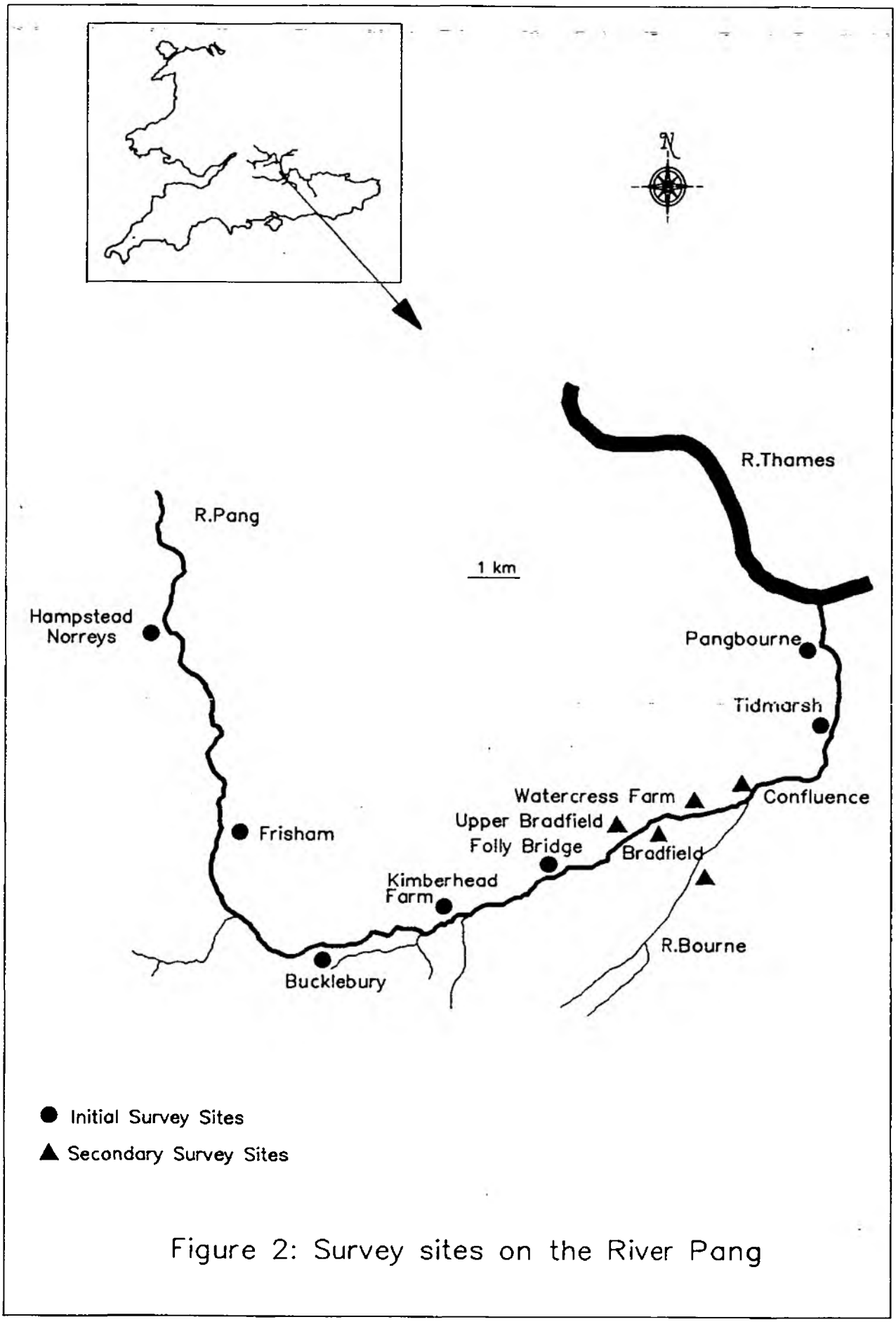



Figure 2: Survey sites on the River Pang

to be present in the *E. danica* larvae at the confluence but absent at Folly Bridge (discussed in more detail in Results Section 1). It was originally planned to also sample the fish communities at these sites, however this could not be arranged in the time available.

## 2:2 Macroinvertebrate Sampling Procedure.

The macroinvertebrate communities were sampled using an FBA pond net and the standard sampling methodology outlined by Armitage (1983). This sampling technique involves a 3 minute kick/sweep sample followed by a one minute stone search, which ensures that all the habitats present are examined. The macroinvertebrates collected were brought back to the laboratory where they were identified to family level. Each site was then given a score using the BMWP scoring system (Armitage *et al*,1983) and finally, the similarity of the sites was examined using Sorensons Quotient of Similarity (Hellawell 1986).

## Section 3:Experimental Materials & Methods

The *E. danica* larvae used in the experimental work were all collected from the confluence site (Fig. 2), as *S. adipophila* was known to be present at this location. The larvae were brought back to the laboratory alive and kept in water at 15°C, oxygenated by an aquarium air pump. This method of storage proved effective at keeping the larvae alive for short periods of time (2-3 days). The larvae were not preserved as viable *S. adipophila* specimens were required for the experimental work.

### Experiment 1. Examination of *S. adipophila* at different temperatures

The individual *E. danica* larvae used for this experiment were measured, then killed by decapitation. Three larvae were placed into a petri dish which contained sterilised River Pang water. In all twelve such petri dishes were required such that the experiment could run over the required period of time. Four dishes were placed in a fridge set at 8°C, four into an incubator set at 14°C, and the final four were placed into an incubator set at 40°C. One dish from each set of conditions was removed after ½,1,2 and 3 days, such that the larvae could be dissected and the number of *S. adipophila* recorded. The total number of *S. adipophila* present was not as important as the form of the parasite. *S. adipophila* were classified as either 'crinkled', 'smooth' or 'uncoiled', and the number of each form recorded. Three freshly killed larvae were also examined as controls at the start of the experiments. Photographs were taken of some of the prepared slides.

**Experiment 2. Examination of the same *S. adipophila* specimens at 40°C**

A freshly killed nymph was dissected and a slide prepared using the method outlined in Section 1:1. A small rectangle (2cm by 4cm) was marked with an indelible pen on the centre of the slide and the number of each form of *S. adipophila* inside was recorded. By permanently marking a small area on the slide, the same *S. adipophila* specimens could be monitored. In this case as only a small area was being examined all the *S. adipophila* present were counted. The slide was placed in a small petri dish containing some wet tissue paper, then placed in an incubator set at 40°C. The wet tissue was in the petri dish in an attempt to slow the drying out of the slide. The marked area of the slide was then examined after various time intervals and each time the number of each form of *S. adipophila* present was recorded.

**Experiment 3. Examination of *S. adipophila* form when exposed to internal and external fish secretions**

*S.adipophila* specimens were removed from four freshly killed *E. danica* larvae and slides prepared using the dissection technique outlined in methods section 1:1. A Bullhead (*Cottus gobio*) was dissected using the technique outlined in method section 1:4. The isolated skin mucus was placed on one of the prepared slides. The gall bladder was ruptured above the second slide so exposing the *S. adipophila* to the bile fluid. The stomach acid and mucosal cell scrape were placed on the third slide and the intestinal scrape added to the final slide. Each slide was labelled and then marked with a small square using an indelible pen. This ensured that the same *S. adipophila* specimens were examined each time. The slides were

then examined immediately and again after an hour with the number of 'crinkled', 'smooth' and 'uncoiled' forms of *S. adipophila* being recorded. This procedure repeated using a Nine-spined stickleback (*Pungitius pungitius*), a Stone Loach (*Noemacheilus barbatulus*) and the combined material from three Three-spined sticklebacks (*Gasterosteus aculeatus*).

## Section 4: Transmission Electron Microscope Studies on *S. adipophila*

The ultrastructure examination was carried out in conjunction with Kings College, London. *E. danica* larvae were collected from the confluence site (Fig.2), and then transported live to Kings college, London. The larvae were dissected using the standard technique, with the alimentary tract removed and cut into small sections. These sections were placed into 2.5% gluteraldehyde in 0.1M phosphate buffer at pH 7.4 for 1 hour at 4°C. The remainder of the work was completed by Dr Whitfield at Kings College. After several washes with the same phosphate solution, regions of the fat bodies containing *S. adipophila* were dissected away from the alimentary tract. These fat bodies were subject to secondary fixation using 1% osmium tetroxide in 0.1M phosphate buffer for 1 hour at 4°C. Specimens were dehydrated in increasing ethanol concentrations and embedded in spur's resin. Block curing was achieved by 2 days at 60°C after which thin sections were obtained with a LKB ultramicrotome and stained with uranyl acetate and lead citrate. The grids were examined with a Joel JEM-1000X11 transmission electron microscope.

# Results

## Section 1: Survey of *E. danica* and *S. adipophila* in the River Pang Catchment

### Distribution of *E. danica* and *S. adipophila*

Figure 6, representing both surveys, shows the distribution of both *E. danica* larvae and *S. adipophila* within the Pang catchment. In the initial survey, *E. danica* larvae were abundant at all sites below Kimberhead Farm but were absent from all the sites sampled upstream of this point, although potentially ideal habitat was present. The distribution of *S. adipophila*, which is related to the distribution of its *E. danica* host, was also be seen to be limited in that it was present in the *E. danica* larvae at Tidmarsh but absent from the larvae at Folly Bridge. From the secondary survey, which involved a more extensive examination of the Pang between these two sites, *S. adipophila* was found to be present in the *E. danica* larvae as far upstream as Bradfield. Figure 8 shows the small weir which appeared to be the only influence on the river between the sites where *S .adipophila* was present (Bradfield) and where it was absent (Upper Bradfield). Both *E. danica* and *S. adipophila* were present in the river Bourne.

Figure 7 outlines in more detail the change which occurred in the prevalence of *S. adipophila* in the *E. danica* larvae along the length of the Pang. Prevalence is defined as the number of individual *E. danica* larvae containing *S. adipophila*, divided by the number of hosts examined which is then expressed as a percentage (Margolis *et al*, 1982). A marked decrease in the prevalence of *S. adipophila* occurred between Watercress Farm and Bradfield (100% prevalence - 40% prevalence). *S. adipophila* was then found to be absent from all

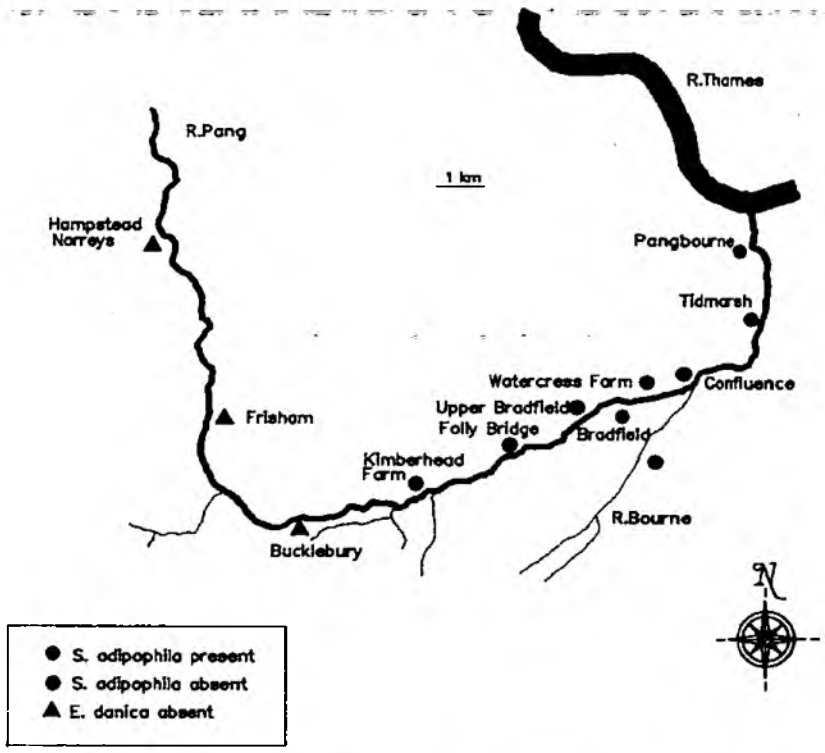



Figure 6: Distribution of E. danica and S. adipophila within the Pang catchment

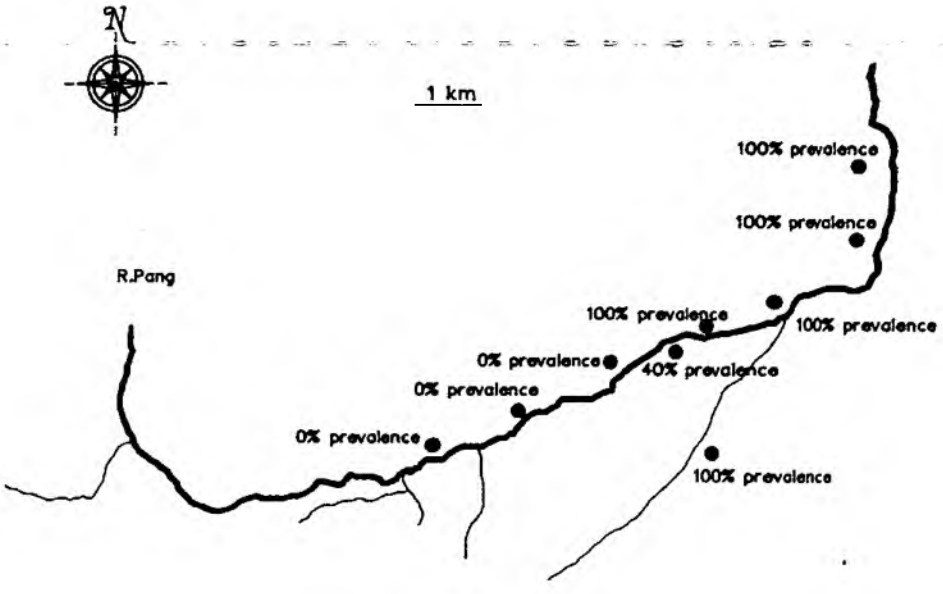



Figure 7: Prevalence of S. adipophila in E. danica in the Pang catchment

sites upstream of Bradfield. A decrease in the mean number of *S. adipophila* per larva was observed to begin upstream of the confluence of the Pang and the Bourne, (Table 2).

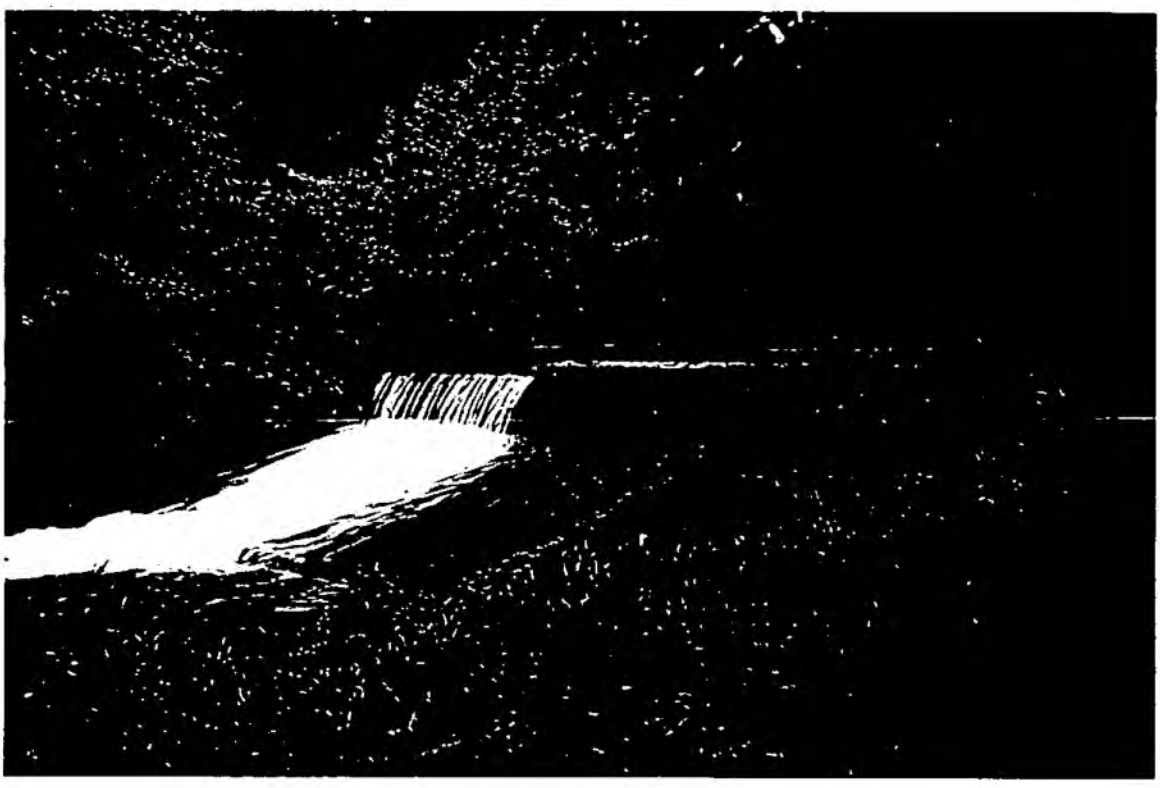


**Figure 8. The weir at Bradfield**

## Comparison of macroinvertebrate communities

Comparisons were made between the macroinvertebrate communities at Folly Bridge, where *S. adipophila* was absent, and at the confluence site where *S. adipophila* was present.

### Macroinvertebrates

The macroinvertebrate communities at Folly Bridge and the confluence site are shown in Table 1. From these results the similarity of the macroinvertebrate communities was calculated using Sorensons Quotient of Similarity. Each site was also scored using the BMWP score system (Armitage 1983).

Sorensons Quotient of Similarity (I):

$$I = \frac{2c}{(a+b)}$$

where a= number of families in community A
b= number of families in community B
c= number of families common to both

The calculated value of I ranges between 0 and 1, where 0= complete dissimilarity between sites, and 1= complete similarity. For Folly Bridge and the confluence site, the calculated value of I= 0.83, indicating that the macroinvertebrate communities at these two sites were very similar in composition.

**BMWP scores:** Folly Bridge 105, Confluence site 114

Both the calculated BMWP scores indicate good water quality.

**Table 1.** Macroinvertebrate Communities at the Folly Bridge and Confluence Sites

| TAXA | FOLLY BRIDGE | CONFLUENCE |
|---|---|---|
| Ephemerellidae | * | * |
| Ephemeridae | * | * |
| Odontoceridae | * | * |
| Goeridae | * | * |
| Sericostomatidae | * | * |
| Agriidae | * | |
| Rhyacophilidae | * | * |
| Limnephilidae | * | * |
| Ancylidae | | * |
| Gammaridae | * | * |
| Dytiscidae | * | * |
| Elmidae | | * |
| Hydropsychidae | | * |
| Tipulidae | * | * |
| Simuliidae | * | * |
| Piscicolidae | | * |
| Hydrobiidae | | * |
| Sphaeriidae | * | |
| Glossiphoniidae | * | * |
| Chironomidae | * | * |
| Oligochaeta | * | * |

## Relationship between *E.danica* and *S. adipophila*

### *E. danica* larval length and numbers of *S. adipophila* per larva

Figure 9 shows the relationship between larval length and the number of *S. adipophila* per larva. This graph represents the combined data from all of the infected *E. danica* individuals encountered. The data was examined to see whether a linear relationship existed between these two variables. As the data is not normally distributed a parametric test such as Pearsons product-moment correlation could not be applied without first performing a transformation (Elliot 1977). Spearman's rank correlation provides a suitable non-parametric test under such circumstances.

**Spearman's rank correlation ($r_s$)**

$$r_s = 1 - \frac{6(\sum d^2)}{n(n-1)}$$

where : $d^2$ = square of the difference between each pair of ranked values
n = number of pairs of observations

The value of $r_s$ ranges between -1 and +1 (-1= complete discordance between the ranked variables and +1= complete concordance.)

The calculated value of $r_s = -0.01$.

For $n > 10$, the null distribution of $r_s$ is similar to that of Pearsons product-moment correlation co-efficient (r). As n=120, the significance of the calculated $r_s$ value is determined by comparing it against critical tabulated values of r. The calculated value of $r_s$ is less than the critical value of r (r= 0.176, p=0.05), from which it can be concluded that, in this case, there was no significant relationship between larval length and the number of *S. adipophila* per larva.

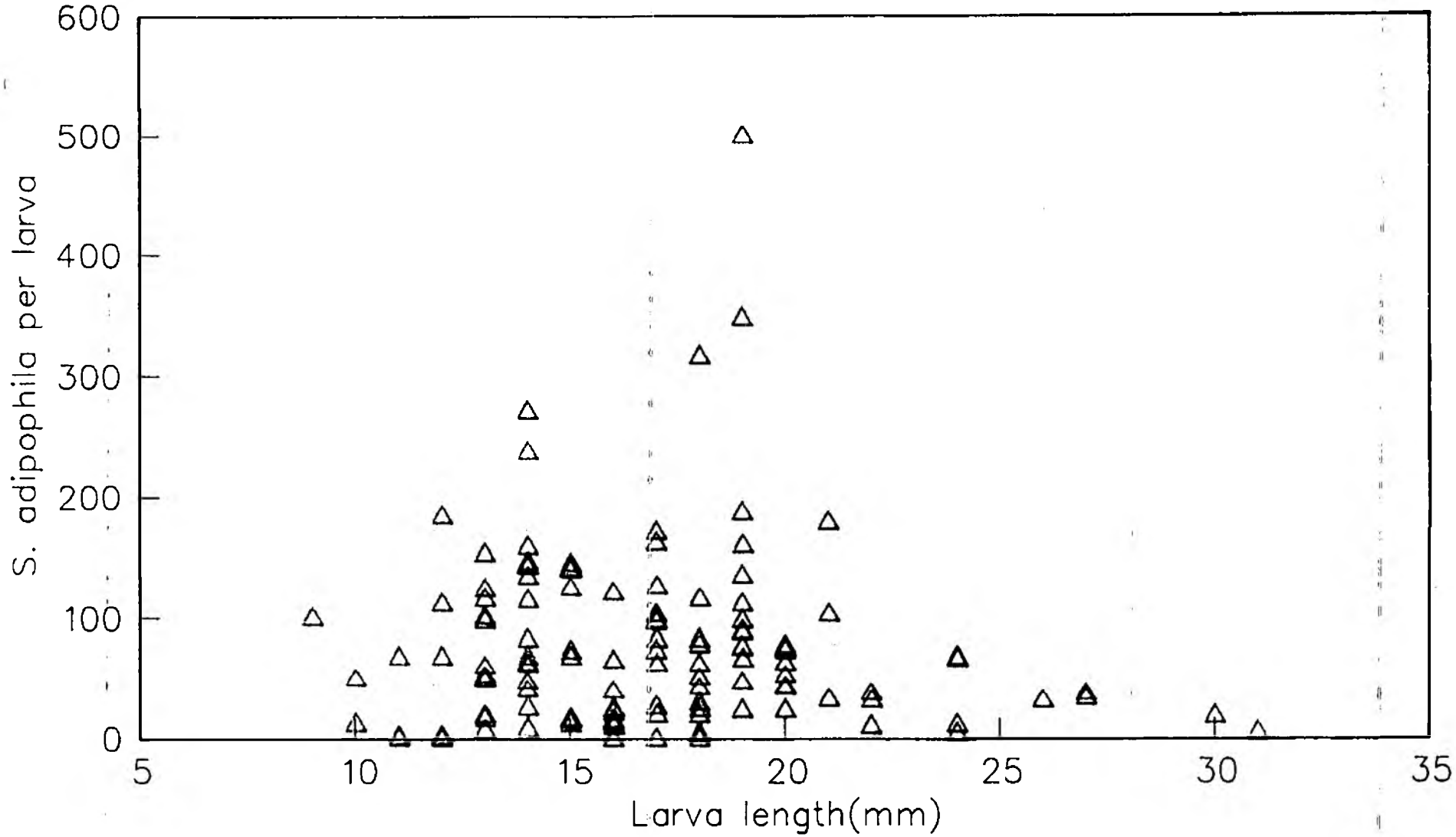



Figure.9: Relationship between S. adipophila number and E danica length

**_S. adipophila_ distribution within the _E. danica_ larval community**

Table 2 shows the distribution of *S. adipophila* within the *E. danica* larval community at each site surveyed. The distribution was examined by calculating the variance to mean ratio ($s^2/\bar{x}$), also referred to as the *index of dispersion*, where:

$$Variance(s^2) = \frac{\sum x^2 - \frac{(\sum x)^2}{n}}{N-1}$$

$$Mean(\bar{x}) = \frac{\sum x}{n}$$

n = number of sampling units in a sample
x = counts for a series of sampling units

The $s^2/\bar{x}$ ratio will approximate to unity when a distribution is random. For each site the variance to mean ratio was > 1 indicating a contagious distribution of *S. adipophila* within the *E. danica* larval community, a distribution commonly referred to as an aggregated distribution or overdispersion (Whitfield 1979).

Distribution of *S. adipophila* within the *E. danica* larvae can also examined by approximating the variance to mean ratio to Chi-squared using the equation:

**Chi-squared** = $I$**(n-1)** where $I$ = index of dispersion

For each site the calculated Chi-squared values lay outside the appropriate 5% (and 1%) significance level again indicating a contagious distribution of *S. adipophila* within the *E. danica* larval community. This overdispersion of *S. adipophila* in the *E. danica* larval community at each site is seen more clearly in Figure 10, and is especially obvious at the Tidmarsh site where the most extensive survey work was carried out. The high *S. adipophila* numbers found in certain larvae would only have a very small probability of occurring if *S. adipophila* were distributed at random through the host larval population.

**Table 2.** Distribution of *Spiriopsis adipophila* within *Ephemera danica* larvae

| Site (Grid Ref.) | *E. danica* sample size | *S.adipophila* mean number per larva | Variance | Variance/mean ratio | Chi-Squared Test | Distribution |
|---|---|---|---|---|---|---|
| Pangbourne (SU/63507650) | 20 | 106 | 2210 | 24.8 | 471.8 | Contagious |
| Tidmarsh (SU/63307370) | 50 | 89.1 | 5444 | 51.3 | 2513.7 | Contagious |
| Confluence (SU/62407350) | 10 | 61.4 | 832 | 13.5 | 121.5 | Contagious |
| Watercress Farm (SU/60707300) | 10 | 18.1 | 162 | 9.0 | 81.0 | Contagious |
| Bradfield (SU/60307280) | 10 | 1.4 | 5.6 | 4.0 | 36.0 | Contagious |
| R. Bourne (SU/62207320) | 20 | 69.2 | 11618 | 167.9 | 3189.9 | Contagious |

**Variance to mean ratio:** > 1 suggests a contagious distribution
= 1 suggests a random distribution
< 1 suggests a regular distribution

**Chi-squared ($\chi^2$):** The $\chi^2$ values all clearly lie outside the 5% significance levels (Appendix Fig.1). In each case a contagious distribution is indicated.

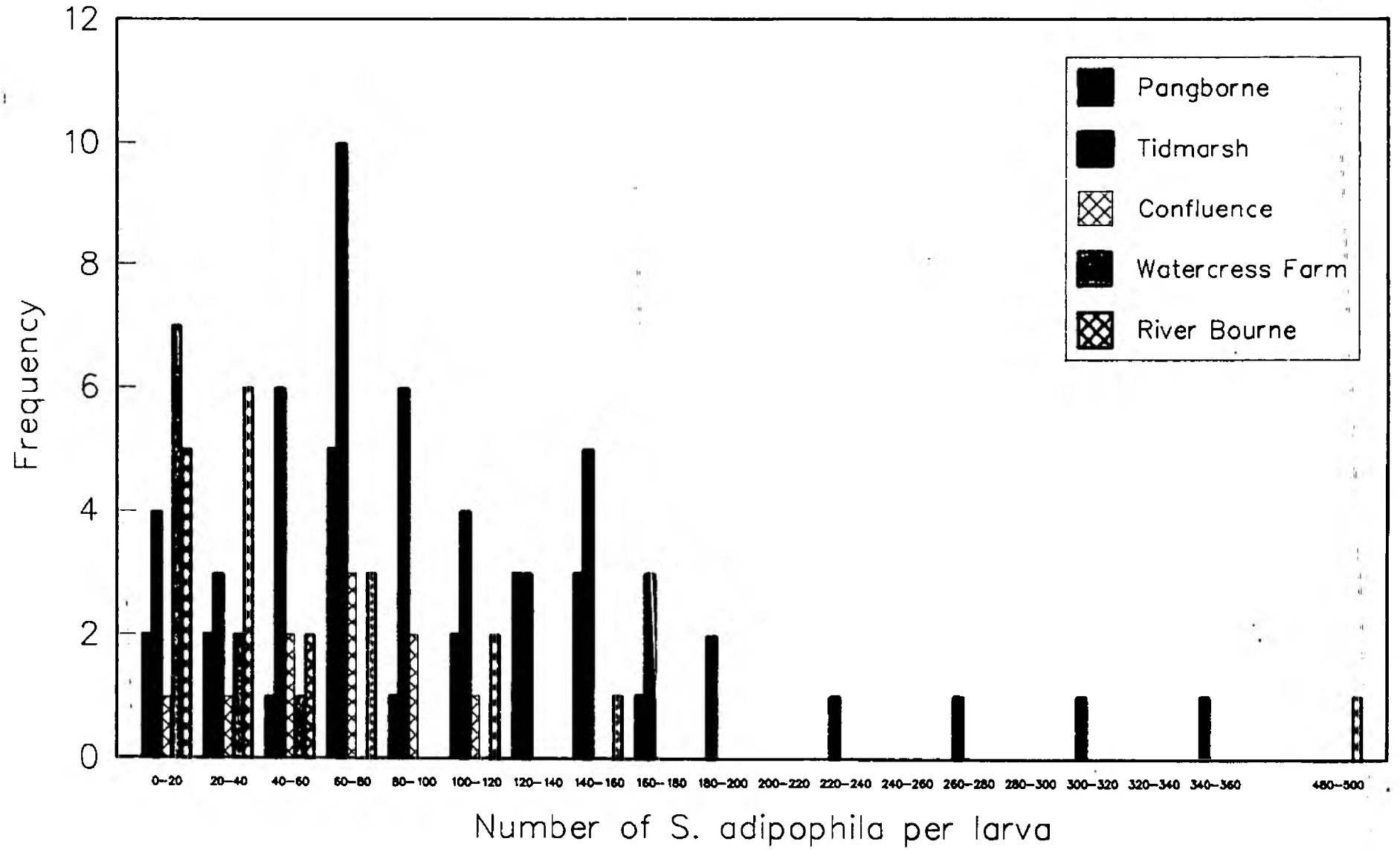



Figure 10: Frequency of the number of S.adipophila per E.danica larvae

## Section 2: Experimental results

### Experiment 1. Examination of *S. adipophila* form at varying temperatures

The results from experiment 1 are represented by Figures 11,12 and 13. The 'crinkled', 'smooth' and 'uncoiled' forms of *S. adipophila* are shown in Figures 15 and 16. The 'crinkled' form of *S. adipophila* accounted for 90% of the total in the initial count (Figures 11-13), so would appear to be the form commonly adopted by *S. adipophila* in live *E. danica* larvae.

The results for the form of *S. adipophila* at 8°C and 14°C follow a similar pattern. As the length of time of exposure to these temperatures increases, the percentage of 'smooth' *S. adipophila* found in *E. danica* larvae increases, with an associated decrease occurring in the presence of the 'crinkled' form. The extent of this change is greater at 14°C. At both temperatures it took 72 hours for the 'smooth' form to become more abundant than the 'crinkled' form. The percentage of 'uncoiled' *S. adipophila* remained relatively constant throughout.

At 40°C, there was a similar increase in the percentage of the 'smooth' form of *S. adipophila* again associated with a decline in the percentage of the 'crinkled' form. This change occurred over a much shorter time period than at 8°C or 14°C. After 24 hours at 40°C, over 80% of the *S. adipophila* isolated from the larvae had uncoiled. After 48 hours at 40°C, only the uncoiled form of *S. adipophila* was present in the larvae.

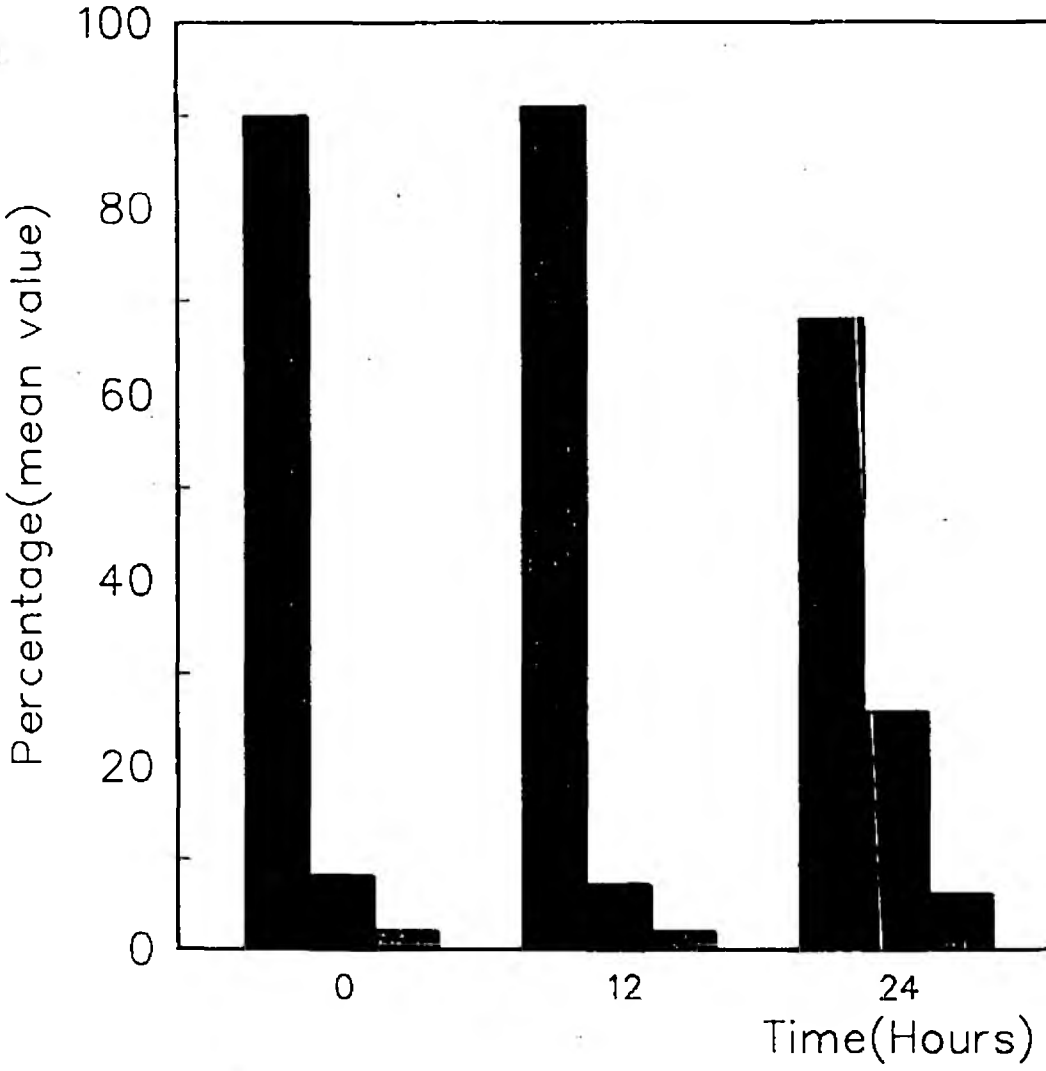
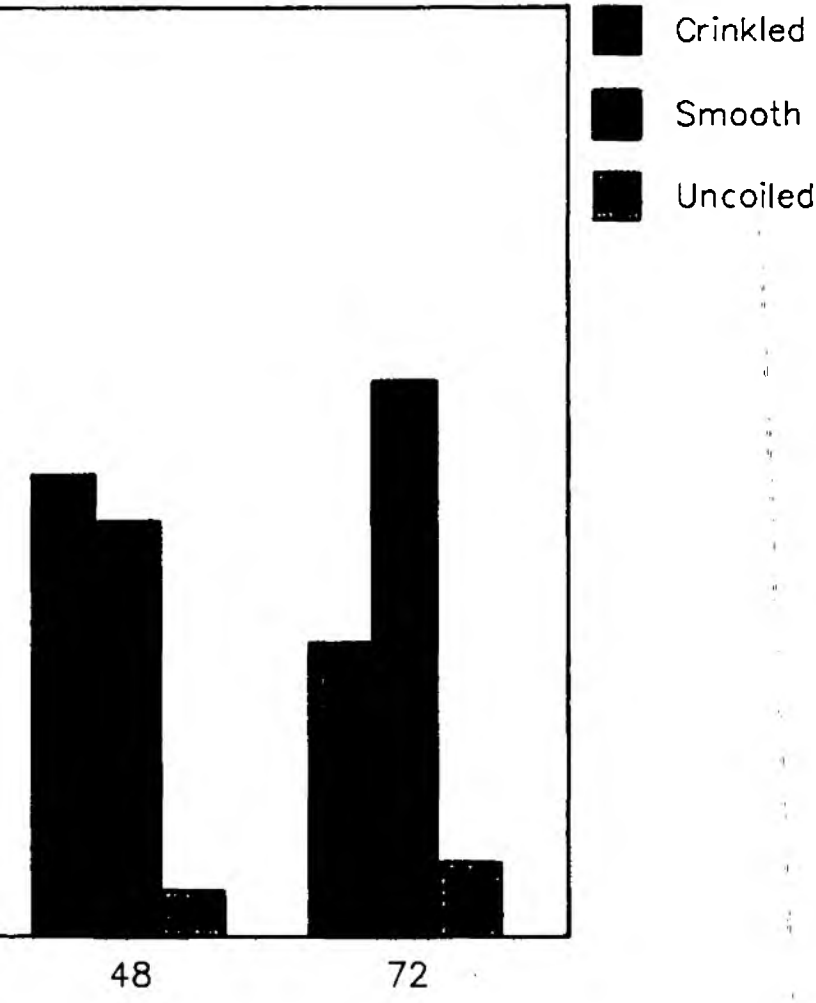



Figure 11: Morphology of S.adipophila at 8'C


Crinkled
Smooth
Uncoiled
48
72

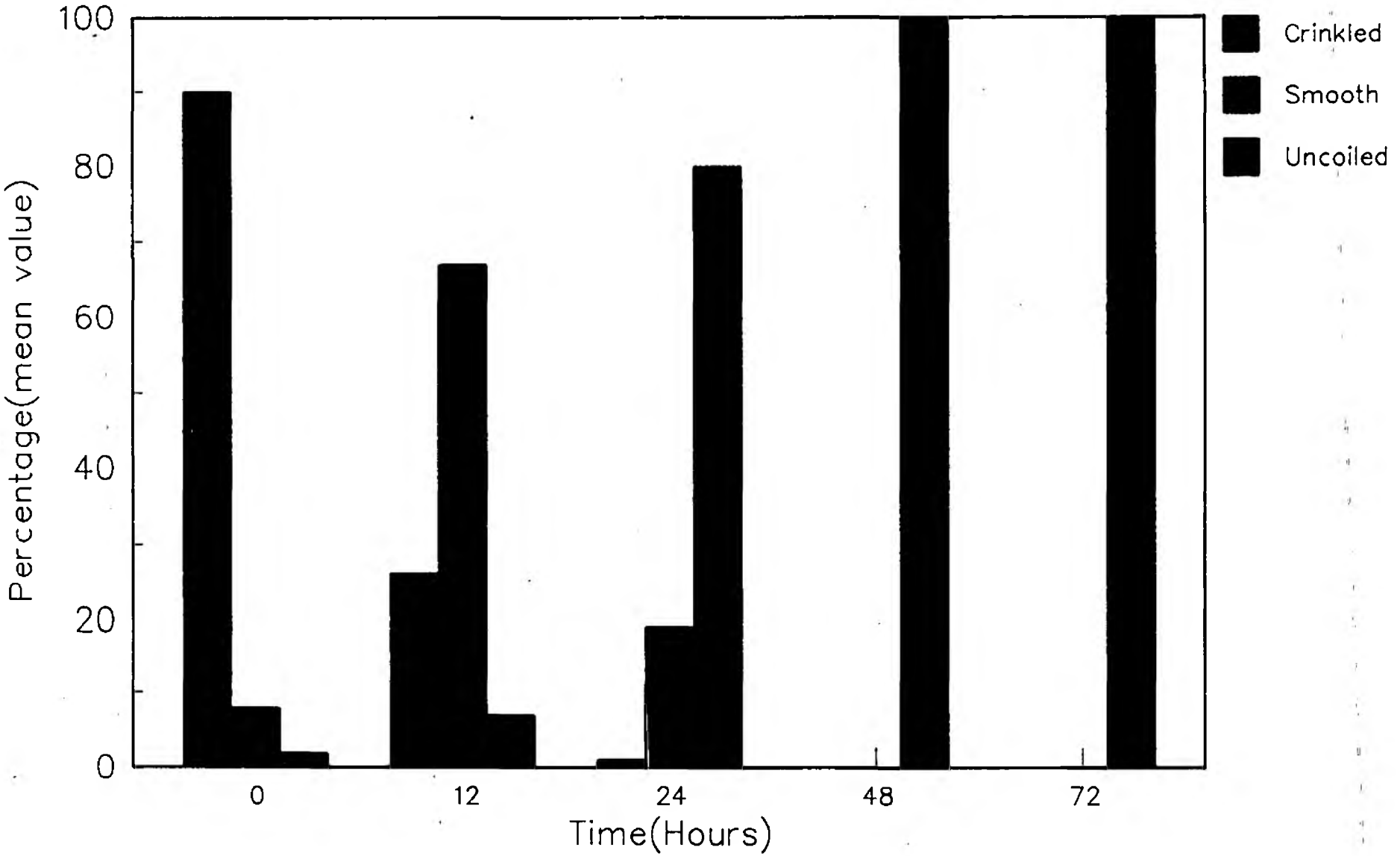



Figure 13: S.adipophila morphology at 40'C

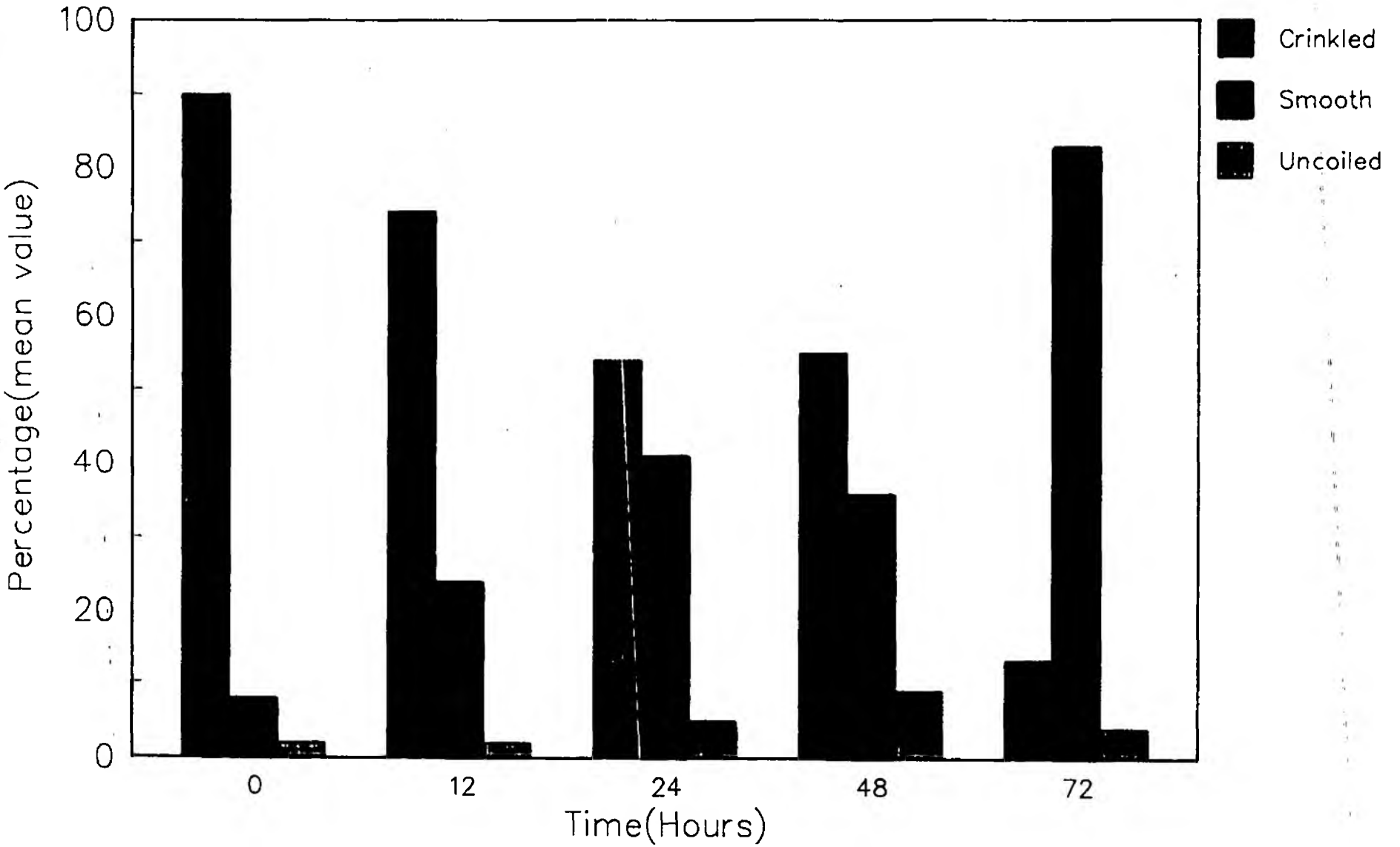



Figure 12. Morphology of S.adipophila at 14'C

**Experiment 2. Examination of the same *S. adipophila* specimens at 40°C**

In this experiment, which lead on from experiment 1, the form adopted by the same *S. adipophila* specimens at 40°C was recorded after various time intervals. This contrasts with experiment 1 in which different *S. adipophila* specimens were examined at each temperature and time interval. The experiment was originally designed to run over 48 hours, however the prepared slide dried out after only 24 hours.

The results from experiment 2 are represented in Figure 14. As with experiment 1, initially (t=0) over 90% of the *S adipophila* individuals present were 'crinkled' in form. This situation remained the same for the first two hours, such that no immediate changes in *S. adipophila* form were observed. After 12 hours exposure to 40°C a change in the *S. adipophila* specimens had occurred in that 89% of the specimens were now present in the 'smooth' form. After 24 hours exposure to 40°C, all the *S. adipophila* were either 'smooth' or 'uncoiled'. As the same individuals were monitored throughout the experiment, the results suggest that individual *S. adipophila* specimens are capable of adopting the three described forms.

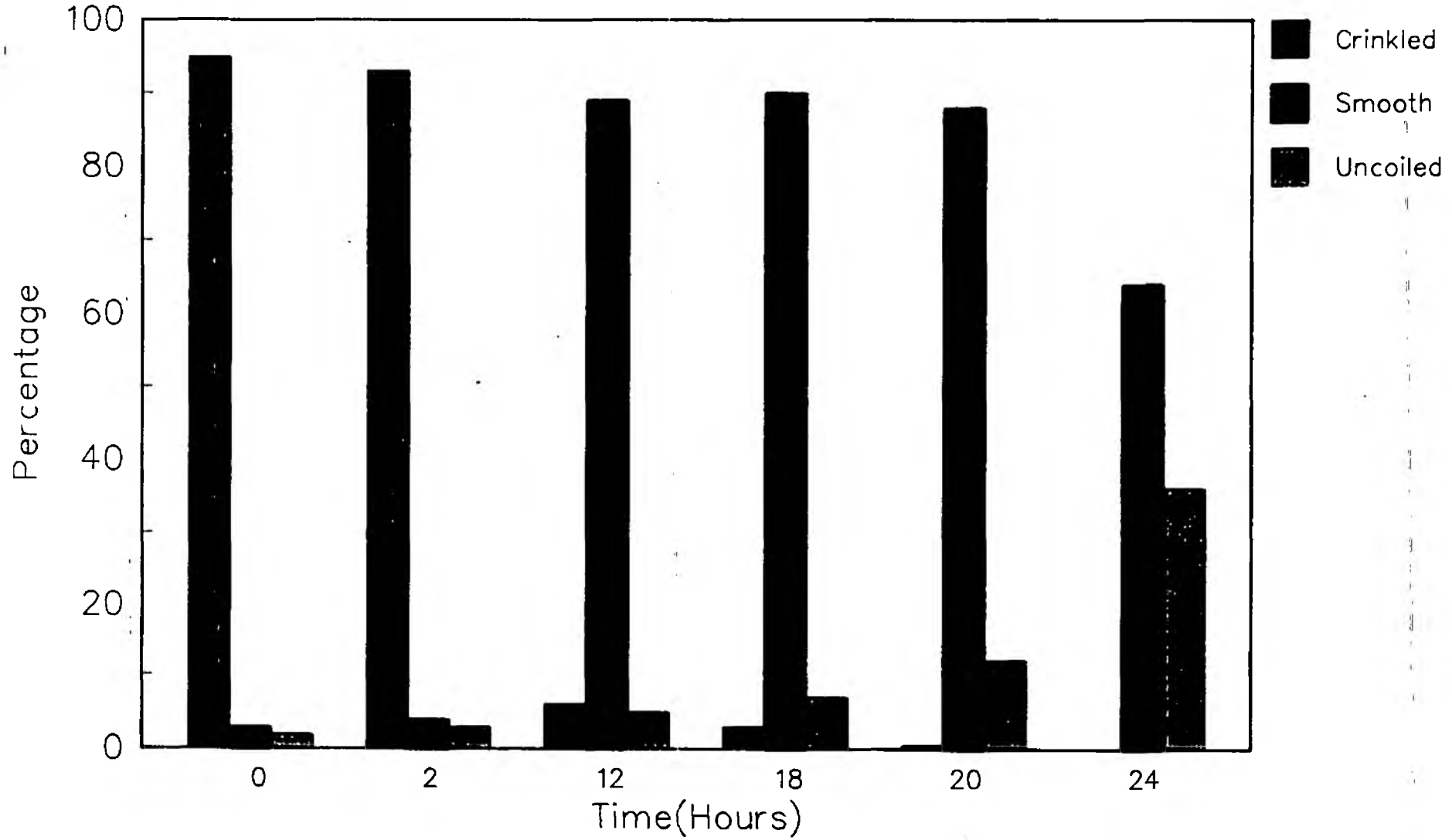



Figure 14: Change in Morphology of Individual S. adipophila Specimens at 40'C

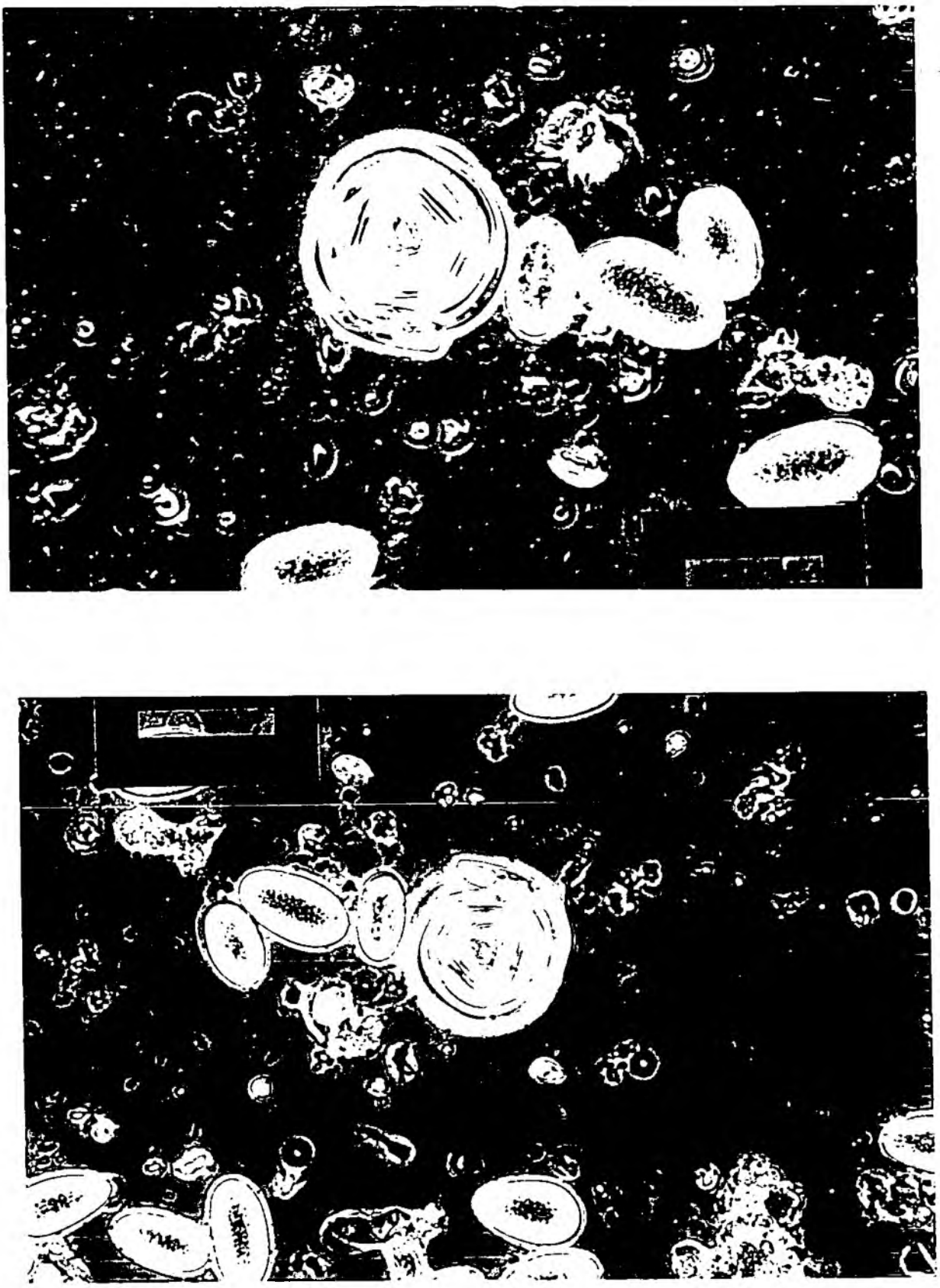


Figure 15: 'Crinkled' form of *S. adipophila*

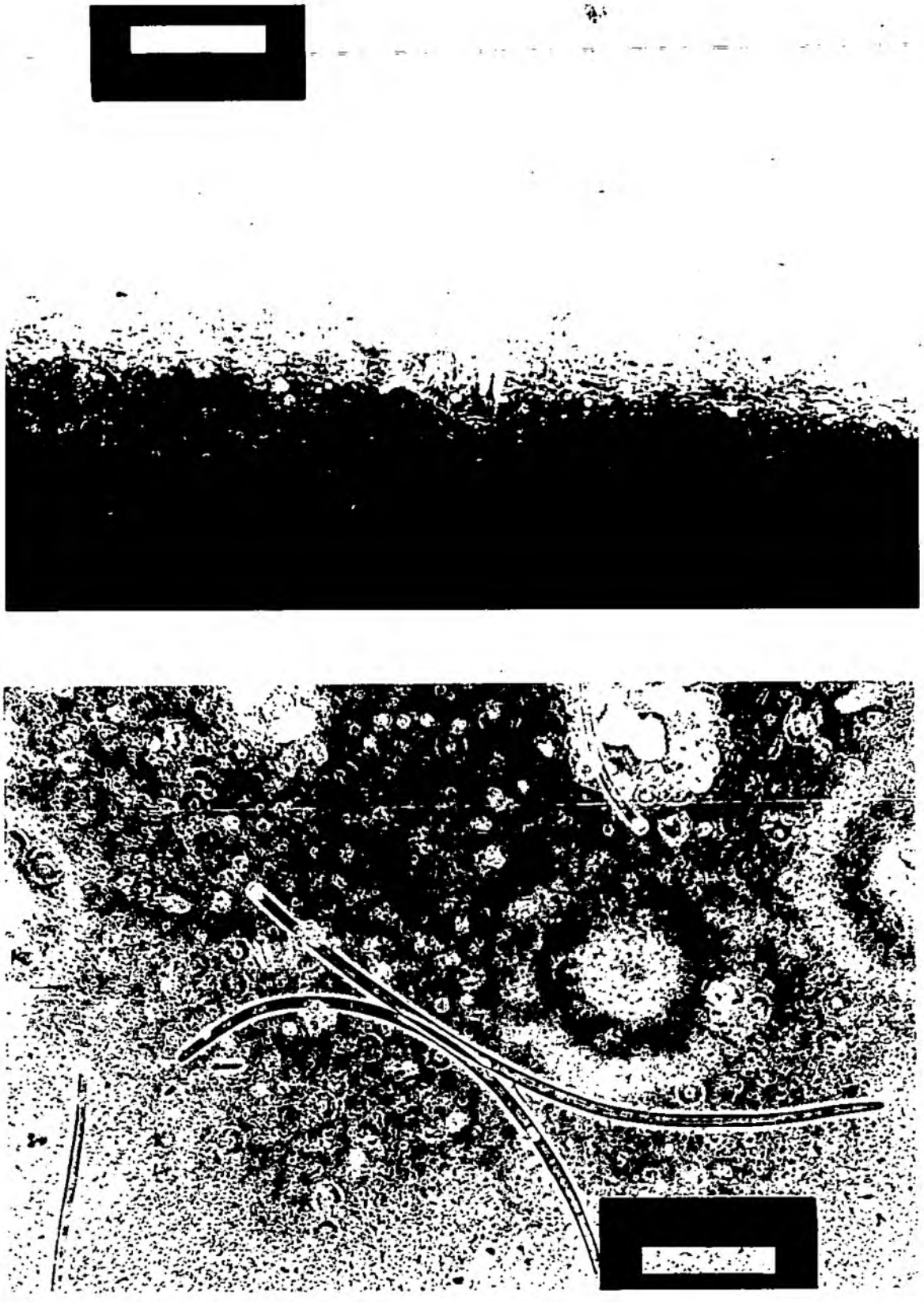


**Figure 16: The 'smooth' and 'uncoiled' forms of *S. adipophila***

**Experiment 3. Examination of *S. adipophila* morphology when exposed to internal and external fish secretions**

Tables 3-6 represent the results from this experiment. The *S. adipophila* specimens exposed to the internal and external secretions of all four fish remained unchanged in form over the length of the experiment. As with the previous experiments, in the initial count (t=0 hours) the 'crinkled' form of *S. adipophila* is the most abundant on each prepared slide. This again suggests that this is the commonest form of *S. adipophila* in *E. danica* larvae. This situation remained the same in the final counts (t=1 hour). On no occasion did a significant change in *S. adipophila* morphology occur in response to the fish secretions.

The experiment was originally designed to run over a longer time period but one problem encountered during this experiment was that only a small amount of each secretion could be obtained from each fish, such that the slide dried out very quickly. A second identified problem was that the fish had been kept captive for at least seven days, which may have affected their metabolism, in turn possibly affecting the composition of their body fluids and secretion.

**Table 3. Effect of the secretions of a Three-spined stickleback (*Gasterosteus aculeatus*) on the morphology of *S. adipophila***

| Secretion | Initial Count (t=0 hours) | | | Final Count (t=1 hour) | | |
|---|---|---|---|---|---|---|
| | crinkled | smooth | uncoiled | crinkled | smooth | uncoiled |
| Skin mucus | 22 | 3 | 1 | 19 | 1 | 0 |
| Bile | 21 | 2 | 0 | 22 | 1 | 0 |
| Stomach acid | 16 | 4 | 0 | Slide dried out | | |
| Intestinal fluid | 34 | 11 | 0 | 23 | 8 | 0 |

**Table 4. Effect of the secretions of a Bullhead (*Cottus gobio*) on the morphology of *S. adipophila***

| Secretion | Initial Count (t=0 hours) | | | Final Count (t=1 hour) | | |
|---|---|---|---|---|---|---|
| | crinkled | smooth | uncoiled | crinkled | smooth | uncoiled |
| Skin mucus | 31 | 0 | 1 | 31 | 0 | 1 |
| Bile | 34 | 0 | 0 | 38 | 1 | 0 |
| Stomach acid | 75 | 4 | 0 | 71 | 2 | 1 |
| Intestinal fluid | 19 | 1 | 0 | 19 | 2 | 0 |

**Table 5. Effect of the secretions of a Stoneloach (*Noemacheilus barbatulus*) on the morphology of *S. adipophila***

| Secretion | Initial Count (t=0 hours) | | | Final Count (t=1 hour) | | |
|---|---|---|---|---|---|---|
| | crinkled | smooth | uncoiled | crinkled | smooth | uncoiled |
| Skin mucus | 82 | 2 | 1 | 91 | 2 | 1 |
| Bile | 50 | 1 | 0 | 53 | 3 | 0 |
| Stomach acid | 65 | 3 | 1 | 57 | 3 | 1 |
| Intestinal fluid | 41 | 1 | 0 | 39 | 1 | 1 |

**Table 6. Effect of the secretions of a Nine-spined stickleback (*Pungitius pungitius*) on the morphology of *S. adipophila***

| Secretion | Initial Count (t=0 hours) | | | Final Count (t=1 hour) | | |
|---|---|---|---|---|---|---|
| | crinkled | smooth | uncoiled | crinkled | smooth | uncoiled |
| Skin mucus | 36 | 1 | 0 | 41 | 0 | 0 |
| Bile | 29 | 2 | 1 | 28 | 4 | 1 |
| Stomach acid | 16 | 1 | 0 | Slide dried out | | |
| Intestinal fluid | Slide dried out | | | Slide dried out | | |

## Section 3: Transmission Electron Microscope studies on *S. adipophila*

Figures 17-22 represent the images obtained from the transmission electron microscope work that was carried out in collaboration with Dr Whitfield at Kings College, London.

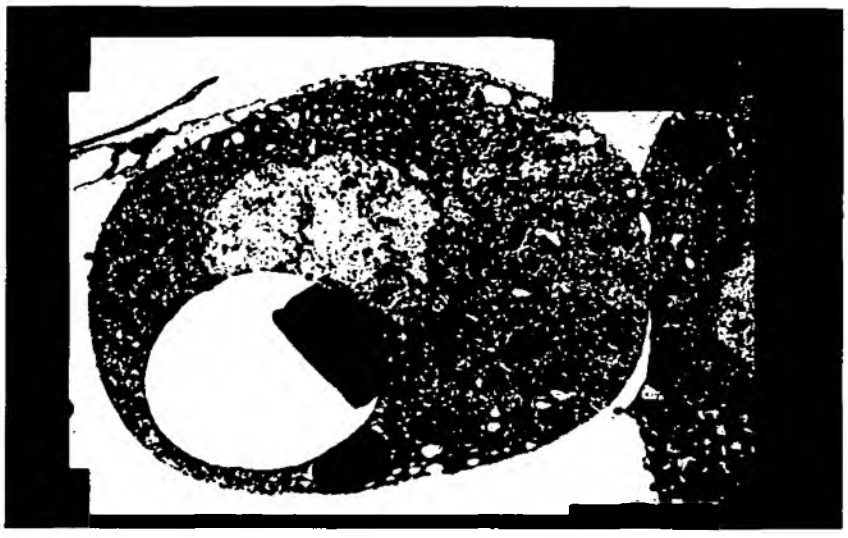


**Figure 17: Fat body cell (x4,800)**

The rounded fat body cell is approximately 15$\mu$m in diameter with one large lipid inclusion (with artefactual damage) approximately 6$\mu$m in diameter.

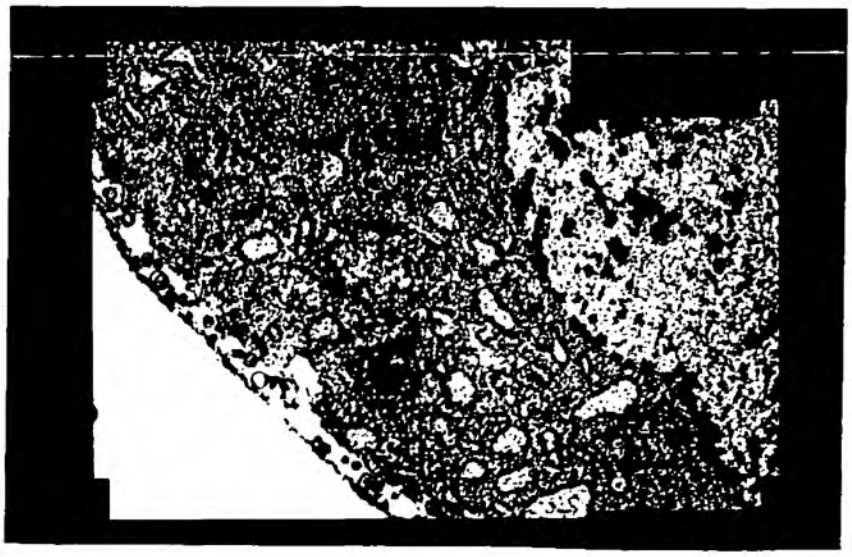


**Figure 18: Detailed fat body cell (x19,000)**

In this image the cell margin, rough endoplasmic reticulum and part of the nucleus can be seen.

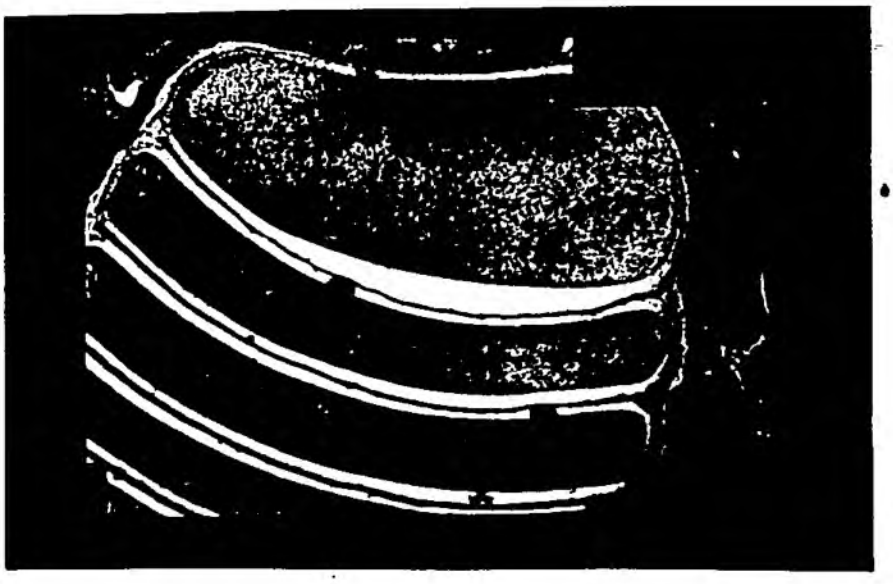


**Figure 19: *S. adipophila* spiral *in situ* surrounded by fat body cells (x3,600)**

The image shows a transverse profile of parts of 5 turns of the parasite enclosing spiral. The spiral disk thickness is between 20-25$\mu$m.

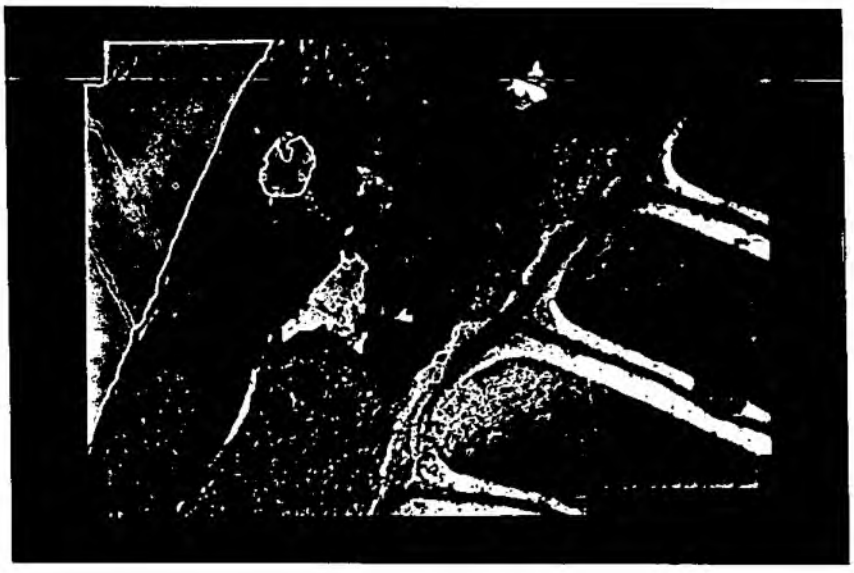


**Figure 20: *S. adipophila* spiral and surrounding fat body cells (x7,200)**

This image shows the transverse profiles through parts of 4 turns of the parasite enclosing spiral and the interface between the spiral an intact surrounding fat body cells.

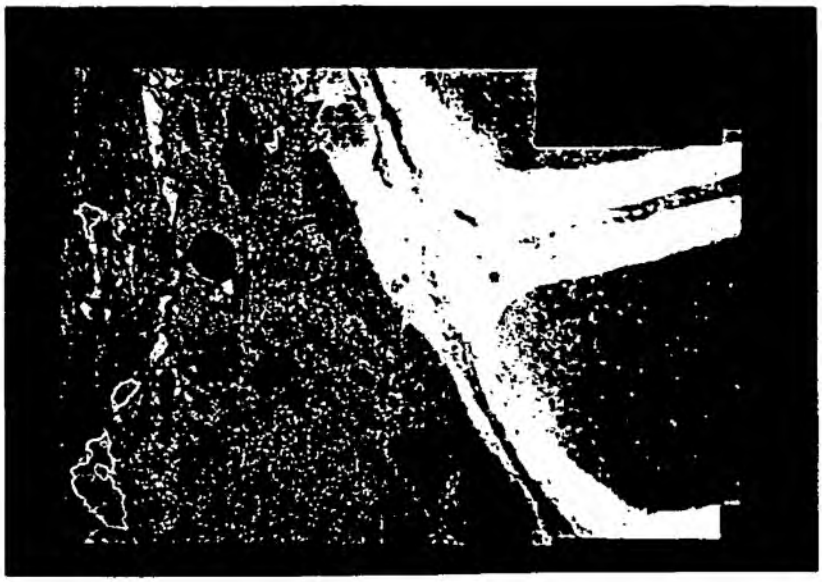


**Figure 21: Detail of the interface between the spiral and surrounding cells (x14,000)**

The image shows in more detail the interface between the spiral and surrounding fat body cells. From this image the presence of an outer boundary layer around the spiral, approximately 0.15$\mu$m in thickness can be detected. The drawing below shows this same image diagrammatically.

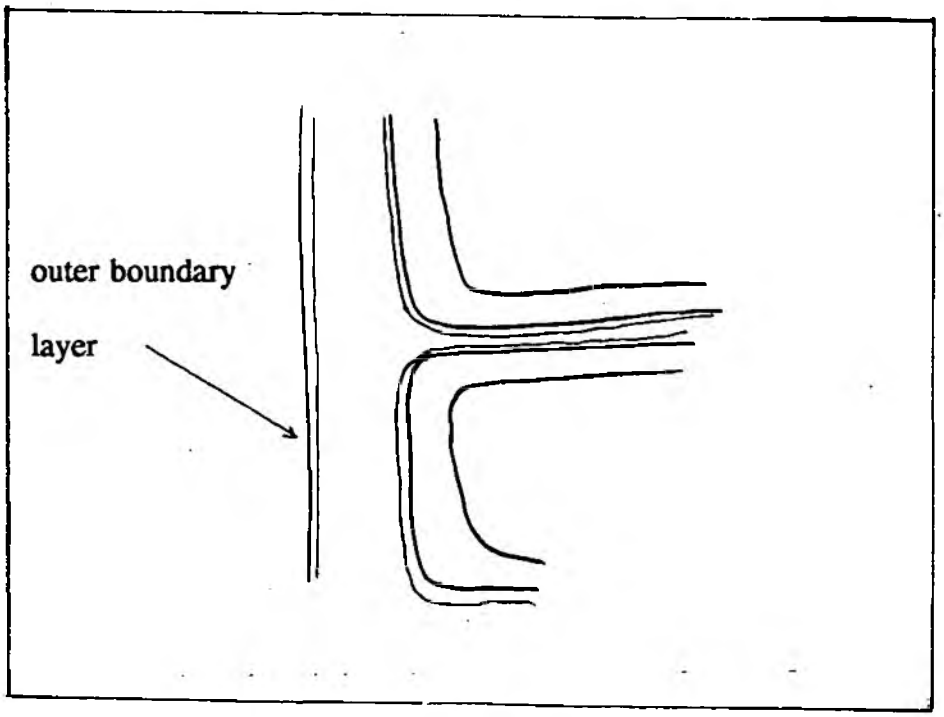


**Figure 22: Possible developmental phase of *S. adipophila* (x7,200)**

This final image shows the transverse section through a possible intracellular developmental phase of the parasite enclosing spiral. It is important to note that the observed parts of the spiral are enclosed within one continuous volume of fat cell cytoplasm.

# Discussion

**Distribution of *E. danica* and *S. adipophila* within the River Pang catchment**

From the survey work carried out during the first section of the project, both *Ephemera danica* and *Spiriopsis adipophila* were found to have a limited geographical distribution within the River Pang catchment. *E. danica* is generally widely distributed throughout Europe, Elliot *et al.*(1988). Ideal conditions for *E. danica* larvae are unpolluted lakes, ponds, streams and rivers where appropriate sandy/gravelly substrate is present (Williams *et al.*1992). In the River Pang, *E. danica* larvae were abundant in the lower reaches but absent upstream of Kimberhead Farm even though appropriate habitat was present. This limited geographical distribution was not expected. As *E. danica* larvae are sensitive to even low level pollution (Williams *et al.*1992), it is possible that poor water quality in the upstream areas was responsible for this absence of larvae. There are, however, few sources of pollution on the Pang with the only 'major' sewage treatment works being at of Bradfield (Ruse 1993), downstream of which *E. danica* larvae were found in abundance. Over the 1989 summer period the Pang dried up upstream of Bucklebury, Ruse (1993), so the most likely explanation for the lack of *E. danica* in the upstream areas is that these area have yet to be recolonised.

An uneven geographical distribution of *Spiriopsis adipophila* was also recorded throughout the Pang catchment. Although intrinsically linked to the distribution of its host, the percentage of larvae infected with *S. adipophila* decreased in the upstream sites, with the parasite being absent from the *E. danica* larvae upstream of Bradfield. Such an uneven geographical distribution in the intensity of infection of *S. adipophila* in mayflies (where

intensity is defined as the number of individuals of a particular parasite species in each infected host, Margolis *et al.*1982) along the length of 'infected' rivers was also recorded by Avry and Delage (1966) and Bennett (1994b). Bennett examined the geographical distribution of *S. adipophila* in *E. danica* larvae taken from the north and south branches of the River Wey (also a tributary of the River Thames). As with the Pang, infection levels were recorded at a much greater intensity in the lower part of the river (100% prevalence was also recorded) and the numbers of *S. adipophila* per nymph had been steadily increasing since the survey began in 1991. Numbers of *S. adipophila* per nymph were then found to decline such that *S. adipophila* was only found in very low numbers in both of the upper branches. The *E. danica* from the north branch were found to be more heavily infected than those from the south branch. Arvy and Delage, who carried out the original work on *Ephemera vulgata*, also recorded that the percentage of nymphs infected with *S. adipophila* was extremely variable along the rivers studied in the Eyzies region of France.

From all three studies, it would appear that for some reason the intensity of infection of *S. adipophila* in mayflies is extremely variable along the length of the rivers where it is found. In this survey, the apparent disappearance of *S. adipophila* from the *E. danica* larvae occurred over a very small stretch of river (approximately 1km in length), such that the River Pang offers an ideal site to examine what is responsible for the rapid decline and disappearance of this parasite from its host.

In the River Pang the only observed influence on the river between the sites where *S. adipophila* is present and apparently absent from the *E. danica* larvae, was the weir at Bradfield (Fig.8). As the transmission of *S. adipophila* is to date unknown it is possible that

this weir is in some way associated with the apparent disappearance of *S. adipophila* from the mayfly larvae through acting as a physical barrier and thus preventing it moving upstream. In Bennett's (1994b) survey of the River Wey, no such physical barrier was observed between the downstream and upstream sites. Preserved *E. danica* specimens from an upstream site on the River Pang (Folly Bridge) were supplied to Bennett as, although the dissection techniques used in both surveys were the same, Bennett's counting procedure involved recording absolute numbers of *S. adipophila* per nymph. Bennett's examination of these preserved samples detected very low numbers of *S. adipophila* at this site (A total of 5 *S. adipophila* individuals were found in 10 *E. danica* larvae) which suggests that the weir does not act as a physical barrier preventing *S. adipophila* from moving upstream, but that by this stage in the river the intensity of infection was so small in the nymphs that it was not detected using the counting procedure adopted in this survey.

*S. adipophila* had not therefore disappeared completely from the *E. danica* larvae over this short stretch of river, but the intensity and prevalence of infection had decreased significantly. It has been suggested that some form of low level pollution, often exacerbated by low flows, may be making these Ephemeroptera species susceptible to infection by *S. adipophila* (Arvy and Delage 1966, Bennett 1994a). Arvy and Delage (1966) suggested that pollution (the specific type was not specified) appears to increase the number of *S. adipophila* per nymph, such that the increased intensity of infection coupled with the pollution leads to a dramatic decline in nymph numbers. In the Pang, the greatest intensity and prevalence of infection was found in nymphs downstream of the only 'major' sewage discharge, and *S. adipophila* then declines in intensity and prevalence upstream of this point. The Pang has also been subject to low flows over the past years due to over abstraction of groundwater,

Ruse (1993). It is, however, unlikely that pollution coupled with low flows is responsible for the geographical distribution of *S. adipophila* within the Pang catchment. The discharge from the sewage treatment works at Bradfield is only approximately $91m^3$ per day (Ruse 1993) and as the catchment is mainly rural the remainder of the discharges are minor. The River Pang has historically scored well using the BMWP score system so indicating good water quality, and has been described as one of the best rivers, in terms of water quality, in the Thames catchment (Ruse 1993). This was reflected during this survey where the two sites surveyed and given a BMWP score both indicated good water quality. The site where the *E. danica* larvae were more heavily infected with *S. adipophila* was actually judged to be of marginally better water quality than the site where the number of *S. adipophila* per nymph was very low.

The macroinvertebrate communities were examined in detail at a site where *S. adipophila* was present and at a site where it was absent from the mayfly larvae, in an attempt to understand this uneven geographical distribution of the parasite. As the life cycle of *S. adipophila* is to date unknown, it is possible that another host, or hosts, are required for it to complete its development (i.e the mayfly nymphs only act as an intermediate host). The absence of such a host upstream could therefore restrict the geographical distribution of *S. adipophila*. There was however no significant difference found between the macroinvertebrate communities at the sites examined, so this again appears not to be the case. It was speculated that the fish communities at these sites would have been the same in composition, as the sites are in close proximity to each other, thereby ruling out another potential host group, however, as the fish populations were not surveyed this area requires further examination. Finally, it has been speculated that the stocking of salmon smolts may

be in some way related to the distribution of *S. adipophila* with salmon acting as another host in the life cycle (Bennett, personal communication). The hypothesis is that the stocking increases the density of the other host, in turn causing an explosion in *S. adipophila* numbers. This again would not seem to be accountable for the geographical distribution of *S. adipophila* in the Pang due to the close locality of the 'infected' and 'uninfected' sites and the fact that the river has been stocked with salmon (fry and parr) as far upstream as Kimberhead Farm (NRA Fisheries data obtained through personal communication) where *S. adipophila* was absent from the mayfly larvae.

To conclude this section of the project, a limited geographical distribution of both *E. danica* larvae and *S. adipophila* were observed in the River Pang. Although the distribution of *E. danica* can be accounted for, the reason for the limited distribution of *S. adipophila*, which was also found in work by Arvy and Delage (1966) on rivers in the Ezyies region of France and Bennett (1994b) in the River Wey, remains unknown.

**Relationship between *E. danica* and *S. adipophila***

The data collected during this survey of the River Pang allowed an examination of the relationship between 'parasite' (*S. adipophila*) and 'host' (*E. danica*). This was intended to further the limited knowledge of the biology of *S. adipophila*. The first area examined involved looking for a relationship between the intensity of infection (i.e number of *S. adipophila* per nymph), and larval length. Bennett (1994b) observed that initially the medium size larvae (8mm-15mm) were the heaviest infected and that over time the infection became greatest in the larger larvae (15mm-30mm). In this survey, when the data from all the infected larvae was combined (which involved data for nymphs ranging from 9mm-37mm

in length), no significant relationship was found to exist between these two variables. In this case at least, the intensity of infection of *S. adipophila* appears to bear no relation to nymph size. Although not measured here, Bennett (1994b) found that no relationship existed between the size of *S. adipophila* and the nymph length, as the size of *S. adipophila* remained relatively constant regardless of nymph length.

The second area examined was the distribution of *S. adipophila* within the *E. danica* larval community. This differs from the initial survey work in that in this examination the distribution involved is not a geographical one but a frequency distribution of intensity of the *S. adipophila* infection among the individuals of the host population, *E. danica* larvae. At each site examined on the River Pang, an overdispersed (also commonly referred to as contagious or aggregated) distribution existed within the *E. danica* larval community. An overdispersed distribution implies that the chance of infection is not randomly assorted between the members of the host community (i.e the regular presence of host individuals with high parasite numbers only has an extremely small probability of occurring if the parasites were distributed at random through the host population, Whitfield 1979).

Crofton 1971, cited from Whitfield (1979) considered the within host distribution of parasites from a general view point. He concluded that it is diagnostically characteristic of parasitism to produce overdispersion such as that observed by *S. adipophila* in the mayfly communities. Unfortunately such a contagious distribution can be generated by many different mechanisms. Almost any morphological, physiological or behavioral difference between the members of the host population can generate an overdispersed distribution (Whitfield 1979), therefore such a distribution predicts nothing specific about the biology of any given parasite.

This part of the project was aimed at discovering something more of the relationship of *S. adipophila* and its mayfly host, which in turn would further the understanding of its biology. There is apparently no relationship between parasite intensity and larval length, and the pattern of distribution of *S. adipophila* within its mayfly host is one which occurs frequently in nature (Whitfield 1979). Little additional information about the biology of *S. adipophila* has therefore been accrued from this study.

**Exposure of *S. adipophila* to a change in environmental conditions**

The response of *S. adipophila* when exposed to changing environmental conditions was investigated in an attempt to provide more information on its life cycle. There has to date been little such work done in this area. Delvaux (1975) believed that *S. adipophila* was transmitted to the next generation of its mayfly host by invading the eggs before they were laid, and Arvy and Delage (1966) recorded that *S. adipophila* is carried through the imago stage of the adult fly. Such observations could lead to the view that *S. adipophila* completes its entire life cycle within its mayfly host, a view subscribed to by Arvy and Delage. An alternative life cycle could involve *S. adipophila* passing through another host, or hosts, such that the mayfly only acts as an intermediate host. The aim of these crude experiments was to attempt to generate some response from the apparently inert form of *S. adipophila* encountered in mayflies, by simulating some of the conditions likely to be experienced by the parasite upon attempting to establish itself in other potential hosts.

Examination of freshly killed larvae found that the 'crinkled' form of *S. adipophila* was the most common form present in this host. Allowing infected larvae to decay at temperatures representing the internal body temperatures of a bird (40°C), fish (ambient water temperature,

14°C) and at an arbitrary low temperature (8°C), found that the morphology of *S. adipophila* within the mayfly larvae changed, with the most significant change occurring at the highest temperature. Furthermore it was discovered that individual *S. adipophila* specimens were capable of adopting all three described forms, and at high temperature would progress from 'crinkled' to 'smooth' and then uncoil.

Interpretation of these results is difficult as the experiments were crude in design. It is possible that the observed change in morphology is important in the life cycle of *S. adipophila* and, since it occurs at high temperature, indicates that a warm blooded animal which preys on mayfly larvae, such as a duck, could act as another host. Such a change in morphology is not uncommon among protozoan parasites on entering other hosts (Lee 1985). Desportes *et al.*(1975) reported that *S. adipophila* was stimulated to uncoil when exposed to a physical stress, which in that experiment was increased light intensity, therefore it is also possible that the uncoiling which occurred in response to increasing temperature was a response initiated purely because of the physical stress placed on the parasite by the higher temperature.

In the final set of experiments, the response of *S. adipophila* when exposed to internal and external secretions of four species of fish was examined. These secretions represent the first points of contact which the parasite would encounter in a potential fish host. Again it is not uncommon for protozoan parasites to pass through an intermediate macroinvertebrate host before completing their life cycle within a secondary fish host. (El-Matbouli *et al.*1992 documented that the myxosporean *Hoferellus carassi* has a two host life cycle involving a fish, in this case goldfish *Carassius auratus*, and an oligochaete, *Tubifex tubifex*). The fish

chosen for these experiments were those which were expected to be present within the Pang (although the survey work was never completed), and are widely distributed throughout the British Isles. These fish were all potential hosts to *S. adipophila* as they all predate mayfly larvae (Maitland and Campbell 1992). On no occasion was a change in the morphology of *S. adipophila* observed when exposed to these secretions. Such a result may suggest that *S. adipophila* does not pass through a fish host so completes its life cycle in the mayfly as Arvy and Delage (1966) speculated. It must be stressed that these experiments were crude in design and that the results are by no means conclusive. As was mentioned in the results section the fish used were small in size, which made it difficult to extract anything other than a very small quantity of each secretion.

In conclusion to this section, the results obtained do not shed much light on the life cycle of *S. adipophila*. The lack of response of *S. adipophila* when exposed to the simulated internal condition of a potential fish host may suggest that it completes its life cycle in mayfly larvae, or may this have been an artifact of the experimental system

The final section of the project was involved with an ultrastructural examination of *S. adipophila*. The work was carried out in conjunction with Dr.Whitfield at Kings College, London. From the images obtained, two main points were highlighted. The first area involved the discovery of an extremely thin boundary layer (estimated at 0.15$\mu$m in thickness) which surrounds the parasite. This layer has not previously been documented. It is possible that this membrane is responsible for maintaining the coil shape of the ribbon. It is also possible that this thin membrane ruptured when the parasites were exposed to the high temperature, thus allowing the ribbon to uncoil. Such a process could be important in

the life cycle of *S. adipophila*. The second discovery was that the parasite appears to be both intracellular and extracellular within its mayfly host. In Figures 19 and 20 the segments of the parasite enclosing spiral were surrounded by intact fat body cells and the spiral was large in comparison, suggesting that here the parasite is extracellular. In the final image (Figure 22), a much smaller parasite enclosing spiral section can be seen to be surrounded by, and contained within, fat cell cytoplasm (i.e the parasite here appears to be intracellular). This is perhaps the first recorded image of a developing ribbon. With these results it is possible to speculate something of the development of *S. adipophila*. It is possible that the parasite (without the ribbon) enters the fat cell of its mayfly host, or is taken up within a vacuole, which is not uncommon for protozoan parasites (Whitfield, personal communication), and once inside the cell begins to the process of developing the ribbon. This would be the intracellular part of the development of *S. adipophila*. As the ribbon develops the fat cell becomes enlarged and eventually ruptures and the development is completed outside of the fat body cell. This is, however, only speculation on the development of *S. adipophila* although the parasite does appear to be present in both intra and extracelluarly.

In conclusion to this final section, the transmission electron microscope examination of *S. adipophila* may have provided some information on the development of the parasite. There is however, much which still remains unknown. The method of entry to the mayfly host has yet to be determined, and it is still unknown whether a few individual parasites penetrate the mayfly and divide to produce the large numbers observed, which have been recorded as great as 4,000 parasites per nymph (Bennett 1994b), or whether each individual parasite infects the host separately. It is also unknown whether or not there are further stages in the development of *S. adipophila*, once the characteristic ribbon has been formed.

There is still much scope for work on the mayfly parasite *Spiriopsis adipophila*. Unfortunately, due to the limited time available to this project, some proposed work was left uncompleted. The development, life cycle and transmission of *S. adipophila* still remain a mystery but further work along the lines of the experiments tried here could clarify the potential involvement of another host. Finally, the distribution of *S. adipophila* throughout the British Isles is unknown. *S. adipophila* has yet to be recorded outside the Thames catchment (Bennett personal communication). It can however be speculated that the reason that *S. adipophila* has yet to be found outside the Thames catchment is because it has yet to be looked for in most other regions. It is my personal belief that the mayfly parasite *Spiriopsis adipophila* will be found to be widespread in streams and rivers throughout the British Isles.

# Appendices

**Appendix 1: Raw data from the River Pang survey**

**Site: Pangbourne**

| Number | Larval length(mm) | No.of *S.adipophila* | Number | Larval length(mm) | No. of *S.adipophila* |
|---|---|---|---|---|---|
| 1 | 19 | 67 | 11 | 19 | 160 |
| 2 | 22 | 10 | 12 | 16 | 24 |
| 3 | 17 | 126 | 13 | 14 | 143 |
| 4 | 14 | 159 | 14 | 17 | 74 |
| 5 | 18 | 79 | 15 | 14 | 134 |
| 6 | 11 | 69 | 16 | 14 | 83 |
| 7 | 20 | 23 | 17 | 19 | 112 |
| 8 | 9 | 100 | 18 | 15 | 74 |
| 9 | 15 | 140 | 19 | 15 | 125 |
| 10 | 22 | 37 | 20 | 18 | 42 |

**Site: Folly Bridge**

| Number | Larval length(mm) | No. of *S. adipophila* | Number | Larval length(mm) | No. of *S .adipophila* |
|---|---|---|---|---|---|
| 1 | 17 | 0 | 11 | 18 | 0 |
| 2 | 18 | 0 | 12 | 17 | 0 |
| 3 | 24 | 0 | 13 | 18 | 0 |
| 4 | 18 | 0 | 14 | 21 | 0 |
| 5 | 27 | 0 | 15 | 20 | 0 |
| 6 | 17 | 0 | 16 | 26 | 0 |
| 7 | 21 | 0 | 17 | 13 | 0 |
| 8 | 16 | 0 | 18 | 14 | 0 |
| 9 | 15 | 0 | 19 | 17 | 0 |
| 10 | 15 | 0 | 20 | 19 | 0 |

**Site: Tidmarsh**

| Number | Larval length(mm) | No. of *S. adipophila* | Number | Larval length(mm) | No. of *S. adipophila* |
|---|---|---|---|---|---|
| 1 | 14 | 62 | 26 | 15 | 145 |
| 2 | 24 | 69 | 27 | 17 | 97 |
| 3 | 21 | 32 | 28 | 16 | 66 |
| 4 | 14 | 146 | 29 | 17 | 162 |
| 5 | 20 | 42 | 30 | 16 | 116 |
| 6 | 18 | 116 | 31 | 17 | 271 |
| 7 | 20 | 76 | 32 | 14 | 171 |
| 8 | 15 | 140 | 33 | 17 | 347 |
| 9 | 19 | 89 | 34 | 19 | 78 |
| 10 | 12 | 184 | 35 | 20 | 153 |
| 11 | 16 | 121 | 36 | 21 | 179 |
| 12 | 12 | 112 | 37 | 13 | 19 |
| 13 | 19 | 98 | 38 | 19 | 135 |
| *14 | 14 | 118,116,110, 115,122 | 39 | 18 | 316 |
| 15 | 18 | 63 | 40 | 10 | 50 |
| 16 | 20 | 43 | 41 | 16 | 39 |
| *17 | 20 | 53,43,49,55, 51 | 42 | 14 | 237 |
| 18 | 19 | 89 | 43 | 15 | 12 |
| 19 | 15 | 69 | 44 | 13 | 124 |
| 20 | 16 | 13 | 45 | 20 | 74 |
| 21 | 13 | 53 | 46 | 15 | 16 |
| 22 | 17 | 98 | 47 | 14 | 61 |
| 23 | 19 | 187 | 48 | 12 | 143 |
| 24 | 18 | 24 | 49 | 13 | 50 |
| 25 | 17 | 83 | 50 | 12 | 69 |

* Slides used to evaluate counting procedure

**Site: River Bourne**

| Number | Larval length(mm) | No. of *S. adipophila* | Number | Larval length(mm) | *No. of S. adipophila* |
|---|---|---|---|---|---|
| 1 | 27 | 37 | 11 | 24 | 4 |
| 2 | 19 | 499 | 12 | 22 | 31 |
| 3 | 13 | 49 | 13 | 17 | 19 |
| 4 | 16 | 21 | 14 | 31 | 5 |
| 5 | 19 | 47 | 15 | 15 | 143 |
| 6 | 19 | 77 | 16 | 30 | 18 |
| 7 | 27 | 33 | 17 | 21 | 103 |
| 8 | 24 | 67 | 18 | 24 | 11 |
| 9 | 17 | 63 | 19 | 26 | 31 |
| 10 | 13 | 101 | 20 | 14 | 25 |

**Site: Kimberhead Farm**

| Number | Larval length(mm) | No. of *S. adipophila* | Number | Larval length(mm) | No. of *S. adipophila* |
|---|---|---|---|---|---|
| 1 | 13 | 0 | 11 | 19 | 0 |
| 2 | 22 | 0 | 12 | 15 | 0 |
| 3 | 22 | 0 | 13 | 14 | 0 |
| 4 | 14 | 0 | 14 | 20 | 0 |
| 5 | 20 | 0 | 15 | 23 | 0 |
| 6 | 21 | 0 | 16 | 21 | 0 |
| 7 | 26 | 0 | 17 | 18 | 0 |
| 8 | 28 | 0 | 18 | 33 | 0 |
| 9 | 19 | 0 | 19 | 21 | 0 |
| 10 | 27 | 0 | 20 | 17 | 0 |

**Site: Confluence of the Pang and Bourne**

| Number | Larval length(mm) | No. of *S .adipophila* | Number | Larval length(mm) | No. of *S. adipophila* |
|---|---|---|---|---|---|
| 1 | 18 | 83 | 6 | 17 | 103 |
| 2 | 20 | 64 | 7 | 17 | 26 |
| 3 | 13 | 16 | 8 | 13 | 98 |
| 4 | 18 | 51 | 9 | 14 | 64 |
| 5 | 14 | 69 | 10 | 14 | 40 |

**Site: Watercress Farm**

| Number | Larval length(mm) | No. of *S. adipophila* | Number | Larval length(mm) | No. of *S. adipophila* |
|---|---|---|---|---|---|
| 1 | 19 | 23 | 6 | 12 | 2 |
| 2 | 14 | 8 | 7 | 14 | 47 |
| 3 | 16 | 15 | 8 | 18 | 19 |
| 4 | 18 | 29 | 9 | 16 | 10 |
| 5 | 15 | 15 | 10 | 10 | 12 |

**Site: Bradfield**

| Number | Larval lenght(mm) | No. of *S. adipophila* | Number | Larval length(mm) | No. of *S. adipophila* |
|---|---|---|---|---|---|
| 1 | 16 | 0 | 6 | 17 | 0 |
| 2 | 12 | 0 | 7 | 11 | 2 |
| 3 | 18 | 4 | 8 | 12 | 1 |
| 4 | 11 | 0 | 9 | 18 | 0 |
| 5 | 12 | 0 | 10 | 13 | 7 |

**Site: Above Bradfield**

| Number | Larval length(mm) | No. of *S. adipophila* | Number | Larval length(mm) | No. of *S. adipophila* |
|---|---|---|---|---|---|
| 1 | 20 | 0 | 11 | 16 | 0 |
| 2 | 18 | 0 | 12 | 20 | 0 |
| 3 | 19 | 0 | 13 | 14 | 0 |
| 4 | 17 | 0 | 14 | 19 | 0 |
| 5 | 14 | 0 | 15 | 14 | 0 |
| 6 | 12 | 0 | 16 | 14 | 0 |
| 7 | 15 | 0 | 17 | 14 | 0 |
| 8 | 14 | 0 | 18 | 13 | 0 |
| 9 | 17 | 0 | 19 | 24 | 0 |
| 10 | 16 | 0 | 20 | 12 | 0 |

**Appendix 2: Experimental raw data**

**Experiment 1**
**Controls**

| Number | Larval length(mm) | 'smooth' | 'Uncoiled' | 'crinkled' | Total |
|---|---|---|---|---|---|
| 1 | 14 | 8 | 2 | 42 | 52 |
| 2 | 13 | 1 | 0 | 36 | 37 |
| 3 | 19 | 12 | 2 | 101 | 145 |

***S. adipophila* form at 8°C**

| Number | Larval length(mm) | 'smooth' | 'uncoiled' | 'crinkled' | Total |
|---|---|---|---|---|---|
| Form after 12 hours | | | | | |
| 1 | 17 | 6 | 0 | 53 | 59 |
| 2 | 19 | 2 | 1 | 23 | 26 |
| 3 | 14 | 3 | 2 | 98 | 103 |
| Form after 24 hours | | | | | |
| 1 | 12 | 5 | 0 | 8 | 13 |
| 2 | 18 | 17 | 1 | 58 | 76 |
| 3 | 15 | 7 | 6 | 26 | 39 |
| Form after 48 hours | | | | | |
| 1 | 16 | 2 | 10 | 22 | 34 |
| 2 | 14 | 3 | 31 | 20 | 54 |
| 3 | 13 | 1 | 10 | 10 | 21 |
| Form after 72 hours | | | | | |
| 1 | 13 | 14 | 4 | 13 | 31 |
| 2 | 11 | 15 | 2 | 7 | 24 |
| 3 | 16 | 66 | 4 | 23 | 93 |

**Form of *S. adipophila* at 14°C**

| Number | Larval length(mm) | 'smooth' | 'uncoiled' | 'crinkled' | Total |
|---|---|---|---|---|---|
| **Form after 12 hours** | | | | | |
| 1 | 13 | 4 | 0 | 9 | 13 |
| 2 | 14 | 17 | 1 | 92 | 110 |
| 3 | 13 | 11 | 3 | 30 | 44 |
| **Form after 24 hours** | | | | | |
| 1 | 15 | 20 | 4 | 21 | 45 |
| 2 | 13 | 39 | 3 | 147 | 189 |
| 3 | 13 | 52 | 4 | 36 | 92 |
| **Form after 48 hours** | | | | | |
| 1 | 13 | 10 | 8 | 27 | 45 |
| 2 | 14 | 28 | 2 | 28 | 48 |
| 3 | 14 | 27 | 3 | 66 | 96 |
| **Form after 72 hours** | | | | | |
| 1 | 16 | 53 | 4 | 5 | 62 |
| 2 | 14 | 56 | 2 | 9 | 65 |
| 3 | 12 | 54 | 2 | 12 | 70 |

Form of *S. adipophila* at 40°C

| Number | Larval length(mm) | 'smooth' | 'uncoiled' | 'crinkled' | Total |
|---|---|---|---|---|---|
| **Form after 12 hours** | | | | | |
| 1 | 12 | 86 | 4 | 16 | 106 |
| 2 | 11 | 40 | 3 | 17 | 60 |
| 3 | 12 | 194 | 37 | 128 | 359 |
| **Form after 24 hours** | | | | | |
| 1 | 19 | 87 | 98 | 1 | 186 |
| 2 | 15 | 6 | 88 | 1 | 95 |
| 3 | 15 | 10 | 171 | 0 | 181 |
| **Form after 48 hours** | | | | | |
| 1 | 22 | 0 | 67 | 0 | 67 |
| 2 | 17 | 0 | 156 | 0 | 156 |
| 3 | 11 | 0 | 34 | 0 | 34 |
| **Form after 72 hours** | | | | | |
| 1 | 11 | 0 | 73 | 0 | 73 |
| 2 | 15 | 0 | 121 | 0 | 121 |
| 3 | 15 | 0 | 105 | 0 | 105 |

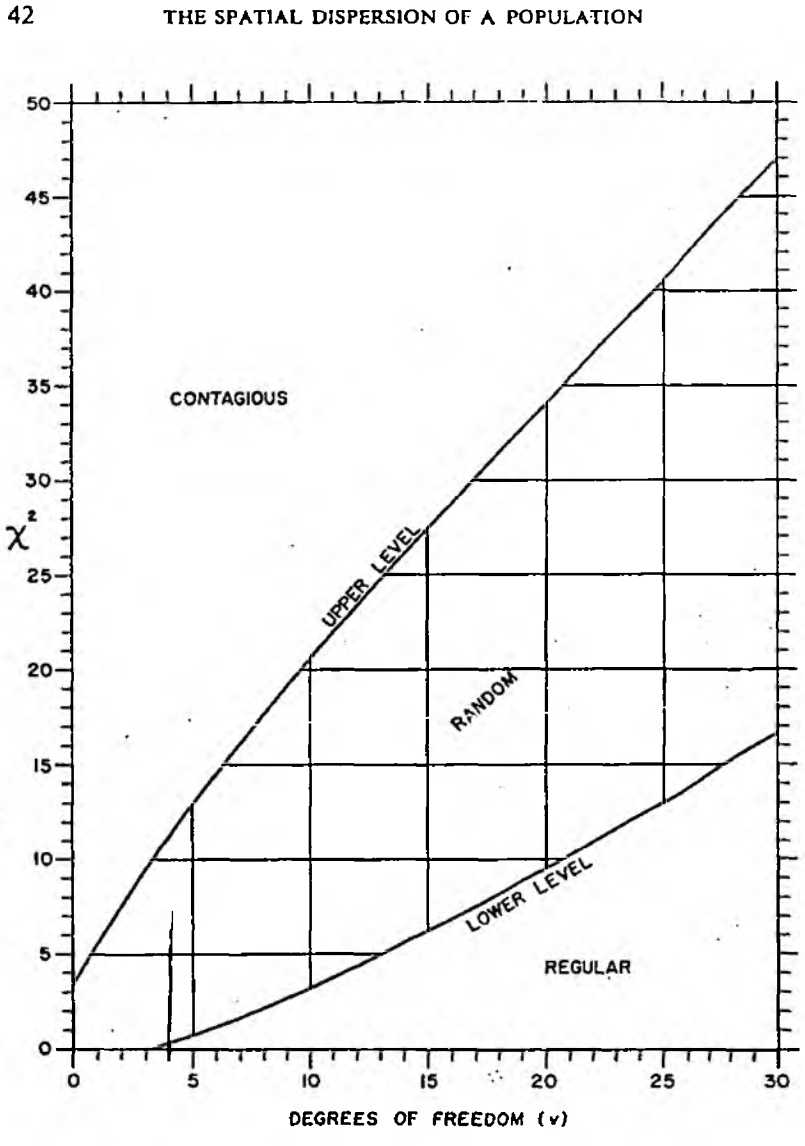
**Experiment 2**

**Form of the same *S. adipophila* individuals exposed to 40°C**

| Time (hours) | 'Crinkled' | 'Smooth' | 'Uncoiled' | Total |
|---|---|---|---|---|
| 0 | 247 | 9 | 5 | 261 |
| 2 | 238 | 11 | 5 | 254 |
| 12 | 16 | 227 | 13 | 256 |
| 18 | 9 | 242 | 26 | 277 |
| 20 | 1 | 197 | 46 | 244 |
| 24 | Slide dried out | | | |
| | | | | |

## Appendix 3

**Figure 1: The 5% significance levels for Chi-squared**



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