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A CONTROL STRATEGY FOR *Tanytarsus barbitarsis* Freeman (Diptera: Chironomidae), A SMALL-SCALE PEST ORGANISM

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SUMMARY

A control-orientated research programme was conducted on the chironomid, *Tanytarsus barbitarsis* Freeman, in the saline lagoons at Port Augusta over a four year period. In this locality, adult flies are frequently pestiferous. The research had two main aims: i) to develop a stable, long-term control strategy which was environmentally sound; ii) to establish whether it was possible to apply the philosophy of Integrated Pest Management (IPM) to a small-scale pest organism through a biological research programme of limited resources.

The physical environment and the nature of the problem at Port Augusta are described. A morphological description of *T. barbitarsis* and *T. semibarbitarsus* head capsules is given which indicates that, although specimens of *T. barbitarsis* from Western Australia and south eastern Australia differ in their salinity tolerance, they belong to the same morphospecies.

A two-year population study shows *T. barbitarsis* to be multivoltine with the length of generation being temperature dependent. Observations indicate that predation on eggs and 1st instar larvae by juvenile and larval fish (*Atherinosoma microstoma*) exercises natural control on the *T. barbitarsis* population during spring and early summer. Experimental exclusion of fish gives support to the observations.

The eggs of *T. barbitarsis* are highly impermeable until the pharate 1st instar has developed rectal excretory tissue for osmoregulation. *T. barbitarsis* 4th instar larvae are able to osmoregulate in media between 5 and 95 g l^{-1} NaCl; their LC_{50} is 100 g l^{-1} NaCl. Ion excretion is effected through the rectal tissue in larvae, but in pupae this site occurs on the dorsum of the thorax.

Adults are strongly attracted to two regions of the electromagnetic spectrum, 490-510nm and 370-400nm, the latter being more attractive.

A pathogen with some potential for control of *T. barbitarsis* is described as *Tipula* iridescent virus (TIV).

An integrated control strategy using, fish predation, hypersaline water (300 g l^{-1}) and light traps is proposed. A mathematical model is used in a computer simulation to predict the results of control measures and the future nuisance outbreaks of *T. barbitarsis* adults at Port Augusta.

The concept of IPM is discussed and the results of this research programme are evaluated: It is possible to derive control strategies for small-scale pests through programmes of basic biological research.

DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University and, to the best of my knowledge and belief, it contains no material previously published or written by another person except where due reference or acknowledgement is made.

I give my consent to this thesis being made available for photocopying and loan.

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The guidance, enthusiasm and sense of humour of Prof. W.D. Williams has made this thesis possible.

The project was funded on grants from The Corporation of The City of Port Augusta and The Electricity Trust of South Australia. I thank their liaison officers Mr R. Rutter and Dr R. Ainslie for their help and co-operation.

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CHAPTER 1

Introduction

As part of the biosphere, man competes with a variety of organisms for finite resources. The word 'pest' is used to describe a certain group of organisms including competitors for food, fibre and shelter, as well as those which cause discomfort or ill health. Methods of combatting pests have reflected human cultural and technological development.

Pre-agricultural (10,000 a B.P.) humans were nomadic and tolerated a variety of ectoparasites (lice, fleas and bugs), their only defence being personal grooming (Southwood, 1976). The development of agricultural settlements led to the acquisition and cultural transmission of pest control practices. Such practices were discovered by trial and error, and consequently they were often steeped in mysticism and ritual (Philip and Rozeboom, 1973). However, with the passage of time, the transition to rational pest control was made. The Chinese, for example, understood the biology of many pest organisms and applied highly appropriate and ingenious control methods (Konishi and Ito, 1973; Flint and Van den Bosch, 1981).

Despite control measures, pests, and particularly insects, have had a profound influence upon human history, affecting the outcome of wars (for example, bubonic plague and malaria halted Hannibal at Syracuse) and causing mass human migrations (the Israelites from Egypt) (Howard, 1930; Cloudsley-Thompson, 1976).

The Renaissance in Europe led to a new emphasis upon scientific understanding. Entomology and especially systematic entomology were initiated by Ulysse Aldrovandi (1522 - 1605 A.D.) (Beier, 1973). Accompanied by the growth in systematics and the development of the microscope by Leeuwenhoek (1632 - 1723), scientific investigation became the basis for pest control. This ethic continued into the 20th century, culminating in the publication of E.D. Sanderson's 'Insects of Farm, Garden and Orchard' (1915) which stressed the need for sound biological information and advocated the integration of a range of methods. In fact, the same approach (although not universally applied) is central to current pest control philosophies, but a major diversion has intervened.

During World War II (1940s), the threat to troops from insect-borne diseases (chiefly typhus borne by lice) prompted an intensive screening of chemical compounds for insecticidal activity in the United States (Philip and Rozeboom, 1973). The chlorinated hydrocarbons, among them DDT (dichloro-diphenyl-trichloroethane) - a compound first synthesized in 1874 and later discovered to have insecticidal activity by Meuller in 1948 - were found to be highly effective. In response to the same problem, the organophosphates were discovered in wartime Germany.

These discoveries gave impetus to a perception that chemicals were the most effective means of countering pests. Consequently, the post war years saw strong emphasis placed upon pesticides and by 1962, 2.3×10^8 kg of insecticide were being manufactured annually in the United States alone (Flint and Van den Bosch, 1981). This was essentially a technological phenomenon generally lacking in appreciation of mechanism or consequence; indeed the mode of action of many insecticides remains unclear (Gerolt, 1983).

The idea of reviving integrated methods of pest control was put forward by Stern _et al_.(1959). They introduced the term, 'Integrated Pest Management' (IPM), and outlined a systematic approach for dealing with pest organisms. By the 1970s when problems such as environmental contamination, resistance, target pest resurgence and secondary pest outbreak had become evident (Hagen and Franz, 1973; Pal and Brown, 1974; Carter, 1976; Luck _et al_., 1977), IPM had gained widespread acceptance.

Since 1972, the year when DDT was banned in the United States, a growing number of published accounts has advocated the implementation of IPM (Wood, 1973; Glass, 1975; Metcalf and Luckmann, 1975; Huffaker and Croft, 1976; Telford, 1977; Flint and Van den Bosch, 1981). For many economic entomologists, IPM is now the preferred strategy for counteracting pest organisms.

The method of IPM (Flint and Van den Bosch, 1981) is based upon a series of requirements. Included are: an understanding of the biology of the pest organism, a knowledge of key environmental factors which affect the pest population, a knowledge of the range of possible control measures available, a knowledge of the 'weak links' in the life cycle of the pest, an effective monitoring programme and a knowledge of the economics of the situation.

The economics of pest management is a 'normative' phenomenon, i.e. criteria are decided by subjective means (Mumford and Norton, 1984), and therefore funding depends upon human attitudes to problems. Southwood (1976) has pointed out that about one in six of mankind is suffering from insect borne diseases and that one fifth is malnourished, whilst insects consume enough food to feed that fifth. Adequate funding for effective long-term research and the implementation of IPM can be justified for these large-scale agricultural or health pests. On the other hand, there are numerous small-scale pest organisms which do not warrant sufficient expenditure to fulfil the prerequisites of IPM.

Such small-scale pest problems are often of a local nature and commonly involve nuisance arthropods such as mayflies, caddisflies, ants, wasps, millipedes, lice, and ticks, and especially the Diptera (Jamnback, 1969). Both biting dipterans (Culicidae, Psychodidae, Ceratopogonidae, Rhagionidae, Simuliidae, Muscidae, Tabanidae), many of which, being vectors of disease, are major pests (Metcalf and Flint, 1962; Duckhouse pers. comm.), and non-biting dipterans (Chironomidae, Chaoboridae) are common small-scale pests (Jamnback,1969).

The group of interest here, the Chironomidae, is a dipteran family which is widespread across the globe. Species occur in a range of aquatic habitats such as streams, lakes, temporary pools, intertidal pools (Bryce and Hobart, 1972) and microenvironments such as water in flowers (Arum lillies, Davies, pers. comm., 1983; pitcher plants, Paterson and Cameron, 1982). Chironomids are the only holometabolous insects which occur in the Antarctic (Belgica antarctica Jacobs, Sugg et al., 1983; Parochlus steineni (Gercke) Edwards and Usher, 1985). As larvae, they occupy a number of niches including that of predator, detritivore, grazer and parasite (Bryce and Hobart, 1972).

Certain genera within two subfamilies, the Chironominae and the Tanypodinae, are widely implicated as pests (Grodhaus, 1963). Nuisance outbreaks of chironomids have been reported from the United States (see Grodhaus, 1963 for review), Nicaragua (Bay, 1964), England (Mundie, 1956a), Holland (Beattie, 1981), South Africa (De Meillon and Gray, 1937), The Sudan (Wülker, 1963, Lewis, 1956 & 1957; Kay et al., 1978; Cranston et al., 1983) and Australia (Edward, 1986).

Chironomid adults emerge from water bodies and gather in mating swarms over the adjacent land. Under certain conditions, particularly in naturally or artificially productive standing waters, chironomid populations may reach high numbers (100,000 larvae per square metre of lake bottom [Learner and Edwards, 1966; Freeman, 1973]) and emergent adults pose severe nuisance problems to proximal human settlements (Grodhaus, 1963).

Records exist of chironomid swarms affecting livestock (Burril, 1913; Bonnel and Mote, 1941), soiling newly-painted surfaces, contaminating molten plastics (Grant, 1960), causing allergic responses in humans (Weil, 1940; Lewis, 1956; Kay *et al.*, 1978; Bellas, 1982; Wirtz, 1984), attracting spiders (Grodhaus, 1963) and generally spoiling outdoor activities.

It is, therefore, chiefly as adults that chironomids pose a problem, although reports exist of larvae and egg masses contaminating water supplies and swimming pools (Grodhaus, 1963). The nuisance caused by the adults is considerably increased by their attraction to lights at night. The marine chironomids, in particular, are strongly attracted (L. Cheng pers. comm., 1983), increasing their nuisance levels around artificial light sources along coastlines.

The subject of this thesis, *Tanytarsus barbitarsis* Freeman, which was first identified in eastern Australia in 1966 (Bayly and Williams, 1966), was originally described by Freeman (1961). Freeman suggested that the species may be better placed in a new genus because of its reduced tibial combs. Subsequent work has not addressed this suggestion (Glover, 1973), but in terms of ecological specialization, *T. barbitarsis* appears to be quite distinct from the rest of the

Australian chironomid fauna by virtue of its ability to inhabit hypersaline (30 - 140 g l^{-1}) waters.

T. barbitarsis is distributed across southern Australia, its most northerly record being from Dampier in north-west Australia ([collected by D.H. Colless], Glover, 1973). There are no records of the species from the eastern seaboard, but it has been collected near Lake Buchanan in Queensland (B.V. Timms, pers. comm.). Large populations occur in saline lakes such as those in the craters of western Victoria (Werowrap, Gnotuk - Bayly and Williams, 1966; Walker, 1973; Paterson and Walker, 1974a, 1974b), Rottnest Island (Edward, 1983), Lake Eyre (Ruello, 1976; Williams and Kokkinn, 1986, in press) and the south lagoon of the Coorong in South Australia (Glover, 1973; Kokkinn, 1986).

The study of chironomid biology in Australian fresh waters is confounded by problems of identification; the fresh water fauna is diverse and remains largely undescribed in the larval state. It is therefore not surprising to find that T. barbitarsis, which inhabits saline waters with low species richness, is one of the few Australian chironomids which has been the subject of an autecological study (Paterson and Walker, 1974a). Based upon a monthly sampling programme over the course of a year, Paterson and Walker (1974a) were able to investigate the seasonal dynamics of T. barbitarsis in Lake Werowrap (a saline, crater lake in western Victoria) and provide an estimate of its annual production. In a subsequent palaeolimnological study (Paterson and Walker 1974b), they examined chironomid head capsules in the sediments of the lake and made an interpretation of its recent history.

The combatting of small-scale pest organisms such as T. barbitarsis is often in the hands of local authorities or city councils where, frequently, one person, for example the health officer, is faced with the task of pest control. At this level, pesticides are still widely used because their application often has immediate results, although little consideration is given to the impact of heavy and continued use (Von Rumker et al., 1972).

With the common acceptance that alternatives to chemical control are more desirable, there is an increasing trend towards referring small-scale pest problems to biologists for solution. Biologists undertaking such research are often subject to constraints such as a lack of knowledge of the basic biology of the pest, a shortage of funding, an urgent need to produce effective control measures and a shortage of time. Is the method of Integrated Pest Management applicable under such circumstances? And if not, how should such research be conducted to make it applicable?

These are important questions, not least because they focus the attention of both the scientific and lay community upon biologists. They reflect a requirement to solve biological problems by 'strong inference' (Platt, 1964). That is, based upon strong evidence, to formulate and test hypotheses which could lead directly to the solution of problems. With agricultural entomologists currently seeking safe alternatives to toxic chemicals (Jones, 1985), the solution of small-scale pest problems will not only render a service to society, but serve as an avenue for pure biological research which could be supported by funds currently used for pesticides.

The nuisance outbreak of T. barbitarsis at Port Augusta (South Australia) offered an opportunity to address the questions outlined above. The City Council and the State Electricity Authority (ETSA) provided funds for the employment of a biologist for three and a half years to undertake a programme of research which it was hoped would lead to the solution of the midge problems.

This thesis has as its core a two-year sampling programme which sought to elucidate the biology of *T. barbitarsis* at Port Augusta. The aim was to produce an integrated control strategy for *T. barbitarsis* in the fly-ash disposal lakes at Port Augusta, South Australia. Peripheral to the investigation of basic biology was a series of questions, some of them arising during the course of the project, which sought to test hypotheses relevant to the control of *T. barbitarsis*. In effect, then, since time and manpower were limited, the project was an attempt to apply the philosophy of Integrated Pest Management to a small-scale pest organism by gathering the basic biological information and, concurrently, seeking appropriate control measures.

The thesis begins by describing the physical environment at Port Augusta and the nature of the nuisance. This is followed by a description of larval *T. barbitarsis* using its close relative, *T. semibarbitarsus*, as a comparison. Results of the two-year sampling programme are reported and discussed in Chapter 4. Subsequent chapters describe a series of experimental investigations relating to: fish predation, the eggs of *T. barbitarsis*, salinity tolerance and osmoregulation of the larvae and the attraction to light by the adults. A viral infection which shows some potential for control of chironomids, as well as a range of other arthropod taxa, is discussed in Chapter 9.

The general discussion (Chapter 10) outlines the control strategy for *T. barbitarsis* at Port Augusta and points to several promising avenues for further research. The thesis concludes with an evaluation of the applicability of IPM to a small-scale pest organism.

CHAPTER 2

The physical environment and the nature of the problem

Port Augusta is a city (population 16,360 in 1985) at the head of Spencer Gulf in South Australia (32°30', 137°46'). The climate is indicated by data supplied by The Electricity Trust of South Australia for the period 1/7/82 to 7/7/83.

Air temperatures during this period ranged from 2°C (4/7/83) to 45°C (17/2/83). The hottest period occurred between 20/1/83 and 17/3/83, the coldest between 1/7/82 and 12/8/82. Fig. 2.1 gives air temperature information.

The rainfall was sporadic with heavy falls recorded in November, March and June (Fig. 2.2). Most annual precipitation falls in late summer and autumn (between February and May).

Windstars in Fig. 2.3 summarize wind direction data for summer and winter months. Summer winds blow chiefly from the south, whereas in winter, winds have some northerly component. Wind velocities are highest during summer when strong southerly winds prevail. Commonly, during summer, the only calm period of the day is in the early morning.

The variation in daylight hours over the 1982/1983 period is shown in Fig. 2.4. Daylight hours exceeded hours of dark between 26/8 and 14/4.

The main industries at Port Augusta are railway related activities and power generation. In a series of power stations south of the city - Thomas Playford A and B, Northern Power Station 1 and 2 (commissioned in 1984) - 40% of South Australia's electricity is generated by burning low-grade coal from Leigh Creek, SA (30°29', 138°25').

Figure 2.1 Maximum and minimum air and lake water temperatures at Port Augusta for the period 1/7/82 to 7/7/83.

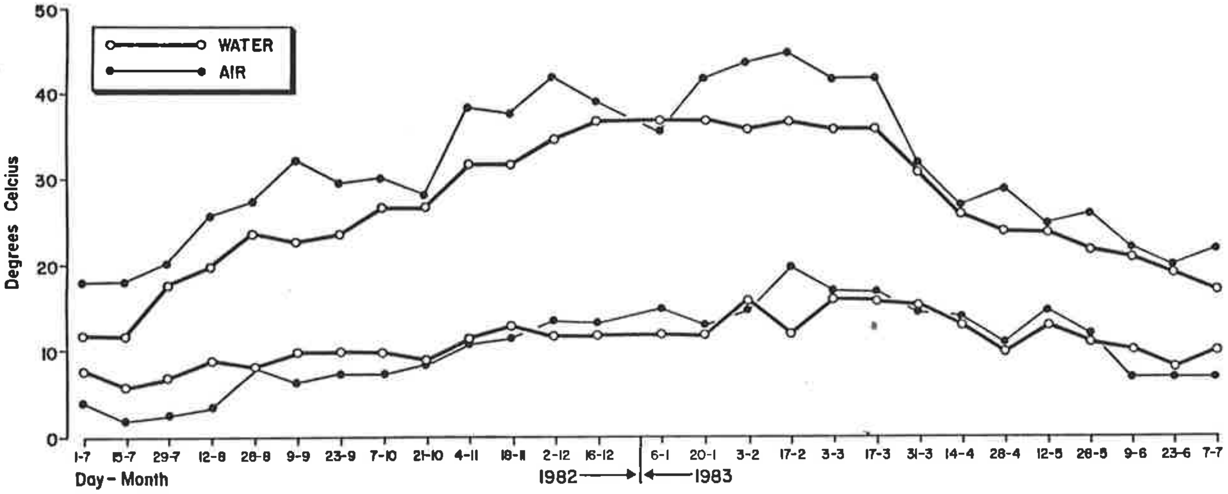


Figure 2.2 Rainfall at Port Augusta over the period 1/7/82 to 7/7/83.

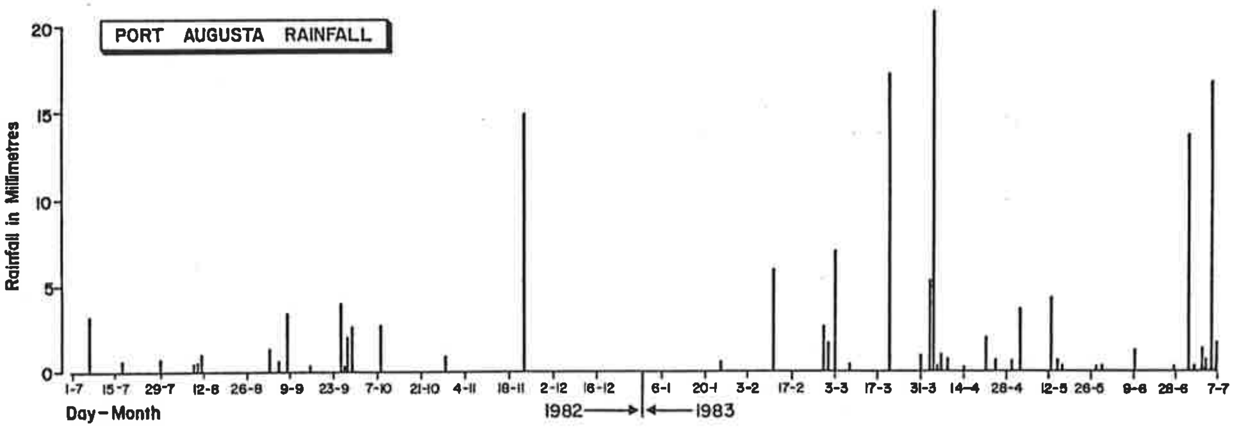


Figure 2.3 Wind stars showing the prevailing wind directions at Port Augusta during the summer and winter of the 1982/83 period.

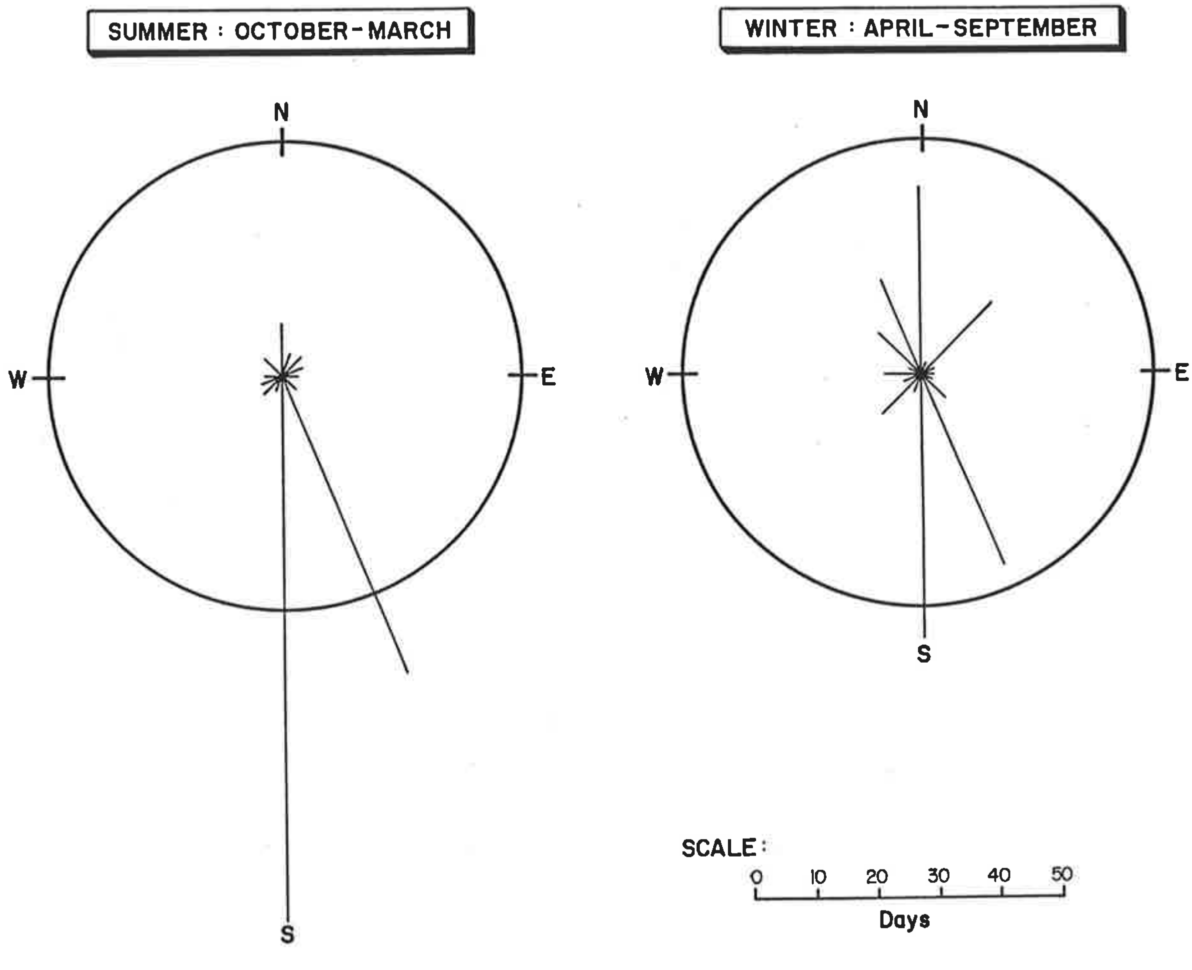
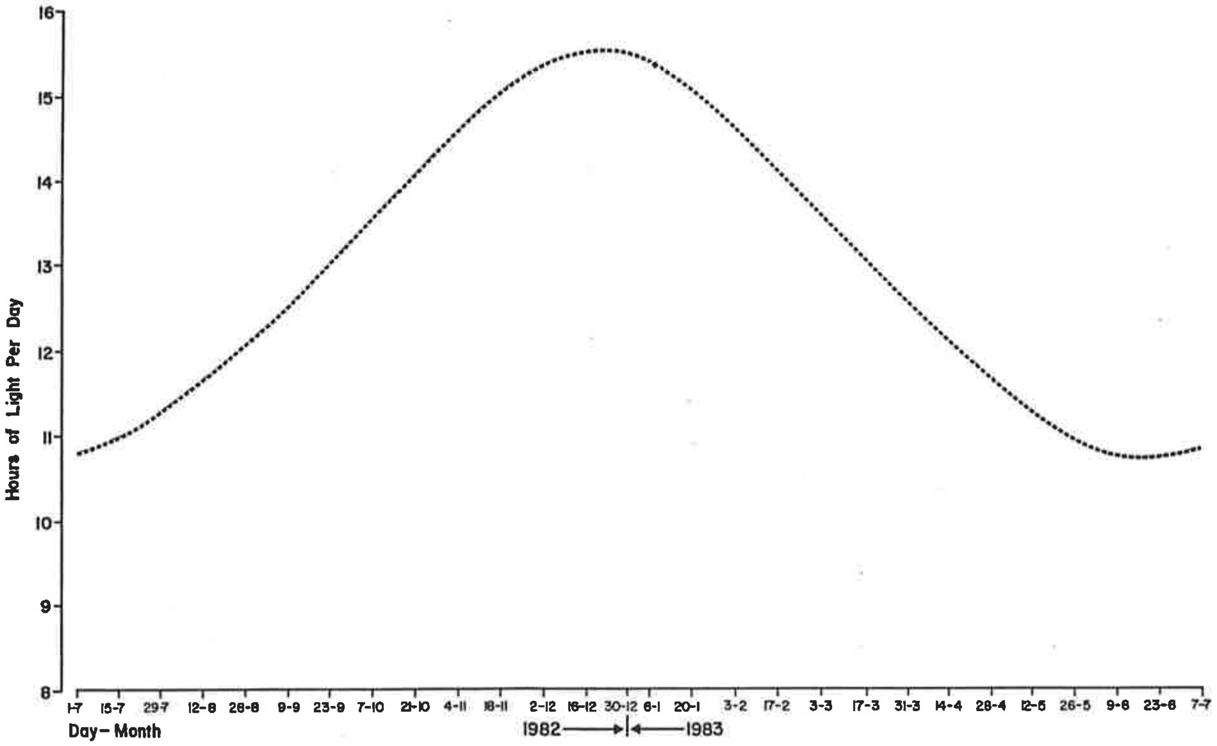


Figure 2.4 Variation in daylight hours at Port Augusta over one year. (Data supplied by Australian Meteorological Bureau.)



Approximately 20% of the coal consists principally of refractory silicates and alumina (Al_2O_3). These emerge from the furnaces as ash (see Table 2.1 for typical ash analyses). Ash ranges in size from large, porous fragments 2 cm in diameter to very fine, silty particles - "fly-ash". The fly-ash is collected from the power station chimneys by electrostatic precipitation. All ash accumulates in a series of bins from where it is flushed into a large collecting sump by seawater drawn from Spencer Gulf. Approximately 30,000 cubic metres of ash-laden water is produced each day.

Table 2.1 A typical chemical analysis of ash from Leigh Creek coal. (Data supplied by ETSA Chemistry Department.)

Average ash content of coal is 19.5%
Eight ash analyses are tabulated to show variation.
Components are expressed as percentages.

Compound	Analysis Number							
	1	2	3	4	5	6	7	8
SiO_2	51.8	56.1	51.9	49.7	41.9	32.0	56.8	57.9
Al_2O_3	26.2	20.9	25.8	22.1	23.9	26.5	24.7	21.1
TiO_2	2.1	1.8	2.2	1.8	2.0	1.3	2.1	1.2
Fe_2O_3	5.1	3.4	3.7	6.1	9.8	6.6	4.1	2.9
CaO	4.1	3.4	4.8	5.5	6.9	13.4	3.3	3.6
MgO	2.6	1.3	2.2	3.1	2.4	3.3	2.1	0.9
Na_2O	4.1	2.3	3.7	4.9	5.5	3.0	2.9	3.0
P_2O_5	N/A	0.7	N/A	N/A	1.8	4.2	N/A	N/A

Note: K_2O and Mn_3O_4 being less than 0.5% in ash, are not reported; the balance is mostly SO_3.
N/A indicates not analysed.

An elongated north-south depression on the eastern margins of the city is occupied by a series of three artificial lakes which constitute the ash disposal system. The southern lake, "ETSA Lake" (Fig. 2.5), receives ash-laden water from the power stations and it is the primary site of ash sedimentation. The central lake, "Bird Lake", is separated from ETSA Lake by a narrow ash causeway (Fig. 2.5 b). Still bearing some ash, water enters Bird Lake through a culvert at the eastern end of the causeway where it deposits fine "greasy" sediments (mainly on the eastern side of the island). The northernmost artificial lake, "Railway Lake", lies between the highway in the south and the railway line in the north. Railway Lake receives a limited inflow of water from Bird Lake via a culvert underneath the highway. To the north of the railway line is a salt-encrusted depression, "Salt Lake", which is seasonally filled by rainwater. It too connects to the lake system via a culvert.

Water flows from the power station outlet through ETSA Lake, into Bird Lake and is returned to the sea via Hospital Creek at the south-western corner of Bird Lake (see Fig. 2.5 for flow directions).

Some physiographic data from the lake system are summarized in Table 2.2.

Table 2.2 Physical parameters of the Port Augusta fly-ash lakes.

Lake	Area	Volume	Salinity*	
			Summer, 1983	Winter, 1983
ETSA Lake	566,000m^2	283.000m^3	60g/litre	50g/litre
Bird Lake	701,000m^2	351,000m^3	80g/litre	60g/litre
Railway Lake	377,000m^2	189,000m^3	160g/litre	140g/litre

*Spencer Gulf salinity - 42g/litre.

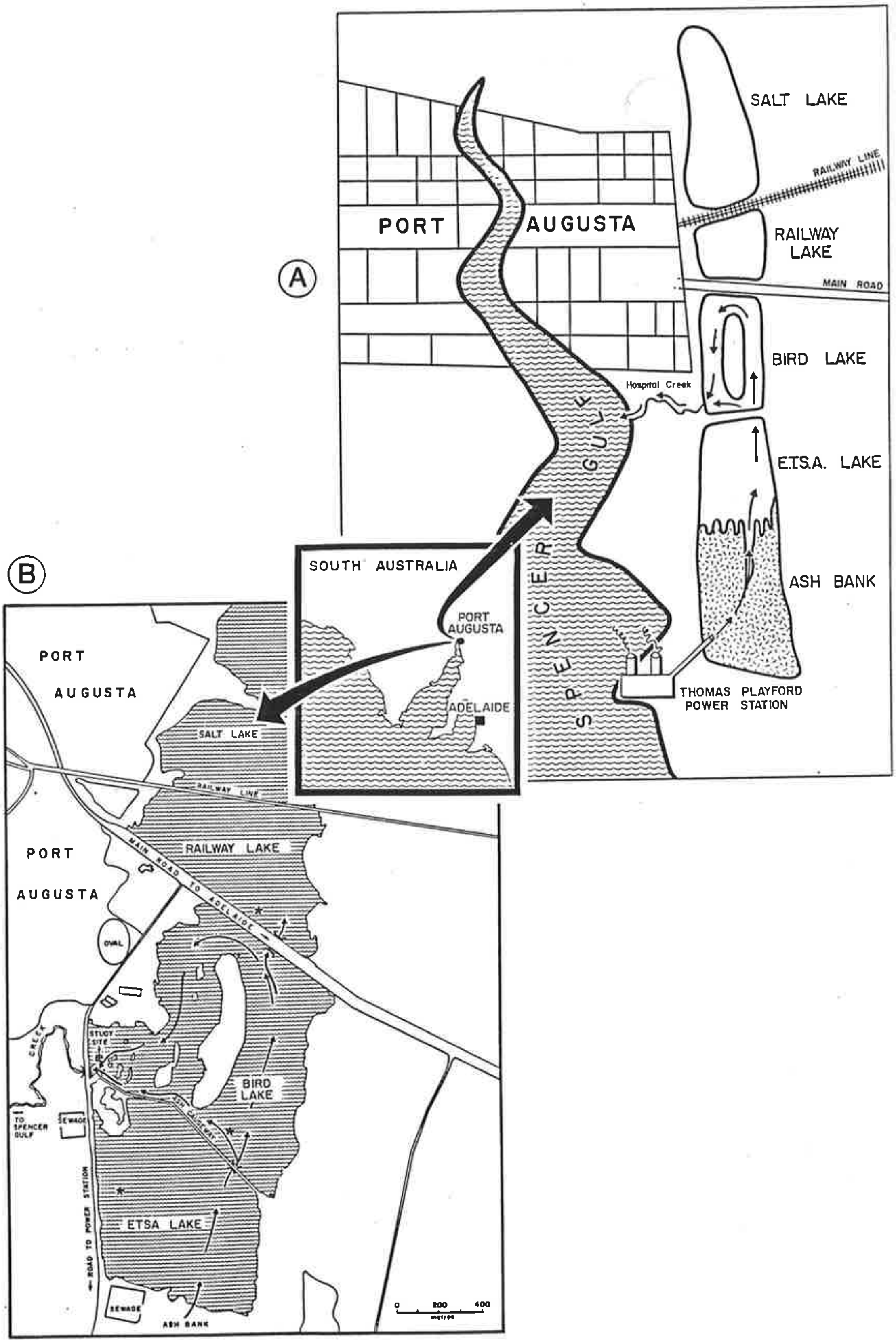
Prior to November, 1972, the eastern approach road to Port Augusta was flanked by dry clay pans. In order to improve the scenic appearance of this approach, the Port Augusta City Council requested that the Electricity Trust of South Australia release water from its fly-ash lagoon (ETSA Lake, Fig. 2.5) so as to inundate the clay pans. By the beginning of 1973, the eastern approach to Port Augusta was flanked by salt water lakes.

In late 1973, residents bordering the western shores of the lakes reported the occurrence of chironomids in nuisance numbers. At times the number of midges is so high around the fringes of the lakes that breathing is difficult. The midges are strongly attracted to artificial light, consequently they enter homes at night, posing a serious nuisance to residents. Clothing and furnishings become soiled, and food is contaminated. Some reports indicate allergic responses in children, particularly when midges lodge in the eyes.

Attracted by the abundant source of insect prey, numerous spiders colonize the eaves and walls of houses. At times the density of spider webs on houses is so high that it gives the walls a brown appearance.

Such problems have recurred each summer, and in 1974, with the construction of several new homes in areas near to the lakes (east of Edinburgh Terrace), the number of complaints from residents increased. In May, 1978 the City Council requested assistance from the Engineering and Water Supply of South Australia. In response, a biologist, Mr Brenton Peters, made a brief survey and concluded that all of the lakes supported large populations of chironomid larvae. He recommended that the lakes flanking the highway be drained (Railway Lake and Bird Lake).

Figure 2.5 A diagram (A) and map (B) indicating the lake system at Port Augusta. Arrows indicate current direction.



During the years after the initial flooding of the old clay pans, a large and diverse bird population colonized the lakes, feeding mainly on the chironomid larvae. Bird Lake has a large island in its centre (Fig. 2.5) which became the focus of the Port Augusta Bird Club activities. Members of the club are anxious that predators should not gain access to the island which is a refuge for breeding and roosting birds. The club, therefore, has opposed suggestions to drain the lakes.

As an alternative to draining, in November 1979 the lakes were treated with the insecticide Malathion (Shell Chemicals) at a rate of 360ml per hectare of water. Two applications of the insecticide, one week apart, brought about no apparent reduction in chironomid numbers.

In November, 1980 Railway Lake (Fig. 2.5) was isolated from the other lakes and allowed to dry by evaporation. The smell of the decomposing, organic-rich muds was so offensive and the number of complaints so high that after two months the lake was refilled. Subsequently, ETSA has indicated that Bird Lake is now an essential part of the ash disposal system; thus the option of draining the lakes flanking the highway is precluded.

In an attempt to cover unsightly roadside edges in January, 1981, the control weirs were raised and as the level rose, water passed into the salt-encrusted pan to the north of the railway line (Salt Lake, Fig. 2.5). The raised water level became a threat to the stability of the railway and it was therefore decided to lower the weirs and allow the water level to drop to its previous mark. During this operation, highly saline water (160 g l^{-1}) which was apparently beyond the range of salinity tolerance of the chironomid larvae, filled Railway Lake. Owing to the very low input of water from Bird Lake and the high evaporation rate, the Railway Lake remained highly saline and free from chironomid larvae until some time after the beginning of the research programme.

In June, 1981 the City Council of Port Augusta resolved to seek the assistance of The University of Adelaide. After negotiations, it was decided to provide a grant for a biologist to undertake basic research over a period 18 months with the aim of formulating effective control measures for the midges. As a result, the research programme commenced on 1st February, 1982. At the end of the initial 18-month period, ETSA agreed to provide funding for a further period of 2 years, extending the duration of the project to a total of three and a half years.

CHAPTER 3

Establishing the identity of the problem organism

INTRODUCTION

A population study of larval chironomids involves considerable problems associated with the sorting of benthic samples. The two major ones are separating organic from inorganic fractions, and sorting larvae (particularly smaller instars) from plant detritus. Over and above this, difficulties arise when thousands of individuals must be identified from obscure morphological features.

Webb and Scholl (1985) suggested that the following features can be used to separate larvae of Chironomus species: tubules on abdominal segments, colour and shape of the teeth of the mentum, size of mentum, colour of clypeus, colour of gula, number and patterns of striae on ventromental plates, size of the head capsule, colour of mandibular teeth, ratio of antennal segments i.e. L1/L2, W and MS. Their antennal ratios refer to relationships between the length of first antennal segment (L1), the length of second antennal segment (L2), the width of the first antennal segment measured at the ring organ (W) and the distance between the teeth adjacent to the central tooth of the mentum (MS). Some of these characters are not easily discernible in unmounted specimens.

More conspicuous morphological features are required for identification of chironomid larvae for ecological work. Frequently, however, after the identities of the species present have been verified by mounting of head capsules, they can be recognized by certain gross features such as colour, overall length, shape or even their posture after preservation.

In diverse communities where species can only be distinguished by subtle morphological differences, the specific resolution of the chironomid larval fauna for population studies is not practicable. The salt water chironomid fauna, however, is apparently much less diverse, and therefore, autecological studies are much more feasible.

Fundamental to any ecological investigation involving a pest organism is the need to have a clear idea of its identity (Flint and Van den Bosch, 1981). Two problems relating to the identity of *T. barbitarsis* were evident at the outset: firstly, although a description of the adult was available (Freeman, 1961; Glover, 1973), there was no published description of the larva - the target of proposed ecological research; secondly, distribution data indicated two distinct groups of *T. barbitarsis* with differing salinity tolerances.

Historically, chironomid taxonomy has developed on two fronts. One, inspired by Thienemann, concerned itself with the description of immature stages, whilst a second, the so-called "English School", worked almost exclusively with adults (Pinder, 1983). Collection methods favoured adult males which established a foundation upon which subsequent, broader taxonomic and biogeographical analyses could be based. Taxonomic work with adults was also favoured because they generally provide the best characters. The initial descriptions of Australian chironomid fauna were made by Freeman (1961) who used adult material exclusively. This taxonomic emphasis upon adult material has generally inhibited ecological studies involving chironomid larvae.

An inability to resolve chironomid communities to the species level has resulted, for example, in studies such as that of Wilda (1984), who measured the production of chironomid *genera* in a North Carolina reservoir. The fact that fish are able to forage selectively for particular chironomid *species* from a diverse community in bottom muds (Mackey, 1979), indicates that such gross analyses are inadequate.

Recently the need for work on the taxonomy of chironomid larvae has been recognized. As a consequence, several keys to larvae of species in the Chironomidae have been published (Bryce and Hobart, 1972; Mason, 1973; Geiger *et al*., 1978; Webb & Scholl, 1985; Wiederholm, 1983). However, few morphological descriptions of Australian species are available (Womersley, 1936; Edward, 1963, 1964; Martin, 1963, 1964, 1971, 1974, 1975; Atchley & Martin, 1971; Hashimoto, 1973; Roback, 1982a, 1982b) and the published keys only separate genera (Martin, 1974, 1975).

There are no formal descriptions of *T. barbitarsis* larvae. Unpublished information, however, has made larval identification possible (pers. comm. D. Edward, J. Martin, B. Glover, P. Waller and P. Suter). Adult specimens of *T. barbitarsis* were identified as the nuisance organism at Port Augusta prior to the commencement of the project (Glover pers. comm.). The important larval phase, however, which proliferates in the peculiar conditions provided by the fly-ash lakes, had not been unequivocally identified as *T. barbitarsis*.

Given the suggested lack of congruence between the adult and larval taxonomic systems (Pinder, 1977, 1978; Webb, 1980) and the fact that the generic concept in the larval system has a much narrower sense than that of the adult system (Martin, 1971; Pinder, 1983), it was evident that there was a need to describe the larvae at Port Augusta. This need is supported by the observation that the subdivisions based on adult characters of the genus *Tanytarsus* do not always correspond with those based on the larvae (Bryce and Hobart, 1972).

In addition to the task of identifying the larvae, distribution data suggested that there are two larval "physiotypes". One, distributed throughout south-eastern Australia, has an upper limit of salinity tolerance approaching 100 g l^{-1} (Williams, 1978). The other, collected from Rottnest Island (Western Australia) and northern Queensland, is able to tolerate salinities exceeding 140 g l^{-1} (Edward, 1983; B.V. Timms pers. comm.)

The manipulation of water salinities in the Port Augusta lagoons was considered to be a feasible control measure. Thus the upper limit of salinity tolerance of larval chironomids would have important implications for their taxonomy and distribution, as well as control. Investigation of the two physiotypes was, therefore necessary. Could they be distinguished as different morphotypes? Did both occur in south-eastern Australia?

The aims of the taxonomic investigation were threefold: to describe and confirm the identity of the larval chironomids in the Port Augusta Lakes; to investigate specimens of both physiotypes and compare their morphologies in order to make some judgement about their taxonomic affinity; to make a collection of larvae from saline waters in south-eastern Australia to broaden the distributional data base.

MATERIALS AND METHODS

In order to make a reasonable judgement about the identity of the Port Augusta larvae, putative specimens of T. barbitarsis (some of which had been reared and identified in adult form) were collected from Port Augusta and compared with the larvae of the closely-related salt water species, Tanytarsus semibarbitarsus Glover (verified by P.Suter on adult material), obtained from Noora (South Australia). Putative specimens of T. barbitarsis larvae were then collected from the following salt lakes:

Lake Gnotuk, Lake Kielambete, Little Dip Lake, Serpentine Lake (see Table 3.3 for details and localities), and the variation within the *barbitarsis* group was investigated. In this way, the characters useful in separating *T. barbitarsis* from *T. semibarbitarsus* could be distinguished from the variation within the *barbitarsis* group and any morphological distinctiveness of the Serpentine "physiotype" (from Rottnest Island, W.A.) could be ascertained.

Salt water chironomid larvae have reduced post-cephalic features such as abdominal tubules; therefore the study was restricted to head capsule morphology. The head capsules of fourth instar larvae (according to the convention of Pinder (1983) who has used fourth instars for larval description) were viewed by light and scanning electron microscopy (SEM). Specimens were prepared for light microscopy by treatment with a 4% potassium hydroxide solution and mounting on glass slides in polyvinyl-lactophenol to which a few drops of acid fuchsin in glacial acetic acid had been added. Head capsules were viewed at 400X and drawn with the aid of a camera lucida. Representative types were photographed using a Zeiss III RS photomicroscope.

Preparation for SEM involved critical point drying, mounting on stubs and double coating with carbon (i.e. 2 X 15nm) and gold/palladium alloy (i.e. 2 X 20nm) in a Denton Vacuum Model 502 high-vacuum evaporator. Specimens were viewed in an ETEC Autoscan scanning electron-microscope fitted with a tungsten filament at 20KV, operating in secondary electron mode. Photomicrographs were obtained using Kodak FXP 120 Panatomic-X film.

In order to compare data with that of Paterson and Walker (1974a) maximum dorsal head capsule width was measured for approximately 50 individuals from each instar in the Port Augusta population. The following morphological features were used in both a qualitative and quantitative comparison: mental teeth, mandibular teeth, antennal tubercles, proportions of the head capsule (length vs breadth), antennal ratio i.e. the relative lengths of segments one and two and the length of the petioles of the Lauterborn organs. The terminology used follows Saether (1971, 1980).

RESULTS

From studies of electron micrographs followed by verification on a number of individuals under light microscopy, a series of distinct morphological differences between the Port Augusta *T. barbitarsis* population and *T. semibarbitarsus* has been identified.

The most distinctive difference is in the overall shape of the head capsule. In *T. semibarbitarsus*, the head capsule is subrectangular (width/length = 0.82, n = 27) while in *T. barbitarsis* it is stouter and subquadrate (width/length = 0.97, n = 36) (Figs 3.1a, 3.2a & 3.3).

The antennal tubercles of *T. semibarbitarsus* are prominent and elongate (Width/Length = 0.56, n= 18) (Figs 3.2a & b) while those of *T. barbitarsis* are short and flared outwards (Width/Length = 1.11, n = 20) (Figs 3.1a & b). The first antennal segment of *T. semibarbitarsus* specimens is slender and generally longer than that of *T. barbitarsis* (Fig. 3.1b & 3.2b).

Another clear distinguishing feature is the length of the petiole of the Lauterborn organ. In *T. barbitarsis* it is approximately as long as the 3rd antennal segment (Fig. 3.1c) whereas in *T. semibarbitarsus* it is much more elongate, being as long as the 3rd, 4th and 5th antennal segments combined (Fig 3.2c).

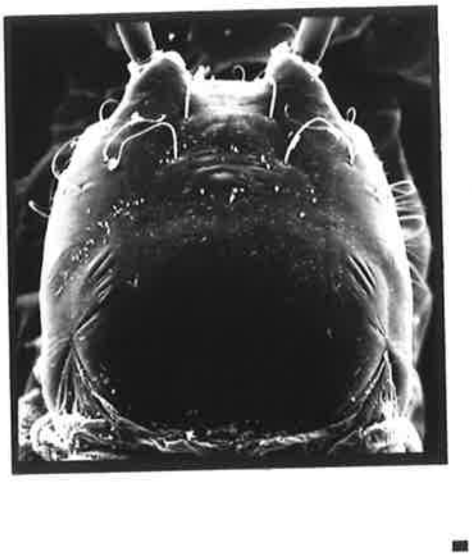
Although the size of the mentum is generally very similar, some differences are noted. The mental teeth of *T. semibarbitarsus* are equal in size and project upwards (Fig. 3.2e). The central tooth is comprised of three rounded denticles (Fig. 3.2f). In *T. barbitarsis*, the outer mental tooth is flared outwards (not in all individuals, Fig. 3.5a) and, in approximately 20% of individuals, the central tooth had several pinnacles on each of the three denticles (the "ornate morph") (Fig. 3.1fii).

The mandibular teeth of *T. semibarbitarsus* are generally more elongate than those of the Port Augusta specimens of *T. barbitarsis* (Figs 3.1d & 3.2d).

Table 3.1 summarizes the characters which can be used to distinguish Port Augusta *T. barbitarsis* from *T. semibarbitarsus*.

Maximum head capsule widths of the four instars of *T. barbitarsis* from the Port Augusta population are given in Figure 3.4. Fourth instar head capsules ranged in width from 0.313 mm to 0.359 mm, showing some 15% variation in size. Significantly, the distributions for the first and second instar head capsule widths were very close, making it difficult at times to distinguish one from the other without measurement.

The investigation of specimens from the 6 localities (including *T. semibarbitarsus* from Noora) revealed that there is considerable within-group variation. Mean head capsule widths (Table 3.2) ranged from 0.337 in the Port Augusta specimens to 0.257mm in the Little Dip specimens. Head capsule widths differed significantly between most populations except Serpentine & Little Dip Lake, Serpentine & *T. semibarbitarsus*, and Little Dip Lake & *T. semibarbitarsus* (One way analysis of variance (5 and 36 degrees of freedom, F = 48.28, P .001) with Tukey's HSD test. See Sokal and Rohlf, 1981).



a

b

c

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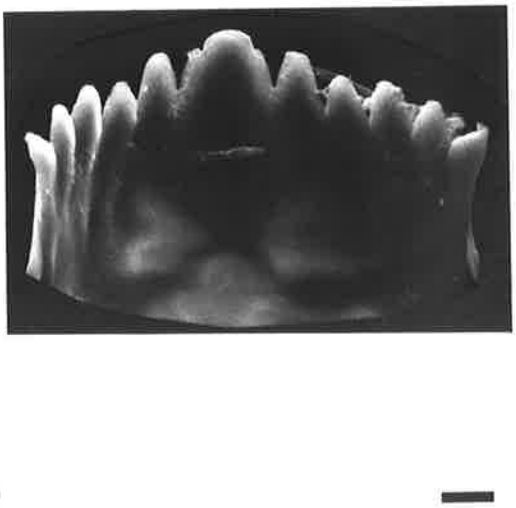
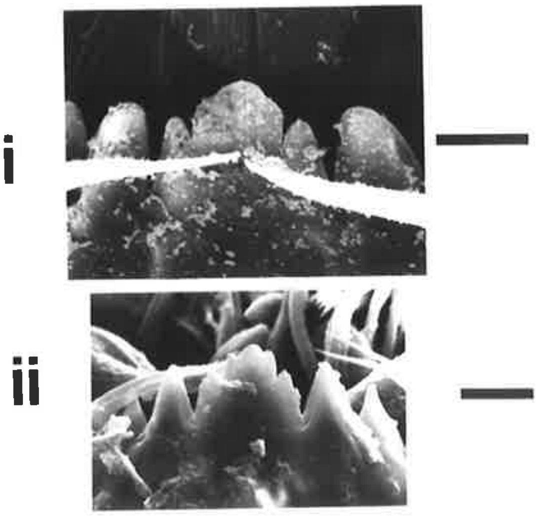


e



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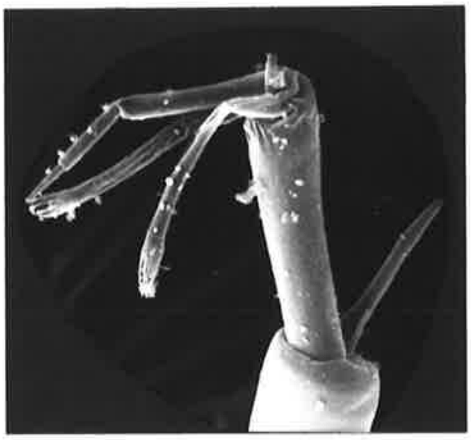

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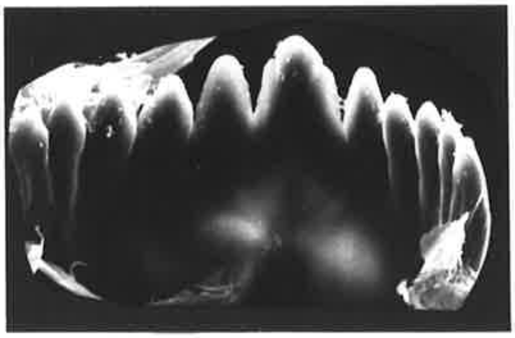


e

f

Figure 3.3 Ventral views of the head capsules to show length breadth ratios. a) *T. semibarbitarsus* , b) *T. barbitarsis*
L = labrum, M = mandible, S = ventromental plates,
H = mentum

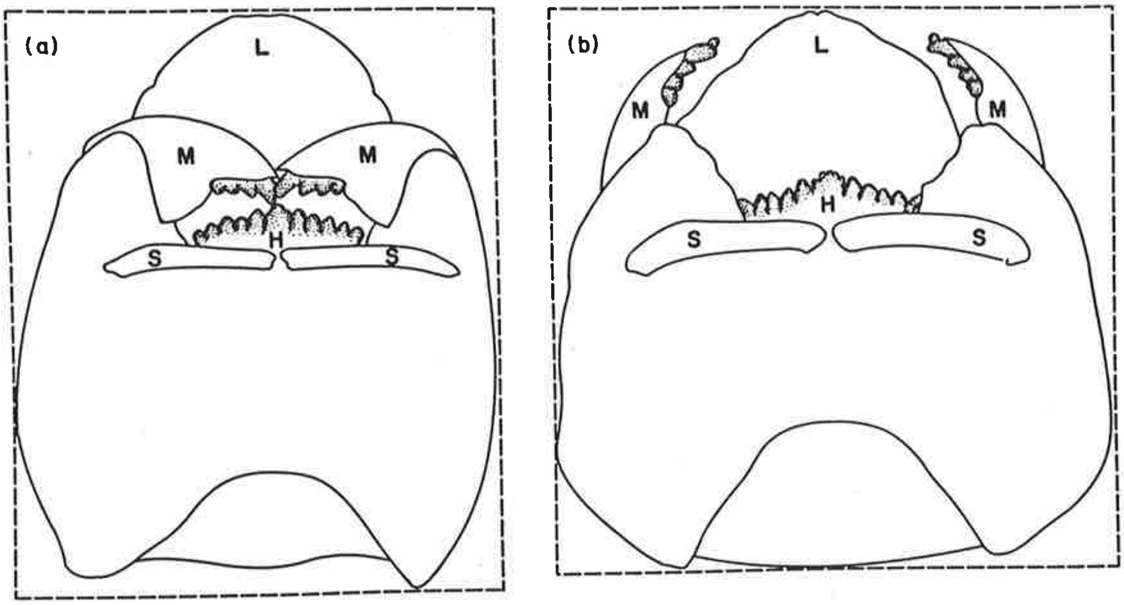


Figure 3.4 Head capsule widths of the four instars at Port Augusta.

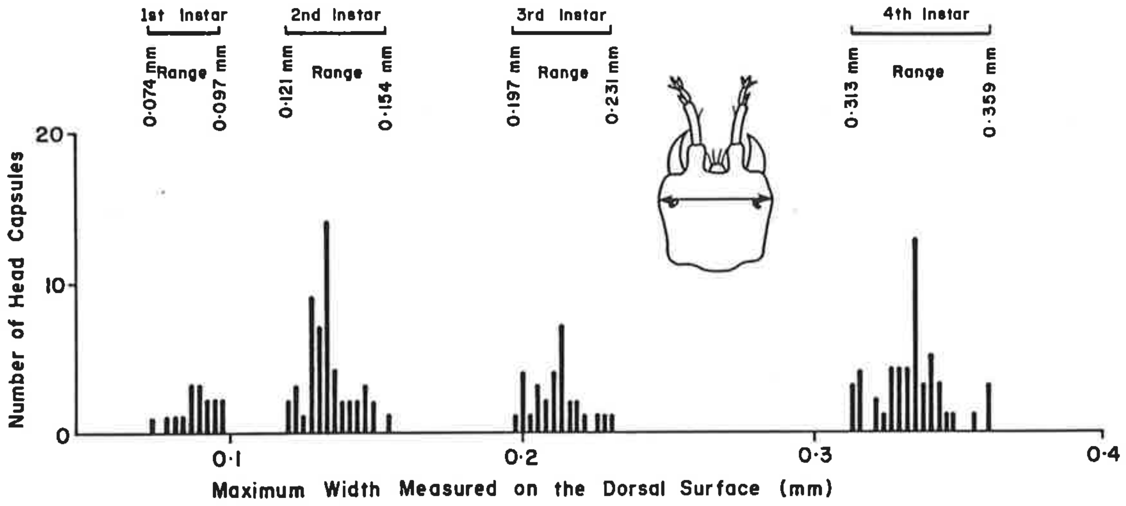


Table 3.1 Distinctive morphological features of Port Augusta *Tanytarsus barbitarsis* and Noora *Tanytarsus semibarbitarsus* from larval head capsules.

TAXONOMIC UNIT	NAME	DISTINGUISHING FEATURES
Sub-family	Chironominae	Striated plates present
Tribe	Tanytarsini	Antennal tubercles prominent, approximately as long as wide or longer, 1st antennal segment long and curved.
Genus	*Tanytarsus*	Antennal tubercles without a spur. Petioles of Lauterborn organs shorter than or equal to last three antennal segments together.
Species	*barbitarsis*	*The antenna* Antennal tubercles flared laterally and separated by a curved line (Fig. 3.1a&b). Petioles of Lauterborn organs approximately equal in length to 3rd antennal segment (Fig. 3.1c). *The mentum* Outer tooth deflected outwards (Fig. 3.1e). Central tooth may be ornate (Fig. 3.1fii). *The mandible* Mandibular teeth generally blunt and rounded (Fig. 3.1d). *The head capsule* As wide as it is long when viewed from the ventral surface (Figs 3.1a & 3.3a).
Species	*semibarbitarsus*	*The antenna* Antennal tubercles prominent. Twice as long as wide, project straight forward. Separated by a rectangular line (Figs 3.2a & b). Petioles of Lauterborn organs as long as the last three antennal segments together (Fig. 3.2c). *The mentum* Lateral teeth even (Fig. 3.2e). Outer tooth not markedly reflected outwards (Fig. 3.2e). Central tooth simple with 3 rounded denticles (Fig. 3.2f). *The mandible* Mandibular teeth generally elongate (Fig. 3.2d). *The head capsule* Elongate (Figs 3.2a & 3.3b).

Absolute lengths of segments 1 and 2 of the antenna vary consistently with head capsule size. However, the antenna of *T. semibarbitarsus* are considerably longer, in absolute terms, than those of *T. barbitarsis* (Table 3.2). Antennal ratios (1/1+2 : 2/1+2) are consistently close to 0.8 : 0.2 for all specimens (Table 3.2). An analysis of the length of the first antennal segment of specimens from the different localities (one way analysis of variance (5 and 36 degrees of freedom, F = 147.98, P .001) with Tukey's HSD test) shows a significant difference between most groups except: Kielambete & Serpentine, Kielambete & Gnotuk, and Serpentine & Gnotuk.

The mentum shows considerable variation. This appears to be mainly due to wear (Fig. 3.5d). In addition, although most *T. barbitarsis* specimens have the outer tooth deflected outwards (Fig. 3.1e), several did not (Fig. 3.5a). The dimensions and relative sizes of the mental teeth in the 6 sample groups showed no useful distinctive features which would override the variation due to wear (Fig. 3.5). Similar observations could be made for the mandible. The mandibular teeth showed so much variation between individuals, mainly due to wear, that it was of no value as a distinguishing character.

An interesting morph was observed in both the Little Dip and Kielambete specimens. It had what appeared to be spatulate teeth on both the mentum and the mandible (Fig. 3.5e). This could simply be a wear phenomenon or perhaps an artifact under the microscope. Such scraper-like appendages, however, may be valuable in Lake Kielambete where larvae live on rock slabs and feed by scraping material from the hard surfaces.

Table 3.2 Head capsule widths, antennal lengths and antennal ratios of 4th instar *Tanytarsus* from six localities.

PA = Port Augusta Lakes
LD = Little Dip Lake
KB = Lake Kielambete
GN = Lake Gnotuk
SP = Serpentine Lake
SEMI = *semibarbitarsis*

Width of head capsule (mm)

	PA	KB	SP	LD	GN	SEMI
Mean	0.337	0.294	0.271	0.257	0.293	0.261
S.D.	0.0138	0.005	0.012	0.013	0.013	0.009
(n=7)						

Total means:	*barbitarsis*	n=35	Mean = 0.291	S.D. = 0.030
	semibarbitarsus	n=7	Mean = 0.261	S.D. = 0.009

Length of 1st antennal segment (arbitrary units)

	PA	KB	SP	LD	GN	SEMI
Mean	21.4	25.3	25.4	16.7	26.3	36.1
S.D.	2.0	1.1	1.3	0.95	0.95	1.8
(n=7)						

Total means:	*barbitarsis*	n=35	Mean = 23.0	S.D. = 3.80
	semibarbitarsus	n=7	Mean = 36.1	S.D. = 1.77

Length of 2nd antennal segment (arbitrary units)

	PA	KB	SP	LD	GN	SEMI
Mean	6.1	7.3	6.9	4.0	7.1	9.0
S.D.	0.7	1.1	1.1	0.6	0.7	1.4
(n=7)						

Total means:	*barbitarsis*	n=35	Mean = 6.29	S.D. = 1.5
	semibarbitarsus	n=7	Mean = 9.00	S.D. = 1.4

Ratio of 1st and 2nd antennal segment (1/1+2 : 2/1+2)

PA	KB	SP	LD	GN	SEMI
0.80:0.20	0.77:0.23	0.79:0.21	0.81:0.19	0.79:0.21	0.80:0.20

(S.D. = standard deviation)

Figure 3.5 Profiles of menta and mandibles from a) Port Augusta b) Serpentine c) *T. semibarbitarsus* d) Gnotuk and e) Little Dip.

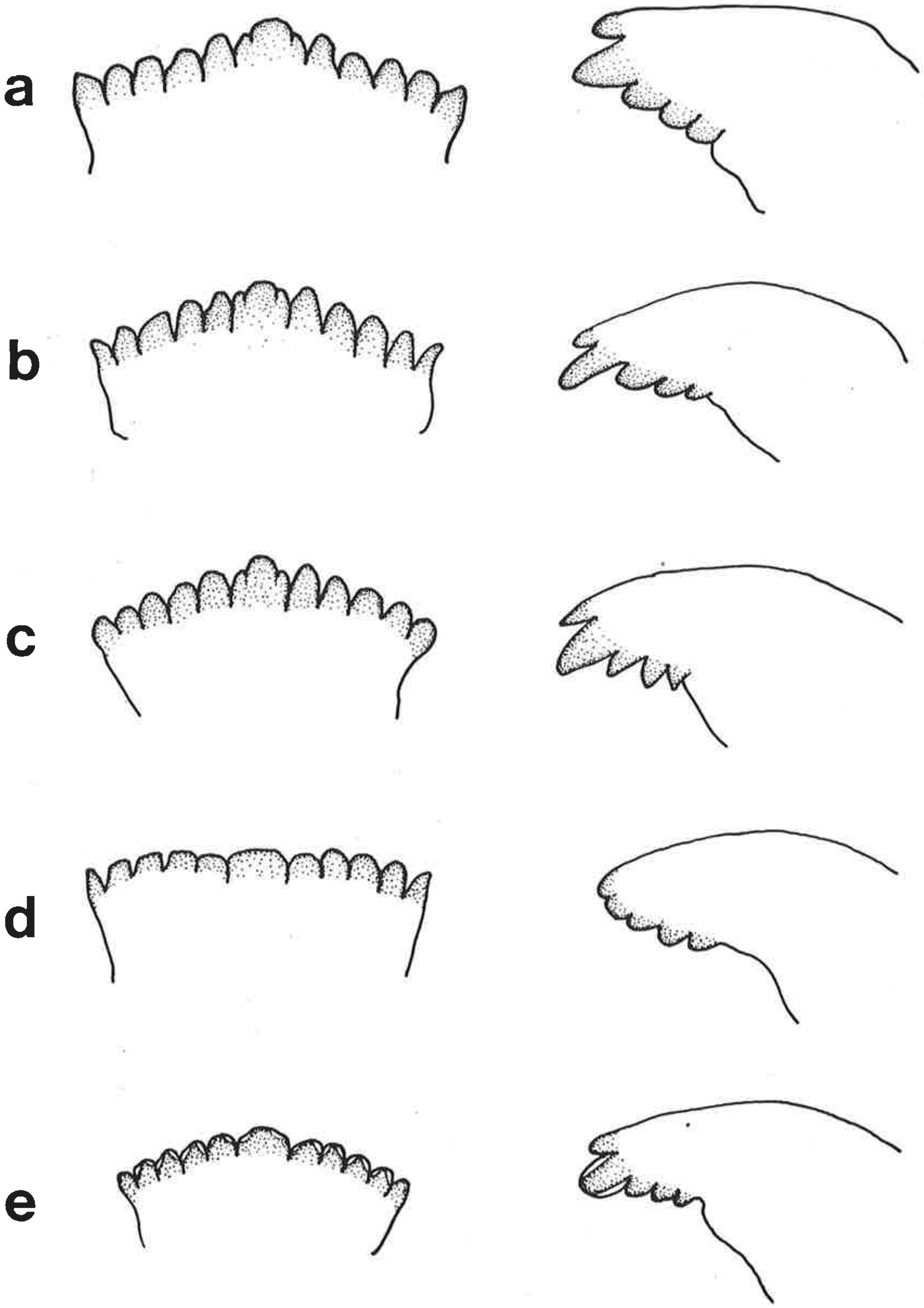


Table 3.3 A summary of collection data for *T. barbitarsis* and *T. semibarbitarsus* in Australia.

Locality	Latitude	Longitude	Environment	*T. barbitarsis*	*T. semibarbitarsus*	Reference
SOUTH AUSTRALIA						
Bolivar	34°46'	138°30'	Sewage works	+	+	Glover, 1973
Coorong - south lagoon	36°15'	139°38'	Salt lagoon	+		Kokkinn unpubl., Glover, 1973
Berri	34°17'	140°36'		+	+	Glover, 1973
Blanchetown	34°21'	139°37'		+	+	Glover, 1973
Devlin Pound	34°10'	140°11'		+	+	Glover, 1973
Port Augusta	32°30'	137°46'	Salt lake	+		Kokkinn unpubl.
Little Dip Lake	37°10'	139°45'	32^{o}/oo TDS	+		Kokkinn unpubl.
ICI Saltfields	34°48'	138°36'	Brine ponds	+		Glover, 1973
Port Broughton	33°36'	137°56'	Mangrove swamp	+		Glover, 1973
Thompson's Beach	34°52'	138°30'		+	+	Glover, 1973
Middle Beach	34°36'	138°25'	Samphire swamp	+		Glover, 1973
Waterfall Gully	34°48'	138°30'		+		Glover, 1973
Ramco	34°10'	139°57'		+	+	Glover, 1973
Milang	35°24'	138°58'		+	+	Glover, 1973
Arkaroola	30°20'	139°22'			+	Glover, 1973
Big Swamp	34°39'	135°42'			+	Glover, 1973
Kersbrook Creek	34°47'	138°51'			+	Glover, 1973
Happy Valley	35°09'	137°09'	Sewage works		+	Glover, 1973
Inman River mouth	35°30'	138°31'			+	Glover, 1973
Bremer River, Callington	34°58'	139°02'			+	Glover, 1973
Barmera-Lake Bonney	34°15'	140°28'			+	Glover, 1973
Morgan	34°02'	139°40'			+	Glover, 1973
Swan Reach	34°34'	139°36'			+	Glover, 1973
Reedy Creek Falls-Caloote	34°58'	139°16'			+	Glover, 1973
Whyalla	33°02'	137°35'	Mangrove swamp	+		Kokkinn unpubl.
Lake Eyre South	29°18'	137°30'	Salt lake	+		Ruello, 1976
Noora	34°26'	140°52'	Evaporation basin	+	+	Suter, pers.comm.
VICTORIA						
Lake Werowrap	38°15'	143°29'	54^{o}/oo TDS	+		Paterson & Walker, 1974a
Lake Keilambete	38°13'	142°53'	72^{o}/oo TDS	+		Kokkinn unpubl.
Lake Gnotuk	38°13'	143°06'	57^{o}/oo TDS	+		Bayly & Williams, 1966
Cheetham Saltworks	37°55'	144°35'	Brine Pond	+		Glover, 1973
Red Rock, Alvie	37°31'	144°39'		+		Glover, 1973
NEW SOUTH WALES						
Barmah	36°01'	144°57'			+	Glover, 1973
Moruya Heads	35°55'	150°10'			+	Glover, 1973
WESTERN AUSTRALIA						
Rottnest Island	32°00'	115°30'	Salt lake	+		Freeman, 1961
Dampier	20°39'	116°43'		+		Glover, 1973
Bremer Bay	34°23'	119°23'		+		Freeman, 1961
Tambrey Station	21°38'	117°36'		+		Freeman, 1961
Dalyup River	33°44'	121°34'		+		Freeman, 1961
Millstream Station	21°35'	117°04'		+		Freeman, 1961
Nedlands	31°57'	115°51'		+		Glover, 1973
Needilup Creek	33°57'	118°46'		+		Glover, 1973
Lake Grace	33°06'	118°28'		+		Freeman, 1961
Esperance	33°52'	121°54'		+		Freeman, 1961
Toodyay	31°33'	116°28'			+	Glover, 1973
Marchagee			Salt lake	+		Halse, 1981
NOTHERN TERRITORY						
Mount Hay Bore	23°24'	133°09'			+	Glover, 1973

NOTE: Where no indication of the environment is given, specimens have been recorded as adults.

Head capsule shape (length-breadth ratio), the prominence of antennal tubercles, and the relative length of the Lauterborn petioles are consistent in the barbitarsis group where all specimens conform to the ratios described above for the Port Augusta population (Figs 3.1a and 3.3a).

Table 3.3 summarizes collection data for barbitarsis and semibarbitarsus.

DISCUSSION

Although it is probable that further species of salt water Tanytarsini will be discovered, the lack of diversity among the described fauna simplifies taxonomic work. Apart from the highly-specialized marine species, Pontomyia cottoni Womersley, Telmatogeton australicus Womersley and Clunio martini Hashimoto (Hashimoto, 1973), the only described Australian species besides T. barbitarsis which occur in salt waters exceeding 10 g l^{-1} are T. semibarbitarsus, and T. neosydneyensis Glover, which is an estuarine species (Glover, 1973).

The morphological characters which distinguish T. barbitarsis from T. semibarbitarsus, such as shape of the head capsule, antennal tubercles and Lauterborn organs, are all characters which are commonly used in the major keys (Bryce and Hobart, 1972; Mason, 1973; Wiederholm, 1983). Characters such as mental and mandibular teeth, which are also widely used in keys, are not appropriate for this group because of the considerable variation due to wear. It is likely that similar differential wear occurs in most other chironomid species depending upon the environment from which they were taken. The value of mental and mandibular teeth can, therefore, be called into question as useful taxonomic characters for the discrimination of larval chironomid species by ecologists.

An added advantage of the three main distinguishing characters for *T. barbitarsis* and *T. semibarbitarsus* is that it is possible to separate preserved specimens without clearing and mounting the head capsules.

The morphometric analysis shows considerable within-group variation of head capsule size or antennal length for *barbitarsis*. Such variation, however, is not unexpected in the light of the considerable range in size of head capsule width in the Port Augusta population (Fig. 3.4). It is conceivable that varying environmental factors (sediment grain size, size of food particles) select for particular size classes of larvae.

The constancy of the antennal ratio (despite the fact that it was 0.8:0.2 for both species) indicates that such relationships would be better diagnostic features than absolute magnitudes of anatomical features. Indeed, this has been found to be so in the difficult *Chironomus* group (Webb and Scholl, 1985). Once again, such characters are not appropriate for ecological work.

Morphological variation in the putative *T. barbitarsis* group shows no clear pattern. There are no consistent differences between the Serpentine Lake (Rottnest Island, W.A.), high-salinity "physiotype" and the low-salinity specimens from south eastern Australia. All were consistently identified as *T. barbitarsis* using the three major distinguishing features described above. This work has not shown the existence of a distinct morphotype from Western Australia which would indicate the presence of another closely-related species. Observed differences in salinity tolerance, therefore, would have to be ascribed to long-term acclimatization of larvae in permanent salt lakes where salinities are consistently in the upper range (100 - 140 g l^{-1}).

The distribution of *T. barbitarsis* (Table 3.3) extends from southern Australia into the tropics. The species has not been collected from the eastern seaboard. Its presence in the intertidal pools of the South Australian gulfs indicates that it could be found in similar environments around Australia. However, its distribution may be restricted to sheltered, low-energy coastlines. Larvae of *T. barbitarsis* are only collected from salt waters, indicating their halobiontic habit.

Collection data for *T. semibarbitarsus* indicate a similar wide distribution. The species, however, appears to be restricted to waters in the low salinity ranges (less than 25 g l^{-1}, P.Suter pers. comm.). Such differential tolerance to salinity would render the head capsules of the two species useful to palaeolimnologists as indicators of palaeosalinities.

Tanytarsus barbitarsis larvae have been described from reliable and constant features of the 4th instar head capsule. There is no indication from larval morphology that a second species with high salinity tolerance exists. The possibility still exists, however, that an examination of all life stages may reveal a second species (see Lindberg, 1967).

CHAPTER 4

Population Studies

INTRODUCTION

There is a large volume of literature which deals with the ecology of the Chironomidae (see bibliographies by Fittkau et al., 1976; Davies and Davies, 1976c & d, 1977, 1978, 1979 and 1980; Pinder, 1986). Many ecological examinations include population studies of varying aims.

In some cases, chironomid populations are studied to elucidate life history adaptations to extreme or unusual environments (e.g. Gordon et al., 1978; Butler, 1982; Paterson and Cameron, 1982). In others, their influence upon community structure provides the focus, for example, their role as predators (Jones, 1974), or as food for fish (Ponyi et al., 1983) and birds (Maher, 1984; Maher and Carpenter, 1984; Tait-Bowman, 1980).

The development of the trophic-dynamic model of ecosystems (Lindeman, 1942) and the International Biological Programme, in particular, stimulated many population studies of chironomids concerned chiefly with the measurement of secondary production (e.g. Jónasson, 1964, 1965; Jónasson and Kristiansen, 1967; Kimerle and Anderson, 1971; Charles et al., 1973; Paterson and Walker, 1974a; Menzie, 1981), and the role of chironomid larvae in the release of limiting nutrients (Gardner et al., 1983).

Investigations of the impact of pollution on both running and standing waters formed the central theme to a further category of population studies. One sort was concerned with the effects of pollutants such as heavy metals and raised temperatures upon chironomid populations (Koehn and Frank, 1980), another dealt with the usefulness of chironomids in biological surveillance (e.g. Saether, 1979; Morris and Brooker, 1980; Armitage and Blackburn, 1985).

Mention should be made in this brief review of the proposal by Thienemann (1913) that particular chironomid taxa are indicative of lake trophic status because it has stimulated several studies mainly of a palaeolimnological nature (e.g. Deevey, 1942; Brundin, 1958; Saether, 1975; Warwick, 1975; Hofmann, 1983).

Chironomids as nuisance organisms have also generated a variety of population studies concerned with the influence of: substrate type (Ali and Mulla, 1976a), seasonal effects (Ali et al., 1977), nutrients (Provost and Branch, 1959), insecticides (Norland et al., 1974; Shan-ching, 1978; Ali and Lord, 1980a), biological control agents (Bay and Anderson, 1966; Hershey, 1985) and pathogens (Molloy and Jamnback, 1981; Treverrow, 1985) upon problem species.

With some exceptions (for example, Kimerle and Anderson, 1971), population studies of chironomids have suffered from two universal weaknesses: a failure to deal with the significance of sampling data (i.e. how well does sampling data reflect actual changes in populations), and poor taxonomic resolution resulting from a lack of larval descriptions (see Chapter 3).

A study of T. barbitarsis at Port Augusta had the advantage of being free from taxonomic problems, there being only one chironomid species present at the prevailing water salinities (50 to 100 g l^{-1} NaCl). Hence it was possible to embark upon research with the following aim: to study with acceptable accuracy the influence of biological and physico-chemical factors upon the variation in generation time and abundance of T. barbitarsis in the Port Augusta fly-ash lagoons.

The need for such a study in terms of pest management is indicated by two main points. Firstly, a knowledge of the seasonal dynamics of _T. barbitarsis_ is essential for the timing of effective control measures, either chemical or biological. And secondly, a population study which reveals the influence of biotic and abiotic factors upon the abundance of an organism will offer a series of opportunities for control via the manipulation of such factors.

I. PILOT SURVEY

Materials and Methods

Before the sampling programme proceeded, it was appropriate to investigate both the vertical and horizontal distribution of _T. barbitarsis_ larvae within the lake system. This information was required before decisions could be made on depth, number and position of samples required to return data of acceptable accuracy.

The vertical distribution of larvae within the sediments was investigated by taking a series of ten 300 mm cores with a plastic coring pipe (diameter 100 mm) in a variety of substrates, ranging from coarse (1 mm grain size) ash to fine, organic ooze. These cores were divided into 50 mm segments and preserved separately. In the laboratory, the relative number of larvae in each segment was recorded.

Horizontal distribution was investigated in a separate sampling survey. At each of 19 randomly chosen sites at three levels within the lake system (ETSA Lake, Bird Lake and Railway Lake - see Fig 4.1), a series of 5 samples was taken within a 25 m^2 area. A brass coring device (constructed after consideration of the results of the vertical survey) was inserted 100 mm into the mud surface and its upper end sealed to facilitate the withdrawal of poorly compacted sediments. The choice of a core device for sampling was made in the light of several favourable comparisons with other sampling implements (Flannagan, 1970; Milbrink and Wiederholm, 1973; Paterson and Fernando, 1971) particularly in shallow waters (Hellawell, 1978).

Apart from considerations of sampling accuracy, the preliminary survey was undertaken to estimate sample treatment time. A major difficulty in many studies of benthic organisms is the amount of effort required to get results with reasonably narrow confidence limits. It appeared from the work of Paterson and Walker (1974a) that the generation time for *T. barbitarsis* was approximately 30 days at 17°C. A sampling interval of 14 days, therefore, was chosen with the intention of tracing the development of individual generations. The number of benthic samples which could be taken each fortnight would depend primarily upon treatment time and the time available for sorting of samples during each 14-day period. To this end, a record was kept of the time it took to treat each sample from the initial survey.

Results

The vertical distribution of larvae within the 10 cores indicated unequivocally that all larvae were within the top 50 mm of substrate, and most within the top 10 mm, even in relatively well-oxygenated sediments. This is consistent with the observations of Oliver (1971) who found that more than 95% of chironomid larvae normally occur within the top 10cm of the substrate. On the strength of this finding, a more permanent brass coring device (diameter 44 mm), which took a standard 100 mm vertical core from the substrate, was constructed.

The results of the horizontal survey are given in Table 4.1. A one-way analysis of variance on the transformed data ($\log_{10} X + 0.5$ to render the mean and variance independent), with 18 and 75 degrees of freedom, gave an F value of 31.70 which was highly significant. This confirmed that there were significant differences between sampling sites. A multiple range test (Duncan's) on the untransformed data revealed which sites were not significantly different from one another (at the 5% level) and could, therefore, be grouped as strata or subunits.

The variances for each lake were far greater than the means (Table 4.1), suggesting a negative binomial distribution (Elliott, 1971). When tested for goodness of fit to a negative binomial distribution, the data from ETSA and Bird Lakes yieloed a Chi^2 value of 15.058 with 16 degrees of freedom and a k value (the clumping factor Elliott, 1971) of 2.72675. This indicated that the larvae in these two lakes had a clumped or negative binomial distribution (numbers of larvae in Railway Lake were so low that it was not considered in these analyses). The implications of this indication were that, before statistical treatments requiring normally distributed data could be applied, sampling data would have to be transformed (Log_{10} (x+1)).

Figure 4.1 plots the 5 areas within the lakes which are represented by samples not significantly different from one another (Duncan's multiple range test). The water flow directions, indicated by arrows in Fig. 4.1, were derived from a survey of water salinities across the lake system. The correspondence between flow and distribution of *T. barbitarsis* larvae suggests that salinity or substrate may be exerting some influence upon the presence of larvae.

Average sample treatment time was 1h 32min (range 23 min to 2h 40min) (for procedure see p. 49). The presence of plant material was a considerable impediment to the sorting process.

Table 4.1 Number of fourth instar *T. barbitarsis* larvae collected on the same day from 19 randomly-selected sites within the Port Augusta lake system.

Site No.	Replicates					Mean	Locality of sample
1	36	37	47	53	60	47	ETSA Lake
2	48	45	42	100	38	55	Salinity 50 g l^{-1}
3	41	128	268	109	165	142	Mean = 91.2
4	150	271	139	116	114	158	s = 60.9
5	37	91	41	62	82	63	s^2 = 3715.5
6	112	77	79	65	82	83	
7	12	12	22	11	11	14	Bird Lake
8	65	64	80	35	73	63	Salinity 75 g l^{-1}
9	83	88	76	46	60	71	Mean = 38.3
10	16	5	26	30	19	19	s = 24.5
11	20	21	17	19	13	18	s^2 = 598.6
12	35	34	51	49	31	40	
13	28	17	16	18	23	20	
14	68	74	60	59	45	61	
15	1	1	1	0	2	1	Railway Lake
16	3	1	2	0	1	1	Salinity 140 g l^{-1}
17	1	2	0	8	10	4	Mean = 3.68
18	9	7	13	11	6	9	s = 3.96
19	7	1	4	0	1	3	s^2 = 15.60

Discussion

From the survey results it seemed that to monitor effectively the chironomid population in the entire lake system on a fortnightly basis sampling could be restricted to each of the five indicated strata (Fig. 4.1). However, there was considerable uncertainty as to the reality, extent and permanence of such subdivisions. In addition, the sampling of five strata, each with a minimum of five samples, would require the treatment of 25 samples per fortnight, yielding a total treatment time (37.5h) exceeding that available for benthic samples (18h).

Clearly the width of the confidence limits is inversely related to the number of samples, thus in order to decide upon the required number of samples (in terms of accuracy) the following approximation was devised: The precision of knowing how closely the sample mean resembles the population mean can be calculated from the expression:

$CL = t.s/\sqrt{n}$ .... (1) (Elliot, 1971)

Where:

CL - 95% confidence limits

t - Student's t

s - standard deviation

n - number of samples

Confidence limits can be converted to give the approximate coefficient of variation from: $CV = 50.CL.\sqrt{n}/X$ .... (2)

Where:

CV - coefficient of variation

CL - 95% confidence limits

n - number of samples

X - sample mean

This is based on the assumption that, for values of n greater than 15, t is close to 2. The variation of the CV with different numbers of samples has been calculated for each of the three Port Augusta lakes, based upon the results of the preliminary survey. This relationship is presented in Table 4.2.

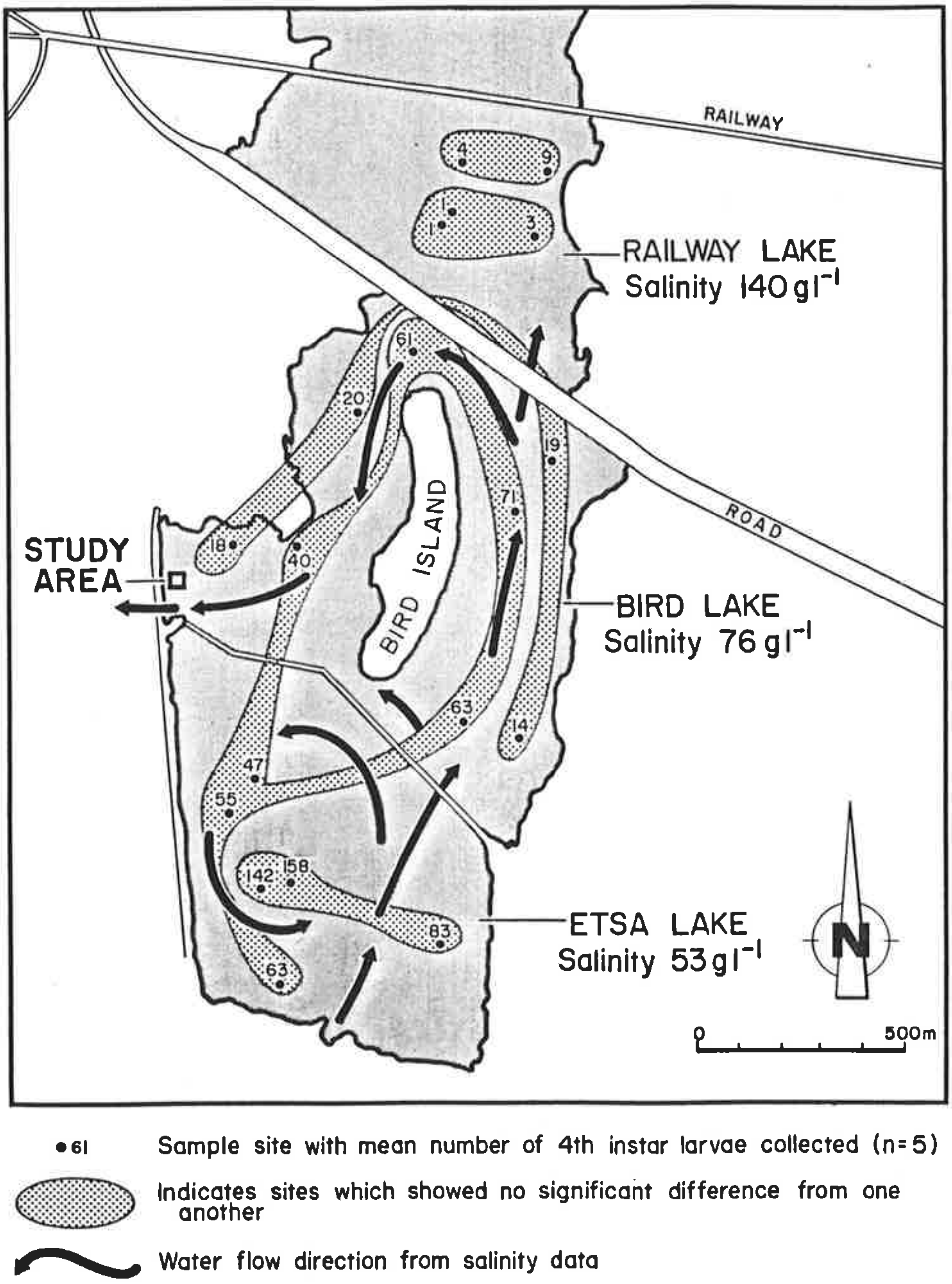
Table 4.2 Relationship between the coefficient of variation and the number of samples required in each lake.

ETSA Lake		Bird Lake		Railway Lake	
No. of samples	CV	No. of samples	CV	No. of samples	CV
1224	10%	1567	10%	2773	10%
309	20%	395	20%	693	20%
138	30%	175	30%	311	30%
78	40%	100	40%	175	40%
51	50%	65	50%	113	50%
36	60%	45	60%	79	60%
27	70%	34	70%	58	70%
21	80%	27	80%	45	80%
17	90%	21	90%	36	90%
14	100%	18	100%	30	100%

From Table 4.2 it appears that, to sample the three lakes randomly with a coefficient of variation of only 50%, it would require 229 samples each fortnight. This represents a treatment time of 340h or approximately 40 days. Clearly this was not a viable option.

The only option remaining was to choose subjectively a single study site which was a representative microcosm of the chironomid habitat at Port Augusta. Since Railway Lake had very low numbers of chironomids at that time it was excluded from consideration. A study site measuring 10,000 m^2 (100 X 100m) was thus chosen with three criteria in mind: it was representative of the two southern lakes, it was sheltered from strong winds and it was readily accessible. A series of test samples indicated that 12 benthic samples (sorting time approximately 18h) taken randomly within this area would return confidence limits narrow enough to detect significant changes in the population.

Figure 4.1 Diagram of the Port Augusta lake system indicating distribution of T. barbitarsis larvae, the study area and the water flow direction.



II. THE FIRST YEAR OF SAMPLING (1/7/82 to 7/7/83)

Materials and Methods

a) Monitoring the physical environment

i) Temperature Air temperature information was supplied by The Electricity Trust of South Australia from their Port Augusta weather station. In addition, fortnightly maximum and minimum water temperatures were monitored by a maximum and minimum thermometer placed midway between surface and bottom in the study area and read fortnightly.

ii) Water level A graduated staff was placed in the study area and water level readings were made each fortnight.

iii) Water salinity Water samples were taken in the study area each fortnight and the salinity was determined conductometrically using the formula of Williams (1986).

iv) Dissolved oxygen Water samples were collected from mid-water on 10 occasions during the year. Dissolved oxygen content was determined by the unmodified Winkler method.

v) pH The pH of the water was measured on two occasions, once in summer and once in winter.

vi) Phosphate Orthophosphate and total phosphorus determinations were made at my request by the Thomas Playford power station laboratory on a fortnightly basis. Water samples were collected from the following sites: Spencer Gulf (marine water), the ash inflow pipe and the study site. They were delivered at 8.30 a.m. every second Friday morning for immediate analysis. Orthophosphate determinations were made by the vanadomolybdophosphoric acid-colorometric method (APHA, 1975).

vii) Nitrate Nitrate determinations commenced in February, 1983, and continued until the end of the first year of sampling. These were determined for the same sites as the phosphate by the cadmium reduction method (APHA, 1975). These analyses, too, were undertaken in the laboratory of the power station.

viii) Chlorophyll *a* Determination of chlorophyll *a* content of water from the study site was undertaken each fortnight by the acetone-spectrophotometric method (APHA, 1975).

b) Sampling the biota

i) Benthos Fortnightly sampling of the benthos was undertaken by extracting 12 cores (100 mm deep, 44 mm diameter, volume 152 cm^2) at random positions within the study area. These were placed in plastic containers and preserved in a 4% formalin solution. Care was taken to shake the containers after they were sealed in order to mix the preservative throughout the sediments.

In the laboratory, samples were washed in a 125 micron sieve to remove silt particles and formalin before the lighter fraction (including the biota) was separated from the heavier fraction (inorganic) by floatation in a magnesium sulphate solution (S.G. 1.20 - Flannagan, 1973). A check of the settled fraction indicated that less than 5% of the biota was lost after 30 minutes of floatation.

Larval instars were recognized from head capsule size (see Fig. 3.4) and counted along with pupae in a maze tray under a binocular microscope at 60X magnification.

ii) Adult emergence A series of 8 submerged funnel traps (Mundie, 1956a; Rosenberg *et al.*, 1980; Rosenberg and Wiens, 1983) was placed in two rows of four across the study area. These consisted of 1 litre plastic bottles with 200 mm diameter funnels inserted, stem first, into their caps. The stems had an internal diameter of 10 mm and they projected at least 100mm into the bottle to prevent loss of emergents after death and settling.

The traps were emptied every fortnight and material preserved in a 4% formalin solution. Counting of emergents was undertaken in the same way as the larvae. It was found that the resistant thoracic case of the adult remained intact for at least a fortnight after the animals died. Thus it was possible to account for all adults that had emerged during the previous fortnight by counting thoracic cases.

iii) The plankton The 1st instar of many chironomid species is planktonic (Davies, 1972; Davies, 1976b) and preliminary work revealed that *T. barbitarsis* was no exception. It was therefore necessary to sample the water column for the planktonic instars. To this end, a modification of Pieczynski's (1961) water mite trap was used (see Davies, 1976a). The device consisted of a series of 250 ml plastic bottles with 75 mm diameter plastic funnels set, stem first, into the caps. Each of two plankton traps had 5 such bottles pointing in different directions. The traps differed from one another in that one had a bottle which projected upwards whereas the other had a bottle which projected downwards - this because of limitations of water depth (500 mm average depth in study site). The other four traps faced the points of the compass in the horizontal plane.

The two traps were deployed in opposite corners of the study area. They were emptied once a fortnight and their contents preserved in a 4% formalin solution. Samples were washed in a 125 micron sieve and counted in the same way as the benthos.

iv) Observations on the lake community During the sampling programme, it became evident that the plankton traps were good samplers of three other organisms which occurred in the lakes, namely, the fish

Atherinosoma microstoma (Gunther, 1861), the hydrophilid beetle Berosus devisi, and the amphipod Allorchestes compressa. Records were kept of the abundance of these species in the plankton traps each fortnight.

In addition, the growth and degeneration of the macrophytes, Ruppia tuberosa (sea grass), Enteromorpha prolifera, Cladophora sp. and Oedogonium/Oocardium sp. (all filamentous algae) was monitored during the course of the year.

Results

a) The physical environment

i) Temperature A plot of air and water maxima and minima (1/7/82 to 7/7/83) is presented in Fig. 2.1. Air temperatures ranged from 2°C (4/7/83) to 45°C (17/2/83). The maximum air and water temperatures were recorded between 20/1/83 and 17/3/83; minima were recorded between 1/7/82 and 12/8/82. Predictably, the range of water temperature (31°C) was narrower than that of air temperature (43°C).

An effect which was not forseen was that, since the particular area where the thermometer was erected was not overgrown by Ruppia, water temperature records do not reflect the effect of shading.

ii) Water level did not fluctuate by more than 30 mm during the entire study period. This is because water leaves the lake system by spilling over a weir which maintains a constant water level.

iii) Water salinity fluctuations during the 1982/83 sampling period are shown in Fig. 4.11. During this period, water flow through the lake system was constant (30,000 m^3 per day - Electricity Trust of S.A.). Salinity differences between summer and winter could therefore be ascribed to the effects of differential evaporation rates. During mid-summer salinity rose to a maximum of 78 g l^{-1} and it fell to a minimum of 48 g l^{-1} during winter.

iv) Dissolved oxygen values are shown in Table 4.3. In all cases, the water column was saturated with dissolved oxygen as would be expected in such a shallow, turbulent water body. However, levels of saturation may not be realistic because of the dubious assumption that the decrease of oxygen solubility with increasing salinity is a linear function (De Deckker and Williams, in press).

Table 4.3 Dissolved oxygen content of water from the Port Augusta study site.

Date	Dissolved O_2 (mg l^{-1})	Saturation %
3/ 6/82	11.2	229
17/ 6/82	11.0	213
1/ 7/82	11.4	207
15/ 7/82	10.8	193
12/ 8/82	12.5	245
26/ 8/82	10.7	223
9/ 9/82	10.1	220
23/ 9/82	13.9	290
2/12/82	8.8	205
6/ 1/83	8.4	205

v) pH The following values were recorded: 8.6 on 7/10/82 and 8.8 on 7/7/83.

vi) Orthophosphate A graphical comparison of orthophosphate-P in the water from the three sampling sites is made in Fig. 4.2. It is evident that orthophosphate-P values in Spencer Gulf water are consistently low. In contrast, water flowing into the lakes from the ash disposal system had elevated values, indicating a contribution of this nutrient by the fly-ash.

Consistently high (circa 50 µg l^{-1}) orthophosphate-P values from the study site suggest that it is not a limiting nutrient there.

vii) Nitrate Values for dissolved nitrate are presented in Fig. 4.3. Generally the measurements show strong fluctuations between sampling periods. However, there is evidence that nitrate is introduced to the lakes by the fly-ash disposal system. Consistently low values in the study area suggest that nitrate is a limiting nutrient.

viii) Chlorophyll a values were relatively low (10 µg l^{-1}) during the winter months, but increased during summer when the system was not shaded by macrophytes. Values fluctuated considerably from month to month (Fig. 4.4).

b) The biota

i) Benthos Data on 2nd instars showed no trends and are not presented here. The relative abundance of 3rd instars in the benthos is presented in Fig. 4.5. Initially (1/7 to 12/8/82), 3rd instar numbers were in the region of 30,000 m^{-2}. Numbers fell sharply in late August (26/8/82) to 5,000 m^{-2} where they remained until late spring (21/10/82). In spring, there was a steady increase which reached an apparent peak in summer (3/2/83) whereafter numbers declined once more to levels of 5,000 m^{-2} (17/3/83 to 28/4/83). During late autumn and winter (12/5/83 to 7/7/83), numbers of 3rd instars increased to levels not seen previously (90,000 m^{-2}).

A plot of 4th instars is presented in Fig. 4.6. During winter (1/7/82 to 26/8/82), 4th instar numbers in the benthos reached 60,000 m^{-2}. With the onset of spring there was a steady decline (26/8/82 to 4/11/82) until numbers were in the vicinity of 10,000 m^{-2}. Between 8/11/82 and 3/3/83, 4th instar numbers appeared to remain in the region of 25,000 m^{-2}. This was followed by a fall in numbers between 17/3/83 and 26/5/83 to approximately 10,000 m^{-2} once more. During the early part of winter (9/6/83 to 7/7/83), 4th instar numbers increased to 30,000 m^{-2}.

ii) Adult emergence data are presented in Fig. 4.7. Prior to the deployment of submerged funnel traps (23/9/82), emergence was estimated from adults trapped in the downwardly directed plankton trap. This estimate revealed a spring adult emergence (26/8 to 9/9/82). Subsequent to this (23/9 to 2/12), emergence remained at a low level (circa 500 m^{-2}). Although not recorded, a large emergence was reported on 22/12/82 (R. Rutter, pers. comm.). This trend was continued during January and February, 1983 when emergence remained at a high level (circa 6,000 m^{-2}). A peak emergence (13,000 m^{-2}) was recorded on 20/1/83. The fortnight ending 17/3/83 reflected a sharp fall in the number of emergents (circa 1,000 m^{-2}). Subsequently (31/3/83), there was another peak in emergence (9,000 m^{-2}). During autumn and early winter (28/4 to 7/7/83), the number of emergents decreased but remained at a plateau of 2,000 m^{-2}.

Figure 4.2 Orthophosphate-phosphorus concentrations in Spencer Gulf, in ash inflow and at the study site.

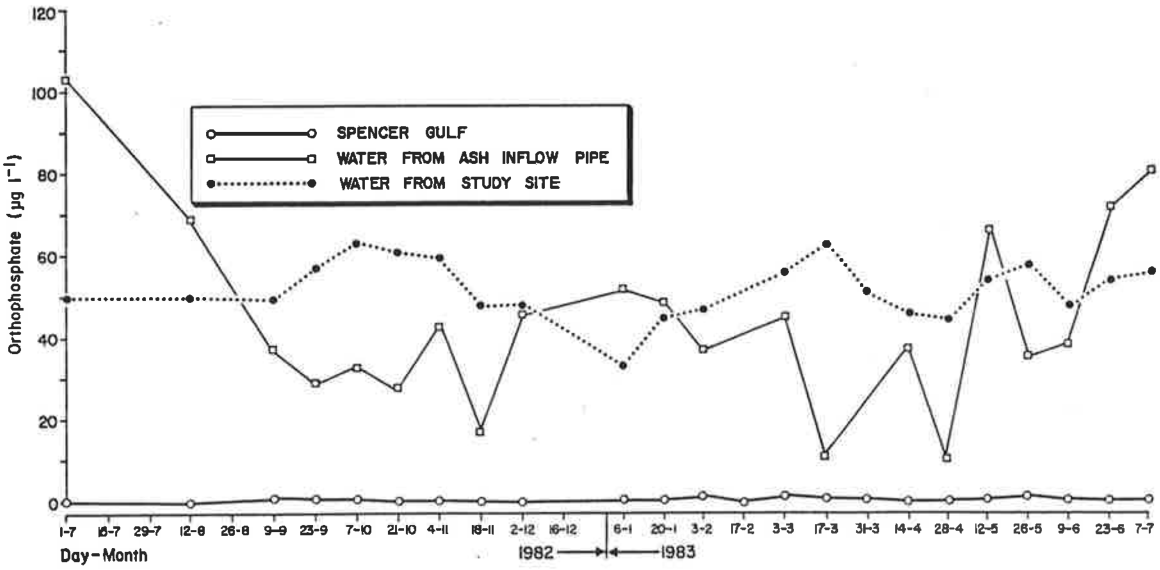


Figure 4.3 Nitrate concentrations in Spencer Gulf, the ash inflow and at the study site.

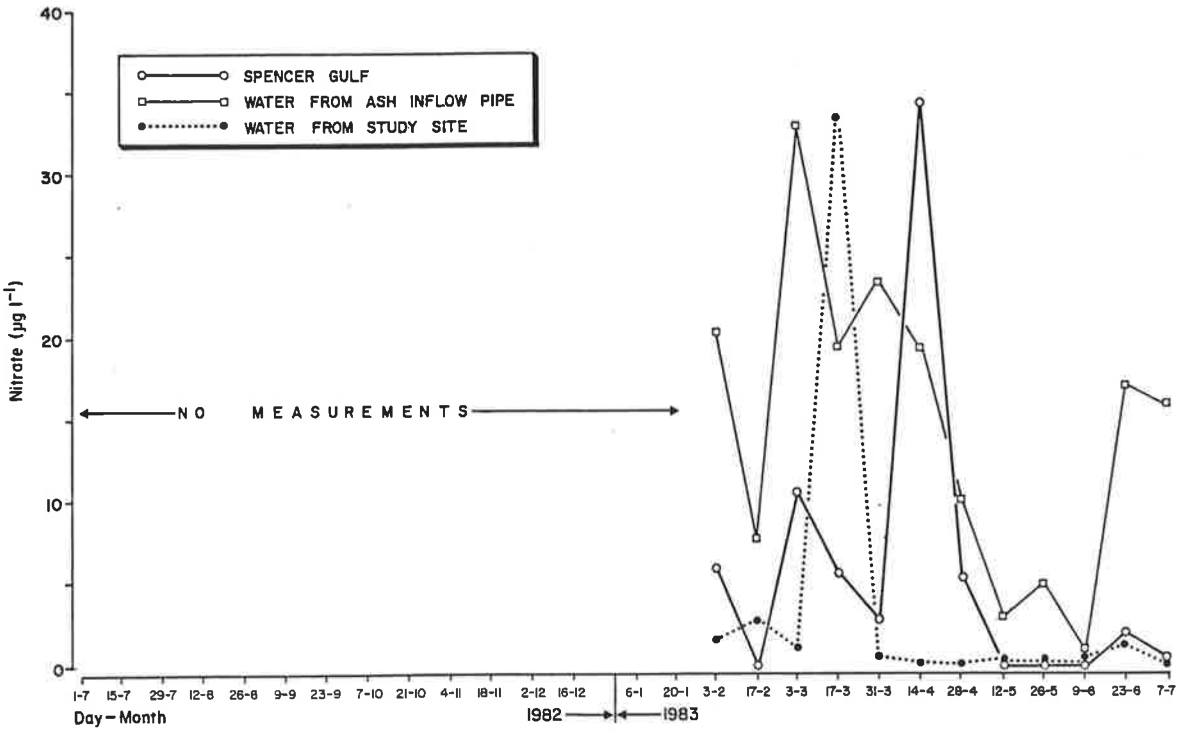
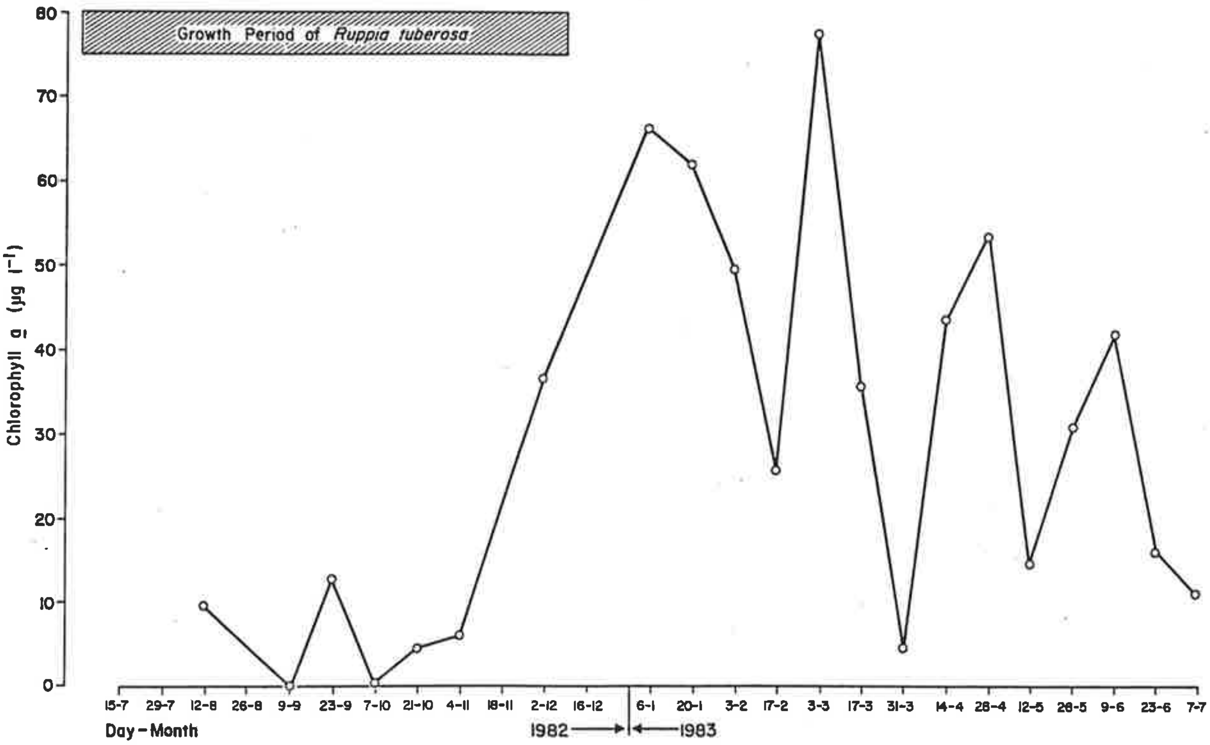


Figure 4.4 Abundance of chlorophyll a and major occurrence of Ruppia in the study site.



iii) *The plankton* The relative abundance of *T. barbitarsis* larvae and pupae in the plankton is presented in Fig. 4.8. Total numbers are given because of the high variability between individual components of each trap. In general, Fig. 4.8 shows that peaks in the numbers of 1st instars are reflected by 2nd instars except in early winter when some lag occurs - probably due to temperature effects (12/5 to 23/6/83). Abundances of the larger instars (3rd and 4th) show discernible lags consistent with a pattern of developing cohorts. This is particularly evident between 6/1/83 and 14/4/83.

iv) *Observations on the lake community* Total numbers of the fish, *Atherinosoma microstoma*, and the beetle *Berosus devisi*, as well as the presence of the amphipod *Allorchestes compressa* over the study period are given in Fig. 4.9. *A. microstoma* was present all year round, but its population received a major recruitment in November. *B. devisi* was recorded between 18/11/82 and 7/7/83 with peak abundance in summer. *A. compressa* presence was strongly correlated with the presence of *Ruppia tuberosa* (see Fig. 4.4 and below).

During July/August, there was a rapid growth of *R. tuberosa* in the two southern lakes. By the end of August, the water column was pervaded with this aquatic macrophyte, its long (1 metre) stems rising to the surface and forming a continuous mat. Filamentous algae (*Enteromorpha prolifera*, *Cladophora* sp. and *Oedogonium* ? *Oocardium* sp.) became conspicuous during September, overgrowing *R. tuberosa* and forming a covering mat.

The disappearance of the abundant plant material was rapid and coincided with the first hot spell (30°C, 4/11/82). By January, the lake bottom was once more relatively bare mud.

Figure 4.5 Pattern of abundance of 3rd instar larvae in the benthos -1982/83

NOTE: The pattern of adult emergence plotted as horizontal bars above each of the 3rd, 4th instar and Adult figures (Figs 4.5, 4.6, 4.7) was derived from the 4th instar data. This interpretation of emergence pattern is given to assist in comparison between this and Figs 4.6 and 4.7.

Vertical bars on figures represent 95% confidence limits.

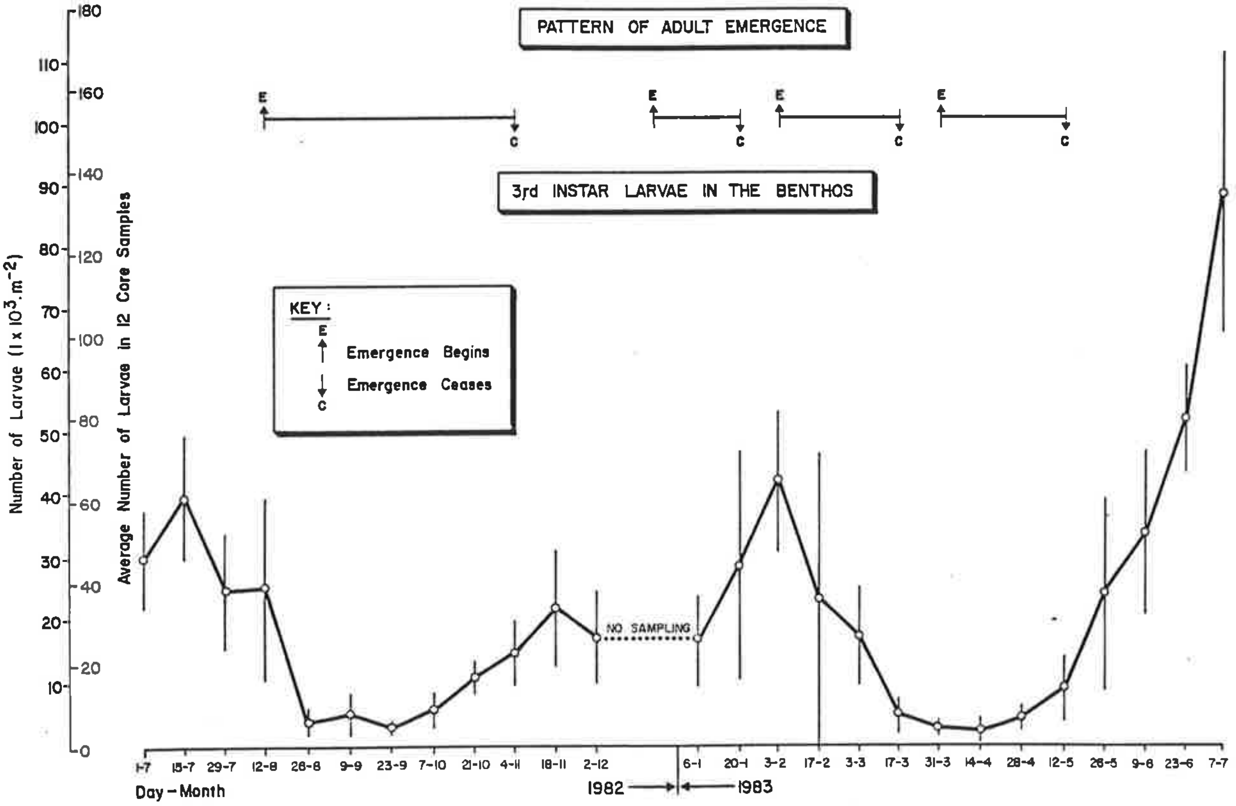


Figure 4.6 Pattern of abundance of 4th instar larvae in the benthos -1982/83

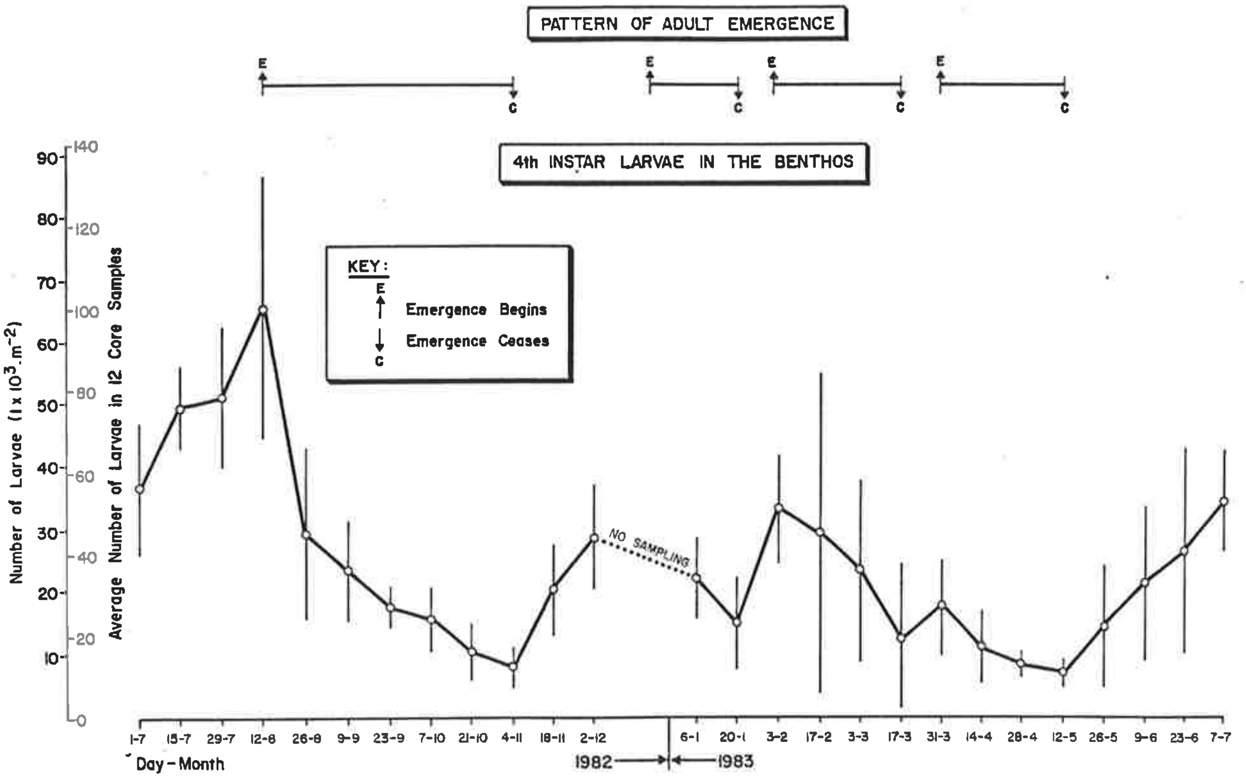


Figure 4.7 Adult emergence 1982/83

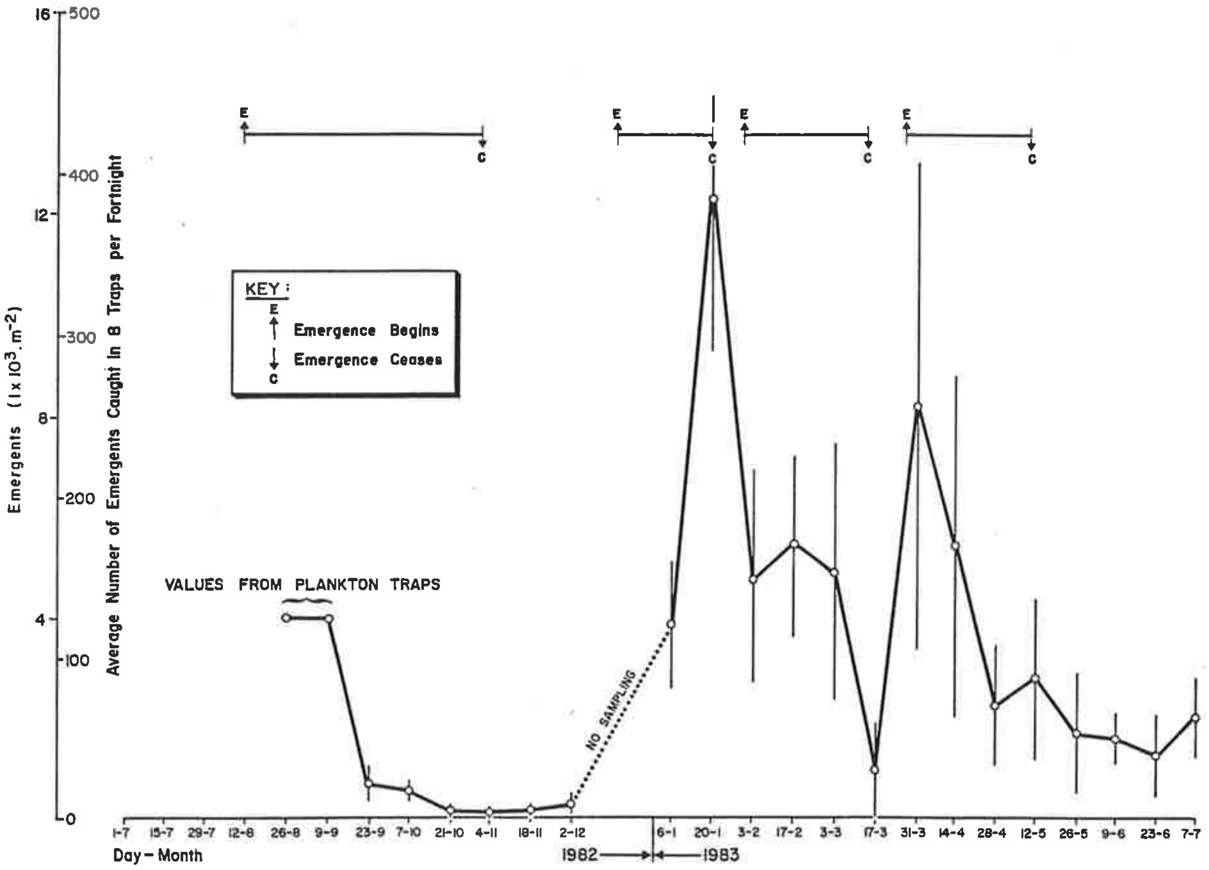


Figure 4.8 Larvae caught in plankton traps.

I	1st instars
II	2nd instars
III	3rd instars
IV	4th instars
P	pupae

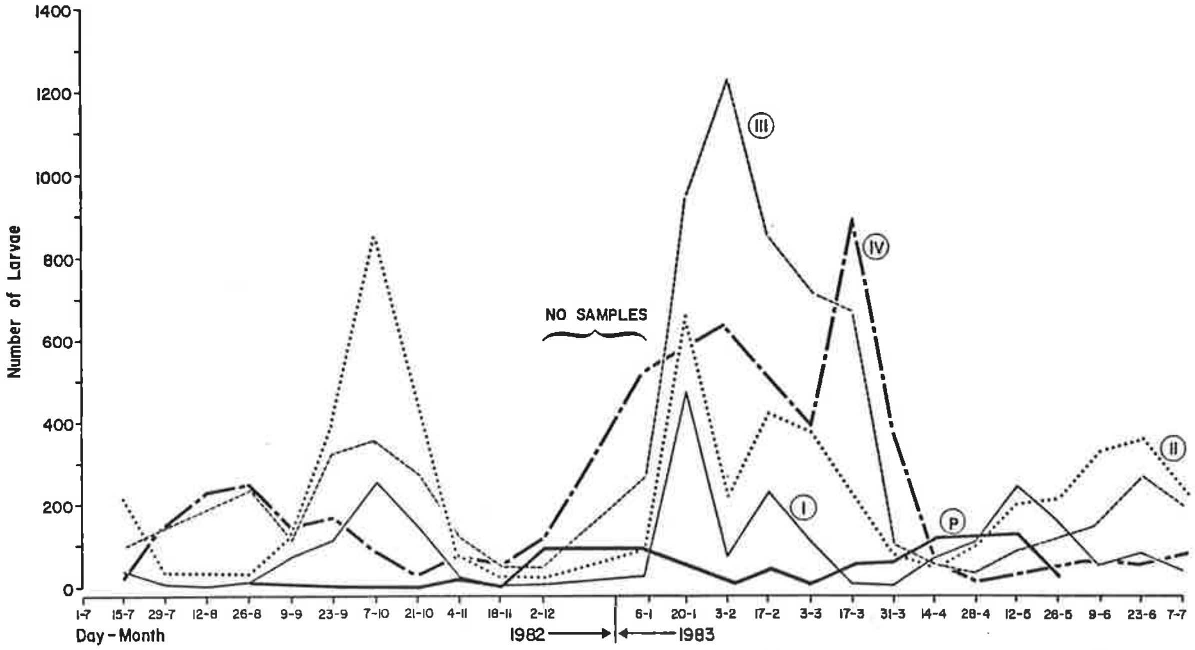
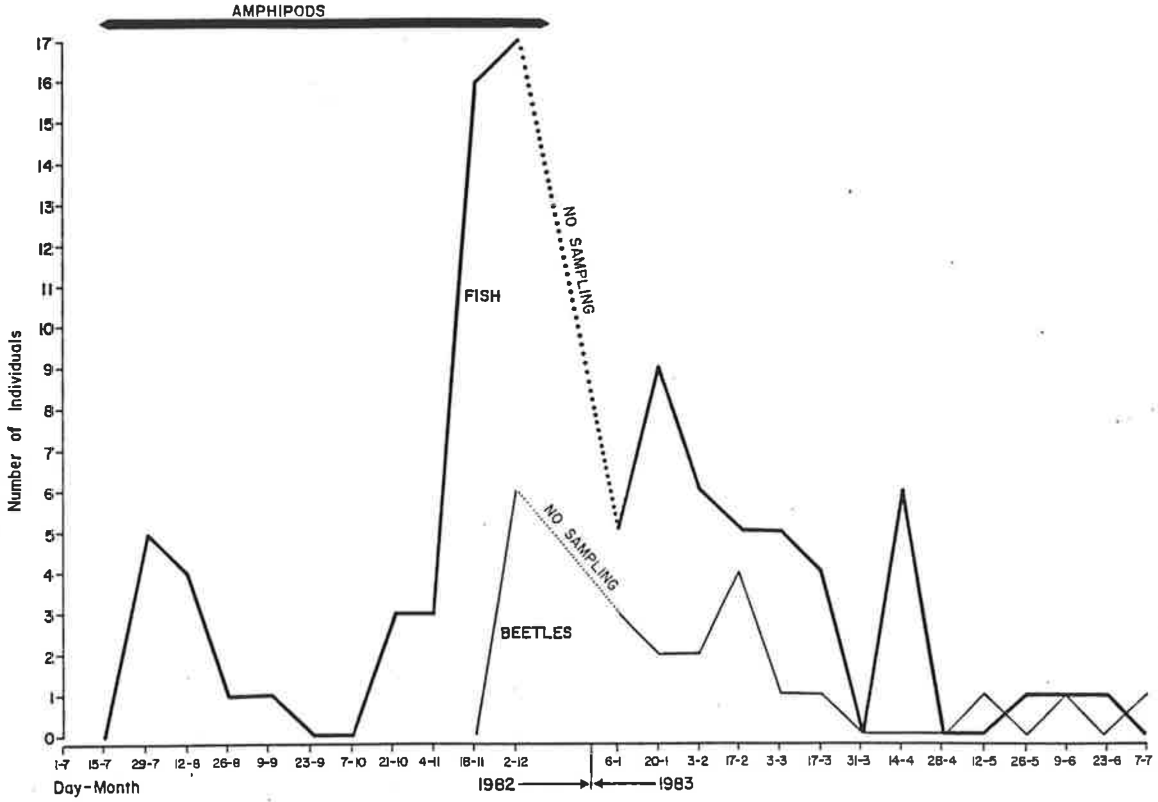


Figure 4.9 The abundance of fish (*Atherinosoma microstoma*), beetles (*Berosus devisi*) and the occurrence of amphipods (*Allorchestes compressa*) during 1982/83. Data from plankton traps.



III. THE SECOND YEAR OF SAMPLING (13/9/84 to 12/9/85)

Materials and Methods

a) Monitoring the physical environment

i) Temperature Based upon the observation during the 1982/83 sampling programme that there was a significant temperature difference between surface and bottom water (particularly in the presence of Ruppia mats), two maximum and minimum thermometers were deployed in the study area. One was placed with its bulbs just above the mud surface, where the majority of chironomids settle, and the other was placed with its bulbs 200 mm below the water surface. Both thermometers were read once a fortnight.

ii) Water salinity Water samples were collected once a fortnight and the salinity determined as before.

b) Sampling the biota

i) Larval settlement A technique for monitoring the larval chironomid population was developed on the basis of an observation that larvae settle readily upon any available surface in the water column. Since the emphasis during the latter phase of the study was upon experimental work, the new technique was an attempt to continue the monitoring of larvae without labour-intensive mud sampling.

A series of 12 plastic rods was erected randomly within the study area. Upon these rods at 200 mm from the mud surface, were suspended 25 mm, PVC plumbing end caps with the cup facing upwards.

Each fortnight, the caps were gently removed from the water column to ensure that larvae remained within their tubes, and transferred to plastic containers for removal to the laboratory.

Scrubbed replacement caps were then deployed as settlement surfaces for the following fortnight.

ii) Adult emergence Three rows of four emergence traps (12 in total) were placed on lines across the study area. These were identical to those used during the first sampling year. They were emptied on a fortnightly basis and the emergent adults counted as before.

iii) Fish The two sets of plankton traps were retained as fish traps. However, instead of simply recording the total number of fish present as during the first year, fish were recorded as either larvae (visible yolk sac), juveniles (less than 30 mm in length), or adults (greater than 30 mm).

Results

a) The physical environment

i) Temperature Maximum and minimum fortnightly water temperatures for surface and bottom waters are documented in Fig. 4.10. Maximum bottom temperatures tended to fluctuate more markedly than surface ones. Generally, summer bottom temperatures had a narrower range than surface temperatures. Between 26/4 and 12/9, there was little vertical temperature difference.

ii) Water salinity in the study site during the second year of sampling was the single most important physical difference between the two years (Fig. 4.11). An explanation for this difference would seem to relate to the testing of a large new power station (The Northern Power Station) between 22/11/84 and 5/3/84. This involved a considerable increase in water flow through the lake system (from 30,000 to 60,000 m^3 d^{-1}). The effect of this increased flow was to hold water salinity in the study area between 50 and 60 g l^{-1} during a period when it would previously have risen to levels between 70 and 80 g l^{-1} (Fig. 4.11).

b) The biota

i) Larval settlement data are shown in Figs 4.12 and 4.13. The settlement technique did not live up to expectations. There was a high degree of variability between the numbers of larvae settling on the plates. Confidence limits overlapped to the extent that most observed fluctuations did not appear significant. However, one important comment is possible. It appears that the numbers of smaller instars, particularly 1st instars, remained at a low level throughout the summer.

ii) Adult emergence was significantly different from that of the 1982/83 sampling season (Fig. 4.14). After a small initial emergence (25/10), there were no peaks of adult emergence during the summer (8/11/84 to 11/4/85). It was only in late autumn that a significant emergence took place (9/5/85). Subsequently, there were two minor peaks in emergence during winter (6/6/85 and 30/8/85).

iii) Fish Resolution of the fish population into age classes showed that there was a marked peak in the abundance of larvae in early summer (22/11/84) (Fig. 4.15). This was followed by a smaller peak in the abundance of juveniles between 31/1 and 26/4/85. A minor peak in larval abundance occurred in mid-winter (18/7/85). It should be noted that the abundance of larvae (110) in spring was significantly higher than that measured during the spring of 1982 (17).

Figure 4.10 Maximum and minimum water temperatures 1984/85

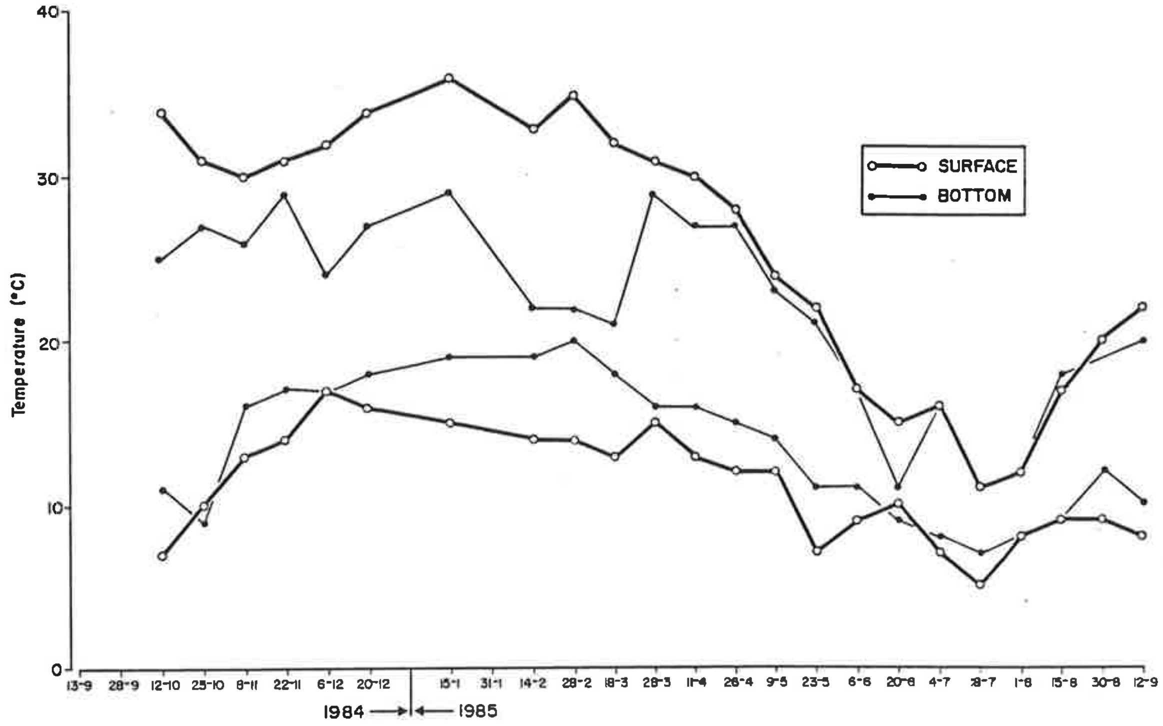
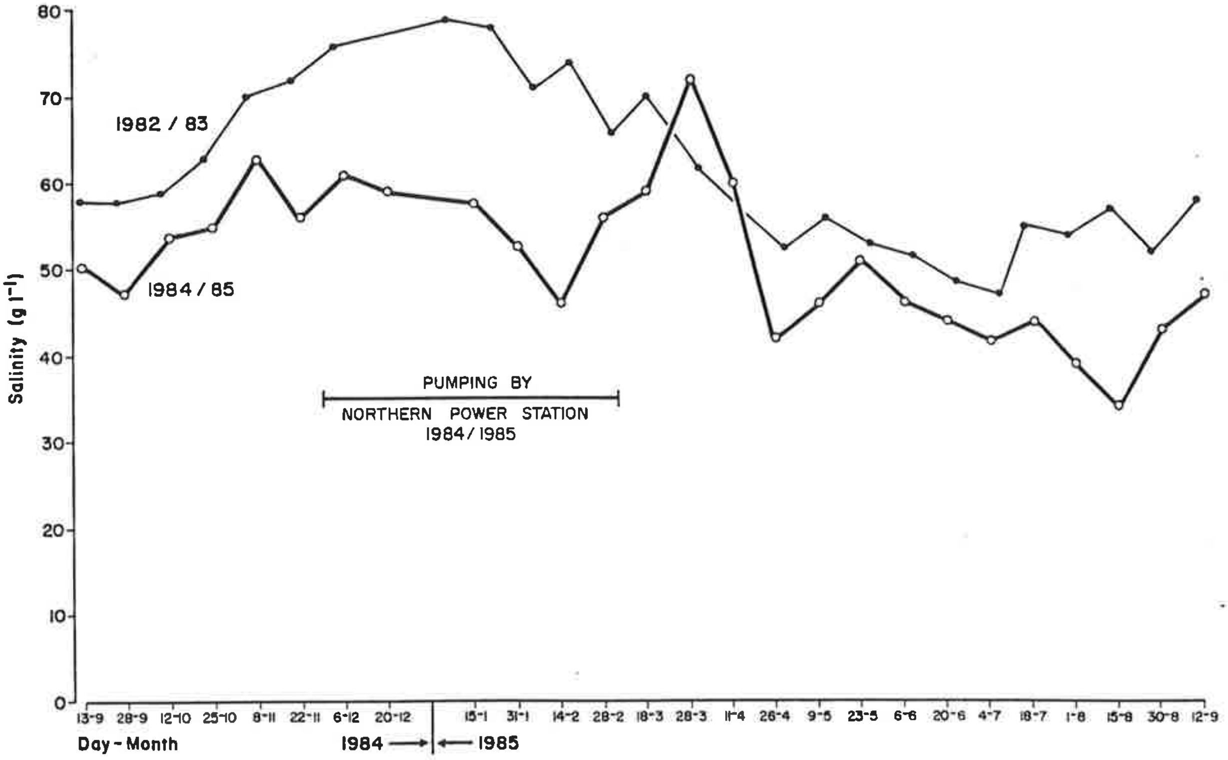


Figure 4.11 Water salinities in the study site, 1982/83 and 1984/85



iv) The lake community At the beginning of this study period (13/9/84), the lakes were covered with mats of *R. tuberosa* and filamentous algae. The first hot spell of summer occurred on 15/1/85 and by 14/2/85 no macrophytes were visible in the study area.

The onset of macrophyte growth took place in mid-May 1985, but *R. tuberosa* remained as fine, green stubble on the mud surface throughout winter and spring. It was only in mid-November 1985 that *Ruppia* had formed continuous cover on the mud surface. Filamentous algae grew in small, isolated patches during 1985. This contrasted strongly with the previous sampling season when the system was overgrown with macrophytes during winter and spring (31/7/82 to 4/11/82).

The effect of the increased throughput of water could be seen upon the salinity of Railway Lake which is proximal to suburban Port Augusta. Under the old pumping regime (1982/83), Railway Lake remained at salinities above 140 g l^{-1}. With the dilution of the system during 1984, salinities in Railway Lake fell to levels below 90 g l^{-1}.

Figure 4.12 Larval settlement of smaller instars

I - 1st instars

II - 2nd instars

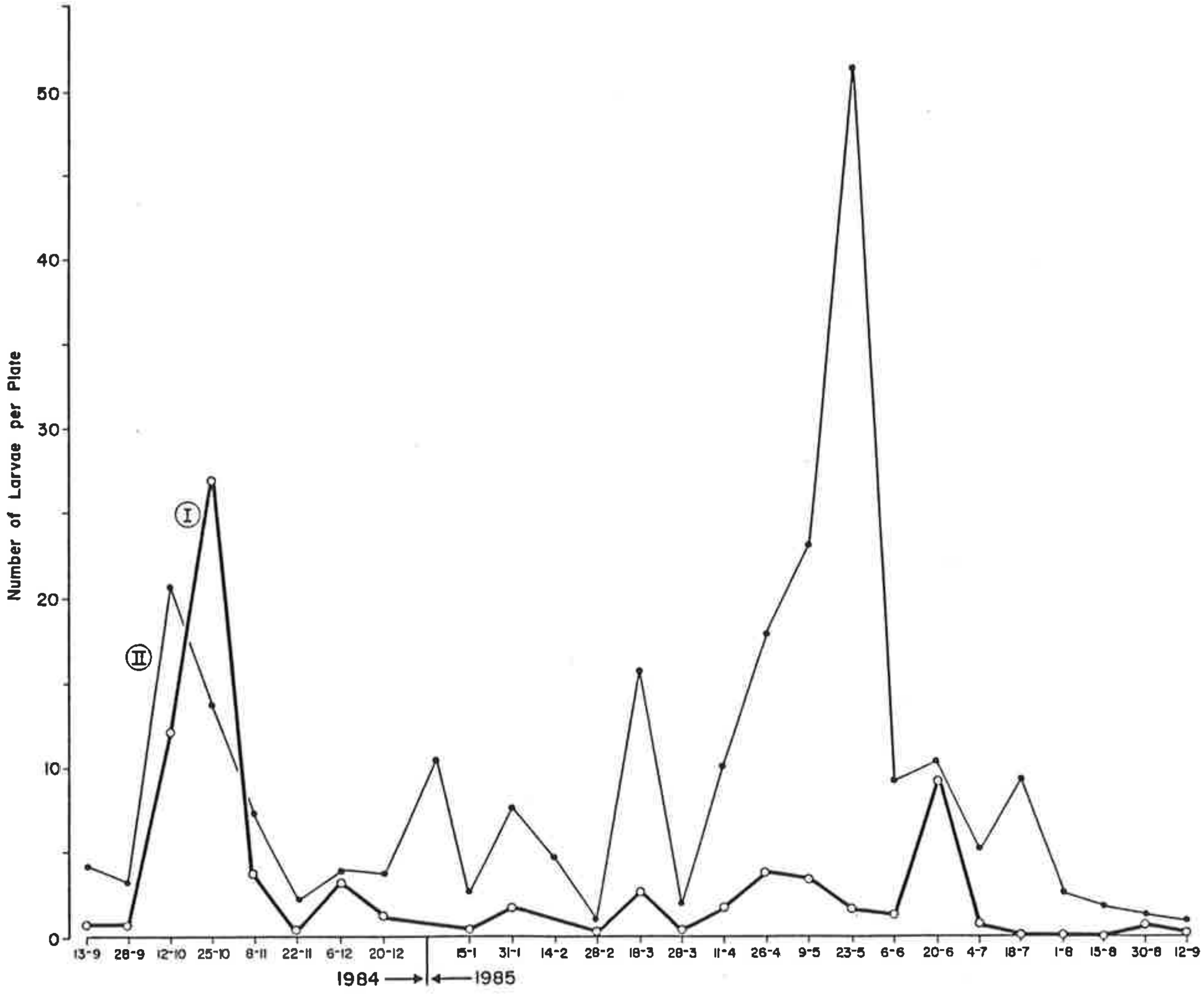


Figure 4.13 Larval settlement of the larger instars, 1984/85

III - 3rd instars

IV - 4th instars

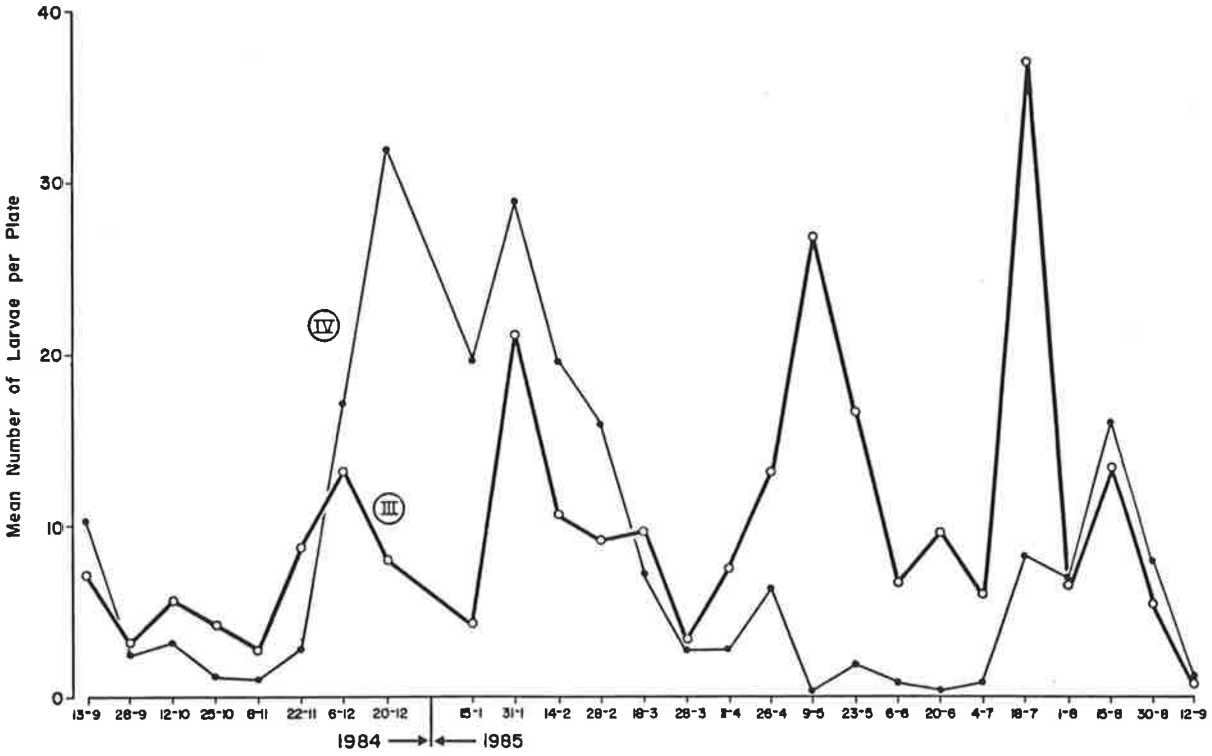


Figure 4.14 Adult emergence 1984/85

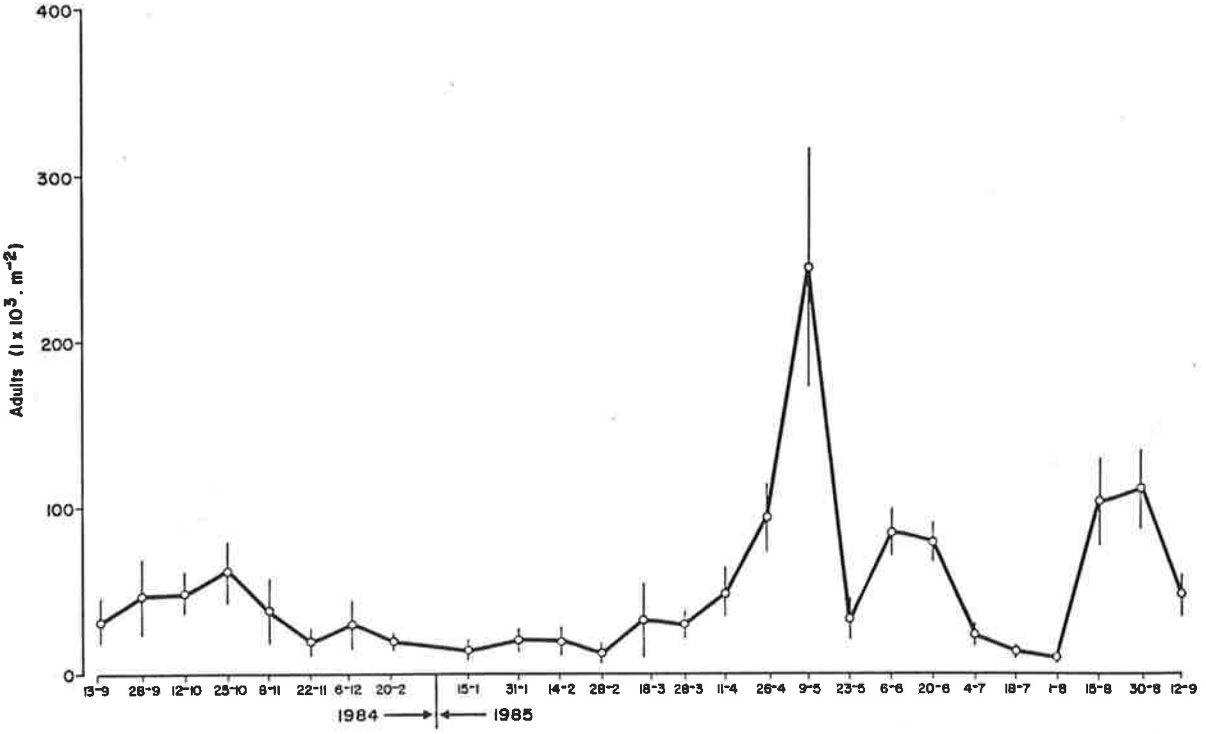
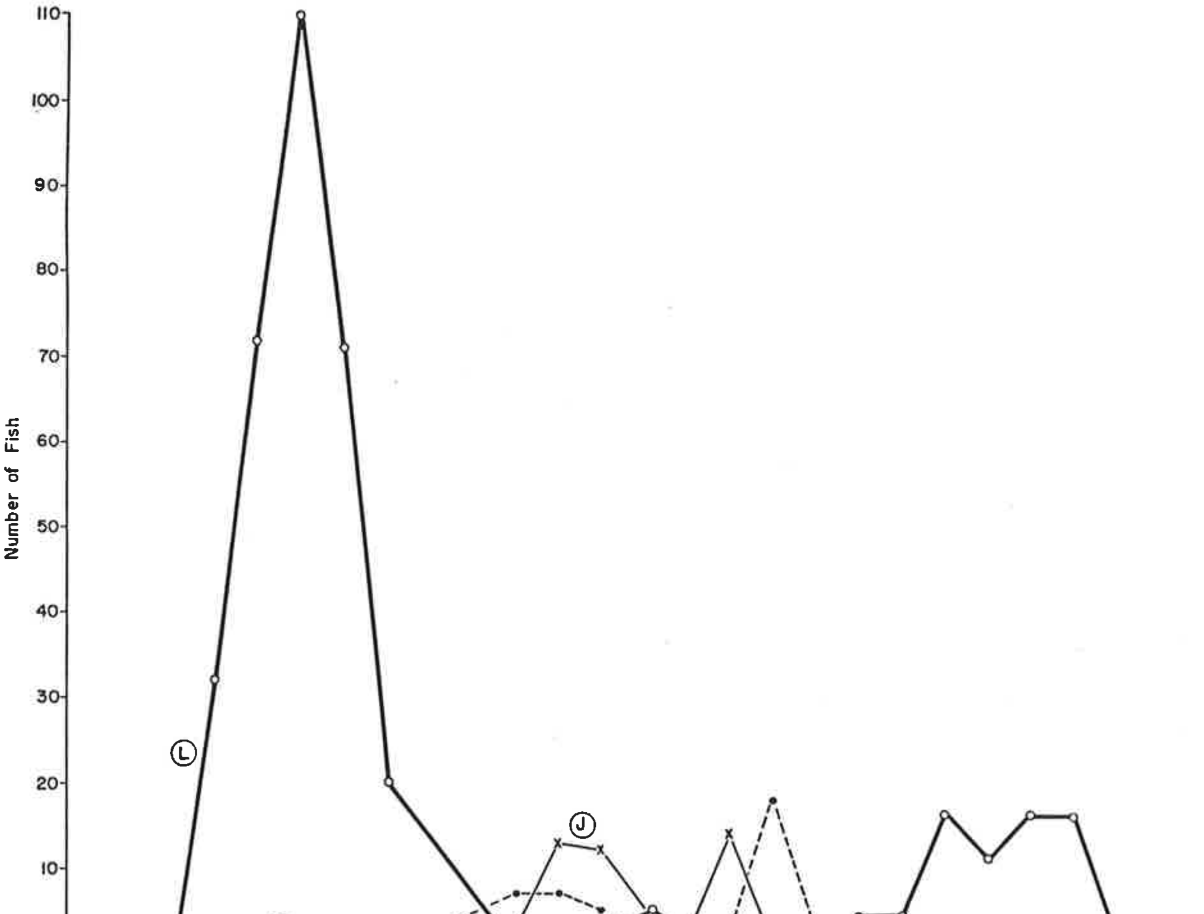


Figure 4.15 Fish (Atherinosoma microstoma) abundance, 1984/85

L - larvae
J - juveniles
A - adults



DISCUSSION

Physical Factors

Results of nutrient sampling (Figs 4.2 and 4.3) indicate that there is a constant input of plant nutrients (phosphates and nitrogen) from the ash disposal system to the lakes. Orthophosphate levels are frequently high in saline waters (Ryther and Dunstan, 1971; Williams, 1984), but nitrate does often appear to be limiting in these environments (De Deckker and Williams in press).

The patterns shown in Fig. 4.3 strongly indicate that there is a constant addition of nitrate to the Port Augusta lake system and that it is rapidly utilized. When N/P ratios were calculated using the nitrate and phosphate data the values were consistently lower than 5. This gave some indication that nitrogen was limiting (Forsberg *et al.*, 1978) despite the fact that nitrite-N and ammonia-N had not been included in the calculation. In fact, the ratios were so low that it is doubtful whether contributions of nitrogen from these two sources would have altered the conclusion. The eutrophying effect of nutrient input is manifest in the dense growth of *Ruppia tuberosa* and filamentous algae at certain times of the year.

Chlorophyll *a* values (Fig. 4.4) suggest that phytoplankton production is limited by the shading effect of the macrophytes (see Fig. 4.4). During December when there is rapid die-back of the macrophytes, chlorophyll *a* values rise sharply although they do fluctuate widely subsequently.

Temperature data from 1984/85 (Fig. 4.10) showed that there is a significant temperature differential between surface and bottom waters. Since it was decided that mean bottom temperatures would be used in all considerations of *T. barbitarsis* generation time, it was necessary to convert the mean surface temperatures of 1982/83 to mean bottom temperature values. In order to do this, the summer and winter mean surface and mean bottom temperatures from 1984/85 were regressed against one another by the least squares method. The two regression equations,

$$y = 0.67x + 5.78 \quad (r^2 = 0.61) \quad \text{Summer } (12/9 - 26/4)$$

$$y = 0.63x + 4.86 \quad (r^2 = 0.58) \quad \text{Winter } (26/4 - 12/9)$$

where y = mean bottom temperature and x = mean surface temperature, returned rather low coefficients of determination. Nevertheless the relationships are significant and sufficiently accurate for ecological purposes.

The single most important physical difference between the two sampling years was water salinity. Figure 4.11 shows the significant effect of the increased throughput of "fresher" water, particularly during the summer months. The fresher water is likely to have played a major role in the increased number of fish larvae recorded during 1984/85. An investigation of larval and juvenile salinity tolerance in *A. microstoma* would throw more light on this likelihood.

The Fauna

It will be recalled that the object of the population study was, in the absence of taxonomic problems, to study with acceptable accuracy the variation in generation time and abundance of *T. barbitarsis* in the study site at Port Augusta. In addition, it should be noted that this work has not used production studies because they lack relevance to the problems at hand (see Kokkinn and Davis, 1986). It is surprising to find, however, that even with a concentrated effort in a relatively small area, the data are highly variable. The sources of this variation may include the distribution of *T. barbitarsis*, sampling errors, and sorting methods. Since a great deal of care was taken to minimize error due to sampling, it can be stated with assurance that the bulk of the variation derives from distributional factors.

Analysis of Data

The fact that population fluctuations with time are plotted as untransformed means with 95% confidence limits (Figs 4.5 - 4.7 and 4.14) does not reflect an uncritical way of representing the data. Indeed, several alternatives were considered before this method of presentation was chosen.

Consideration of the literature on chironomid populations showed that it is common to depict the data in one of three different ways: without any mention at all of the variability about the mean (e.g. Titmus and Badcock, 1980; Graham and Burns, 1983); by plotting one standard error about the mean (e.g. Jones, 1974); by plotting 95% confidence limits about the mean (e.g. Kimerle and Anderson, 1971; Charles et al., 1973; Menzie, 1981).

The practice of using the standard error, which is given by,

$$SE = \frac{s}{\sqrt{n}} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \ (1)$$

(where: SE = standard error, s = standard deviation and n = number of samples), although not rendering the variation in an intuitively obvious form, is quite acceptable as long as the number of samples exceeds 10. In this case, the 95% Confidence Limits, which are given by,

$$95\%\ CL = \pm \frac{t.s}{\sqrt{n}} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \ (2)$$

(where t = Student's t) would approximate 2 X SE (i.e. for greater than 10, t approximates 2). However, where n is small (less than 6), the use of the standard error in graphical representations could be construed as an attempt to minimize the apparent error. This practice is particularly questionable where the standard error bars are used to indicate that apparent population fluctuations represent statistically significant events.

It was therefore decided, in the light of data variability and difficulties in distinguishing cohorts in previous studies (e.g. Kimerle and Anderson, 1971), to adopt a conservative attitude. In all cases, 95% confidence limits would be plotted and real population fluctuations would be taken as the points where there was no overlap of these limits. In addition, it was possible to deduce fluctuations from harmonic increases or decreases in means and corresponding 95% confidence limits over several sampling dates.

When calculating 95% Confidence Limits, the clumped (negative binomial) distribution of _T. barbitarsis_ at Port Augusta was borne in mind. The calculation of 95% confidence limits for a negative binomial distribution is given by:

$$95\%\ \mathrm{CL} = \mathrm{antilog}\ (\bar{y} \pm t \sqrt{\frac{0.1886\ \mathrm{trigamma}\ k}{n}} - \frac{k}{2}) \quad \ldots\ldots (3)$$

(where: $\bar{y}$ is the sample mean and k is the exponent of the binomial equation and trigamma k is a function derived from tables) (Elliot, 1971).

However, when goodness-of-fit tests were conducted for individual sampling dates in order to estimate the parameter k, it was discovered that more than half the data was best described by a Poisson distribution, i.e. the variance was approximately equal to the mean. The calculation of 95% confidence limits for samples from a Poisson distribution are derived from:

$$95\%\ \mathrm{CL} = \bar{X} \pm t.\sqrt{\frac{\bar{X}}{n}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Where $\bar{X}$ is the sample mean

It will be seen that this approximates the formula for normally distributed data where the central-limit theorem applies i.e.:

$$95\%\ \mathrm{CL} \quad = \quad \bar{X} \pm t.\sqrt{\frac{s^2}{n}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (5)$$

(where s^2 is the variance). Note that equation 5 is the equivalent of equation 2.

It would be possible to develop a unifying statistical treatment for the whole data set by building into the model a factor which would account for the increasing variance with the increasing size of the mean (Venables, pers. comm.). However, it was decided that it would be acceptable to use equation (2) to calculate 95% confidence limits in the light of two matters: First, the intention to discern broad fluctuations in the data, not inflections between individual points; and, second, patterns of abundance for the different life history stages, while not individually unequivocal, are mutually supportive (see later).

Initially, the data were transformed (log (n+1)) and the 95% confidence limits were plotted about each mean. However, for the purposes of this analysis, it was found that the data in this form had no advantage over a plot of untransformed data. In addition, the untransformed data had the advantage of being more obvious. It was, therefore, decided to present the untransformed data with 95% Confidence Limits (equation (5)) plotted about each mean.

It should be mentioned, before passing to an interpretation of population data, that two other methods of data analysis were investigated. These were time series analysis (spectral analysis) and splined polynomial regression (cubic) (Parsons and Hunt, 1981). Neither method gave analyses which could be treated with more confidence than the method described above.

Generation Time

Figures 4.16 and 4.17 represent an analysis of sampling data from the two sampling years. During 1982/83, T. barbitarsis passed through five generations in the study site. These are represented in Fig. 4.16 as A-B, B-C, C-D, D-E, and E-F. Generation D-E remains a matter for interpretation since it is not reflected in the 3rd and 4th instar larval data, possibly because of a recruitment failure reflected in 1st instar numbers. Generation E-F, although not represented in adult emergence or 1st instar data, is confirmed from the observation of a mass emergence of adults on August 12, 1983.

Two factors made the duration of generation A-B uncertain. These were the possibility that fish predation had obscured the true pattern and the fact that no sampling was undertaken during December (see dotted lines Fig. 4.16). The duration of generation A-B (Fig 4.16) given as 147 days at 19.5°C mean bottom temperature was therefore not included for consideration.

The two generations of 1985 (Fig. 4.14 & 4.17) are clearly discernible and their duration can be estimated with reasonable accuracy. These are G-H and H-I (Fig. 4.17). Generation time and mean bottom water temperature are given in Table 4.4.

Figure 4.16 Generations of 1982/83 traced through their developmental stages.

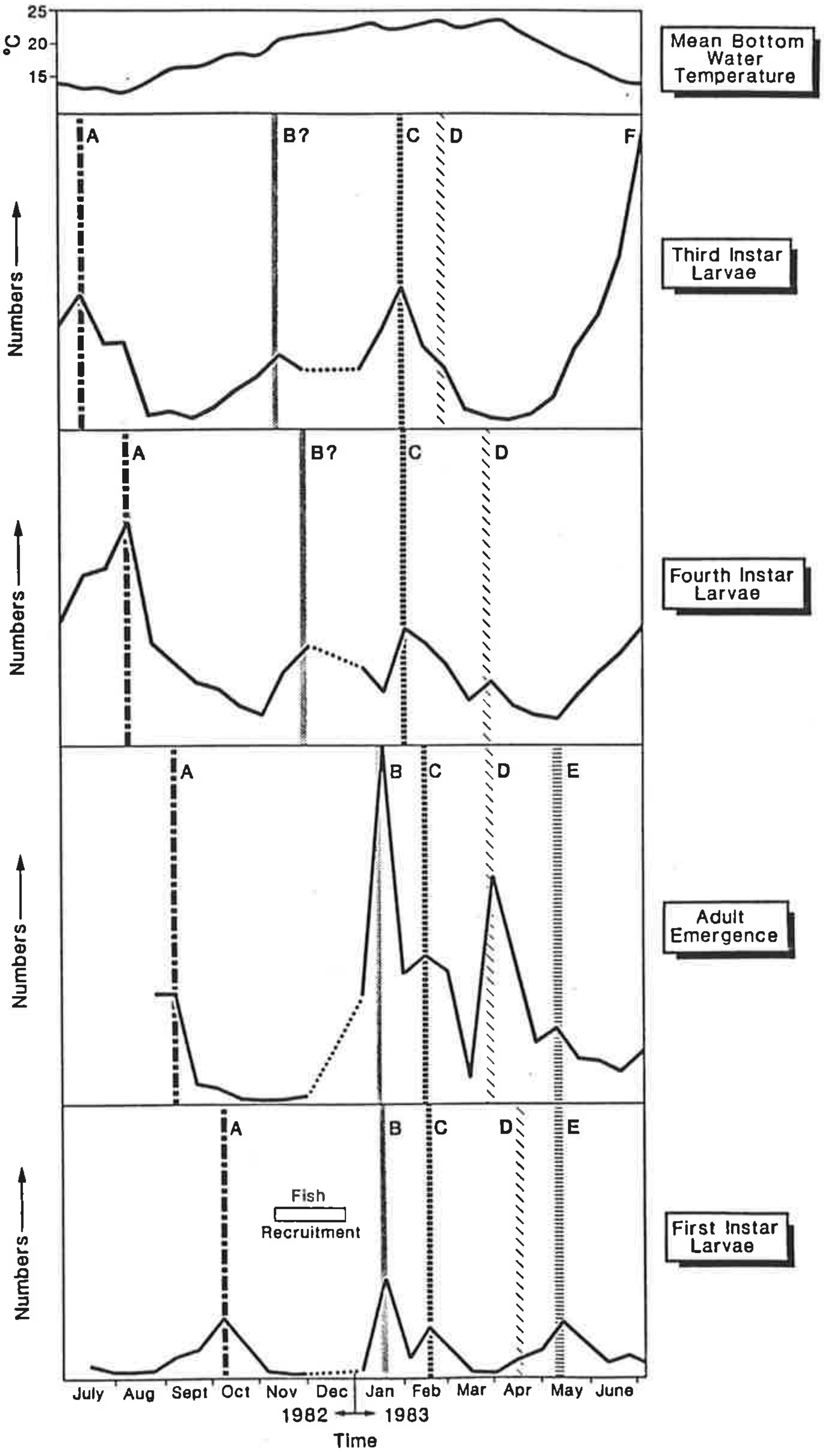


Figure 4.17 Generations of 1984/85, the mean water temperatures and fish abundance.

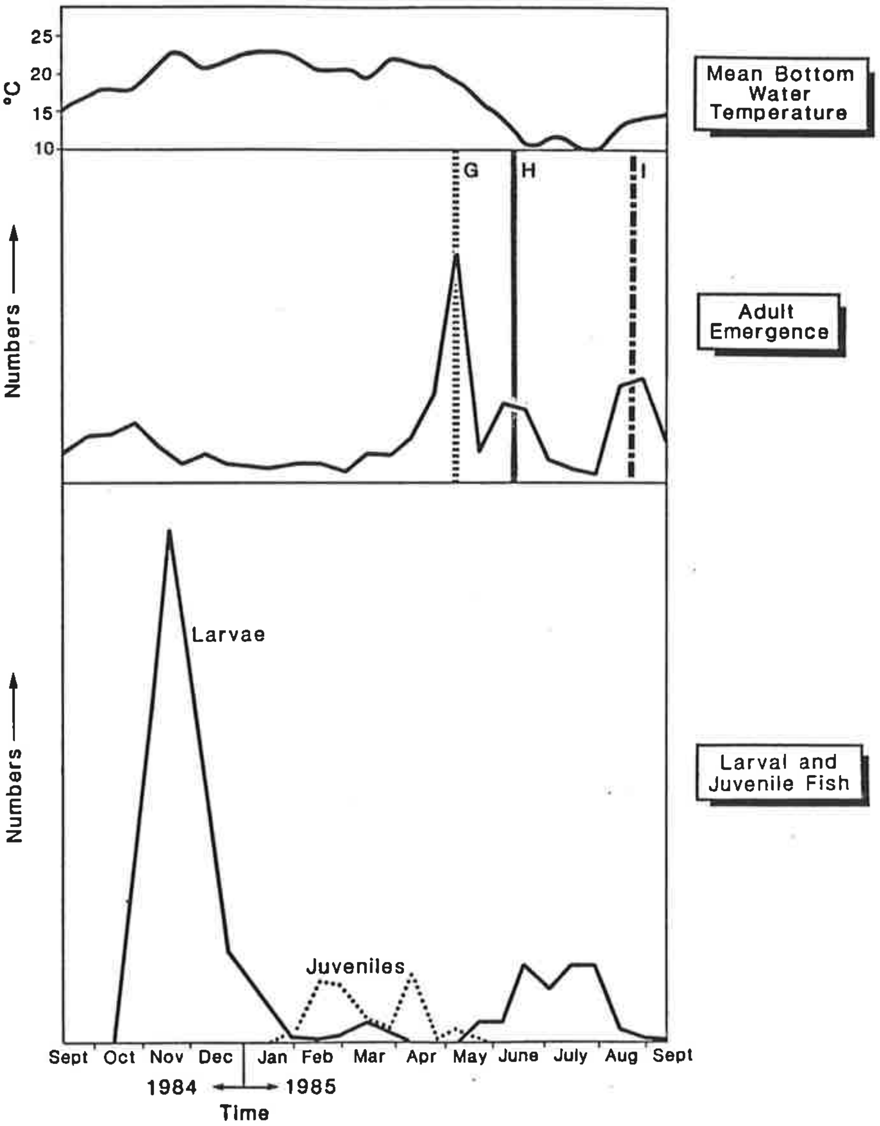


Table 4.4 Generation time of T. barbitarsis and mean water temperature.

Generation	Length in Days	Mean Bottom Water Temperature °C
B-C	28	22.5
C-D	42	22.0
D-E	42	17.2
E-F	92	13.5
G-H	36	15.0
H-I	70	12.0

The matrix of temperature and generation time (Table 4.4) data was subjected to a goodness-of-fit test by a least squares regression. A power curve represented by the equation, $y = 1682.61x^{-1.27}$, where y = generation time in days and x = mean bottom water temperature in °C, was shown to represent the relationship best. The regression coefficient (r) was calculated as 0.76. When tested for significance by a one way analysis of variance (Sokal and Rohlf, 1981), an F value of 5.64 was returned. This was significant at the 0.1 (90%) ($F_{1,5}$ = 4.06), but not the 0.05 (95%) level ($F_{1,5}$ = 6.61). This level of significance was not unexpected bearing in mind that there were only 6 data points.

Table 4.5 The relationship between generation time and temperature in some chironomid species

Species	Temperature °C	Generation Time (Days)	Reference
Chironomus sylvestris	22	14	Konstantinov, 1958
C. sylvestris	18	21	Konstantinov, 1958
C. strenzkei	28	11	Syrjamaki, 1965
C. zealandicus	15	41	Robb, 1966
C. zealandicus	20	32	Robb, 1966
C. zealandicus	25	20	Robb, 1966
C. zealandicus	30	20	Robb, 1966
C. riparius	25	15.5	Scharf, 1972
Thalassosmittia clavicornis	10	110	Morley & Ring, 1972
T. marinus	10	150	Morley & Ring, 1972
Paraclunio alaskensis	10	204	Morley & Ring, 1972
Cricotopus sylvestris	22	10	Menzie, 1981
Polypedilum nubifer	12.5	70	Edward, 1986
P. nubifer	16	36	Edward, 1986
P. nubifer	22	25	Edward, 1986
Dicrotendipes conjunctus	12.5	70	Edward, 1986
D. conjunctus	16	60	Edward, 1986
T. barbitarsis	10	90	This study
T. barbitarsis	15	53	This study
T. barbitarsis	20	37	This study
T. barbitarsis	25	25	This study

When compared with other multivoltine species, it can generally be said that *T. barbitarsis* has a relatively long generation time. However, such comparisons should be viewed with care because generation time may be dependent upon a range of factors including, acclimation and acclimitization history, food supply and water chemistry. Despite this note of caution, examples (mostly from laboratory determinations) given in Table 4.5 support the above generalization. For example, of the ten species given for comparison, six had shorter generation times than T. barbitarsis, three had longer generation times, and one was roughly the same. The three species with apparently longer generation times (S. clavicornis, S. marinus and P. alaskensis) were investigated at 10°C only (Morley and Ring, 1972). Generation times of P. nubifer and D. conjunctus (Table 4.5) were estimated from the graphs given by Edward (1986). However, the data for these Australian species is very similar to values for T. barbitarsis. Further discussion of the length of generation time can be found in Chapters 6 and 7.

A laboratory investigation of the effect of temperature upon the length of generation would have been an appropriate method of collecting more accurate data. However, despite several attempts to rear T. barbitarsis under laboratory conditions, the animals failed to grow and develop in vitro. This, indeed, was a perplexing result for an organism which is a nuisance in the field.

Failure to develop under artificial conditions may be attributable to some specific dietary requirement. Gut content analysis, for example, revealed the presence of the diatom, Surirella sp., in high numbers. Observations of the dietary habits of other marine chironomid larvae reveal that they select for certain groups of diatoms from the substrate (Morley and Ring, 1972). Other studies have confirmed the effect of food availability upon growth and development of chironomid larvae (Hilsenhoff and Narf, 1967; Biever, 1971; Jones, 1974; Ward and Cummins, 1979).

Apart from the failure to rear T. barbitarsis, it would appear that had such an exercise been possible, it would not have been representative of field values because of an inability to duplicate field trophic conditions. Menzie (1981), for example, did not use laboratory estimates to calculate production because he regarded them as unrepresentative. Growth and development rate in the field is controlled by a complex set of factors including the carbon, phosphorus and nitrogen content of the sediments (Iyengar et al., 1963), number of bacteria (Toscano and McLachlan, 1980), and the presence of Cyanobacteria (Provost and Branch, 1959).

It would seem, therefore, that generation times are best estimated under field conditions. However, before suggesting the ideal circumstances for such estimates, the proposition that it is largely temperature (under appropriate trophic conditions) which determines generation time, should be examined.

This is certainly not the case among some univoltine and bivoltine species. For example, the growth of Chironomus anthracinus in Lake Esrom is limited to two periods, i.e. the spring maximum of phytoplankton and the autumn turnover (Jónasson, 1964, 1965; Jónasson and Kristiansen, 1967). Rasmussen (1984) has found that none of the growth or production parameters of Chironomus riparius and Glyptotendipes paripes showed correlation with temperature. Rather they were correlated with algal blooms. In addition, Koskinen (1968) has found the growth and development of Chironomus salinarius to be different for Norwegian and German populations.

A further confusing factor is that an increase in temperature may cause an increase in growth and development in some species, but only result in an increase in the development of others (Mackey, 1977).

It seems likely, however, from work on multivoltine chironomid species that generation time is largely dependent upon mean water temperature (Konstantinov, 1958; Oliver, 1971; Morley and Ring, 1972;

Menzie, 1981). This assumption was applied to T. barbitarsis, but the attenuating effect of high salinity upon generation time was not overlooked (see Chapters 6 & 7).

Although there are some problems with the accurate determination of generation time from field data, the major deficiency in this study has been the lack of accurate temperature data. This has undoubtedly resulted in the low correlation of the data (see above). It would be desirable in further studies to collect accurate temperature data, preferably using a submerged continuous recorder.

Abundance of T. barbitarsis

During the first year of sampling (1982/83) there was some suggestion that the presence of predatory fish (A. microstoma) and beetles (B. devisi) during spring and early summer may have reduced the number of emergent adult T. barbitarsis (Figs 4.9 & 4.16). The absence of good fish data and the lack of sampling during December rendered this an uncertain suggestion.

However, during 1984/85 the lower salinities (Fig. 4.11) appear to have resulted in much higher numbers of fish larvae and juveniles between October 1984 and April 1985 (Figs 4.14, 4.15, 4.17). This association between the presence of large numbers of fish and the absence of any problem chironomid emergence throughout the spring and summer gave support to an hypothesis that predation effects natural control of T. barbitarsis for part of the year at Port Augusta.

CHAPTER 5

Fish predation

INTRODUCTION

Chironomid larvae may form a major food item for several species of inland fishes (Bryce and Hobart, 1972). Indeed, the diversity of fish feeding habits is matched by the diversity of chironomid habitats (Oliver, 1981). Several studies of fish diets related the effects of predation to chironomid communities (Gilinsky, 1984; Walde and Davies, 1984; Hershey and Dodson, 1985; Hershey, 1985). Bohanan and Johnson (1983), for example, showed a reduction in the number of free-living predatory chironomids. Rarely do studies involve a quantitative assessment of the impact of predation upon a single chironomid species (e.g Iwakuma and Yasuno, 1983).

Investigations of fish as control agents for chironomids have generally shown them to be of limited value in this regard. Thus, Bay and Anderson (1965) found that carp, *Cyprinus carpio*, and goldfish, *Carassius auratus*, both bottom feeders, reduced chironomid numbers in ponds when stocked at 168 - 560 kilograms per hectare, but over the long term in water spreading basins other natural enemies tended to maintain larval populations at the same level as did the carp. Further, in a study of the mosquitofish, *Gambusia affinis*, a surface feeder, Bay and Anderson (1966) found that no reduction in chironomid numbers occurred even when *G. affinis* reached densities of more than 280 kilograms per hectare.

Results of the ecological study (Chapter 4) indicate the possibility that *Atherinosoma microstoma* exercises a natural control upon the *T. barbitarsis* population at Port Augusta during spring and early summer. Three life stages of *T. barbitarsis* are particularly vulnerable to predators: the egg, which is laid singly and settles out to the bottom, the first instar larva, which is planktonic in habit and the pupa which rises up through the water column during emergence.

This investigation sought to clarify the extent of fish predation upon the various life stages of *T. barbitarsis* and to gain some idea of its role as a natural control agent, particularly with a view to increasing its effect as a pest control measure.

Although *A. microstoma* has been described as primarily a benthic feeder (Cadwallader and Backhouse, 1983), its dorso-terminal mouth and the fact that its relatives, *A. elongata*, *A. presbyteroides* and *A. wallacei* feed from the nekton as well as the benthos (Prince *et al.*, 1982) suggests an ability to feed efficiently on both resources.

A. microstoma is an estuarine species frequently found in coastal lagoons and salinas across southern Australia (Potter *et al.*, 1986). Along the mangrove coasts of Spencer and St. Vincents Gulfs (South Australia), individuals move up creeks in schools on the incoming tide and feed mainly in the water column. Along such coasts there is a discernible vertical zonation with regard to the distribution of dipteran larvae. An upper zone consists of infrequently-inundated (weekly) pools which are occupied by the salt water mosquito, *Aedes vigilax*. The middle zone, which is inundated frequently (most days) by gently-flowing water, is occupied by *T. barbitarsis*. The lower zone is inundated on a daily basis by relatively swiftly-flowing water. *A. microstoma* is active in the lower and middle zone.

The exclusion of the planktonic *A. vigilax* from the middle zone appears to be mediated by fish predation. *T. barbitarsis*, however, possibly by virtue of its benthic tube-dwelling habit, escapes heavy predation and is able to occupy the organic rich sediments. In the lower zone, where high water velocities are reflected in the relatively coarse sediments, *T. barbitarsis* was not collected (personal observations).

The Port Augusta fly-ash lakes essentially provide similar conditions to those found in the middle zone of the gulf coasts described above. The chief differences such as dense macrophyte growth and anoxic sediments are brought about by the eutrophying effects of the fly-ash.

MATERIALS AND METHODS

I. Feeding Trials

A series of feeding trials was conducted in the laboratory. They consisted of three treatments:

a) Ten sub-adult (30 to 40 mm) *A. microstoma* were introduced into a glass fish tank (L-350 mm, W-200 mm, Ht-200 mm) containing water collected from the study site. The fish were starved for 3 days, after which, 100 third and fourth instar *T. barbitarsis* larvae were introduced into the tank. After 3 days, the fish were removed and the number of remaining larvae in the tank was recorded.

b) In the second trial, 100 third and fourth instar larvae were introduced into the tank 3 days prior to the introduction of the fish. The larvae established themselves in tubes constructed chiefly on the glass bottom. Once more, 3 days after the introduction of starved fish, the remaining number of chironomid larvae was recorded.

c) The third trial involved the inclusion of a layer of sediments (20 mm deep), collected from the study site, prior to the introduction of the chironomid larvae as in the second trial.

In a separate feeding trial, _T. barbitarsis_ eggs were introduced into a fish tank containing 5 juvenile (average length circa 20 mm) _A. microstoma_ by means of a dropper. Approximately 30 eggs were introduced at a time and fish behaviour was observed from a point out of their view. During these observations, an attempt was made to record the number of eggs, if any, which were taken by the fish. This trial was also performed using 10 sub-adult fish (see section above).

II. Gut Contents

A total of 42 adult fish collected during November and December 1983 was dissected and gut contents analysed. In addition, the gut contents of 8 post-larvae were examined.

III. Incidental observations on the abundance of _T. barbitarsis_ eggs

The eggs of _T. barbitarsis_ were collected as follows:- A circular sample (area 0.0452 m^2) was obtained by pushing a segment of PVC stormwater pipe into the mud. The enclosed surface sediments were then pumped into a plastic bucket with a hand-pump and removed to the laboratory.

Floatation of the sediments was undertaken in a $MgSO_4$ solution (S.G. 1.20). The lighter fraction was placed in a sucrose gradient according to the method of Marquardt (1961) and the eggs separated from other material by centrifugation. Final separation and estimation of abundance by subsampling was done under a binocular microscope.

A series of samples was collected during each of four periods at fortnightly intervals: May/June 1984 (3 samples), July/August 1984 (3 samples), November/December 1984 (4 samples) and November/December 1985 (3 samples).

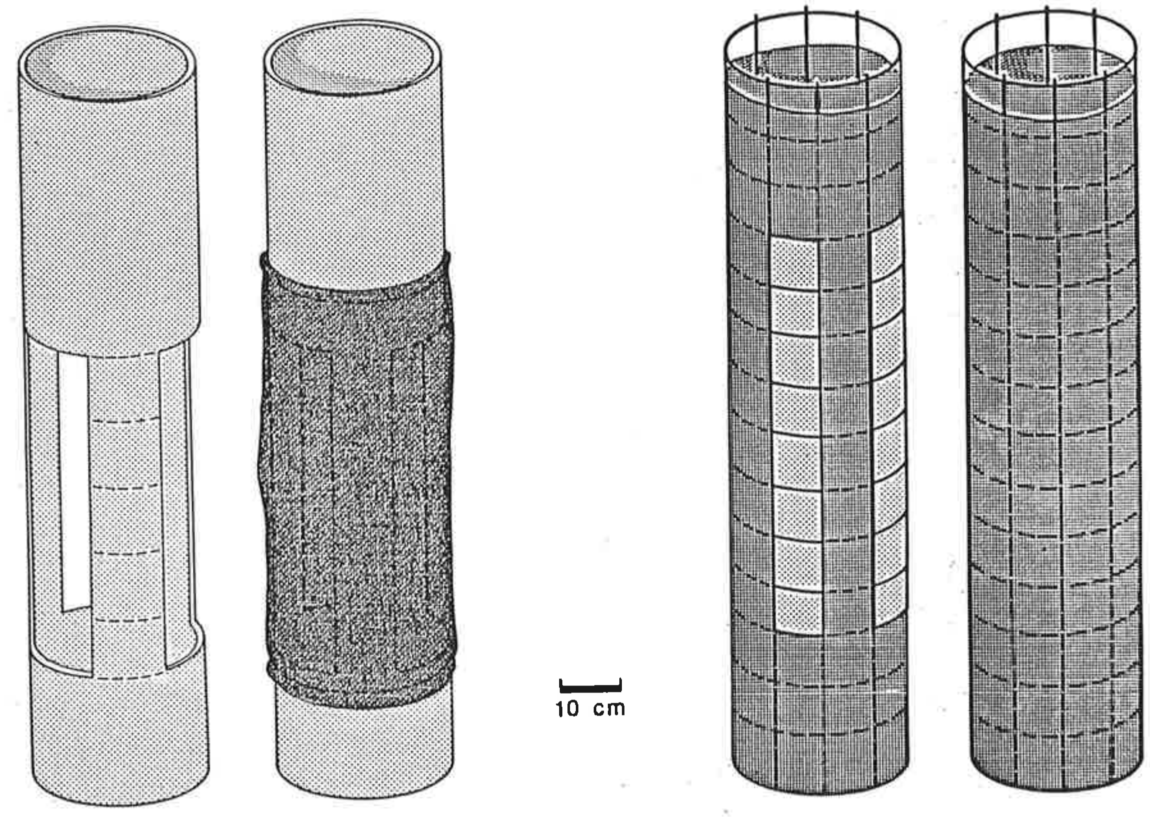
IV. Fish exclusion experiments

In an attempt to quantify the effect of predation by juvenile (less than 20 mm in length) and adult *A. microstoma*, two types of exclosure were employed: fine and coarse-mesh exclosures.

Fine-mesh exclosures Three rectangular panels (500 X 100 mm) were cut from each of twelve 1.5 m lengths of PVC stormwater pipe (diameter 240 mm) so that two solid hoops at either end (500 mm long) were supported by intervening plastic struts (100 mm wide). The open panels of six of the lengths were covered with fine nylon gauze (0.75 mm maximum aperture). Panels of the remaining six lengths were left uncovered so as to allow access to fish and therefore to serve as controls (Fig. 5.1). The twelve exclosures were deployed by forcing the lower solid end into the sediments. Emergence of *T. barbitarsis* adults was monitored by floating an emergence trap (see Chapter 4) on the water surface within the solid, upper end of the plastic pipe which protruded from the water.

Coarse-mesh exclosures A series of ten cylindrical wire frames (diameter 350 mm) was covered with plastic mesh (2.5 mm maximum aperture). In five of these, panels were cut to allow access to fish and therefore to serve as controls. The lower end of the frame was pushed into the sediments and an emergence trap was floated on the enclosed water surface at the upper end (Fig. 5.1).

Figure 5.1 Diagrams of: (from left to right) fine-mesh control; fine-mesh exclosure; coarse-mesh control; coarse-mesh exclosure



Measurements taken from 50 *A. microstoma* (size range - 7.3 mm to 53 mm in length) returned a significant linear relationship between total length and maximum body width (usually just behind the operculum except in post larvae which are broadest in the gut region).

The regression equation was: $y = 0.1563x$ (95% CL $\pm$ 0.0150) - 0.5377 mm Where; y = maximum body width, x = total length and CL = 95% confidence limits. From this expression it was conservatively estimated that the 2.5 mm mesh would exclude all fish over 20 mm in length.

Exclosure experiments

i) The 5 coarse-mesh exclosures and their controls were deployed randomly within the study area on May 9, 1985 and the emergence traps emptied at the end of each of four consecutive fortnights.

ii) In anticipation of the spring fish recruitment (see Chapter 4), both fine and coarse-mesh exclosures and controls were deployed in October 1985. Emergence traps were emptied on a fortnightly basis.

In both of the above experiments, fish populations were monitored in the submerged funnel traps as before (Chapter 4).

iii) In response to a late recruitment of *A. microstoma* in the South Lagoon of the Coorong (a system with an animal community closely resembling that at Port Augusta) both sets of exclosures (coarse and fine-mesh) were deployed on February 4, 1986 at Policeman's Point and the emergence traps emptied on a weekly basis for five consecutive weeks.

The fish population was monitored using a series of eight baited, cylindrical fish traps.

RESULTS

I. Feeding Trials

The three different treatments indicate unequivocally that the ability of *T. barbitarsis* to build its tubes within sediments provides considerable protection from predation by *A. microstoma* (Table 5.1).

Table 5.1 Results of predation by *A. microstoma* on *T. barbitarsis* under different physical circumstances.

Trial No.	Number of larvae surviving after 3 days		
		Treatment	
	Larvae in water column	Larvae on bare glass	Larvae in sediments
1	2	7	53
2	6	11	41
3	7	16	60

Similarly, observations of both juvenile and sub-adult fish during the egg-feeding trial showed that all fish actively consumed the eggs of *T. barbitarsis* from the water column. It was estimated that fish consumed in excess of 70% of the eggs introduced into the water each time.

II. Gut Contents

i) Adult fish Chironomid remains, chiefly head capsules, were recovered from the gut of all but two individuals dissected. Third and fourth instar T. barbitarsis formed the major items in the gut contents of the 42 individuals examined. When the relative abundance of the four life stages (2nd, 3rd and 4th instars as well as pupae) was calculated from sampling data for the same period of the previous year, indications were that 2nd instars were under-represented in gut contents and pupae over-represented. Both 3rd and 4th instars appeared in gut contents close to their relative abundance proportions (Table 5.2).

ii) Larval gut contents No quantitative analysis was possible. Gut contents were generally without recognizable fragments. One individual had fragments of a cyclopoid copepod in its stomach. No chironomid remains were found.

III. Abundance of T. barbitarsis eggs

Numbers of eggs during November/December 1984 were lower than during the other periods of sampling. Table 5.3 gives estimates of egg abundance.

Table 5.3 Indicated egg abundance

Sampling period	Number of eggs per m^{-2}			
	Sample 1	Sample 2	Sample 3	Sample 4
May/June 1984	800	500	500	
July/Aug 1984	600	300	500	
Nov/Dec 1984	80	90	110	140
Nov/Dec 1985	500	800	1200	

Table 5.2 Gut contents of adult <u>A</u>. <u>microstoma</u> collected in Nov./Dec., 1983

Size of fish (mm)	Number of larvae in the gut			
	2nd instar	3rd instar	4th instar	Pupa
19	1			
22	2	2		
26			2	
28		2		
29		3	1	
29	1			1
29	No chironomid remains found			
31		2	2	
32		3		
33	1	2		
33		4		
33			1	
34		1	1	
34		1		1
34		1	1	2
36			3	
37			4	
37				1
37		1		2
38		1		3
38				2
38		6	8	4
39		2		
40		4	1	
40			1	3
41		1	3	
41			2	
41			3	3
42		1		1
43			3	
43	No chironomid remains found			
44		5		
46			3	2
46		3	3	2
46			4	2
47		1		4
49		5	1	
53			2	3
54			5	1
54		3	3	
55		4	3	3
Mean	0.12	1.40	1.7	1.0
Percentage	2.8%	33%	41%	23%
Relative abundance Nov./Dec., 1982	13%	37%	46%	3.3%

IV. Fish exclosure experiments

i) Coarse-mesh exclosures - May to July, 1985

On all but one fortnight (4/7) no significant difference between exclosure and control treatments was detected (one way ANOVA - Table 5.4). When the results for all four fortnights were combined, analysis showed no significant difference between treatments and controls.

Fish trapping (Fig.4.15, Chapter 4) indicated that the number of adults was very low during this period. However, there was an increase in the number of fish larvae between 23/5 and 15/8 in an unusual (Potter pers. comm.) winter recruitment.

ii) Coarse and Fine-mesh exclosures, October 1985

The unexpected alterations to the water level of the lakes over this period seriously interfered with the experiments and they were, therefore, abandoned.

Council officials undertook to alter water salinities in the lakes by these manipulations prior to the end of the research project. The necessity for them to do this was brought about by severe pressure from residents who complained about chironomid infestations.

However, had the levels not been tampered with it is certain that the experiment would have failed because during this time there was no recruitment of A. microstoma. In fact, only two adult fish were collected during October and November, 1985.

iii) Coarse and fine-mesh exclosures - The Coorong, February/March, 1986

Results from one week showed a significant treatment effect for fine-mesh exclosures (25/2/86) (Table 5.4). When all fine-mesh data were combined and analysed (one way Anova), a significant treatment effect was returned (Table 5.4).

No treatment effect could be shown for coarse-mesh exclosures (Table 5.4).

Fish trap results are shown in Fig. 5.2. It will be seen that there was considerable variation in the total number of fish trapped from week to week. Numbers were particularly low during the weeks 25/2-4/3 and 4/3-10/3. However, with the exception of the penultimate week, there was a general reduction in the proportion of fish smaller than 20 mm in length over the period of sampling (18/2 - 27%, 25/2 - 20%, 4/3 - 13%, 10/3 - 33% and 18/3 - 9%).

There was an apparent shift in the modal value over the 5-week period from the 20-25 mm to 25-30 mm size class. At no stage was there a large proportion of larval or post larval *A. microstoma* as was observed at Port Augusta during November 1982 and November 1984 (Chapter 4).

Table 5.4 Results of fish exclosure experiments at Port Augusta and The Coorong.

<u>Coarse-mesh exclosures, Port Augusta May to July 1985</u>

Date	Controls					Mean	Exclosures					Mean	ANOVA
23 May	14	5	2	9	7	7.4	10	6	6	9	13	8.8	$F_{(1,8)} = 0.338$ NS
6 June	34	20	24	32	29	27.8	40	26	33	30	45	34.8	$F_{(1,8)} = 2.666$ NS
20 June	32	23	17	21	27	24.0	20	21	21	24		21.5	$F_{(1,7)} = 0.690$ NS
4 July	7	6	6	4	4	5.4	9	10	5	7	9	8.0	$F_{(1,8)} = 5.828$ **
	Grand mean controls					<u>16.2</u>	Grand mean exclosures					<u>18.1</u>	$F_{(1,37)} = 0.028$ NS

<u>Coarse and fine-mesh exclosures, The Coorong February to March 1986</u>

Coarse-mesh Exclosures

Date	Controls					Mean	Exclosures					Mean	ANOVA
11 Feb	13	18	5	4	11	10.2	17	18	9	6	27	15.4	$F_{(1,8)} = 1.326$ NS
18 Feb	3	2	1	5	9	4.0	23	3	4	10	9	9.8	$F_{(1,8)} = 2.282$ NS
25 Feb	6	7	3	1	2	3.8	4	2	7	10	6	5.8	$F_{(1,8)} = 1.258$ NS
4 Mar	1	1	1	3	2	1.6	21	1	3	5	2	6.4	$F_{(1,8)} = 1.655$ NS
11 Mar	11	2	10	0	1	4.8	2	8	1	7	2	4.0	$F_{(1,8)} = 0.838$ NS
	Grand mean controls					<u>4.9</u>	Grand mean exclosures					<u>8.3</u>	$F_{(1,48)} = 1.953$ NS

Fine-mesh exclosures

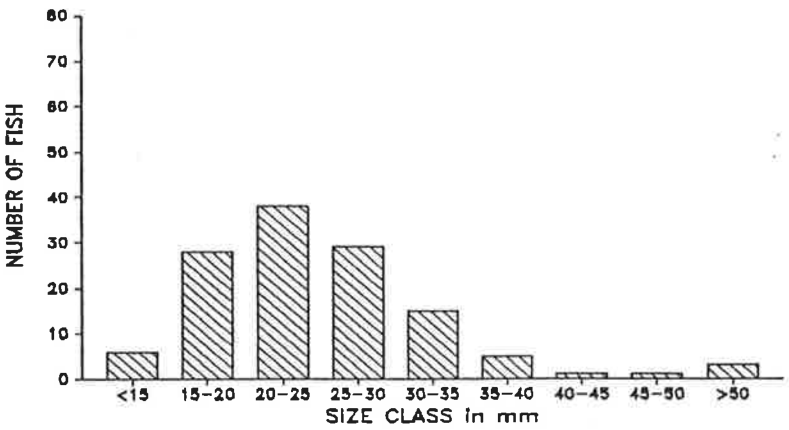
Date	Controls						Mean	Exclosures						Mean	ANOVA
Feb	1	3	12	5	9	1	5.2	28	14	11	4	10	3	11.7	$F_{(1,10)} = 2.485$ NS
Feb	3	1	6	0	2	4	2.7	1	7	1	5	3	11	4.7	$F_{(1,10)} = 1.216$ NS
Feb	1	1	0	0	1	3	1.0	3	2	5	3	1	6	3.3	$F_{(1,10)} = 7.000$ **
Mar	0	0	4	3	1	2	1.7	1	7	1	1	2	4	2.7	$F_{(1,10)} = 0.703$ NS
Mar	4	2	0	0	1	0	1.2	1	0	1	2	0	3	1.2	$F_{(1,10)} = 0.000$ NS
	Grand mean controls						<u>2.3</u>	Grand mean exclosures						<u>4.7</u>	$F_{(1,58)} = 4.176$ **

NOTE Data was checked for heteroscedasticity by log-ANOVA prior to analysis
$F_{(1,8)} = 5.32$ $F_{(1,10)} = 4.96$ $F_{(1,37)} = 4.08$
$F_{(1,48)} = 4.04$ $F_{(1,58)} = 4.00$ All F values are at the 0.05 level.

Figure 5.2 Results of fish trapping at the Coorong over the duration of the exclosure experiments.

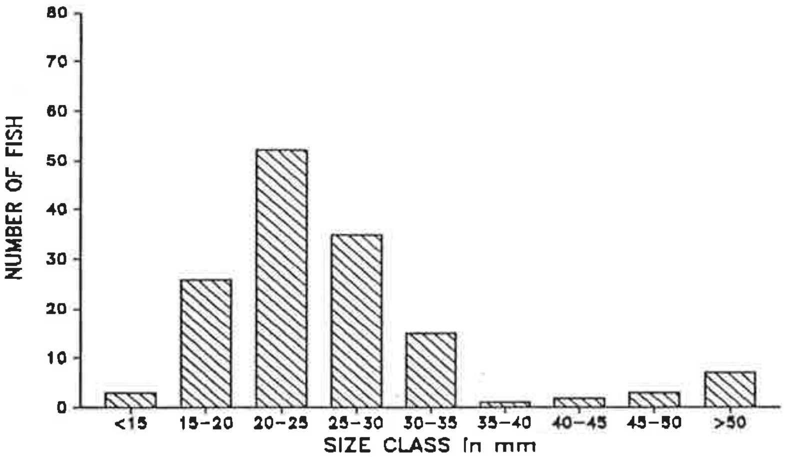
a

11th to 18th Feb, 1986



b

18th to 25th Feb, 1986



c

25th Feb to 4 Mar, 1986

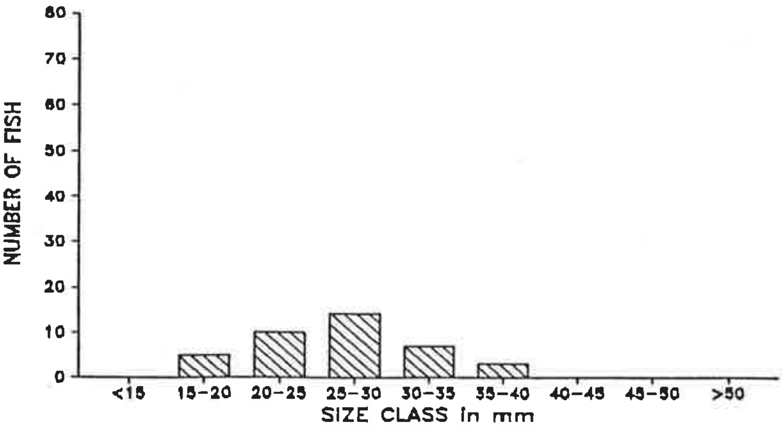


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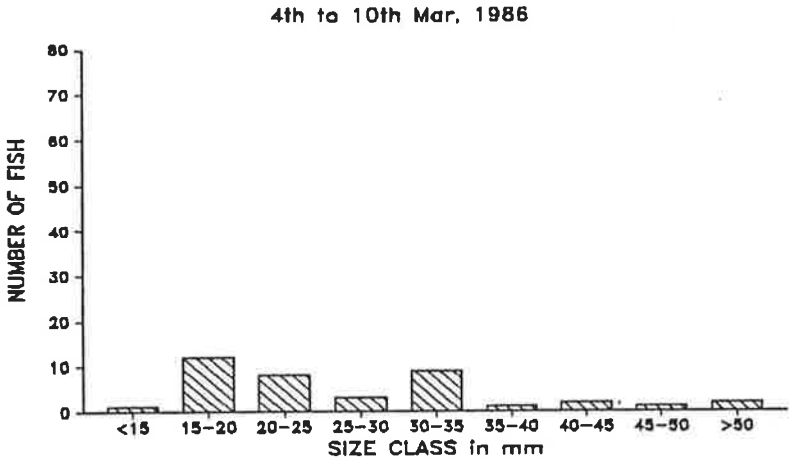
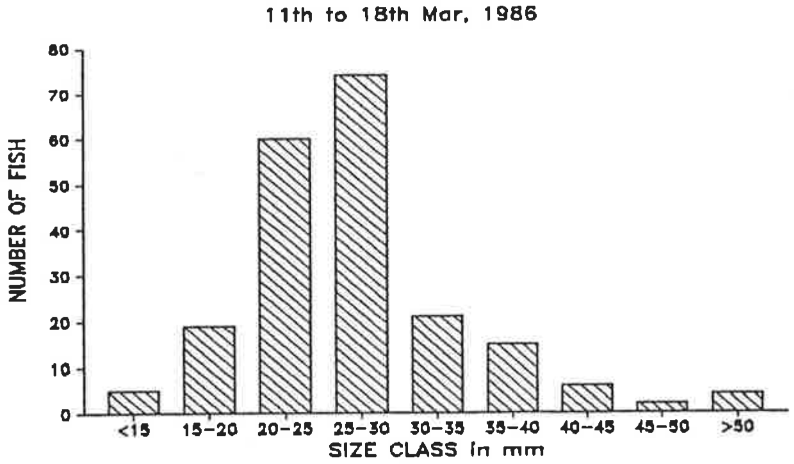
Figure 5.2 (cont.)

d



e



DISCUSSION

Results of the feeding trials clearly indicate that tube-dwelling T. barbitarsis are afforded protection, in sediments, from predation by A. microstoma. The feeding method of A. microstoma involves detection of prey by sight and ingestion by a sucking action of the mouth (Alexander, 1970). It seems likely that the construction of its tube within sediments gives T. barbitarsis a better purchase on the substrate and enables it to resist the suction pressure exerted by feeding A. microstoma. In addition, T. barbitarsis larvae show avoidance behaviour by moving to the opposite end of the tube in response to mechanical stimulation . An alternative possibility is that, since A. microstoma has the anatomical features of a plankton feeder (dorso-terminal mouth, fusiform shape), its benthic feeding habit may be a recent adaptation. The consequence of this may be that it is not able to take in large volumes of sediment with its prey. Observations along the South Australian gulf coasts suggest that it relies upon the turbulence of transgressing tides to provide it with the opportunity to prey upon dislodged benthic animals on the mud surface or in the water column.

Two observations at Port Augusta support the proposition that benthic infaunal prey is largely unavailable to A. microstoma. It was particularly noticeable that whenever the surface sediments were disturbed by walking, the feeding activity of A. microstoma upon dislodged chironomid larvae was evident from the visible fish activity. The second observation involved a situation where dense, saline water (150 g l^{-1}) was being pushed through the culvert from Railway Lake into Bird Lake. The salt water, visible by its red colour, moved as a wedge over the sediments inducing T. barbitarsis larvae to leave their tubes in the sediments and enter the water column. Along the front of this "intrusion" the water surface was visibly disturbed by feeding *A. microstoma*. These observations led me to believe that *A. microstoma* preys chiefly upon *T. barbitarsis* which either enter the water column or are not securely anchored within the sediments. An analysis of gut contents leaves no doubt that *T. barbitarsis* larvae form the major dietary item of *A. microstoma* in the Port Augusta fly-ash lakes. The observation that smaller instars are under-represented in the ingesta whereas pupae are over-represented (Table 5.2) with regard to their abundance in the lakes, has two possible explanations. Food size selection has been found to be positively correlated with fish size (Wurtsbaugh *et al.*, 1980) in *Gambusia affinis*. This is likely to apply to *A. microstoma* and one would therefore not expect fish of the size dissected to prey upon 1st and 2nd instar chironomid larvae. However, the fact that pupal *T. barbitarsis* are over-represented in gut contents (23% in gut contents, 3% in samples) indicates that availability of prey in the water column may dictate the diet of *A. microstoma*.

The absence of chironomid remains in the gut contents of the 7 post-larvae dissected does not have great significance. The small size of eggs (0.2 mm maximum length) and 1st instar head capsules (0.1 mm maximum length) and their friability would make them difficult to detect in gut contents unless fish specimens had fed on large numbers within minutes of collection. It is clear, from the simple feeding trials, however, that *A. microstoma* of all ages will readily feed upon *T. barbitarsis* eggs.

Indications from the egg sampling (which was undertaken to collect material for various experiments) are that, at the time of high fish larval numbers (November 1984), egg numbers were low. Absolute confidence cannot be placed in the egg abundance data because of a lack of replication. However, the fact that a great deal of effort was expended during Nov/Dec 1984 to procure sufficient eggs for experiments, lends support to the assertion that egg numbers were indeed low during this period.

Ideally, fine and coarse-mesh fish exclosures would have been deployed in the study site at Port Augusta during a time of fish recruitment which matched the November, 1984 event in intensity (Fig. 4.15). Apart from the fact that the experiments were spoiled by changes in water level during November 1985, it appears that the complete absence of a spring fish recruitment would have had the same effect.

Fish biologists in South Australia generally reported an anomalous year with regard to recruitment (Puckridge, Pierce pers. comm.). I am unable to speculate on the cause of this recruitmnet failure, but the unusual presence of larvae during mid-winter may hold some clue. Despite a failure to conduct exclosure experiments under conditions which were hypothesized to cause a period of natural control of T. barbitarsis at Port Augusta, (numbers of juvenile A. microstoma in the Coorong did not reach those of November 1984 at Port Augusta) some useful conclusions can be drawn from the experiments which were conducted.

Coarse-mesh exclosures at Port Augusta gave a significant treatment effect during the last fortnight of the experiment (4/7). As has been noted before, this experiment coincided with an anomalous winter recruitment of A. microstoma. The number of juvenile fish was roughly one seventh of the November 1984 peak. However, since predation upon smaller instars and eggs would show a lag before being reflected in emergence, the effect seen on 4/7 may indeed have been caused by juvenile fish. The fact that this effect was seen in coarse-mesh exclosures would indicate that either, fish larger than 20 mm in length prey upon eggs and 1st instars or that the coarse-mesh excluded most post larval A. microstoma.

Coarse-mesh exclosures deployed in the Coorong for five weeks (at least one generation of T. barbitarsis) showed no treatment effect despite relatively high numbers of fish (Fig. 5.2).

Fine-mesh exclosures returned a treatment effect for one particular week (25/2) as well as when all data was pooled (Table 5.4). This indicated that juvenile fish affect the numbers of emerging T. barbitarsis. The grand means for controls (2.3) and exclosures (4.7) suggest that adult emergence was halved by fish predation.

There is no way of comparing fish numbers between Port Augusta and The Coorong from the data gathered. Ideally, identical fish traps would have been used in each locality, however, heavy turbulence at the Coorong led me to use the alternative fish traps there.

The exclosure experiments indicate that fish predation may indeed have been the cause of the anomalously low level of T. barbitarsis emergence during spring and early summer of 1984/1985 at Port Augusta.

A distinctive feature of the pattern of fish recruitment at Port Augusta, particularly during 1984/1985 (Fig. 4.15), is that there appears to be high mortality among post larvae. This is reflected in the fact that some two months after a peak in larval numbers (110 larvae - 22/11/84), the number of juveniles trapped had fallen by an order of magnitude (11 juveniles - 14/2/85).

The cause of this high mortality among post-larval A. microstoma is likely to be two-fold. Firstly, the very high numbers of recruiting larvae prey heavily upon T. barbitarsis eggs and 1st instar larvae, rapidly depleting this food resource. The Port Augusta fly-ash lakes are particularly depauperate in zooplankton and there is, consequently, no alternative food source for post larval A. microstoma; this results in heavy mortality. Secondly, although the eggs and 1st instar larvae provide an ideal prey size for post larvae, there is no readily-available intermediate-sized prey to sustain juvenile A. microstoma. They must rely on the smaller benthic T. barbitarsis larvae which are not easily captured.

The following sequence of events is therefore postulated. Recruitment of A. microstoma results in heavy predation upon the eggs and planktonic 1st instars of the spring generation of T. barbitarsis. Once this food resource is depleted, the lack of an appropriate-sized planktonic prey results in heavy mortality of larval and juvenile A. microstoma. The absence of large numbers of planktivorous fish releases predation pressure upon T. barbitarsis and a post-predation generation appears as a synchronized mass emergence of adults (see adult emergence pattern Fig. 4.15).

Although some adult and juvenile A. microstoma are still present in the lakes, their numbers are not high enough to exercise control by consuming a significant proportion of the emerging pupae. It is also probable that synchronous emergence is an adaptation by aquatic dipterans to oversupply predation pressures and to ensure mating success in the short-lived, swarming species.

Attempts to extend the natural control exercised by A. microstoma should, therefore, involve some measures which will considerably improve the survival rate of recruiting larvae. Such measures may include the introduction of an intermediate sized "bridging" food source (natural or artificial) or, more practically, the regular, mechanical disturbance of sediments at the time of fish recruitment to suspend the benthic larvae in the water column and make them available to post-larval and juvenile fish as food.

Chapter 6

Eggs

INTRODUCTION

The habit of laying individual eggs of T. barbitarsis, being a fully-aquatic species, suggests some form of specialization and presents itself as a subject worthy of investigation as a potential 'weak point' in its life cycle. The idea here is that areas of specialization within the life cycle of pest organisms offer the best opportunities for manipulation and consequently control (Flint and Van den Bosch, 1981).

Several areas relating to the egg phase offer potential for manipulation. Firstly, a knowledge of oviposition behaviour, egg sinking rate, egg anatomy and embryology would give background information which could either suggest avenues for control or validate the feasibility of other measures. Secondly, since it is possible to manipulate water salinities within the lakes at Port Augusta, a knowledge of egg permeability and embryonic salt balance would indicate the vulnerability of this stage of the life cycle to such measures. Thirdly, knowledge of the effect of temperature upon the rate of embryonic development would not only indicate egg-hatching rates at different times of the year, but give some idea of the effect of temperature upon the development of the organism as a whole.

Knowledge of egg-laying, egg structure and physiology is also of interest at a less pragmatic level. It is possible that knowledge of these matters can throw light upon the evolutionary pathway taken by T. barbitarsis.

A major proportion of the chironomids that can tolerate salt water inhabit marine intertidal areas. There they occupy a variety of habitats ranging from moist algal mats to tidal pools and subtidal waters (see Neumann, 1976 for review). The group is of polyphyletic origin and derives from several sub-families (Brundin, 1966; Hashimoto, 1976). Two major groupings, however, seem to be involved: freshwater and terrestrial. It has been suggested that species which lay their eggs in masses have evolved from freshwater ancestors; those that lay eggs singly have terrestrial antecedents (Morley and Ring, 1972). From which group does it seem likely that *T. barbitarsis* arose?

Species from the genus *Tanytarsus*, *Pontomyia*, *Chironomus* (Chironominae), *Thallosmittia* and *Clunio* (Orthocladiinae) are reported to lay their eggs in masses (Tokunaga, 1932; Caspers, 1951; Morley and Ring, 1972; Neumann, 1976; Hashimoto, 1976), and can, therefore, be assumed to derive from freshwater ancestors. It is unlikely that the single egg-laying habit is a specific adaptation to salt water. For example some highly specialized marine forms such as *Pontomyia cottoni* and *Clunio martini*, which belong to the above group, lay eggs in masses.

Species from the genus *Telmatogeton* and *Paraclunio* (Telmatogetoninae) lay individual eggs. *Paraclunio* has been reported to oviposit into filamentous algae (Hinton, 1981). Such habits suggest a terrestrial origin. It is therefore striking that *T. barbitarsis*, which belongs to a sub-family (Chironominae), where eggs are usually laid in masses, should deposit its eggs singly (Edward, pers comm.).

There are three likely explanations for the evolution of *T. barbitarsis* when taking account of its mode of egg laying. The first is that it may have evolved directly from a terrestrial ancestor; in which case it would be reasonable to expect that the eggs retain some resistance to desiccation. Secondly, T. barbitarsis may have evolved from freshwater ancestors which moved first into marine and then into semi-terrestrial intertidal habitats, acquiring its mode of egg laying , and thence back into saline aquatic environments. If this explanation applies, then T. barbitarsis is a highly derived member of the Australian Chironomidae. Finally, T. barbitarsis may have evolved directly from a freshwater species into saline waters, acquiring its egg-laying habit in response to a particular selective pressure there.

This chapter, therefore, investigates oviposition, sinking rate, anatomy, embryology, ion excretion and hatching of T. barbitarsis eggs with a view to the control of the population at Port Augusta and throwing some light upon its evolutionary pathways.

MATERIALS AND METHODS

I. Descriptive aspects

Four matters are of particular interest in this context: the nature of oviposition; the rate of egg sinking; the anatomy of the eggs and the identification of distinct stages in embryological development.

Observations of oviposition during the course of this project were undertaken in the field with two aims in mind: one, to establish its timing and, two, its method. These field observations were then compared with those of Edward (pers comm.), who observed the oviposition behaviour of T. barbitarsis within glass containers.

To determine the egg sinking rate the following method was used. A glass coverslip was glued to a length of glass tube (internal diameter 20 mm). The tube was clamped in a retort stand with the open end projecting upwards and filled with distilled water. An inverted microscope (Leitz-Diavert) was placed beneath the coverslip and focussed on the internal surface. Individual T. barbitarsis eggs were introduced into the top of the water column using a dropper and the settling time measured with a stopwatch by observation of their time of arrival on the coverslip below.

In order to make an anatomical study several hundred eggs were prepared for scanning electron microscopy (SEM) by dehydration through an alcohol series, critical point drying and double coating with carbon and gold/palladium alloy (i.e 2 X 15nm). Specimens were viewed and photographed in a Philips 505 scanning electron microscope.

Rather than make a detailed study of the embryological development, the purpose of this investigation was to identify easily-recognizable stages which could be used as reference points during experimental work. To this end, several hundred eggs, which are transparent to transmitted light, were viewed under a Zeiss III photomicroscope and representative stages photographed.

II. Ion excretion in eggs and embryo

Investigations of sites of chloride ion passage across the chorion of T. barbitarsis eggs was undertaken using the silver staining technique of Conte et al.(1972). Viable eggs were briefly washed in distilled water to remove external NaCl. They were then placed in a 0.1M silver nitrate solution under bright light for 8 to 10 min. Stained specimens were subsequently washed in distilled water and then placed in a 0.1M sodium thiosulphate solution for 5 min.

The material was dehydrated through an alcohol series, critical point dried and single-coated (15 nm) with carbon as a preparation for SEM. The areas of silver staining were mapped on a Philips 505 SEM which was linked to a Tracor Northern (TN 5500 EDS) X-Ray analyser and image enhancement system.

This technique was applied to eggs containing embryos at 3 stages of development, early ("yolky"), mid-term ("developing") and late-stage ("waiting").

Sites of ion excretion in the embryo were sought using the silver staining technique described above. Several methods for forcing premature eclosion were used: (immersion in) i) distilled water plus ultrasound for periods ranging from 2 to 8 min., ii) sodium hypochlorite, iii) sodium hypochlorite plus ultrasound and iv) the 1M silver nitrate solution plus ultrasound. In each case, several eggs (approx. 50) were subjected to forced eclosion before the application of the silver staining technique while the embryos were still theoretically viable.

III. Egg hatching experiments

To investigate whether the eggs of *T. barbitarsis* can withstand desiccation, a factor which would have an important influence upon the distribution of the species, three treatments were used: i) desiccation of eggs at 4°C, ii) desiccation of eggs at 20°C and iii) immersion of eggs at 4°C. The experiment was designed in this way to allow for adequate control. Desiccation of eggs at 20°C could not be controlled for with immersed eggs because of the rapid hatching rate at this temperature. The eggs were therefore desiccated at 4°C and their controls kept immersed at this temperature, which is below the developmental threshold. A further set was desiccated at 20°C as a cross-control.

In each treatment, 50 eggs were divided into batches of 5. Each batch for the two Desiccation treatments was deposited on single hollow-ground glass slides (10 slides each with 5 eggs) and placed in a glass desiccator. For the Immersion treatment, batches were placed in separate 50 mm petri dishes containing water from the study site (salinity 46 g l^{-1}). Each desiccator was placed in separate controlled temperature cabinets, one at 4°C and the other at 20°C. The Immersion treatment eggs were placed in the 4°C cabinet.

One batch of 5 eggs from each treatment was removed at 5-day intervals and placed in separate 90 mm petri dishes (to accommodate slides) containing study site water (46 g l^{-1}). In the case of the Immersion treatment, eggs were removed and re-immersed in the larger petri dishes. Hatching success of each treatment was monitored at 20°C.

The permeability of _T. barbitarsis_ eggs was investigated by placing them in distilled water and monitoring hatching success. Approximately 20 eggs were placed in each of 6 petri dishes. In 4 of the dishes, the water was exchanged four times with triple-distilled water. The remaining 2 dishes, containing water from the study site (salinity 57 g l^{-1}) served as controls. The initial number of eggs present and their stage of development (according to the arbitrary scheme) was recorded for each dish. The dishes were placed in a constant temperature cabinet at 20°C and monitored regularly for embryonic development and egg-hatching. After the experiment, the water from the 4 distilled water petri dishes was pooled and its final salinity checked.

The final series of hatching experiments involved an investigation of the effect of temperature on the rate of embryonic development. Batches (approx. 20) of early-stage (Yolky) eggs were placed in 50 mm plastic petri dishes and immersed in filtered lake water (salinity 50 g l^{-1}). The antibiotic Kanamycin sulphate was added to the water at a rate of 0.1 mg l^{-1} to prevent infections of the eggs during long hatching periods. This step was taken because surface growths of bacteria and fungi, while apparently not affecting the eggs, obscured them from view during daily counting. Replicate petri dishes were incubated in constant temperature cabinets at each of the following temperatures: 4°C (7 reps), 8°C (7 reps), 9°C (3 reps), 12°C (7 reps), 16°C (3 reps) and 20°C (7 reps). The number of eggs which hatched in each petri dish was recorded daily. In order to overcome problems caused by differential egg viability, the point at which 50% of the eggs had hatched (50% Hatch) was used as a measure of embryonic development rate. In each case, results from all replicates was pooled and plotted as a cumulative hatching curve. Any analysis of variability between individual dishes would have attracted criticism on the grounds that it was pseudoreplication _sensu_ Hurlbert (1984).

RESULTS

I. _Descriptive aspects_

a) _Oviposition_ Two methods of oviposition were observed on the lakes at Port Augusta. The first corresponds to that observed by Edward (pers. comm.) where the female settles on the water surface and lays individual eggs sequentially in rhythmical fashion by lowering the abdomen through the surface film. The second mode of oviposition involved a dipping of the abdomen into the water during flight. The female _T. barbitarsis_ would fly low over the calm surface and drop rhythmically to the water, penetrating it momentarily with the abdomen to effect oviposition. The average clutch size of 209 (n = 43, SD = 46) was determined by dissection of female pupae.

The chief period of oviposition at Port Augusta was at dawn. This was probably because the waters were calmest at this time of day. During rare calm periods, oviposition was seen at all times of the day.

It was noticeable that females remained on the water after the entire clutch of eggs had been laid. Many were collected from the surface after it was observed that they were being swept into the culvert without attempting to fly off. Laboratory observations confirmed that these specimens (approx. 100) had all completed oviposition and were devoid of eggs.

b) Egg sinking rate Some difficulty was experienced with this experiment. In several cases the eggs were introduced into the top of the column and they had not appeared on the coverslip within an hour. It was assumed that they had been trapped on the glass by adhesive forces.

The mean settling time through 1480 mm of distilled water was 1461s (n = 12, SD = 242s). Using Stokes Law (Smith, 1975) and assuming a spherical shape (i.e. the coefficient of form resistance = 1 (McNown and Malaika, 1950 (a simplification because the eggs of *T. barbitarsis* are elongate))), the density of the eggs was calculated from:

$$V = \frac{g\ D2\ (p' - p)}{18\ n\ o}$$

Where: V = terminal sinking velocity

g = acceleration due to gravity

D = diameter of a sphere with equal volume to the egg

p' = density of the egg

p = density of the water

n = viscosity of the water

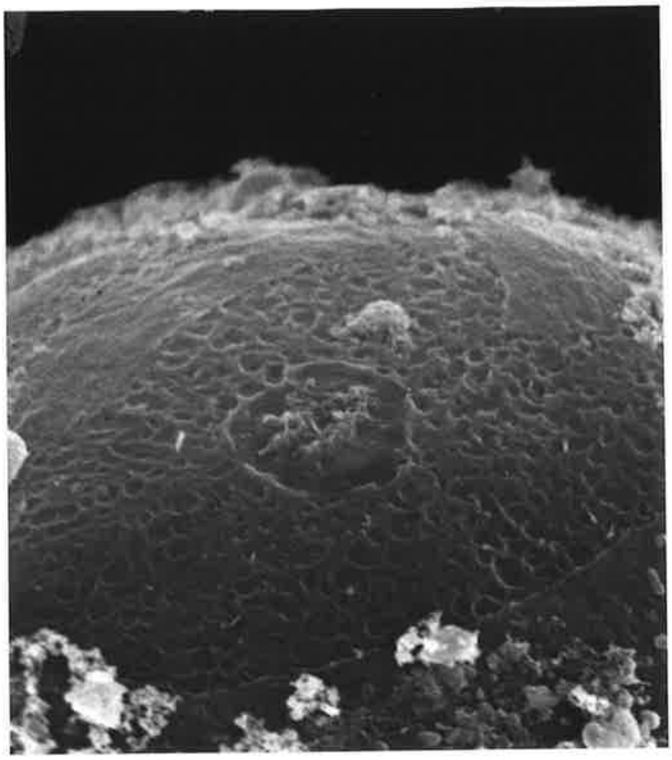
o = coefficient of form resistance

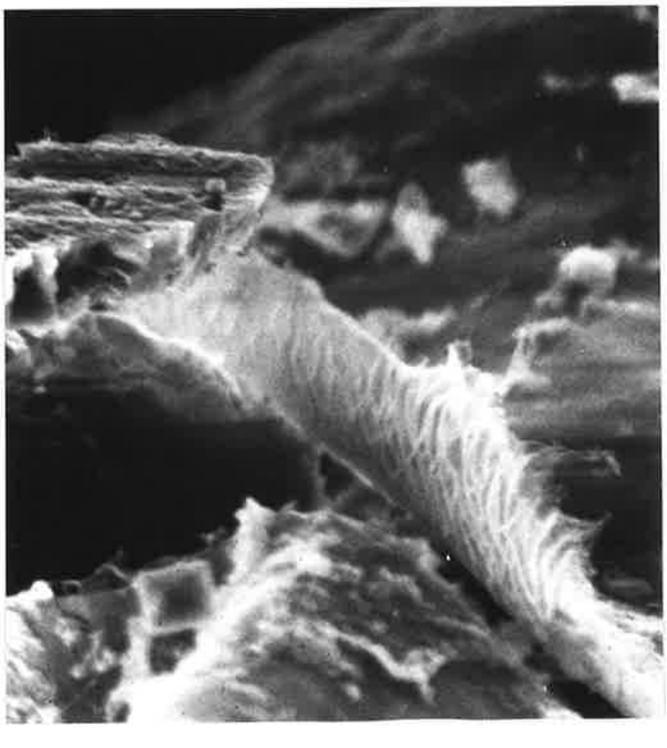
This gave a value of 1.069 g cm^{-3}. When the density and viscosity for saline water at 50 g l^{-1} was substituted into the equation, it was calculated that eggs would settle at the rate of 3.968 X 10^{-4} m s^{-1}, i.e they would take 2520s (42 min.) to settle through 1 m of saline water (50 g l^{-1}).

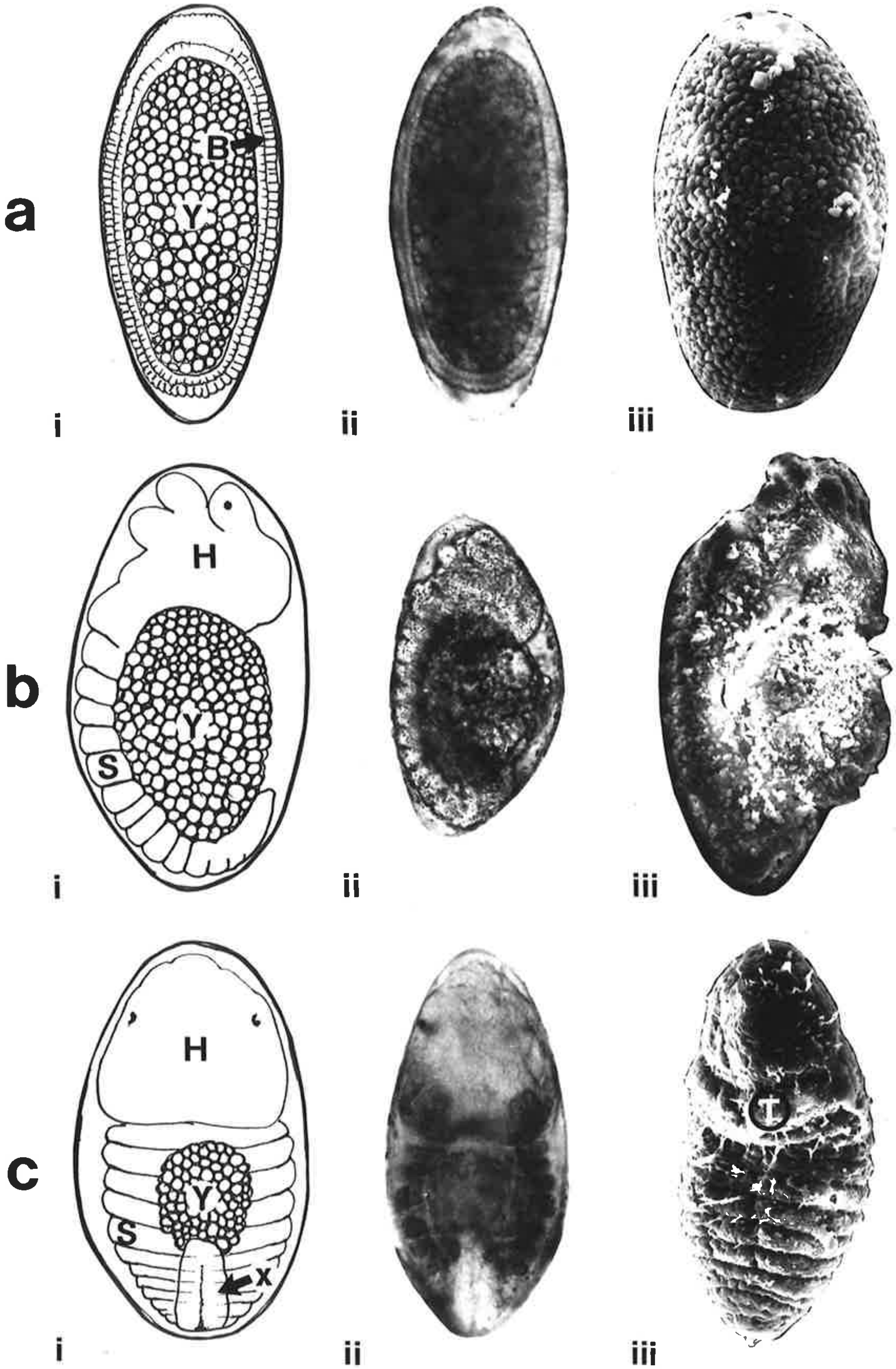
c) Egg anatomy The eggs are oval in shape (maximum width - 0.11 mm (n = 42, SD = 0.02), maximum length - 0.21 mm (n = 42, SD = 0.03)) and they have a featureless, fibrous surface (Fig. 6.1). The anterior end bears a micropyle surrounded by a vestigial, heart-shaped plastron (Fig. 6.2). No pores are visible (Hinton, 1980). Certain pale patches which show up as features on the egg surface in the micrographs (Fig. 6.1a) are thought to be shrinkage artifacts. In cross-section the chorion has a porous, fibrous appearance. No aeropyles or hydropyles were seen despite thorough searching of several cross-sections under high power (SEM) (Fig. 6.3).

d) Embryology Four distinct stages could be distinguished under low power (approx. 100X): i) no discernible embryonic cells, only yolk visible, ii) blastoderm cell layer over yolk visible, iii) developing embryo with visible somites coiled over the yolk surface and iv) fully developed 1st instar with post-cephalic somites compressed longitudinally ("Jack-in-a-box") so that they occupy one half of the available space while the head capsule takes up the other half; yolk appears as a small ball (0.05 mm diameter) attached to the ventral side at the posterior; embryonic movement is visible.

For the purpose of experimental work, eggs in which only the yolk was visible were not useful because there was no indication of their viability. It was therefore decided that three arbitrary stages would be used: "yolky", "developing" and "waiting" stage embryos corresponding to ii), iii) and iv) above (Fig. 6.4).



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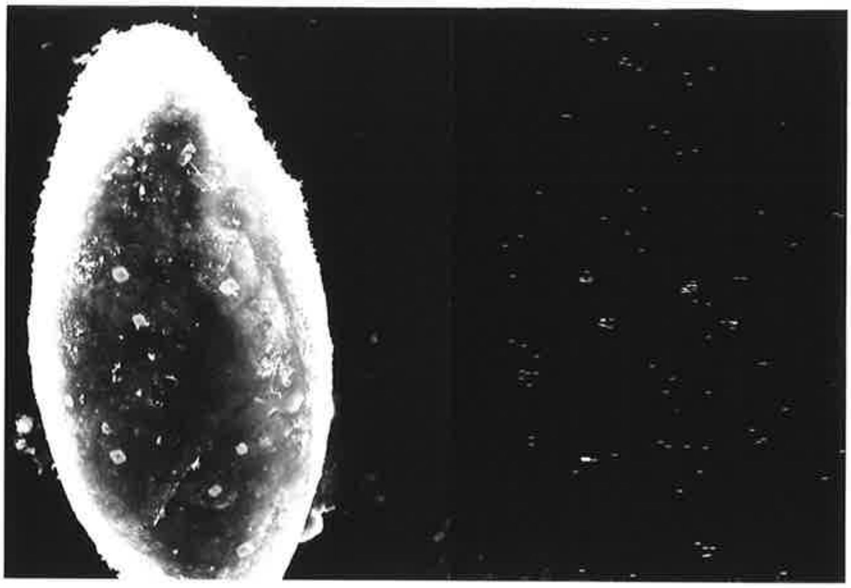
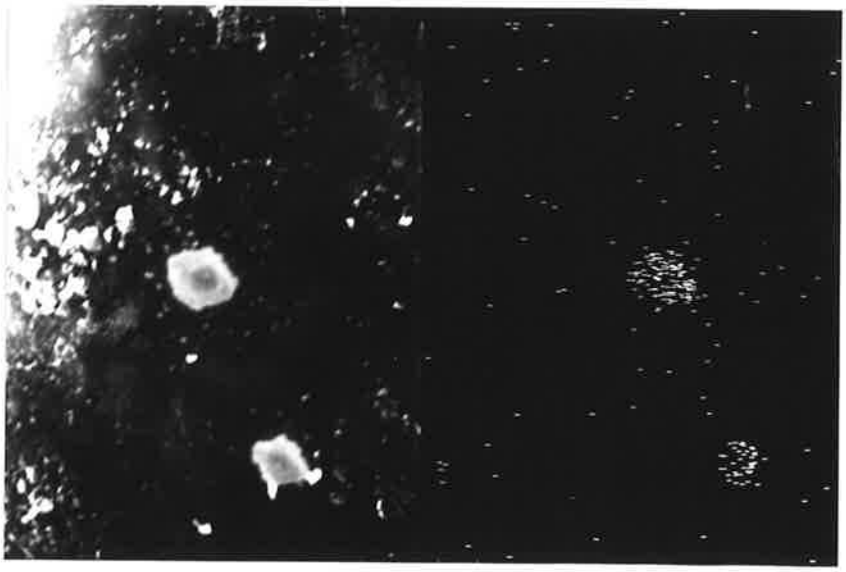
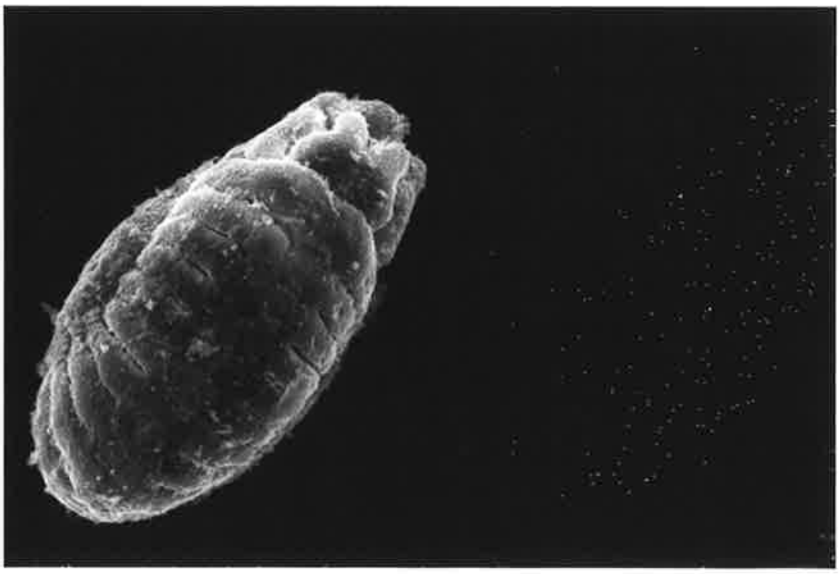
II. Egg permeability and embryonic ion excretion

a) Sites of ion passage through the chorion Of the three egg stages (yolky, developing and waiting) treated with silver for sites of chloride ion passage, only waiting stage eggs stained strongly in localized patches on the chorion, indicating areas of permeability or pores (Fig. 6.5a). The stained areas consisted of irregularly-spaced, circular patches averaging some 5.6 microns in diameter. At the centre of each patch was a small, dark core which suggested the presence of a pore (Fig. 6.5b). It was estimated that there were some 50 patches on each of the eggs viewed (n = 9).

b) Sites of ion excretion on the embryo All four methods of forcing eclosion proved moderately successful, with some 10% of original material appearing as embryos in the prepared (SEM) material. Of the embryos which were stained after successful forced eclosion, none showed evidence of chloride ion excretion sites (Fig. 6.6).







III. Egg hatching experiments

a) Hatching after desiccation Table 6.1 gives the hatching rate of dehydrated and control eggs at 5-day intervals after the commencement of the experiment.

Table 6.1 Viability of dehydrated *T. barbitarsis* eggs

Days after commencement of experiment	Number of eggs hatching after re-immersion		
	Desiccation at 4°C	Desiccation at 20°C	Immersion at 4°C
5	2	1	4
10	0	0	3
15	0	0	5
20	0	0	5
25	0	0	2
30	0	0	4
35	0	0	1
40	0	0	2
45	0	0	0
50	0	0	0

After the initial period of 5 days, 2 larvae were found to have hatched from the 4°C desiccated eggs and 1 from the 20°C desiccated eggs. None of these larvae was alive when they were viewed. In each case, however, 1st instars which hatched from the control series were alive upon inspection. No further larvae were seen to hatch from desiccated eggs.

b) <u>Hatching in distilled water</u> Results of the distilled water hatching experiment are given in Table 6.2. There was no significant difference between development and hatching success of eggs in distilled water and those in lake water. No bursting effects were observed. The 1st instar larvae in the distilled water medium, however, died soon after eclosion whereas those hatching in the control media survived to the end of the experiment. The final salinity of the pooled water from the 4 treatment petri dishes was 0.022 g l^{-1}. Hatching success in distilled water was 67% (53 out of 79 eggs) whereas in the controls it was 47% (23 out of 49 eggs).

Table 6.2 Egg hatching in distilled water

DATE/TIME		Experiment																Control							
		DW 1				DW 2				DW 3				DW 4				SW 1				SW 2			
		Y	D	W	H	Y	D	W	H	Y	D	W	H	Y	D	W	H	Y	D	W	H	Y	D	W	H
20/11/85	1500 h	4	16	0	0	7	13	0	0	8	0	11	0	10	10	0	0	8	15	0	0	6	20	0	0
21/11/85	1700 h	1	8	11	0	3	11	6	0	2	4	10	5	1	6	13	0	2	17	4	0	1	24	1	0
22/11/85	1700 h	1	4	13	2	3	5	12	0	0	1	9	9	1	3	14	2	2	0	20	1	0	2	23	1
23/11/85	1700 h	1	2	8	9	2	4	5	9	0	1	2	16	1	3	5	11	2	0	14	7	0	2	17	7
24/11/85	1700 h	1	2	4	13	2	3	4	11	0	1	5	17	1	2	5	12	2	0	8	13	0	2	14	10

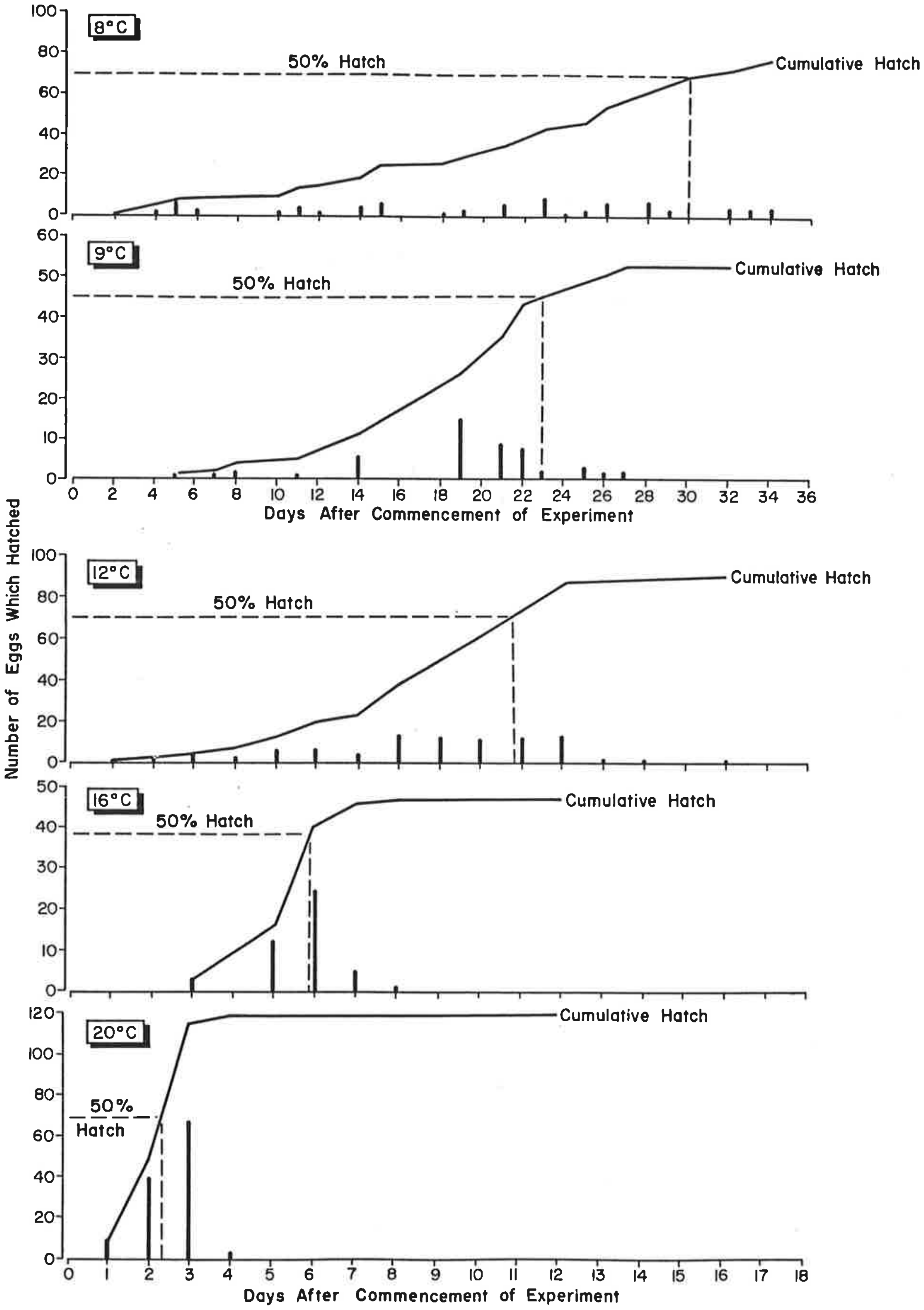
Final salinity - 0.022^{o}/oo. DW - Distilled water SW - Salt water

Y - Yolky egg D - Developing egg W - Waiting egg H - Hatch

Distilled water 53 out of 79 eggs hatch - 67% hatching success

Salt water 23 out of 49 eggs hatch - 47% hatching success

Figure 6.7 Cumulative hatch curves for five different temperatures.
NB. Note the change in horizontal scale after the 9°C graph.



c) The effect of temperature on the rate of embryonic development

Cumulative egg-hatching curves at 5 of the experimental temperatures are given for pooled data from all replicates in each experiment. No hatching was observed at 4°C after 50 days. The following times to 50% hatch were returned by experiments: 8°C - 30 d, 9°C - 22.8 d, 12°C - 10.8 d, 16°C - 5.8 d, 20°C - 2.3 d (Fig. 6.7).

DISCUSSION

The results described above concerning the nature of eggs and oviposition strategy show that T. barbitarsis lays individual, transparent, featureless eggs which sink slowly through the water column and settle out on the sediment surface. Clutch size is small (209) when compared with some freshwater species [Chironomus plumosus - 1676 (Hilsenhoff, 1966) note, however, that it is similar in size to Paratanytarsus parthenogenetica another member of the Tanytarsini, (Edward, 1963)]. The eggs are unable to withstand even brief periods (5 days) of desiccation although they are highly impermeable when immersed in distilled water.

It seems reasonable to conclude, from these findings, that the habit of laying single eggs has evolved in T. barbitarsis not in a terrestrial setting nor even as a strategy to place the eggs in a situation favourable to newly-hatched larvae as in Paraclunio (Hinton, 1981), but rather as a response to some other selective pressure.

If one were to assume, from the lack of tolerance of the eggs to desiccation, that T. barbitarsis evolved directly from a freshwater ancestor, then an important selective pressure in tidal pools, apart from osmoregulatory problems, would come from predation by fish. Perhaps the single-most important characteristic of tidal pools, particularly for visual predators, is the clarity of the water. This places enormous pressure upon all planktonic life phases of prey species. The adaptations of *T. barbitarsis* with regard to eggs and oviposition behaviour may well reflect a response to such predation pressure.

Thus, the habit of depositing single eggs would effectively increase the cost of predation by fish because of the need for energy-expensive searching behaviour. The previous work which attempted to quantify the effects of fish predation upon *T. barbitarsis*, although not conclusive, did indicate the strong likelihood that predation by larval and juvenile fish upon eggs and 1st instars exercises temporary control. Predation upon individual eggs is likely to become less energetically viable as the fish grow in size. It is this constraint which causes size selection for prey as fish grow (Wurtsbaugh *et al.*, 1980). Adaptations by *T. barbitarsis* to avoid fish predation seem to have been sufficient to ensure success in saline intertidal pools despite intense pressure from small fish during recruitment.

Again, the dipping oviposition habit, which has also been observed in aquatic Tipulidae (Hinton, 1981), would serve to make egg dispersal more efficient as well as protecting the female fly from predation during oviposition. It may be noted that *C. plumosus* lays the egg mass onto its hind legs and deposits them in a similar dipping flight over the water surface; it too appears to remain on the water after the completion of oviposition (Hilsenhoff, 1966).

Three points can be made with regard to the slow rate of egg sinking. Firstly, since most intertidal pools, salinas and salt lakes are relatively shallow (less than 1m), the time spent in the water column is not extensive. Secondly, a slowly-sinking object is less likely to attract the attention of a visual predator than is a rapidly-moving one (see Healy, 1984). The fact that the eggs are transparent and have a featureless chorion may contribute to their concealment. Thirdly, the slow sinking rate of the eggs makes them vulnerable to water currents which may beach them in littoral areas or sweep them into less favourable situations. It can be deduced from this, therefore, that oviposition in still waters is likely to be favoured.

Small clutch size is theoretically inversely related to larger egg size (Salthe and Duellman, 1973). The investment of more energy in fewer individual offspring is the alternative to a strategy of laying large numbers of small eggs. However, data for clutch size within the Chironomidae suggest a degree of bimodality. Species such as *Chironomus plumosus* and *Chironomus tentans* lay thousands of eggs in each clutch (range 1400 - 3300), whereas others such as *Corynoneura scutellata* and *Smittia gracilis* lay tens of eggs (range 20 - 99) (Thienemann, 1954). Such patterns imply that there are indeed two alternative strategies.

Comparison between the egg size of *T. barbitarsis* and and that of *C. plumosus* reveals dimensions which run contrary to expectations. *T. barbitarsis*, with its small clutch size (approx 200), lays eggs which are considerably smaller (0.11 X 0.21 mm) than those of *C. plumosus* (0.2 X 0.5 mm) with its large clutch size (approx 1500) (Hilsenhoff, 1966). However, a complicating factor in the discussion about the relative size of insect eggs is the assumption that large egg size reflects larger yolk size and therefore more energy content. This may not be the case. Even so, and bearing such reservations in mind, some comments about the size of *T. barbitarsis* eggs would form a basis for further experimental work of a biochemical nature.

It is clear that egg viability in T. barbitarsis is very high. An analysis of the data from egg hatching experiments yielded a viability in excess of 80%. This figure, in fact, is a conservative estimate because collection was from the sediments where non-viable eggs would accumulate. Such high viability allows for a reduction in clutch size and a more efficient use of energy reserves, making the species more competitive.

The small size of the egg may also reflect a high density giving the advantage of increasing the sinking rate through saline waters. Selection pressure may have favoured more dense storage products for the yolk. The fact that 1st instar larvae retain some yolk on the posterior after hatching from which they feed, attests to the nutritional efficiency of this storage product.

Further, constraints may have been placed upon the nature of the storage material in that the yolk should require little oxygen for catabolism and yield non-toxic waste products. These constraints follow from the fact that for most of embryonic development there appears to be no communication with the outside milieu.

Efforts to locate sites of ion excretion in the embryo were influenced by two previous discoveries concerning ionic balance in saline invertebrates. Firstly, the knowledge that the site of ion excretion is in a 'neck' gland in larval Artemia (Conte et al., 1972). Secondly, knowledge that the site of ion excretion in pupal T. barbitarsis is on the dorsum of the thorax (see Chapter 7). Such knowledge, taken in conjunction with the observation of a thickened collar in late stage T. barbitarsis embryos (Fig. 6.4c iii), led to an hypothesis that this collar was the site of specialized ion-excretion tissue.

The silver staining technique applied to the embryos after forced eclosion was unsuccessful probably because the embryos were not alive at the time of exposure to the silver nitrate solution. However, evidence from other sources indicates that Waiting stage embryos use rectal tissue to excrete ions and thereby maintain osmotic homeostasis.

There is a strong indication from the absence of stained sites on the chorion of Yolky and Developing stage eggs and from hatching experiments in distilled water, that the chorion is impermeable during most of embryonic development. Conversely, the discovery that there are sites of ion extrusion in the chorion of Waiting stage eggs suggests that the embryo is actively maintaining osmotic balance. The need for communication with the external medium during the latter stages of embryonic development probably arises due to increased oxygen demands and excretory imperatives.

It is possible then to propose that eggs remain impermeable until the late stages of embryonic development, by which time the rectal tissue has differentiated. At this stage the developing embryo is able to exchange oxygen and waste products with the external medium while still maintaining its osmotic balance. Support for this proposal comes from light micrography of Waiting stage embryos. In each case the rectal tissue showed up as a clear halo at the posterior end of the embryo (Fig. 6.4c ii) giving a strong indication of its activity. It should be noted, however, that in order to extrude ions through pores in the chorion, the concentration of the fluid within the egg has to be raised to levels above that of the medium outside. Consequently, the Waiting stage embryo has always to be able to tolerate water salinities higher than those which prevail in the lake.

If a prescribed mathematical relationship between temperature and rate of embryonic development could be shown to have general validity, then developmental patterns could be deduced by the measurement of a few points (Howe, 1967). The search for such a relationship has occupied both entomologists, who are concerned with many practical aspects of development rate, and aquatic biologists, who require such information for the calculation of production (Edmondson, 1974).

There have been several reviews of the equations proposed for the description of the temperature/development relationship (Howe, 1967; Nielsen and Evans, 1960; Bottrell, 1975). These have pointed out the limitations of the various methods.

Despite Howe's (1967) assertion that there is little basis for choice between the different mathematical relationships, some consensus has been reached in recent times. This can be summarized as a general acceptance of Belehradek's (1935) equation:

$$D = a(t - b)^{-c}$$

Where: D = development time;

t = temperature;

a, b, and c are fitted parameters.

Bottrell (1975) pointed out that it is not necessary to introduce the term b into the computations unless development continues at 0°C and below. This being so, the relationship is simply a power function i.e. $D = at^{-c}$.

In recent times the temperature/development relationship has been described using a power function (or Belehradek's equation) for rotifers (Herzig, 1983), Ephemeroptera (Humpesch, 1980) and entomostracan zooplankters (Hart, 1985); thus its widespread use is emphasized.

When the data from hatching experiments with T. barbitarsis eggs were regressed using the least squares method, the power function, $y = 8,712.32x^{-2.70}$ was returned with a coefficient of determination (r^2) of 0.99, where: y = time to 50% hatch in days and x = mean water temperature in °C.

The convention of using the time to 50% hatch overcomes problems of egg viability and is commonly used in other similar studies (e.g. Elliot, 1978; Humpesch, 1980).

This relationship is represented in Fig. 6.8 where egg hatch time becomes asymptotic for temperatures below 5°C. This is in good agreement with the finding that at 4°C no hatching occurred after 50 days.

The different life stages of insects often have slightly different temperature optima and limits (Huffaker, 1944). The concept of rate of development for the whole life cycle, however, even when the various instars are known to differ, may be useful to estimate the number of generations annually or the potential rate of increase (see Chapter 4).

The temperature/generation time relationship which was derived from the population data fell just short of being significant at the 0.05 level. However, a comparison of this curve with that derived from egg hatching experiments (Fig. 6.8) raises some interesting points of discussion.

The Q_{10} is a factor which reflects the increase in the rate of a biological process with the change of 10° in temperature. It is defined as:

$$Q_{10} = \left(\frac{K_1}{K_2}\right)^{\frac{10}{(t_1 - t_2)}}$$

Where: k_1 is the rate of function 1, k_2 is the rate of function 2, t_1 is the temperature at which function 1 was measured and t_2 the temperature at which function 2 was measured.

As a comparison between the generation time and the rate of embryonic development Q_{10}, values were calculated over the temperature range, 12 to 15°C. The choice of this range was influenced by the fact that the two generations of 1985 were clearly-defined and their associated parameters could be estimated with confidence. Development rates for embryos were taken from the egg-hatch curve in Fig. 6.8. The values were as follows:

Whole generation

$$Q_{10} = \left(\frac{0.027}{0.014}\right)^{\frac{10}{(15-12)}} = 8.79$$

Embryonic development

$$Q_{10} = \left(\frac{3.5}{1.8}\right)^{\frac{10}{(15-12)}} = 9.1$$

The similar results returned by this single comparison for the two rates of development suggest that embryonic development may be used as a guide to the length of generation time.

When the egg-hatch curve was extrapolated to pass through the points represented by the two 1985 generations, it was found that, as temperatures increased, the theoretical (extrapolated egg-hatch) curve diverged considerable from the generation time curve (Fig. 6.8).

Since higher temperatures were accompanied by higher salinities in 1982/83, this divergence probably reflects the influence of this factor upon generation time. Organisms which live in highly saline media have to expend energy to maintain osmotic homeostasis. Consequently, their growth and development is slowed (see Dana and Lenz, 1986 for a discussion of this phenomenon in Artemia and Teitjen and Lee, 1972 in nematodes). However, in 1984/85 the whole lake system was diluted, giving Q_{10} values which indicate a close correspondence between embryonic development/temperature and generation time/temperature relationships.

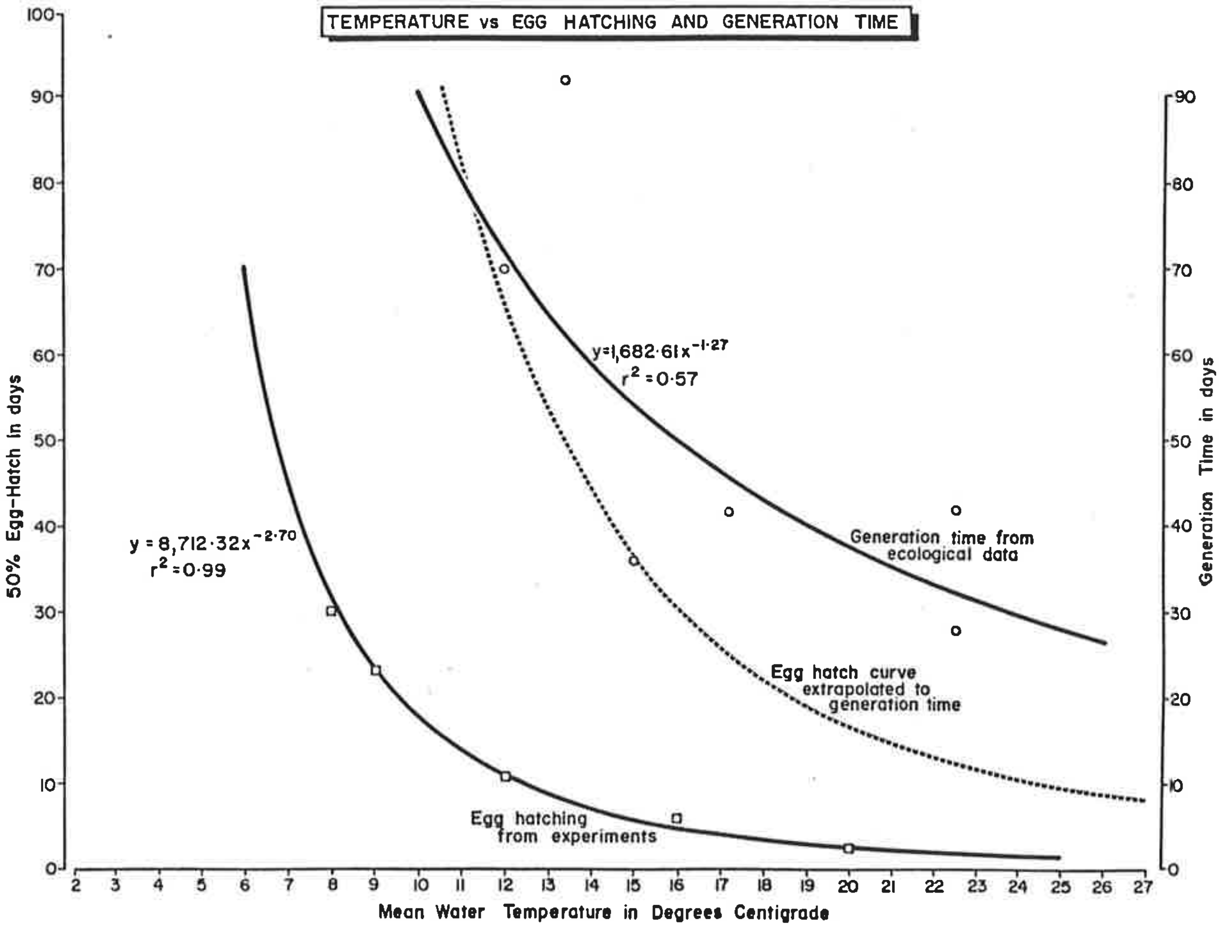
It is evident from the various investigations described above that T. barbitarsis eggs are well adapted to succeed under all but severe predation pressure. The eggs are highly impermeable throughout most of embryonic development and, hence there is little opportunity for reducing embryonic survival. A greater opportunity is perhaps presented in later stages when embryos are in contact with the external medium.

It has been suggested that the late stage embryo is able to excrete excess ions via its rectal tissue. If this is indeed so, the implications are that it must always be subject to salinities higher than that of the external medium in order to create a gradient and induce an outward movement of ions across the chorion.

A logical follow-up would be to investigate the viability of T. barbitarsis eggs in a range of salinities. If experiments confirm that eggs have a lower tolerance to salinity than do other life stages then the manipulation of lake salinities at times of heavy oviposition would be a logical control measure. This would be particularly useful because the mass emergences of adults would be a clear signal to begin such control measures.

The comparison between the rate of embryonic development and the generation time suggests that there should be a prolongation of generation time with the increase in water salinity. The implication of the permanent lowering of water salinity by the operation of a new power station is, in the absence of competing factors, that there will be more generations of T. barbitarsis per year thus exacerbating the midge problem.

Figure 6.8 Graphical comparison between (curves from left to right):
i) The effect of temperature on egg hatching time
ii) (dashed line) The curve from (i) moved to pass through data points from 1984/85 where the generation time temperature relationship could be estimated with maximum confidence.
iii) The effect of temperature on the length of generation (see Chapter 4).



CHAPTER 7

Osmoregulation, salinity tolerance and the site of ion excretion

INTRODUCTION

In addition to saline coastal lakes such as those at Port Augusta, the larvae of *T. barbitarsis* occur widely in inland salt lakes as well as intertidal marine habitats across southern Australia (Glover 1973; Edward 1983; Kokkinn 1986). There, waters range in salinity from 20 to 170 g l^{-1}. Although it has been claimed that the species is able to exploit freshwater environments in the absence of competition from other chironomids (Paterson and Walker, 1974b), it is clear that it is essentially an organism of saline waters.

The ability of *T. barbitarsis* to survive in hypermarine waters, it seems, is the key to its success and hence, its activity as a problem organism at Port Augusta. Permanent waters in the salinity range 40 to 200 g l^{-1} are not common, and there has therefore been little opportunity for organisms to adapt to such loci. As a result, competition is much reduced in these environments for pre-adapted species such as *T. barbitarsis*. However, at present, the extent of its salinity tolerance can only be deduced from distributional data.

The need to gain a more accurate idea of halotolerance in *T. barbitarsis* became apparent when it was discovered that water salinity in the Port Augusta lakes could be manipulated. Flow to various parts of the lake system could be cut off and the salinity allowed to rise by evaporation. Subsequently, the highly saline water could be released into adjoining lakes to act as an insecticide. To investigate the feasibility of this potentially cheap control measure, certain background information relating to the salinity tolerance of *T. barbitarsis* was sought.

Three lines of research were undertaken: an investigation of the pattern of osmoregulation; a determination of halotolerance; and the location of ion excretion sites. The primary aim of this research was to quantify the effects of salinity upon *T. barbitarsis*. In addition, a better understanding of the processes of ionic regulation was sought with the idea that it would, perhaps, lead to other control measures.

With regard to osmoregulation, previous investigations of euryhaline dipteran larvae have indicated that most, if not all, are capable of regulation (e.g. Beadle 1939; Sutcliffe 1960; Lauer 1969; Phillips & Meredith 1969). Although there were no indications that *T. barbitarsis* would be an exception, it was prudent, as a first step, to establish that it was not.

The need to investigate halotolerance of Port Augusta *T. barbitarsis* arose from strong evidence that different populations of *T. barbitarsis* have different salinity tolerances. Thus, records exist from Western Australia (Edward, 1983) of collections in waters of salinity 140 g l^{-1}, but in South Australia and Victoria the species has been collected from waters reaching only 100 g l^{-1} in salinity (Walker 1973; Paterson & Walker 1974 a, 1974 b).

The site of ion excretion tissue in nematocerans, particularly Culicidae, has been identified by several authors who have found that it is located in the rectal tissue (Ramsay 1950, 1953; Bradley & Phillips 1975, 1977a, 1977b, 1977c; Strange *et al*. 1982). However, it was not known that *T. barbitarsis* employs its rectal tissue in the same way as other euryhaline nematocerans. In addition, although previous work on sites of ion excretion have concentrated on the larva, none has identified a site in the pupa. Yet the pupa is subject to the same stresses from an hyperosmotic external milieu as the larva, but no longer has rectal tissue to maintain ionic balance.

This chapter, therefore, examines the following aspects of T.barbitarsis biology: the pattern of osmoregulation, salinity tolerance of the Port Augusta population and the site of ion excretion in the larva and pupa.

MATERIALS AND METHODS

Osmoregulation

Fourth instar larvae were placed in solutions of the following salinities at 20°C: 5 g l^{-1}, 27 g l^{-1}, 36 g l^{-1}, 62 g l^{-1} and 94 g l^{-1}. Salinities were determined conductometrically using the formula of Williams (1986). The solutions were essentially concentrated sea water. To allow for salinity equilibration, the larvae were held in the respective solutions for at least 24 h before any measurements were made. Individual larvae were then briefly washed in distilled water, blotted dry, and immersed in paraffin oil. Haemolymph was extracted by piercing the integument with a fine needle, care being taken to avoid inclusion of gut fluids. Six droplets from each individual were suspended in emmersion oil in the holes of a copper grid which was placed in a Clifton Technical Physics Osmometer. The osmotic pressure of each droplet was calculated from its freezing point depression and an average value was then derived.

Salinity Tolerance

By convention, salinity tolerance can be expressed in terms of the concentration of NaCl at which 50% mortality occurs (viz. LC_{50}). Since control was being proposed by applying high salinities it was only the upper limit which was of interest. Determinations of the upper LC_{50} were undertaken on two separate occasions and protocol followed the American Society for Testing Materials (ASTM, 1980). Fourth instar larvae were exposed in 100 mm glass Petri dishes to water from the Port Augusta lakes which had been concentrated by evaporation to the levels indicated below. Three replicates, each containing 20 fourth instars, were exposed to a series of salinities. Each of two experiments were allowed to run for 96 hours (according to ASTM convention) and survival was monitored daily. In each experiment, there was a control series which contained water from the parent lakes (salinity of 50 g l^{-1}).

Solutions of the following salinities were used:

Experiment 1. 100 g l^{-1}, 110 g l^{-1}, 120 g l^{-1}, 130 g l^{-1}, 140 g l^{-1}, 50 g l^{-1} (control).

Experiment 2. 90 g l^{-1}, 95 g l^{-1}, 100 g l^{-1}, 105 g l^{-1}, 110 g l^{-1}, 50 g l^{-1} (control).

All experiments were run at 20°C. Petri dishes were kept covered to minimize evaporation, and the salinity was checked conductometrically after each experiment to establish the extent of the increase in concentration. Data were analysed by plotting probit response against log dose according to the method of Hewlett and Plackett (1979).

Sites of Ion Excretion

The sites of chloride ion excretion were located by staining larvae and pupae with silver according to the method of Conte *et al.*

(1972). Live specimens (fourth-instar larvae and pupae) were washed in distilled water to remove external NaCl. They were then placed in a 0.1M silver nitrate solution under bright light for several minutes. Stained specimens were subsequently bathed in a 0.1M sodium thiosulphate solution for 5 min.

The material was dehydrated through an alcohol series, critical point dried and single-coated (15 nm) with carbon as a preparation for scanning electron microscopy (SEM). The areas of silver staining were mapped on a Philips 505 SEM which was linked to a Tracor Northern (TN 5500 EDS) X-Ray analyser and image enhancement system.

RESULTS

Variations in haemolymph osmotic pressure are indicated in Fig. 7.1. It can be seen that the fourth-instar larvae of _T. barbitarsis_ are able to regulate the concentration of their body fluids in a wide range of external media. Between 5 and 29 g l^{-1} regulation appears to be very efficient. As the external salinity increases to above 29 g l^{-1}, however, there is a gradual increase of internal haemolymph concentration.

Table 1 sets out the results of the salinity tolerance experiments. The LC_{50} values obtained were 96.9 ±20.6 g l^{-1} (95% Confidence Limits (CL)) NaCl and 89.4 ±5.2 g l^{-1} (95% CL) NaCl respectively. This indicated that at salinities approaching 90 g l^{-1} NaCl and temperatures of 20°C, there was 50% mortality of 4th instar _T. barbitarsis_ larvae from the Port Augusta population.

The sites of ion excretion in fourth-instar larvae and pupae are illustrated by means of split-image scanning electron micrographs

(Fig. 7.2 and 7.3). On the left-hand side, the secondary electron image is displayed, on the right, the corresponding X-ray map.

In the fourth instar larva, only the perianal rectal tissue stained strongly with silver, clearly indicating the main site of chloride ion excretion (Fig. 7.2).

In the pupa there was no staining of the posterior abdomen. Instead, four distinct areas, located on the dorsum of the mesothorax were identified: two linear anterior areas lying on either side of the midline each measuring 0.27 by 0.04 mm, and two posterior circular regions set symetrically slightly further from the midline with a diameter of 0.09 mm (Fig. 7.3). A close-up view of the cuticle showed the silver to have stained numerous circular patches each measuring approximately 3.4 microns in diameter.

Figure 7.1 Variation of osmotic concentration of 4th instar haemolymph with changing osmotic concentration of the external medium at 20°C. Values are means with 95% confidence intervals indicated by bars. N = 5.

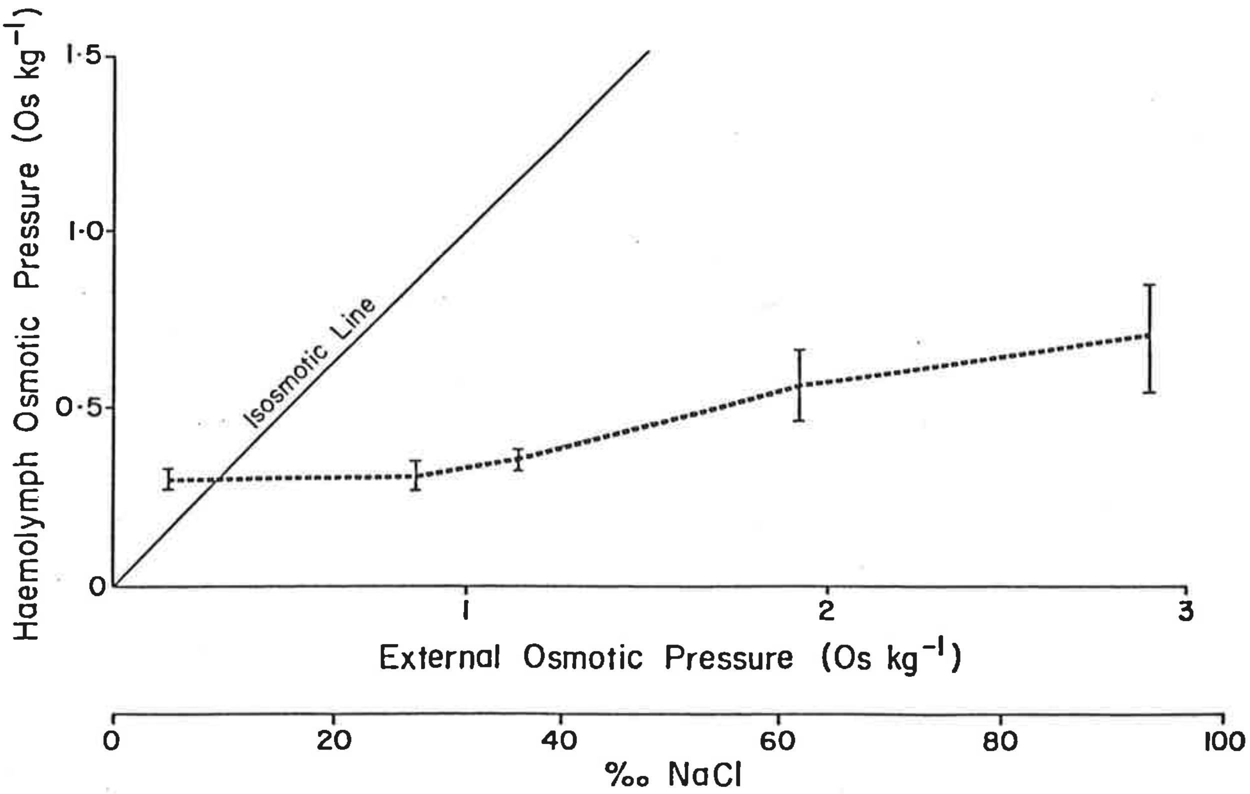


Table 7.1 Results of two experiments to determine the LC_{50} of <u>T. barbitarsis</u>. The first using large increments in the concentration of the medium and the second concentrating on the range indicated by the former.

Treatment		Final	Mean number of	Controlled
Probit	Salinity $(g\ l^{-1})$	Salinity $(g\ l^{-1})$	Larvae Surviving (± S.E.)	Mortality (%)
Experiment 1.				
4.432	100	104	11.0 (1.5)	28.5
5.345	110	113	4.0 (1.0)	63.5
5.915	120	124	0.3 (0.3)	82.0
5.915	130	132	0.3 (0.3)	82.0
	140	140	0	100
Control	50	56	16.7 (0.3)	

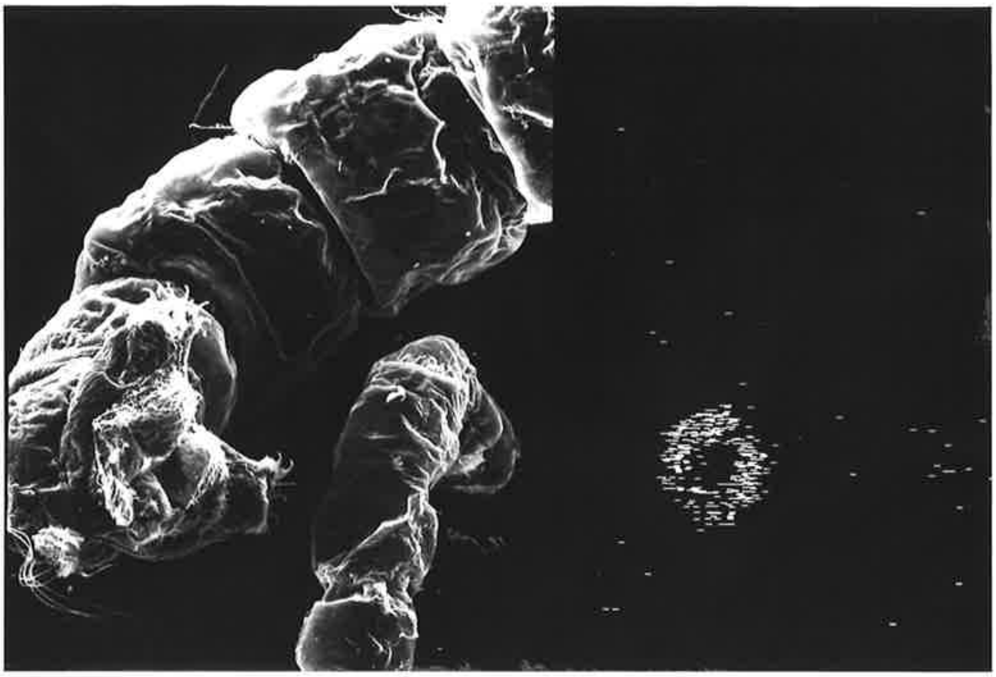
Equation for Log Dose (y) vs Probit (x) : $y = 1.703 + 0.066\ (\pm 0.73)x$
$r^2=0.88$

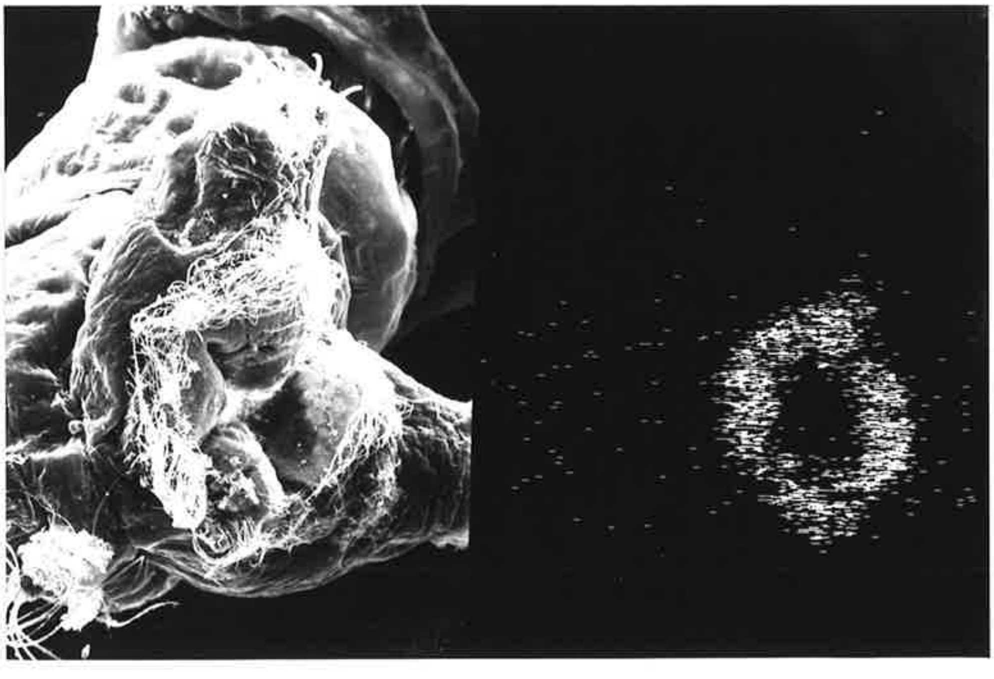
Treatment		Final	Mean number of	Controlled
Probit	Salinity $(g\ l^{-1})$	Salinity $(g\ l^{-1})$	Larvae Surviving (± S.E.)	Mortality (%)
Experiment 2.				
4.372	90	93	13.7 (0.7)	26.5
4.786	95	97	10.7 (0.7)	41.5
5.292	100	104	6.7 (1.8)	61.5
5.896	105	107	2.7 (1.3)	81.5
5.841	110	112	3.0 (0.6)	80.0
Control	50	56	19.0 (0.6)	

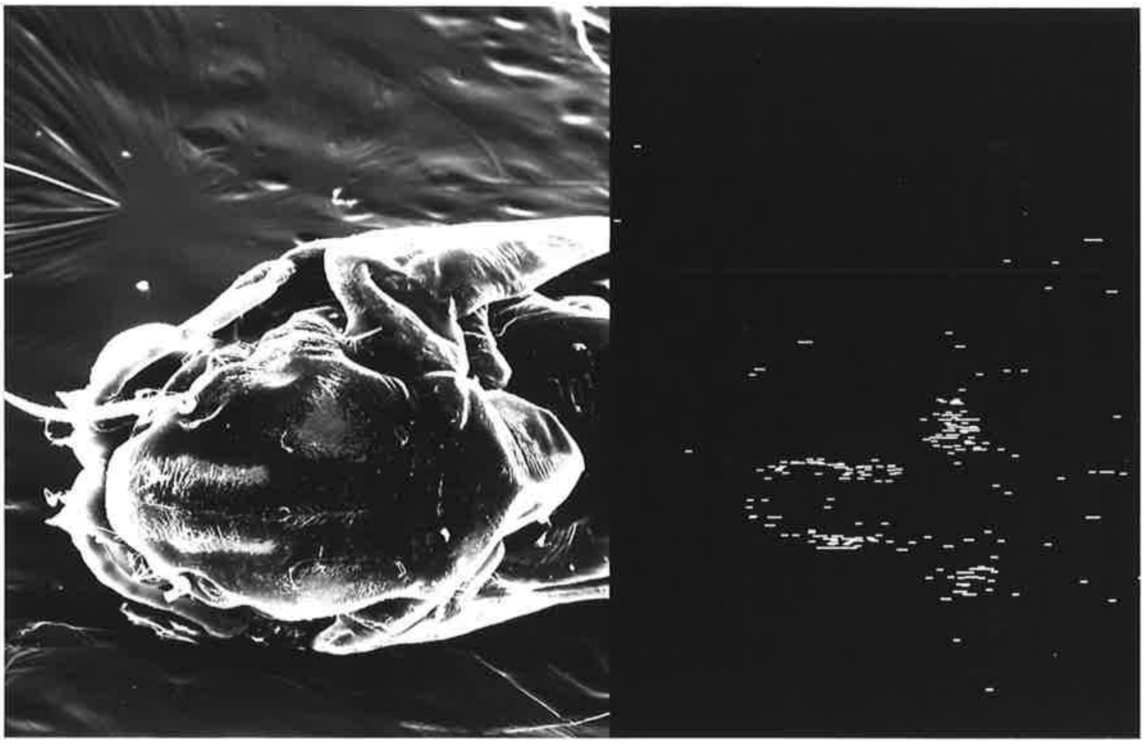
Equation for Log dose (y) vs Probit (x): $y = 1.734 + 0.051\ (\pm 0.023)x$
$r^2 = 0.94$

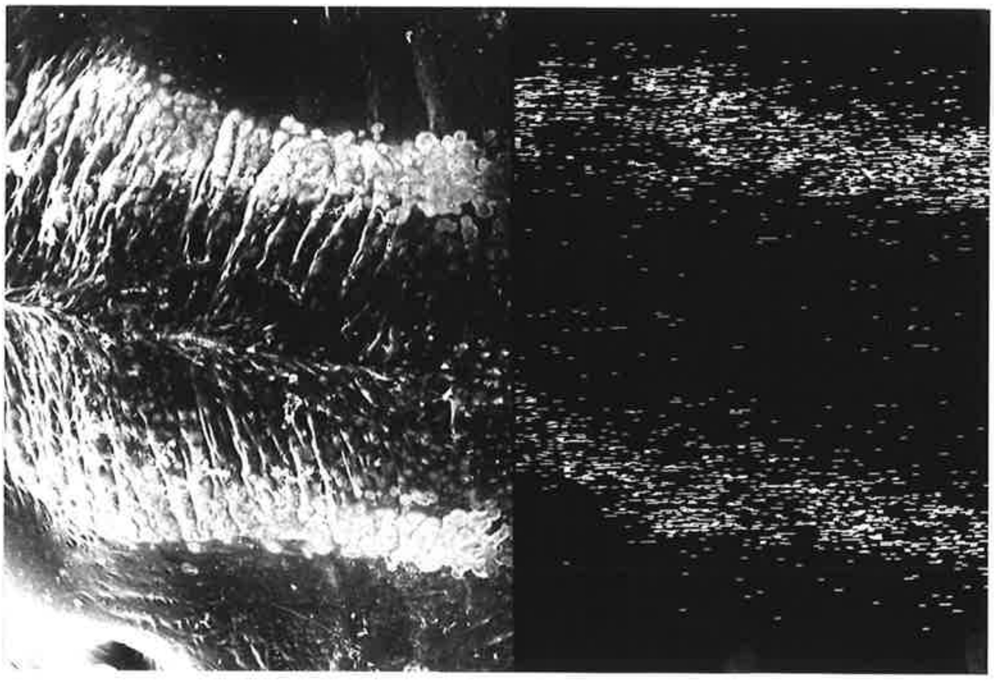
$LC_{50} = 96.85 \pm 20.6\ g\ l^{-1}$ (95% CL) NaCl (expt 1);

$89.45 \pm 5.2\ g\ l^{-1}$ (95% CL) NaCl (expt 2)









DISCUSSION

Two osmoregulatory categories can be recognized among larval nematocerous dipterans which occur in saline waters. Members of the first category are unable to hyporegulate; their upper limit of salinity tolerance is equal to the initial concentration of the haemolymph (Bayly 1972). Examples from this group include: Aedes aegypti (Wigglesworth, 1938), Chironomus plumosus and Tanypus nubifer (Lauer, 1969). Species belonging to the second category have evolved the ability to hyporegulate in concentrated media; this includes the classical example of Aedes detritus (Beadle 1939). Results reported here unequivocally indicate that T. barbitarsis is both an efficient hyper- and hyporegulator. Moreover, it is unrivalled among chironomids for its ability to exploit hypersaline waters.

Despite its apparent adaptation to hypersaline waters, the relatively low concentration of its haemolymph (c. 0.3 Osm per kilogram of water (Osm kg^{-1}) or 10 g l^{-1} NaCl) may be taken to indicate the freshwater origins of T. barbitarsis. A similar observation was made for the euryhaline mosquito Aedes detritus by Beadle (1939). The dilution of haemolymph to one-third of seawater in freshwater organisms is considered an adaptation to minimize the gradient across the integument. The value, 0.3 Osm kg^{-1}, appears to reflect a minimum in terms of evolutionary possibility for insects; further dilution of body fluids would preclude essential physiological processes (Krogh 1939; Burton 1973). Retention of such dilute internal media in T. barbitarsis and A. detritus has imposed an even more severe and energetically costly gradient across the integument in hypersaline waters.

Organisms which hyporegulate against strong gradients must expend considerable energy extruding ions, it has been estimated that for every three sodium ions transported across the cell membrane, one molecule of ATP is required (Burton 1973). There is also evidence for the high cost of hyperregulation (e.g *Chironomus salinarius*). Thus, *C. salinarius* normally occurs in waters up to 37 g l^{-1} in salinity, but when reared in dilute media it has an extended development time (Neumann, 1961).

The energetic costs of invading hyperosmotic environments may explain several life history characteristics of *T. barbitarsis*. Firstly, it may explain why, even at *mean* water temperatures of 25°C (at salinities of 70 g l^{-1}), the generation time is 25 days, a relatively long period for such insects - compare this, for example, with *Chironomus thummi* which has a generation time of 15.5 days at 25°C (Scharf 1972) (see Table 4.5, Chapter 4). Secondly, it may explain why *T. barbitarsis* is distributed in highly productive waters and only reaches great numbers (200,000 m^{-2}) in eutrophic systems, natural or otherwise, indicating its need for high food levels. Because *T. barbitarsis* can closely regulate haemolymph concentrations across a wide range of salinities (5 to 94 g l^{-1}, Fig. 7.1), it can exploit highly saline waters (80 g l^{-1}) where there is little competition for benthic detritus. And thirdly, it may explain why it requires an extended period of inundation, including high summer temperatures, to allow its numbers to build up to any significant level. Such explanations regarding the energetic costs of osmoregulation require experimental investigation.

The above life history traits have important consequences for the distribution of *T. barbitarsis*. It restricted to productive waters and, because of its long generation time, it is seems unable to exploit water bodies inundated only in winter (but see Edward, 1983). This is exemplified in the south east of South Australia where a series of seasonally filled salt lakes lies south of a large, permanent, hypersaline water body, The Coorong. The south lagoon of the Coorong supports a large population of T. barbitarsis whereas the species is not present in the seasonally filled adjacent salt lakes (De Deckker and Geddes 1980; Williams 1985). These patterns were repeated in Lake Eyre South, an episodically filled salt lake which was recently inundated (February 1984 to January 1985). It contained water for an extended period, including the hot summer months, and T. barbitarsis was recorded from the lake in large numbers (Williams and Kokkinn 1986).

Regarding its pattern of osmoregulation, there is a slight increase of internal concentration above 29 g l^{-1}, but no strong indication of a breakdown of the regulatory ability as external salinities approach 94 g l^{-1}. One must therefore assume from the LC_{50} results a rather sharp failure of regulation near 100 g l^{-1}.

This upper limit of salinity tolerance is supported by collection data from eastern Australia. However, the occurrence of populations of T. barbitarsis on Rottnest Island, which survive in waters at 140 g l^{-1} (Edward, 1983), may be explained by: the fact that they belong to a different taxon from the species found in South Eastern Australia or, alternatively, a process of acclimatization by populations which have been consistently exposed to higher salinities in the permanently filled salt lakes found on the island (see Chapter 3).

The absence of T. barbitarsis from freshwater environments was ascribed by Paterson and Walker (1974b) to the fact that they are unable to compete with other detritivores under freshwater conditions. It is also feasible to postulate that during some part of the aquatic life history dilute media are not tolerated. However, this 'weak link' does not appear to be the fourth instar larva. Of course, T. barbitarsis may inhabit fresh waters, but in such low numbers as to have been missed in collections to date.

An important part of ionic and water balance in hyperosmotic media is the excretion of concentrated 'urine'. The site of this excretion in the larvae of euryhaline aquatic Diptera is the posterior rectal tissue (Sutcliffe 1960; Phillips & Bradley 1977). Similarly, it has been shown here, using the silver staining technique of Conte *et al.* (1972), that the major site of chloride ion excretion in *T. barbitarsis* 4th instar larvae is the rectal tissue. In addition, the activity of this tissue in first-instar larvae has also been demonstrated. Although the technique was limited to staining *chloride* ions, it must be presumed that sodium ions are excreted at the same rate and probably from the same site. Further work now remains to elucidate fully the system of ionic regulation in *T. barbitarsis* as has been done for certain mosquitoes (see Phillips and Bradley 1977 for review).

To date, all work on sites of ion excretion in aquatic Nematocera has concentrated upon the larvae. However, the pupa, which is just as important a part of the aquatic life cycle, has not been investigated. This work has shown that, upon pupation, the site of chloride ion excretion is switched to tissue on the dorsal thorax in *T. barbitarsis*, an apparently improbable location for such a process. This discovery raises a range of new questions about such an array of adaptations. For example, is this dorsal site of ion excretion active in the embryo (see Chapter 6)? Do similar adaptations occur in euryhaline species belonging to other dipteran families? It should be recalled, in this context, that the switching of sites of ion excretion has been reported among other aquatic animals. The brine shrimp *Artemia salina* has been shown to switch this site from the 'neck' in the larva to the metepipodite segments of the branchiae in the adult (Croghan 1958; Copeland 1966; Conte *et al.* 1972). Furthermore, the apparently unusual site of chloride ion excretion in the *T. barbitarsis* pupa may not be as improbable as it initially appears. Asakura (1978) assayed different regions of larval *Aedes togoi* Theobald, a euryhaline mosquito, for phosphatase activity which is an indicator, *inter alia*, of active transport across membranes. He discovered that phosphatase activity was high in the luminal border of the gastric caecae, which lie in the thoracic region. The indication was that the larvae drink their hyperosmotic medium, absorb ions in the caecae and induce water to follow. Hyporegulation is achieved by the excretion of ions from the rectum. The discovery that dorsal thoracic tissue is employed to excrete such ions in the pupa of *T. barbitarsis*, which has no flux of ions through the rectum, then seems to be quite sensible. Quite possibly, the site of ion uptake in the pupa is also in the gastric caecae which are proximal to the excretory tissue of the thorax. Such a system does, however, require that the pupa is able to drink the medium.

On the strength of such observations, two hypotheses are proposed: that the site of chloride ion excretion (absorbtion) in the pupae of euryhaline culicids is probably in the same region of the thorax; secondly, in the pre-pupal stage of *T. barbitarsis*, which is characterized by a swelling of segments 2 and 3, excretory tissue has already begun to differentiate in order to facilitate the changeover in sites from larva to pupa. Indeed, some as yet inconclusive silver staining of pre-pupae indicates this.

Close inspection of the excretory areas on the pupa revealed that the cuticle had stained in numerous circular patches each measuring some 3.4 microns across (Fig. 7.3b). These disc-like patches resemble those described from the cuticle of mayfly nymphs (Wichard and Komnick, 1971; Komnick and Abel, 1971). The discs, which are of a similar size to the circular patches on T. barbitarsis pupae, overlie the so-called ephemerid chloride cells and have been demonstrated to absorb chloride ions actively from the medium (Komnick et al., 1972).

The chloride cells described from mayflies resemble those found in the gills of fish (Wichard and Komnick, 1971). Such cells in fish, however, are active in either excretion or absorbtion of chloride ions depending upon the osmotic milieu (Potts and Parry, 1964).

The silver stained patches on the cuticle of T. barbitarsis pupae, therefore, could possibly indicate the presence of chloride cells which are analagous to those referred to above. Furthermore, close scrutiny of micrographs of the anal tissue in 4th instar larvae suggests the presence of disc-like patches there too (Fig. 7.2b).

In their description of ephemerid chloride cells from mayfly nymphs, Filshie and Campbell (1984) noted the presence of bacteria adhering to the external surface of the chloride cell discs. They speculated that these bacteria were utilizing either ammonium and bicarbonate ions which were extruded as by-products, or sodium and chloride ions which were passively released. In fact, if one bears in mind their dual role in fish, it is possible that the polarity of chloride cells can be reversed facultatively; thus the bacteria would benefit during periods of ion extrusion.

It appears that ionic regulation can be achieved by regulating the cellular concentrations of chloride alone. Kirschner (1979) has suggested that the net extrusion of sodium ions is accomplished by electrical coupling with the active transport of chloride ions. Thus, if it could be demonstrated that T. barbitarsis posessed cells which could both excrete and absorb chloride ions facultatively, there would be no need to show the presence of any other excretory tissue. However, some cautionary evidence comes from studies of osmoregulation in the nauplii of *Artemia* suggesting the active extrusion of sodium ions from the salt gland tissue (Conte, 1984).

The fact that anal papillae, which are the site of ion uptake in freshwater chironomids (Koch, 1938), are absent or reduced in saltwater species (Neumann, 1976) indicates, at least, that chloride cells alone may not be sufficient for ionic regulation in hypotonic media. The structure of the epidermal cells responsible for the uptake of ions in the anal papillae resembles more closely those found in the 'neck' organ of *Artemia* nauplii than ephemerid chloride cells (Credland, 1975; Conte, 1984). An anatomical and histological study of excretory tissue in *T. barbitarsis* would, therefore, provide a much firmer basis for theories about ion regulation systems in this species.

In the absence of specialized tissue such as that found in the anal papillae of freshwater chironomids it would seem that *T. barbitarsis* would be unable to survive for long periods in dilute media. Its ability to hyperregulate (Fig. 7.1) may therefore be a short-term phenomenon only.

The implication of findings reported here for the control of nuisance *T. barbitarsis* populations is that, where possible, the holding of permanent or semi-permanent water bodies in the salinity range between 35 and 100 g l^{-1} should be avoided. Clearly, *T. barbitarsis* is preadapted to exploit waters in this salinity range where competition for benthic resources (algae and detritus) appears to be reduced. Manipulations of water salinity should aim to concentrate waters to lethal levels above 90 g l^{-1} or to dilute them below 35 g l^{-1} where inceased competition from other organisms will result in reducing the larval population.

More specifically, it would be possible to block the outlet from the system (i.e. the culvert to Hospital Creek (Fig. 2.5)) and allow Salt Lake and Railway Lake to fill. These northern lakes are not presently required for ash disposal. Once they are full of salt water, they can be isolated by the closure of the Main Road culvert between Railway Lake and Bird Lake (Fig. 2.5). In this way, a concentrated brine can be accumulated by evaporating and refilling the lakes. This will be available for backflushing through the system at a time when it will have the maximum effect upon the larval population of T. barbitarsis so as to reduce nuisance adults in summer months.

To check the feasibility of repeated backflushing of the lake system with highly saline water (higher that 200 g l^{-1}), Railway Lake was isolated during early summer of 1984 and the fall in water level was monitored. This rough experiment indicated that it would be possible to back-flush in spring and at least on two subsequent occasions during summer - evaporation rate being the constraint to the rapid accumulation of the strong brine.

It should be borne in mind, however, that the raising of salinity is likely to cause high mortality among the fish, Atherinosoma microstoma. In spring, when it appears that large numbers of juvenile A. microstoma control T. barbitarsis via egg and 1st instar predation, it would be expedient to hold back salinity manipulation measures. If sampling reveals an absence of fish recruitment (as in 1985) then the introduction of the toxic, hypersaline water could proceed.

In this way, a natural control agent can be combined with the application of a freely-available toxin (NaCl) as part of an integrated strategy of pest management.

CHAPTER 8

Attraction to Light

INTRODUCTION

Were it not for a single feature of its behaviour, T. barbitarsis would be regarded as benign or even beneficial at Port Augusta. It is the strong attraction to artificial light which renders the species a serious nuisance: On calm, warm evenings the adults fly towards suburban lights and, being small, they pass through mesh on open windows to enter houses in large numbers. An investigation of this positive phototactic behaviour, therefore, became a logical part of the control-orientated research programme.

The central question of the investigation was to determine whether any particular spectral component of artificial light attracted adult flies. Further, it was important to find out whether the "intensity" of the light played a role in attraction. The question was important because the discovery of a particularly attractive light source would open the way for two relatively cheap and environmentally acceptable possible control methods: first, decoy lights to divert midges from residential areas; second, light traps to intercept midges before they reached the city.

A study of spectral phototaxis in T. barbitarsis requires information from two separate bodies of knowledge, namely on the underlying physiological process involved in colour perception, and, second, of behavioural responses to particular colours. Knowledge of the former does not always lead to increased understanding of the latter. Attraction to monochromatic light is not constant for a given species, but varies with stage of development, sex, foraging behaviour and flight or fight behaviour (Menzel, 1979). For example, bees have been found to be positively phototactic when leaving hives, but negatively so when returning (Von Frisch, 1965), despite having a similar spectral sensitivity at both times (Wendland, 1977).

Since, principally, this study concerns the pragmatic issue of finding one particularly attractive wavelength for control purposes, the underlying principles, although of considerable interest, are of secondary importance. Even so, some background information was essential to effective experimental planning and the interpretation of results. In addition, any understanding of underlying principles will considerably enhance the value of biological information collected for purposes of control (see Chapter 11).

The Nematocera have been largely ignored in studies of insect colour vision. However, work on one nematoceran, *Aedes aegypti*, has shown that the visual pigments in larvae and adults are similar, i.e. Rhodopsin 520/ Metarhodopsin 480 (Brown and White, 1972; Stein *et al.*, 1979). Colour vision in brachycerans such as *Calliphora* and *Musca* is much better studied, probably because these flies are large, and more available and convenient as experimental subjects (e.g. Burkhardt, 1964; McCann and Arnett, 1972).

Insect visual pigments comprise a variety of rhodopsins, each with differing molecular weights and absorbtion spectra (see White, 1985 Table 2). These rhodopsins range in wavelength of maximum absorbance from 345 to 610 nm. In addition to the nature of the visual pigments, there are several other factors which modify the spectral sensitivities of insect receptors. These include tapeta and inteference corneal filters, accessory screening pigments, self-screening by rhodopsin, and electrical coupling of receptor cell systems (Goldsmith and Bernard, 1974; White, 1985).

In general, there appear to be three principal classes of insect photoreceptors, namely, with sensitivity peaks in the ultraviolet (340-360 nm), violet to blue (420-460 nm), and green (490-550 nm) regions of the spectrum (Menzel, 1979). Bernard (1977, 1979) has discovered a fourth set of red receptors (610 nm) in butterflies. The mosquitoes, closest relatives of the Chironomidae that have thus far been studied, appear to possess only one class of receptor, green ones (Goldman, 1971; Brown and White, 1972).

Regarding knowledge of insect phototaxis, i.e. the behavioural aspects, one important generalization can be made: most insects are strongly attracted to ultraviolet light: Bees, flies and other insects appear to be much more sensitive to ultraviolet than to green light. High sensitivity to this part of the spectrum may compensate for the relatively low intensity of ultraviolet radiation in natural light (Laughlin, 1976).

Nuisance chironomids have frequently been reported to show strong attraction to artificial light (e.g. De Meillon and Gray, 1937; Grodhaus, 1963; Hilsenhoff, 1966; Beattie, 1981; Graham and Burns, 1983). The marine species appear to be strongly attracted to light (L. Cheng pers. comm., Morley and Ring, 1972). This positive phototaxis is so pronounced that it led Cheng and Hashimoto (1978) to propose that the widespread dispersal of <u>Pontomyia</u> species throughout the western Pacific was due to their attraction towards the lights of fishing vessels to which they subsequently attached.

Despite the strong movement of chironomids towards artificial light and their consequent nuisance value, there has been but one experimental study regarding the effect of wavelength and light intensity upon phototaxis. Ali _et al_. (1984) presented a range of coloured, incandescent lights to three chironomid species in a darkened room. They found that white lights were preferred over yellow, blue, green, red and orange lights. In addition, they found that, when white lights of varying intensity were offered to the midges, preference was positively correlated with intensity. They concluded that midge species responded more to the quantity (power or intensity) than to the quality (colour or wavelength) of light.

This chapter begins with a pilot experiment to establish an area of spectral preference of _T. barbitarsis_. It goes on to examine experimentally the spectral preference between 400 and 900 nm of the species and the effect of "intensity" upon this preference. Finally, a comparison is made between the attractiveness of the preferred wavelength and white light with a varying ultraviolet component.

MATERIALS, METHODS AND RESULTS

I. Pilot Experiment

Materials and Methods

In order to gain an approximate idea of the region of the spectrum to which _T. barbitarsis_ is attracted, a pilot experiment was conducted involving a set of seven lantern-type light traps constructed from acetate sheeting (Fig. 8.1). The light source, a 50W incandescent globe, was masked with a coloured theatrical gel. These gels were chosen to represent seven colours: violet, indigo, blue, green yellow orange and red. Figure 8.2 gives the spectral transmission of these filters. Although none was monochromatic, it was assumed that an attractive region could be identified from trapping results.

The seven traps were set out on calm evenings, each in a separate stall at the edge of Bird Lake. The stalls allowed for a screening between each trap and also, since the stalls could only be approached from the front, a reasonably equal choice between the lights for approaching _T. barbitarsis_.

Trapping was conducted on three nights for at least 4 hours on each occasion. Adult flies, which were trapped in the glycerol in the jar at the bottom of each trap (Fig. 8.1), were counted on the following day in the laboratory.

Results and Conclusions

After three nights of trapping, it became evident that the use of "impure" light sources would not yield the pattern of colour preference in _T. barbitarsis_; it was clear that the question would only be answered by using monochromatic filters. Table 8.1 sets out the results of the pilot investigation.

A one-way analysis of variance yielded an $F_{(6,\ 14)} = 1.55$ which was not significant. This indicated that _T. barbitarsis_ showed no particular preference for any single filter.

This pilot investigation is documented rather fully because it gave an unexpected result which could have some significance in terms of life history: On trap nights 1 ($t = 3.885$) and 3 ($t = 2.445$), there was a significant difference between the numbers of male and female _T. barbitarsis_ trapped. The imbalance was not always skewed in one direction; on night 1 the males outnumbered the females (83 to 17%), whereas on night 3 the females outnumbered the males (88 to 12%).

Figure 8.1 Photograph of lantern trap used in pilot investigation
Scale: 100 mm

Figure 8.2 Spectral transmission ranges of the seven theatrical gels used in the pilot investigation

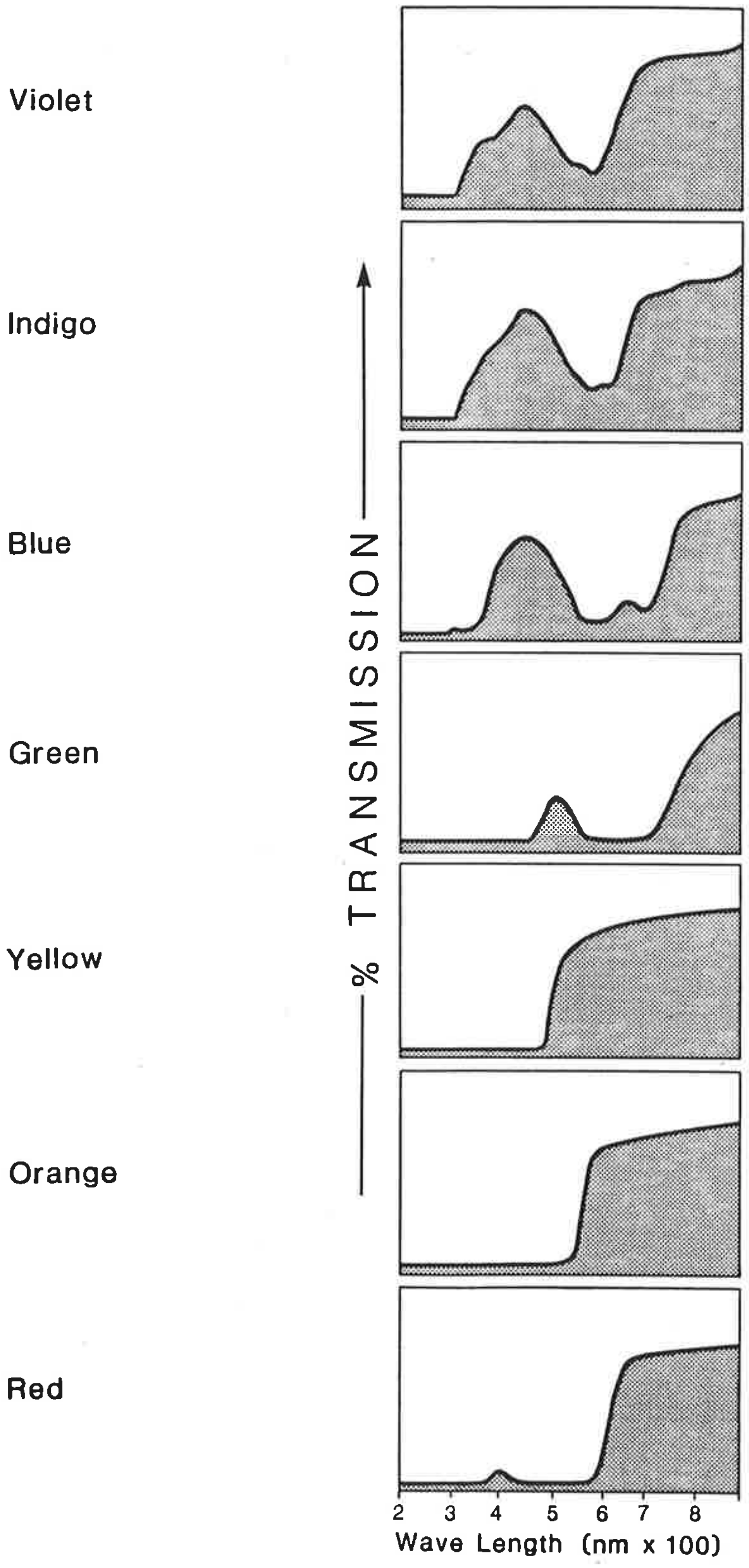


Table 8.1 Results of pilot investigation with theatrical gels.

	Trap Night 1			Trap night 2			Trap night 3		
Trap	Females	Males	Total	Females	Males	Total	Females	Males	Total
Violet	8	40	48	15	9	24	372	41	413
Indigo	12	131	143	13	11	24	310	44	352
Blue	-	-	-	64	5	69	97	11	108
Green	5	80	85	27	12	39	36	4	40
Yellow	30	94	124	31	6	37	474	72	546
Orange	25	59	84	11	5	16	82	7	89
Red	9	38	47	4	0	4	13	2	15
TOTALS	89	442	531	165	48	213	1384	181	1563
%	17%	83%		77%	23%		88%	12%	

II. Spectral Preference Experiments

The apparatus and its calibration

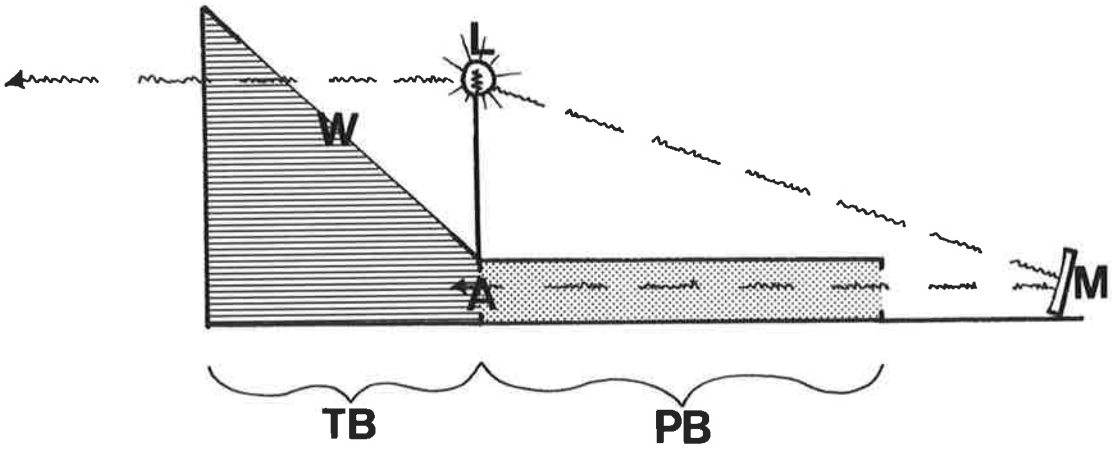
Following the pilot investigation, a light trap apparatus was constructed which provided for the production of monochromatic light (Fig. 8.3). Essentially, it consisted of two elements: a) A Trap Box into which midges were attracted from the lake surrounds; b) A Preference Box which presented midges with seven different choices without directional bias. The two units were connected by a single slot-aperture so that midges which had been attracted into the Trap Box were forced to enter the Preference Box by this aperture (Fig. 8.3).

In order to avoid the problems of heat and varying intensity from the use of several light sources, a single, 100W tungsten/halogen globe was reflected off seven concave mirrors focussed on the aperture through circular holes (50 mm diameter) at the front of each of the seven partitions of the Preference Box. To midges entering the Preference Box at the slot-aperture, the whole mirror surface would appear to glow at the same intensity as the filament.

The experimental procedure was as follows: Light from the tungsten globe was allowed to pass out of the Trap Box by removing an opaque slider over a perspex window (Fig. 8.3). Once several hundred midges had been attracted into the trap box, the slider was replaced, blocking out the white light source. The only visible light in the Trap Box then emanated from the slot-aperture connecting it to the Preference Box. The midges then flew to the slot-aperture and there were presented with an array of lights in the Preference Box. Those individuals which were trapped in the various partitions of the Preference Box were counted through a transparent window from above after the insertion of a plastic slide to prevent their escape.

a) Calibration of the light trap and the filters

In order to ensure that the various light options presented to the midges had the same "intensity", both the light trap and the filters had to be calibrated. "Intensity" here is taken to mean irradiance (E) i.e. a measure of the energy which falls upon a planar surface (Watts per square metre). Thus, the irradiance is the amount of energy falling on a unit area of surface, regardless of how it is perceived.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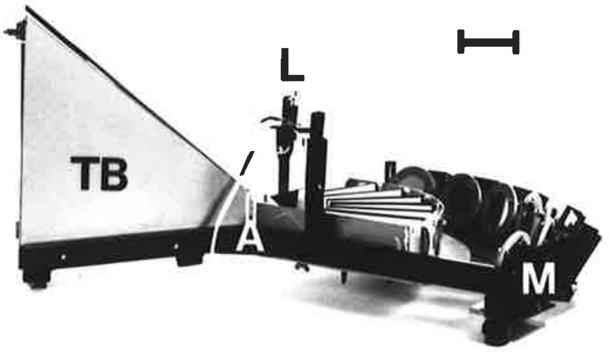
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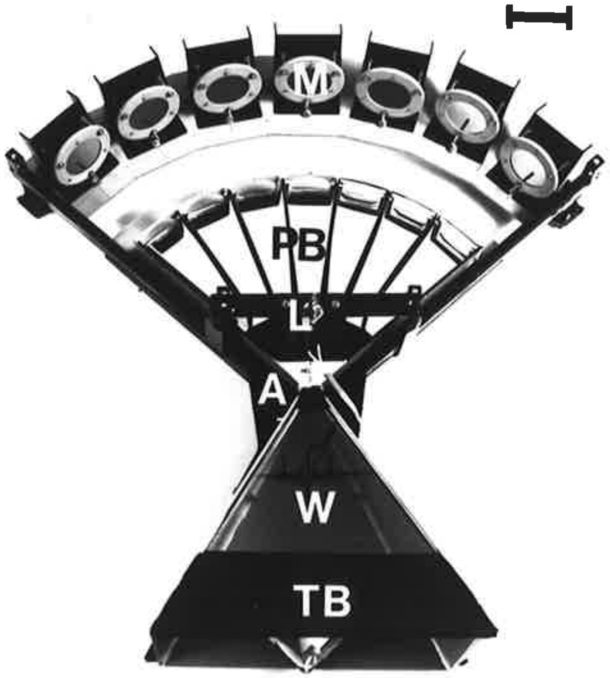
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i) Calibrating the 7 compartments of the Preference Box

Because of inconsistencies in the mirror surfaces and the varying position of each compartment with regard to the filament, there was a variation in irradiance received by the different compartments of the Preference Box. Using a UDT light measuring instrument, fitted with a radiometric filter with a flat response (±7%) from 450 to 950 nm , the irradiance was recorded at each of the 7 positions (compartments of the Preference Box). Having recorded these values, which ranged from 10.28 to 13.54 (arbitrary units), different neutral density filters (Kodak #96) were placed between the mirror and the sensor until all positions received equal irradiance (Table 8.2).

ii) Calibrating the Kodak Wratten filters

A series of Kodak Wratten filters each of which transmits light over a narrow wave-length range (Figs 8.4 & 8.5) (apart from the opaque and infra-red filters), were placed over the circular apertures at the front of the Preference Box. Each of these filters transmits different amounts of energy (Fig. 8.4). Calibration was necessary to equalize the irradiance of all filters used for spectral preference experiments.

The light measuring instrument (in the same configuration as above) was used to measure the irradiance of light transmitted through each filter. These values ranged from 6.81 to 9.84 (arbitrary units). Neutral density filters were then added to reduce all filters to the same "intensity" (Table 8.2).

Figure 8.4 The spectral transmission ranges of the various Kodak Wratten filters used in phototaxis experiments of *T. barbitarsis*

Colour	Kodak Wratten Filter Number
Blue	50
Blue	94
Blue-green	45
Dark-green	74
Green	93
Yellow-green	73
Orange	72B
Red	70
Infra-red	88A

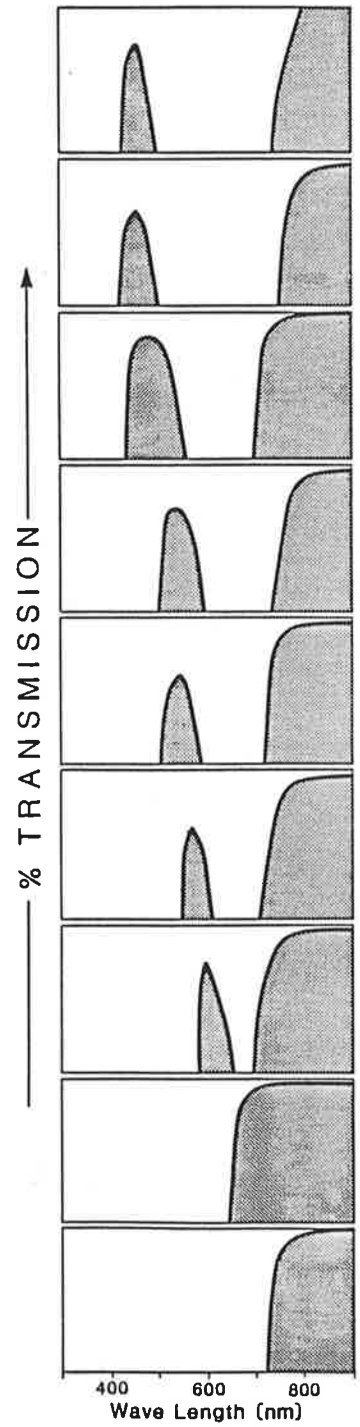


Figure 8.5 The limits of transmission for the Kodak Wratten filters.

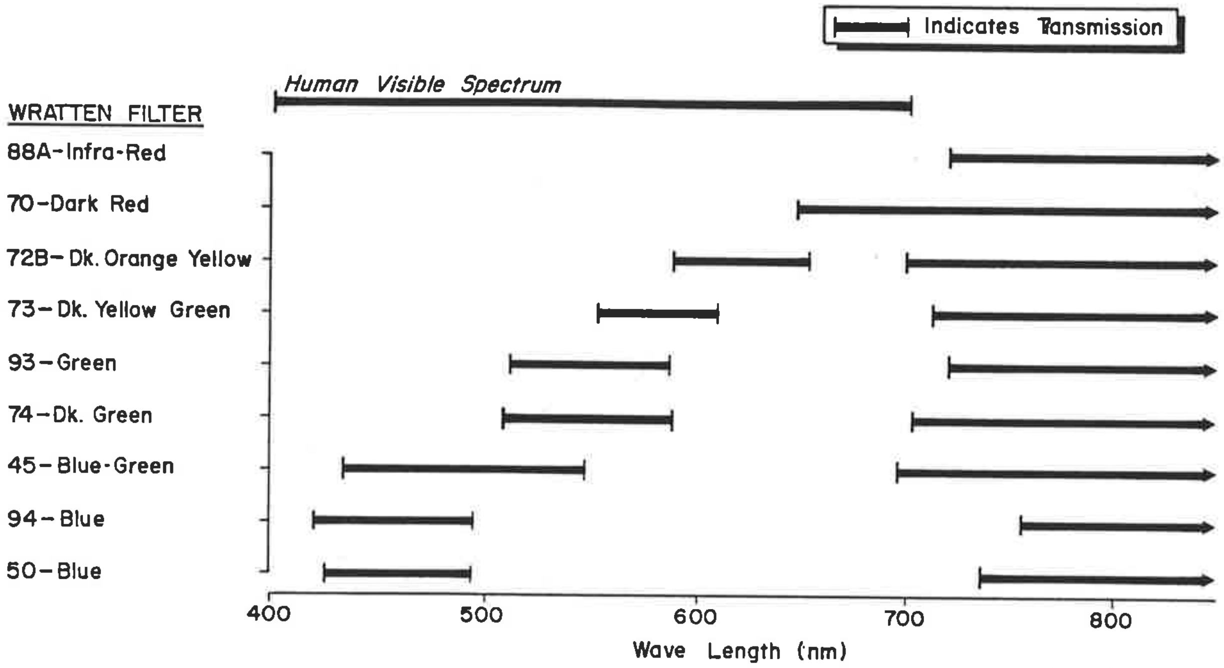


Table 8.2 Calibration for Irradiance

Preference Box		Wratten Filters	
Position No.	N.D. Filter required	Wratten filter No.	N.D. Filter required
1	0.1	50 Blue	none
2	0.2	94 Blue	none
3	0.1	45 Blue-Green	0.1
4	0.2	74 Dark Green	none
5	0.1	93 Green	none
6	none	73 Yellow-Green	none
7	0.1	72B Orange	0.1
		70 Red	0.2
		88A Infra-Red	0.1

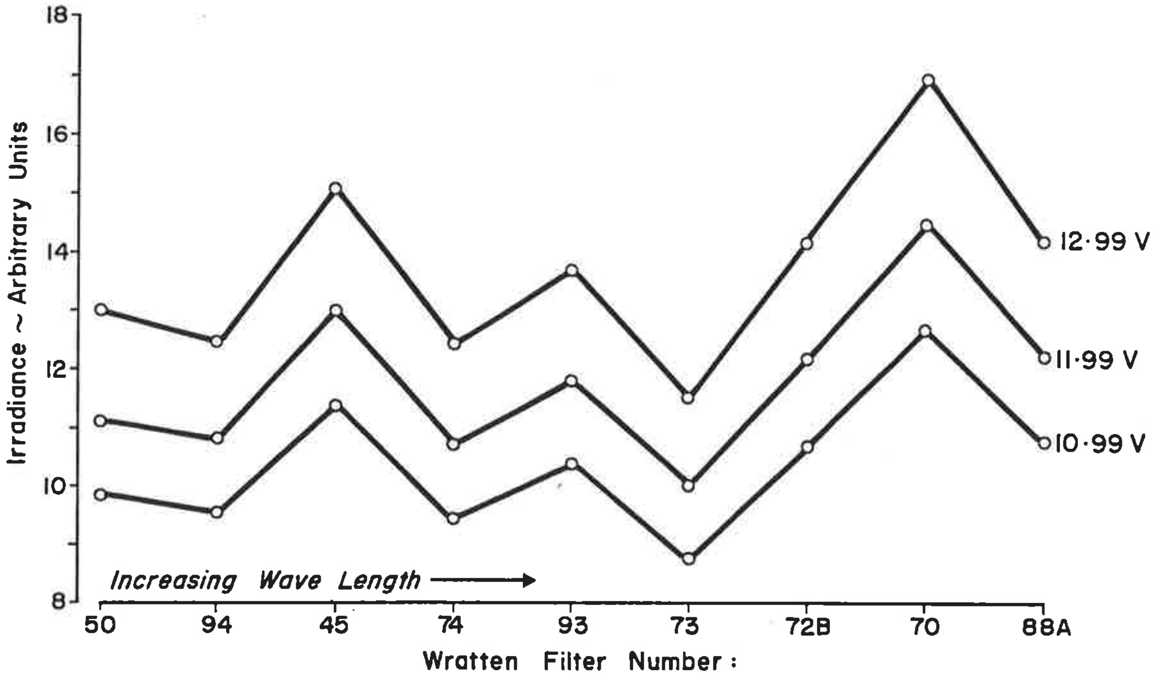
b) Colour temperature

A further concern, bearing in mind that voltages are likely to vary when running the light source off a generator in the field, was the possibility of an uneven change in irradiance across the spectrum with changing filament temperature; i.e. the higher the filament temperature, the more short wavelength radiation is emitted.

To investigate such variation, the irradiance of all filters was measured at three different voltages. These were plotted (Fig. 8.6) to check for non-systematic variation.

Results indicated that the variation in irradiance between 10.99 and 12.99V was remarkably consistent (Fig. 8.6). It was therefore possible to assume that, if the globe were operated between 10.99 and 12.99 volts, non-systematic variation in irradiance of the different filters would not be a problem.

Figure 8.6 The variation of irradiance with voltage



The Experiments

A series of coloured filters was used in each experiment; some blanks constructed from opaque, black paper were always included. Between each run the position of the filters with respect to one another and to the Preference Box was changed. In this way experimental errors would be randomized.

Voltage was monitored by connecting a portable voltmeter across the terminals of the globe.

Five spectral preference experiments were conducted, each following logically from the results of its predecessor.

Although it was initially assumed that the light trap could be used under any weather conditions, this proved not to be the case. In the presence of a slight breeze (more than 2 knots), midges will not leave their roosting positions and fly towards a light source. Consequently, the light trapping experiments could only be conducted on calm evenings. These, surprisingly, are rather rare at Port Augusta. For example, in the period, July 20 to December 4, 1985, trapping was possible on only 5 nights and indeed, the number of nuisance nights reflects this.

Experiment 1: The spectral preference of *T. barbitarsis* between 400 and 900 nm.

The following non-overlapping Wratten filters were used in a series of 12 preference trials: #94 - Blue (420 - 490 nm), #74 - Dark green (510 - 590 nm), #72B - Orange-yellow (590 - 655 nm), #70 - Red (645 - into infra-red), #88A - infra-red (720 nm into infra-red) Refer to Figs 8.4 and 8.5 for ranges of transmission.

Table 8.3 presents results of experiments conducted with five non-overlapping filters ranging from the blue to the infra-red region of the spectrum.

Table 8.3 Spectral preference between 400 and 900 nm

No. = Number of midges caught in the partition
% = Percentage of the midges caught in that "run"

Blue #94		Dark Green #74		Orange-Yellow #72B		Red #70		Infra-Red #88A		Blank		Blank	
No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
2	11	12	63	3	19	0	0	1	5	1	5	0	0
8	13	32	50	12	19	2	3	3	5	2	3	5	8
4	20	9	45	0	0	1	5	4	20	2	10	0	0
2	8	15	60	2	8	1	4	3	12	1	4	1	4
12	39	8	26	1	3	1	3	4	13	4	13	1	3
2	22	5	56	0	0	2	22	0	0	0	0	0	0
2	8	9	36	4	16	3	12	6	24	1	4	0	0
12	18	26	39	8	12	11	17	2	3	4	6	3	5
4	14	11	38	3	10	3	10	2	7	3	10	3	10
2	10	12	60	2	10	1	5	1	5	2	10	0	0
2	20	3	30	2	20	0	0	1	10	1	10	1	10
2	9	11	48	5	22	2	9	3	13	0	0	0	0
Total													
54		153		42		27		30		21		14	
Mean													
4.5	16%	12.8	45%	3.5	12%	2.3	8%	2.5	9%	1.8	6%	1.2	4%

An analysis of variance yielded an $F_{(6,\ 77)}$ value of 25.2 which was highly significant (F.01 = 3.12). In order to discover where significant differences in preference for different filters occurred, an unplanned pairwise comparison of means was conducted. The minimum significant difference (MSD) was compared with a critical value to determine whether that particular pair of means was statistically different from one another (Sokal and Rohlf, 1981). This showed the following: Dark-green (#74) was preferred above all other filters in the experiment. Blue was preferred to the blanks. There was no sign of preference between the other treatments including the blanks.

In all trial results the percentages were transformed using (arc sin $\sqrt{\text{proportion}}$) to remove unacceptably high levels of heterogeneity (Cochran's test for heterogeneity).

From the results of this first experiment, it may be concluded from positive phototactic behaviour that the strongest attraction of _T. barbitarsis_ is to the region of the spectrum between 510 and 590 nm. _T. barbitarsis_ did not distinguish between infra-red filters and blanks suggesting an inability to percieve infra-red.

Experiment 2: The relative attraction of _T. barbitarsis_ to green (490 to 510 nm) and white light of equal intensity?

In order to equalize irradiance between the two options, measurement with the UDT instrument indicated that the addition of a 0.5 N.D. Filter would reduce the white light light to the level of #74 - dark green filter. In this case, the two filters were used with 5 blanks and their positions changed as before.

Table 8.4 presents results of trials conducted with white light and #74 - dark green light with equal irradiance.

Table 8.4 Preference between #74 - dark green and white light of the same intensity.

White		Dark-green		Blank		Blank		Blank		Blank		Blank	
No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
33	87	4	11	0	0	1	2	0	0	0	0	0	0
14	88	1	6	0	0	0	0	0	0	0	0	1	6
9	70	0	0	0	0	2	15	2	15	0	0	0	0
55	93	4	7	0	0	0	0	0	0	0	0	0	0
68	91	3	4	0	0	0	0	1	1	3	4	0	0
Totals													
179		12		0		3		3		3		1	
Means													
35.8	89%	2.4	6%	0	0%	0.6	1%	0.6	1%	0.6	1%	0.2	5%

This experiment showed that white light was clearly more attractive than #74 - dark green or any of the blanks.

Experiment 3: To determine whether there is there a narrow band within the blue to green region (420 to 610 nm) of the spectrum which is particularly attractive to *T. barbitarsis*? The experiment was designed to indicate if there is an attractive subregion within the range of #74 - dark green

The following Wratten filters were used: #94 - Blue (420 - 490 nm), #50 - Blue (425-490 nm), #45 - Blue-green (434-550 nm), #73 - Dark yellow-green (555-610 nm) (refer to Figs 8.4 and 8.5).

Table 8.5 presents results of experiments conducted with five filters within the blue to green range of the spectrum.

Table 8.5 Preference within the blue to green region of the spectrum (420 to 610 nm).

Blank		Blank		Blue #94		Blue #50		Blue-green #45		Dark green #74		Yellow-green #73	
No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
9	6	5	3	46	28	9	6	46	28	32	20	16	10
5	6	4	5	14	18	10	13	23	30	12	16	9	12
6	3	2	1	46	22	33	15	61	29	42	20	23	11
2	4	0	0	12	21	10	18	13	23	13	23	7	12
1	8	0	0	0	0	3	25	3	25	3	25	2	17
0	0	0	0	3	18	1	6	11	65	0	0	2	12
0	0	0	0	0	0	1	9	4	36	5	45	1	9
0	0	0	0	1	25	0	0	2	50	0	0	1	25
Totals													
23		11		122		67		163		107		61	
MEANS													
2.9	4%	1.4	2%	15.2	22%	8.4	12%	20.4	29%	13.4	19%	7.6	11%

A one way analysis of variance of the transformed percentages yielded $F_{(6,49)} = 9.8513$ which was highly significant at the .05 level. A pairwise multiple comparison of means (Sokal and Rohlf, 1981) yielded a complex pattern of differences between filters. One filter, however, gave results which showed it to be preferred above all other choices, the #45 - blue-green.

By a process of deduction, using the spectral transmission ranges of the filters (see Fig. 8.5), it was concluded that wavelengths between 490 and 510 nm constituted the particularly attractive region.

Experiment 4: The intensity of the attractive wavelength (490 to 510 nm) with respect to the phototactic response in *T. barbitarsis*

Five, identical #45 - Blue-green Wratten filters had the levels of transmission altered by the addition of neutral density filters as follows (in descending order): No.1 (no N.D. filter), No.2 (N.D. 0.2), No.3 (N.D. 0.5), No.4 (N.D. 1.0), No.5 (N.D. 1.6).

Table 8.6 presents results of experiments conducted with five identical blue-green filters, each masked with a different N.D. filter to reduce irradiance.

Table 8.6 Attraction to #45 - blue-green filters of varying intensity.

Blank		Blank		No.1		No.2		No.3		No.4		No.5	
No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
0	0	0	0	1	33	0	0	1	33	0	0	1	33
0	0	1	4	5	19	9	37	3	12	2	8	6	23
0	0	0	0	2	33	4	67	0	0	0	0	0	0
0	0	0	0	1	6	7	44	3	19	3	19	2	13
0	0	0	0	1	20	0	0	0	0	2	40	2	40
0	0	0	0	6	38	3	19	2	13	2	13	3	19
0	0	0	0	4	27	1	7	7	47	2	13	1	7
0	0	0	0	0	0	0	0	0	0	3	60	2	40
TOTALS													
0		1		20		24		16		14		17	
MEANS													
0	0%	0.1	1%	2.5	22%	3.0	26%	2.	17%	1.8	16%	2.1	18%

A one way analysis of variance (excluding blanks, which were obviously not attractive) yielded $F_{(4,\ 35)} = 0.4254$ which was not significant.

From the experiment it can be concluded that the intensity of the preferred wavelength plays no role in the phototactic response of T. barbitarsis.

Experiment 5: Attraction within the ultraviolet region.

The experiment was designed to address two questions: Since T. barbitarsis appears to prefer white light to the green region of the spectrum (Experiment 2), is the region of attraction in the far-U.V. (300 to 370 nm) or in the near U.V. (370 to 400 nm)? Secondly, does the brightness of the ultraviolet light play a role in phototaxis?

The following choices were given in this experiment: Blank, A. - U.V. screen plastic, B. - U.V. screen plastic + 0.3 N.D. filter, C. - 2 mm soda glass, D. - 2 mm soda glass + 0.4 N.D. filter, E. - 0.5 N.D. filter only, F. - #45 - blue-green.

For spectral transmission of glass and U.V. screen plastic refer to Fig. 8.7.

Table 8.7 gives the results of preference experiments with far-U.V. white, near-U.V. white and blue-green filters.

Table 8.7 Attraction to #45 - blue-green and white light of varying U.V. content.

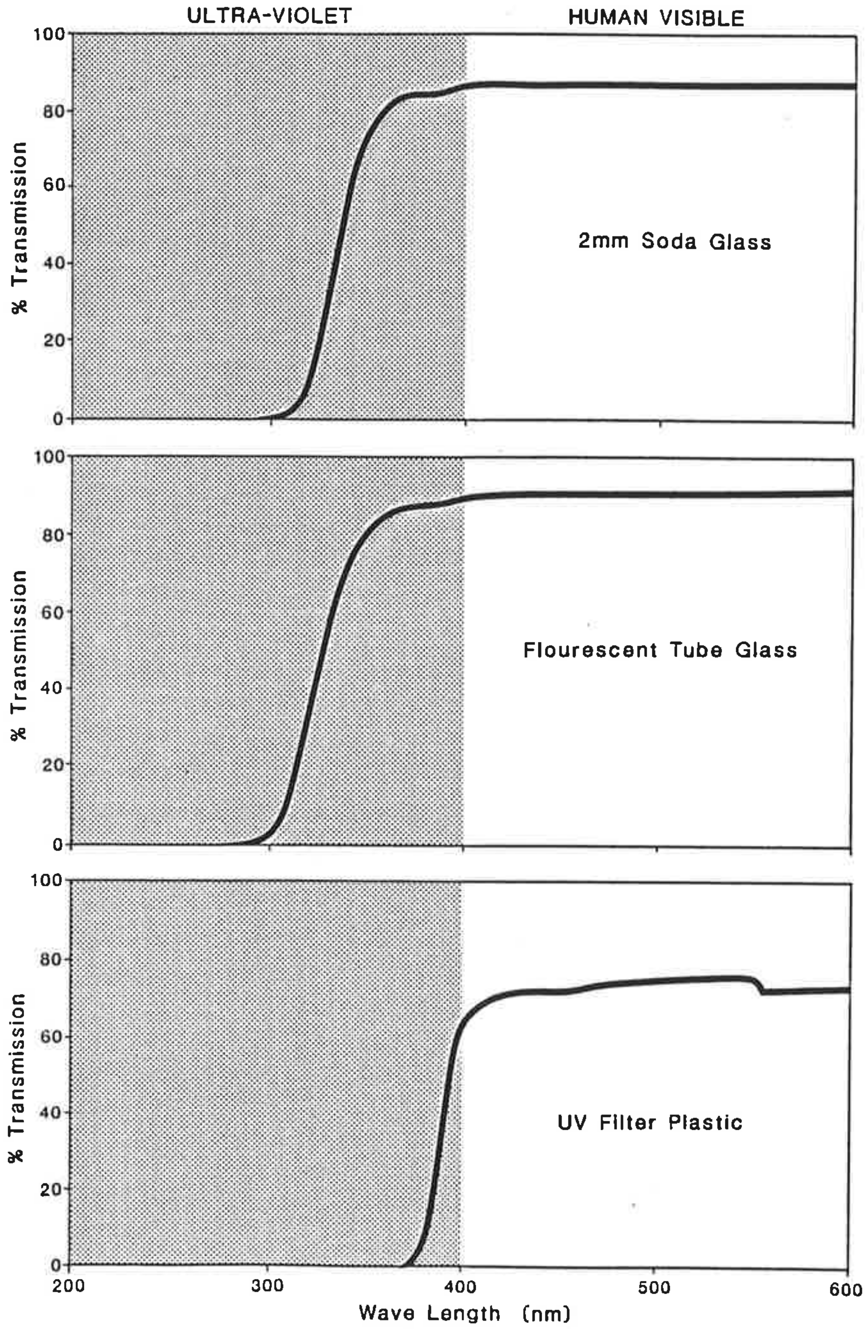
Blank
A. - U.V. screen plastic
B. - U.V. screen plastic + 0.3 N.D. filter
C. - 2 mm soda glass
D. - 2 mm soda glass + 0.4 N.D. filter
E. - 0.5 N.D. filter only
F. - #45 - blue-green

Blank		A.		B.		C.		D.		E.		F.	
No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
0	0	12	24	6	12	4	8	11	22	6	12	10	20
3	10	10	32	4	13	1	3	7	23	6	19	0	0
1	3	5	15	4	12	6	18	9	27	7	21	1	3
0	0	3	15	5	25	8	40	2	10	1	5	1	5
0	0	7	19	10	28	8	22	5	14	3	8	3	8
2	6	4	11	8	22	5	14	2	6	10	28	5	14
0	0	14	30	7	15	8	17	9	19	4	9	5	11
1	4	7	25	6	21	3	11	2	7	3	11	6	21
0	0	3	33	2	22	2	22	0	0	2	22	0	0
TOTALS													
7		65		52		45		47		42		31	
MEANS													
0.8	2%	7.2	22%	5.8	18%	5.0	16%	5.2	16%	4.7	15%	3.4	11%

A one way analysis of variance (excluding the blank) yielded $F_{(4,\ 35)} = 3.0988$ which was significant. This indicated that there was differential preference between the treatments. A multiple pairwise comparison of means (Sokal and Rohlf, 1981) indicated that only one pair was significantly different from one another i.e. the U.V. screen was preferred to #45 - blue-green.

From this experiment it is clear that the attractive peak is in the near-U.V. region. The intensity of the light plays no role in phototaxis.

Figure 8.7 Transmission of 2 mm soda glass, glass from a fluorescent tube (Philips TLD 36W/33 White) and ultraviolet filter plastic.



DISCUSSION

Although it was not possible to identify a restricted and attractive region of the spectrum from the preliminary light trapping, the exercise revealed an interesting phenomenon: there is a differential trap rate for male and female *T. barbitarsis* at different times. When a correlation was sought between the lunar cycle and trap success, the limited data were inadequate for such an analysis. It is not known, therefore, whether this pattern reflects differential abundance of the sexes or their differential attraction to light.

Other observations, however, support the proposal that it is the result of some life history-related behavioural trait. For example, it has been noted that 90% of *Culicoides sanguisuga* (Ceratopogonidae) caught in light traps are non-gravid females (Belton and Pucat, 1967). In addition, Barr *et al*. (1960) observed that *Culex pipiens quinquefasciata* females were not attracted to light on moonlight nights.

Further investigation of this phenomenon may not only lead to a better understanding of *T. barbitarsis* swarming behaviour, but provide new avenues for control in the future.

The spectral preference experiments were able, by a process of elimination (and deduction), to identify specific areas of the electromagnetic spectrum which are particularly attractive to *T. barbitarsis*.

An initial major concern was that *T. barbitarsis* would be attracted to light in the infra-red range (longer than 700 nm). Some work on the Culicidae, for example, suggests that this family can perceive infra-red radiation (Callahan, 1965; Magnum and Callahan, 1968). However, this is contradicted by the findings of Peterson and Brown (1951) who concluded that mosquitoes were able to follow conducted heat, but not infra-red radiation. Goldsmith and Bernard (1974) stated that, in their judgement, reports of insect eyes acting as infra-red detectors were not supported by adequate evidence.

The concern about the ability of *T. barbitarsis* to perceive infra-red radiation sprang from the fact that all the Kodak Wratten filters used, although monochromatic between 400 and 700 nm, transmitted infra-red radiation (see Fig. 8.4). If *T. barbitarsis* could perceive and was attracted to infra-red radiation, then the Wratten filters would not have been suitable for this work.

Results from Experiment 1, while not indicating whether *T. barbitarsis* has the ability to perceive infra-red radiation, demonstrated that the phototactic response which it elicited, was not different from blank (opaque) filters. This result indicated that it was the particular monochromatic regions of the filters between 400 and 700 nm which were the source of the phototactic stimulus for *T. barbitarsis*.

The initial experiment (No. 1) identified the green region (510-590 nm) of the spectrum as being particularly attractive to *T. barbitarsis* (here it should be pointed out that reference to spectral colours relates only to human perception). At this stage, preference was tested between the green Wratten filter (#74, 510-590 nm) and a white light of equal intensity. Results (Experiment 2) gave a strong indication that the white light was more attractive.

Logically, it was considered that if a particularly attractive subregion within the green (510-590 nm) wavelengths could be isolated, then perhaps it would prove more attractive than white light of equal intensity. When presented with a series of different blue and green filters (Experiment 3), one did indeed prove more attractive to

T. barbitarsis than all others, i.e. the blue-green (#45, 430-550 nm). The blue-green filter (#45) has only one small region of the spectrum which is not represented in other "adjacent" filters (see Fig. 8.5) used in Experiment 3. It was proposed, therefore, that this region (490-510 nm) constituted the particularly attractive part of the green wavelengths.

A further preference experiment was conducted to determine whether the intensity of the particular wavelength played a role in attraction. The results (Experiment 4) indicated that the intensity of the attractive wavelengths (490-510 nm) did not increase the phototactic response.

By this point in the programme of spectral preference experiments, it had been established that there was only one region within the human visible spectrum (400-700 nm) to which T. barbitarsis was strongly attracted, viz. the blue-green (490-510 nm). Preference for white light over the blue-green would indicate a stronger attraction to ultraviolet radiation. The final experiment (No. 5), therefore, used a plastic ultraviolet screen which cut out wavelengths below 370 nm (Fig. 8.7), 2 mm soda glass which cut out wavelengths below 300 nm and the blue-green filter (Wratten #45) in an experiment to determine whether the region of strongest attraction for T. barbitarsis was the blue-green (490-510 nm), the near-ultraviolet (370-400 nm) or the far-ultraviolet (300-370 nm). In addition, the white light filters were presented as two different intensities (see Experiment 5).

Results (Experiment 5) indicated that it was the near-ultraviolet region (370-400 nm) which was marginally more attractive to T. barbitarsis. As in Experiment 4, the intensity of the light played no role in the phototactic response.

How do these results agree with previous work? Indeed, reference to several comprehensive reviews (Goldsmith, 1961; Burkhardt, 1964; Goldsmith and Bernard, 1974; Menzel, 1979; White, 1985) revealed that, in general, for the Diptera, there are two major peaks of sensitivity: one in the ultraviolet (350-360 nm) and one in the blue-green (480-500 nm).

Conclusions drawn from this work differ in one major respect from results obtained with other Diptera (mainly Brachycera). Whereas the major peak of ultraviolet sensitivity in other dipterans so far studied is around 350 nm (for example Musca and Calliphora (McCann and Arnett, 1972)), T. barbitarsis appears to have its sensitivity peak between 370 and 400 nm.

The rather weak preference shown by T. barbitarsis for near-ultraviolet (370-400 nm) light over blue-green (490-510 nm) is consistent with data collected for higher Diptera. It seems that spectral sensitivity may depend upon the intensity of the stimulus as well as the state of adaptation of the eye (i.e. light or dark). Consequently, some authors report a stronger attraction to blue-green while others report the ultraviolet as being more attractive (see Goldsmith and Bernard, 1974 for discussion). In Drosophila, for example, at low light intensities there are two preference peaks of nearly equal height at 360 and 500 nm. However, at higher intensities the peaks shift to 350 and 480 nm and the ultraviolet sensitivity is about 45 times the green sensitivity (Schumperli, 1973).

Heisenberg and Buchner (1977) demonstrated phototactic behaviour in D. melanogaster which corresponds to the patterns observed for T. barbitarsis at Port Augusta. Drosophila in a chamber containing two compartments move towards the half illuminated with green light. If an equal ultraviolet illumination is superimposed on both compartments, the flies leave the area illuminated with the green light and move to the area solely lit by ultraviolet light. This behaviour was not affected by increasing the intensity of the green light.

There are several records of strong phototactic behaviour of chironomids toward ultraviolet light. For example, Frost (1957) found that, while mosquitoes and tipulids were caught in much larger numbers by white light traps, chironomids were trapped more efficiently by a "black light" (ultraviolet). Belton and Pucat (1967), on the other hand, found that white light and ultraviolet light traps were equally efficient in trapping Chironomidae. Despite such discrepancies, it is widely accepted that ultraviolet light will attract chironomids and it is routinely used for their capture (for example, Tait-Bowman, 1980).

If _T. barbitarsis_ is strongly attracted to near-ultraviolet light (370-400 nm), as this work suggests, why then do they move towards domestic and street lights in Port Augusta? In fact, specifications given for both common tungsten filament incandescent lights and fluorescent tubes confirm that they emit near-ultraviolet light (Kaufmann, 1984). A test on glass taken from a Philips white fluorescent tube indicated its permeability to wavelengths down to 350 nm (Fig. 8.7). Presumably, as in many insect species (Menzel, 1979), _T. barbitarsis_ has heightened sensitivity to ultraviolet light and displays a strong phototactic response to even the weak stimuli emitted by common suburban light sources.

The biological significance of the strong positive phototaxis of insects to ultraviolet light, although subject to speculation, remains unexplained. The proposal put forward by Mazokhin-Porshnyakow (1969) that ultraviolet light signifies "open space" so that the phototactic response represents an escape behaviour, is frequently quoted as being the most plausible (e.g. Menzel, 1979; White, 1985).

The results of spectral preference experiments on *T. barbitarsis* stand in contrast to those reported by Ali *et al*. (1984), the only other such study on chironomids. They did not report the spectral qualities of the incandescent light bulbs which they used and it is, therefore, difficult to comment upon the preferences shown by the chironomids involved in their study. Their conclusion that it is the intensity of light which affects the extent of the phototactic response may be explained in two ways. Firstly, there is supporting evidence which indicates that, indeed, the intensity of light *does* play a role in the attraction of insects, for example in mosquitoes (Barr *et al*., 1960). However, evidence to the contrary is equally common (e.g. Heisenberg and Buchner, 1977; Harris and Miller, 1983). Secondly, the phototactic response observed by Ali *et al*. (1984) may be explained by the fact that, as the intensity of a white, incandescent bulb increases (e.g. 25 to 100W) so do emissions extend into the ultraviolet region of the electromagnetic spectrum (or, at least, the magnitude of emissions increases). The phototaxis towards "brighter" lights may simply signify a preference for a particular ultraviolet wavelength which was not emitted by the other "cooler" filaments.

The results reported here make it possible to specify the sorts of light sources which will be particularly effective as trap lights or decoys at Port Augusta. Ideally, the lights should be commercially available, but further work will indicate whether they will have to be specially constructed. The trap lights should emit strongly in the blue-green (490-510 nm) and near-ultraviolet (370-400 nm) regions of the spectrum.

An examination of published spectral power distribution curves for a range of light sources reveals that a Clear Mercury (5900K) high intensity discharge lamp emits two monochromatic bands within the near-ultraviolet i.e. 345 to 360 nm and 385 to 405 nm (Kaufman, 1984). When a source of blue-green (circa 500 nm) light was sought, it was discovered that few commercially-available lights emit strong peaks in this region of the spectrum. The General Electric SPX 27 fluorescent lamp (General Electric, 1985) and the High Pressure Sodium Lamp, both of which have emission peaks in the blue-green, are the most suitable sources of 490 to 510 nm radiation.

It would be possible, therefore, to conduct field trials with the abovementioned commercial light sources in order to establish the feasibility of using them to mitigate chironomid problems at Port Augusta.

In addition, further experiments are required to investigate a series of questions raised by this work. For example:- Can the region of peak attraction within the ultraviolet be defined more closely? What is the effect of polarized light upon phototaxis? Will further, more rigorous work with intensity confirm its lack of influence in phototaxis? How do dark-adapted *T. barbitarsis* and light adapted *T. barbitarsis* differ in their spectral preference?

This work has exploited fully the possibilities of field experimentation and it is likely that further progress will only be made under laboratory conditions where light sources can be controlled more rigorously. However, laboratory oriented work will be hampered by the problems of acquiring the appropriate *T. barbitarsis* adults to act as subjects.

In summary, it can be stated that this research, one of only two such studies on chironomids, has revealed that *T. barbitarsis* has two regions of preference in the electromagnetic spectrum, one between 370 and 400 nm and the other between 490 and 510 nm. The fact that the species is more strongly attracted to the ultraviolet peak (370-400 nm) provides an explanation for its movement towards suburban lights which emit radiation in this region. It is suggested that commercially-available lamps such as Clear Mercury, General Electric SPX 27 and Sodium High Pressure, which emit strong peaks in the preferred regions, be used in pilot experiments to attract *T. barbitarsis* away from suburban Port Augusta on potentially problematic evenings.

CHAPTER 9

A Viral Infection

INTRODUCTION

Early during the larval sampling programme at Port Augusta, it was noticed that approximately 1% of individuals, usually 4th instars, were iridescent green instead of the more usual russet brown colour. No immediate explanation of this phenomenon was available and it was first thought to have been either the effect of copper ions from the power station boilers or caused by an infection.

The matter did not warrant serious attention because it had so little impact upon the population as a whole. However, further information about the iridescent green larvae emerged when C.M. Manwell, at my request, compared enzyme patterns of green individuals with "normal" larvae using gel electrophoresis. It was discovered that several major enzyme loci were absent in the green larvae; this absence confirmed that they were diseased.

Further microscopial observations showed that in infected larvae the green colouration appeared to be localized in the fat bodies (lateral, longitudinal structures stretching along the length of the body).

No further interest in the matter was taken until samples from another population of *T. barbitarsis* revealed the same infection. It then seemed worthwhile to identify the pathogen. This second infected population was found in Lake Werowrap, a maar lake in western Victoria (see Walker, 1973). Here, it was estimated from semi-quantitative sampling that approximately 10% of the population was infected with the green pathogen. In addition, it was evident from their behaviour that the green larvae were diseased; they floated passively in the water column, moving sluggishly.

This chapter briefly describes work which identified the pathogen and discusses its potential as a control agent for *T. barbitarsis* as well as for other problem arthropods.

MATERIALS AND METHODS

Several iridescent green *T. barbitarsis* larvae were fixed in 3% glutaraldehyde for 2 hours at 4°C. Subsequently, the animals were cut into short segments (approx. 2mm) longitudinally, and washed three times (3 X 10 min.) in Sorenson's buffer at 4°C. Tissue was then transferred to a 1% osmium tetroxide solution for 1 hour at 4°C to effect secondary fixation.

Dehydration of the tissue pieces was undertaken through an ethanol series using 10 min. immersion in each of the following concentrations: 30%, 50%, 70%, 90%, 95%, 100%, 100%, 100%.

The tissue was then transferred to Spurr's standard embedding medium (firm) via increasing concentrations of propylene oxide i.e. 100% propylene oxide, 75:25 propylene oxide - Spurr's, 50:50, 25:75, 100% Spurr's.

Infiltration of the tissue with Spurr's medium was effected over 24 hours under a vacuum with the solution being changed at the 12 hour interval. Embedding took place over 48 hours at 70°C.

Resin blocks containing tissue were trimmed manually and sectioned on glass knives in a Reichert OM3 ultramicrotome to a thickness of between 50 and 60 nm. The tissue was stained with uranyl acetate and lead citrate and viewed in a JEOL 100S transmission electron microscope. Representative sections were photographed.

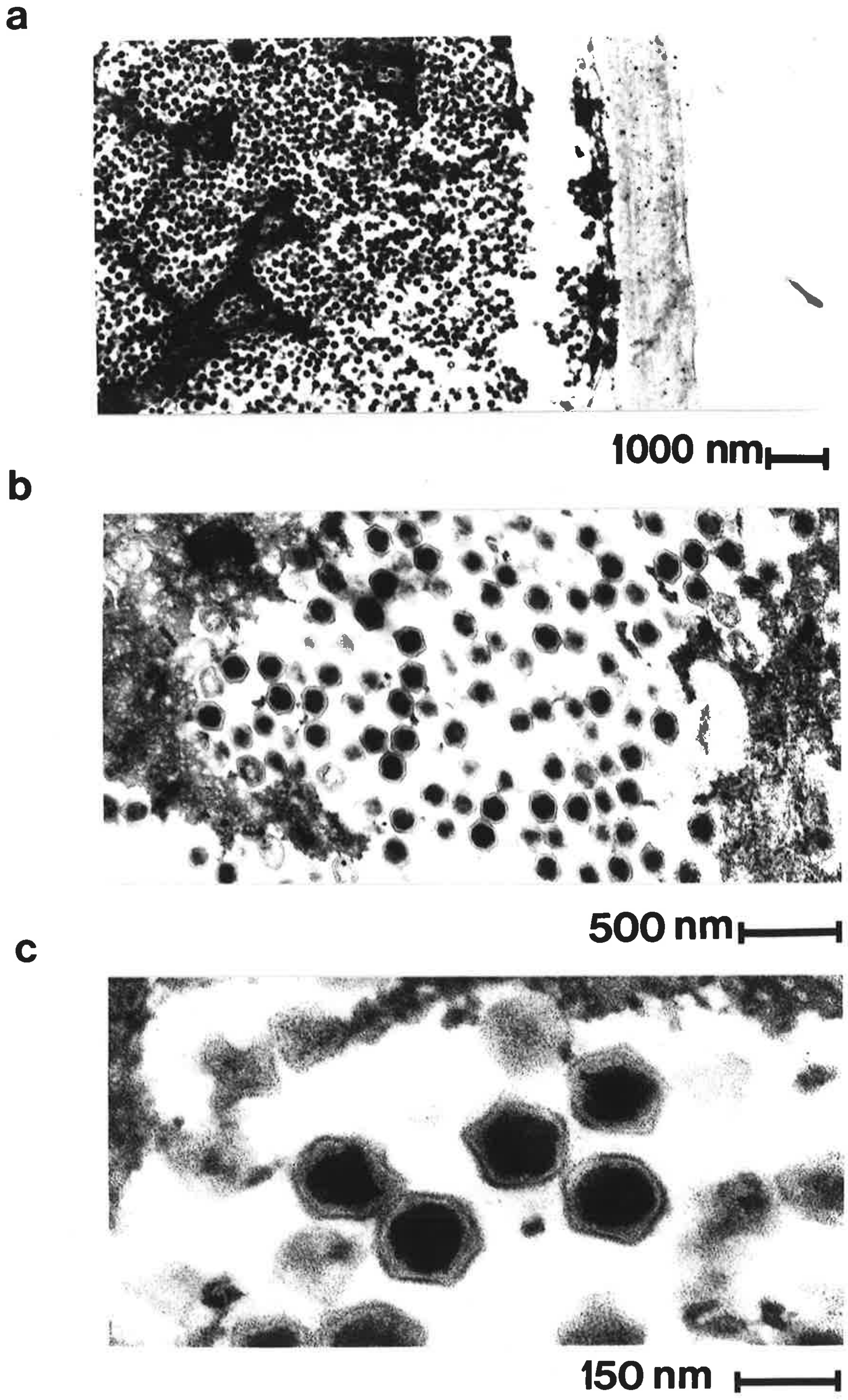
RESULTS

Micrographs of tissue from the fat body of a 4th instar *T. barbitarsis* larva are given in Figure 9.1. The organism which is responsible for the iridescent green colouration of some *T. barbitarsis* larvae was identified as *Tipula* iridescent virus (TIV). Virus particles are very numerous and measure some 150 nm in diameter (Fig. 9.1a).

DISCUSSION

The identification of TIV as a pathogen of *T. barbitarsis*, although interesting, is in itself not of great significance. Such viruses have been described from several dipteran taxa including Tipulidae (Smith, 1973; Carter *et al.*, 1983), Culicidae (Clark *et al.*, 1965; Linley and Nielsen 1968 a & b), Simuliidae (Avery and Bauer, 1984) and Chironomidae (Stoltz *et al.*, 1968). It is the potential of *T. barbitarsis* as a naturally-occurring culture population for the production of an insect pathogen that makes this discovery interesting.

Although efforts are currently underway to develop tissue cultures for virus production (e.g. Lynn and Hink, 1980; Kloc *et al.*, 1983; Kloc *et al.*, 1984), most viruses used in biological control programmes derive from large insect colonies which are reared under controlled conditions. Large laboratory colonies are subject to problems such as infections by other pathogens or simply high costs due to their labour-intensive nature (Vaughn, 1981).


a
1000 nm
b
500 nm
c
150 nm

Very little attention has been paid to the idea of concentrating viruses from naturally-occurring insect populations. Frequently, large numbers of a single insect species occur in particular situations; these are often in artificial systems such as plant monocultures. The *T. barbitarsis* populations in the fly-ash lakes at Port Augusta and in Lake Werowrap represent good examples of insect monocultures which would be suitable for the culture of viruses potentially pathogenic to other pest organisms. It was this idea which presented itself when the differential levels of TIV infection were observed between Port Augusta and Lake Werowrap.

In considering the idea, it seems that four requirements must be met before natural insect populations can be exploited for useful pathogenic viruses:

1. The pathogenic organism should be artificially transmissable to other pest taxa.
2. There should be an exploitably high level of infection in the host population.
3. The insect host species should be abundant and consistently available for exploitation in this way.
4. Infected individuals should have characteristics which make them amenable to mechanized separation from healthy individuals.

Each of the above requirements will be considered in turn.

The usefulness of *T. barbitarsis* TIV as a pathogen of other pest taxa as well as the possibility of its artificial transfer are fundamental, but are unknown factors at present. In this regard, it should be noted that the iridoviruses appear to be a diverse and specific group of pathogens with even some taxa being reported from

Amphibia (Lunger, 1966). Matta (1970), for example, found that R MIV could not be regarded as simply a strain of other iridescent viruses. Such apparent specificity would be disadvantageous to any idea of using *T. barbitarsis* TIV for the purpose of pest biocontrol.

However, early work by Smith *et al.* (1961) in which the idea of high specificity for all insect viruses is disputed, appears less discouraging. By experimental cross-innoculation with TIV, the successful infection of 7 species of Diptera, 11 species of Lepidoptera and 3 species of Coleoptera was achieved. Smith *et al.* (1961) stressed the need for high concentrations of the virus in innocula and its freedom from contaminating microorganisms.

Regarding the requirement for relatively high rates of infection in the culture population, the fact that there are differential levels of infection between Port Augusta and Lake Werowrap indicates that there is the potential to manipulate environmental parameters to increase the infection rate.

The need for large, naturally-occurring host populations is well-fulfilled by *T. barbitarsis*. In both the natural (The Coorong, Lake Werowrap) and artificial (Port Augusta, Dampier Salt Works) permanent, hypersaline lakes of Australia, *T. barbitarsis* populations reach high densities (100,000 m^{-2}), particularly in the absence of fish. The consistently high numbers of *T. barbitarsis* (see population data) renders the species and its particular habitat, which is relatively free from competitors and pathogens, ideal for viral culture.

Unlike terrestrial species which cannot easily be harvested for infected host individuals, *T. barbitarsis* has two important characteristics which fulfil this requirement: firstly, they are distinctively different in colour and secondly, infected individuals tend to occur in the water column of shallow lakes, being unable to re-establish themselves in tubes on the bottom once they are dislodged by turbulence. Using these characteristics, it would be possible to devise a mechanized system with pumps and photoelectric sensors to remove infected *T. barbitarsis* larvae from the water column continuously.

In this regard, however, the question may be raised as to whether constant removal of TIV infected individuals would not, in the longer term, reduce the level of infection in the population as a whole. This would require investigation, but some previous work indicates otherwise. For example, Linley and Nielsen (1968 a & b) have reported transovarial transmission of mosquito iridescent virus (MIV) in nature, suggesting that the removal of 4th instar larvae may not reduce the overall level of infection. Additional support for this idea comes from Stoltz *et al*. (1968) who concluded that, although infection of *Chironomus plumosus* with TIV probably occurs in the early stages of development, it does not become manifest until the fat bodies develop when the larva has reached a length of 21 mm.

Work on the mode of infection by viruses in insects often indicates that it is ingested orally (e.g. Grimstad and Haramis, 1984; Sikorowski and Tyson, 1984). If this is the case for *T. barbitarsis* TIV then the infection rate could depend upon the number of virus particles in the substrate. Constant removal of infected individuals may indeed have the effect of lowering virus concentrations and reducing infection levels.

The hypersaline lakes of Australia offer ideal loci for the monoculture of the TIV host species, *T. barbitarsis*. They are low in species diversity and productive, providing large populations free from the problems of many laboratory colonies. Two major lines of research need to be pursued in order to investigate the feasibility of TIV production from such lakes.

Of primary importance is to investigate, experimentally, the cross-infectivity of *T. barbitarsis* TIV with other pest insect taxa. Without this fundamental prerequisite knowledge there would be no point in continuing the idea any further.

The second line of work involved would be an ecophysiological study to identify parameters which increase the level of TIV infection in naturally-occurring *T. barbitarsis* populations. A comparison of Lake Werowrap and the Port Augusta Lakes from this point of view would provide a good starting point for such investigations.

This work, therefore, draws attention to the potential of *T. barbitarsis* for the culture of pathogenic viruses for use in biological control programmes against problem insects. It suggests the possibility that virus infected *T. barbitarsis* larvae could be removed from the culture population and the TIV viruses stored for future use as insecticides.

CHAPTER 10

General Discussion

Two major questions have been addressed in this thesis. The first of these is the question of what is a suitable control strategy for the nuisance midge, Tanytarsus barbitarsis Freeman in the Port Augusta fly-ash lakes. It required a practical solution, but to reach this it was necessary to test a range of scientific hypotheses. The second is the question of whether the method of Integrated Pest Management (IPM) is applicable to small-scale pest organisms where knowledge of basic biology is absent and research funding is limited. Although largely relating to methodology, this question raises several, pertinent philosophical points.

Possible solutions to the above questions, in the light of research results and experience, form the basis for this chapter.

I. SOME POSSIBLE CONTROL STRATEGIES

This research has provided information on some apects of the basic biology of T. barbitarsis which enables some management plans to be devised as likely to be effective. Four biological features, drawn from the research results, provide a foundation for the development of control strategies. These are:

i) The relationship between generation time and mean water temperature for T. barbitarsis is best described by the equation, $y = 1682.61x^{-1.27}$, where: y = generation time in days and x = mean water temperature in degrees Celcius (see Chapters 4 and 6).

ii) During years when there is high fish (Atherinosoma microstoma) recruitment, predation exercises control upon T. barbitarsis in spring and early summer (see Chapter 5).

iii) The 4th instar larvae of T. barbitarsis have an LC_{50} of 98g l^{-1} NaCl (see Chapter 7).

iv) Adult T. barbitarsis are strongly attracted to blue-green (490 - 510 nm) and near ultraviolet light (370 - 400nm) (see Chapter 8).

There follows a description of some management strategies which have been devised using knowledge of the above features.

a) A management plan based upon fish predation and salinity tolerance

i) Basis for the management plan

At the core of this management plan is a mathematical model which relies upon certain assumptions.

Firstly, generation time is solely a function of average water temperature over the lifetime of any one particular generation. The relationship, $G_i = aT_i^{-b}$, is used to estimate the generation time by fitting the model to the real data.

Where: G_i = generation time for generation i
T_i = average water temperature for generation i
a = generation time constant (120.11 fortnights)
b = generation time index (1.27)

Secondly, the size of the adult population is a function of 3 variables: numbers of adults in the previous generation ($N_{a(i-1)}$), average water salinity for the current generation (S_i) and number of A. microstoma present during the current generation (F_i). The logistic equation (Lotka, 1925; Volterra, 1926) is used as a basis to describe the population size of any particular generation in the equation,

$$Na_i = \frac{r\ Na_{(i-1)}\ S_i\ -c\ F_i\ -d}{1 + (r/K)\ Na_{(i-1)}}$$

Where: c and d are constants which quantify the effects of salinity and fish predation, r is the intrinsic growth rate and K is the carrying capacity of the environment. These parameters are all estimated from historical data.

ii) Use of the model in a management plan

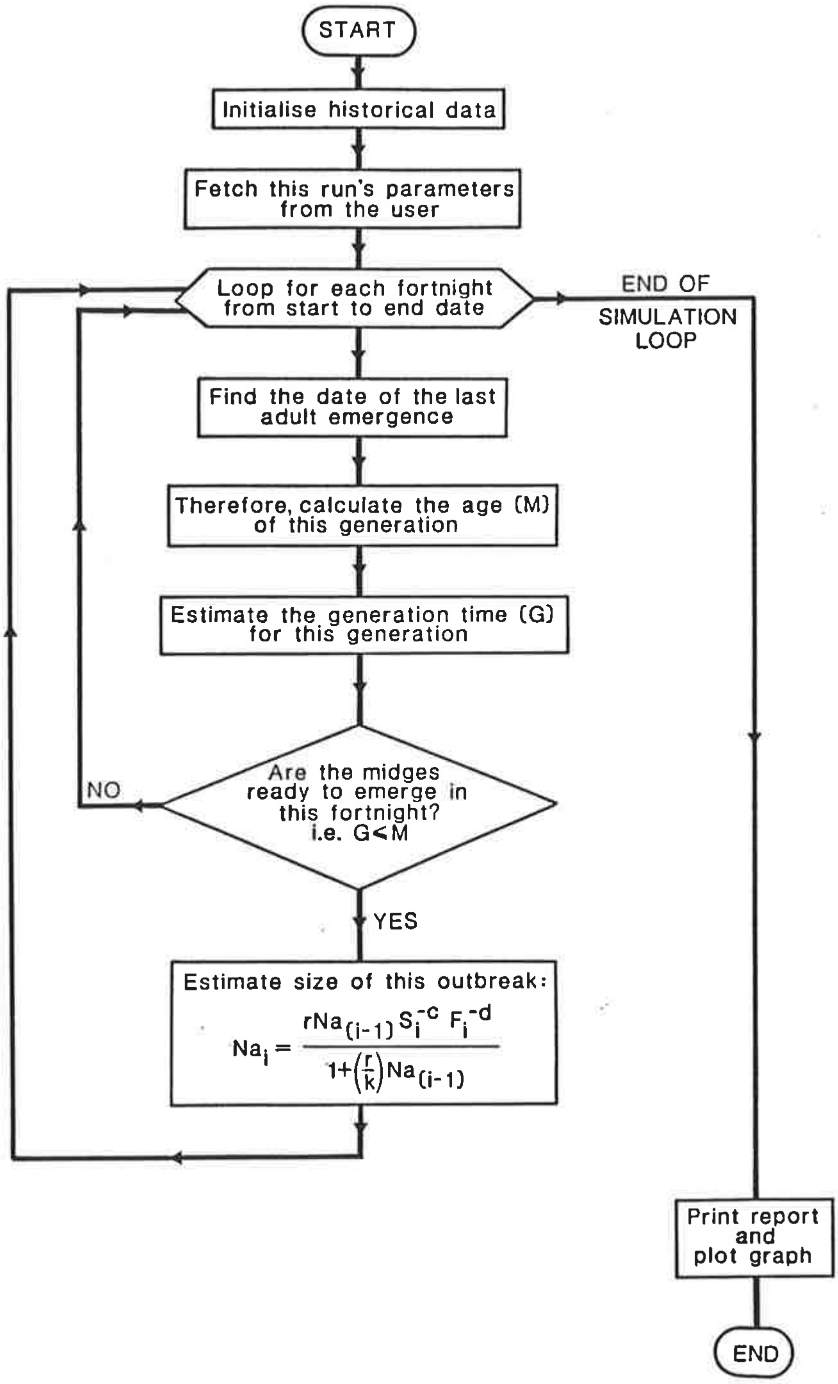
The model is incorporated in a computer simulation which predicts levels of adult abundance on a fortnightly basis. A logical flow chart for the computer simulation is presented in Figure 10.1. The beginning of the first fortnight of the simulation is assumed to be the first mass emergence of spring (usually in late August). It is intended that the pest managers will monitor adult emergence so that the simulation can be commenced at the correct time.

As the year progresses, temperature and salinity data are used to update the model, which combines this input with an historical data set to predict the timing and extent of future adult emergences. Adult emergence, being the cause of the problem, is the only event reflected in the simulation predictions.

iii) Application of the strategy

It is proposed that control of the *T. barbitarsis* population using natural fish predation and highly saline water will be exercised in the following way: Firstly, a routine sampling programme will monitor a series of parameters on a fortnightly cycle. These are: water salinity, mean water temperature, fish numbers, *T. barbitarsis* adult emergence. These data, which can be collected without much effort, will be used - in conjunction with the computer simulation - to take appropriate measures to control *T. barbitarsis*.

Figure 10.1 A logical flow chart for the computer simulation.



Management of the population will essentially involve the decision of when to apply hypersaline water to the system. If fish numbers are high during spring and early summer then this natural control will be allowed to take its course.

Once the period of natural control due to fish predation is over, then highly saline water, which has been concentrated by evaporation in the northern lakes (Salt Lake and Railway Lake (Fig. 2.5)), will be released at intervals to reduce the larval population. The average salinity of Bird Lake (Fig. 2.5) will be raised above 110 g l^{-1} during this exercise.

The volume of Bird Lake is 3.5 X10^5 m^3 and its summer salinity is 55 g l^{-1}. Therefore it is calculated from the relationship:

$$y = \frac{\text{lethal salinity} \times \text{volume of Bird Lake}}{\text{salinity of Bird Lake} + x}$$

Where: y = volume of water which must be displaced from Bird Lake

x = salinity of the saline water to be added.

$$\text{i.e.} \quad y = \frac{110\text{g l-1} \times 3.5 \times 10^5\ \text{l}}{55\text{g}\ l^{-1} + 300\text{g}\ l^{-1}} = 1.1 \times 10^5 \text{ litres}$$

The release of 1.1 X 10^5 litres of water, some 25% of the volume of Railway and Salt Lakes combined, at 300 g l^{-1} is feasible. It should also be borne in mind, that the hypersaline water, by virtue of its density, will flow underneath the fresher Bird Lake water causing high mortality at the mud-water interface where the great majority of _T. barbitarsis_ larvae occur. In addition, this lack of mixing will have the effect of displacing the fresher water from Bird Lake and thus reducing the dilution effect. It follows that the release of the hypersaline water from the northern lakes should be undertaken during a time of low water turbulence.

iv) Examples to illustrate the use of the computer simulation

Note: A summary of ecological data used for the computer simulation is given in Table 11.1.

Figure 10.2 shows the predicted pattern of emergence during one year in the absence of fish predation (d = 0) or the introduction of hypersaline water (c = 0). Spring emergence is arbitrarily set at 1 September for all simulations shown here. The abundance of the first generation is set at 100. The carrying capacity K is set at four times this value i.e. 400; this factor is consistent with historical data. The intrinsic growth rate, r, is arbitrarily taken as 10. The pattern shown by Figure 10.2 is simply a series of nuisance outbreaks varying in their spacing according to the mean water temperature. Temperature data from 1984/85 were used in all simulations (the 'Fresher' set).

If the monitoring programme reveals a fish recruitment equivalent to that of 1984 (Fig. 4.15), there is no intervention, leaving this period of natural control to take its course. The computer simulation for this event (Fig. 10.3) with all other parameters set as in Fig. 10.2, assumes that there was 0.7 mortality due to fish predation (i.e. $F_i^{-0.7}$). The 1984 fish data is used in the simulation and it will be seen that the predicted pattern resembles the actual pattern from sampling data (Fig. 4.14, Chapter 4).

Figure 10.4 shows the results of the computer simulation when parameters were the same as for Fig. 10.2 (i.e. 0.7 fish predation using 1984 fish data set), but two releases of hypersaline water are applied after the controlling effect of fish predation has ceased. *T. barbitarsis* larval mortality is assumed to be 50% (i.e. c = 0.5) when the effective salinity at the bottom is raised to 150 g l^{-1} (a conservative estimate). In fact, the application of hypersaline water, at my recommendation, after the completion of the research programme caused larval mortality estimated at 70%. It will be seen from Fig. 10.4 that this combined strategy has the potential to exercise effective control over the *T. barbitarsis* population.

From Fig. 10.5, however, where the effect of fish predation is removed from the simulation, it appears that the use of hypersaline water alone will not exercise satisfactory control over *T. barbitarsis* at Port Augusta.

b) A control strategy involving the use of attractive lights

A second strategy involves the use of lights which emit wavelengths in the attractive range to divert midges from residential areas. The erection of lights would not only form the basis for an effective control strategy, but it would supplement the control measures described above.

A conspicuous series of lights could be erected at 100 m intervals along the length of Bird Island (Fig. 2.5). Each light would comprise a combination of mercury vapour and GE SPX 27 fluorescent tubes. Erection would be so that the lights are visible from the surrounding waters.

Switching on of these "decoy" lights would only be necessary during periods of high adult emergence and particularly on calm, summer evenings. Data from the fortnightly monitoring programme will indicate the levels of adult emergence.

A refinement to the decoy lights would be to incorporate a trapping mechanism in their design. The fact that approximately 40 tonnes of *T. barbitarsis* adult biomass emerges from the lakes annually suggests that there may be a viable harvest of animal tissue available for little extra effort. Possible examples of uses for this chironomid material include as compost or fish food. Indeed, there is currently a pilot study at Port Augusta which is investigating the commercial rearing of the penaeid prawn, *Penaeus latisulcatus* in heated water from the power station. The material from the light traps could well serve as a suitable food for this species.

c) Other potential control measures

There are a range of further control measures which remain largely unproven. Potentially the most useful of these is the bacterium, *Bacillus thuringiensis* var *israelensis*. It remains only for successful field trials to be conducted with this "insecticide" before it will become available for use in conjunction with the measures described above.

Other potential control measures such as use of the pathogen, *Tipula* iridescent virus (TIV) and insect growth regulators remain distant possibilities.

Table 11.1 A summary of ecological data which provided an historical basis for the computer simulation.

	Salty Data Set 1982/83				Fresher Data Set 1984/85			
Fortnights from 1/9/85	Mean Bottom Water Temperature	Salinity (^{o}/oo)	Fish	Midge Emergence (m^{-2})	Mean Bottom Water Temperature	Salinity (^{o}/oo)	Fish (*larvae)	Midge Emergence (m^{-2})
1	16.5	58	1	3947	15.0	50	0	335
2	17.0	58	0	656	17.0	47	2	507
3	18.5	59	0	528	18.0	54	3	518
4	18.0	63	3	131	18.0	55	35 *	673
5	20.5	69	3	92	21.0	63	73 *	408
6	21.0	71	16	146	23.0	56	110 *	206
7	21.5	70	17	283	20.5	61	72 *	314
8	22.0	73	10	2000	22.5	59	20 *	213
9	23.0	76	5	5000	23.0	57	17 *	200
10	22.0	79	5	3819	23.5	58	15 *	154
11	22.0	78	9	12223	22.0	53	10 J	213
12	23.0	71	6	4711	20.5	46	20 J	197
13	22.0	74	5	5379	21.0	56	17 J	116
14	23.0	66	5	4806	19.5	59	10 J	616
15	23.0	70	4	933	22.5	72	5 J	318
16	21.0	62	0	8117	21.5	60	16 J	529
17	19.0	56	6	5379	21.0	42	18	1025
18	17.5	53	0	2228	19.0	46	5	2690
19	16.5	56	0	2737	16.0	51	4 *	629
20	15.0	53	1	1623	14.0	46	4 *	926
21	14.5	52	1	1560	10.0	44	16 *	849
22	13.5	49	1	1210	12.0	47	11 *	233
23	13.5	50	1	1942	9.0	44	16 *	127
24	12.5	54	5	1700	10.0	39	16 *	915
25	14.0	57	4	2000	14.0	34	2 *	1132
26	15.0	52	1	6000	15.0	43	0	1213

Figure 10.2 Results of computer simulation showing the annual pattern of T. barbitarsis adult emergence in the absence of fish predation or salinity effects.

```
ENTER ENVIRONMENT CONDITIONS (SALTY(=S)/FRESHER(=F)) F
ENTER GENERATION TIME CONSTANT (A>0)  120.11
ENTER GENERATION TIME INDEX (B>0)     1.27
ENTER MIDGE INTRINSIC GROWTH RATE (R>0)  10
ENTER MIDGE CARRYING CAPACITY (K>0)400
ENTER MIDGE SALINITY INDEX (C>=0)      0
ENTER MIDGE PREDATOR FISH INDEX (D>=0) 0

                  SIMULATION SUMMARY REPORT

                              (N)     (T)
              DATE          MIDGES    TEMP

 *  1       1/ 9/84          100      15
    2      15/ 9/84            0      17
    3      29/ 9/84            0      18
    4      13/10/84          286      18
    5      27/10/84            0      21
    6      10/11/84          351      23
    7      24/11/84            0      21
    8       8/12/84          359      23
    9      22/12/84            0      23
   10       5/ 1/85          360      24
   11      19/ 1/85            0      22
   12       2/ 2/85          360      21
   13      16/ 2/85            0      21
   14       1/ 3/85            0      20
   15      15/ 3/85          360      23
   16      29/ 3/85            0      22
   17      12/ 4/85            0      21
   18      26/ 4/85          360      19
   19      10/ 5/85            0      16
   20      24/ 5/85            0      14
   21       7/ 6/85            0      10
   22      21/ 6/85            0      12
   23       5/ 7/85          360       9
   24      19/ 7/85            0      10
   25       2/ 8/85            0      14
   26      16/ 8/85            0      15
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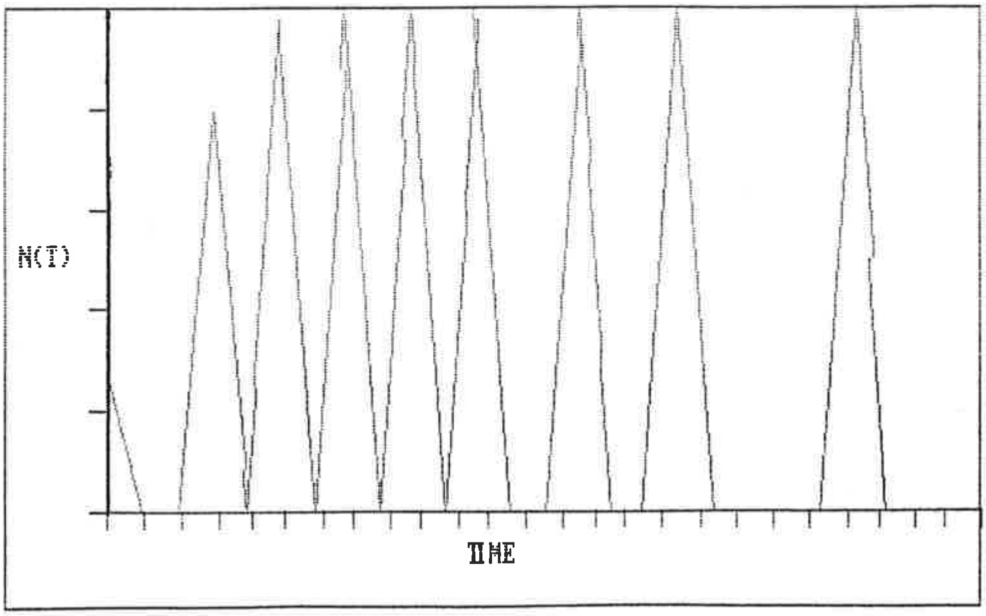


Figure 10.3 Results of computer simulation showing the effect of fish predation on the annual pattern of _T. barbitarsis_ adult emergence.

```
ENTER ENVIRONMENT CONDITIONS (SALTY(=S)/FRESHER(=F)) F
ENTER GENERATION TIME CONSTANT (A>0)  120.11
ENTER GENERATION TIME INDEX (B>0)      1.27
ENTER MIDGE INTRINSIC GROWTH RATE (R>0)  10
ENTER MIDGE CARRYING CAPACITY (K>0)400
ENTER MIDGE SALINITY INDEX (C>=0)      0
ENTER MIDGE PREDATOR FISH INDEX (D>=0) .7


                SIMULATION SUMMARY REPORT

                           (N)      (T)              (F)
            DATE         MIDGES    TEMP             FISH

*  1      1/ 9/84         100       15                0
   2     15/ 9/84           0       17                0
   3     29/ 9/84           0       18                0
   4     13/10/84         286       18               35
   5     27/10/84           0       21               73
   6     10/11/84          22       23              110
   7     24/11/84           0       21               72
   8      8/12/84           6       23               20
   9     22/12/84           0       23               17
  10      5/ 1/85           7       24               15
  11     19/ 1/85           0       22                0
  12      2/ 2/85          14       21                0
  13     16/ 2/85           0       21                0
  14      1/ 3/85           0       20                0
  15     15/ 3/85         104       23                0
  16     29/ 3/85           0       22                0
  17     12/ 4/85           0       21                0
  18     26/ 4/85         289       19                0
  19     10/ 5/85           0       16                4
  20     24/ 5/85           0       14                4
  21      7/ 6/85           0       10               16
  22     21/ 6/85           0       12               11
  23      5/ 7/85         216        9               16
  24     19/ 7/85           0       10               16
  25      2/ 8/85           0       14                2
  26     16/ 8/85           0       15                0
```

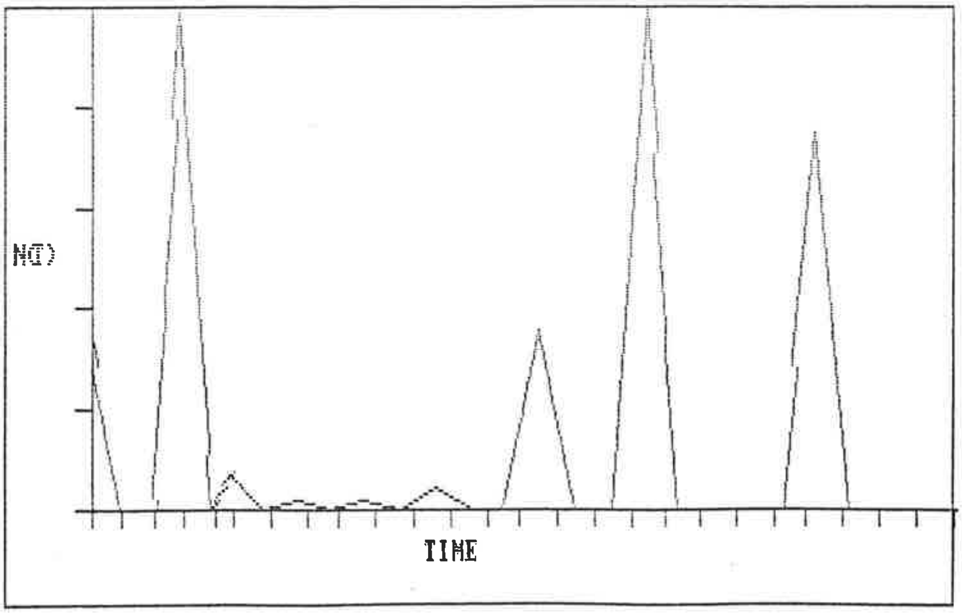


Figure 10.4 Results of computer simulation showing the effect of fish predation and the application of hypersaline water on the annual pattern of _T. barbitarsis_ adult emergence.

```
ENTER ENVIRONMENT CONDITIONS (SALTY(=S)/FRESHER(=F)) F
ENTER GENERATION TIME CONSTANT (A>O)  120.11
ENTER GENERATION TIME INDEX (B>O)     1.27
ENTER MIDGE INTRINSIC GROWTH RATE (R>O)  10
ENTER MIDGE CARRYING CAPACITY (K>O)400
ENTER MIDGE SALINITY INDEX (C>=O)      .5
ENTER MIDGE PREDATOR FISH INDEX (D>=O) .7


                   SIMULATION SUMMARY REPORT

                            (N)      (T)    (S)          (F)
              DATE        MIDGES    TEMP  SALINITY      FISH

*  1       1/ 9/84         100      15       57           0
   2      15/ 9/84           0      17        0           0
   3      29/ 9/84           0      18        0           0
   4      13/10/84         286      18        0          35
   5      27/10/84           0      21        0          73
   6      10/11/84          22      23        0         110
   7      24/11/84           0      21        0          72
   8       8/12/84           6      23        0          20
   9      22/12/84           0      23        0          17
  10       5/ 1/85           7      24        0          15
  11      19/ 1/85           0      22      150           0
  12       2/ 2/85           2      21        0           0
  13      16/ 2/85           0      21        0           0
  14       1/ 3/85           0      20        0           0
  15      15/ 3/85          16      23        0           0
  16      29/ 3/85           0      22      150           0
  17      12/ 4/85           0      21        0           0
  18      26/ 4/85          16      19        0           0
  19      10/ 5/85           0      16        0           4
  20      24/ 5/85           0      14        0           4
  21       7/ 6/85           0      10        0          16
  22      21/ 6/85           0      12        0          11
  23       5/ 7/85          70       9        0          16
  24      19/ 7/85           0      10        0          16
  25       2/ 8/85           0      14        0           2
  26      16/ 8/85           0      15        0           0
```

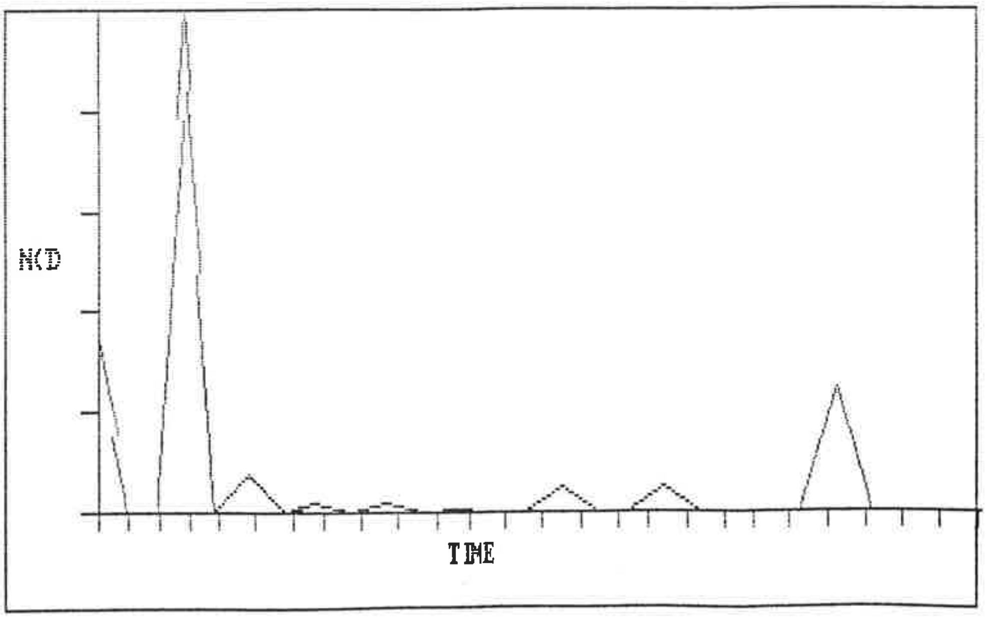


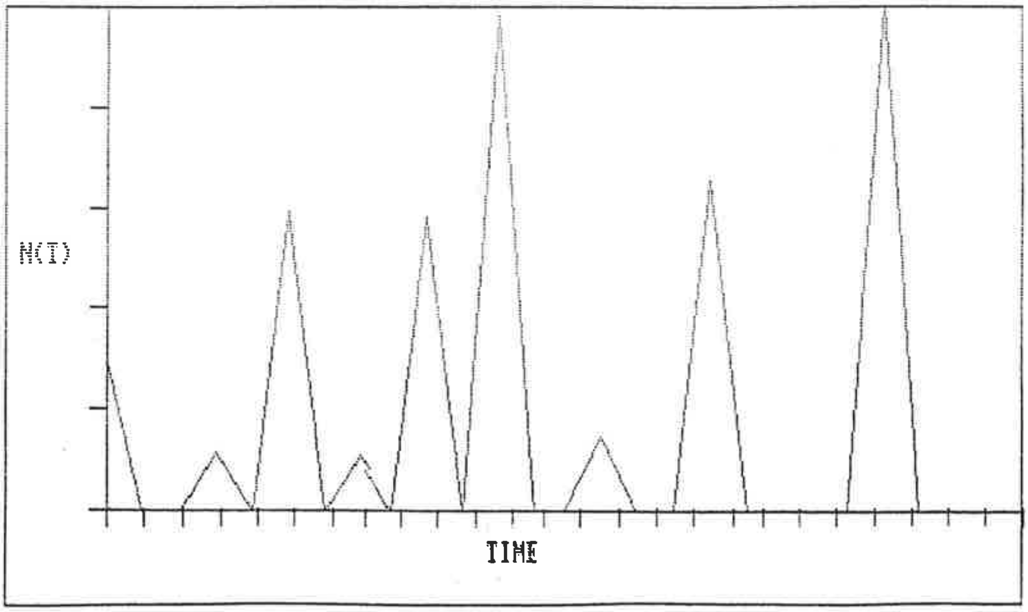
Figure 10.5 Results of computer simulation showing the effect of hypersaline water alone on the annual pattern of *T. barbitarsis* adult emergence.

```
ENTER ENVIRONMENT CONDITIONS (SALTY(=S)/FRESHER(=F)) F
ENTER GENERATION TIME CONSTANT (A>0)  120.11
ENTER GENERATION TIME INDEX (B>0)      1.27
ENTER MIDGE INTRINSIC GROWTH RATE (R>0)   10
ENTER MIDGE CARRYING CAPACITY (K>0)400
ENTER MIDGE SALINITY INDEX (C>=0)      .5
ENTER MIDGE PREDATOR FISH INDEX (D>=0)  0

                     SIMULATION SUMMARY REPORT

                                (N)      (T)     (S)
                DATE          MIDGES     TEMP   SALINITY

 *   1      1/ 9/84           100       15       57
     2     15/ 9/84             0       17      150
     3     29/ 9/84             0       18        0
     4     13/10/84            40       18        0
     5     27/10/84             0       21        0
     6     10/11/84           201       23        0
     7     24/11/84             0       21        0
     8      8/12/84            39       23      150
     9     22/12/84             0       23        0
    10      5/ 1/85           196       24        0
    11     19/ 1/85             0       22        0
    12      2/ 2/85           332       21        0
    13     16/ 2/85             0       21        0
    14      1/ 3/85             0       20      150
    15     15/ 3/85            50       23        0
    16     29/ 3/85             0       22        0
    17     12/ 4/85             0       21        0
    18     26/ 4/85           223       19        0
    19     10/ 5/85             0       16        0
    20     24/ 5/85             0       14        0
    21      7/ 6/85             0       10        0
    22     21/ 6/85             0       12        0
    23      5/ 7/85           339        9        0
    24     19/ 7/85             0       10        0
    25      2/ 8/85             0       14        0
    26     16/ 8/85             0       15        0
```



II. FURTHER WORK

There are several areas within the control programme for *T. barbitarsis* which require further work. Each of these will be discussed in turn.

i) Fish Predation

While a combination of field exclusion and laboratory experiments indicated that fish predation does exercise control over *T. barbitarsis*, the extent of this effect remains unquantified. It is suggested, therefore, that experimental exclosures and controls, similar to those described in Chapter 5, be deployed during times of fish recruitment in order to quantify the effect of fish predation upon adult emergence of *T. barbitarsis*.

ii) Attractive light source

The light preference experiments indicated two attractive ranges within the electromagnetic spectrum, 490-510 nm and 370-400 nm. The spectral power curves of mercury vapour and GE SPX 27 lamps, include peaks within these ranges or marginal to them. It is possible, however, that the area of preference could be defined more narrowly and the effect of intensity further elucidated. In the event of a refinement of knowledge in this area, the two recommended light sources may be replaced by others with peaks which correspond more closely to the attractive wavelengths. Further work with light would involve a continuation of the experimental programme, probably in the laboratory.

iii) Bacillus thuringiensis var israelensis (BTI)

BTI remains the only relatively cheap, appropriate insecticide which is currently available for use against *T. barbitarsis*. It is suggested that further work will involve the testing of this pathogen at Port Augusta.

iv) Tipula iridescent virus (TIV)

The suggestion that TIV is a potential control agent for *T. barbitarsis* at Port Augusta would require an extended programme of research for confutation. Such research is beyond the scope of a small-scale control programme and its associated research activities. It is hoped, however, that this idea may form the basis for future research.

III. THE APPLICABILITY OF INTEGRATED PEST MANAGEMENT TO A SMALL-SCALE PEST

As a basis for further discussion, the definition of IPM as stated by Flint and Van den Bosch (1981) is given: "Integrated Pest Management (IPM) is an ecologically based pest control strategy that relies heavily on natural mortality factors such as natural enemies and weather and seeks out control tactics that disrupt these factors as little as possible. IPM uses pesticides, but only after systematic monitoring of pest populations and natural control factors indicates a need. Ideally, an integrated pest management program considers all available pest control actions, including no action, and evaluates the potential interaction among various control tactics, cultural practices, weather, other pests, and the crop to be protected."

It will be seen that this definition lays down principles, but lacks any general methodology. Despite this, IPM has assumed, in the minds of some field workers and health officers, a status beyond its present limitations. The use of jargon and abbreviations has further contributed to the misplaced expectations.

In order to understand the philosophy of IPM and the zeal with which it has been promoted, it is necessary to know that it reflects a reaction to the excessive use of chemical pesticides. The history of this reaction, where terms such as: "integration", "harmonious control", "pest management" and "integrated pest management" have evolved in a 'band-waggoning' sequence, has been described by Van Emden (1983) who suggests that "rational integrated pest management" (RIP) is the logical next step!

The active promotion of an alternative to reliance upon pesticides can perhaps be justified in terms of a need to convert the uninformed crop managers to its methods. Despite this persuasive effort, it seems unlikely that the use of insecticides in the USA will decline before the year 2000 (Pimentel, 1985). There are several reasons for this: i) There is insufficient ecological information for the implementation of IPM. ii) Economic damage thresholds for each pest population are difficult to obtain. iii) There is a lack of interdisciplinary co-ordination. iv) The application of insecticides, still strongly promoted by chemical companies, is simpler and gives immediate, visible results (Pimentel, 1985).

Despite these factors, the amount of insecticides used on some crops has declined, for example, on peanuts and sorghum (Frisbie and Adkisson, 1985) and apples (Tette _et_ _al_., 1981). This decline reflects increased use of IPM in these areas as a response to pesticide resistance and the high cost of replacement compounds (Pimentel, 1985).

There would be no need to define IPM at such length if the principle were accepted that pesticides, like any other control measure, must fulfil a range of criteria before being deemed suitable for use in any particular situation. Indeed, the term, "integrated" would also become redundant. The concept of pest management would then be, that all appropriate control measures should be combined to give a stable, effective and environmentally-harmonious control strategy.

IPM, as practised, has two main components, the concept and the methodology. The concept is essentially what has been stated above. However, if one seeks general IPM methods, as would health officers faced with small-scale pests such as *T. barbitarsis*, numerous specific case histories can be found in the literature (e.g. Hoy and Herzog, 1985; Laird and Miles, 1985), but few studies yield widely-applicable methods. Some attempts at generalization do occur: For example, Curtis (1985) has suggested models for the use of insecticide mixtures to manage resistance, and Van Emden (1983) has proposed an experimental sequence for developing a pest management programme using pesticides.

How should control strategies for small-scale pests proceed when there is a general lack of universal method? The key, it seems, lies in an understanding of the prevailing systems, particularly their biology. This understanding can only be improved by the appropriate research. If one bears in mind that pest management is a technology which has drawn heavily upon insight and accident for its methods, then the advantages of biological research can be appreciated. Accidental discoveries such as the effect of the insect growth regulator, CGA-72662, which was originally synthesised as a potential herbicide (Friedel, pers. comm.), lead to new control measures but lack the logical cause and effect feedback system. On the other hand, the proposal that near-ultraviolet light will attract adult *T. barbitarsis* is an hypothesis which can be tested and referred back to the principles from which it was drawn. The added advantage of biological research is that it too draws information from technological and serendipitous sources, making it at least their equal. However, it is able to integrate such information into a body of knowledge based upon understanding and theory.

It follows, therefore, that the recommended approach to small-scale pest problems is via a programme of basic biological research. The success of this approach depends upon the quality of the research and the available resources. The researchers involved require: freedom to follow hypotheses; access to a wide range of expertise; generalist skills and experience in a wide variety of techniques.

Van Emden (1983) sees the ultimate solution to particular pest problems as a nebulous goal and he adds that there is not sufficient time to collect enough background data on the ecosystem to reach this solution. However, it seems likely that, in the case of small-scale pests, a biological approach will yield solutions superior to current technology-based methods which are transplanted from unsimilar situations and often involve the heavy use of insecticides. The more comprehensive the biological research, the more likely it will be to yield an effective control strategy.

As has been stated above, a major reason for the failure of IPM has been its lack of interdisciplinary co-ordination. The scheme set out below represents, heirarchically, the various levels of activity of a biologically-based pest control programme.

FUNCTION	PERSONNEL	INSTITUTION
Management and Co-ordination	Administrative staff	Government/ University
Scientific supervision Hypothesis formation	Senior research staff	Government/ University
Hypothesis formation Hypothesis testing	Junior research staff Technicians	Government/ University
Data collection Implementation of measures	Health officers Agricultural officers Field staff	Government Local authorities

It will be seen that both government research institutions and universities are deemed suitable loci for small-scale pest control programmes. However, the universities have one major advantage in that they provide a wide range of equipment and expertise in one locality. The present project, for example, required advice from mathematicians, physicists, taxonomists, electron microscopists, botanists, engineers, chemists and biochemists. In addition, it had the advantage of critical supervision from academic staff.

A reluctance by some universities to support projects of an applied nature seems unwarranted when one bears in mind the pure research, including taxonomic, physiological and ecological goals, that have been achieved by this programme.

It is significant that not all work was directly related to potential control measures. Much of it was of a basic nature, for example the description of *T. barbitarsis* and *T. semibarbitarsus* larvae which contributes to the framework of biological knowledge upon which potential control measures rely.

The methods advocated for Integrated Pest Management caused scepticism at the outset of this project because they required extensive biological knowledge for their implementation. However, the concept of pest management, as redefined above, has served as a guide to the rational development of a basic control strategy for T. barbitarsis at Port Augusta through biological research.

No doubt, a large proportion of the funds currently spent on insecticides could be redirected into biological research projects which would yield effective long-term solutions to small-scale pest organisms. In addition, much basic biological information would be gained from such projects.

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