ADAPTATION TO TEMPERATURE IN CADDIS LARVAE (TRICHOPTERA)

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ABSTRACT

The occurrence of temperature acclimation was investigated in a range of caddis species.

A closed bottle method was used for the majority of the respiration experiments, a flow-through respirometer being designed for the remaining experiments.

Temperature acclimation was demonstrated in eight out of twelve species studied. Increased ability to compensate was associated with increased ecological distribution.

The hydropsychids were shown to have a greater ability to acclimate than polycentropodids with similar distributions.

No relationship was apparent between the interspecific differences in the respiration rate and the distribution of the species.

For some species maintenance at a warmer temperature for 4-5 weeks caused a decrease in the undulatory activity of the larvae when compared, at a constant temperature, with larvae maintained at a cooler temperature. For eight of the ten species for which both sets of data were obtained the metabolic and undulatory data were compatible. A relationship between undulatory activity and distribution was suggested.

For two cased caddis species a positive correlation was demonstrated between the number of gills and the body weight. Maintenance at the warmer of two temperatures over a period including a moult caused an increase in the number of gills on larvae and pupae. Intraspecific differences were found in the number of gills on larvae from different field sites, the number increasing with increased stream temperature. No relationship was demonstrated between the oxygen consumption of the larvae and the number of gills.

No evidence was obtained for a difference in metabolism following maintenance of larvae at fluctuating temperatures of differing amplitudes.

Decreased undulatory activity was demonstrated in larvae of *Hydropsyche contubernalis* maintained under conditions of greater temperature fluctuations.

Field acclimatisation was demonstrated in two species, *Sericostoma personatum* and *Potamophylax cingulatus*, larvae from the warmer sites having a lower respiration rate than larvae of the same species from cooler sites, when both were measured at the same temperature.

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CHAPTER 1 INTRODUCTION

Water temperature has a diverse and far reaching influence upon many aspects of aquatic life. It affects, for example, egg development (Humpesch and Elliott, 1980; Elliott, 1984), growth and development (Markarian, 1980; Brittain, 1983; Abdullahi and Laybourn-Parry, 1985; Oemke, 1987), fecundity (Vannote and Sweeney, 1980) emergence (Nebeker, 1971; Perry et. al., 1987) and metabolism. Many investigations have been made into the relationship between water temperature and oxygen consumption (Huebner, 1973; Robinson et. al., 1983; Laybourn-Parry and Tinson, 1985; Al-Dabbagh and Luka, 1986). These have generally demonstrated an increase in oxygen consumption with an increase in temperature. The form of the relationship varies but many studies have demonstrated the presence of plateaux in the temperature-metabolism curve which have been related to the temperatures encountered by the animal in the field (Hildrew and Edington, 1979; Epp and Lewis, 1980a). The majority of experiments investigating the relationship between oxygen consumption and temperature have tended to measure the acute response to temperature but it may be more useful to consider the response of animals acclimated to the experimental temperature. The occurence of temperature acclimation (a response to a single factor, in this case temperature) or acclimatisation (a response to a number of factors in the field) in poikilotherms in general and aquatic insects in particular are not well documented. This lack of information on this important aspect of the respiratory physiology of these animals provided the initial impetus for this research. There are some doubts about the occurrence and importance of temperature acclimation in the invertebrates. Kulkarni (1978) states that there is irrefutable evidence that poikilothermic invertebrates undergo metabolic compensation in response to thermal variations but Keister and Buck (1974) state that insects in general have little ability to compensate for environmental temperature. Hayashi and Yoshida (1987)

demonstrated seasonal changes in the respiration of the Dobsonfly, *Protohermes grandis*, indicating an ability to acclimatise in this species. A preliminary study by Harrison and Badcock (1981) demonstrated an ability for larvae of *Chaetopteryx villosa* and *Potamophylax cingulatus* to compensate their metabolism, in response to different temperatures, in the field and laboratory respectively. In both cases a reverse acclimatory response was reported, larvae from the warmer conditions having a higher respiratory rate than those from the cooler conditions.

The importance of the relationship between oxygen consumption and water temperature, and the influence of acclimation on this relationship, is largely due to the differences in thermal regimes encountered in the field. Considerable data has been accumulated concerning the temperature of streams (Macan, 1958; Edington, 1965; Crisp and LeCren, 1970), rivers (Langford, 1970; Boon and Shires, 1976) and lakes (Brittain, 1976). The distributions of closely related species have been related to the differences in the thermal regime along streams and rivers. In Britain one of the best known downstream sequences is that of the hydropsychids whose distributions are well documented (Edington and Hildrew, 1973; Badcock, 1974 and 1975; Hildrew and Edington, 1979). There is a clear pattern in the distribution of species from cool headstreams to the lower reaches of a river system which appears in part to be related to temperature. Edington and Hildrew (1973), Philipson and Moorhouse (1974) and Hildrew and Edington (1979) demonstrated differences in the metabolism of larvae of a number of hydropsychid species which were apparently related to the thermal conditions to which they are subjected in the field. Similar conclusions were drawn by Philipson and Moorhouse (1976) for four polycentropodid species. However, as suggested by Harrison and Badcock (1981), if caddis larvae are capable of compensating their metabolisms in response to different temperatures the observed interspecific differences in the metabolism may reflect differences in the thermal history of the species,

caused by the different field distributions, rather than being a factor controlling the distribution of the species.

The aim of this study was twofold. Of paramount interest was to discern whether or not the larvae of a range of caddis species were capable of acclimating to temperature in the laboratory. Secondly if temperature acclimation was shown to occur,interspecific comparisons of the extent of the acclimation ability were to be made in order to determine whether this varied from species to species and to investigate any possible relationship between field distributions and acclimation ability.

The most relevant measure of an ability to acclimate was thought to be the oxygen consumption at physiologically 'normal' temperatures, rather than investigating factors such as lower or upper lethal temperatures.

The investigation is essentially divided into seven sections. Despite the apparent ease with which oxygen consumption can be determined for aquatic insects,there are many problems associated with each method. In particular,care must be taken that the experimental conditions allow relevant and meaningful data to be obtained. The advantages and disadvantages of a number of methods of measuring respiration are discussed in Chapter 3. The methods chosen,including a modified form of the micro-Winkler technique and a new design for an automatic flow through respirometer, are described.

Obviously many factors, including body size, activity, oxygen concentration, diel rhythms, container size and light may influence the oxygen consumption of an animal. A number of these factors have implications for the experimental protocol used while others have relevance to the interpretation of data from experiments described in the other Chapters. These factors are discussed in Chapter 4.

The ability to acclimate to constant temperatures of 6 and 18^{o}C is investigated in Chapter 5. Data are presented for larvae of six hydropsychids, three polycentropodids, two limnephilids and a

sericostomatid, and for pupae of one limnephilid, and the results discussed in relation to the distribution of the species.

The undulatory behaviour of larvae acclimated to 6 and 18^{o}C is discussed in Chapter 6, the data being considered in conjunction with the respiratory data presented in Chapter 5, differences in the ventilation rate having implications for the allocation of energy between respiratory costs and growth/development.

In Chapter 7 a limited investigation is made into the ability of three species to acclimate to diurnally varying temperatures of different amplitudes. The influence of different temperatures, both in the field and experimentally, upon the number of gills present is discussed in Chapter 8 and finally evidence is presented in Chapter 9 for two species for the occurrence of field acclimatisation.

The work is reviewed in Chapter 10, suggestions being made for further research in the area.

CHAPTER 2 MATERIALS AND METHODS

2.1 Collection and Identification of the Larvae

The larvae were collected from various sites by visual searching. In most cases the larvae were collected from streams or rivers, the larvae being found on the underside or topside of stones, or on the substratum previously covered by the stone. One species, *Anabolia nervosa* (Curtis), was collected from a shallow lake by picking the larvae from the sandy bottom. The larvae used in this study are listed in Table 2.1, with the site or sites where they were collected.

The larvae collected from Keele University campus (*Hydropsyche angustipennis* (Curtis), *Potamophylax cingulatus* (Stephens), *A. nervosa*, *Sericostoma personatum* (Spence) and *Plectrocnemia conspersa* (Curtis)) were transported back to the laboratory in water in collecting jars. The other species, collected from more distant sites were packed in muslin packets, filled with wet water weed or moss, which were placed in vacuum flasks. This proved to be a suitable method, mortalities on the journey generally being minimal. On arrival back at the laboratory the larvae were introduced into well aerated pond water, at a temperature similar to that at which they had been collected, and left for between 15 and 20 hours.

Table 2.1 Larvae studied and the sampling sites from which they were obtained

Species	Sampling Site	Map Reference
Diplectrona felix McLachlan	Tumbling Brook above confluence with Coombes Brook.	SK003519
	Tributary to Nant Menial, Llanerfyl.	SJ032093
Hydropsyche instabilis (Curtis)	River Dove at Milldale.	SK140548
Hydropsyche siltalai (Dohler)	River Gam, Llanerfyl.	SH971070
Hydropsyche pellucidula (Curtis)	River Derwent below weir at Belper.	SK345482
	Nant Menial, Llanerfyl.	SJ031096
Hydropsyche angustipennis (Curtis)	Dell Stream, University of Keele.	SJ823442
Hydropsyche contubernalis McLachlan	River Derwent below Bassenthwaite Lake.	NY200322
Plectrocnemia conspersa (Curtis)	Springpool stream, University of Keele.	SJ821439
Polycentropus flavomaculatus (Picket)	Endrick water, near Carron Valley Resr.	NS722838
Neureclipsis bimaculata (L.)	River Derwent below Bassenthwaite lake.	NY200322
Potamophylax cingulatus (Stephens)	Dell Stream, University of Keele.	SJ823442
Anabolia nervosa (Curtis)	Lakes, University of Keele.	SJ823442
Sericostoma personatum (Spence)	Dell Stream, University of Keele.	SJ822445

The net-spinning larvae were identified using the keys produced by Hildrew and Edington (1981) and Badcock (unpublished). The larval identification was sufficiently certain to make rearing to adults unnecessary. The two limnephilid species were identified with the keys published by Hiley (1976) and Wallace (1980) and the sericostomatid, *Sericostoma personatum* by the key in Hickin (1967). The identification of *P. cingulatus* and *S. personatum* was confirmed by rearing larvae to adults, using the key of Macan (1971) for the identification of the adults.

2.2 Maintenance of the larvae at the desired experimental conditions

In general terms this study required a system by which the larvae could be maintained for a period of weeks or months under controlled temperature conditions. Initially, bearing in mind the flowing nature of the habitat of most of these species, attempts were made to maintain the larvae under flowing water conditions. Various systems were tried but all had problems. Most critically, in terms of the aims of the experiment, difficulties arose in reliably maintaining the required temperatures. In addition in linear designs, where water passed from one chamber to another, partitions between chambers became clogged and conditions varied from chamber to chamber. Eventually survival was observed to be as high in still water as in flowing water, provided that the water was well aerated. The final system used was to maintain the larvae in perspex dishes 175mm long, 115mm wide and 50mm deep with a layer of substratum, collected from the sampling site, on the bottom. The water was aerated by bubbling compressed air through an aquarium stone and in addition a slight water current was produced by directing a steady jet of air through fine bore piping horizontally into the water, in a similar way to the method used by Mackay (1981). Under these conditions nets were spun by the net-spinning larvae and particles of food were seen to be trapped by the nets as they passed around the chamber. The chambers were inspected frequently, every day where

possible, and dead larvae removed. The water was replaced by fresh aerated pond water, at the required experimental temperature, at weekly intervals. Food was added after the water was replaced. The choice of food differed for the different families. The Hydropsychidae are generally considered to be omnivorous, members of the family occupying all of the trophic levels from detritivore to carnivore (Wallace et al, 1977). The importance of different food types varies for different species and therefore a variety of foods were provided for each species. This consisted of 1) Ground up preconditioned *Alnus glutinosa* leaves (conditioning being achieved by immersing packs of leaves in one of the campus streams for 4 weeks to allow natural microbial colonisation). 2) Animal material consisting of finely chopped chironomids, 3) Fine detritus was obtained from the sites where the larvae were collected by sieving the substratum with a 250 um sieve. For the three polycentropodids, in addition to the food provided for the hydropsychids, small *Gammarus pulex*, which are particularly favoured by *N. bimaculata* (Otto, 1985) and small *P. cingulatus* (removed from their cases) were added. These were all seen to be consumed by all three species. The limnephilids and *S. personatum* were fed on a mixture of preconditioned *Alnus glutinosa* and *Quercus robur* leaves, the former being particularly valuable food (Iversen, 1974; Otto, 1981). All species grew satisfactorily on these diets and succesfully pupated.

Temperature control was achieved by placing the perspex animal containers on a thermogradient bar. Two thermobars were available, one 1.15x1.15m and one 0.65x0.5m. The temperature control was comparable on the two thermobars, both maintaining the set temperature $\pm$ 0.5^{o}C. A greater temperature range was possible on the larger thermobar, but both were capable of achieving a range in excess of 6 to 18^{o}C which was the temperature range used in the experiments described in Chapters 5 and 6. The small thermobar was only capable of maintaining a constant temperature gradient but the larger thermobar was also capable of maintaining a

gradient of diurnal variations of increasing amplitude. This was used in the experiments discussed in Chapter 7.

An artificial light regime of 14 hour light and 10 hour dark was maintained during the acclimation period.

CHAPTER 3 MEASUREMENT OF RESPIRATION

3.1 INTRODUCTION

Methods for measuring the oxygen consumption of aquatic macro invertebrates can be divided into three types, volumetric, closed and flow through systems.

Volumetric methods, of which the Gilson and Warburg apparatus and Cartesian Divers are the most widely used, depend on animals being kept in water in contact with a known volume of air. Oxygen consumed by the animal is replaced by oxygen diffusing from the air, while the carbon dioxide produced is removed by an absorbent. The resultant change in gas volume or pressure is then measured.

Closed respirometers, which include any system which enclose animals in a fixed volume of water, have been widely used, for example Daniels and Armitage (1969), Calow (1975), Sweeney (1978), Roux (1979) and Harrison and Badcock (1981). The method depends upon the decrease in the oxygen concentration during the experimental period, the reduction being measured either chemically or polarographically at the end of the experimental period or continuously by a polarographic method.

Flow through systems depend upon water passing through an experimental chamber containing the animal. The oxygen consumption is calculated from the reduction in the dissolved oxygen concentration and the flow rate. These systems can be simple, for example Feldmeth (1971) describes an apparatus where the water flow is produced by a siphon system. However the systems are typically more complex, accurately controlled flow rates usually being produced by peristaltic pumps (Dries et al, 1979; Wrona and Davies, 1984).

Each of the methods for measuring oxygen consumption have advantages and disadvantages in terms of accuracy and precision, simplicity of use, operating time and cost. The choice of method depends upon the animal being

studied and the aims of the experiment. Edmondson and Winberg (1971) recommend using the simplest method that will give the desired results.

Whichever type of respirometer is chosen, the dissolved oxygen must be determined. In volumetric methods this is an integral part of the respirometer but with closed or flow through systems the dissolved oxygen must be determined independently. For a closed system it may be determined chemically by the Winkler method (Winkler, 1888) or polarographically, while for a flow through system a polarographic method is normally used, the electrode either being an integral part of the apparatus (Hart, 1980; Ademek and Fischer, 1985; Lampert, 1986) or a sample of water is obtained from the outflow and injected into an electrode chamber (Wrona and Davies, 1984).

For the reasons discussed in Chapter 3.3 a closed bottle method was considered suitable for the investigation of acclimation to constant temperature (Chapter 5) and for measuring the respiration of larvae from the field (Chapter 9) while a fully automatic flow through respirometer was designed to investigate the response of larvae to changes in oxygen concentration (Chapter 4), the ability of larvae to acclimate to diurnal temperature variations of different amplitudes (Chapter 7) and diel variations in the respiration rate (Chapter 4).

3.2 Methods and Results

3.2.1. Closed Bottle Method

Many external factors may inflence the respiration rate of the larvae, some of which are investigated in Chapter 4, and this experimental procedure is designed to take these into account when pertinent.

Larvae were placed singly in glass stoppered bottles ranging in size between 15 and 65 ml, the size of bottle used being determined by the experimental temperature and the size of the larvae. The bottles were half filled with well oxygenated sterilised pond water and left unstoppered for

approximately 12 hours. This allowed the larvae to become accustomed to the bottle and in the case of net-spinning larvae for a retreat to be built. At the end of this period the water was carefully tipped out and the bottle refilled with fully aerated and sterilised pond water at the desired experimental temperature,and the bottle carefully stoppered ensuring that no air bubbles were trapped. The bottles were placed randomly in a water bath, the water being maintained at the desired temperature + 0.5^{o}c by a Grant heater pump in conjunction with a Grant cooler unit. The experiments were run for between 10 and 14 hours, all the larvae in a single experiment being left for the same length of time.

3.2.2. Measurement of Dissolved Oxygen

Modified micro-Winkler method

The chemical determination of oxygen described by Winkler (1888) is still widely used. When manganous sulphate solution (Winkler reagent A) and sodium hydroxide solution and potassium iodide (Winkler reagent B) are added to the water being tested, manganous hydroxide is formed which reacts with the dissolved oxygen to form a precipitate of manganic hydroxide.

$MnSO_4 + 2NaOH \rightarrow Mn(OH)_2 + Na_2SO_4$

$4Mn(OH)_2 + 2H_2O+O_2 \rightarrow 4Mn(OH)_3$

Upon acidification potassium hydroxide is oxidised, free iodine being liberated.

$2Mn(OH)_3+2KI+6H^+ \rightarrow 2Mn^{++} + 2K^+ + 6H_2O + I_2$

In the standard Winkler method the iodine is titrated with sodium thiosulphate to yield a measure of the oxygen present in the sample. The standard method has been critiscised on a number of counts. Caritt and Carpenter (1966) demonstrated a large variation in results obtained by different workers. They suggested this was mainly a result of photochemical oxidation of iodide and due to the loss of iodine by volatilization. A lack of sharpness in the starch-iodine end-point causes a further difficulty

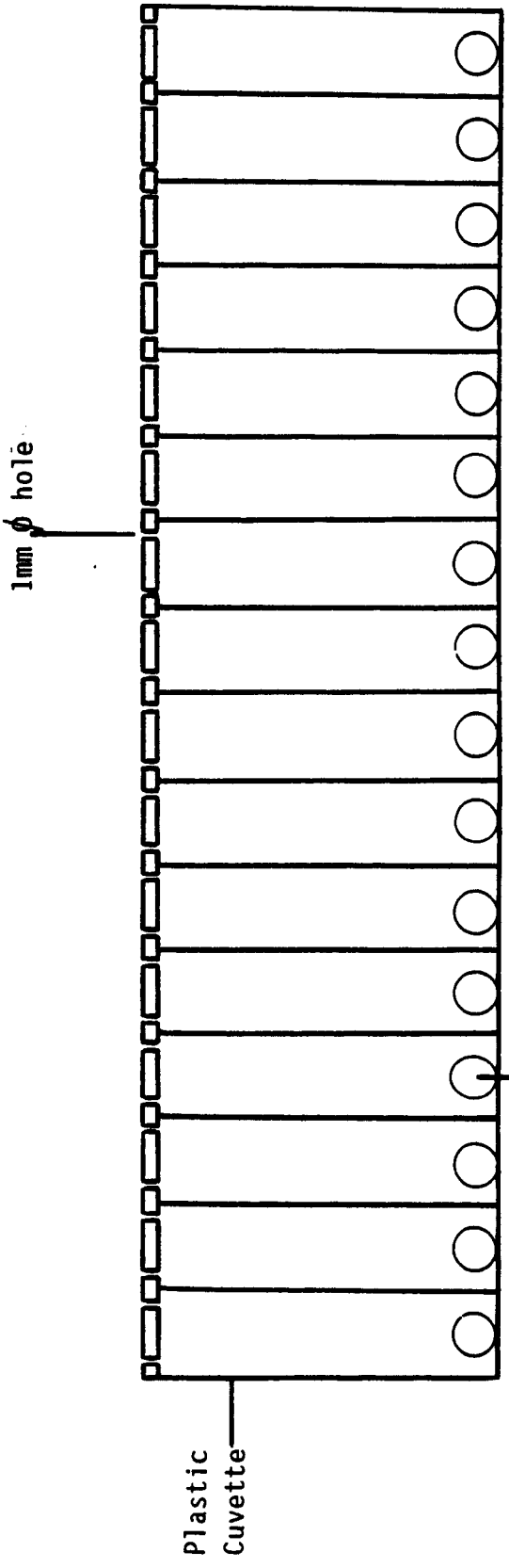


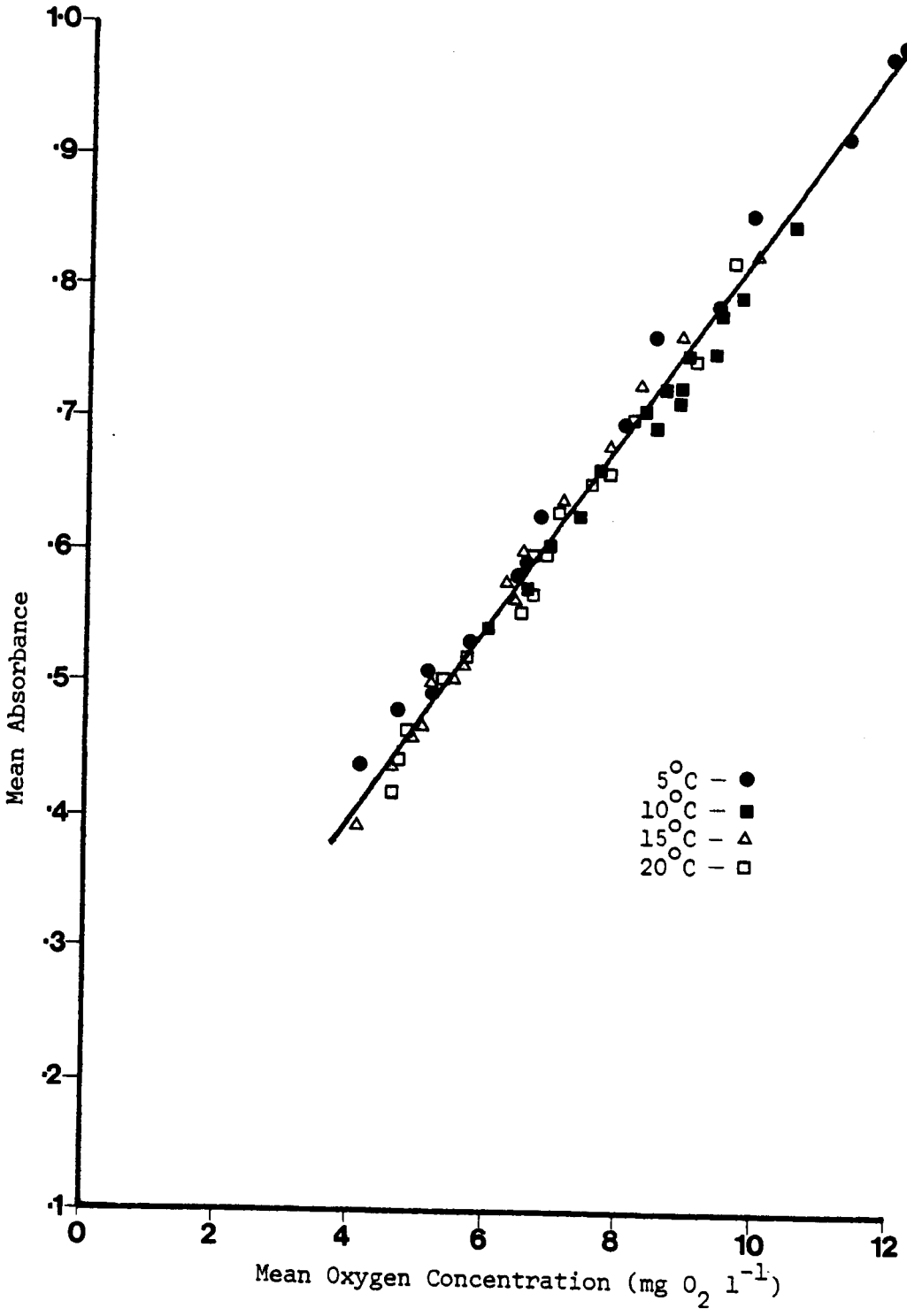
Fig. 3.1. Design of Cuvette apparatus.

(Bryan et al 1976) and the repeated titrations are time consuming and tedious. Many of these problems are overcome by the use of a micro-Winkler method (Fox and Wingfield 1938) and of a spectrophotometer to measure the iodine levels (Rees and Hilton 1977). The micro-technique typically involves the use of a syringe fitted with a means of accurately withdrawing the required amounts of reagents. The contents of the syringe can then be determined by titration, using micro-burettes, or more conveniently by the use of a spectrophotometer to measure the amount of iodine in the solution.

Initial work with a micro-syringe fitted into a Zipette apparatus indicated that the method was time consuming and that great care was needed to ensure standardisation of the mixing procedure in order to achieve a high degree of accuracy and precision. Consequently a simple alternative method was developed. The apparatus, shown in Fig.3.1, consists of 16 plastic cuvettes (4.5ml capacity) with a lid through which two 1mm diameter holes are drilled into each cuvette. A single styrocell bead with a diameter of 4-5mm was added to each cuvette in order to ensure efficient mixing of the reagents. In operation the sample of water to be tested was removed, with a 5ml syringe fitted with a needle, and injected into a cuvette, care being taken to ensure that no air was trapped. The required amounts of Winkler reagents A (50µl) and B (50µl), together with sodium azide to prevent interference by nitrates, were injected into each cuvette using a 50-250µl Finnpipette and the holes plugged using the tips of cocktail sticks. The reagents were fully mixed by invertion of the cuvettes once every 10 seconds for 90 seconds. The required amount of phosphoric acid (100µl) was then added, the cuvettes replugged and inverted once every 10 seconds for 60 seconds in order to dissolve the precipitate fully. The solution, now containing free iodine, was removed using a syringe fitted with a needle and the amount of iodine determined using a spectrophotometer at 450nm.

To calibrate and check on the accuracy of this method, it was compared

Fig. 3.2. The mean absorbances determined by the cuvette method compared with the mean oxygen concentrations determined by the standard Winkler method at 5,10,15 and 20°C.



with the standard Winkler method. Fifteen 65ml glass bottles were filled with water at a wide range of oxygen concentrations. The oxygen concentration was determined using the standard Winkler method and the absorbance recorded following the use of the cuvette method. Two replicates were performed for each method and the experiment repeated at 5,10,15 and $20^{o}c$ to investigate any temperature effect. The mean absorbances determined by the cuvette method were plotted against the mean oxygen concentrations of the water determined by the standard Winkler method (Fig.3.2). The results show a high correlation between the two methods and no apparent temperature effect. Combining the data for the four temperatures produces a single equation $x = (y-0.118)/0.0718$

where x = Oxygen concentration (mg $O_2 l^{-1}$)

y = Absorbance.

From this it can be seen that a decrease in the absorbance of 0.1 units is equivalent to a decrease in oxygen concentration of 1.393 mg$O_2 l^{-1}$.

A possible source of error in this method is a difference between the results obtained from the 16 cuvettes, either caused by differences in the cuvettes or in the amounts of reagents dispensed. Three replicates were performed using the same water sample in each of the 16 cuvettes. Comparison of the absorbance values obtained from each of the cuvettes, the data being shown in Table 3.1, indicates an acceptable degree of variation between the 16 cuvettes, the coefficient of variation being just over one.

Table 3.1 Absorbance values obtained by each of the cuvettes on three separate water samples

Chamber	Water Samples 1	2	3
1	0.73	0.64	0.71
2	0.72	0.64	0.71
3	0.72	0.64	0.72
4	0.72	0.64	0.72
5	0.73	0.63	0.73
6	0.72	0.62	0.73
7	0.72	0.64	0.72
8	0.74	0.63	0.71
9	0.74	0.64	0.71
10	0.73	0.62	0.72
11	0.74	0.63	0.73
12	0.73	0.62	0.72
13	0.73	0.62	0.71
14	0.72	0.62	0.71
15	0.73	0.62	0.72
16	0.72	0.63	0.73
$\bar{x}$	0.728	0.63	0.719
σ_n	0.0075	0.0867	0.0078
Coef.of Var.	1.031	1.375	1.086

A second possible source of error is a difference in results between repeated replicates, a difference in the amount of mixing being the most likely source of such an error. The procedure was repeated five times on five water samples. The results, shown in Table 3.2, again indicate an acceptable degree of variation,the mean coeffecient of variation being less than 0.8.

Fig. 3.3. Demonstration of typical linear relationship between the oxygen electrode response and the oxygen saturation determined by the standard Winkler method.

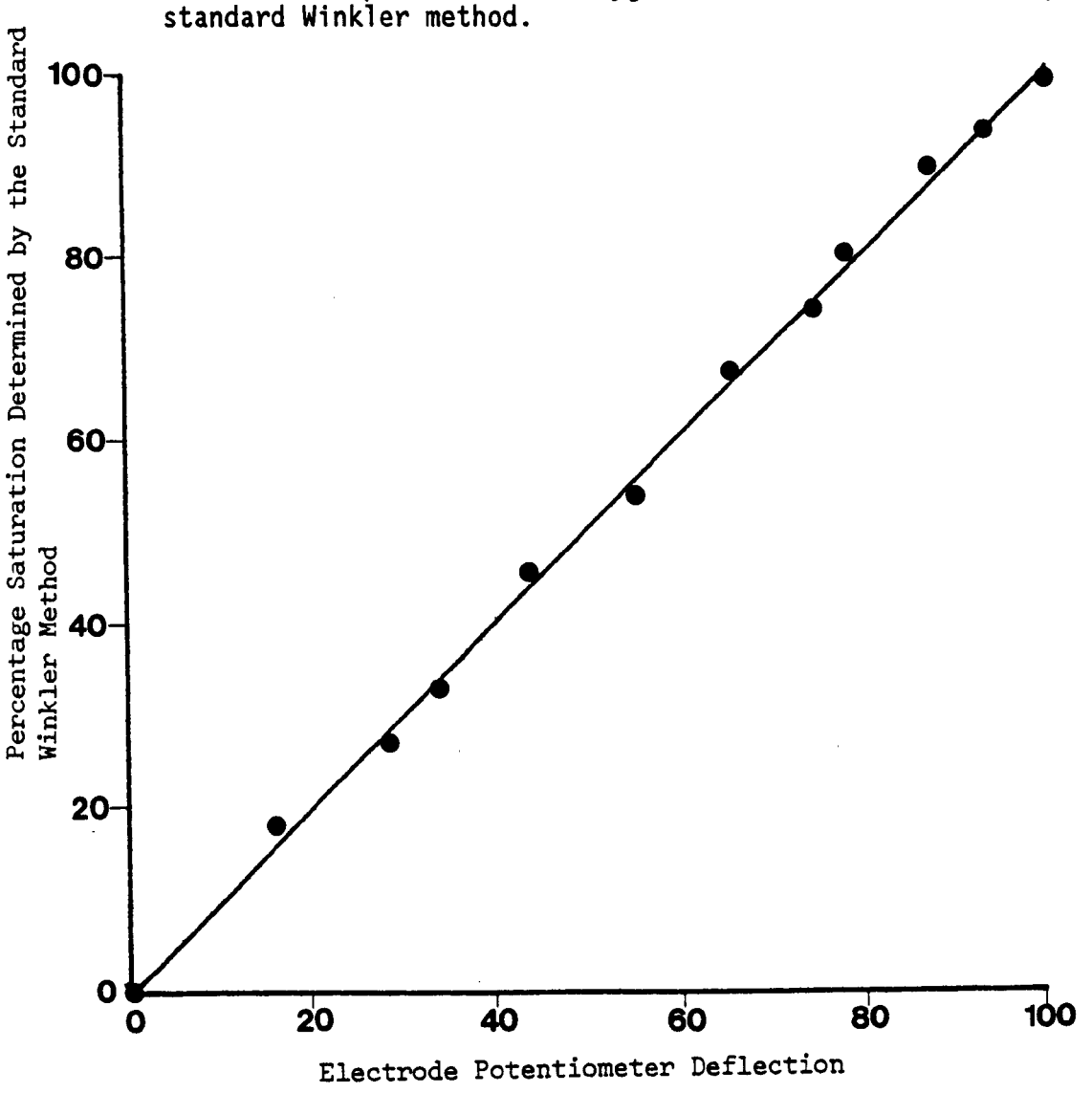


Table 3.2 Comparison of the absorbance values obtained by the cuvette apparatus for repeated replicates on the same water sample

		Sample 1	2	3	4	5
Rep.	1	0.79	0.72	0.69	0.63	0.66
	2	0.78	0.71	0.69	0.63	0.65
	3	0.77	0.72	0.69	0.64	0.65
	4	0.78	0.72	0.7	0.63	0.66
	5	0.78	0.72	0.69	0.64	0.66
	$\bar{x}$	0.78	0.718	0.692	0.634	0.656
	σ_{n-1}	0.007	0.0045	0.0045	0.0055	0.0055
	Coef. of Var	0.91	0.627	0.65	0.868	0.835

3.2.3 Polarographic method

In later experiments a polarographic method was used due to its greater convenience. A Rank Brothers (Bottisham, Cambridge) Clark type oxygen electrode, operating at a 0.7v polarisation potential, was used. Full scale deflection was set at 100% saturation and zero deflection at complete deoxygenation, obtained using a 2% sodium sulphite solution. The electrodes were found to respond in a linear manner, typical data from one experiment being shown in Fig 3.3. Constant diffusion was maintained in the electrode chamber by the use of an internal stirrer and the chamber was maintained at the experimental temperature by water pumped through the water jacket from the experimental water bath. Water from the experimental bottle was removed using a 5ml syringe and injected into the electrode chamber. The short length of time for which the water was in contact with the air prevented significant diffusion of oxygen into the water.

The results obtained by the cuvette and the electrode method were compared. Fifteen 65ml glass bottles were filled with mixtures of air saturated and deoxygenated water to produce a range of oxygen saturations ranging from fully saturated to totally deoxygenated. The data which are

Fig. 3.4. Comparison of the percentage oxygen saturation determined by the cuvette and Rank oxygen electrode methods.

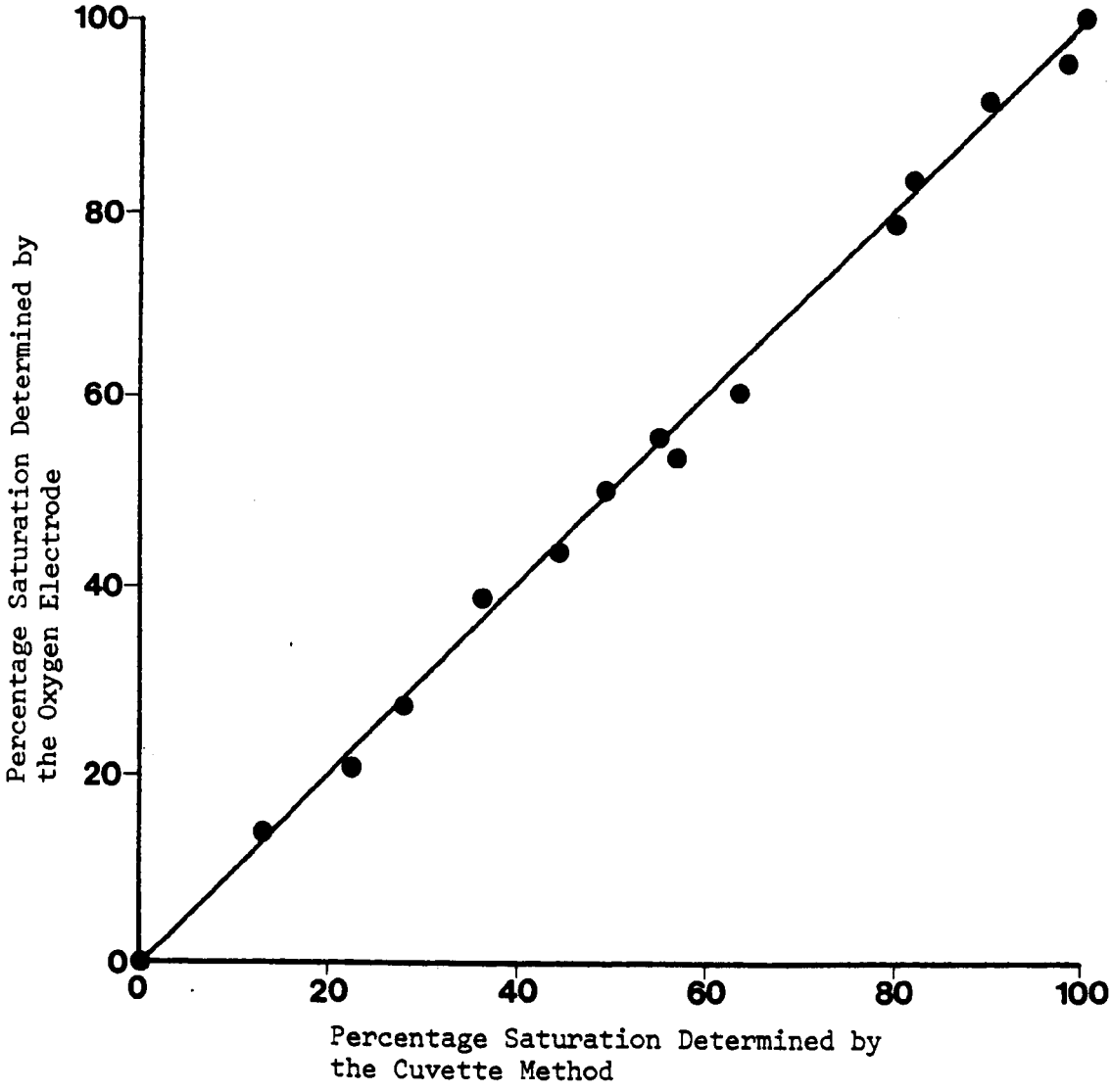
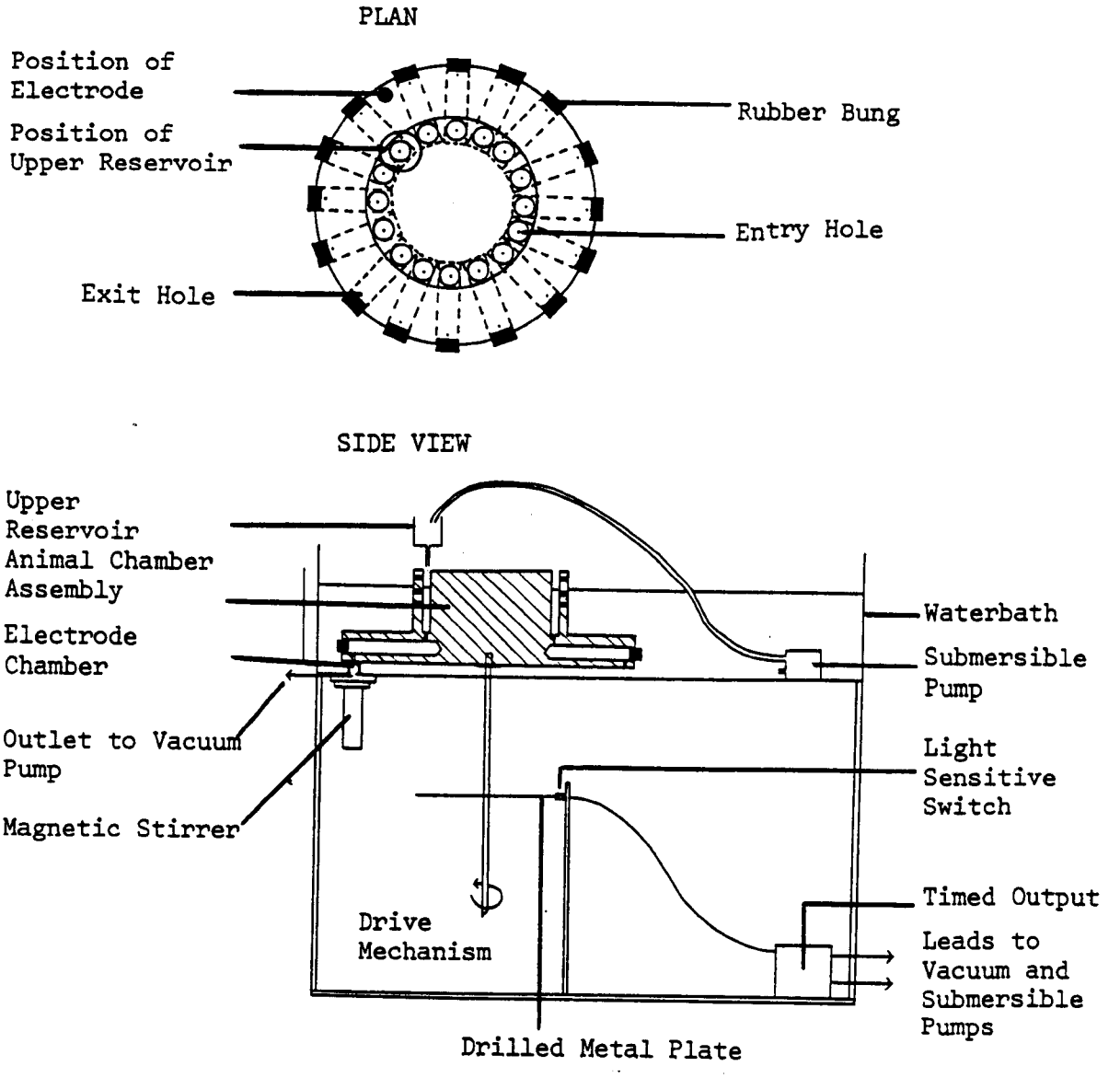


Fig. 3.5. Design of flow-through respirometer.



plotted on Fig. 3.4, indicate that the two methods produce comparable results.

3.2.4 Flow through respirometer

The closed bottle method has been widely critiscised, although it remains a useful method if its limitations are acknowledged. However for some experiments it is a totally unsuitable method, in particular for experiments involving a controlled change in the experimental conditions or where it is necessary to follow respiration over an extended period of time. For these reasons a flow through respirometer was considered more suitable for certain experiments. Ideally the respirometer used needs to satisfy four criteria, it should be capable of performing a reasonable number of replicates (>10),the flow rate and/or the chamber size should be variable, it should work automatically to allow 24 hour data collection and it should be both simple and inexpensive. Existing designs for flow through respirometers failed to satisfy all of these criteria. Relatively inexpensive, simple and automatic designs tend to allow few replicates while respirometers capable of performing a greater number of replicates are more complex, expensive and normally require the samples to be obtained manually. A new respirometer was designed which can operate both in a closed or a flow through mode in a fully automatic way.

3.2.5 Respirometer construction and mode of operation

The general design of the respirometer is shown schematically in Fig. 3.5. The respirometer consists of the following:

Animal chamber assembly

The central part of the respirometer consists of a 24mm high solid perspex cylinder with a diameter of 210mm. Into this 16 animal chambers were formed by drilling holes 63mm long with a diameter of 13mm. Holes with a diameter of 1.5mm were drilled into each of the animal chambers, an entry

hole through the upper surface of the perspex block into the inner end of the chamber and an exit hole through the lower surface 8mm from the outer edge of the cylinder. In operation the chambers are sealed with rubber bungs inserted as far as the exit hole, resulting in the chambers having a volume of 7.3ml. A second perspex cylinder, 45mm high and with a diameter of 110mm, was fixed on to the upper surface of the first cylinder. 16 holes with a diameter of 10mm drilled through the second cylinder were aligned with the small entry holes to the animal chambers, to act as water reservoirs. Holes with a diameter of 1.5mm were drilled into each of these reservoir chambers at heights of 21,27 and 33mm.

Motor drive.

The animal chamber assembly is driven by the use of a constant speed motor geared down to a rate of one revolution in 75 minutes.

Electrode chamber/oxygen electrode.

The electrode chamber is formed as shown in Fig. 3.5, with the bottom half of a standard Rank Brothers oxygen electrode screwing into position under the chamber. The chamber is connected to two small bore pipes (1mm internal diameter) one situated at the top to act as an air vent and the second at the bottom to act as a drain.

Submersible pump.

A Eheim model 1012 rotary pump capable of delivering up to 4.5l min^{-1} was used. In operation the actual rate of delivery was restricted by the use of a screw clamp so as to allow 50ml of water to be pumped in 5 seconds.

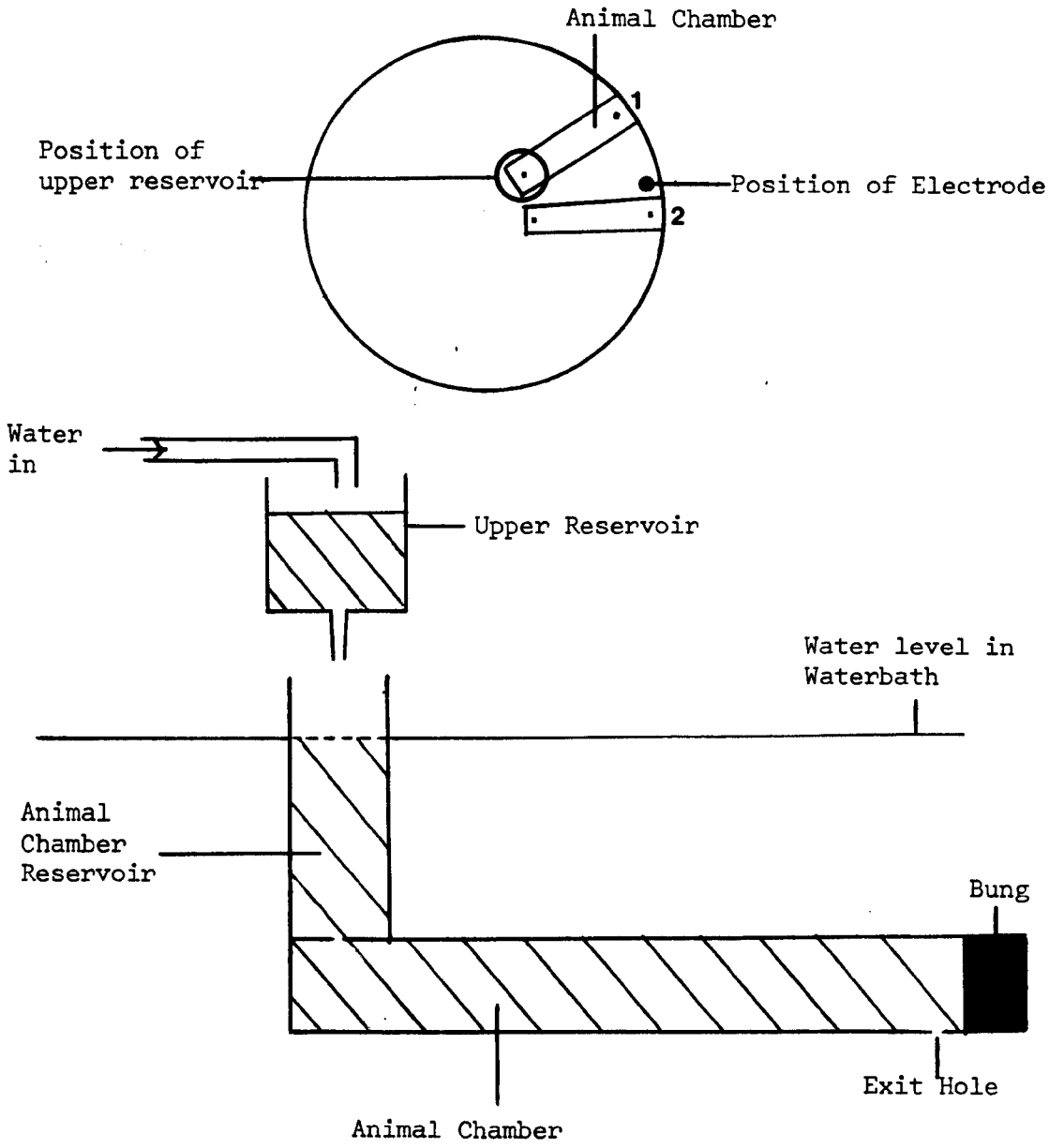


Fig. 3.6. Refilling the chamber which has just been sampled and emptying the electrode chamber.

Peristaltic pump.

A Gilson miniplus 2 pump, capable of pumping upto 500ml $hour^{-1}$ was used.

Vacuum pump

A small vacuum pump was connected through a one litre glass bottle by small bore plastic tubing to the drain hole of the electrode chamber.

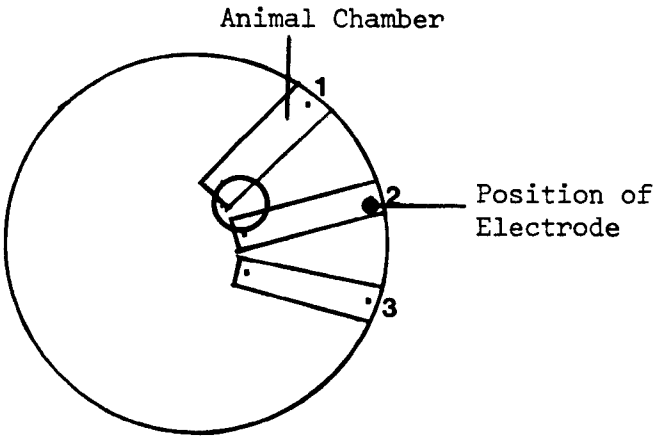
Timed output

A timed output, activated by a light sensitive switch controlled by a drilled metal disc connected to the animal chamber drive mechanism, causes the submersible pump and the vacuum pump to run for five seconds.

3.2.6 Mode of operation - Closed system

The operation is in three stages, firstly at the start of the cycle the water in the animal chamber must be replaced with fresh oxygenated water, then after the required time period a sample of the water is taken from the animal chamber into the electrode chamber and thirdly the electrode chamber must be emptied ready for the next sample. These three stages are achieved in two actions.

As chamber two approaches the sampling position (Fig. 3.6) the light sensitive switch is activated causing the submersible and vacuum pumps to run for five seconds. Water pumped into the upper reservoir runs into the reservoir of chamber one from which a water sample has previously been taken, flushing out the old water. At the same time the electrode chamber, which at this time is sealed against the base of the perspex block, is sucked dry by the vacuum pump.



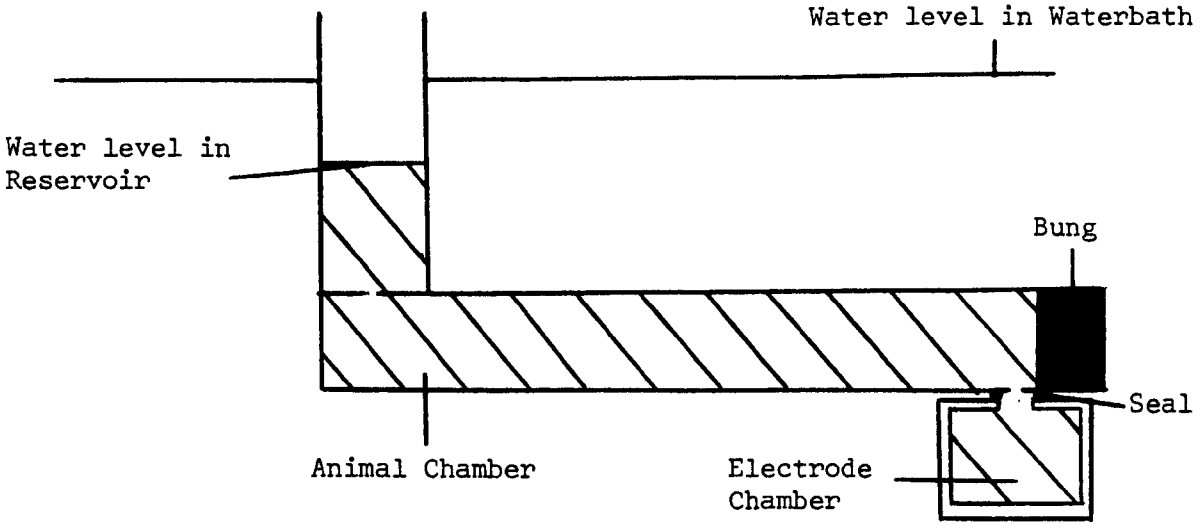


Fig. 3.7 Method of placing the water sample in the oxygen electrode.

As the animal chambers rotate the water is maintained in the chambers by water pressure. As chamber two passes over the electrode chamber (Fig. 3.7) it creates a head of water in the chamber and a sample of water will fill the electrode chamber, the air being expelled through the upper tube.

3.2.7 Mode of operation - flow through system

The procedure for filling and emptying the electrode chamber remains the same as for the closed mode but the mechanism for replenishing the water in the animal chamber differs (Fig. 3.8).

The upper portion of the animal chamber assembly is surrounded by a watertight collar, leaving a gap of 3mm. The water level of the water bath is adjusted until the water is above the uppermost hole into the chamber reservoirs. A variable speed peristaltic pump is used to pump water from the water bath into the bottom of the area enclosed by the collar. This causes water to pass through the animal chambers at a rate determined by the flow rate of the pump. Due to the water sample taken into the electrode chamber the flow rate is not entirely uniform throughout the cycle, there being an additional flow of approximately 1.2ml at the start of the cycle (equal to approx. 1ml/hour).

3.2.8 Testing the respirometer

Important criteria of a respirometer are its precision, the closeness of repeated measures and its accuracy, the closeness of the measured value to the true value. In this system precision may be affected either by differences between the chambers or by differences between the same chamber over time. A comparison was made of the results obtained by the sixteen animal chambers, without animals, over a period of six revolutions. The data, expressed in arbitrary units are shown in Tables 3.3 and 3.4.

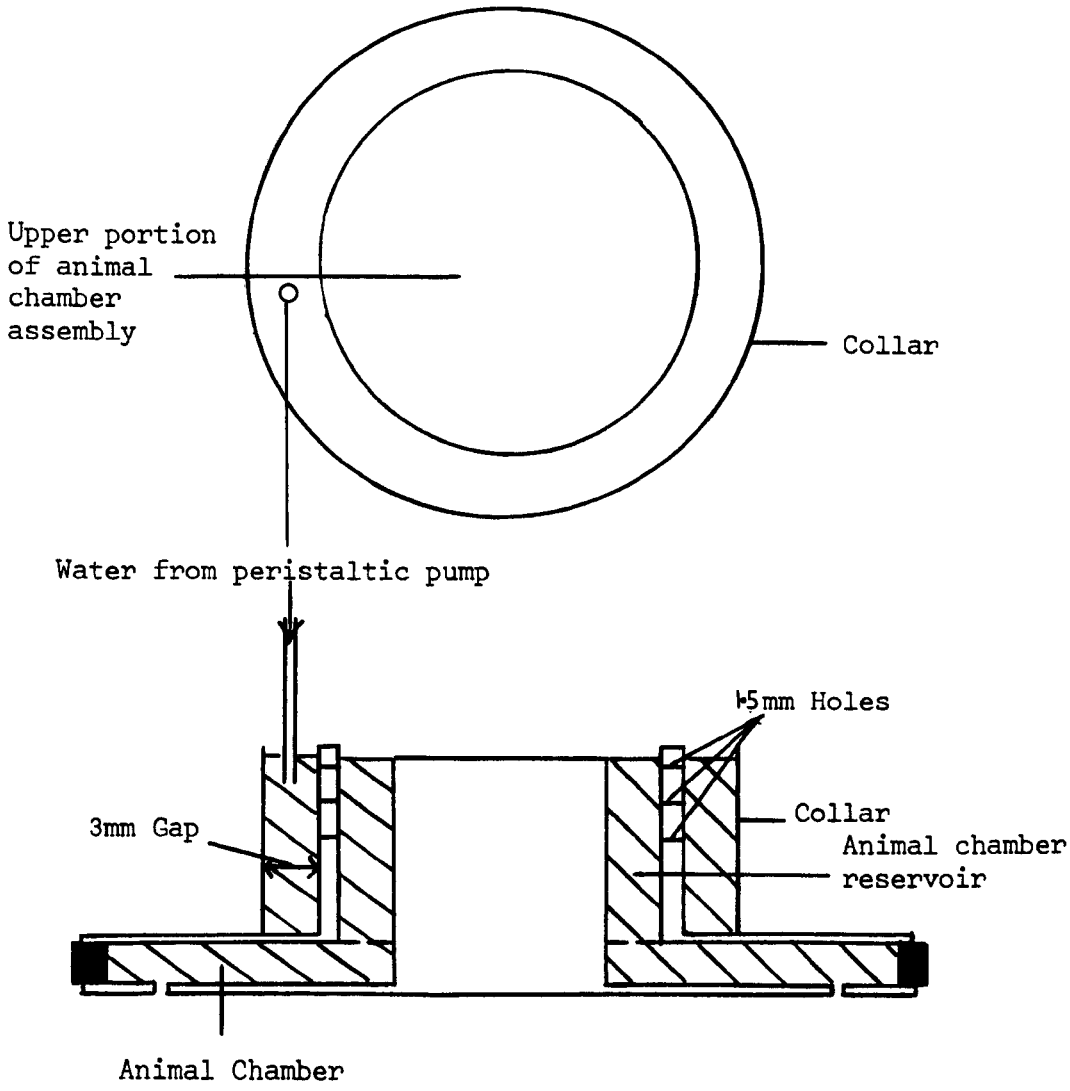


Fig. 3.8 Mechanism for achieving a water flow through the 16 animal chambers.

Table 3.3 Comparison of the dissolved oxygen concentration in the 16 animal chambers. The values being the mean of six replicates expessed in arbitrary units.

Chamber	Mean of 6 replicates	σ_{n-1}
1	45.83	0.516
2	46	0.548
3	45.67	0.408
4	45.42	0.376
5	45.58	0.585
6	45.67	0.606
7	45.83	0.516
8	45.58	0.665
9	45.83	0.753
10	45.92	0.585
11	46.08	0.585
12	45.92	0.665
13	46.08	0.376
14	45.92	0.585
15	46.08	0.376
16	46.17	0.683
$\bar{x}$	45.85	
σ_n	0.208	

Table 3.4 Comparison of the dissolved oxygen concentrations, expressed as the mean and standard deviation in arbitrary units, of the 16 animal chambers over six rotations.

	Rotation							
Chamber	1	2	3	4	5	6	$\bar{x}$	σ_{n-1}
$\bar{x}$	45.25	45.44	45.88	45.94	46	46.59	45.85	0.4711
σ_n	0.306	0.39	0.28	0.428	0.354	0.317		

These data indicate that the precision is good. The differences between the 16 chambers are small and although there is an increasing trend in the values with time, this is small and would probably be compensated for by a similar shift in the control values under experimental conditions.

The accuracy of the system was compared against a standard Clark type oxygen electrode. The upper reservoir was positioned so as to replenish the water in the animal chamber approaching the sampling position. Starting with air saturated water the oxygen concentration was measured in five chambers, and then the motor was stopped. Oxygen was stripped from the water by bubbling nitrogen through it. A further five readings were obtained by restarting the motor and the procedure repeated to produce progressively deoxygenated water until the water was completely deoxygenated. For each oxygen concentration measured, samples were taken from the water bath and injected into the chamber of a Rank Bros. oxygen electrode, calibrated using deoxygenated water for the zero setting and the value obtained with the new respirometer for the 100% saturated setting. The data, shown in Table 3.5, demonstrate a close relationship between the results obtained by the two methods. This indicates that the process of replenishing the water in the chambers is effective, all of the original water being replaced, and the similarity of the first results after a change in oxygen concentration, with subsequent values for that same oxygen concentration indicates a complete removal of the previous sample from the electrode chamber.

Table 3.5 Comparison of the percentage oxygen saturation of eight water samples, determined by the flow through respirometer(a) and a standard Rank Brothers oxygen electrode(b).

	Water sample							
	1	2	3	4	5	6	7	8
a	100	89	83	62	53.5	40	19	0
	98	89	83	59	52.5	39	19	0
	100	89	84	61	52.5	40	18.5	0
	99	89	83	61	52.5	39	19	0.5
	100	89	-	61	52	39	19	0
$\bar{x}$	99.4	89	83.25	60.8	52.6	39.4	18.9	0.1
σ_{n-1}	0.894	0	0.5	1.095	0.948	0.548	0.224	0.224
b	100	89	82.5	60.5	52	39	19.5	0
	99	88	83	62	52	40	17.5	0.5
$\bar{x}$	99.5	88.5	82.75	61.25	52	39.5	18.5	0.25
σ_{n-1}	0.707	0.707	0.354	1.061	0	0.707	1.414	0.354

3.2.9 Testing the respirometer - Flow through method.

As this method depends upon the flow rate through the chambers, differences between the rates, which should be equal in the 16 chambers pose the greatest potential problem to this method. To compare the flow rates in the 16 chambers the respirometer was run for a full rotation with the chambers full of oxygenated water. The motor was then stopped for 30 minutes and the water in the water bath deoxygenated by bubbling nitrogen through it. With the motor restarted the peristaltic pump was switched on, delivering 2.5ml water per minute and left running for 25 minutes. At the end of this period the water in each of the chambers was sampled and the oxygen concentration determined. The data are presented in Table 3.6, where it can be seen that following the period of pumping the oxygen concentrations are reasonably consistent showing that the flow rate through each chamber is approximately the same.

Fig. 3.9. Comparison of the oxygen consumption of *Potamophylax cingulatus* at 17°C, determined using 65ml closed bottles and the flow-through respirometer operated in the closed mode.

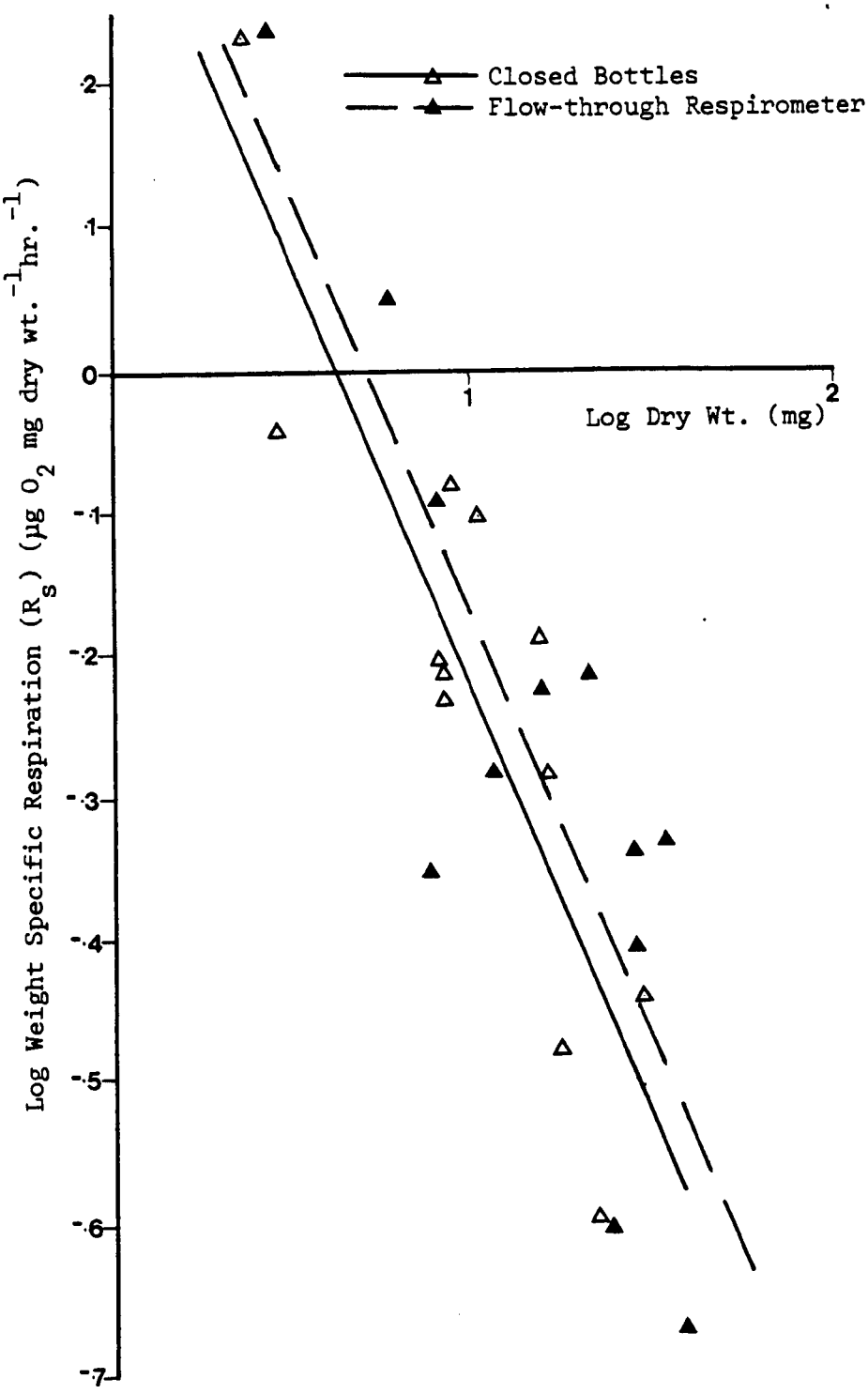


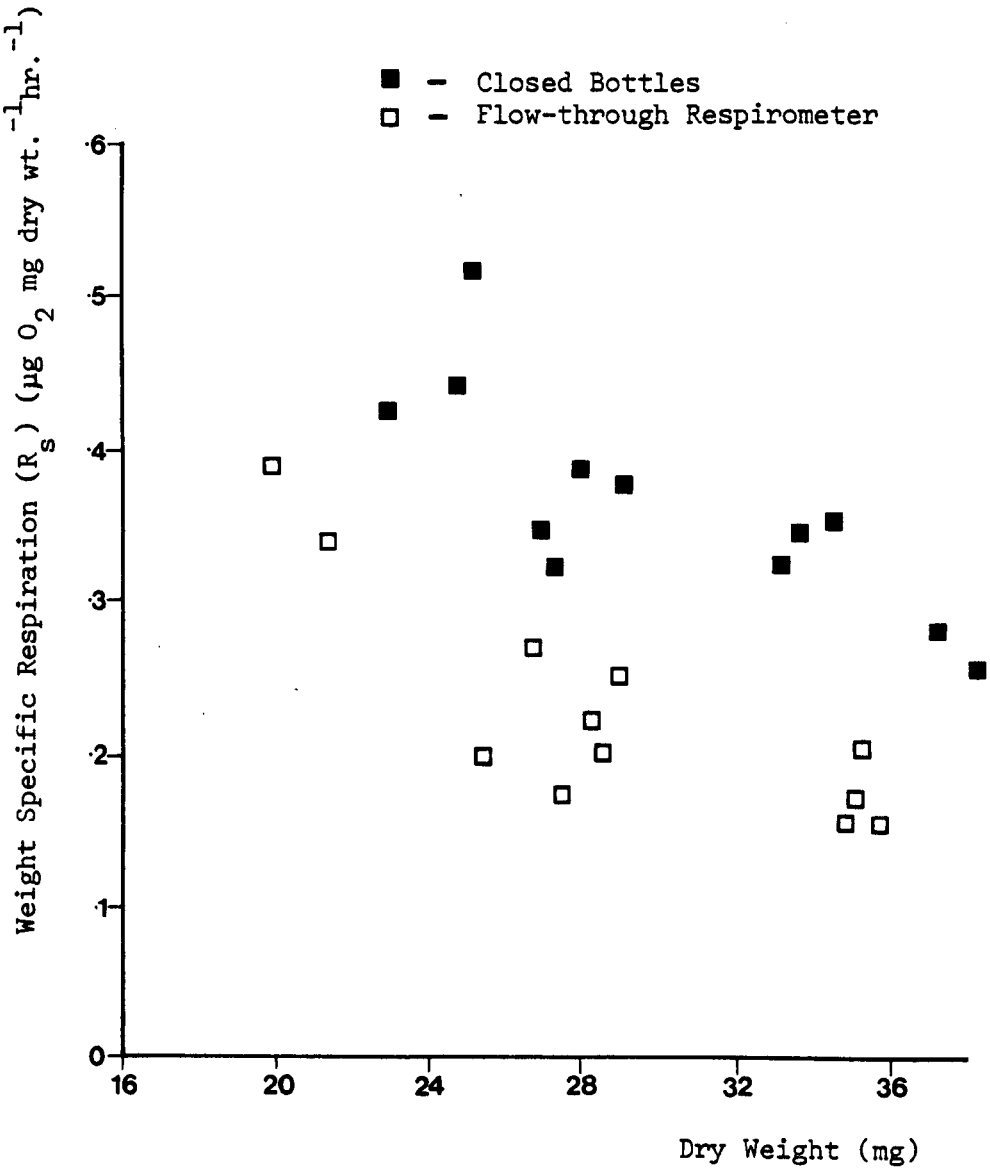
Table 3.6 Dissolved oxygen concentration, expressed as the percentage air saturation, in the 16 animal chambers before and after a 25 minute period of water flow through the chamber

Chamber	dissolved oxygen (% air saturation) before pumping	dissolved oxygen (% air saturation) after pumping
1	94.9	71.7
2	95.1	71.6
3	94.2	71.9
4	94.8	71.1
5	93.9	71.7
6	94.4	71.5
7	94.7	71.5
8	94.0	70.9
9	94.8	70.8
10	95.2	70.8
11	95.2	71.0
12	95	71.4
13	94.4	71.4
14	94.9	70.5
15	93.9	70.3
16	94.7	70.2
$\bar{x}$	94.63	71.14
σ_n	0.431	0.507
coefficient of variation (%)	0.456	0.713

3.2.10 Comparison of the respiration rates determined by the closed bottle method and the flow through respirometer in the closed mode

The two methods were compared using *Potamophylax cingulatus*. 24 individuals of a similar size were split into two groups. Twelve were placed in oxygenated water in 65ml glass stoppered bottles for 19 hours at 17^{o}C. The other twelve larvae were placed in the animal chambers of the flow through respirometer, operated in the closed mode, at 17^{o}C and the oxygen consumption determined over three rotations of the apparatus. From Fig. 3.9 it can be seen that there is no difference in the data determined by the two methods, analysis of covariance indicating that there is no significant difference between either the slope or intercept of the two regression lines.

Fig. 3.10. Comparison of the oxygen consumption of *Potamophylax cingulatus* at 17°C, determined using the closed bottle method and the flow-through respirometer in the flow-through mode.



3.2.11 Comparison of the respiration rate measured by the closed bottle method and by the flow through system in the flow through mode

Results obtained by the two methods were compared using *Potamophylax cingulatus*. 24 individuals of similar size were split into two groups. Twelve were placed in oxygenated water in 65ml glass stoppered bottles for 25 hours at 17°C. The remaining 12 larvae were placed in the chambers of the flow through respirometer, with a flow rate of 9.25ml hr^{-1}, for 24 hours at 17°C The data are shown in Fig. 3.10 which demonstrates a considerable difference between the two methods, the respiration measured in the flow through system being lower than it is when measured by the closed bottle method.

3.3 Discussion

For the experiments on acclimation to constant temperature (Chapter 5) and the measurement of respiration of larvae collected from the field (Chapter 9) a method was desired which allowed a reasonable number of replicates (>24) to be performed simultaneously so as to maintain a constant acclimation, or collection to experiment, period and to minimise extraneous factors which may influence respiration determinations made at different times. The experimental method should also be simple, inexpensive and allow affective temperature control.

The use of a Gilson respirometer was considered as it fulfills these requirements. However the need to shake the flasks to prevent gas stratification produces a highly unnatural, disturbed environment which has been considered to make this method unsuitable for macroinvertebrate studies (Nagell 1975). Initial trials suggested that for the net-spinning larvae, if allowed to spin a retreat, the shaking had no apparent affect. However cased larvae were highly disturbed by the movement and thus this method was discounted.

The use of a flow through system, despite the advantages of this

method, was not considered suitable for these experiments. The major disadvantage of this method being that with existing designs only a limited number of replicates are possible, without the system becoming complex and expensive.

The closed bottle method appeared to offer many advantages in terms of the ease of replication, temperature control, simplicity and inexpensiveness. However the method has been critiscised (Mann 1958, Kamler 1969) on three counts : 1) The environmental conditions vary during the experiment, most important being the decrease in oxygen tension and the build up of metabolites. 2) Water stagnation leads to the developments of oxygen and carbon dioxide gradients which may stress the animals and alter their activity. 3) Inconsistent respiration readings are obtained in relation to the duration of the experiment. The first problem is inherent in the method but can be minimised by reducing the experimental time to the minimum which produces a measurable decrease in oxygen. Lampert (1984) suggests that a 5% reduction may be sufficient if dissolved oxygen is measured by a good system. However in considering the experimental time period used, a compromise must be made between the first and third problems, a reduction in the time may exacerbate the inconsistency of the respiration rates over time. Lawton and Richards (1970) measuring the respiratory rates of damselfly larvae restricted the fall in oxygen tension to between 5 and 10%, while Hildrew and Edington (1979) working on hydropsychid larvae allowed a fall of 30%. In this study the oxygen tension decrease was restricted to below 20-25%. The third problem can be minimised by maintaining a constant experimental duration, although as Wrona and Davies (1984) point out this still poses difficulties in making intra and interspecific comparisons between studies using different time periods. For this reason the duration of the experiments were kept constant at between 10 and 14 hours. Adjustments for variation in oxygen consumption at different temperatures was achieved by altering the size of the bottle,

which was shown to have no affect upon the respiration rate (Chapter 4.8). The second problem of water stagnation can be overcome by the use of a stirring mechanism (Grant and Hawkes, 1977; Sweeney, 1978). However care must be taken that the stirring mechanism itself does not affect the animals and the advantages of simplicity and the ease of using many replicates are sacrificed. For these reasons the bottles were left unstirred.

Obviously the conditions in a closed bottle are unnatural, particularly for species from fast flowing streams. Therefore in these experiments it is hoped that although the measured respiration is likely to have little relevance to the actual respiration rate in the field, it does accurately reflect differences between different experimental groups of larvae. For the determination of the dissolved oxygen a micro-Winkler method was initially considered the most suitable method. The simple apparatus described in Chapter 3.2.2 provided a useful alternative to the traditional micro-syringe methods, being more rapid and producing more consistent results.

Two of the possible errors of this method are caused by loss of precipitate and the trapping of air in with the sample. Careful introduction of reagents A and B ensured that the precipitate formed towards the lower end of the cuvette and therefore none should be lost in the overspill. Care during the introduction of the sample and reagents, and in the plugging of the cuvettes, normally ensured that no air bubbles were introduced. The presence of small air bubbles appeared not to alter significantly the dissolved oxygen but as a precaution the sample was repeated if an air bubble was introduced. This method appears useful in the absence of an alternative method, although later in this study the use of an oxygen electrode was found to be more convenient. The use of the cuvette apparatus and the oxygen electrode were shown to produce comparable results and so there appears to be no difficulty in comparing the data obtained by

the two methods.

Although the closed bottle method was considered suitable for many experiments it was not considered suitable for all types, those involving changes in the experimental conditions or where respiration was to be followed over time, being examples. For these experiments a flow through system was considered more appropriate. Existing designs sufferred from two main drawbacks, construction of a respirometer capable of running a reasonable number of replicates (>10) creates problems in terms of complexity and cost, and in general systems having a reasonable number of replicates require manual operation. The respirometer described here has a number of advantages over previous designs. As constructed the respirometer has 16 animal chambers, normally 12 being used as experimental chambers and 4 as controls. This number could readily be increased by altering the dimensions of the apparatus if more replicates were required. The flow rate can readily be varied, although due to the nature of the water delivery system it must be constant in all of the chambers. The chief advantage is in the fully automatic nature of the respirometer, allowing 24 hour operation which is particularly useful in experiments over an extended time period. As the respirometer requires a single oxygen electrode and a peristaltic pump with a single output and the animal chamber assembly and water bath are easily made, the apparatus is inexpensive. Finally the respirometer has the added advantage in that it can be used as a closed system as well as in the flow through mode, again automatically. The design also has a number of disadvantages. The size of the animal chambers cannot readily be varied, although a number of animal chamber assemblies could be built with animal chambers of different sizes. The rate of rotation of the apparatus is also fixed, restricting the collection of data from each chamber to once every 75 minutes. Ideally the motor would be replaced by a variable speed motor allowing the rate of rotation to be varied. The most serious problem is perhaps that caused by the size of the electrode chamber

which is approximately 1.2ml. This causes no difficulty when the respirometer is used in the closed mode but in the flow through mode it results in an additional flow of approximately 1.0ml hr^{-1} at the start of the cycle. This aberration in the flow rate obviously has more affect when the overall flow rate through the chamber is lower. This difficulty could be reduced by decreasing the size of the electrode chamber or by replacing the Clark type oxygen electrode with a Radiometer type requiring a much smaller sample. This has so far not been tried and therefore it is not known whether the methods for filling and emptying the chamber would still work.

Comparison of the results obtained by this respirometer in the closed mode with the standard closed bottle method (Fig.3.9) show them to produce comparable data. However in contrast the respiration rate measured by the flow through system is considerably lower than that found by the closed bottle method (Fig.3.10). This has been reported before, for example the respiration of *Nephelopsis obscura* and *Erpobdella punctata* measured by Wrona and Davies (1984) using a flow through system was significantly lower than the values obtained for the same species by Osborne et al (1980) and Linton et al (1982) using a Gilson respirometer. This clearly illustrates the difficulty of determining the significance of a measured respiration rate in an ecological sense and highlights the particular problem of making comparisons between data obtained by closed and flow through systems.

CHAPTER 4 ENDOGENOUS AND EXOGENOUS FACTORS AFFECTING OXYGEN CONSUMPTION

4.1 Introduction

The oxygen consumption of an animal can readily be determined within the limits of each method of measuring respiration discussed in Chapter 3. However the interpretation of this value in an ecological sense and in attempting inter-specific comparisons is more problematic, being complicated by many factors. These include endogenous factors (body size, activity and diel rhythms) and exogenous factors including temperature, oxygen tension, light, food, current and animal density.

4.1.1. Endogenous Factors

Body size.

The relationship between the respiration rate and the body size is one of the classical topics of physiology (Bertalanffy 1951). Many studies have demonstrated a general relationship whereby larger individuals consume more oxygen than smaller ones, but the increase in the respiration rate is slower than the increase in weight and therefore the weight specific respiration (oxygen consumption weight^{-1}) decreases with increasing size (Lampert, 1984). The functional relationship between oxygen consumption and body weight is a power function

where $R=aW^{b}$ or $\frac{R}{W}=aW^{(b-1)}$. (R=respiratory rate, W=weight).

Alternatively in logarithmic forms:

$\log R = \log a + b \log W$ and $\text{Log } \frac{R}{W} = \log a + (b-1)\log W$

(Roff, 1972; Huebner, 1973; Calow, 1975; Franke, 1977; Epp & Lewis, 1980; Sutcliffe, 1984; Laybourn-Parry & Tinson, 1985). Considerable attention has been paid to the value of b, the slope of the regression line. This value normally lies between 0.6 and 1.0. A value of approximately 0.67 indicates that the metabolic rate is proportional to the surface area while 1.0 indicates a proportional relationship between the metabolic rate and the

body weight. Hemmingsen (1960) stated that the normal value for b in poikilotherms is 0.75 ± 0.015; however published values suggest that this value is more variable (Lampert, 1984). Although the bulk of attention has been concentrated on the slope, differences have also been demonstrated in the elevation of the regression lines (Schiemer and Duncan, 1974), which Lampert (1984) suggests may be an indication of the metabolic intensity.

Activity

Activity is a complex phenomenon which has not been extensively investigated, despite its obvious implications for the interpretation of other studies on respiration. Increased activity causes an increase in energy expenditure and many studies have demonstrated variations in activity level, caused for example by the time of day (Chapter 4.3), however the significance of this is difficult to evaluate. The presence and type of substratum (Roux, 1979) current (Feldmeth, 1970b) and the effect of disturbance caused by introduction of the animals into the respirometer (Roff, 1973; Sutcliffe, 1984) have all been shown to influence activity.

A number of studies of the relationship between oxygen consumption and the oxygen content or temperature of the water have demonstrated three levels of oxygen consumption (Newell & Northcroft, 1967; Bayne et al, 1973; Nagell, 1974). These levels may be described as standard, routine and active, corresponding to minimum, 'normal' and maximum activity respectively. As will be discussed later, other environmental factors may have different effects upon these levels of oxygen consumption which should be considered when interpreting data.

Diel Activity

Information on diel activity is particularly important in considering experimental protocol and interpreting data obtained. Duval & Geen (1976) demonstrated that significant errors can result from failure to consider

activity, frequently measured in terms of drift rates. Müller (1966) defined 'night active' and 'day active' species depending on whether higher rates occured during the night or day. Studies on caddis larvae have shown *Potamophylax cingulatus*, *Drusus annulatus* and *Odontocerun albicorne* (Elliott, 1970) and *Sericostoma personatum* (Elliott, 1969) to be night active. In contrast *Anabolia nervosa* has been shown to be day active (Lehmann, 1965; Elliott, 1970). Fewer studies have investigated diel variation in oxygen consumption. Studies by Sigmon (1978) and Hart (1980) found no predictable variation in the O_2 consumption of a dipterous larva and a freshwater shrimp (*Caradina nilotica*) respectively. None of these studies have attempted to correlate diel variations in activity with the variation in oxygen consumption.

4.1.2. Exogenous Factors

Temperature

The influence of temperature on the metabolisms of poikilotherms has been widely investigated. (Precht, et al, 1973; Lampert 1984). Laybourn-Parry & Tinson (1985) suggest that the conclusions drawn from these studies vary, but fall into two categories:

(1) The influence is regular and constant and can be expressed using Krogh's normal curve, or

(2) Poikilotherms are capable of maintaining constant rates when confronted by changes in temperature.

In general terms it can be stated that the relationship is not linear (Roff, 1973) but rather respiration increases at an accelerating rate with increasing temperature, with the complication in some instances of a region of temperature change where the respiration remains stable. This plateau has been said to correspond to the thermal range 'normally' encountered (Calow, 1975; Epp & Lewis, 1980; Harrison & Badcock, 1981) and is taken to indicate that poikilotherms have an ability to maintain a preferred level

of metabolism (Epp & Lewis, 1980b).

The relationship between metabolic rate and temperature is generally characterised by the use of Q_{10} values, calculated from

$$\frac{R_2}{R_1}^{\left(\frac{10}{T_2-T_1}\right)}$$ where R_1 and R_2 are the measured respiratory rates and T_1 and T_2 the corresponding temperatures. However the Q_{10} values reported vary considerably within the same species and the Q_{10} is dependent upon body size and experimental or environmental temperature (Rao & Bullock, 1954; Huebner 1973; Tonapi, 1977; Lampert, 1984) and due to the non-linear relationship involved,it depends upon the region of the temperature range over which it is calculated.

To obtain a single numerical value independant of temperature a temperature characteristic (μ) may be used. Assuming that metabolic rate follows the equation: $R = R_0^{-(\mu/r.T)}$

where R = respiration rate at the given temperature, r = gas constant ($8.3 Jmol^{-1} degree^{-1}$), T = absolute temperature (^oK), R_0 = constant reflecting metabolism when T approaches o, μ = activation energy ($Jmol^{-1}$). The value of μ may be obtained from the slope of the log respiratory rate plotted against the absolute temperature, or calculated by the equation:

$$\mu = r. \frac{\log R_2 - \log R_1}{1/T_1 - 1/T_2}$$

Oxygen Tension

A number of studies have investigated the relationship between oxygen consumption and oxygen concentration, and in some instances attempts made to interpret this ecologically. Two extreme responses have been found, the oxygen consumption is proportional to the oxygen concentration (conformers) or the response to decreasing oxygen concentration is to maintain the oxygen consumption rate until some critical point is reached, after which it declines rapidly (Regulators). However the relationship is complex,

Mangum & Winkle (1973) state that the distribution of the response to declining oxygen appears to defy comprehension, the ability to regulate aerobic respiration at different partial pressures of oxygen transcending taxonomic, ecological and morphological patterns. Different responses may be found in closely related species (Lampert, 1984) and data may be interpreted as being indicative of either a conformer or a regulator by different workers. The mathematical relationship between O_2 uptake and O_2 concentration has been tested by Mangum & Winkle (1973) who in comparing a number of models concluded that a quadratic polynomial provided the best fit. The effect of oxygen concentrations above 100% air saturation have been ignored despite the widespread occurrence of such oxygen concentrations.

Light

Few studies have been performed on the effect of the intensity and quality of light. Some investigations on diel rhythms, for example Ulanoski and McDiffett (1972) have demonstrated that light acts as an important exogenous influence upon respiratory rhythms. Konsta ntinov (1971) demonstrated an increase in respiratory rate in chironomids in response to light which may have been a result of increased mobility.

Crowding and Container Size

A number of attempts have been made to test the interaction between respirometer volume, density of animals and the number of individuals. Goss & Bunting (1980) detected no significant crowding effect for *Daphnia magna* and *Daphnia pulex* at 0.5-2.0 individuals ml^{-1}. Roff (1973) demonstrated no significant effect on the metabolic activity of *Limnocalanus macruras* in containers of sizes ranging from 30-330ml (at the same density of 1 individual ml^{-1}. Sweeney (1978) reported no density effect upon the

respiration of the mayfly *Isonychia bicolor*. In contrast Paterson (1983 and 1985) demonstrated a reduced average respiration rate in a freshwater bivalve as the number of individuals increased, and in addition they changed from being regulators as individuals to partial conformers in groups.

Feeding and current will obviously also have a major influence on the respiratory rate but these factors were considered beyond the scope of this study. Two further factors were investigated, the amount of oxygen consumption incurred by the case of cased larvae and the choice of control bottle methods.

Method and Results 4.2 The Influence of Body Size

4.2.1. Method

Larvae of a wide range of sizes were collected and maintained unfed for approximately 24 hours at the experimental temperature. The respiration rate was measured by the closed bottle method, using 65ml glass bottles. The live weight was determined after gentle blotting to remove excess water and the dry weight determined following 48 hours oven drying at 60^{o}C.

4.2.2. Results

Data are presented for two species, a cased caddis, *Sericostoma personatum*, and a net-spinning larva , *Hydropsyche angustipennis*. The functional relationship between the respiratory rate and body size can be expressed in a number of ways, the most commonly used being log R = loga+blogW which gives the individual respiration rate, and logR_s = loga+(b-1)logW which gives the weight specific rate. (R=Respiratory rate, W=Weight, R_s=R/W). The form in which the weight is expressed may influence the relationship between weight and respiration if, for example, the relationship between wet and dry weight varies with size. For *S. personatum* a comparison is made between the relationships using both wet and dry

weights while for *H. angustipennis* only dry weights are used. These data are shown in Table 4.1.

Table 4.1 Regression analysis for log oxygen consumption (R) and log weight specific respiration (R_s) against log weight for (a) *S. personatum* and (b) *H. angustipennis*

	r	slope	inter-cept	SE est.	SE slope	SE intercept
(a)						
Log wet wt/log R	0.702	0.2595	0.4759	0.0592	0.0575	0.09
Log wet wt/log R_s	-0.925	-0.7275	0.4528	0.067	0.0651	0.1019
Log dry wt/log R	0.656	0.2285	0.6813	0.0617	0.0537	0.0469
Log dry wt/log R_s	-0.948	-0.7748	0.682	0.0613	0.0534	0.0466
(b)						
Log dry wt/log R	0.403	0.1232	0.9446	0.072	0.0679	0.0553
Log dry wt/log R_s	-0.953	-0.8768	0.9447	0.072	0.0679	0.0553

These data for *S. personatum* suggest there is little difference in the relationship between weight and R or R_s, irrespective of whether wet or dry weights are used, the correlations and the slope of the lines being similar. In contrast, for both species the correlation is considerably higher when the weight specific respiration is used rather than the oxygen consumption. In all cases the slopes are similar irrespective of whether wet/dry and R/R_s are used. For both species the value for b, in the region of 0.23-0.26 for *S. personatum* and 0.12-0.13 for *H. angustipennis*, is considerably lower than the expected value (in the region of 0.67) if the respiration is proportional to the surface area.

4.2.3. Discussion

These data indicate that either wet or dry weight are equally suitable and therefore the choice depended upon the ease of obtaining the weights.

The ease of obtaining
dry weights indicated that the latter were more suitable.

The higher correlations achieved by the relationship between log weight and log R_s compared with those obtained between log weight and log R suggest that the former provides a closer relationship and therefore this is used in later experiments. The size dependancy of both R and R_s has implications for the interpretation and comparison of data obtained from groups of larvae with different size distributions, the use of mean values for R and R_s not being suitable. This is discussed further in Chapter 5.

4.3 Activity and Oxygen Consumption

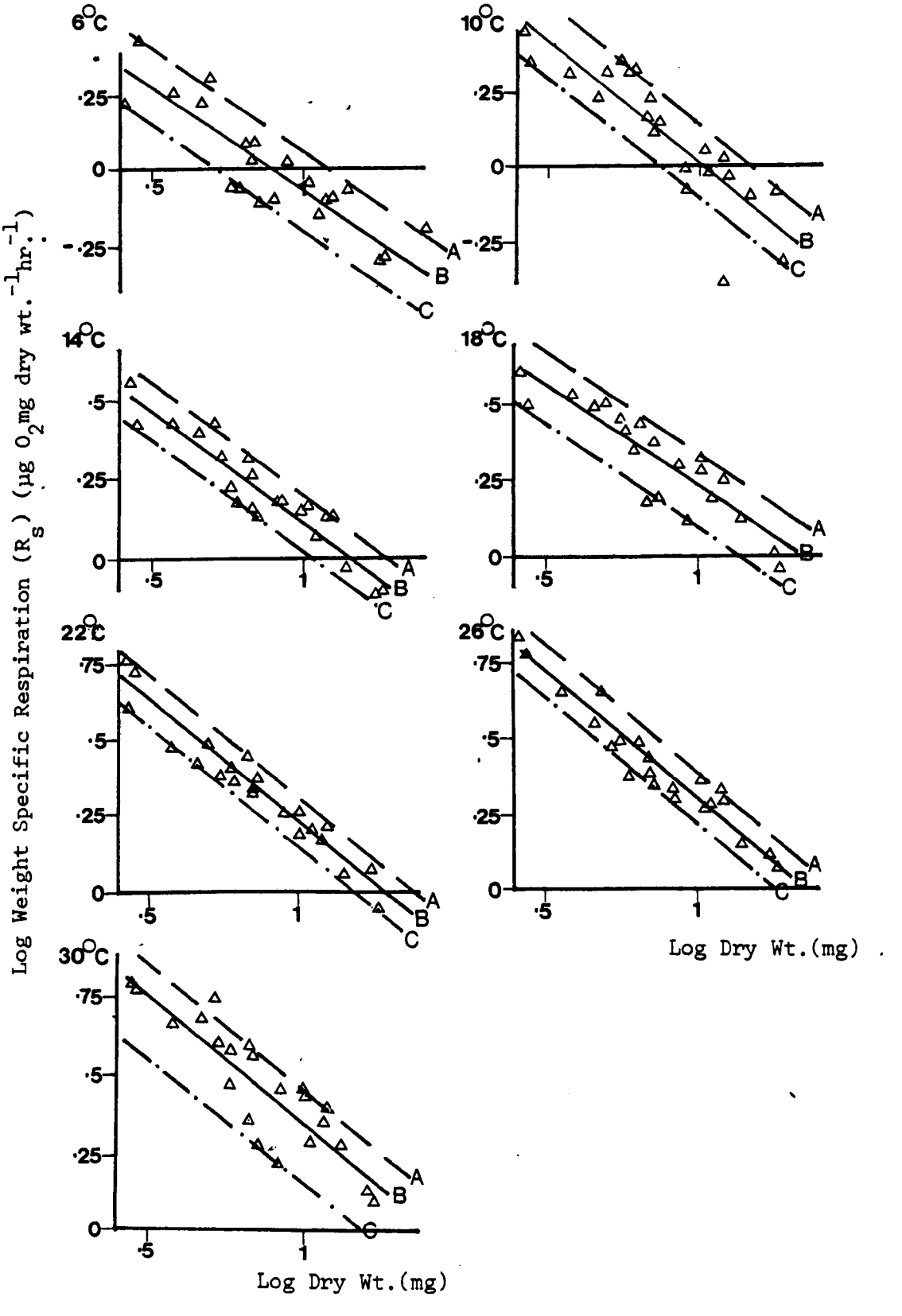
4.3.1 Method

Activity is complex but may be subdivided into respiratory movements and other behavioural movements. Relating oxygen consumption to activity posed difficulties as each period of activity was generally observed to be much shorter than the overall period over which it was feasible to measure respiration. An experiment was performed on *H. angustipennis* in order to attempt to assess three levels of oxygen consumption, standard, routine and active, by measuring the oxygen consumption over as short a time period as possible. Twenty three larvae, with a wide range of body weights, were enclosed individually in 3ml of fully oxygenated water in 5ml glass syringes. After times ranging from 30 minutes to 15 minutes depending upon the experimental temperature, the oxygen content of the water was determined using a Clark type oxygen electrode. The dry weights were determined after oven drying at 60^{o}C for 48 hours. The experiment was repeated at temperatures ranging from 6 to 30^{o}C.

4.3.2. Results

The relationship between log weight specific respiration and log dry weight at each experimental temperature is shown in Fig. 4.1. The continuous lines are least square regression lines calculated from the

Fig. 4.1. The relationship between log weight specific respiration and log dry weight for *Hydropsyche angustipennis*, at temperatures between 6 and 30°C determined over a short time period (15-30 minutes). Line B - least squares regression line. Lines A and C represent the maximum and minimum observed oxygen consumption respectively (fitted by eye).



whole data while the dashed lines, A and C, are fitted by eye to the highest or lowest values respectively, with the slope equal to that of the regression line. A is taken to represent the maximum oxygen consumption, the active rate, while C represents the minimum oxygen consumption, the standard rate. These data indicate a considerable variation in the oxygen consumption of the larvae, some due to intraspecific variations, but in part a result of differences in the level of activity. The range of intermediate points between lines A and C reflect the range of activity that the animals exhibit in the respiration vessel.

4.3.3. Discussion

These data give some indication of the effect of activity on oxygen consumption but it is difficult to draw firm conclusions on the relationship between activity and oxygen consumption when the activity patterns are so short and variable. It should be noted that the fitting of lines A and C is rather subjective and do not necessarily represent either the lowest or highest oxygen consumption or activity levels. Observations during the experiment did suggest that at three temperatures, 6,10 and $30^{o}C$, a number of larvae appeared to show no locomotory or respiratory movements and it was these larvae in which the lowest oxygen consumptions were obtained. At the other temperatures all of the larvae were observed to make some respiratory movements, but these were much reduced in some larvae and so their oxygen consumption may closely approximate a standard consumption rate. It was not evident from this experiment whether or not the higher oxygen consumption represents the physiological maximum value.

Fig. 4.2. *Hydropsyche angustipennis*; Diel oxygen consumption pattern of 12 larvae under natural light. (Ordinate: mean weight specific respiration; Abscissa: time in hours).

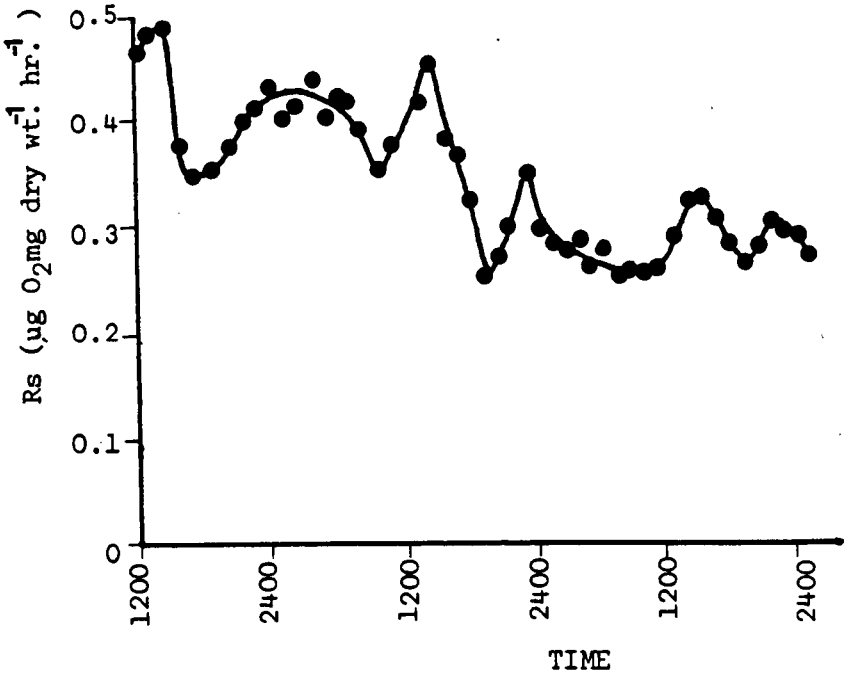
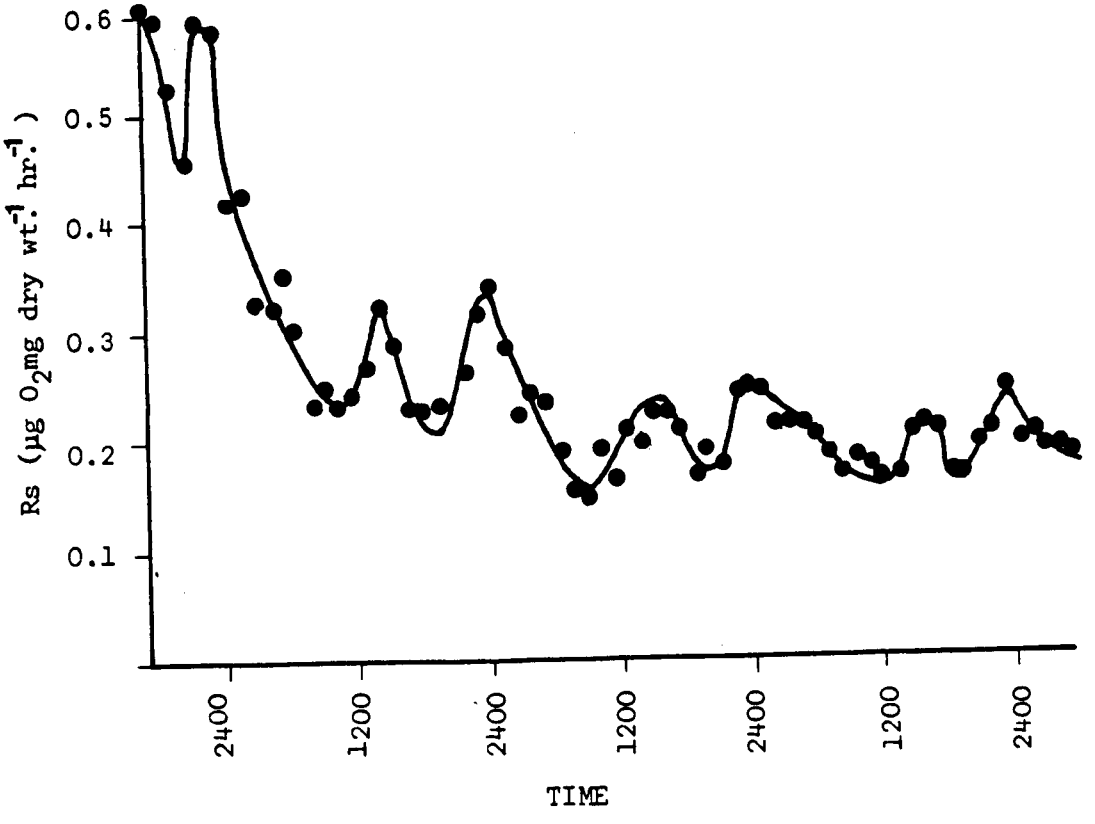


Fig. 4.3. *Potamophylax cingulatus*; Diel oxygen consumption pattern of 12 larvae under natural light. (Ordinate: mean weight specific respiration; Abscissa: time in hours).



4.4 Diel Activity

4.4.1. Method

Twelve larvae of *H. angustipennis* or *P. cingulatus* were placed individually into animal chambers of the rotating respirometer operated in the closed mode as described in Chapter 3.2.6. The remaining 4 chambers were unoccupied and acted as controls. After an initial period of 12 hours, to allow the larvae to settle in the chambers under the experimental conditions, the oxygen consumption was measured over the subsequent 60 hours for *H. angustipennis* and 79 hours for *P. cingulatus*. The experiment was performed at a constant temperature of $15^{o}C$ under natural lighting (Darkness 2130/2200 hours - 0400/0430 hours). The dry weight of the larvae were determined after oven drying at $60^{o}C$ for 48 hours.

4.4.2. Results

The data for the two species are shown in Figs. 4.2 and 4.3. In both species there is a clearly defined repeated pattern, peaks in oxygen consumption occuring around 2400-0200 and 1400-1600 hours. In *H. angustipennis* the larger peak occurs at 1400-1600 hours whilst in contrast for *P. cingulatus* the higher peak occurs at 2400-0200 hours. In both cases superimposed on the peaks and troughs a general decline in the oxygen consumption over the experimental period can be seen.

4.4.3. Discussion

The double peaked nature of these data is perhaps surprising in view of previous work on diel rhythms. Assuming that an increase in oxygen consumption is, partly, at least, a result of increased activity it suggests a bimodal activity pattern. The presence of two peaks of activity has been proposed by Aschoff (1957 and 1966) and Elliott (1970) demonstrated a bimodal activity pattern in one species he studied, *Odontacerum albicorne*, but the remaining species, including *P. cingulatus*,

were shown to have a single peak of activity. The peak of activity was found to be controlled exogenously by light, for *P. cingulatus* activity, being greatest in the dark. This contrasts with these data where one of the peaks in oxygen consumption occurs during the light period. In the field the observed pattern of oxygen consumption, if it does reflect activity, could represent a means of avoiding fish predation, many fish species tending to be least active during the periods of peak larval oxygen consumption. However care must be taken in relating the results obtained in this experiment to the field situation as factors such as diel temperature variations, food and the presence of substrate or shelter could all alter the activity and oxygen consumption pattern.

The general decline in oxygen consumption over the experimental period could represent a settling in period as the larvae became accustomed to the experimental conditions. However this appears unlikely as the larvae spent 12 hours in the chambers before the experiment. Alternatively it may represent starvation effects as the larvae were unfed over the experimental period.

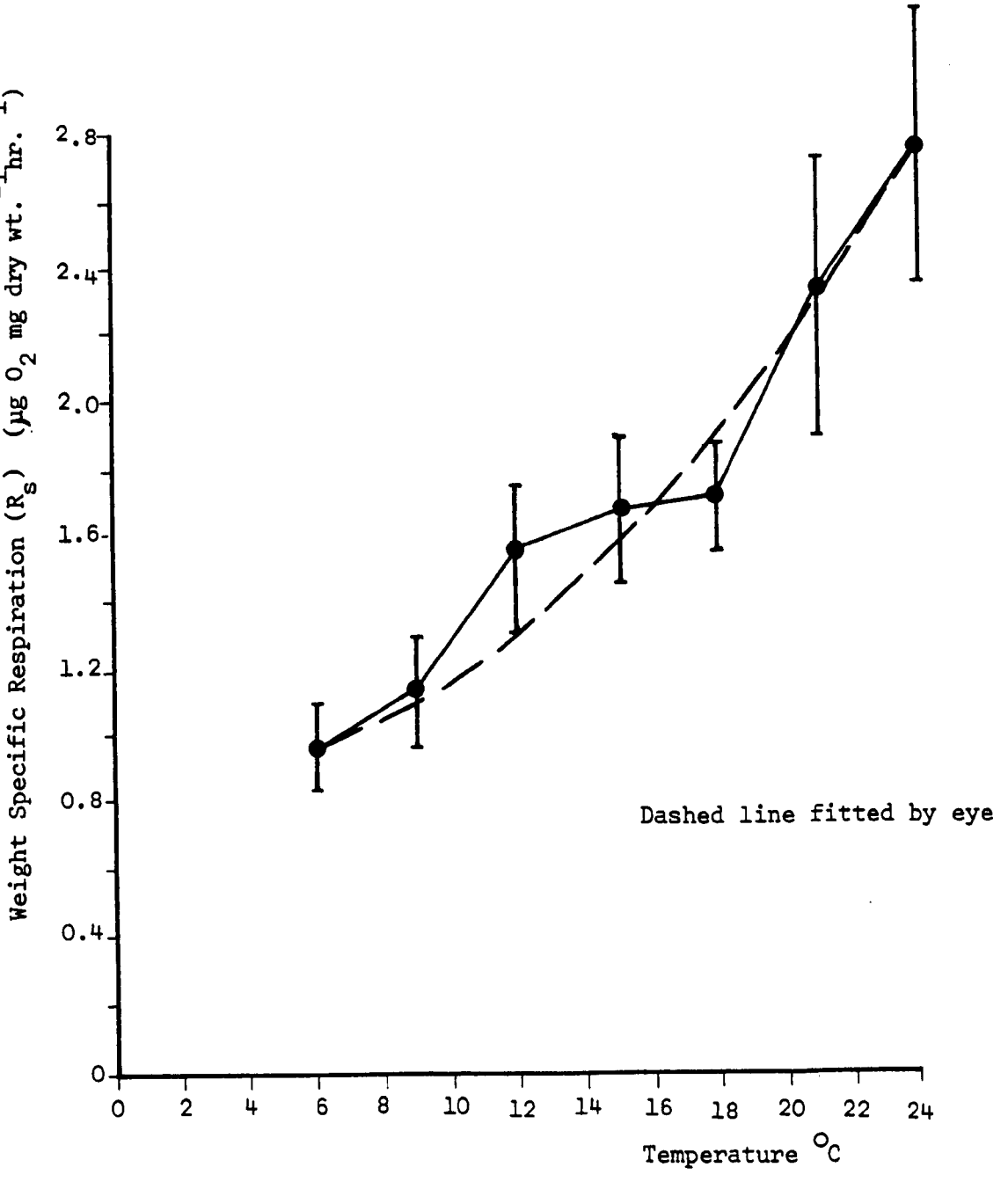
These data demonstrate the disadvantage of not continuously monitoring the rate of oxygen consumption, as occurs in the closed bottle method. For example, an experiment comparing respiration over the period 0600-1800 hours and 1800-0600 hours would show a higher night time respiration for *P. cingulatus* and lower for *H. angustipennis* but it would completely fail to demonstrate the bimodal nature of the relationship with time.

4.5 Temperature

4.5.1. Method

The relationship between oxygen consumption and temperature was investigated on *H. angustipennis*. The oxygen consumption was determined using the closed bottle method, using 32.5ml glass bottles. The experiment was repeated on a single group of 12 larvae at temperatures increasing from

Fig. 4.4. Temperature-metabolism curve for *Hydropsyche angustipennis*. Variation around mean values (n=12), expressed as standard error, is indicated by vertical lines.



6 to 24°C in steps of 3°C. The duration of each stage of the experiment was decreased as the temperature increased, the entire experiment lasting approximately 36 hours. The relatively short time period over which the experiment was run should prevent significant acclimation to temperature occuring. Between each stage of the experiment the bottle was carefully emptied and refilled with fresh fully oxygenated water at the correct temperature. At the end of the experiment the larvae were oven dried at 60°C for 48 hours.

4.5.2. Results

The mean weight specific respiration of the twelve larvae are presented in Fig. 4.4. This clearly shows that the oxygen consumption increases with temperature over the temperature range investigated. There is a suggestion of a plateau in the relationship in the region 12 to 18°C but the relationship could also be represented by the dashed line.

A number of methods are available to characterise the relationship between temperature and metabolism. The most widely used method, despite its limitations, is the Q_{10} value. These are calculated from both the dashed and continuous lines and presented in Table 4.2. The Q_{10} values calculated from the dashed line are apparently constant at around 1.78, showing no dependence upon the temperature range used in the calculation. In contrast the Q_{10} values calculated from the continuous line differ considerably, being over 2.1 at the lower and upper temperature ranges, but only just over one over the range 12 to 18°C, indicating near temperature independence.

Table 4.2 Q_{10} Values over the temperature range for the data presented in Fig. 4.4 for *H. angustipennis* larvae

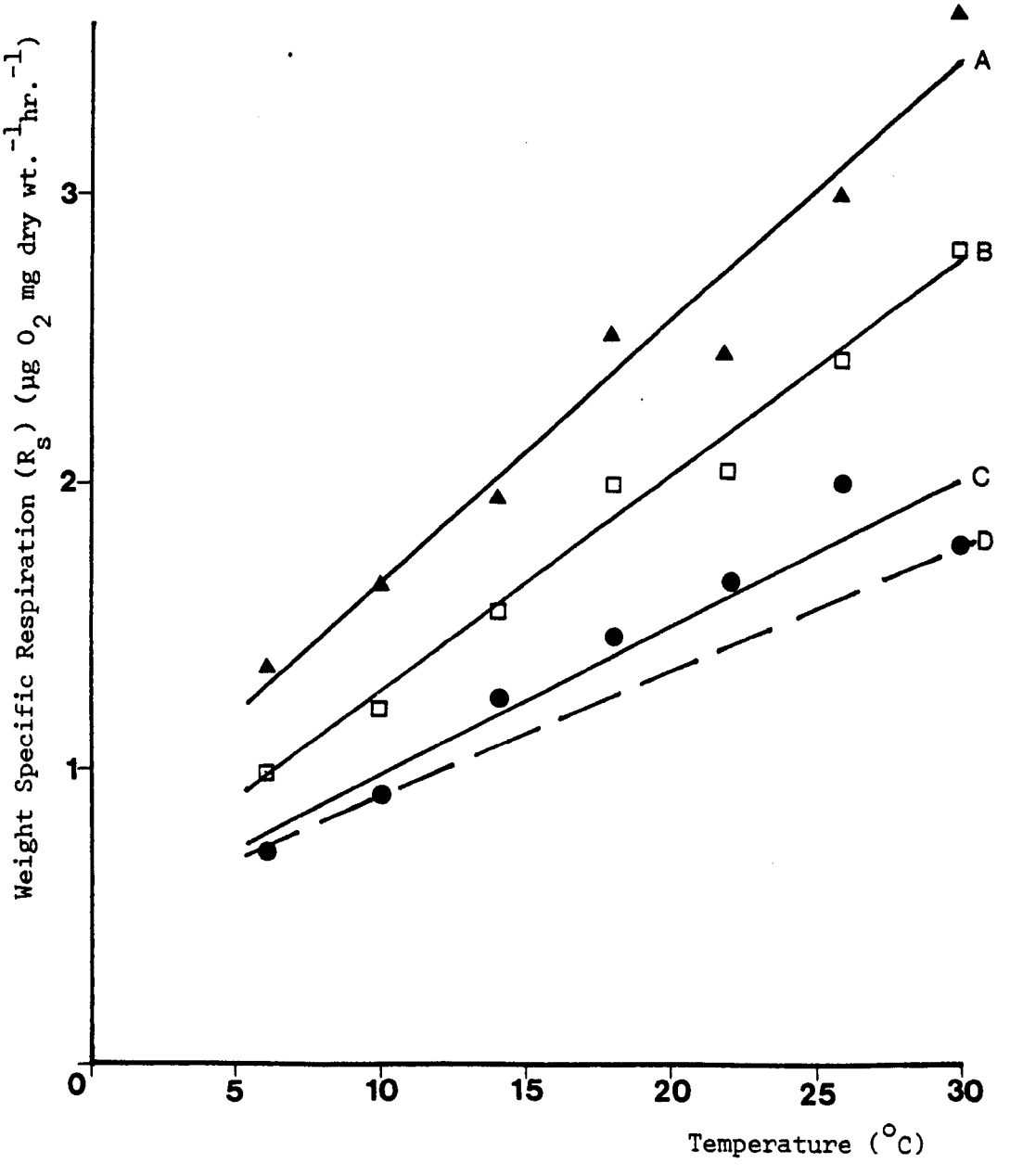
Temperature range (°C)	Q_{10} Values Dashed Line	Continuous Line
6-24	1.7877	1.7877
6-12	1.7349	2.1606
12-18	1.8143	1.214
18-24	1.8152	2.1782

4.5.3. Discussion

These data indicate a typical temperature metabolism relationship. An increase in temperature causes an increase in oxygen consumption, with the rate of increase accelerating as the temperature increases. There appears to be a plateau in the relationship between 12 and 18°C. As in other studies (Hildrew and Edington, 1979) this corresponds to the thermal range normally encountered by the species and indicates an ability to maintain a preferred level of metabolism (Epp and Lewis 1980b) although these data suggest that this may cause increased respiratory costs at the lower end of the temperature range.

A number of studies, for example Newell and Northcroft (1967) have demonstrated a difference in the relationship between 'standard' and 'active' metabolism and temperature, the 'standard' rate often being relatively temperature independant compared with the 'active' rate. From the data presented in Chapter 4.3.2 (Fig 4.2) values are abstracted from lines A,B and C at each experimental temperature, for larvae with a theoretical body weight of 8mg. These data are presented in Fig 4.5. As discussed in Chapter 4.3.3 the 'standard' rate calculated at 14,18, 22 and 26°C included some periods of activity, only at the remaining temperatures was no activity observed. This suggests that line D may more closely

Fig. 4.5. Relationship between 'active' (A), 'average' (B) and 'standard' (C&D) respiration and temperature for *Hydropsyche angustipennis* larvae with a theoretical dry body weight of 8mg. See text for details of abstraction of values from Fig. 4.1.



represent the 'standard' rate rather than line C. From the slopes of the lines it is apparent that a particular increase in temperature causes a larger increase in the 'active' metabolism than it does in the 'standard' metabolism. For example a $10^{o}C$ temperature increase causes an increase of 0.44 and 0.91 μgO_2mg dry wt^{-1} hr^{-1} in the 'standard' and 'active' rate respectively. However if the Q_{10} values for the two lines (A and D) are compared over the range 6-$30^{o}C$ they are 1.51 and 1.45 for the 'active' and 'standard' rate respectively, the difference is relatively small and the 'standard' rate is not temperature independent ($Q_{10} \neq 1$) as has been found in other studies (Newell and Northcroft, 1967).

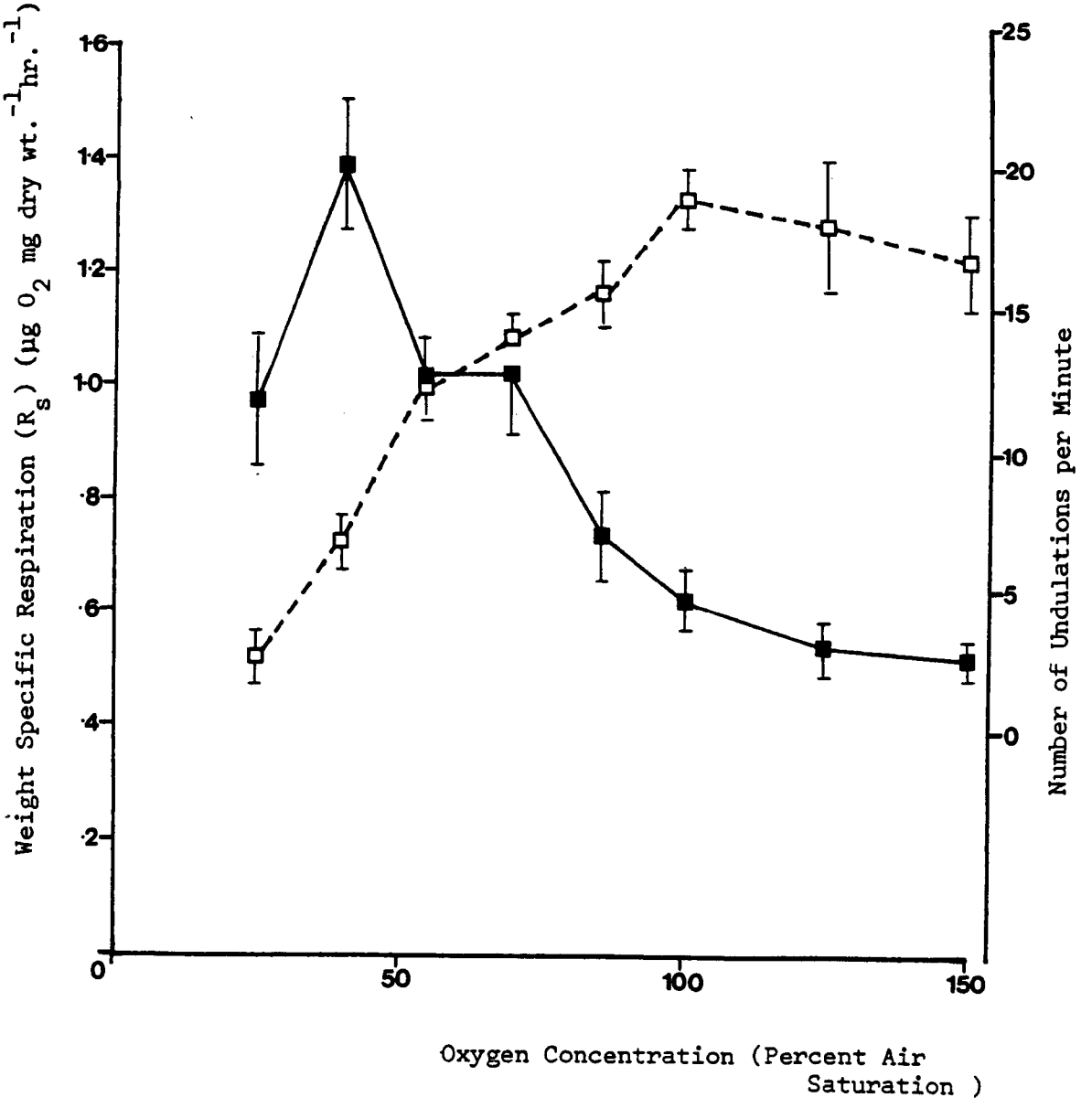
4.6 Oxygen

4.6.1. Method

The oxygen consumption of *H. angustipennis* was determined at a range of oxygen concentrations, from 150 percent air saturation (PAS) to 25 PAS, using the flow through respirometer described in Chapter 3. Twelve 5th instar larvae, of a similar size, were introduced into the animal chamber and allowed to settle overnight at $15^{o}C$ with a flow rate of 9.25ml hr^{-1} $chamber^{-1}$. The oxygen concentration in the respirometer water bath was adjusted to 150 PAS by passing oxygen through the water, the concentration being checked by the use of a Bibby SM01 oxygen electrode. As each animal chamber passed the sampling position 50ml of water from the bath were injected into the upper reservoir, the side holes being blocked off during this time. This resulted in an initial oxygen concentration (C_o) in the chamber equal to the oxygen concentration of the water in the water bath. The final oxygen concentration (C_{tn}) of each chamber was recorded after one revolution of the apparatus the four control chambers providing checks on the value for C_o.

The oxygen concentration of the water bath was reduced, in steps, by bubbling nitrogen through it, the experiment being repeated at 125, 100 and

Fig. 4.6. Variation in oxygen consumption (□) and the number of body undulations (■) with oxygen concentrations between 25 and 150 percent air saturation (Mean values ± standard errors) in *Hydropsyche angustipennis* larvae.



85 PAS. The whole experiment was repeated with a second batch of larvae at 70, 55, 40 and 25 PAS.

The number of body undulations in a 5 minute period were recorded for each larva at a point in the revolution of the animal chamber approximately 180^{o} away from the sampling point.

4.6.2. Results

The weight distribution of the two groups of larvae are similar (Group 1, $x = 7.517; \sigma_n = 0.863$. Group 2, $x = 7.525; \sigma_n = 0.866$) and therefore the comparisons are made by the use of mean values.

As the flow through system was not allowed to reach a steady state, the oxygen consumption was calculated by the use of:

$$R = \frac{V.(C_o - C_{tn})}{1-\exp(-Vt_n w^{-1})}$$

where R = oxygen consumption, V = flow rate, w = chamber volume, t_n = time, C_o = initial concentration and C_{tn} = final concentration. (Propp et al, 1982). The mean weight specific respiration and the number of undulations $minute^{-1}$ are plotted against the oxygen concentration in Fig. 4.6. The weight specific respiration is at a maximum at 100 PAS (approx. $10mgO_2l^{-1}$). As the oxygen concentration decreases the respiration drops, the decrease apparently accelerating below approximately 50 PAS. Above 100 PAS a decrease in weight specific respiration is also observed, although the rate of decrease is less than that observed below 100 PAS.

Above 100 PAS the decrease in weight specific respiration could reflect the slight decrease in body undulations over this change in oxygen concentration. However below 100 PAS the decrease in weight specific respiration is mirrored by an increase in the number of undulations which increased, until the lowest oxygen concentration (25 PAS) when the number decreased.

4.6.3. Discussion

These data indicate that this species is a conformer, the oxygen consumption decreasing as the oxygen concentration decreases. A value of 50 PAS, equivalent to approximately 5.0mgl^{-1} oxygen, appears critical, the rate of decrease in oxygen consumption being double that found above this value. At 40 PAS (Approx. 4.0mgl^{-1} oxygen) the larvae were showing signs of distress and were undulating rapidly. By 25 PAS (Approx 2.5mgO_2l^{-1}) the larvae appeared very distressed, movement and body undulations being reduced and uncoordinated. No previous data are available for caddis larvae relating oxygen consumption to oxygen tension. A number of studies have investigated the relationship between body undulations and oxygen tension (e.g. Leader, 1971; Philipson & Moorhouse, 1974). These data are compatible with those of Philipson & Moorhouse for the same species. They demonstrated, an increase in the number of undulations from 2.5 min^{-1} up to a maximum of just over 20 min^{-1}, as the oxygen concentration decreased from 11 to 3mgl^{-1} oxygen. As the oxygen concentration was reduced further the number of undulations fell to less than 5 minute^{-1} at 1mgO_2l^{-1}.

4.7 Light

4.7.1. Method

The effect of light on the metabolism of *H. angustipennis* was investigated using the closed bottle method. Twenty 5th instar larvae of similar size were placed in 65ml glass bottles, half of which were covered in aluminium foil to exclude light. The bottles were filled with fully oxygenated water, stoppered and placed randomly in a water bath at 17^{o}C. Light was provided by a 60 watt bulb positioned approximately 25-30cm above the experimental bottles. The oxygen content of the water was determined after 18 hours. Dry weight was determined after oven drying at 60^{o}C for 48 hours.

4.7.2 Results

As the weights of the two groups of larvae were similar and larvae were large and showed only a small range of weights, the weight specific respiration is more or less independent of body weight. This allows the two groups to be compared by the use of mean values. The mean and standard deviations of the dry weights and weight specific respiration are shown in Table 4.3.

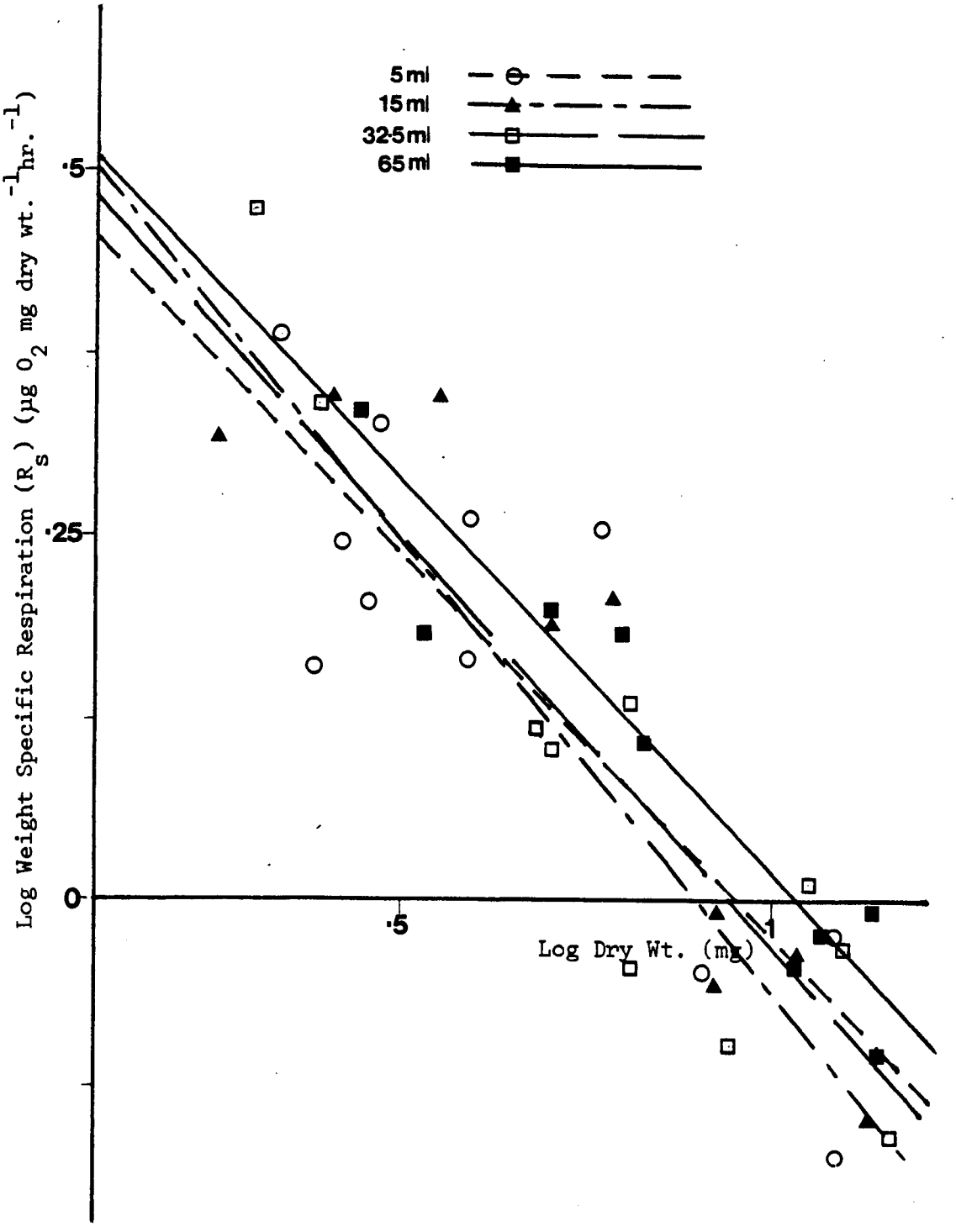
Table 4.3 Mean and standard deviations, calculated from 10 larvae, of the dry weight and the weight specific respiration of *H. angustipennis* determined in the light or the dark. (R_s=$\mu gO_2 mg^{-1} hr^{-1}$; Dry weight = mg).

	Light		Dark	
	Dry wt.	R_s	Dry wt.	R_s
$\bar{x}$	13.68	0.5765	15.87	0.5902
σ_n	3.349	0.0753	2.349	0.1092

4.7.3. Discussion

A value of 0.2047 is calculated for t presenting no evidence for a difference in the mean metabolism, from which it appears that light has no effect upon the metabolism. As before assuming that increased activity causes an increase in the metabolic rate these results differ from those obtained by Elliott (1970) for 5 species of caddis larvae. He demonstrated that light had a controlling effect upon activity, either increasing or decreasing it depending upon whether it was a day or night active species. The light intensity was not measured in this experiment and may have been lower than the 500lx used by Elliott. This could account for the differences between these studies but in the wider context of this study the light intensity used in the other experiments would appear to have no influence upon the respiration rate.

Fig. 4.7. Relationship between log weight specific respiration and log dry weight for *Hydropsyche angustipennis* larvae determined in containers of 65, 32.5, 15 and 5ml. Lines fitted using the method of least squares.



4.8 Crowding and Container Size

4.8.1. Method - container size

The effect of container size was investigated using the closed bottle method. The oxygen consumption was determined in glass bottles of 65, 32.5, and 15ml and 5ml glass syringes, for *H. angustipennis*, and 32.5 and 15ml bottles and 5ml glass syringes for *P. cingulatus*. The duration of the experiment was adjusted so as to cause an approximately equal decrease in oxygen for each container size.

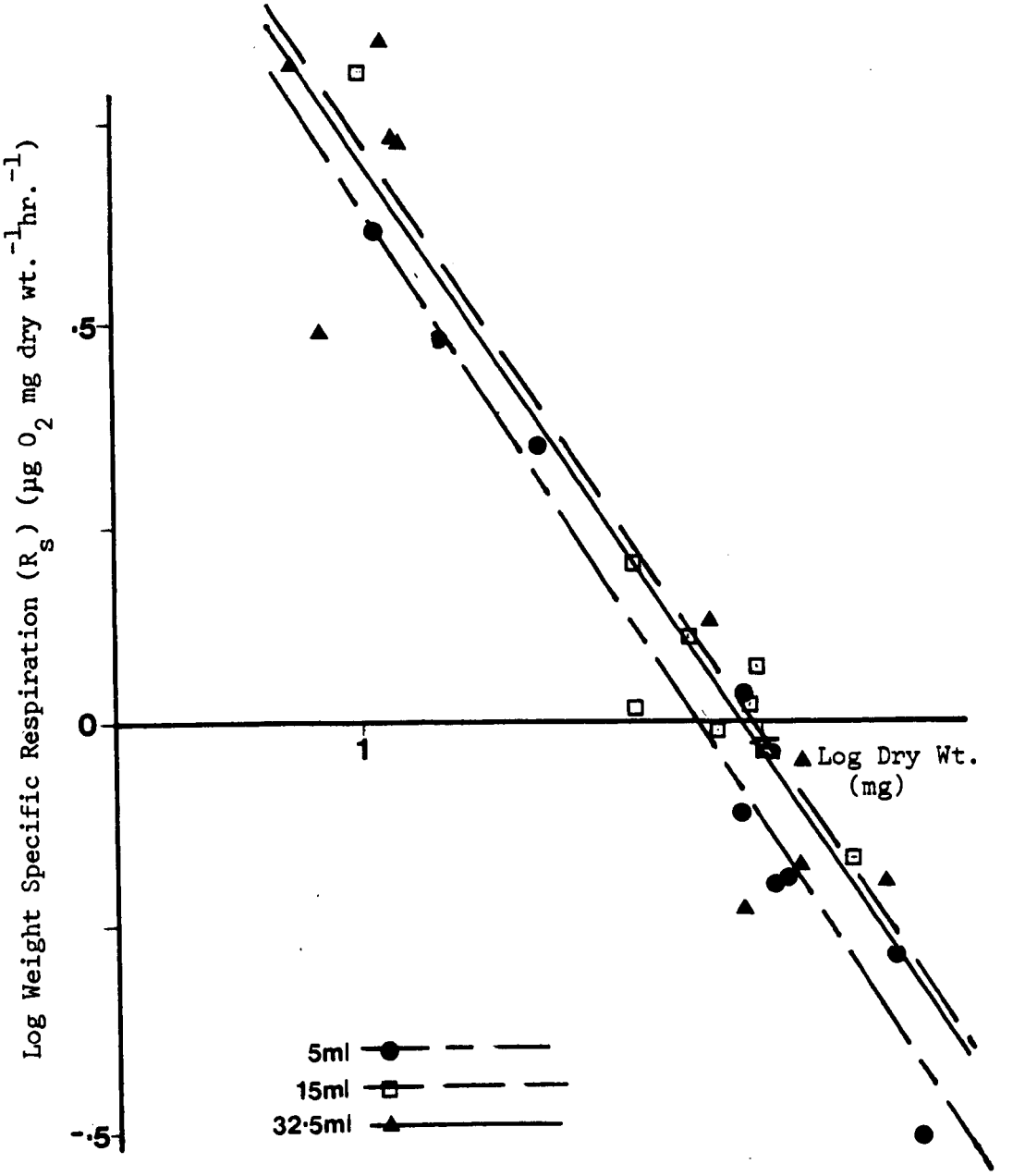
4.8.2. Results

The weight specific respiration of *H. angustipennis* and *P. cingulatus*, determined in containers of a range of sizes, are illustrated in Figs. 4.7 and 4.8. For both species there appears to be no size effect upon oxygen consumption. An analysis of variance on the data, standardised to a dry body weight of 5 and 20mg for *H. angustipennis* and *P. cingulatus* respectively (calculated using ($\ln R_C = b \ln x/w + \ln R_S$ where R_C = standardised weight specific respiration, w = weight, R_S = weight specific respiration, x = body weight to which data is standardised) confirms the lack of a significant difference. (*H. angustipennis* probability of F>2.123 with 3,40df = 0.112; *P. cingulatus*, probability of F>1.87 with 2,24df = 0.176).

4.8.3. Method - crowding

The effect of the number of larvae in the container was investigated using *P. cingulatus* and *H. angustipennis*. For both species ninety five 5th instar larvae of a similar size were divided into groups of 10,5,3 and one individuals, with five replicates for each density. The oxygen consumption was determined at 20^{o}C using 65ml closed bottles, the duration of the measurements being adjusted so as to cause an approximately equal decrease in oxygen in the bottle. The dry weight was determined after oven drying at

Fig. 4.8. Relationship between log weight specific respiration and log dry weight for *Potamophylax cingulatus* larvae determined in containers of 32.5, 15 and 5ml. Lines fitted using the method of least squares.



60°C for 48 hours.

4.8.4. Results

The mean dry weight and the mean weight specific respiration for each density of larvae are given in Tables 4.4 and 4.5 for *H. angustipennis* and *P. cingulatus* respectively.

Table 4.4 Mean and standard deviation of the dry weight and weight specific respiration (calculated from 5 replicates of *P. cingulatus* determined with 10,5,3 or 1 individuals per bottle

		Number of larvae bottle^{-1}			
		10	5	3	1
Dry wt. (mg)	$\bar{x}$	33.4	34.37	36.79	37.36
	σ_{n-1}	2.179	2.869	5.05	4.259
R_s (μgO_2 mg dry wt^{-1} hr^{-1})	$\bar{x}$	0.807	0.674	0.585	0.572
	σ_{n-1}	0.06	0.022	0.036	0.056

Analysis of variance - weight-probability $F > 1.261 = 0.321$

weight specific respiration - probability $F > 27.168 = 0$

Table 4.5 Mean and standard deviations of dry weight and weight specific respiration (calculated from 5 replicates) of *H. angustipennis* determined with 10,5,3 or 1 individuals per bottle

		Number of larvae bottle^{-1}			
		10	5	3	1
Dry wt. (mg)	$\bar{x}$	13.07	13.76	13.95	12.98
	σ_{n-1}	0.917	1.834	2.945	2.581
R_s ($\mu gO_2 mg dry wt^{-1} hr^{-1}$)	$\bar{x}$	0.937	0.875	0.728	0.646
	σ_{n-1}	0.07	0.11	0.137	0.099

Analysis of variance - weight - probability $F > 0.256 = 0.856$

weight specific respiration $F > 7.794 = 0.002$

As at these body weights the influence of weight upon respiration is small and the differences in weights are also not great and non-significant comparisons can be made using the mean weight specific respiration values. In both species there is a clear trend for increasing oxygen consumption as the number of larvae per bottle increase, the mean weight specific respiration increasing by a factor of just over 1.4 as the number of larvae increases from one to 10.

4.8.5. Discussion

The influence of container size alone, rather than the density of individuals has not been widely studied. The lack of a container size effect upon oxygen consumption was also shown by Roff (1973) for the copepod *Limnocalamus macruras*, in containers ranging from 30 to 330ml. Intuitively a size effect would not be expected while the container posed no physical constraints upon the behaviour of the test animal.

A number of studies have reported the lack of a density effect upon

oxygen consumption in, for example, two species of *Daphnia* (Goss and Bunting, 1980) and *Isonychia bicolor* (Sweeney, 1978). These data, indicating a substantial increase in respiration per individual as the number of individuals increase, could be a result of increased activity due to disturbance. Visual observations of both species during the experiment suggested this was true, in particular with *H. angustipennis* there was considerable aggressive behaviour occuring in the bottles containing 10 and 5 individuals.

4.9 Respiration by the case

4.9.1. Method

The oxygen consumption attributable to the case, a result of the presence of epifauna, was investigated for two species, *P. cingulatus* with a mineral case and *Anabolia nervosa* with a case made of vegetable material.

The oxygen consumption of the larvae in their cases was determined at 20^{o}C using 65ml closed bottles. The larvae were then removed from their cases and the oxygen consumption of the cases alone determined, again at 20^{o}C in 65ml bottles. For *Anabolia nervosa* the larvae were divided into two groups which had previously been acclimated to a constant temperature of 6 or 18^{o}C.

4.9.2. Results and Discussion

The mean and the standard deviation of the oxygen consumption of the cases, and the percentage of the total oxygen consumption that this represents, for both species are shown in Tables 4.6 and 4.7.

Table 4.6 Oxygen consumption ($\mu g O_2 hr^{-1}$) by the case of *P. cingulatus* and the percentage of the total oxygen consumption of the larvae and case that this represents.

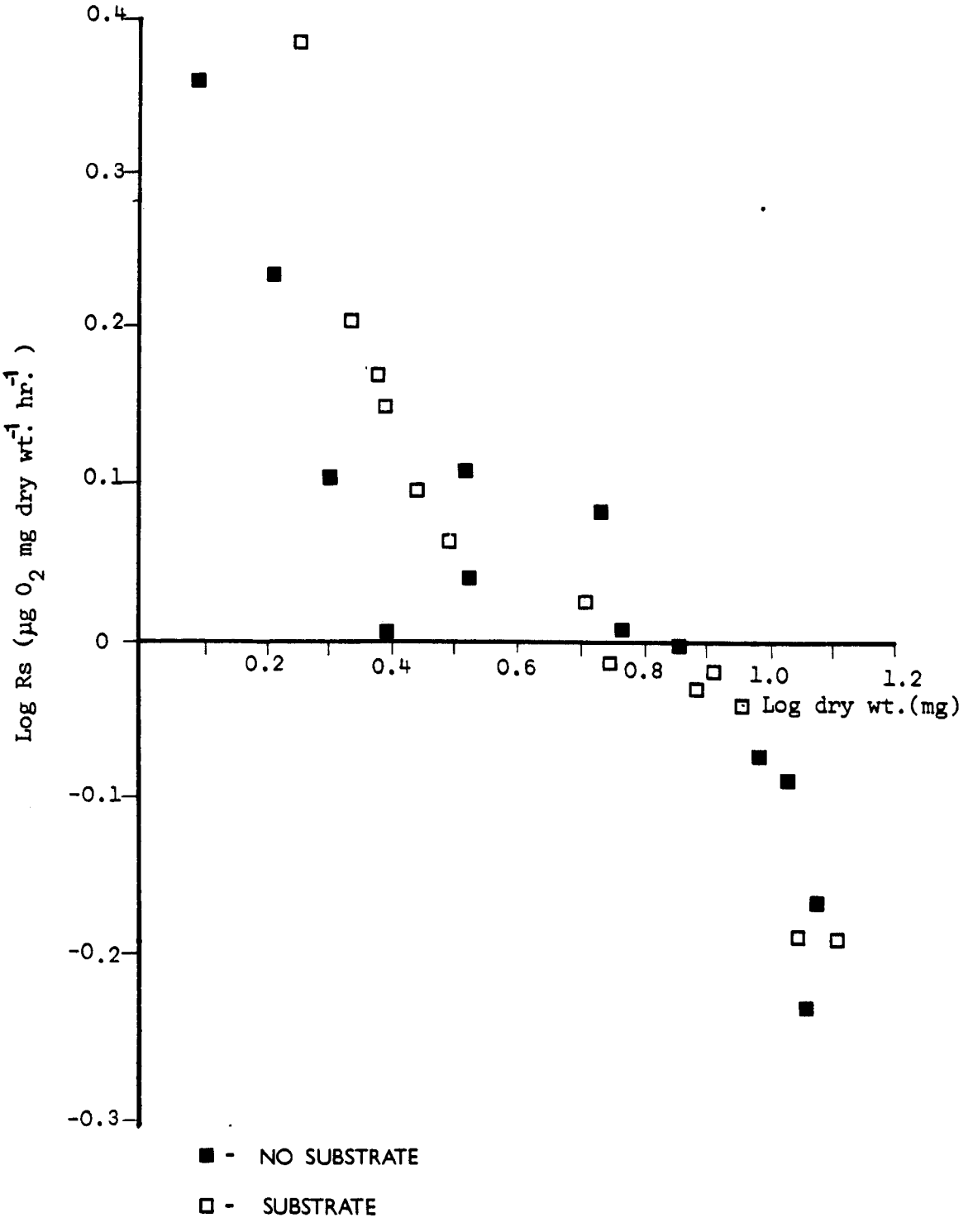
	O_2 consumption of the case	% of the total O_2 consumption accounted for by case
$\bar{x}$	2.427	14.055
σ	0.9602	5.686

Table 4.7 Oxygen consumption ($\mu g O_2 hr^{-1}$) by the case of *A. nervosa* previously acclimated to 6 or 18°C, and the percentage of the total oxygen consumption of the larvae and case that this represents.

		O_2 consumption of the case	% of the total O_2 consumption accounted for by case
6°c acclimated larvae	$\bar{x}$	2.984	10.1597
	σ_n	1.1156	4.6004
18°c acclimated larvae	$\bar{x}$	2.9333	16.3095
	σ_n	1.2338	7.445

For both species there is appreciable oxygen consumption by the empty Case, accounting for something in the region of 10-16% of the total oxygen consumed. The data for *Anabolia nervosa* indicates that the oxygen consumption of the case is not altered by the previous thermal history and therefore, if the oxygen consumption of the larvae decreases following a period of exposure to warmer temperatures, the percentage of the oxygen consumed by the case is higher in the larvae acclimated to the warmer temperature.

Fig. 4.9. The effect of the presence or absence of substrate, in the form of sand/gravel, on the oxygen consumption of *Hydropsyche angustipennis*.



4.10 Miscellaneous factors

4.10.1. Substrate/no substrate method

The presence or absence of a substrate may influence the respiration rate by altering the activity pattern of the larvae. The experiment was performed using twenty six 4th and 5th instar *H. angustipennis* which were divided into two groups matched as closely as possible for size. One group was placed into 65ml glass bottles with no substrate while the remaining group was introduced into 65ml glass bottles containing a layer of substrate in the form of a mixture of sterilised (heated to 105^{o}C for 24 hours) sand and gravel collected from the same site as the larvae. Control bottles were used both with and without substrate.

4.10.2. Results

The log weight specific respiration is plotted against log dry weight for the larvae from bottles with and without substrate in Fig. 4.9. This clearly shows that the presence of the substrate has no effect upon the rate of oxygen consumption.

4.10.3. Elevated oxygen consumption during the early stage of measurement-method

A number of studies have demonstrated an elevated rate of oxygen consumption during the initial period following the introduction of animals to a respirometer vessel (Sutcliffe, 1984). Again, as in the previous section this may reflect an increase in activity during the initial period. This could include searching behaviour and, for net-spinning larvae, possibly the building of a retreat.

The experiment was performed on two species, *P. cingulatus* and *H. angustipennis*. For each species twenty larvae of a similar size were divided into two groups of ten with similar size distributions. One group of larvae was introduced into 32.5ml glass bottles half filled with

oxygenated water. The second group were left unfed in a 135 x 75 x 50mm perspex dish. Both groupsof larvae were maintained at 15°C for 12 hours. At the end of the 12 hours the water was removed from the bottles using a 20ml syringe, causing as little disturbance as possible to the larvae. The oxygen consumption was then determined for both groups of larvae at 15°C using the closed bottle method.

4.10.4. Results

The mean dry weight and weight specific respiration of *H. angustipennis* and *P. cingulatus* larvae are presented in Tables 4.8 and 4.9.

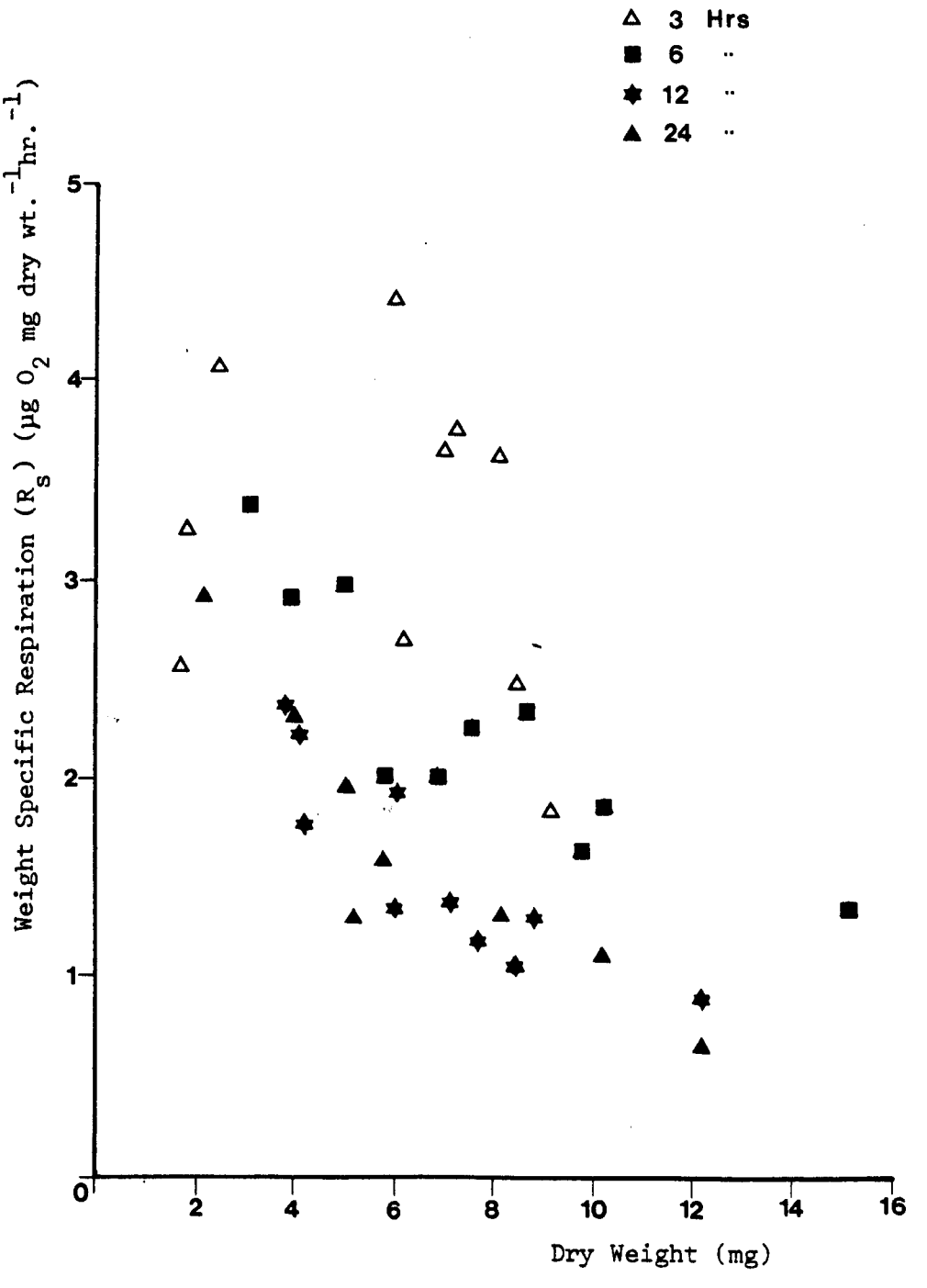
Table 4.8 Mean and standard deviation of the dry weight and weight specific respiration of *H. angustipennis* A) larvae introduced 12 hours before oxygen consumption determination (B) larvae introduced immediately before oxygen consumption determination. (R_s=$\mu gO_2 mg^{-1} hr^{-1}$; Dry weight = mg).

		A	B
Dry wt.	$\bar{x}$	7.14	7.31
	σ_n	1.258	0.916
R_s	$\bar{x}$	1.438	1.621
	σ_n	0.218	0.153

Table 4.9 Mean and standard deviation of the dry weight and weight specific respiration of *P. cingulatus*. A and B as in Table 4.8.

		A	B
Dry wt.	$\bar{x}$	29.06	30.59
	σ_n	4.991	3.606
R_s	$\bar{x}$	0.979	0.926
	σ_n	0.154	0.1399

Fig. 4.10. The influence of the experimental duration on the weight specific respiration of *Hydropsyche angustipennis*. Larvae introduced immediately before the oxygen consumption was determined.



For both species the weight distributions are similar (t test-N.S.) and therefore the comparisons are made using the mean values. For *P. cingulatus* there is no significant difference in the oxygen consumption, while in comparison, for *H. angustipennis* the oxygen consumption is significantly lower ($P<0.05$) in the larvae introduced to the bottles 12 hours before the oxygen determinations were made.

4.10.5. Duration of Experiment

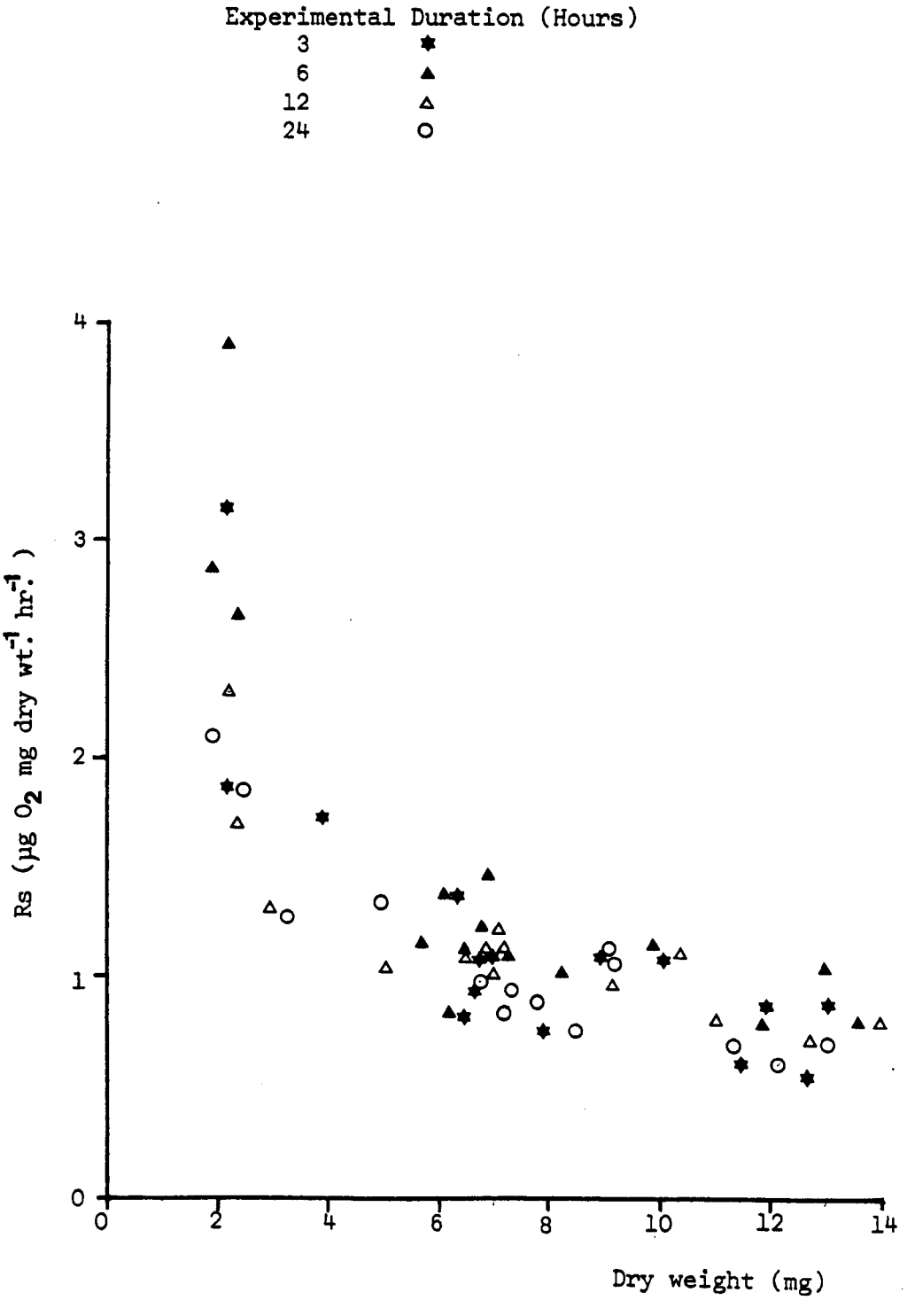
Method

The effect of the length of time over which the oxygen consumption is measured was investigated using *H. angustipennis*. The closed bottle method was used to determine the oxygen consumption of larvae with time periods of 3,6,12 and 24 hours. Two experiments were performed. In the first the larvae were introduced into the bottles immediately prior to the determination of the oxygen consumption. In the second the larvae were introduced into the bottles, which were half filled with oxygenated water, and left for 12 hours before the oxygen consumption was measured (the water in the bottle being carefully removed, so as to cause as little disturbance as possible, and the bottle refilled with fully oxygenated water). During this time rough larval retreats were spun.

4.10.6. Results

The weight specific respiration of the larvae were plotted against dry weight in Figs. 4.10 and 4.11 for the bottles with and without the earlier introduction of the larvae respectively. There is a notable difference in the two sets of data. With the larvae introduced immediately before the measurement of the oxygen consumption (Fig. 4.10) the weight specific respiration is highest and more variable when measured over the shorter time periods (3 and 6 hours). However over longer time periods (12 and 24 hours) there appears to be no difference. In contrast from Fig. 4.11 it

Fig. 4.11. The influence of the experimental duration on the weight specific respiration of *Hydropsyche angustipennis*. Larvae introduced 12 hours before the oxygen consumption was determined.



appears that there is no difference in the weight specific respiration measured after 3,6,12 and 24 hours if the larvae were introduced into the bottle 12 hours before the start of measurements.

4.10.7. Discussion

These data, demonstrating enhanced oxygen consumption when the weight specific respiration was measured over shorter time periods, a phenomenon which disappeared if the larvae were allowed an initial period of time in the bottle. As noted by a number of authors (e.g. Sutcliffe, 1984) this probably reflects increased activity, in this case including retreat building, during the initial period.

4.10.8. Choice of a control method

Introduction

The choice of a suitable control when the closed bottle method is used causes some difficulties. Background bacterial respiration is inevitable in such experiments, particularly over long time periods (e.g. 2-3 days) or if the containers are not cleaned well (Propp et al, 1982). The animal itself may act as the largest source of bacterial innoculation (Sutcliffe et al, 1975) and therefore the use of empty control bottles may introduce errors by underestimating the bacterial respiration.

Method

Twenty four 65ml glass bottles were divided into 3 groups of 8 (A,B and C). One group of bottles (B) were half filled with oxygenated water and a larva introduced into each bottle and left for 24 hours. At the end of this period the water and larvae were removed, all 24 bottles filled with fully oxygenated water and the bottles placed in a water bath at $15^{o}C$. The percentage oxygen saturation was determined immediately for group A and after 24 hours for groups B and C.

4.10.9. Results

The mean and standard deviation of the percentage oxygen saturation of the 3 groups are shown in Table 4.10. These data clearly indicate that there is no difference between the three groups of bottles.

Table 4.10 Comparison of the reduction in oxygen from the initial values (A) in control bottles previously containing larvae (B) or not (C)

	A	B	C
$\bar{x}$ (%)	99.913	98.138	98.038
σ_n	0.203	0.424	0.267

Discussion

On the evidence of this experiment two points are apparent. Firstly the decrease in oxygen is small (<2%) and non-significant in both control methods (B and C). Secondly it appears to make no difference whether the bottles previously contained larvae or not. This suggests that the larvae did not increase the bacterial contamination to any significant degree.

4.11 General Discussion

The results of the experiments discussed in this Chapter, in addition to their intrinsic interest, have a number of implications for the design of later experiments and the interpretation of the data produced. However care must be taken in generalising from these results obtained from just 1 or 2 species. The inherent danger of this is demonstrated by the available data on the effect of oxygen concentration on oxygen consumption, great variations occuring in the relationship between closely related species (Mangum and Winkle, 1973).

A number of the experiments have particular relevance regarding the protocol used in later experiments. The results concerned with crowding and

container size, initial elevated oxygen consumption, the duration of the experiment and the choice of a control method all have implications for the closed bottle method. As the size of the container has no influence upon the oxygen consumption, containers of different size can be used. This allows a constant experimental duration to be maintained at, for example, different temperatures, by adjusting the container size. The duration of the experiment itself may alter the oxygen consumption but as shown in Chapter 4.10.5 this effect disappears if the larvae are allowed an initial period to settle into the bottle before the experiment commences.

Although it has been suggested that the use of an empty control bottle may underestimate respiration attributable to bacteria these data indicate no difference between control values irrespective of whether larvae were previously added to the bottle or not (Chapter 4.10.8).

The diel rhythm observed in the oxygen consumption (Chapter 4.4) requires consideration when the duration of an experiment is chosen. A short time period (4-5 hours or less) may cause difficulty in making inter and intraspecific comparisons as the measurements may coincide with peaks or troughs in the oxygen consumption. An experimental duration of about 12 hours from 0800-2000 or 2000-0800 should cause the variation in oxygen consumption to be averaged out and approximately even for the day and night. The diel variation may pose greater difficulty when the flow through respirometer is used due to the short time period over which the respiration is measured. This difficulty is normally overcome by the use of replicate determinations over a longer time period.

Light (Chapter 4.7) and the presence or absence of substrate (Chapter 4.10.1) were both shown to have no influence upon the oxygen consumption. Later experiments were performed in bottles without substrate, but to maintain consistency all experiments were performed in the dark.

The data presented in Chapter 4.8.3 demonstrate an effect on the oxygen consumption of the number of larvae per bottle. This indicates that

in animals such as caddis larvae, where interactions between individuals are extensive, care should be taken in the number of larvae used per bottle. Within an experiment the number of larvae should be kept constant, in these experiments at one larva per bottle. Comparisons with data from other studies, where the number of individuals per container differ, should be made with caution.

The remaining experiments have implications for the analysis and interpretation of later data. The weight dependence of oxygen consumption (Chapter 4.2) causes difficulties if comparisons are attempted by the use of mean values between groups of larvae with different weight distributions. In such cases it is more suitable to compare the two groups by calculating the regression lines of the data and using analysis of covariance to investigate the significance of the difference in slope and intercept of the lines. This is discussed further in Chapter 5. Dry weights were used as they were easier to obtain than reliable wet weights. Regressions of log weight specific respiration on log body weight were more significant than log oxygen consumption on log body weight and therefore the former was used.

The difficulty involved in determining the level of activity and the short time over which the periods of activity occurred (Chapter 4.3) prevents activity being taken into account. Visual observations during later experiments suggested that there was no difference in the activity levels of, for example, larvae acclimated to different temperatures, and thus their activities are assumed to be the same.

The decrease in oxygen consumption as the oxygen content of the water decreases makes it important not to allow the oxygen concentration of the water to decrease by too great a degree. However in experiments investigating the difference in oxygen consumption between two groups of larvae this factor can only underestimate the difference between the groups.

Finally for cased caddis the respiration due to epifauna or aerobic bacteria present on the case could be important. Chapter 4.9 demonstrates that the oxygen consumption of the case alone is not insignificant. Again, as the oxygen consumption is the same irrespective of the thermal history of the caddis, this can again only underestimate the difference between groups of larvae acclimated to different temperatures.

Where feasible these factors are incorporated in the closed bottle method described in Chapter 3 and the significance of the effect of temperature on respiration is discussed in Chapter 5.

CHAPTER 5 ACCLIMATION TO CONSTANT TEMPERATURE

5.1 Introduction

The inability of aquatic poikilotherms to regulate their body temperatures, together with the fact that thermal diffusion is more rapid than molecular diffusion (Hazel and Prosser, 1974) means that the body temperature of caddis larvae will at all times be close to the ambient water temperature. In the absence of a mechanism to compensate for temperature variation difficulties will arise. A decrease in temperature causes a reduction in the rate of reactions, until a point is reached where the ability to function is lost. An increase in temperature causes an acceleration in the metabolism which is limited by the availability of substrates and energy sources and by the build up of harmful metabolites (Wieser, 1973). This demonstrates that the ability to compensate their metabolism in response to a change in temperature has important implications for individuals of a species to function efficiently at a range of environmental temperatures, vital functions being maintained constant, independant of the environmental temperature. (Precht, 1958).

It is now generally accepted that poikilotherms exhibit a degree of metabolic independence to water temperature. (Bullock, 1955; Anderson and Mutchmoor, 1971; Paravatheswararao, 1972; Kulkarni, 1978). Examples are available from latitudinally separated populations (e.g. Fox and Wingfield, 1937), seasonal (Bayne, 1973; Sutcliffe, 1984; Hayashi and Yoshida, 1987) and experimental acclimation (Calow, 1975; Bulnheim, 1979). A number of studies, for example Bayne (1973), have demonstrated an ability for some species to acclimate their routine metabolic rate but not their standard or active rates. Other work has shown differences between closely related species in their ability to acclimate (Calow, 1975), stenothermal species having limited capacity for regulation (Bullock, 1955).

The time course over which acclimation occurs is extremely variable

(Ivleva, 1973), which could reflect the different mechanisms which may be involved (Bullock, 1955). Bulnheim (1979) demonstrated an acclimation time of 3-6 hours for five euryhaline Gammarus species. A time of 3-4 days was found by Calow (1975) for two species of freshwater gastropods and Harrison and Badcock (1981) suggest a period of 'several' months for acclimation of two species of caddis larvae.

Few studies have investigated the ability of insects to acclimate to temperature. In general insects are said to have a poor ability to compensate (Scholander, 1953; Bullock, 1955; Keister and Buck, 1974). Seasonal compensation has been demonstrated for 2 species of Dobsonfly larvae (Brown and Fitzpatrick, 1978; Hayashi and Yoshida, 1987) and Harrison and Badcock (1981) demonstrated reverse acclimation in two species of caddis larvae.

The aim of this Chapter is to present data on the ability of a range of species of caddis larvae to acclimate to constant temperature, and if possible to relate this to their distributions.

5.2 Method

For each species,larvae were collected from single sites and divided into two groups, matched approximately for size (judged by eye). The larvae were maintained in aerated pond water in 175mm x 115mm x 50mm plastic boxes to which sand and gravel were added to provide substrate and shelter. Temperature control was achieved by placing the plastic containers on a thermogradient bar (see Chapter 2.2 for details) which maintained the water at the desired temperature $\pm$ 0.5^{o}C. The larvae were kept in this way for 4-5 weeks. The water was replaced at weekly intervals and suitable food was provided (see Chapter 2.2).

The oxygen consumption was determined by the closed bottle method, using the protocol described in Chapter 3. This was designed to take into account relevant factors investigated in Chapter 4. The oxygen content of

the water was determined by either the micro-Winkler method (Chapter 3.2.2) or a Clark type oxygen electrode (Chapter 3.2.3) which were shown to produce comparable results (Chapter 3.2.3). The dry weights of the larvae were determined after oven drying at 60°C for 48 hours.

Data are presented for 6 hydropsychid species *Diplectrona felix*, *Hydropsyche instabilis*, *H. siltalai*, *H. pellucidula*, *H. contubernalis* and *H. angustipennis*, 3 polycentropodids (*Neureclipsis bimaculata*, *Plectrocnemia conspersa* and *Polycentropus flavomaculatus*), two limnephilids (*Potamophylax cingulatus* and *Anabolia nervosa*) and the sericostomatid, *Sericostoma personatum*, following acclimation to constant temperatures of 6 and 18°C.

5.3 Results

5.3.1 *Net-spinning larvae - Hydropsychids*

The weight specific respiration, determined at a range of temperatures, is plotted against dry weight on a ln-ln scale for the six species studied in Figs. 5.1 to 5.6 for larvae acclimated to 6 or 18°C. Natural logs (ln) are used in place of the more traditionally used $\log_{10}$ for ease of computation with the statistical package used. This has no effect upon the results, the slope of the regression lines remaining the same and the intercept being equal to 2.303 times the value obtained if $\log_{10}$ was used.

For each species, at all temperatures, and for both groups of larvae, the linear regressions of ln weight specific respiration on ln dry weight are significant at the 0.1% level.

Due to the weight dependancy of the weight specific respiration, and the likelihood of differences in the weight distribution of the two groups of larvae, making comparisons by the use of mean values is not suitable. Instead an analysis of covariance is used to determine the significance of differences between the slope and/or intercept of the regression lines of

Fig. 5.1. Relationship between the ln weight specific respiration of *Diplectrona felix* larvae, determined at 5,10,15 and 20°C, and ln dry weight (■ = 6°C acclimated larvae; □ = 18°C acclimated larvae).

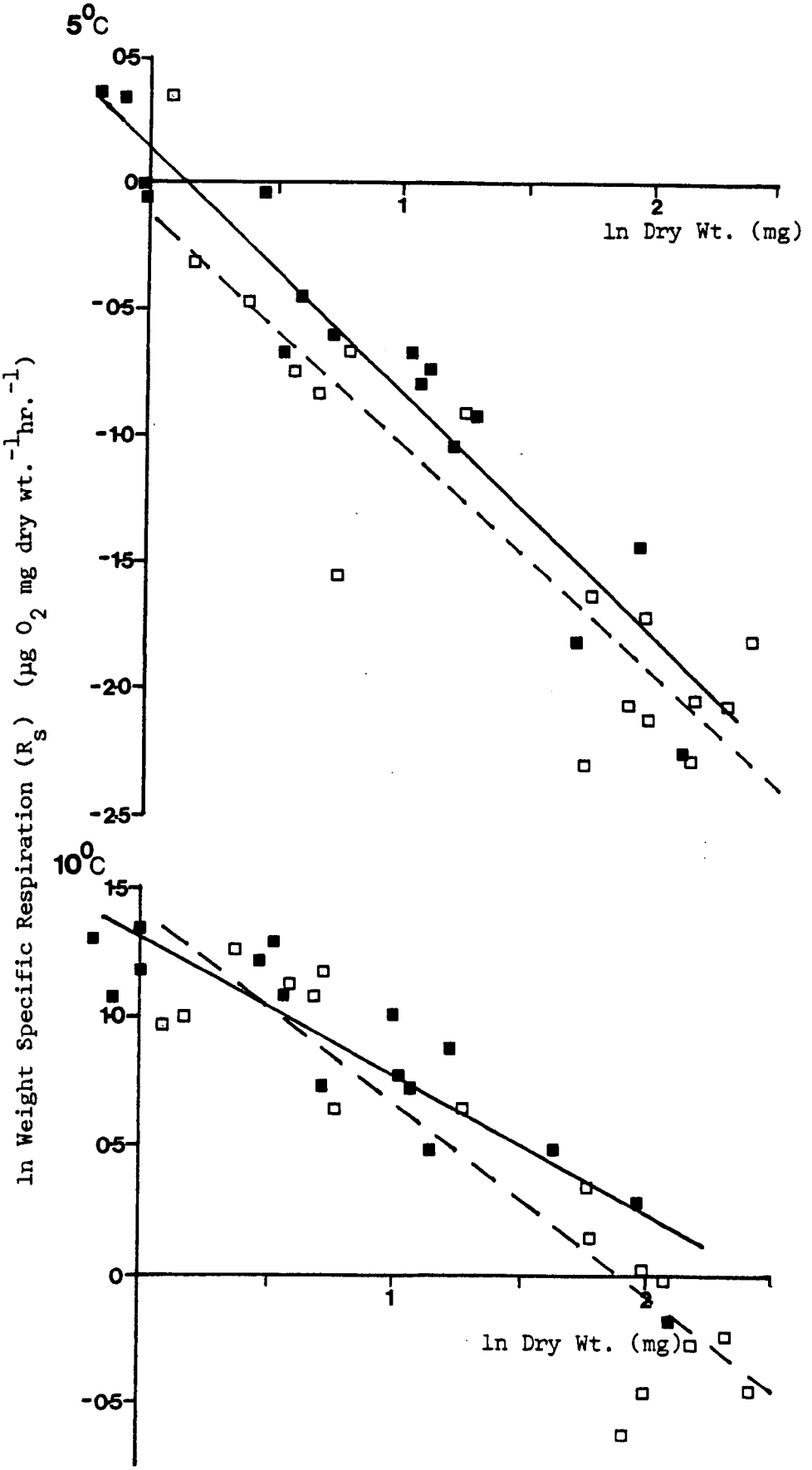


Fig. 5.1. (Cont.)

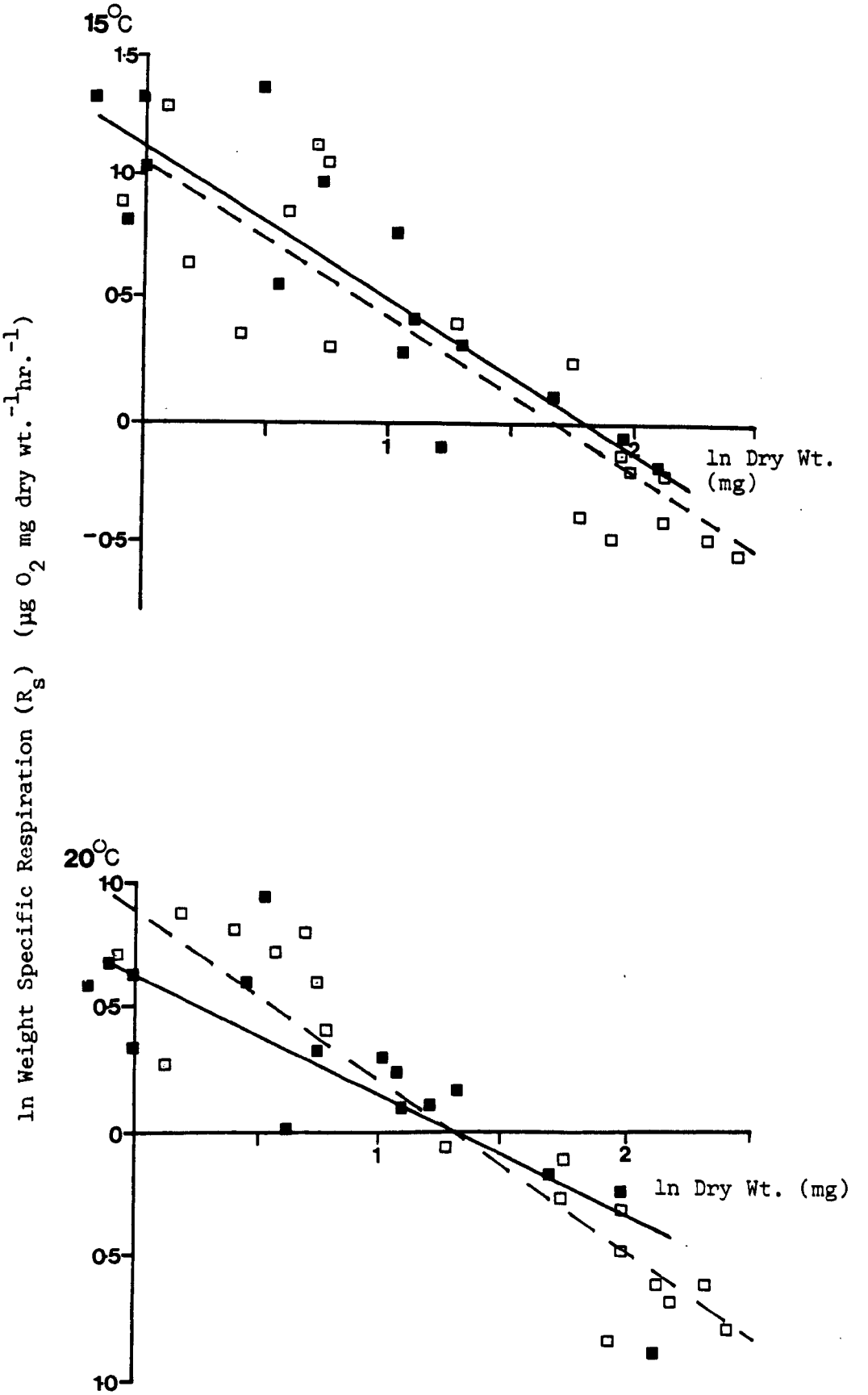

15°C
1·5
1·0
0·5
0
-0·5
1
2
ln Dry Wt. (mg)
ln Weight Specific Respiration (R_s) (µg O_2 mg dry wt.$^{-1}$ hr.$^{-1}$)
20°C
1·0
0·5
0
1
2
ln Dry Wt. (mg)
-0·5
-1·0

Fig. 5.2. Relationship between the ln weight specific respiration of *Hydropsyche instabilis* larvae, determined at 5,10,15 and 20°C, and ln dry weight (■ = 6°C acclimated larvae; □ = 18°C acclimated larvae).

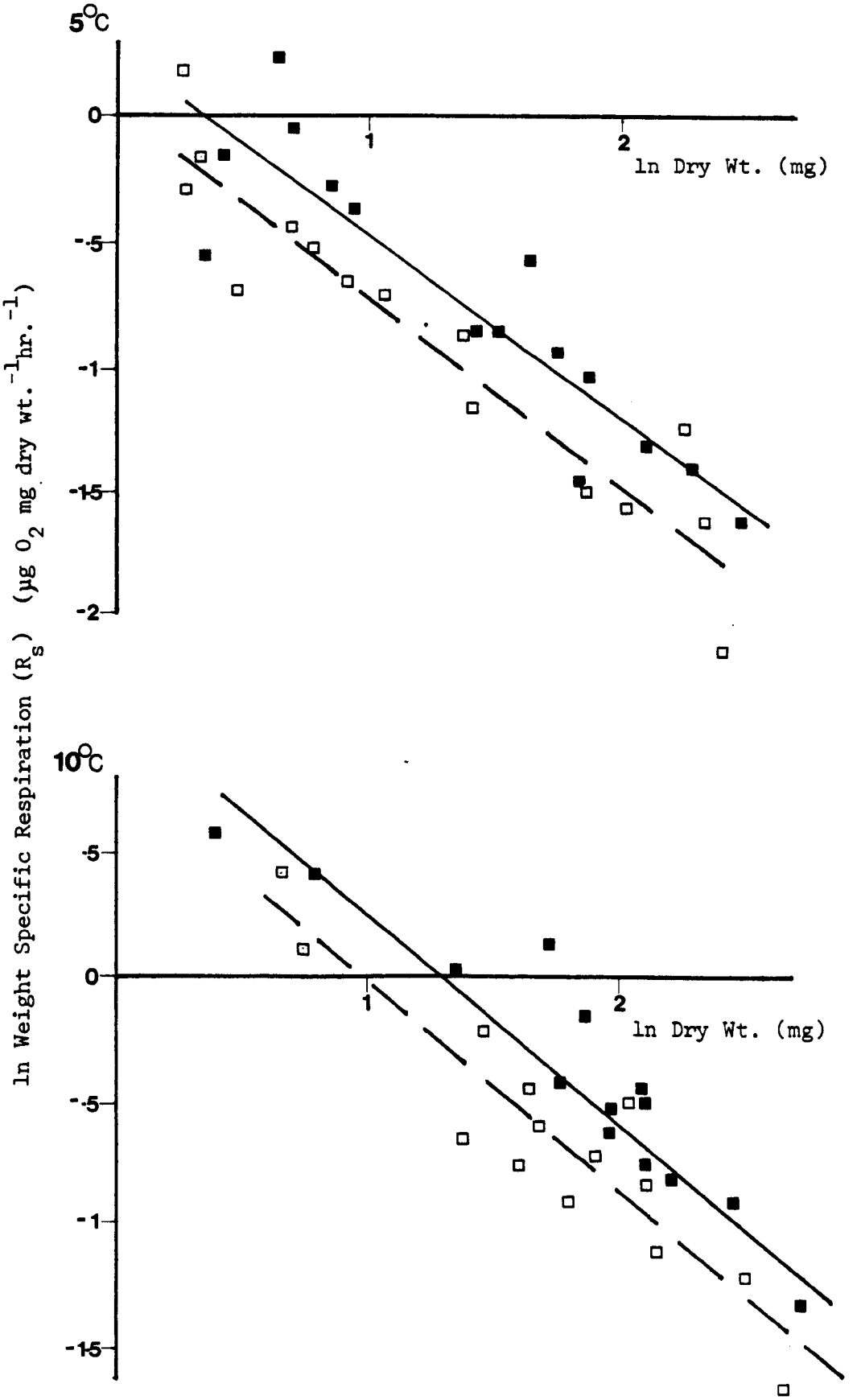


Fig 5.2. (Cont.)

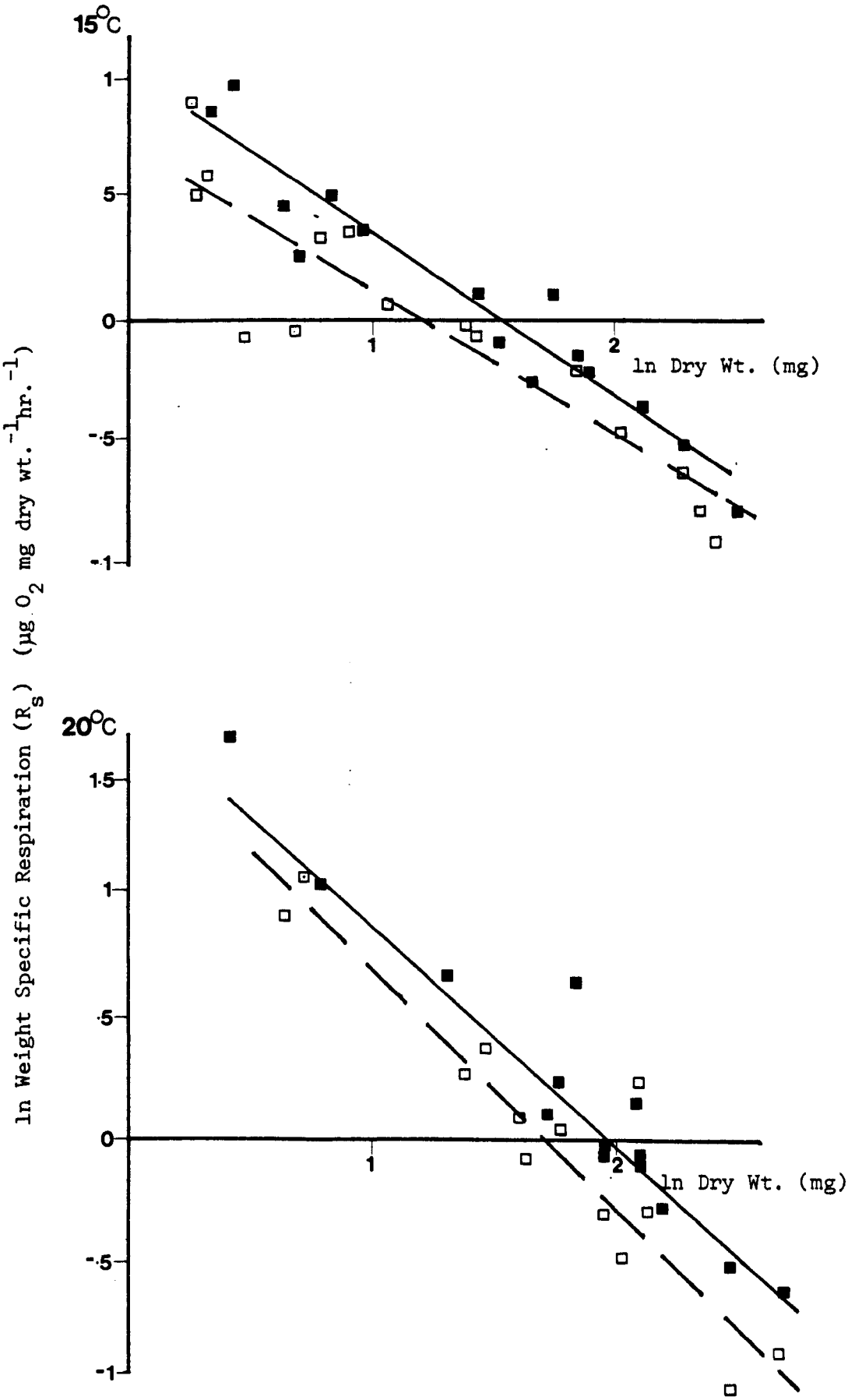

15°C
1
5
0
-5
-1
1
2
ln Dry Wt. (mg)
ln Weight Specific Respiration (R_s) (µg O_2 mg dry wt.$^{-1}$hr.$^{-1}$)
20°C
1·5
1
·5
0
-·5
-1
1
2
ln Dry Wt. (mg)

Fig. 5.3. Relationship between the ln weight specific respiration of *Hydropsyche pellucidula* larvae, determined at 5,10,15 and 20°C, and ln dry weight (■ = 6°C acclimated larvae; □ = 18°C acclimated larvae).

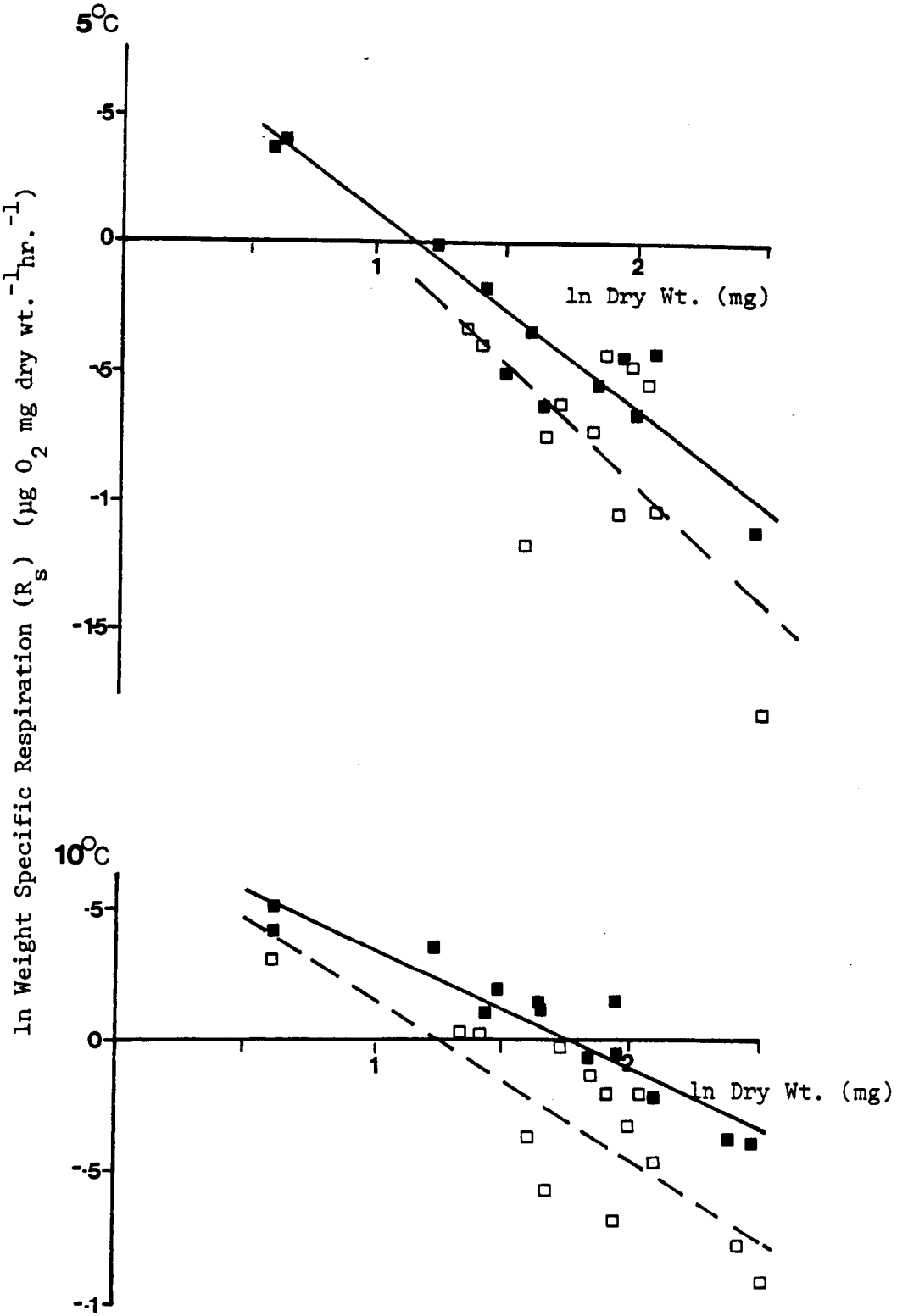


Fig. 5.3. (Cont.)

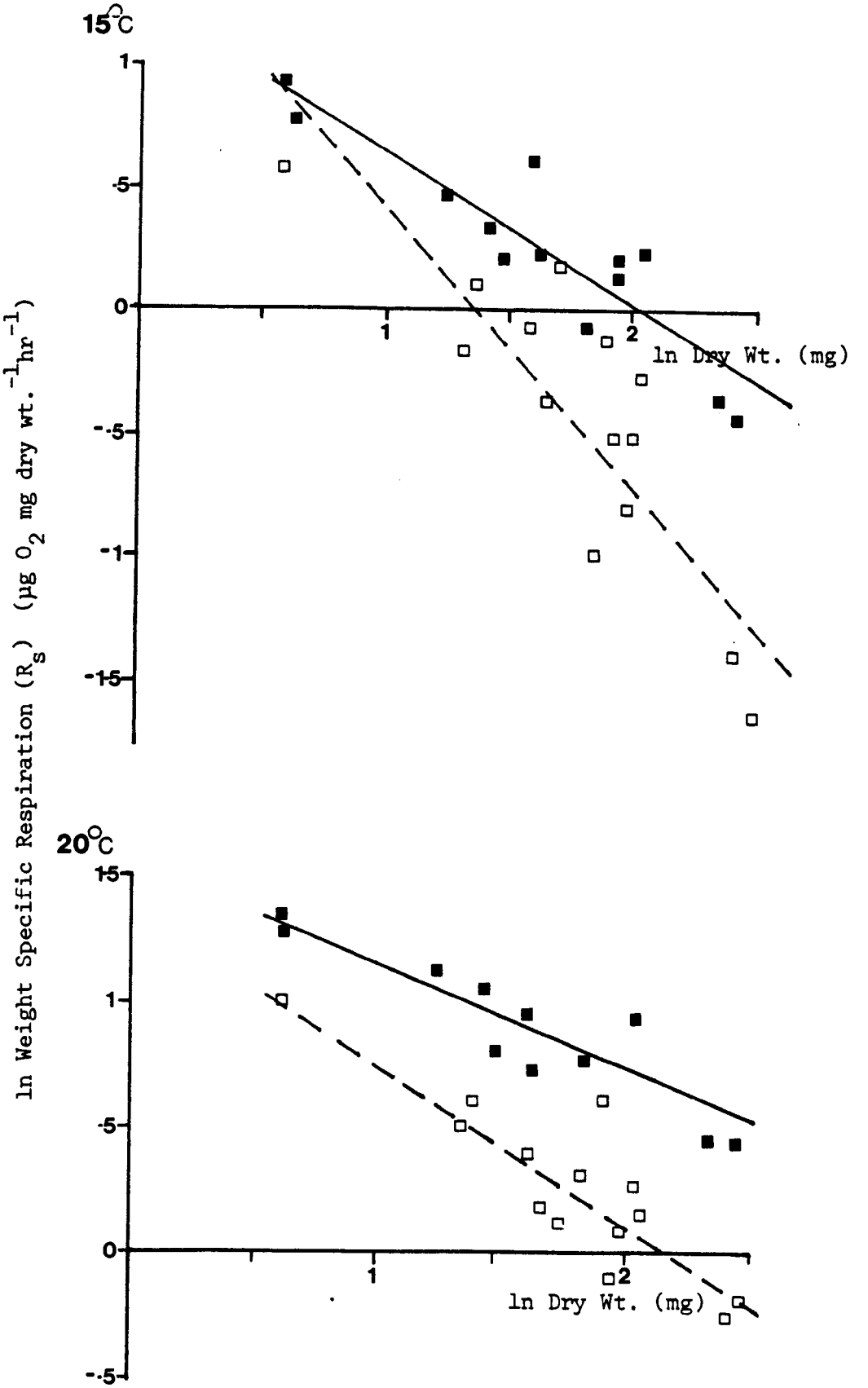

15°C
1
.5
0
-.5
-1
-1.5
1
2
ln Dry Wt. (mg)
ln Weight Specific Respiration (R_s) (µg O_2 mg dry wt.$^{-1}$hr^{-1})
20°C
1.5
1
.5
0
-.5
1
2
ln Dry Wt. (mg)

Fig. 5.4. Relationship between the ln weight specific respiration of *Hydropsyche siltalai* larvae, determined at 5,10,15 and 20°C, and ln dry weight (■= 6°C acclimated larvae; □= 18°C acclimated larvae).

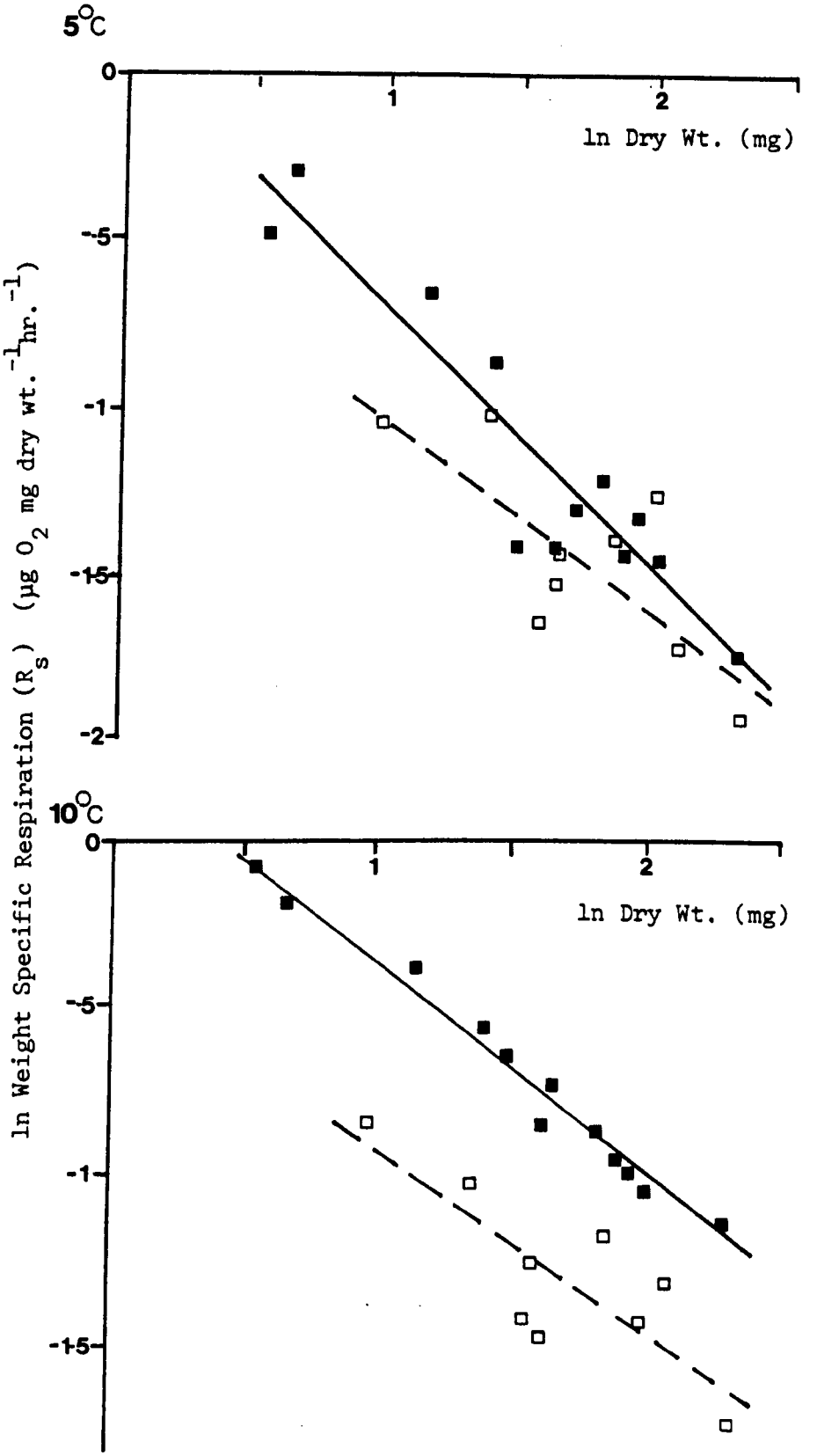


Fig. 5.4. (Cont.)

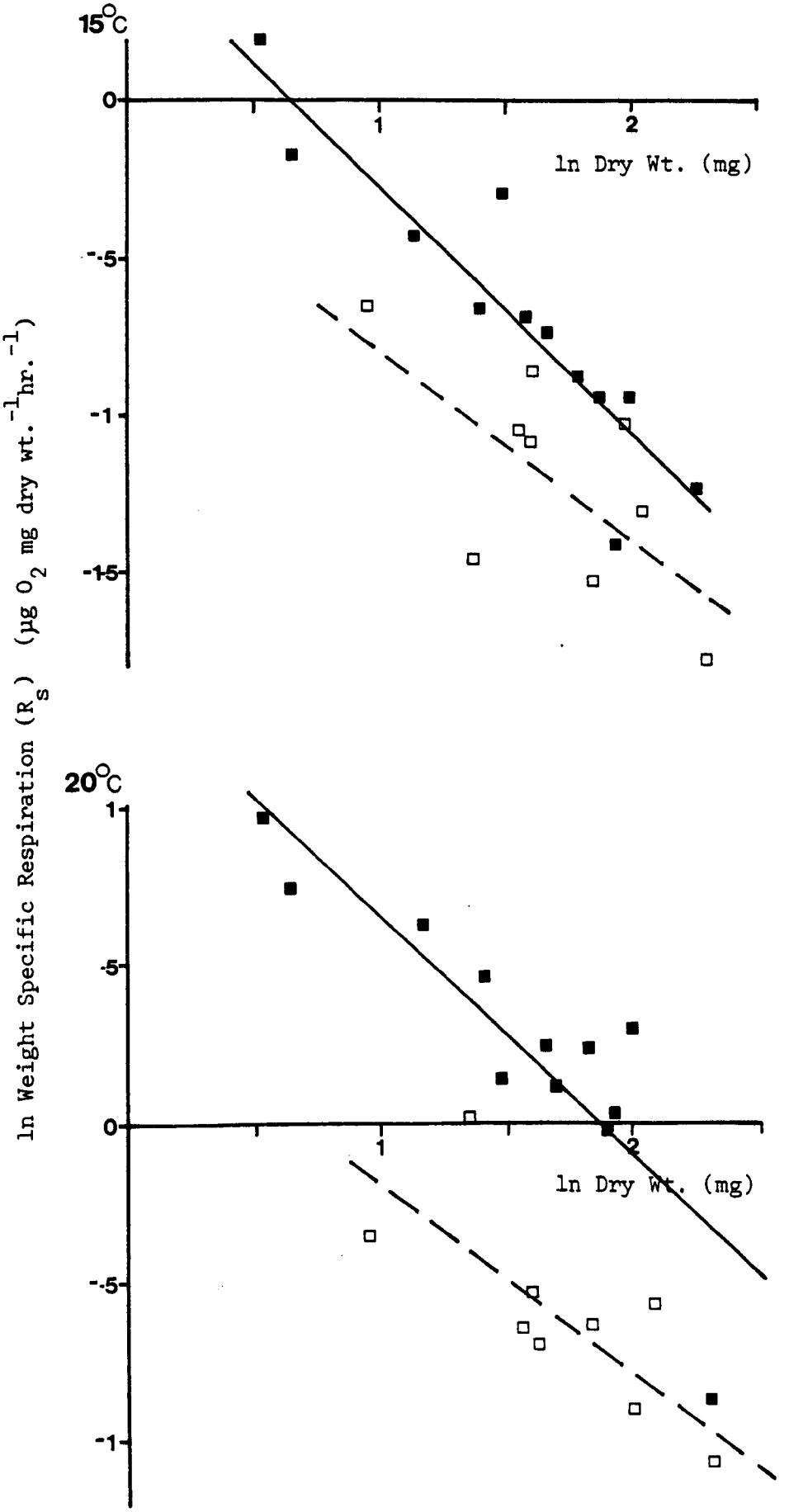

15°C
0
1
2
ln Dry Wt. (mg)
-·5
-1
-15
ln Weight Specific Respiration (R_s) (µg O_2 mg dry wt.$^{-1}$hr.$^{-1}$)
20°C
1
·5
0
1
2
ln Dry Wt. (mg)
-·5
-1

Fig. 5.5. Relationship between the ln weight specific respiration of *Hydropsyche angustipennis* larvae, determined at 8,12,16 and 20°C, and ln dry weight (■ = 6°C acclimated larvae; □ = 18°C acclimated larvae).

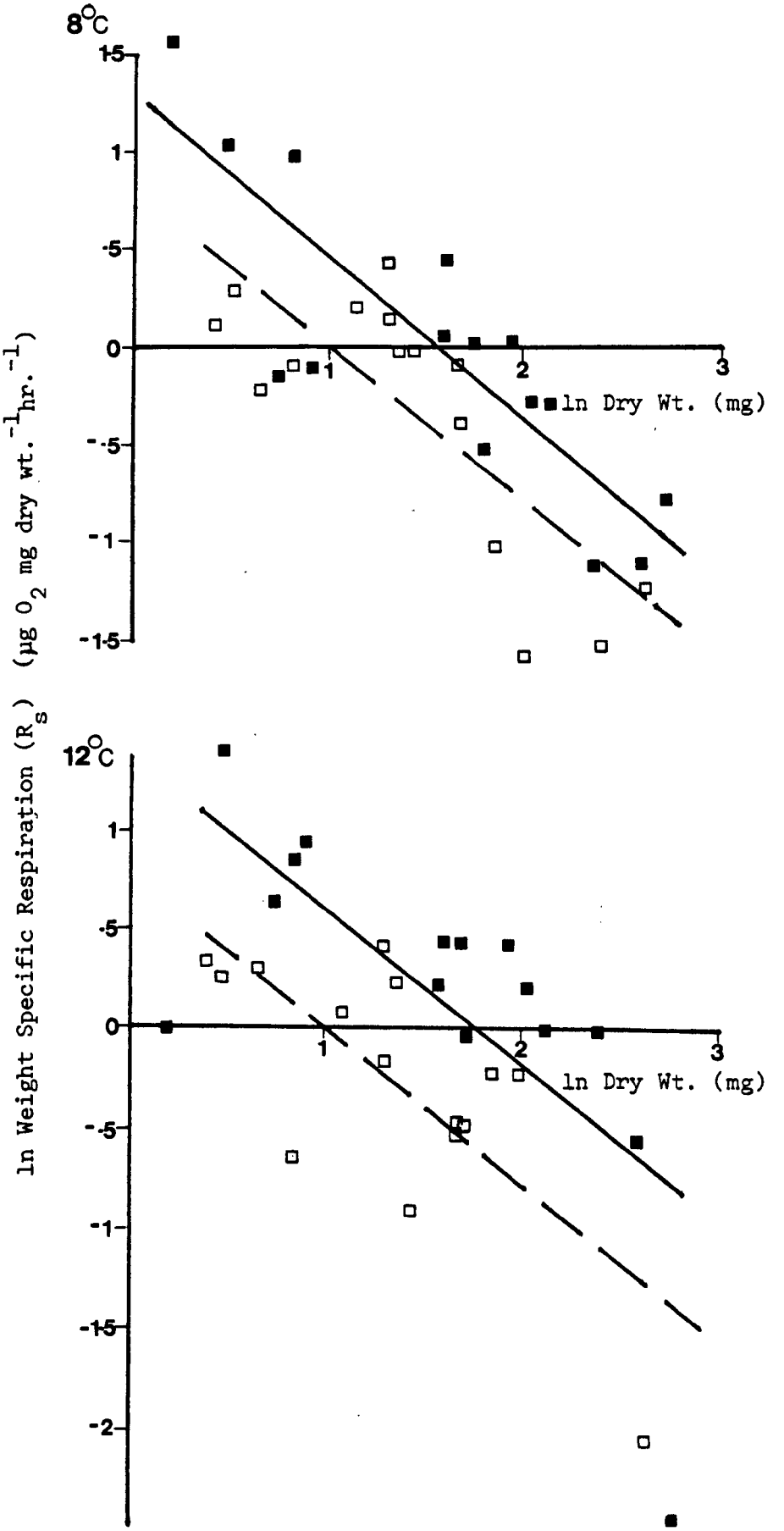


Fig. 5.5. (Cont.)

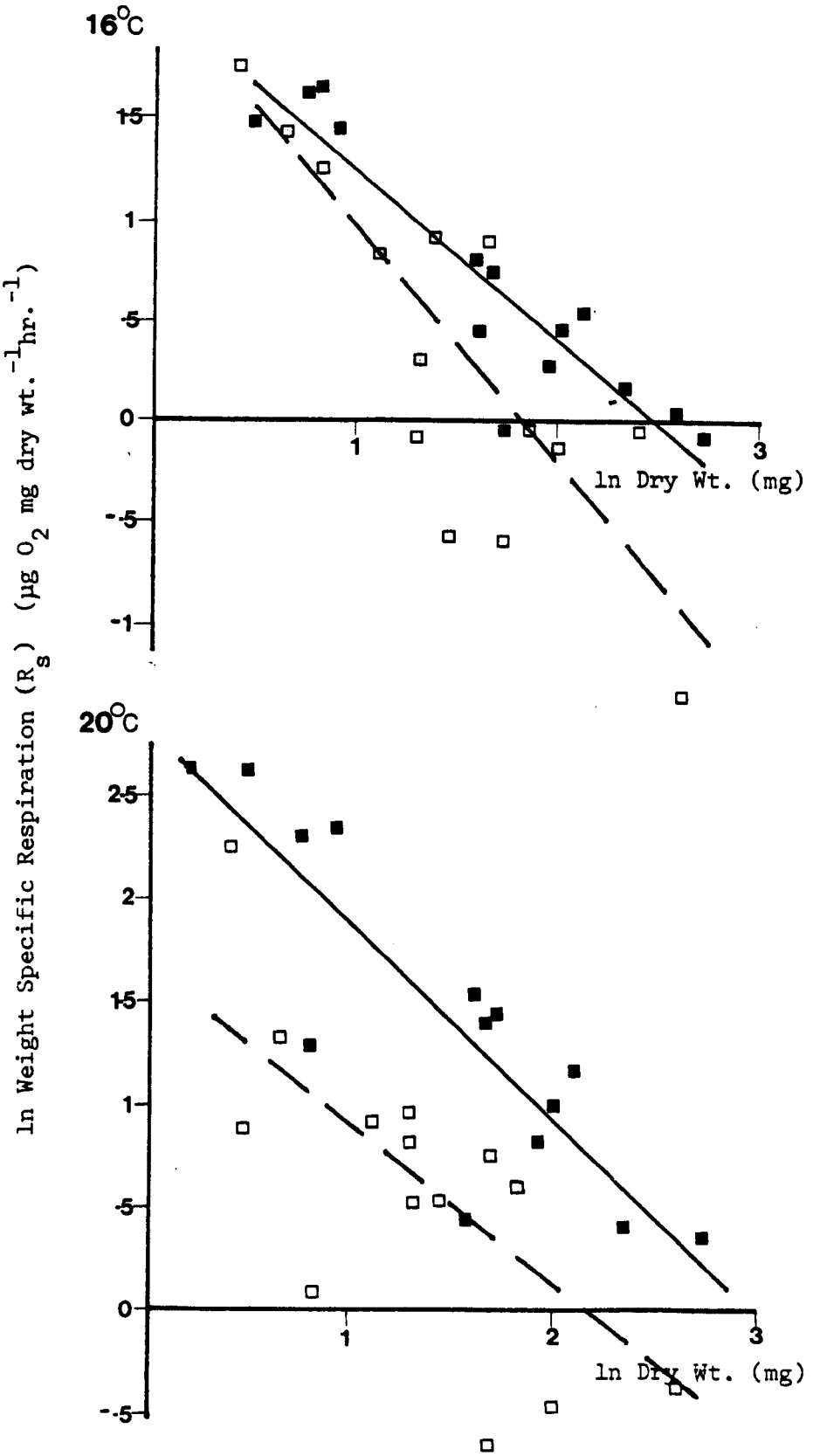
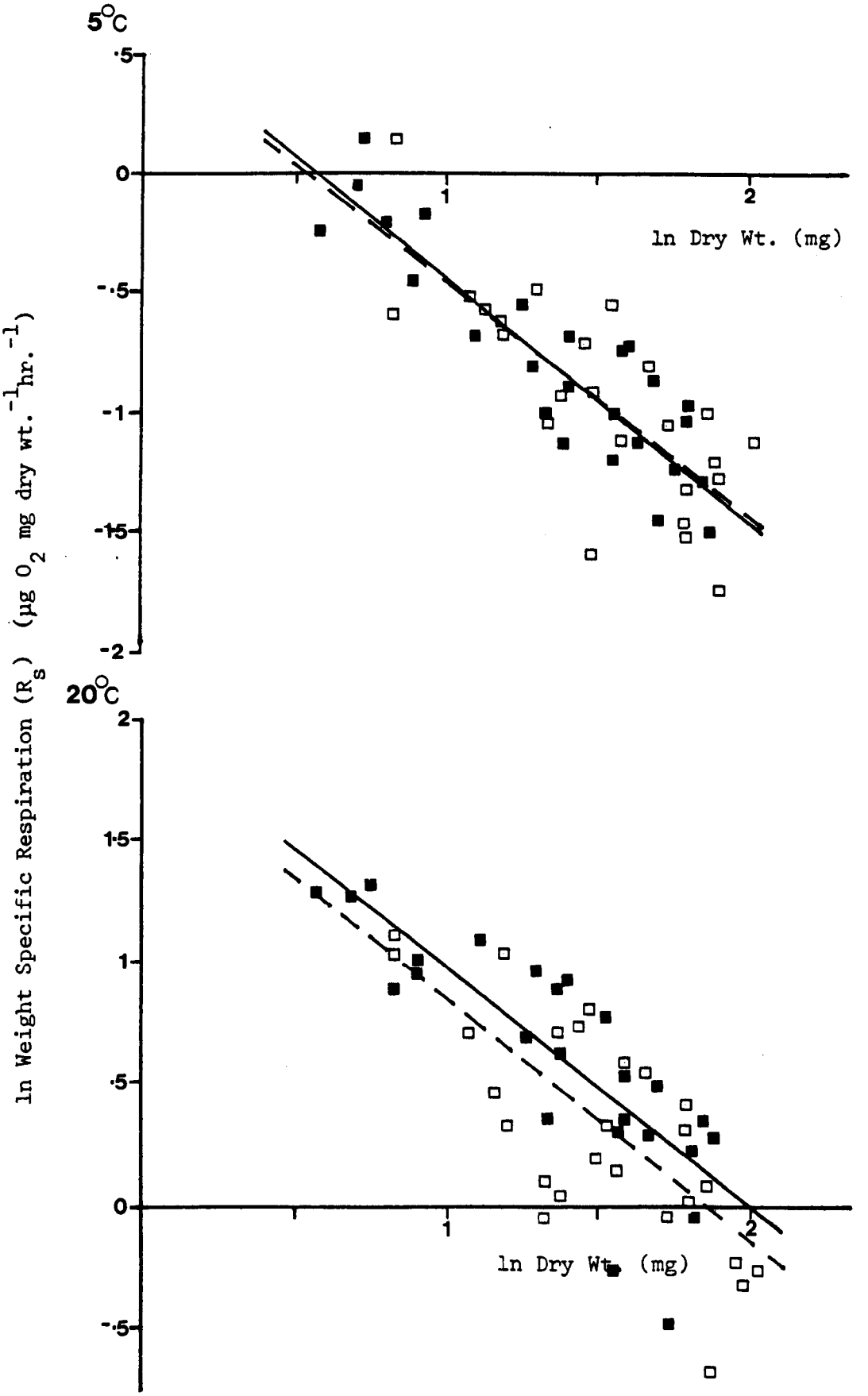

16°C
20°C
ln Weight Specific Respiration (R_s) (µg O_2 mg dry wt.$^{-1}$hr.$^{-1}$)
ln Dry Wt. (mg)
ln Dry Wt. (mg)

Fig. 5.6. Relationship between the ln weight specific respiration of *Hydropsyche contubergalis* larvae, determined at 5 and 20°C, and ln dry weight (■ = 6°C acclimated larvae; □ = 18°C acclimated larvae).



the 6 and 18°C acclimated larvae (Sokal and Rohlf, 1969) The results of the analysis of covariance for the six hydropsychid species are shown in Table 5.1.

Table 5.1 Analysis of the significance of the difference in the slope and elevation of the regression lines of the 6 and 18°C acclimated larvae for the six hydropsychid species. (N.S. = non significant, other values = Probability).

		Temperature °C			
		5	10	15	20
D. felix	slope	N.S.	N.S.	N.S.	2.5
	elevation	N.S.	N.S.	N.S.	N.S.
H. instabilis	slope	N.S.	N.S.	N.S.	N.S.
	elevation	1.0	0.1	1.0	0.1
H. pellucidula	slope	1.0	N.S.	5.0	N.S.
	elevation	2.5	0.1	0.1	0.1
H. siltalai	slope	N.S.	N.S.	N.S.	N.S.
	elevation	5.0	0.1	0.1	0.1
H. angustipennis	slope	0.1	N.S.	N.S.	1.0
	elevation	1.0	1.0	1.0	0.1
H. contubernalis	slope	N.S.	-	-	N.S.
	elevation	N.S.	-	-	N.S.

In general there is no significant difference in the slope of the pairs of regression lines for all of the species, although there are exceptions (*D. felix* at 20°C; *H. angustipennis* at 8 and 20°C; *H. pellucidula* at 5 and 15°C). However the six species may be divided clearly into two groups with regards to the significance of the difference between the elevation of the 6 and 18°C acclimated larvae. In two species, *D. felix* and *H. contubernalis* there is no significant difference in the elevation of the two regression

lines at any temperature, but in the remaining four species there is a significant difference in the elevation, again at all temperatures. In all cases where there is a significant difference in the elevation of the regression lines the larvae maintained at the warmer temperature have a lower weight specific respiration at all temperatures and body weights.

5.3.2 *Polycentropodids*

As with the hydropsychids the weight specific respiration of the three species studied, determined at 20°C, is plotted against dry weight on a ln-ln scale in Figs. 5.7 to 5.9. Again the linear regressions of ln weight specific respiration on ln dry weight are all significant at the 0.1% level. The results of the analysis of covariance for the three species are shown in Table 5.2.

Table 5.2 Analysis of the significance of the difference in the slope and elevation of the regression lines of the 6 and 18°C acclimated larvae for the 3 polycentropodid species. (N.S. = non significant; other values = probability).

		20°C
P. conspersa	slope	N.S.
	elevation	N.S.
P. flavomaculatus	slope	N.S.
	elevation	0.1
N. bimaculata	slope	N.S.
	elevation	N.S.

For all three species there is no significant difference in the slope of the regression lines. For *P. flavomaculatus*, but not the other two species, there is a significant difference, at the 0.1% level, in the elevation of

Fig. 5.7. Relationship between the weight specific respiration of *Plectrocnemia conspersa* larvae, determined at 20°C, and dry weight (■ = 6°C acclimated larvae; □ = 18°C acclimated larvae).

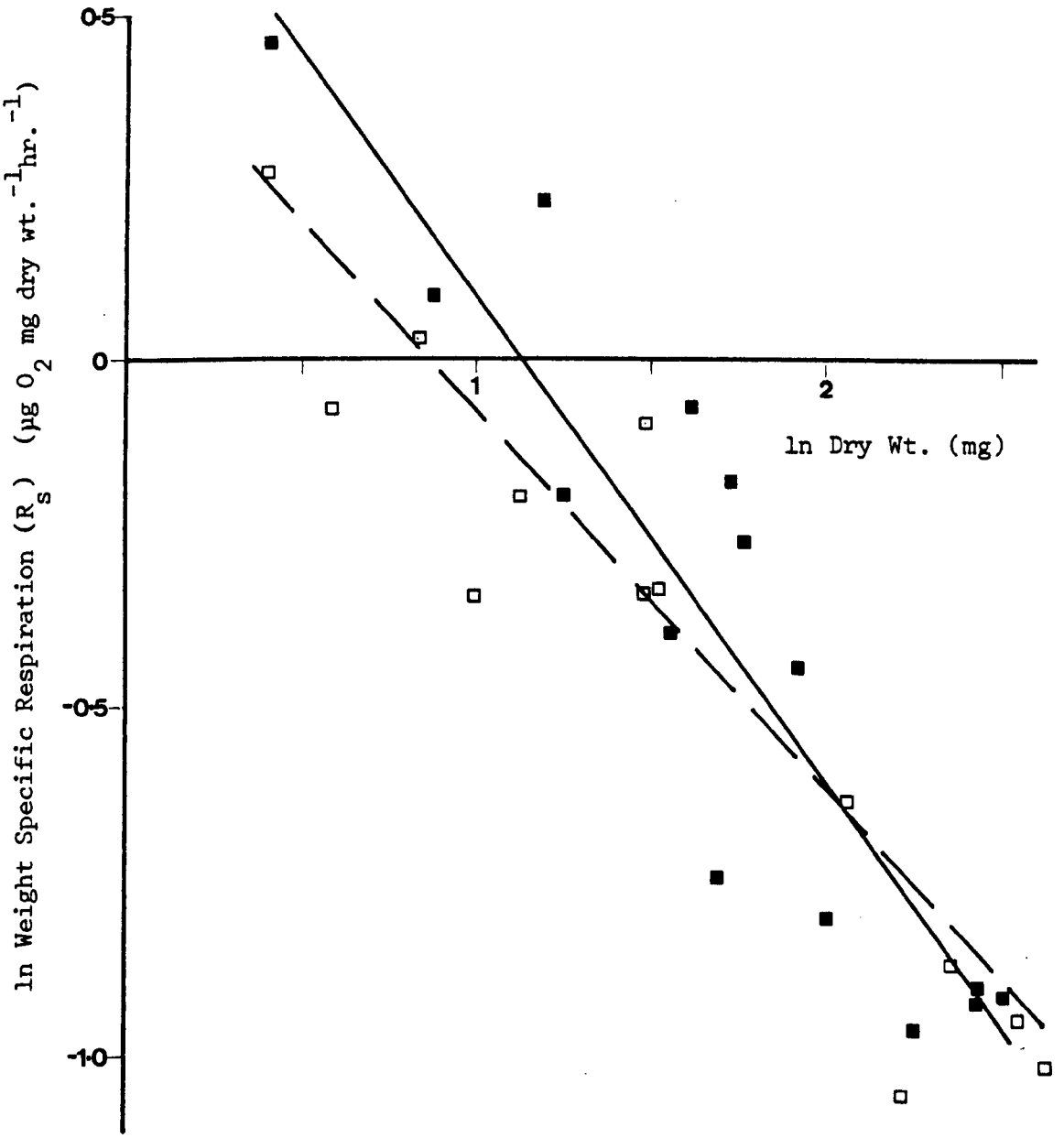


Fig. 5.8. Relationship between the weight specific respiration of *Polycentropus flavomaculatus* larvae, determined at 20°C, and dry weight (■ = 6°C acclimated larvae; □ = 18°C acclimated larvae).

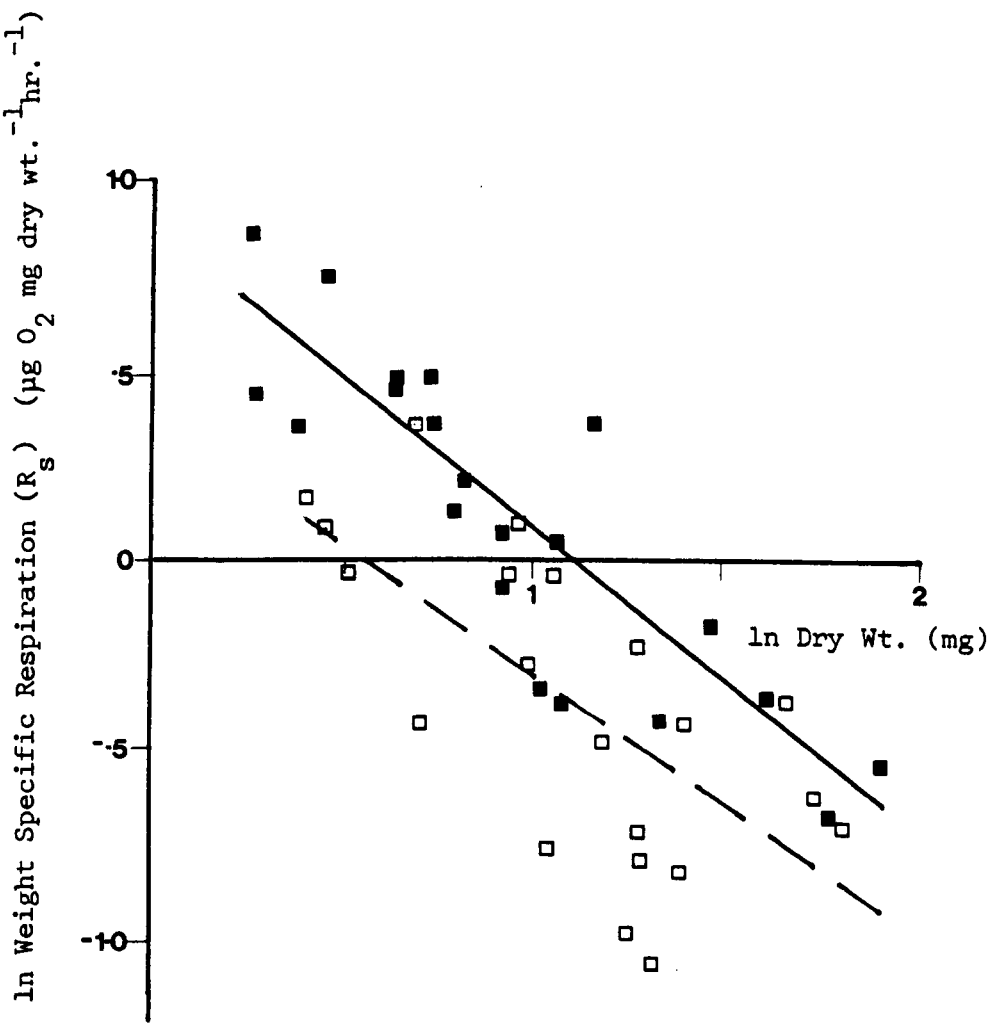
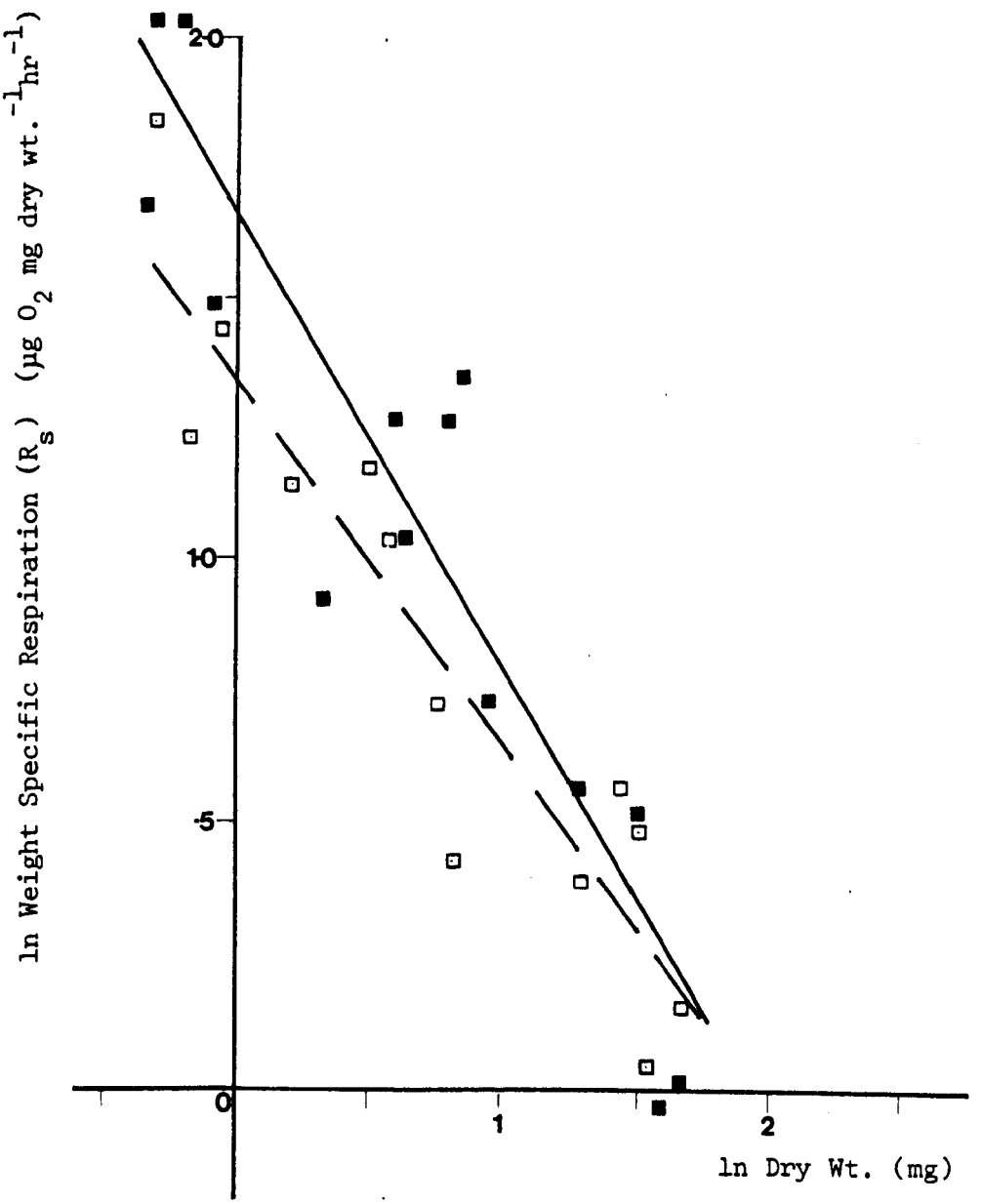


Fig. 5.9. Relationship between the weight specific respiration of *Neureclipsis bimaculata* larvae, determined at 20°C, and dry weight (■ = 6°C acclimated larvae; □ = 18°C acclimated larvae).



the lines. As before the larvae acclimated to the warmer temperature have a lower weight specific respiration at all body weights.

5.3.3 *Cased larvae*

Again as before the weight specific respiration of the two limnephilids and the sericostomatid, determined at a range of temperatures, is plotted against dry weight on a ln-ln scale in Figs. 5.10 to 5.12. The linear regressions of ln weight specific respiration on ln dry weight are again all significant at the 0.1% level. The results of the analysis of covariance for the three species are shown in Table 5.3.

Table 5.3 Analysis of the significance of the difference in the slope and elevation of the regression lines of the 6 and 18°C acclimated larvae, for the three cased larvae. (N.S. = non significant; other values = probability).

		Temperature °C			
		5	10	15	20
P. cingulatus	slope	N.S.	N.S.	1.0	N.S.
	elevation	0.1	1.0	0.1	0.1
A. nervosa	slope	N.S.	-	N.S.	N.S.
	elevation	5	-	0.1	0.1
S. personatum	slope	5	N.S.	N.S.	N.S.
	elevation	0.1	0.1	0.1	0.1

With the exception of the results for *P. cingulatus* at 15°C and *S. personatum* at 5°C there is no significant difference in the slope of the regression lines, but the elevation of the lines are significantly different at all temperatures for all three species. Again the larvae

Fig. 5.10. Relationship between the weight specific respiration of *Anabolia nervosa* larvae, determined at 5, 15 and 20°C, and dry weight (■ = 6°C acclimated larvae; □ = 18°C acclimated larvae).

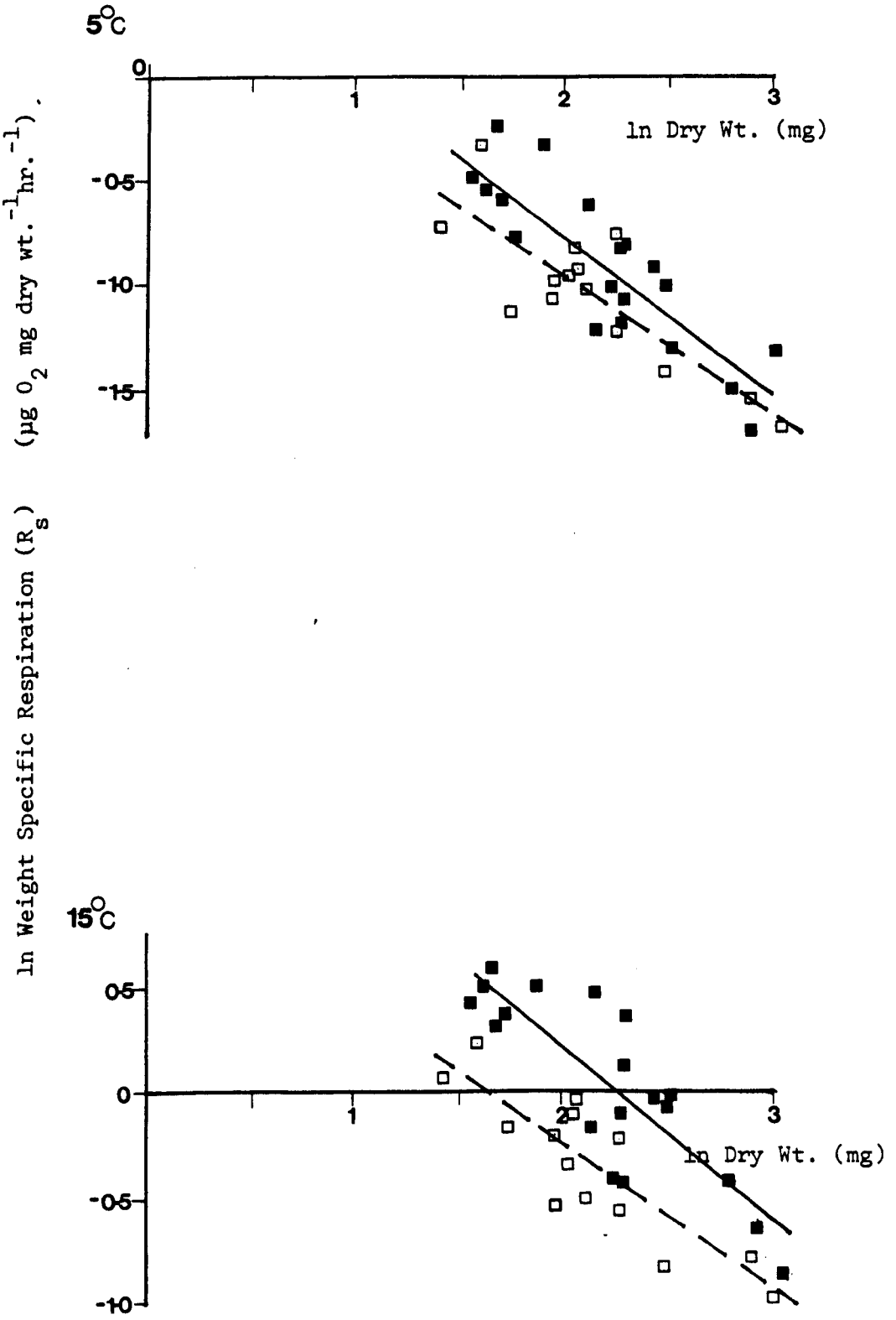


Fig 5.10. (Cont.)

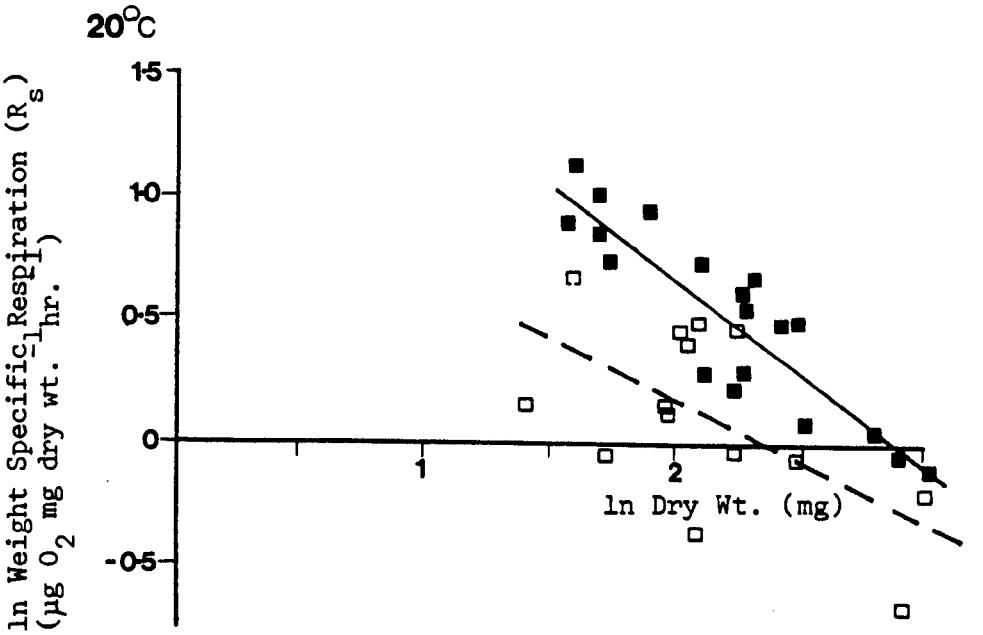

20°C
1·5
1·0
0·5
0
-0·5
1
2
ln Dry Wt. (mg)
ln Weight Specific Respiration (R_s)
($\mu g\ O_2$ mg dry wt.$^{-1}$ hr.$^{-1}$)

Fig. 5.11. Relationship between the weight specific respiration of *Potamophylax cingulatus* larvae, determined at 5,10,15 and 20°C, and dry weight (■ = 6°C acclimated larvae; □ = 18°C acclimated larvae).

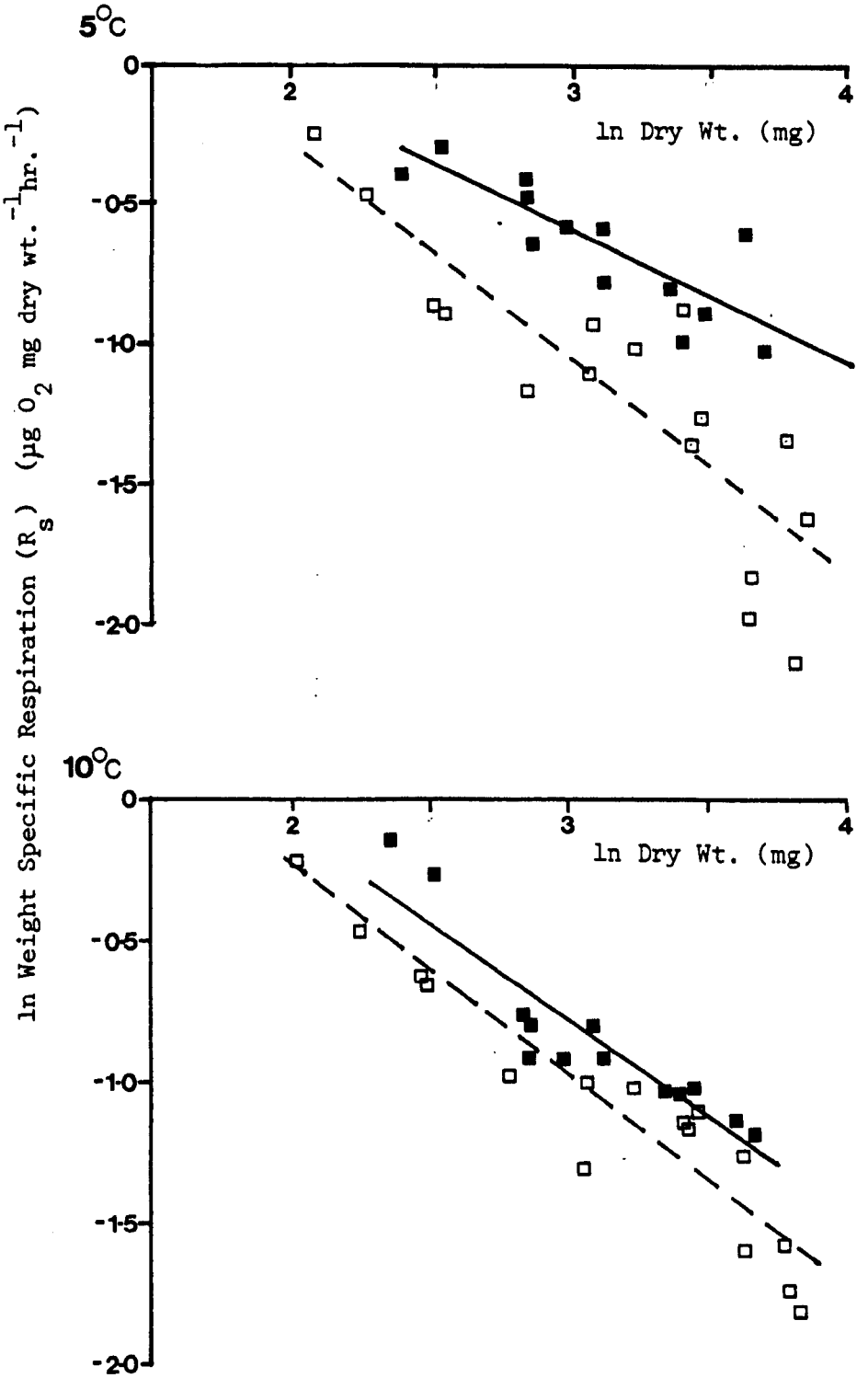


Fig. 5.11. (Cont.)

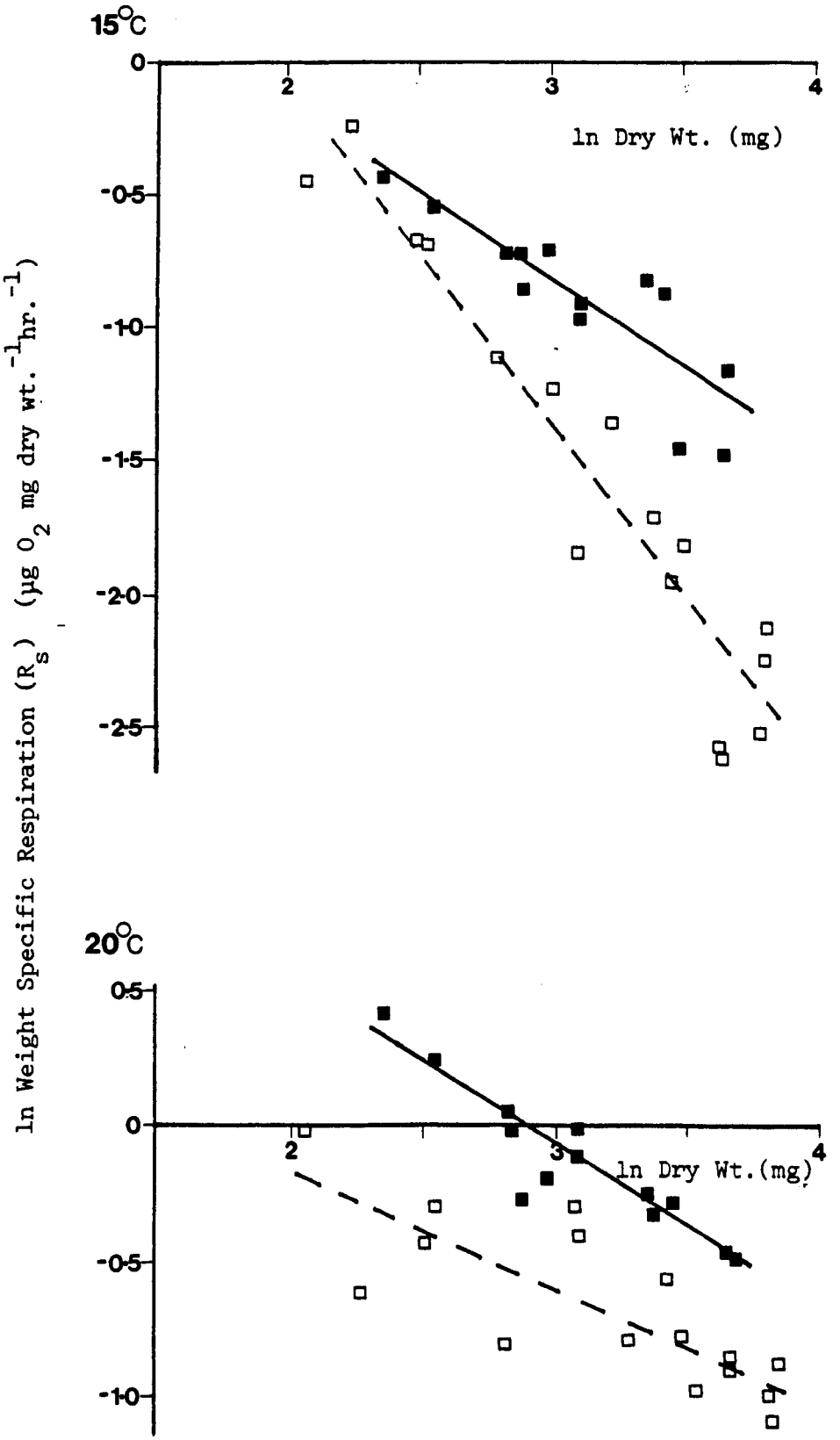

15°C
0
2
3
4
ln Dry Wt. (mg)
-0·5
-1·0
-1·5
-2·0
-2·5
ln Weight Specific Respiration (R_s) (µg O_2 mg dry wt.$^{-1}$hr.$^{-1}$)
20°C
0·5
0
2
3
4
ln Dry Wt.(mg)
-0·5
-1·0

Fig. 5.12. Relationship between the weight specific respiration of *Sericostoma personatum* larvae, determined at 5,10,15 and 20°C, and dry weight (■= 6°C acclimated larvae; □= 18°C acclimated larvae).

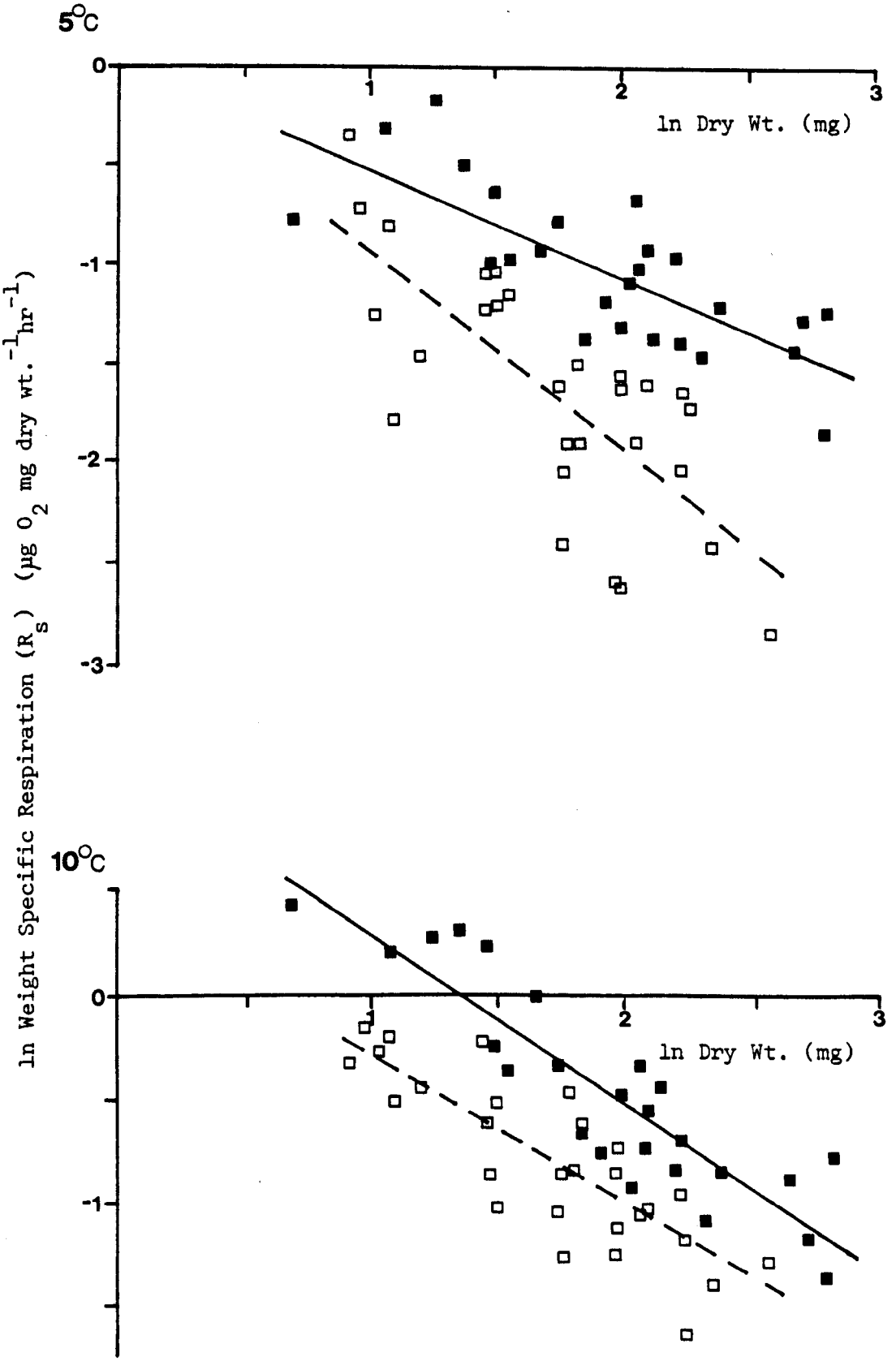


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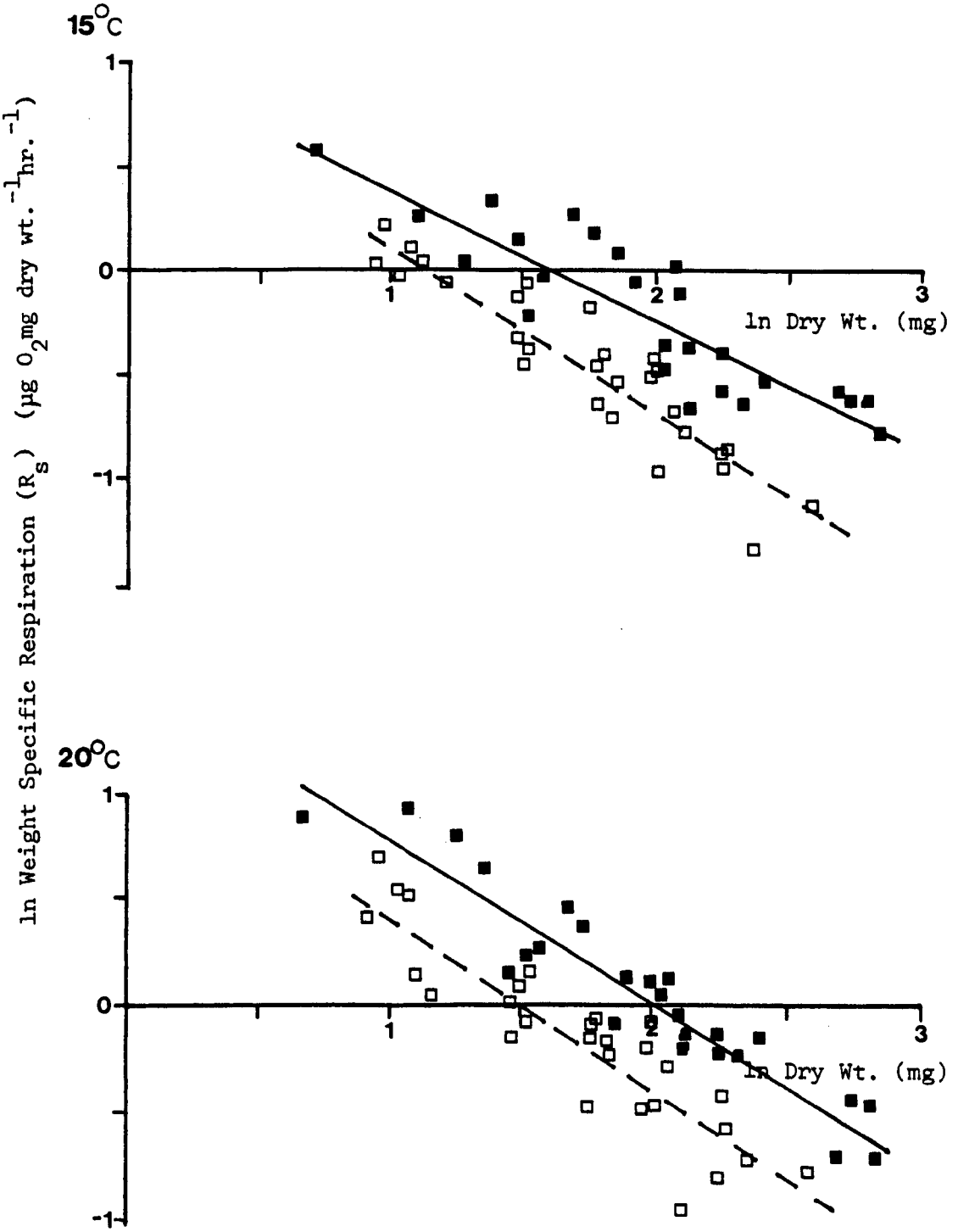
Fig. 5.12. (Cont.)


15°C
20°C
ln Weight Specific Respiration (R_s) (µg O_2mg dry wt.$^{-1}$hr.$^{-1}$)
ln Dry Wt. (mg)
ln Dry Wt. (mg)
1
0
-1
1
2
3

acclimated to the warmer temperature have a lower weight specific respiration at all temperatures and body weights.

5.4.1 Discussion

The slope of the regression lines of ln weight specific respiration on ln dry weight, for all species and at each temperature studied, are summarised in Table 5.4. Three points may be made from these results. Firstly a comparison of the mean values calculated from all of the 6 and 18^{o}C acclimated larvae (all species and at all temperatures) shows there to be no difference in the slope of the regression lines of the two groups of larvae, (t=-0.888, N.S.). This indicates that the relationship between the weight specific respiration of the 6 and 18^{o}C acclimated larvae will remain constant over the size range. Secondly the overall means calculated for each temperature, although varying with lower values at 10 and 20^{o}C, does not follow any trend. Thus it appears that the effect of a temperature increase on the weight specific respiration is to cause an equal upwards shift in the regression lines at all body weights. Thirdly, as previously observed in Chapter 4.2.2, the overall value for b, (slope of ln weight specific respiration against ln dry weight = b-1) being calculated as 0.242, is lower than the value of 0.67 normally quoted for poikilotherms. Harrison and Badcock (1981) obtained similar values for two species of caddis larvae, approximately 0.4 for *P. cingulatus* and 0.22 for *Chaetopteryx villosa*. Calow (1975) points out that the value of b may be dependent upon the form in which the weight is expressed. For example, if large individuals contain proportionally less water than smaller individuals the value for the slope will be larger if wet weight is used rather than dry weight. Calow also proposes that the respiratory rate could be a function of the physiologically active surface. If this is so, the low value of b could reflect a proportionally greater physiologically active surface in smaller individuals compared with larger individuals. This could

occur if the gill systems provide a proportionally larger surface area in small individuals or if the proportion of non-permeable surface (sclerotized areas) is proportionally smaller in small individuals. This was not investigated in the present study.

The major aim of this study was to investigate whether or not caddis larvae are capable of acclimating to temperature. A difference in the elevation of the regression lines, of ln weight specific respiration on ln dry weight, between two groups of larvae maintained under the different temperature conditions is taken to indicate that temperature acclimation has occured. From Figs. 5.1 to 5.14 and Tables 5.1 to 5.3 temperature acclimation, of the 'routine' metabolism, is seen to occur in some species, while others appear to demonstrate no compensatory ability (Table 5.5). In all instances where acclimation was demonstrated it was a partial, type III, response (Precht, 1951), the larvae acclimated to the warmer temperature having a lower weight specific respiration over the range of experimental temperatures. This contrasts with the results of Harrison and Badcock (1981) who demonstrated reverse acclimation, a type V response (Precht, 1951), for *P. cingulatus*. However the authors conceded that the data base was low in that study and may have been subject to a few aberrant results.

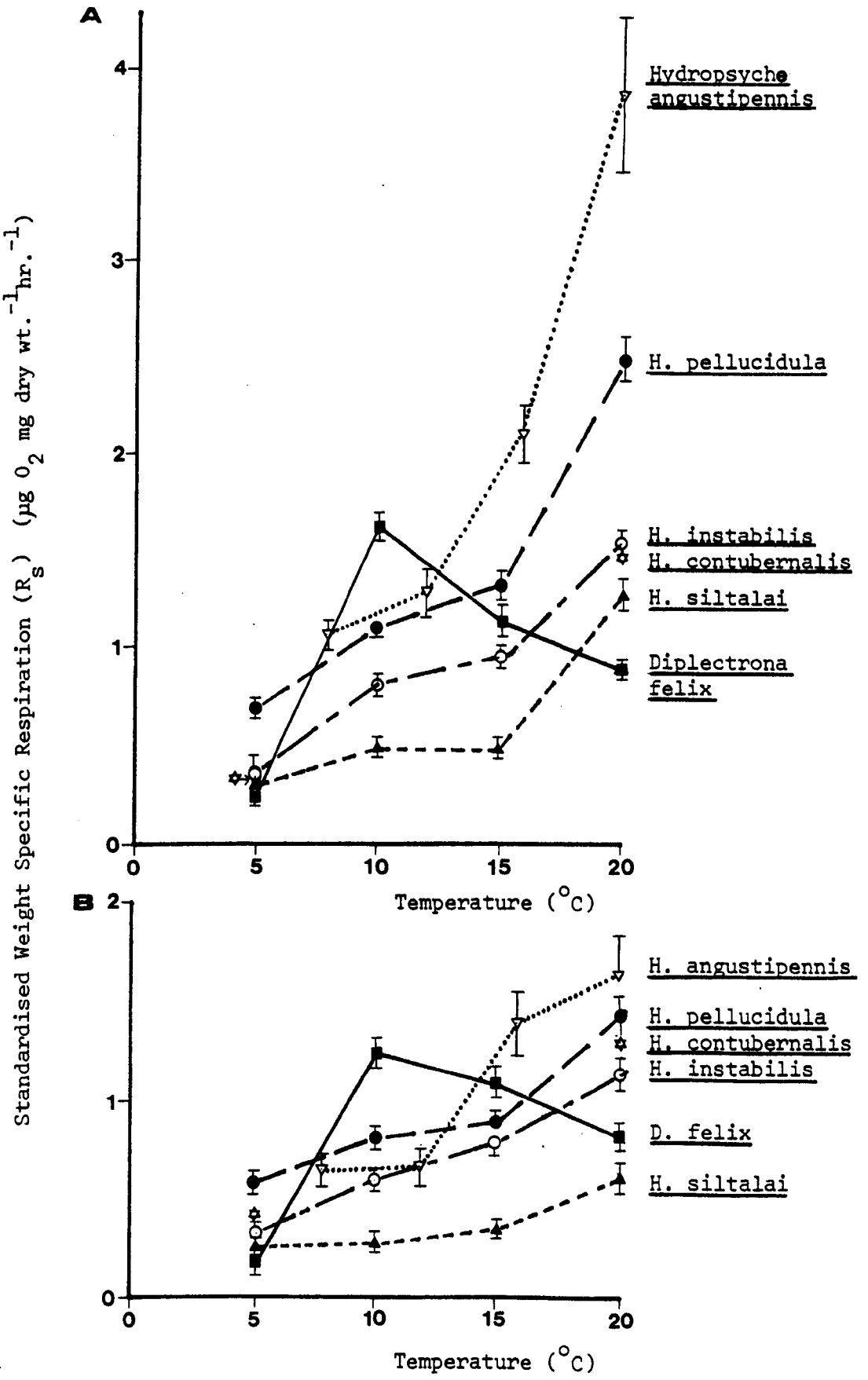
Table 5.5 Species in which an ability to acclimate was and was not demonstrated.

Species showing no acclimatory ability	Species showing some acclimatory ability
Diplectrona felix	*H. instabilis*
Hydropsyche contubernalis	*H. pellucidula*
Plectrocnemia conspersa	*H. siltalai*
Neureclipsis bimaculata	*H. angustipennis*
	Polycentropus flavomaculatus
	Potamophylax cingulatus
	Anabolia nervosa
	Sericostoma personatum

5.4.2 *Interspecific comparisons*

Interspecific comparisons are considered in three ways, a comparison of the weight specific respiration, a comparison of the extent of the ability to acclimate to different temperatures and a comparison of the temperature-metabolism curves. As stated earlier, due to the weight dependancy of the weight specific respiration it is not valid to make comparisons between species or groups of larvae simply by calculating the mean value for the weight specific respiration as this will be influenced by the weight distribution of the larvae. To overcome this the data are standardised to a single body weight for comparative purposes. For the hydropsychids and polycentropodids a dry weight of 5mg was chosen. For the cased larvae a dry weight of 20mg was used for *P. cingulatus* and 5mg for the other two species. These weights were used as they fell towards the midpoint of the weight range for the larvae of all species studied.

Fig. 5.13. Standardised weight specific respiration, of the larvae of the six hydropsychid species. A=6°C acclimated larvae; B=18°C acclimated larvae.



5.4.3 *Comparison of the weight specific respiration of the different species*

The standardised weight specific respiration of the six hydropsychid species studied are shown in Fig. 5.13 For both the 6 and 18°C acclimated larvae a similar pattern occurs. The weight specific respiration is seen to increase in the sequence *H. siltalai*, *H. instabilis*, *H. pellucidula* and *H. angustipennis*. The results for *D. felix* differ from those observed for the other species. At low and high temperatures (5 and 20°C) the respiration rate is lower than that for the other species, with the exception of the 18°C acclimated larvae where the lowest value was obtained for *H. siltalai*. However at 10°C the weight specific respiration of *D. felix* exceeds all of the other hydropsychids and at 15°C lies in an intermediate position. From the incomplete data for *H. contubernalis*, this species appears to have a similar weight specific respiration to *H. instabilis*. Hildrew and Edington (1979) found the weight specific respiration of *H. pellucidula* to be lower than that of *H. instabilis* over the full temperature range, the reverse of what was found in this study. *D. felix* had a weight specific respiration either slightly below or slightly in excess of that found for *H. instabilis* depending upon the temperature considered. No peak, followed by a decrease in the weight specific respiration was demonstrated,as occurred in this study. A major difference in the experimental protocol which could account for these discrepancies is that in the study by Hildrew and Edington respirometers were used with an internal stirring mechanism, which maintained a flow rate of approximately 20cms^{-1}, while in this study unstirred respirometers were used. As the respiration of caddis larvae is affected by flow rate (Feldmeth 1970a) and acclimation can occur in response to different flow rates (Feldmeth, 1970b) the relationship between the weight specific respiration of the different species may reflect differences in the response to water flow.

These data bear no obvious relationship to the field distribution of

these species. Intuitively it might be expected that species with a distribution in warmer water (*H. siltalai*, *H. pellucidula*, (Hildrew & Edington, 1979)), *H. angustipennis* (Badcock, 1975) and *H. contubernalis* would have a lower weight specific respiration, when determined at warmer temperatures than species with a cool water distribution, (*D. felix* and *H. instabilis* (Hildrew and Edington, 1979)), as this would offer a competitive advantage. However two of the 'warm' water species, *H. angustipennis* and *H. pellucidula*, actually had the highest weight specific respiration and *H. instabilis*, a 'cold' water species had a low weight specific respiration which was similar to that obtained for the 'warm' water species, *H. contubernalis*. It therefore appears that there is no simple relationship between the weight specific respiration of the species and their distributions. This possibly reflects interspecific differences in the response to other environmental factors, water flow being likely to be important.

Similar standardised weight specific respirations of the three polycentropodids at 20^{o}C are shown in Table 5.6. The respiration of *Plectrocnemia conspersa*, a headstream species, and *Polycentropus flavomaculatus*, a cosmopolitan species, are similar, although in the latter slightly lower. *N. bimaculata*, a larvae typical of large rivers (Hildrew & Edington, 1981) has a weight specific respiration approximately double that of the other two species.

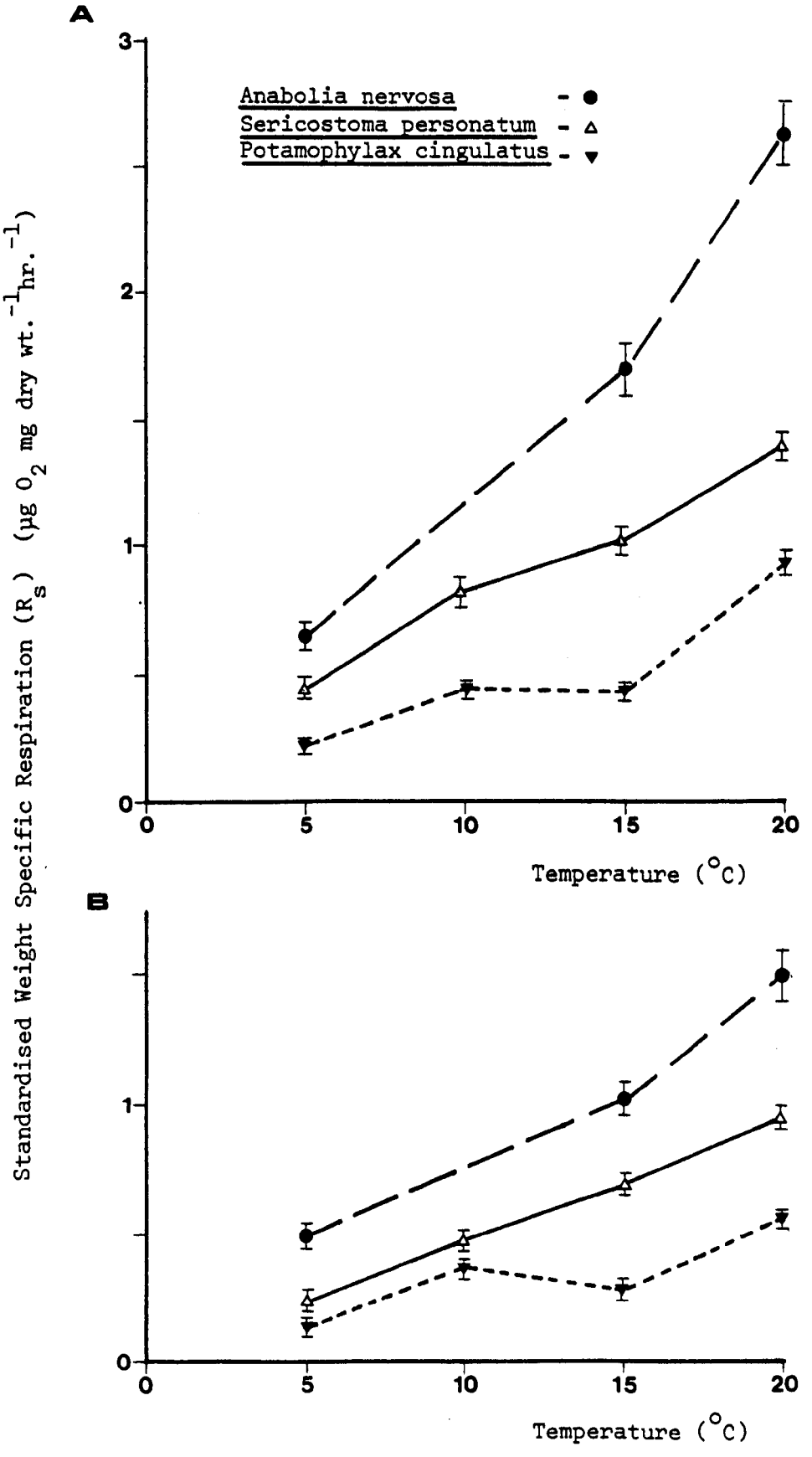
Table 5.6 Standardised weight specific respiration, of the three polycentropodids, at 20°C

	n 6°C acclimated larvae		n 18°C acclimated larvae	
		$\bar{x}$ SE		$\bar{x}$ SE
P. conspersa	15	0.6885 ± 0.0387	15	0.7002 ± 0.0449
P. flavomaculatus	21	0.6736 ± 0.0318	21	0.5098 ± 0.0328
N. bimacalata	14	1.3275 ± 0.0864	13	1.237 ± 0.0614

Judged by these incomplete data the weight specific respirations of these three species are comparable, but slightly lower, than the hydropsychid species with comparable distributions (*P. conspersa*, Rs=0.7; *D. felix*, Rs=0.834; *P. flavomaculatus*, Rs=0.51; *H. siltalai*, Rs=0.6; *N. bimaculata*, Rs=1.24; *H. contubernalis*, Rs=1.31. Values = µg O_2 mg dry $wt^{-1}hr^{-1}$ determined on 18°C acclimated larvae at 20°C). This could possibly reflect the differences in the two families in terms of gills. The polycentropodids having only anal gills while the hydropsychids have many tufted abdominal gills. The respiration of the polycentropodids could have been limited by the rate at which oxygen could be obtained from the water. Obviously as the respiration was only determined at a single temperature it is not possible to say whether the results would be consistent with these species over the temperature range. In particular it would be of interest to determine whether or not the results for *P. conspersa* follow a similar pattern to that observed in *D. felix*.

Finally the standardised weight specific respiration, at a range of temperatures, of the three cased larvae are shown in Fig. 5.14 Due to the greater body size of *P. cingulatus*, direct comparison with the other species is not possible. For the other two species little information is available relating their distributions to water temperature and therefore

Fig. 5.14. Standardised weight specific respiration, of the three cased larvae, against temperature. A=6°C acclimated larvae; B=18°C acclimated larvae.



the respiration data cannot easily be related to distribution. However it is notable that *A. nervosa* which has a fairly cosmopolitan distribution (rapid flowing streams/stagnant pools/lakes (Hickin, 1967) has a weight specific respiration similar to *H. pellucidula* and *H. angustipennis*, while *S. personatum* with a less cosmopolitan distribution (fast streams/rivers or lake outflows (Hickin, 1967)) has a weight specific respiration comparable with *H. instabilis*.

5.4.4 *Interspecific comparisons of the ability to acclimate to temperature*

A measure of the extent to which temperature acclimation has occurred is calculated as the percentage decrease in the weight specific respiration of the 18°C acclimated larvae compared with the 6°C acclimated larvae. This is presented for the hydropsychids in Table 5.7. As shown by the analysis of covariance (Table 5.1), with the exception of the results for two species, *D. felix* and *H. contubernalis*, all of the differences are statistically significant. Although the values vary considerably at different temperatures there is no apparent relationship between the calculated acclimation ability and the temperature at which the respiration was determined.

Table 5.7 Percentage decrease in the weight specific respiration of the 18°C acclimated larvae compared with the 6°C acclimated larvae for the six hydropsychids

	Temperature °C					
	5	10	15	20	$\bar{x}$	On-1
D. felix	12.3	22.6	6.5	6.5	11.98	7.593
H. instabilis	20.9	28.2	16.3	25.3	22.675	5.203
H. pellucidula	16.0	23.8	37.6	41.8	29.8	11.99
H. siltalai	17.9	46.3	29.0	53.2	36.6	16.095
H. angustipennis	37.6	47.0	33.2	57.1	43.73	10.613
H. contubernalis	-2.0	-	-	12.2	5.1	10.041

Note - $\bar{x}$ is Mean Percentage Decrease

It has been noted before that the ability to acclimate varies between related species and that this may influence their distributions (Calow, 1975; Harrison and Badcock, 1981). These data demonstrate a clear relationship between the ability to acclimate and the clearly defined downstream distribution of this family (Badcock, 1975; Hildrew and Edington, 1979). The two species in which no compensation was found both have restricted distributions and occur in habitats with relatively constant temperature regimes, *D. felix* occuring in headstreams with low maxima and a small daily range and *H. contubernalis* occuring in large rivers with high maxima and a small range. The increased ability to compensate in the order *H. instabilis*, *H. pellucidula* and *H. siltalai* corresponds to increasingly widespread ecological distributions and occurence at higher mean water temperatures. The final species, *H. angustipennis*, for which the greatest acclimation ability was found is a widespread species in warmer habitats and is known to be more resistant to sluggish conditions and low oxygen (Badcock, 1975).

Table 5.8 Percentage decrease in the weight specific respiration of the 18°C acclimated larvae compared with the 6°C acclimated larvae for the three polycentropodids

	Percentage Decrease
P. conspersa	-1.7
P. flavomaculatus	24.3
N. bimaculata	6.8

The extent to which acclimation occurred in the polycentropodids, at 20°C, is shown in Table 5.8. From Table 5.2 it is seen that the difference in the respiration of the two groups of larvae is only significantly different for *Polycentropus flavomaculatus*. As before the two species, *Plectrocnemia conspersa* and *Neureclipsis bimaculata*, which have restricted distributions and occur in habitats with relatively constant temperature regimes, are shown to have no ability to acclimate. *P. conspersa* has a similar distribution to *H. instabilis*, and *P. flavomaculatus* a similar distribution to *H. siltalai*. This appears to indicate that the ability to acclimate is greater in the hydropsychids than in the polycentropodids.

The extent to which acclimation occurred in the cased larvae, at a range of temperatures, is shown in Table 5.9. From Table 5.3 it is seen that the difference in the respiration of the two temperature groups of larvae is significant in all three species. There is little difference in the acclimation ability of the three species, the value appearing to be similar to the three more widespread hydropsychids (*H. pellucidula*, *H. siltalai* and *H. angustipennis*).

Table 5.9 Percentage decrease in the weight specific respiration of the 18°C acclimated larvae compared with the 6°C acclimated larvae for the three cased species.

	Temperature °C					
	5	10	15	20	$\bar{x}$	On-1
S. personatum	48.4	51.2	31.6	32.4	40.9	10.345
P. cingulatus	35.3	18.1	39.8	40.6	33.5	10.496
A. nervosa	21.9	-	40.4	43.0	35.1	11.505

5.4.5 *Comparison of temperature metabolism curves*

The temperature metabolism curves of the five hydropsychid species for which data is available at a range of temperatures are shown in Fig. 5.15. In all but one species (*D. felix*) the temperature-metabolism curves are typical and similar to the results presented in Chapter 4. The weight specific respiration generally increases with temperature but there is strong evidence for the occurrence of a plateau in the relationship. This region of relative temperature independence has been related to the temperature regime at which a species occurs (Precht et al., 1973). Hildrew and Edington (1979) demonstrated a progressive extension of the zone of relative temperature independence towards warmer temperatures, which matched the progressively higher temperature regimes experienced by the species in summer for *D. felix*, *H. instabilis* and *H. pellucidula*. No zone of relative temperature independence was found in this study for *H. instabilis*, the increase in respiration being reasonably constant over the temperature range studied. The results for *H. pellucidula* are comparable with those presented by Hildrew and Edington (1979). The respiration is relatively temperature independent in the range 5 to 15°C, after which there is an accelerating increase in the weight specific respiration. *H.*

Fig. 5.15. Temperature-metabolism curves for larvae of five hydropsychid species.

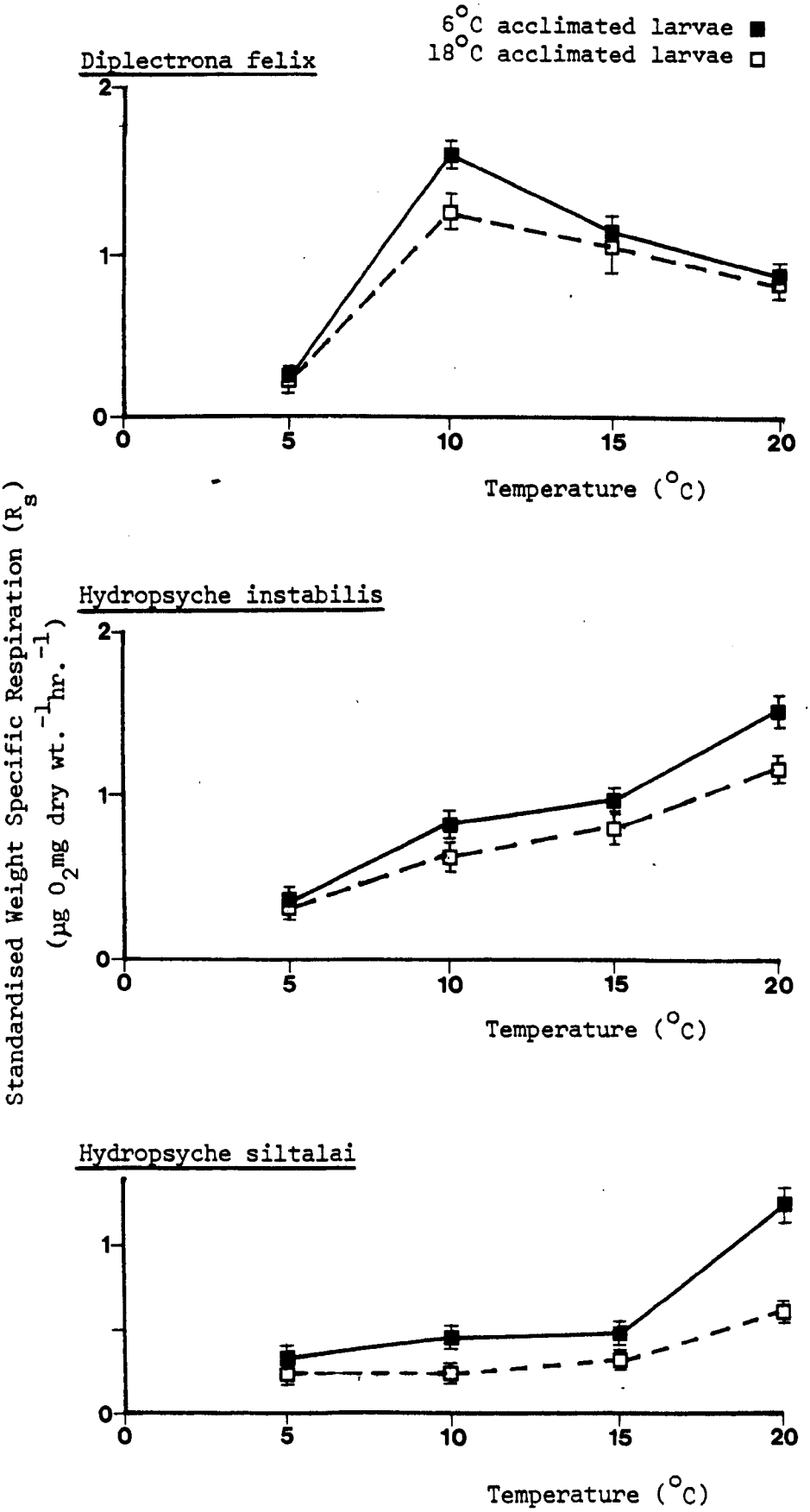
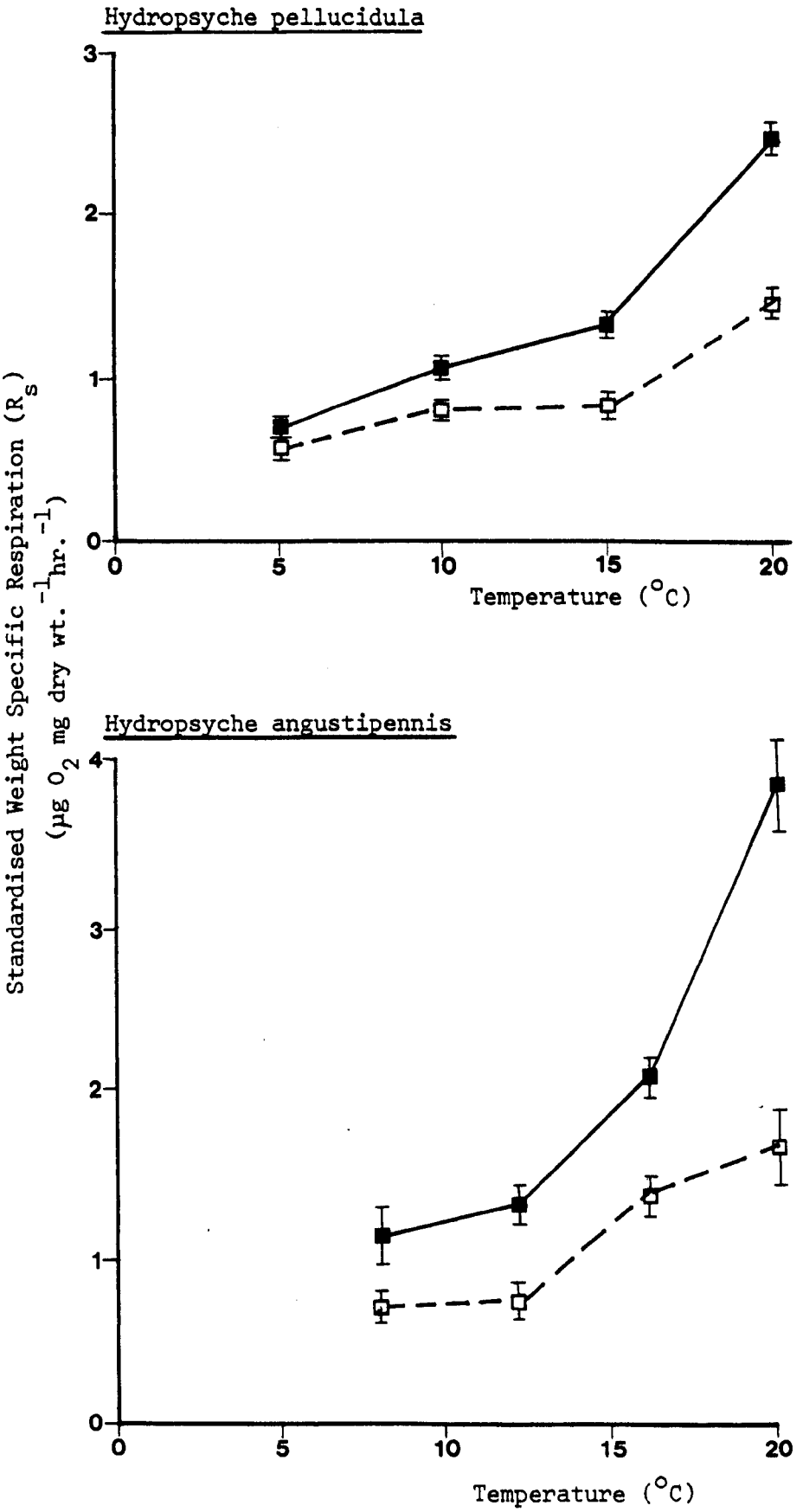


Fig. 5.15. (Cont.)


Hydropsyche pellucidula
Hydropsyche angustipennis
Standardised Weight Specific Respiration (R_s)
(µg O_2 mg dry wt.$^{-1}$hr.$^{-1}$)
Temperature (°C)
Temperature (°C)
0
1
2
3
4
5
10
15
20

siltalai, with a distribution roughly comparable to *H. pellucidula*, also has a zone of relative temperature independence over the same range. For these two species, over the temperature range 5 to 15°C the acclimation is virtually complete (Precht, 1951). The results for *H. angustipennis* are perhaps surprising as they might have been expected to be similar to those obtained for *H. siltalai* and *H. pellucidula*. There is little evidence of a plateau in the relationship, except perhaps below 12°C, and the increase in respiration with temperature is greater than in the other species. This suggests that this species has a different strategy for survival at warmer temperatures than the other species. The results for *D. felix* are unusual and may reflect the onset of respiratory distress, at a temperature between 10 and 15°C, for this species restricted to cooler habitats. The difference between these results and those obtained by Hildrew and Edington (1979), who found an increase in respiration upto 25°C, could be accounted for by the use of stirred respirometer chambers in their study.

Unfortunately, as the temperature-metabolism curves are based upon only 4 temperatures, it is not possible to see whether acclimation to different constant temperatures affects the width or position of the zone of temperature indepedance, in addition to causing a vertical shift in the position of the curve.

It is of interest to compare the weight specific respiration at the temperatures of 6 and 18°C of the cool and warm acclimated larvae respectively. These are estimated from Fig. 5.16 for four hydropsychid species in Table 5.10.

Fig. 5.16. Temperature-metabolism curves of the three cased larvae.

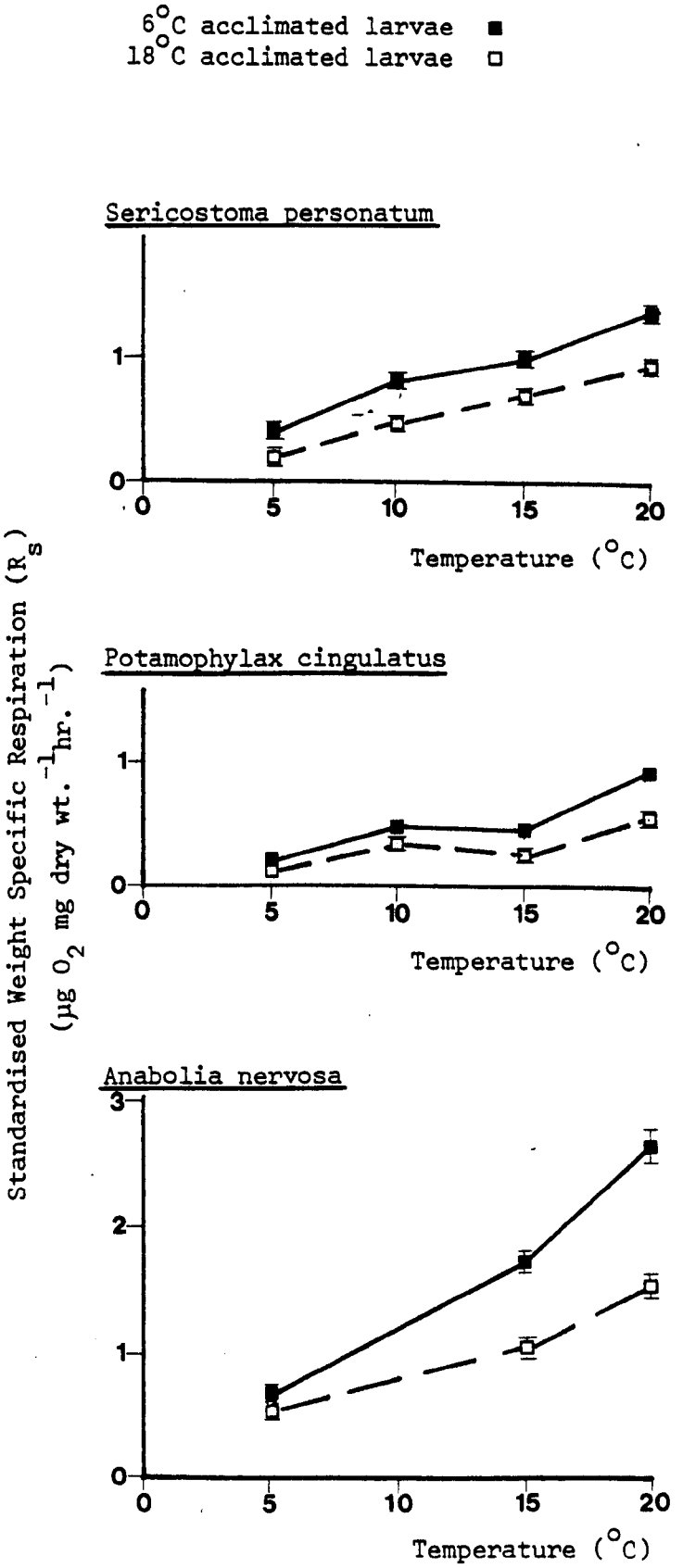


Table 5.10 Weight specific respiration (Rs) at 6 and 18°C of the cool and warm acclimated larvae respectively

	A Rs of 6°C acc. larvae at 6°C	B Rs of 18°C acc. larvae at 18°C	Proportional increase between A and B
H. instablis	0.42	1.00	2.381
H. pellucidula	0.79	1.2	1.519
H. siltalai	0.35	0.48	1.371
H. angustipennis	1.00	1.54	1.54

The degree by which the weight specific respiration increases for an increase in temperature from 6 to 18°C, for larvae acclimated to the same temperatures (6 and 18°C), decreases in the order *H. instabilis*, *H. pellucidula* and *H. siltalai*, which corresponds to the order of increasing ecological distribution. *H. angustipennis* being a highly adapTable species is also shown to have a relatively small increase in its respiration.

The temperature-metabolism curves of the three cased larvae are shown in Fig. 5.16. In all three species there is an increase in the respiration with an increase in temperature. For *P. cingulatus* there is a zone of relative temperature indepdence between 5 and 15°C, while in *S. personatum* the increase in respiration remains constant over the entire temperature range. No firm conclusion can really be drawn for *A. nervosa* due to the lack of data at 10°C. As before the weight specific respiration at 6 and 18°C of the cool and warm acclimated larvae respectively (estimated from Fig. 5.17) are compared in Table 5.11. Although the distribution patterns of these species are less well known than the distributions of the hydropsychids, these values are consistent with them having a distribution intermediate between *H. instabilis* and *H. pellucidula* in terms of the range of temperature regimes at which they live.

Table 5.11 Weight specific respiration (Rs) at 6 and 18°C of the cool and warm acclimated larvae respectively

	A Rs of 6°C acc. larvae at 6°C	B Rs of 18°c acc. larvae at 18°C	Proportional increase between A and B
S. personatum	0.51	0.84	1.647
P. cingulatus	0.25	0.44	1.76
A. nervosa	0.74	1.33	1.797

5.5 Conclusions

The data discussed in this chapter have demonstrated the occurrence of temperature acclimation in some, but not all, species. Assuming that the acclimation has reached a maximum for each of the species, which is thought to be likely following a 4-5 week acclimation period, the degree to which acclimation occurs in each species has been shown to bear a relationship to their field distributions, in terms of the thermal regimes in which they occur. Species with more widespread distributions have a greater acclimation ability than those with restricted distributions.

These data suggest that differences in the temperature-metabolism curves of species may not be a factor controlling distribution, but instead may be a consequence of distribution, if acclimation occurs. The ability to acclimate to temperature may itself have a strong influence upon distribution, increased acclimatory ability allowing a more widespread distribution. Thus rather than, as is suggested, stenothermal species having a low ability to acclimate to temperature (Bullock, 1955) it could be that species with a limited ability to acclimate may be incapable of expanding their range into water of a different temperature.

Acclimation, occuring over a period of days or weeks, may influence the distribution of a species in two ways, spatially or temporally. It

could exert an influence on the spatial distribution, mainly at the population level, movement of individuals being limited. Individuals are more likely to be influenced by changes of temperature with time. Diurnal variations in temperature are too rapid for compensation to occur and in this respect the temperature-metabolism curves are of more relevance, particually the occurence and position of the zones of relative temperature independence.

This study, for the sake of simplicity, fails to take into account other factors, such as acclimation to water flow, which are also relevant to the distribution of species. In addition only acclimation to constant temperature, with the weight specific respiration being determined at constant temperatures, was considered, while the situation in the field is obviously more complex. A limited study of the ability of some species to acclimate to diel temperature ranges of different amplitudes are presented in Chapter 7.

CHAPTER 6 VENTILATORY ACTIVITY FOLLOWING ACCLIMATION AT WARM AND COOL TEMPERATURES

6.1 Introduction

Under conditions of slow or still water the body of an aquatic invertebrate will be surrounded by a larger boundary layer than at a faster flow rate. Within the boundary layer flow is laminar, transport through it being by molecular diffusion, the rate depending upon the thickness of the layer (Feldmeth, 1970a). For invertebrates living under sluggish or still conditions, or for tube dwelling species, the thickness of the boundary layer can be reduced by the creation of additional water movement produced by ventilation of the body.

Respiratory ventilation has been observed in a range of aquatic organisms including chironomids (Konstantinov, 1971; Nagell, 1974; Leuchs, 1986), gammarids (Sutcliffe, 1984), polychaete worms (Coyer, 1973), tube dwelling amphipods (Gamble, 1970) and leeches (Wrona and Davies, 1984). Both cased and net-spinning caddis larvae are known to ventilate their body surfaces by undulation of the abdomen. Van Dam (1938, cited in Feldmeth, 1970b) was the first to investigate the environmental factors controlling the ventilatory behaviour. Later studies by Fox and Sidney (1953), Feldmeth (1970a), Leader (1971) and Philipson and Moorhouse (1974) have demonstrated relationships between ventilation and environmental factors such as temperature, dissolved oxygen and flow rate.

Philipson and Moorhouse (1974) demonstrated differences in the frequency of abdominal undulations of three hydropsychid species, *Hydropsyche siltalai*, *Hydropsyche pellucidula and Hydropsyche angustipennis*, in response to temperature, flow rate and dissolved oxygen. These differences appear to be associated with the ranges of flow rate and temperature in the field distributions of the species. Feldmeth (1970b) demonstrated that acclimation to different flow rates resulted in both a

change in oxygen consumption and differences in the ventilatory activity.

The muscular activity associated with ventilation has a high energetic cost (Philipson and Moorhouse, 1974; Wiley and Kohler, 1984) which will shift the balance of the trade-off between growth and respiratory costs in a detrimental way. In addition for some taxa, including the hydropsychids there is an added cost of ventilation as it precludes other activities including feeding and retreat repair (Wiley and Kohler, 1984).

The ventilatory behaviour of 10 of the species studied in the previous chapter (no data are available for *Anabolia nervosa* or *Sericostoma personatum*) are investigated in respect of temperature. The aims of this study were threefold:

1. To investigate the ventilatory behaviour of the larvae in response to different temperatures.
2. To investigate the influence of acclimation to constant temperatures of 6 and 18^{o}C, on the ventilatory behaviour.
3. To make interspecific comparisons of the ventilatory behaviour and relate this to the respiration results discussed in Chapter 5 and to the distribution of the species.

6.2 Method

The experiments were performed on groups of larvae acclimated to 6 or 18^{o}C for 4-5 weeks under the conditions described in Chapter 5.2.

Acclimated net-spinning larvae were placed individually in 25ml beakers which were fully submerged in aerated temperature controlled aquaria for 12 hours at 10^{o}C. During this period virtually all of the hydropsychids (>95%) built rough retreats but more of the polycentropodids failed to spin a retreat (25-30% failure). For the cased species a small portion of the case material was removed from the upper surface of the case in the abdominal region. Care was taken not to damage the inner lining of the case. This allowed the abdominal movements to be observed while

Fig. 6.1. The effect of temperature on the number of undulations and the time spent undulating in a 5 minute period, and the rate of undulations of *Diplectrona felix* larvae acclimated to 6 and 18°C for 4-5 weeks. Variation about mean, expressed as the SE, is represented by the vertical lines.

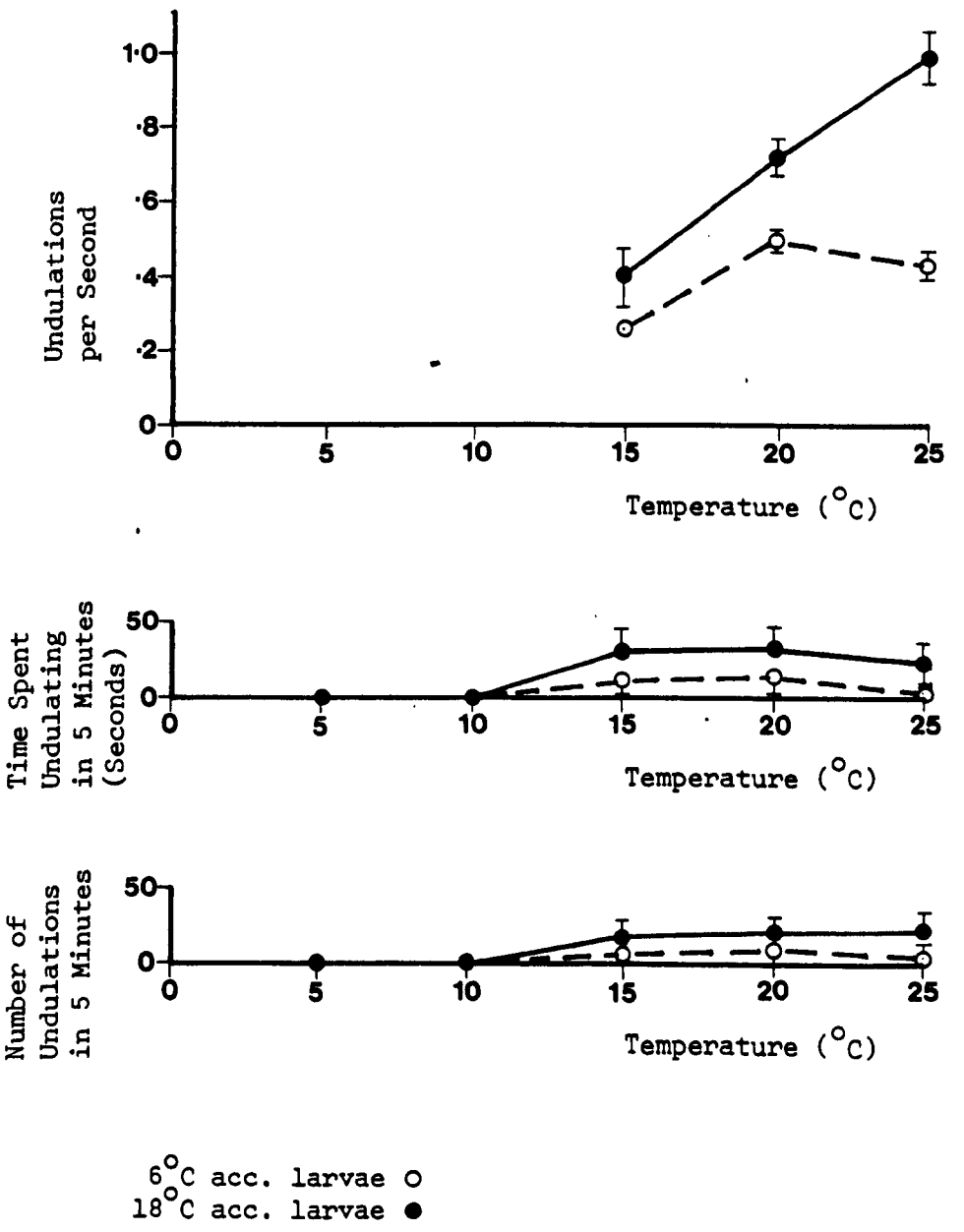


Fig. 6.2. The effect of temperature on the number of undulations and the time spent undulating in a 5 minute period, and the rate of undulations of *Hydropsyche instabilis* larvae acclimated to 6 and 18°C for 4-5 weeks. Variation about mean, expressed as the SE, is represented by the vertical lines.

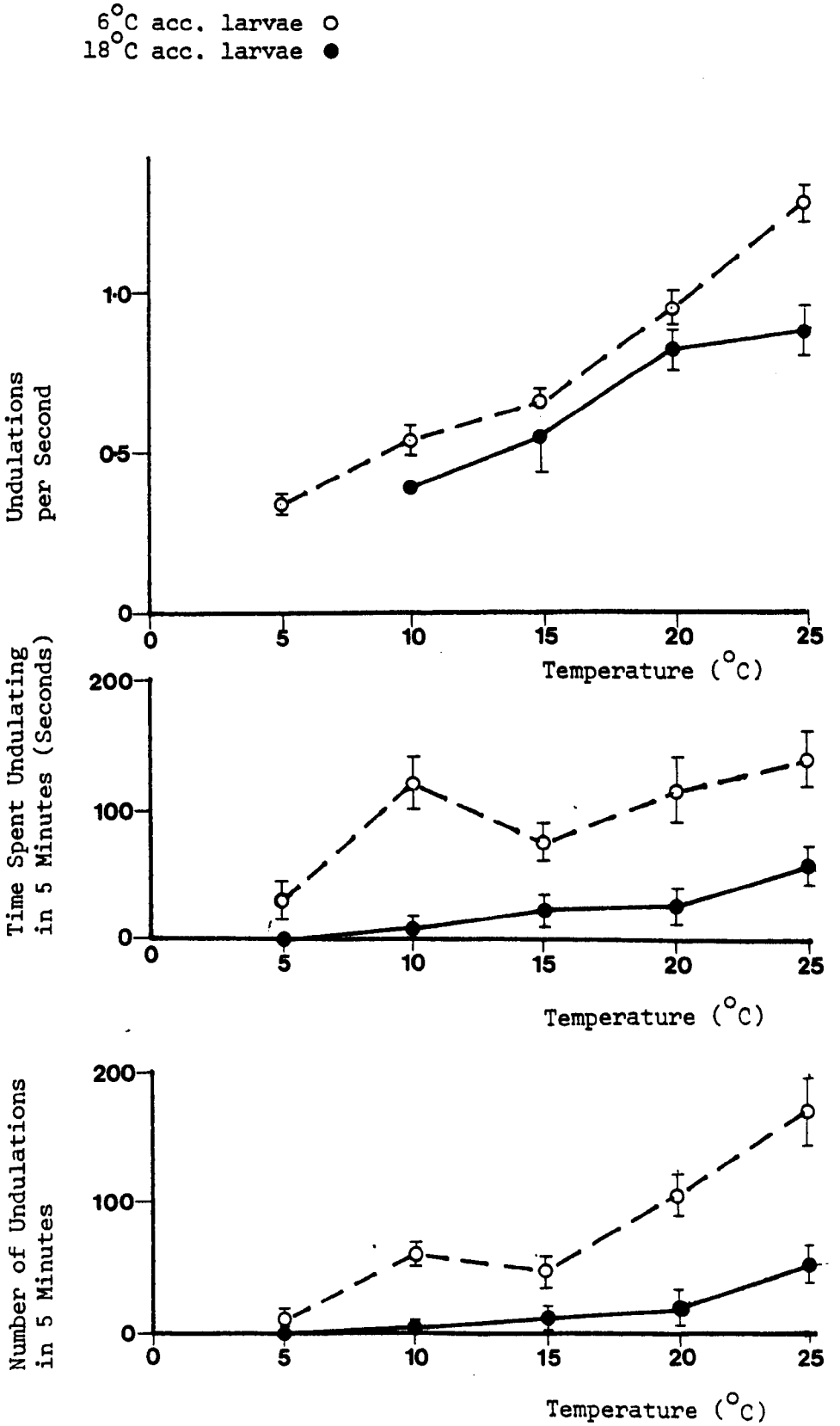


Fig. 6.3. The effect of temperature on the number of undulations and the time spent undulating in a 5 minute period, and the rate of undulations of *Hydropsyche pellucidula* larvae acclimated to 6 and 18°C for 4-5 weeks. Variation about mean, expressed as the SE, is represented by the vertical lines.

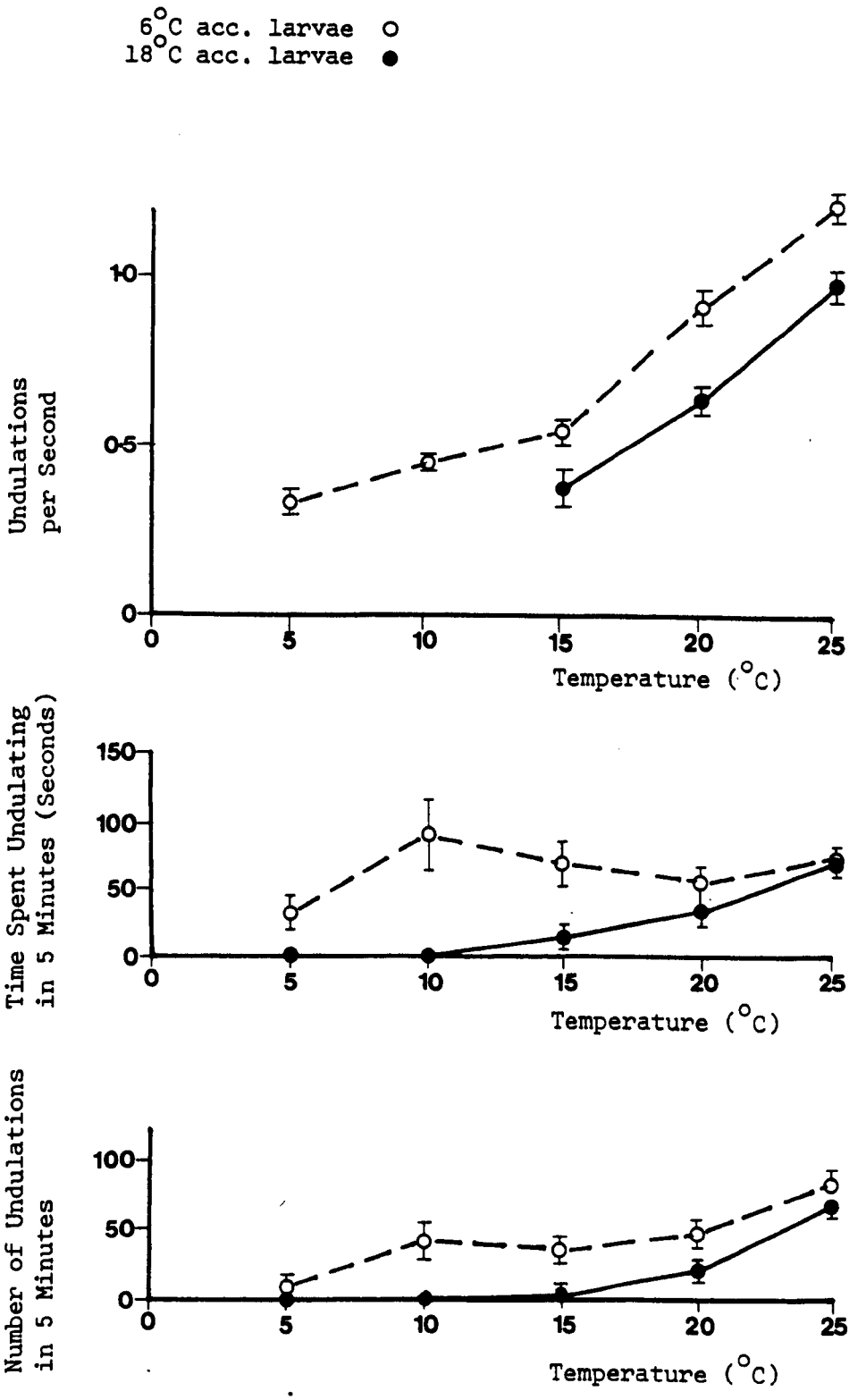


Fig. 6.4. The effect of temperature on the number of undulations and the time spent undulating in a 5 minute period, and the rate of undulations of *Hydropsyche siltalai* larvae acclimated to 6 and 18°C for 4-5 weeks. Variation about mean, expressed as the SE, is represented by the vertical lines.

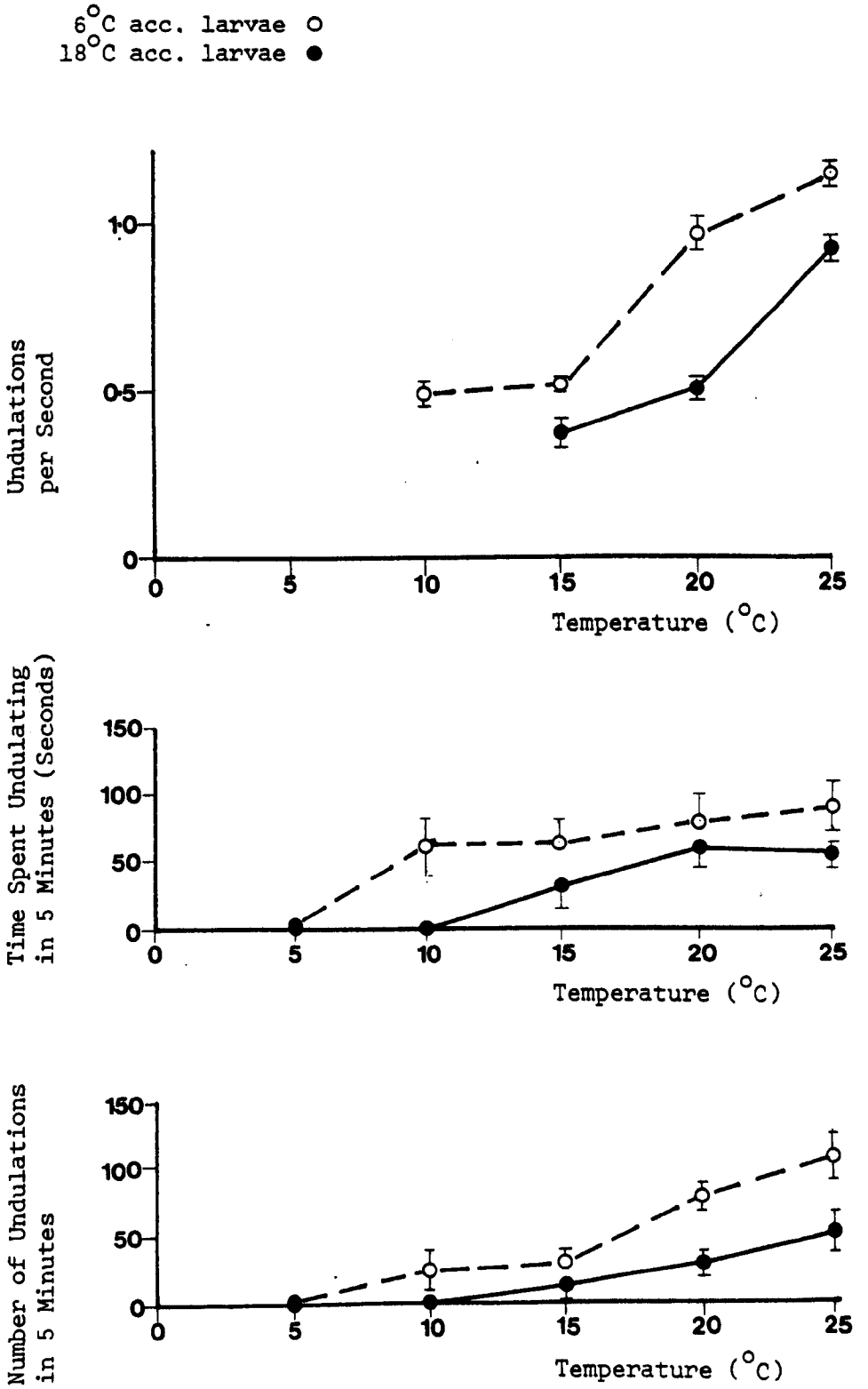


Fig. 6.5. The effect of temperature on the number of undulations and the time spent undulating in a 5 minute period, and the rate of undulations of *Hydropsyche angustipennis* larvae acclimated to 6 and 18°C for 4-5 weeks. Variation about mean, expressed as the SE, is represented by the vertical lines.

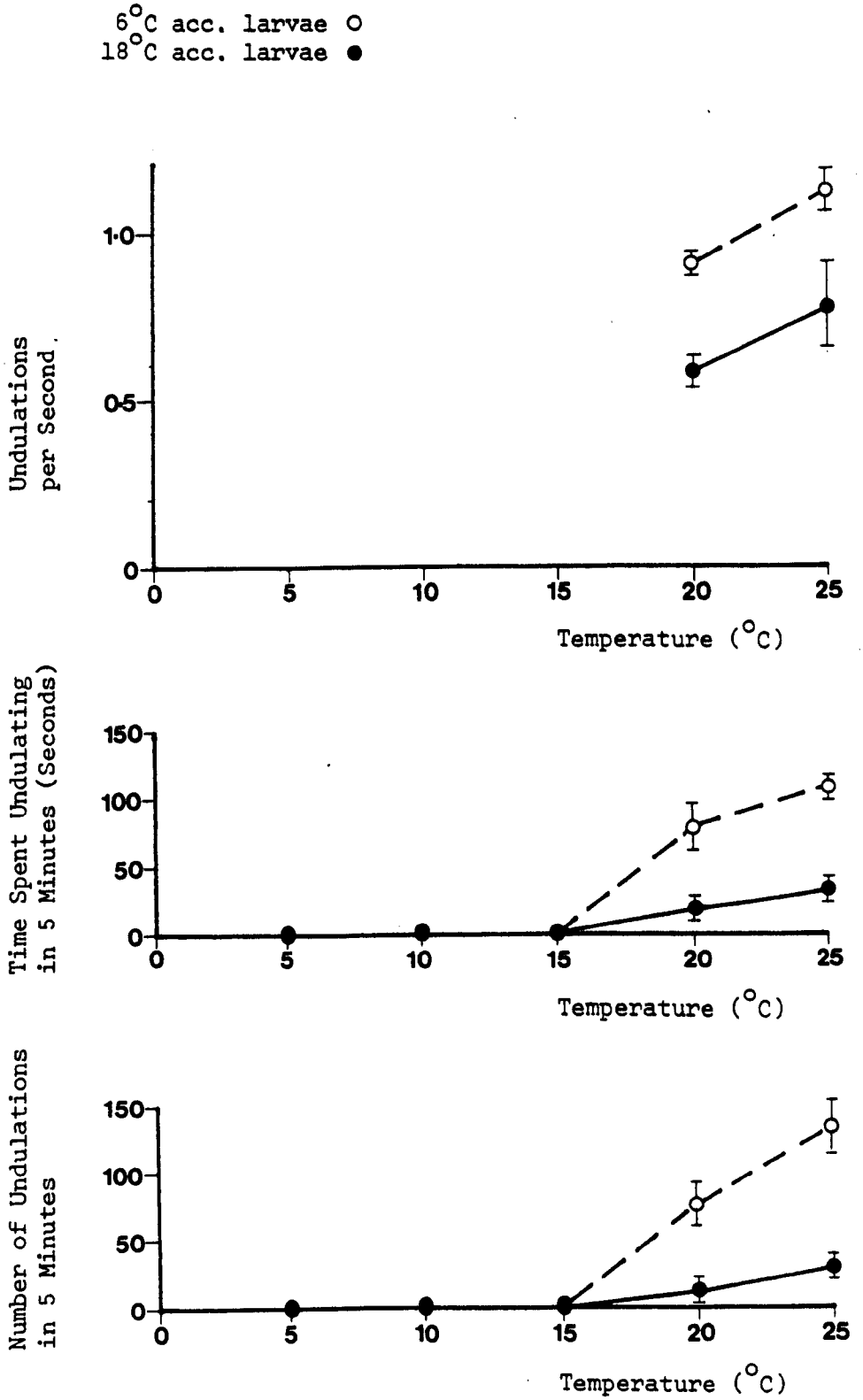
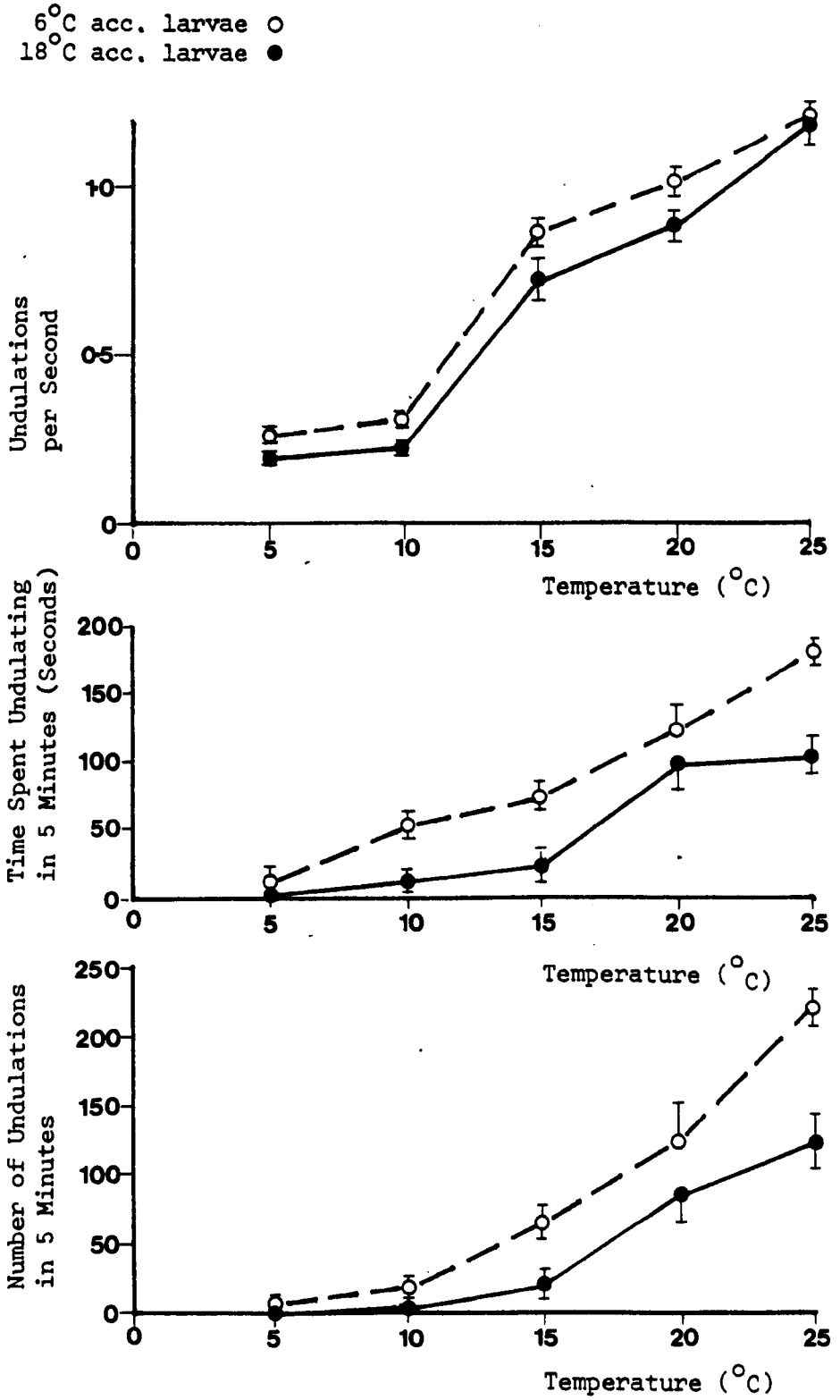


Fig. 6.6. The effect of temperature on the number of undulations and the time spent undulating in a 5 minute period, and the rate of undulations of *Hydropsyche contubernalis* larvae acclimated to 6 and 18°C for 4-5 weeks. Variation about mean, expressed as the SE, is represented by the vertical lines.



allowing the larvae to remain in its natural case. As with the net-spinning species the larvae were introduced into the beakers for 12 hours at 10°C.

The abdominal undulations were observed at temperatures ranging from 5 to 25°C in 5°C increments. The temperature in the aquarium was adjusted to the required temperature by the use of a Grant heater pump in conjunction with a Grant cooler unit. The larvae were maintained at each temperature for one hour before observations began. Between each stage of the experiment the water was allowed to increase gradually up to the next temperature. Each of the larvae was observed for a five minute period, the length of time undulation periods lasted and the number of undulations within each period was recorded. This allowed the time spent undulating, the number of undulations per minute and the rate of undulations during the periods of ventilation to be calculated. Unfortunately no information is gained on the amplitude of each undulation.

Data are presented for 10 of the 12 species for which metabolic data were presented in Chapter 5.

In a further experiment the procedure was repeated on *Hydropsyche angustipennis* larvae collected as 2nd and 3rd instars (initially collected as part of a long term acclimation experiment). The number of undulations in a 5 minute period was determined after 28, 56 and 84 days of acclimation at 9 and 14°C.

6.3 RESULTS

6.3.1 Undulatory behaviour of hydropsychid larvae

The number of undulations, and the total time spent undulating in a five minute period and the rate of the undulations during the periods of ventilation are presented for six hydropsychid species in Figs. 6.1 to 6.6. The number of undulations, the time spent undulating and the rate of undulation all generally increase with an increase in temperature, up to the highest temperature measured. A comparison of the number of undulations

and time spent undulating at any particular temperature, and the change in the ventilatory activity with a change in temperature shows considerable interspecific variation. For example, the increase in the undulatory activity is considerable for *Hydropsyche contubernalis* but relatively negligible for *Diplectrona felix*. In contrast an interspecific comparison of the rate of undulating suggest a high degree of constancy across all of the species.

For five of the six species data for the groups of larvae acclimated to 6 or 18^{o}C demonstrates a difference in all three aspects of the undulatory activity. The larvae acclimated to the warmer temperature undulating less, spending less time undulating and undulating at a slower rate. For the sixth species, *D. felix*, the reverse is true, larvae acclimated to the lower temperature having the decreased undulatory activity. However for this species the data are only significantly different for the rate of undulating.

The data discussed so far include values for larvae failing to ventilate at all during the five minute recording period. (These values were obviously excluded from calculations on the rate of undulating). The percentage of the larvae which did undulate during the five minutes are shown for the six species in Table 6.1. The percentage of the larvae which were observed to undulate increases for all species with an increase in temperature. The interspecific differences are considerable, but comparisons are difficult due to the intra-specific differences between the groups of larvae acclimated to warm and cool temperatures. With the exception of *D. felix* the percentage of the larvae acclimated to the warmer temperature which undulated was lower than for those acclimated to the cooler temperature. For *D. felix* the reverse is true and in addition even at the highest temperature less than a third of the larvae were seen to undulate.

Table 6.1 Percentage of hydropsychid larvae which undulated during the 5 minute observation period.

	Acclimation Temperature (°C)	Experimental Temperature (°C)				
		5	10	15	20	25
D. felix	6	0	0	14.3	29	29
	18	0	0	33	22	33
H. instabilis	6	43	100	86	100	100
	18	0	17	50	50	83
H. siltalai	6	0	75	87.5	100	100
	18	0	0	37.5	100	100
H. pellucidula	6	80	78	100	100	100
	18	0	0	70	100	100
H. angustipennis	6	0	0	0	100	100
	18	0	0	0	67	100
H. contubernalis	6	25	87.5	100	100	100
	18	12.5	37.5	62.5	87.5	100

6.3.2 Undulatory behaviour of the polycentropodid larvae

The number of undulations, and the total time spent undulating, in a five minute period and the rate of the undulations during the periods of ventilation are presented for three polycentropodid species in Figs. 6.7 to 6.9. These data contrast with the data for the hydropsychids in a number of ways. In all three species there is an increase with temperature in the number of undulations and the time spent undulating. However for *Plectrocnemia conspersa* the undulatory activity is small and shows little change with temperature and for *Polycentropus flavomaculatus* the number of undulations and the time spent undulating remained fairly constant between 5 and 20°C after which there was a considerable increase at 25°C. In all three species the rate of undulation increases with increased temperature. For *Neureclipsis bimaculata* the relationship between the rate of undulation and temperature is comparable with the data obtained for the hydropsychid species. For the other two species the rate of undulation is higher over the entire temperature range considered.

Fig. 6.7. The effect of temperature on the number of undulations and the time spent undulating in a 5 minute period, and the rate of undulations of *Plectrocnemia conspersa* larvae acclimated to 6 and 18°C for 4-5 weeks. Variation about mean, expressed as the SE, is represented by the vertical lines.

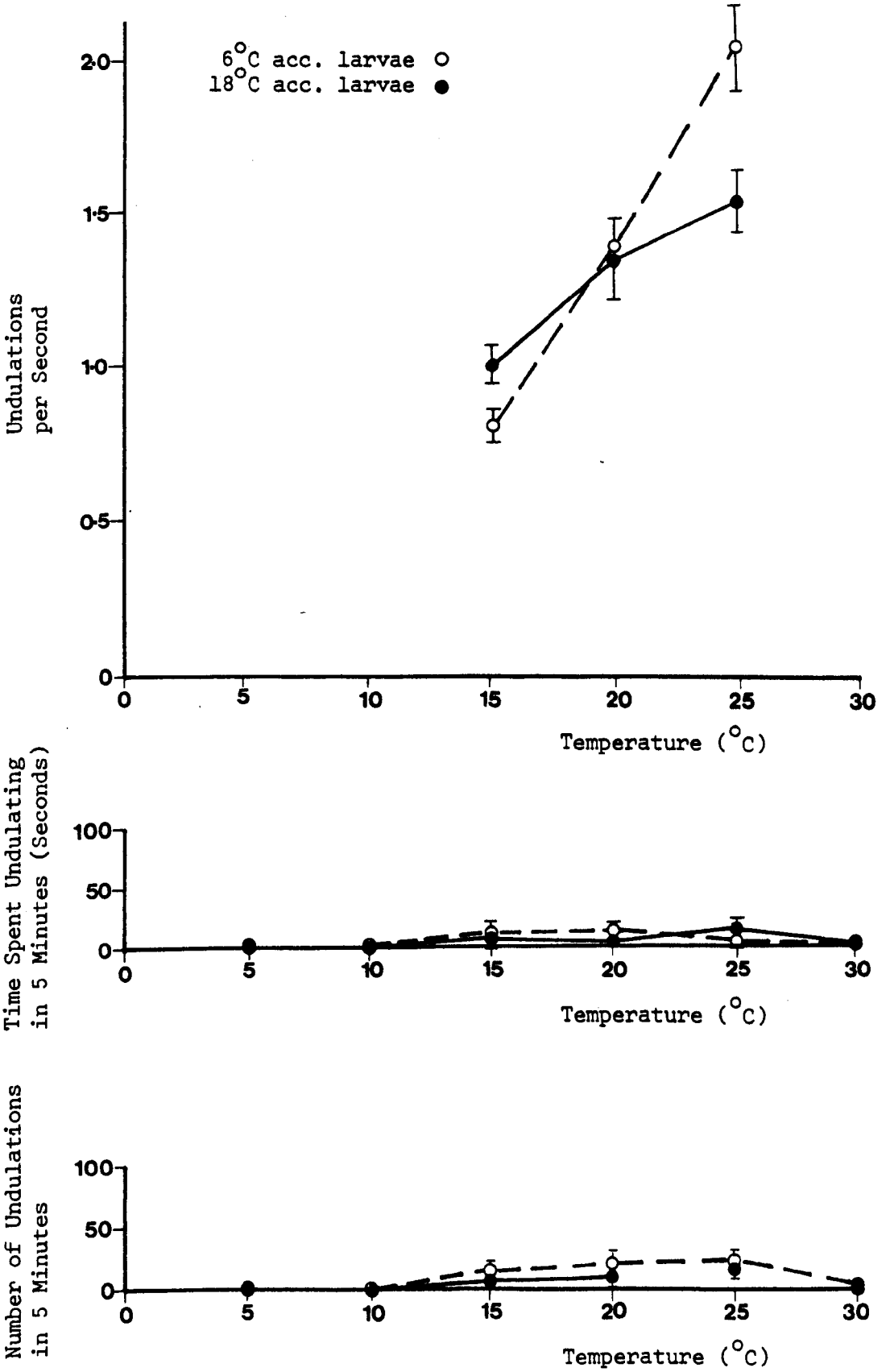


Fig. 6.8. The effect of temperature on the number of undulations and the time spent undulating in a 5 minute period, and the rate of undulations of *Polycentropus flavomaculatus* larvae acclimated to 6 and 18°C for 4-5 weeks. Variation about mean, expressed as the SE, is represented by the vertical lines.

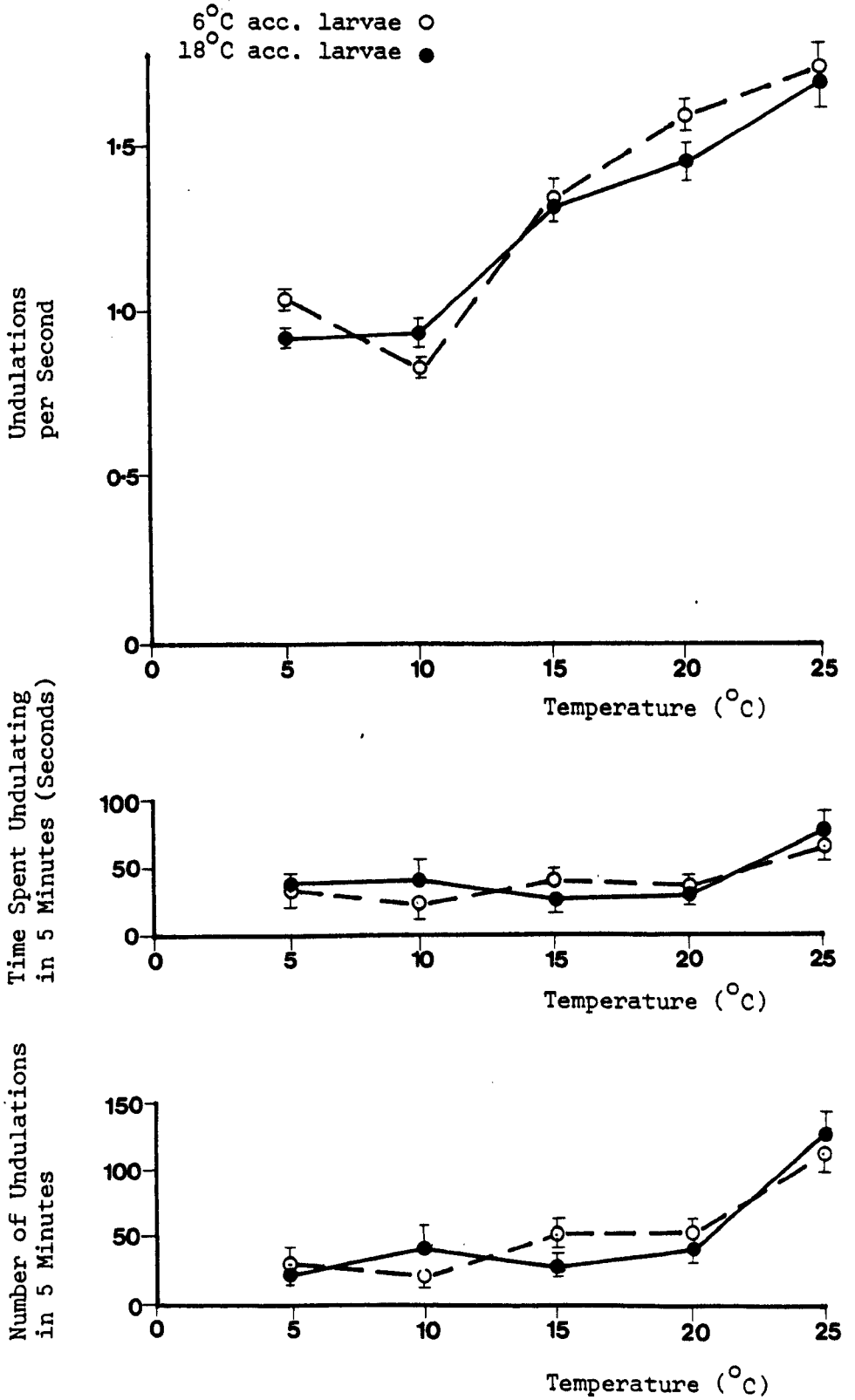
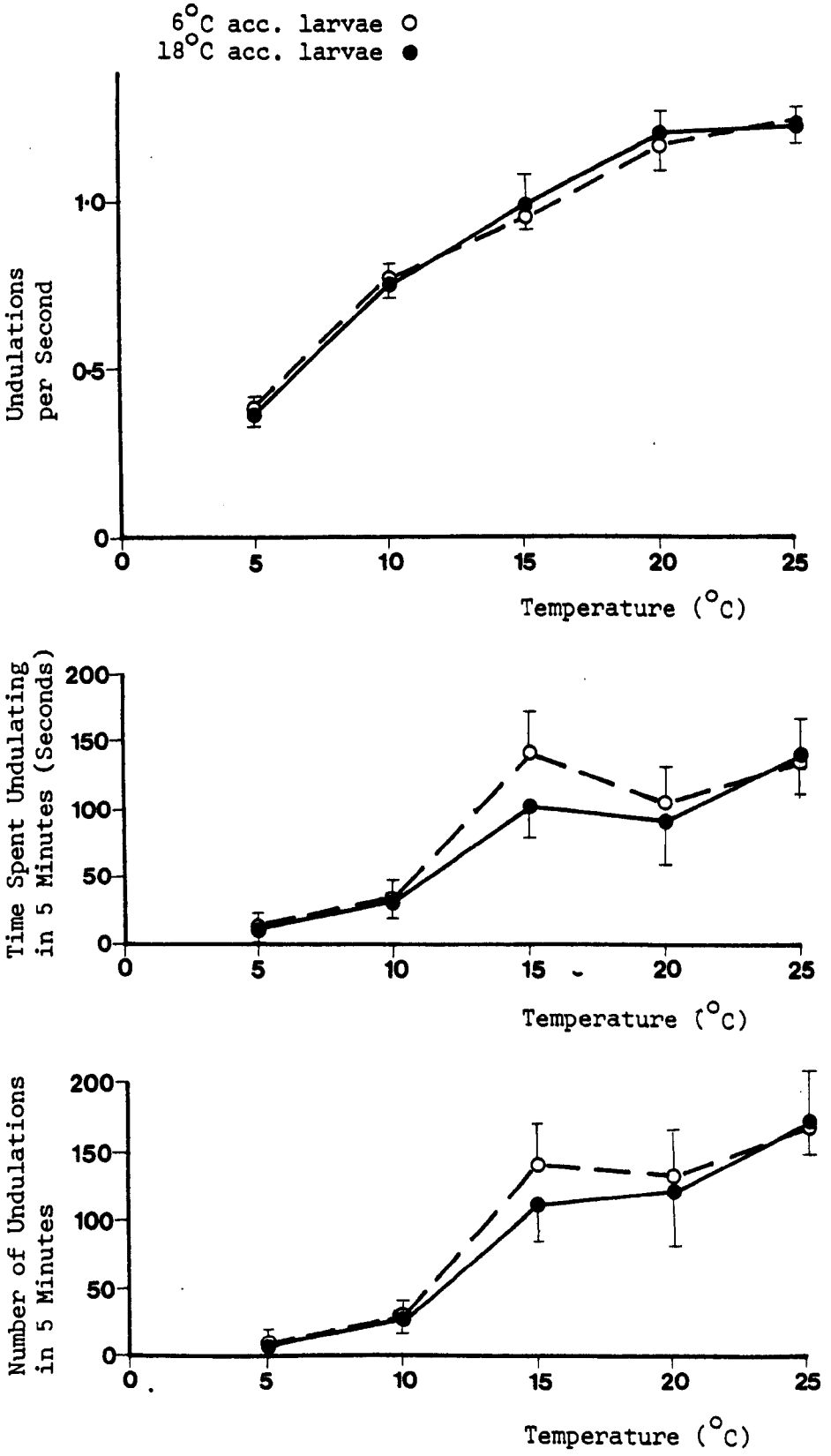


Fig. 6.9. The effect of temperature on the number of undulations and the time spent undulating in a 5 minute period, and the rate of undulations of *Neureclipsis bimaculata* larvae acclimated to 6 and 18°C for 4-5 weeks. Variation about mean, expressed as the SE, is represented by the vertical lines.



With the exception of the rate of undulation data for *P. conspersa* and *P. flavomaculatus* at some temperatures there is no significant difference in the ventilatory behaviour of the 6 and 18°C acclimated larvae.

The percentage of the larvae which did undulate during the five minutes are shown for the three species in Table 6.2.

Table 6.2 Percentage of polycentropodid larvae which undulated during the five minute observation period.

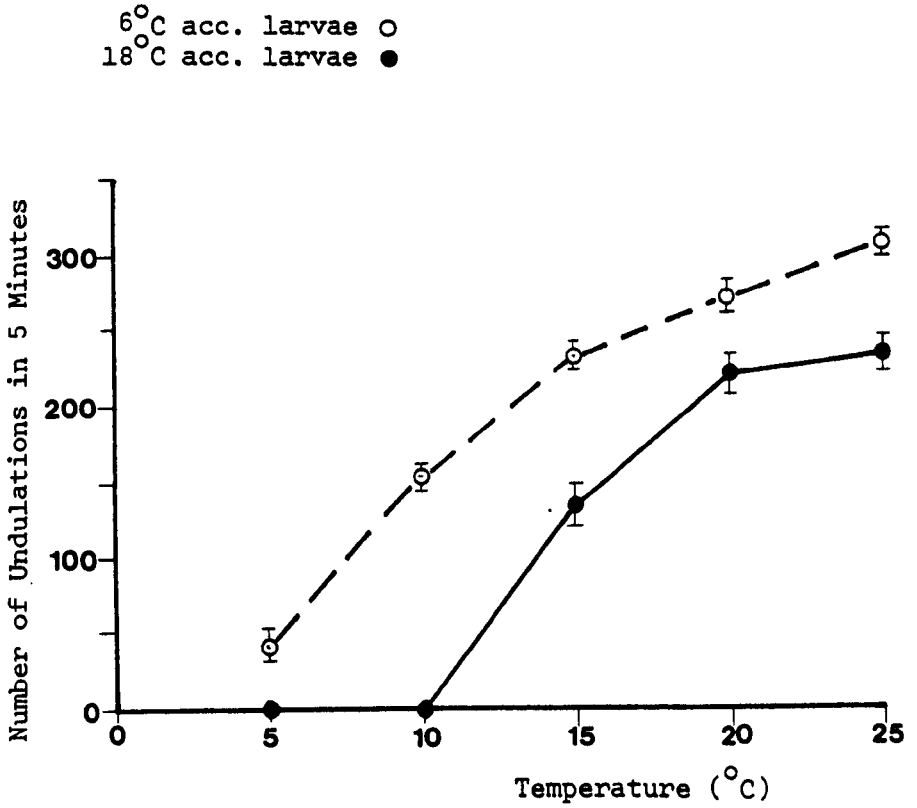
	Acclimation Temperature (°C)	Experimental Temperature (°C)					
		5	10	15	20	25	30
Plectrocnemia conspersa	6	0	0	75	62.5	75	0
	18	0	0	75	62.5	87.5	75
Polycentropus flavomaculatus	6	100	67	100	100	100	-
	18	100	89	91	100	100	-
Neureclipsis bimaculata	6	71.4	71.4	85.7	85.7	100	-
	18	42.9	85.7	85.7	71.4	85.7	-

These data confirm further the lack of a difference between the two groups of larvae acclimated to the cool and warm temperature. They also show that for two of the species, *P. flavomaculatus* and *N. bimaculata*, the former in particular, the percentage of larvae observed to undulate is high even at the lower temperatures. The data for the third species, *P. conspersa*, is more similar to the results obtained for the hydropsychids, the percentage of larvae undulating dropping to nil at the lower temperatures.

6.3.3 Undulatory behaviour of *Potamophylax cingulatus*

Data is only available for one cased larvae, *P. cingulatus*, and for this species data is only available for the number of undulations in a five

Fig. 6.10. The effect of temperature on the number of undulations in a 5 minute period of *Potamophylax cingulatus* larvae acclimated to 6 and 18°C for 4-5 weeks. Variation about the mean, expressed as the SE, is represented by the vertical lines.



minute period. These data are presented in Fig. 6.10. As observed for most of the previous species the number of undulations increases with an increase in temperature. Three points are of note. Firstly the undulations are far more frequent than in the net-spinning larvae already discussed. Secondly, there are significant differences between the number of undulations of the 6 and 18°C acclimated larvae. Thirdly the larvae acclimated to the warmer temperature failed completely to undulate at 5 and 10°C during the five minute period.

The percentage of the larvae which undulated during the five minutes are shown in Table 6.3. Except for the 18°C acclimated larvae at 5 and 10°C, where no larvae at all did, 100% of the larvae undulated.

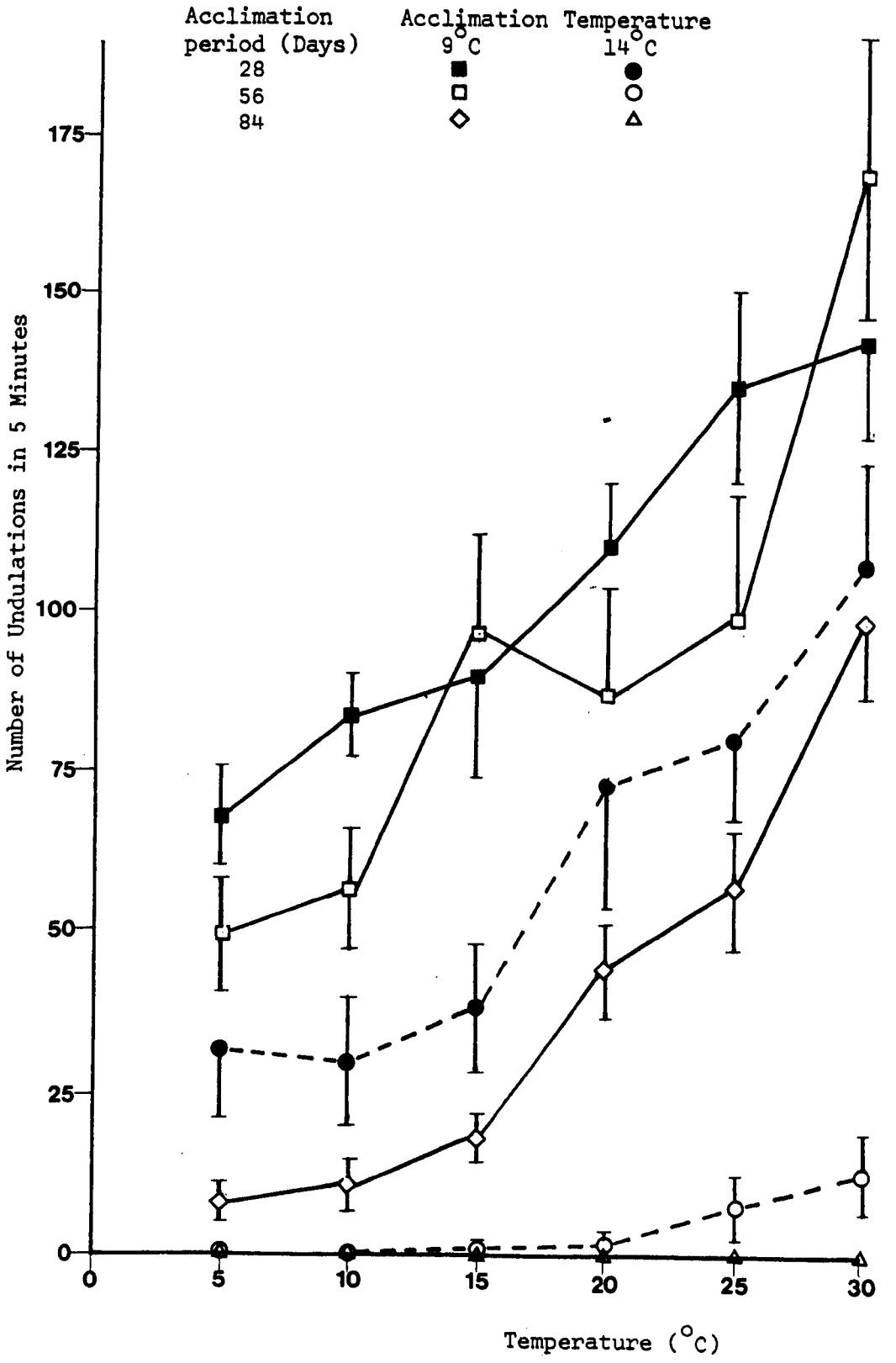
Table 6.3 Percentage of larvae of *Potamophylax cingulatus* which undulated during the five minute observation period.

	Acclimation Temperature (°C)	Experimental Temperature °C				
		5	10	15	20	25
P. cingulatus	6	100	100	100	100	100
	18	0	0	100	100	100

6.3.4 The influence of acclimation time on the undulatory activity of *H. angustipennis*

The mean and the S.E. of the number of undulations in a 5 minute period, of the larvae acclimated to 9 or 14°C after 28,56 and 84 days, are presented in Fig. 6.11. As before for this species (Fig. 6.6) the number of undulations increases with temperature and there are significant differences between the larvae acclimated to the warm and cool temperatures, those acclimated to the warmer temperature undulating less. These data demonstrate a considerable change in the ventilatory activity

Fig. 6.11. The effect of temperature on the number of undulations in 5 minutes of larvae of *Hydropsyche angustipennis* acclimated to 9 and 14°C for 28, 56 and 84 days. Variation about mean values, expressed as the SE, is indicated by the vertical lines.



with the length of the acclimation period. For both groups of larvae the number of undulations decreases as the acclimation time increases. This is most noticeable for the larvae acclimated to the warmer temperature, the undulations becoming negligible after 56 days and non existent after 84 days, although the larvae remained active in other respects.

The percentage of the larvae which undulated during the five minute period are shown in Table 6.4. In contrast although a high percentage of the warm acclimated larvae undulated after 28 days, this dropped considerably after 56 days, only 20% of the larvae undulating even at the warmer temperatures. After 84 days no larvae were seen to undulate during the five minute period, nor during subsequent observations. It should be noted that the larvae were still active after the 84 day period and continued to live for a number of weeks longer.

Table 6.4 Percentage of the 9 and 14°C acclimated larvae of *H. angustipennis* which undulated during the 5 minute observation period.

Acclimation period (days)	Acclimation temperature (°C)	Temperature °C 5	10	15	20	25	30
28	9	90	100	100	100	100	100
	14	70	60	90	100	100	100
56	9	100	100	90	90	90	100
	14	0	0	0	10	20	20
84	9	40	50	60	80	100	100
	14	0	0	0	0	0	0

6.4 Discussion

In all six of the hydropsychid species there is an increase in body ventilation in response to an increase in temperature. This may reflect an enhanced oxygen demand in response to the increased body temperature or it may be necessary to ventilate the body surfaces more as the oxygen content

of the surrounding water decreases. Philipson & Moorhouse (1974) demonstrated that both of these factors affected the number of undulations of three hydropsychid species and therefore the changes are probably in response to a combination of the two factors. Many previous studies on caddis larvae have only presented data for the number of undulations in a certain length of time (Fox and Sidney, 1953; Feldmeth, 1970a and 1970b; Leader, 1971; Philipson & Moorhouse, 1974). These data demonstrate that the increase in ventilation is achieved by both an increase in the time spent undulating and in the rate of undulations. The proportion of time spent undulating may have great importance as it represents the time when feeding and net repair, for example, cannot occur.

The number of undulations and the time spent undulating show considerable interspecific variations but due to the intra specific differences between the larvae acclimated to the warm and cold temperatures comparisons are difficult (Figs. 6.1-6.6). In contrast the rate of undulation during the periods of ventilation appears to be consistent for all of the hydropsychid species, rising to a value of just over one undulation per second at 25^{o}C. This consistency is perhaps surprising, especially in view of the intraspecific differences in the rate of undulation of the larvae acclimated to the different temperatures, which demonstrates that the rate is not fixed.

If the differences between the groups of larvae acclimated to 6 and 18^{o}C are considered it can be seen that there are significant differences in the undulatory behaviour of all six species, although for *D. felix* this is only true for the rate of undulations. For five of the species the larvae acclimated to the warmer temperature undulated fewer times, spent less time undulating and undulated at a slower rate than those acclimated to the cooler temperature. These observations are consistent with a reduced oxygen demand in these larvae. For *D. felix* the reverse was true, the warm acclimated larvae having the increased undulatory activity.

As ventilation of the body is related to the oxygen consumption,it would be expected that those species where a difference in their metabolism was found following acclimation (*H. instabilis*, *H. pellucidula*, *H. siltalai* and *H. angustipennis* (Chapter 5.3.1)) would have comparable differences in their undulatory activity. Similarly for those species in which no respiratory compensation was found (*D. felix* and *H. contubernalis*) it might have been expected that there would also be no difference in the undulatory behaviour. However no such simple relationship is apparent. In an attempt to simplify the situation the number of undulations in a 5 minute period (estimated from Figs. 6.1-6.6) at 6 and 18°C of the 6 and 18°C acclimated larvae respectively are presented for the six species in Table 6.5.

Table 6.5 Number of undulations in a 5 minute period (estimated from Figs 6.1-6.6) at 6 and 18°C of the 6 and 18°C acclimated hydropsychid larvae respectively.

	Number of undulations at 6°C by the 6°C acclimated larvae	Number of undulations at 18°C by the 18°C acclimated larvae
D. felix	0	6
H. instabilis	20	15
H. siltalai	5	17
H. pellucidula	17	17
H. angustipennis	0	5
H. contubernalis	7.5	57.5

H. contubernalis, a species for which no difference was found in the respiration of the two groups of larvae, does show a difference in the number, time and rate of undulations (Fig. 6.6). This indicates that the warm acclimated larvae, although it was not apparent from the measurement of the oxygen consumption, actually did have an advantage over the cool acclimated larvae as the energy expenditure on obtaining the necessary

oxygen was reduced. The high level of the undulatory behaviour of even the warm acclimated larvae at warmer temperatures (Table 6.5) compared with the other species suggests that the survival of this species in warmer temperatures may be energetically expensive. However it should be remembered that other factors, including flow rate (Feldmeth 1970a and 1970b, Philipson and Moorhouse, 1974), affect the ventilatory rates and therefore in the field undulation of the body may be far less important than these data produced in still conditions indicate.

The data for *H. angustipennis* differ from the other species. At 5,10 and 15^{o}C none of the larvae, either warm or cold acclimated, undulated (Table 6.1). At warmer temperatures there was a large divergence between the results for the two groups of larvae. The larvae acclimated to the warmer temperature undulated little even at the highest temperature. (Fig. 6.5 and Table 6.5). This is consistent with the distribution of this species in regions of warmer water and lower oxygen content, as the energy expended in obtaining the necessary oxygen would be minimised.

The data for the three species *H. instabilis*, *H. siltalai* and *H. pellucidula* are similar to each other, with the difference between the groups of larvae acclimated to warm and cool temperature increasing in that order (Figs. 6.2, 6.3 and 6.4). If these data are compared with the respiration data discussed in the previous Chapter (Table 5.7) it can be seen that there was a greater difference in the respiration of the two groups of larvae for *H. siltalai* than for *H. pellucidula*. This is mirrored by the larger difference in the undulatory activity of the groups of larvae in the same order. However *H. instabilis*, which had the lowest compensatory ability when the respiration was measured has the largest difference in the undulatory activity. The results presented in Table 6.5 indicate that for all three species there is a very small change in the number of undulations over the temperature range 6 to 18^{o}C if larvae acclimated to these temperatures are considered. This possibly demonstrates a potential in

these species for distribution over a wide temperature range. This is the case for *H. siltalai* and *H. pellucidula* but *H. instabilis* has a fairly restricted distribution in cooler water (Badcock, 1975; Hildrew and Edington, 1979). Obviously many other factors may prevent a more widespread distribution of this species.

The final species, *D. felix*, differs from all other species tested in that the larvae acclimated to the warmer temperature undulate more. However the differences are only significant for the rate of undulations and therefore are probably consistent with the respiration results, no evidence of metabolic compensation being found for this species (Fig. 5.1). From Fig. 6.1 it is seen that this species undulates very little. Table 6.1 shows that many of the larvae completely failed to undulate within the 5 minute period. If the number of undulations at 6 and 18^{o}C are considered for larvae acclimated to 6 and 18^{o}C (Table 6.5) it again shows that little ventilation occured, the results for this species looking similar to those for *H. angustipennis*. The apparent similarity of the results for these species is surprising but observations during the experiment detected an important difference. The behaviour of *H. angustipennis* remained normal at the warmer temperatures, the larvae maintaining their position within their retreats. In contrast as the temperature increased the *D. felix* larvae became distressed, many left their retreats and the undulations became poorly coordinated. Some larvae appeared to be immobilised at 25^{o}C. It therefore appears that whereas *H. angustipennis* undulates little at warmer temperatures, because it is adapted to life under those conditions, *D. felix* loses its ability to undulate as the temperature rises. This could contribute to the fact that the distribution of this species is restricted to cooler water (Edington and Hildrew, 1973).

As with the hydropsychids,in all three of the polycentropodids investigated there is an increase in body ventilation in response to an increase in temperature. This increase is most marked in *N. bimaculata*, in

P. flavomaculatus the increase is only significant between 20 and 25°C while in the third species *P. conspersa*, there is a relatively small increase. The rate of undulations of *N. bimaculata* are similar to the values obtained for the hydropsychids but for the other two species the rate is faster, reaching values of about 2.0 undulations per second at 25°C.

None of the three species were found to have a significant difference in the ventilatory activity following acclimation to 6 or 18°C. For *P. conspersa* and *N. bimaculata* this is consistent with the lack of compensation in the metabolism of the two groups of larvae (Figs. 5.7 and 5.9). However for *P. flavomaculatus*, which was shown to be capable of compensating its metabolism in response to different acclimation temperatures, the lack of a significant difference in the undulatory activity is surprising.

As *P. conspersa* has a similar, but slightly more extensive, distribution to *D. felix* it is interesting that *P. conspersa* also demonstrated little undulatory activity, although from Table 6.2 it can be seen that a larger percentage of the larvae did undulate than was found for *D. felix*. Again *P. conspersa* showed evidence of distress at the warmer temperatures and as with *D. felix*, this inability to undulate more could contribute to the distribution of this species being restricted to cooler water (Hildrew and Edington, 1981).

The results for *N. bimaculata*, which has a distribution comparable with *H. contubernalis*, are similar to that species. From Table 6.6 it can be seen that at warmer temperatures the undulatory activity of the warm acclimated larvae is very high suggesting, as with *H. contubernalis*, that survival of this species at warmer temperatures is energetically expensive.

From Table 6.6 it can be seen that the undulatory activity of *P. flavomaculatus* varies little over the range 6 to 18°C if larvae acclimated to these temperatures are considered. This result is similar to those

obtained for *H. siltalai* and *H. pellucidula* with which this species has a comparable distribution.

Table 6.6 Number of undulations in a 5 minute period (estimated from Figs. 6.7-6.9) at 6 and 18°C of the 6 and 18°C acclimated polycentropodid larvae respectively.

	Number of undulations at 6°C by the 6°C acclimated larvae	Number of undulations at 18°C by the 18°C acclimated larvae
P. conspersa	0	7.5
P. flavomaculatus	27.5	35
N. bimacultata	10	115

Unfortunately data are only available, for the number of undulations in five minutes, for one species of cased caddis, *P. cingulatus*. As with the other species the number of undulations increase with an increase in temperature, with the number of undulations reaching higher values than for any of the net-spinning species. This may reflect the greater importance of ventilation in cased larvae as it provides the pumping mechanism to create a water flow through the case (Leader, 1971). The values for the number of undulations are slightly higher than values previously published for other species. For example, Fox and Sidney (1953) found a rate of approximately 125 undulations per 5 minutes for *Limnophilus flavicornis* at 20°C and Feldmeth (1970a) found a rate of 60-70 undulations per 5 minutes at 12°C for *Pycnopsyche guttifer* and *Pycnopsyche lepida*.

There are large significant differences in the number of undulations of the 6 and 18°C acclimated larvae (Fig. 6.10), the larvae acclimated to the warmer temperature undulating less. This is consistent with the respiration results which demonstrated an ability to compensate in this species, the warm acclimated larvae having a lower respiration (Fig. 5.11).

It is of interest that in the warm acclimated larvae there are no undulations at 5 and 10°C. This indicates that the larvae can obtain sufficient oxygen within the confines of the case without the need to create a flow of water.

The experiment comparing the undulatory activity of *H. angustipennis* after maintenance for different lengths of time at cool and warm temperatures (9 and 14°C) are interesting. After 28 days the results are comparable with those obtained for this species acclimated to 6 or 18°C after a similar time period (Fig. 6.5), although the number of undulations is higher. This probably reflects the difference in size of the larvae, 2nd and 3rd instar larvae being used in this experiment compared with 4th and 5th instar larvae in the previous experiment.

The decrease in the number of undulations with time may partially reflect the increase in size of the larvae during the experiment. (The weight distribution of the two groups of larvae at each time was kept approximately equal). However the decrease in the number of undulations of the warm acclimated larvae is greater than could be explained by this alone. Apparently the warm acclimated larvae have an advantage over the cool acclimated larvae in terms of the energy expenditure on body undulations. This was probably true after 28 days and possibly after 56 days. However by 84 days, when no undulations occured at all in the warm acclimated larvae, it was observed that the larvae were in a distressed state at warmer temperatures (25 and 30°C) and a further increase in temperature led to immobilisation of the larvae, still without any undulations occuring. This seems to indicate that the warm acclimated larvae eventually completely lose their ability to undulate but as this is based on this single experiment firm conclusions cannot be made as to the relevance of this result.

6.5 Conclusions

In response to an increase in temperature the ventilation of the body surface is increased in all cases by both an increase in the time spent undulating and in the rate of undulations.

For some, but not all, species these data demonstrate significant differences in the undulatory behaviour of larvae acclimated to different temperatures. Larvae acclimated to the warmer temperature undulating for less time and more slowly.

For five of the species studied *Hydropsyche instabilis*, *H. siltalai*, *H. pellucidula*, *H. angustipennis* and *Potamophylax cingulatus*) the decreased undulatory behaviour of the warm acclimated larvae corresponds with their decreased oxygen consumption demonstrated in the previous Chapter.

In a similar way three of the species (*Diplectrona felix*, *Plectrocnemia conspersa* and *Neureclipsis bimaculata*) in which no metabolic compensation was detected also had no significant difference between the undulatory behaviour of the warm and cool acclimated larvae.

However the results for the remaining two species were not as expected. For *H. contubernalis*, which showed no metabolic compensation, the warm acclimated larvae undulated less and more slowly, indicating that they may have had an advantage in terms of energy allocation between respiration and growth. For *P. flavomaculatus* the reverse was found, metabolic compensation occuring but no difference in the undulatory behaviour being detected. This would appear to indicate that the warm acclimated larvae require the same amount of ventilation as the cool acclimated larvae in order to obtain less oxygen.

From an analysis of the undulatory behaviour over the temperature range 6 to 18^{0}C, of larvae acclimated to those temperatures, it is suggested that it may contribute to, or be a consequence of, their distributions. Two species, *D. felix* and *P. conspersa*, were shown to be virtually incapable of undulating at warmer temperatures, even when

acclimated to warmer temperatures, which may prevent their penetration into warmer water. The three species with widespread distributions (*H. pellucidula*, *H. siltalai* and *P. flavomaculatus*) all had undulatory activities which remained constant over the temperature range 6 to 18^{o}C. Thus over a wide habitat temperature range the energetic costs of ventilation remain constant. Similar results were obtained for *H. instablis* but its more restricted distribution could be the consequence of many other factors. The results for *H. angustipennis*, the undulatory activity of warm acclimated larvae being minimal even at warmer temperatures, are consistent with this species distribution in warmer habitats. Finally, the high undulatory activity of *H. contubernalis* and *P. flavomaculatus* at warmer temperatures indicates that the distribution of these two species at warmer sites has severe energetic costs. As previously stated any conclusion made about the relevance of these data to field distributions should be treated cautiously as in the field many other factors, mostly notably water flow, will influence the undulatory behaviour, decreasing its importance, especially for the net-spinning larvae.

CHAPTER 7 ACCLIMATION TO DAILY TEMPERATURE FLUCTUATIONS OF DIFFERENT AMPLITUDES

7.1 Introduction

Virtually all natural waters are characterised by the occurence of a diurnal temperature variation (Edington, 1965; Crisp and LeCren, 1970; Brittain, 1974; Boon and Shires, 1976). Differences in the amplitude of this periodicity may have some influence upon the distribution of species (Precht, 1973; Hildrew and Edington, 1979). Little work has been published with regards to the influence of diurnal temperature variation on oxygen consumption. Sarviro (1977 and 1980) investigated the respiration of the crustaceans *Streptocephalus torvicornis* and *Gammarus lacustris* under conditions of sinusoidal temperature variation, and demonstrated difference between it and the rate at the corresponding constant temperature. To the author's knowledge no work has been published on the effect of acclimation to temperature variations of different amplitudes.

The aim of the work discussed in this Chapter was to make a limited investigation into the effect of maintenance of larvae at temperature regimes ranging from a constant 15^{o}C to a diurnal variation ranging from 10^{o}C at night to 20^{o}C during the day, the oxygen consumption of the larvae being determined at a constant temperature. Data are presented for three species, *Hydropsyche instabilis*, *Hydropsyche contubernalis* and *Potamophylax cingulatus*.

7.2 Method

The larvae were maintained for three weeks on the larger of the thermogradient bars described in Chapter 2.2. Plastic trays 175mm x 115mm x 50mm deep were three-quarter filled with pond water and placed on the bar in the correct position to obtain the desired temperature regime. The water temperature was monitored at weekly intervals over a 24 hour period using a

Grant temperature recorder. In other respects the larvae were maintained as for the larvae acclimated to constant temperature (Chapter 2.2).

At the end of the acclimation period the larvae were maintained at a constant temperature of 15^{o}C for 6 hours, after which their oxygen consumption was determined, in 65ml glass bottles, over a 20 hour period using the closed bottle method described in Chapter 3.2.1.

For one species, *H. contubernalis*, the number of undulations and the time spent undulating, in a five minute period and the rate of undulating during the periods of ventilation, were determined using the method described in Chapter 6.2.

7.3 Results

The ln weight specific respiration of larvae of *H. contubernalis*, maintained at a constant 15^{o}C or at daily ranges of 13.3 <-> 16.7^{o}C, 11.7 <-> 18.3^{o}C and 10 <->20^{o}C, is plotted against the ln dry weight in Fig. 7.1 a-d. The four least square regression lines are plotted together for comparative purposes in Fig. 7.2. There is clearly no significant difference between the data for the four temperature ranges.

The number of undulations, and the time spend undulating, in a five minute period and the rate of undulating at a range of temperatures, for the larvae maintained at the different temperature regimes are presented in Fig. 7.3. With the exception of the data obtained for the rate of undulations at 15^{o}C ($F_{3,16} = 3.422^{*}$) the differences are non-significant, but there is a decreasing trend in all three aspects of the ventilatory activity as the range of the maintenance temperature increases. The ln weight specific respiration of larvae of the second species, *H. instabilis*, maintained at the same temperatures as the first species, is plotted against the ln dry weight in Fig. 7.4a-d. As before, plotting the four regression lines together (Fig. 7.5) shows that there are no significant differences between the data for the four temperature ranges.

Fig. 7.1. Relationship between the ln weight specific respiration and ln dry weight for larvae of *Hydropsyche contubernalis* maintained at daily ranges of (a) constant 15°C, (b) 13.3 - 16.7°C, (c) 11.7 - 18.3°C and (d) 10 - 20°C.

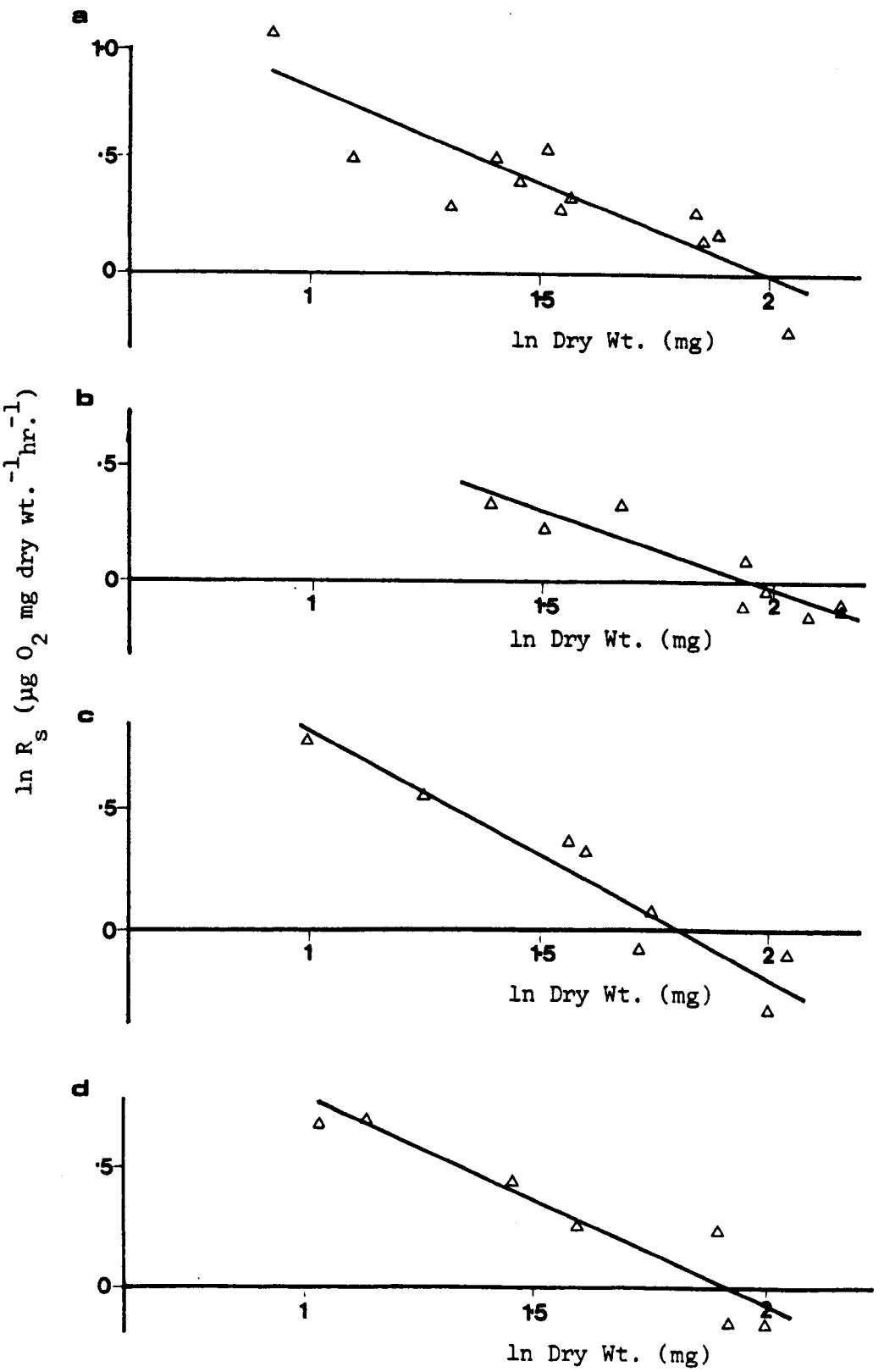


Fig. 7.2. Least square regression lines obtained from the data in Fig. 7.1a-d combined for comparative purposes.

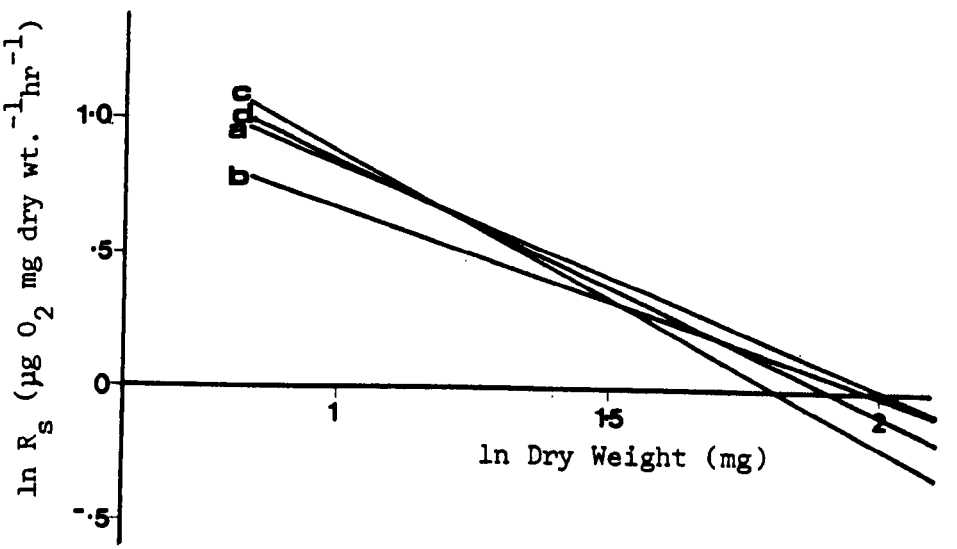


Fig. 7.3. Number of undulations, and the time spent undulating, in a 5 minute period, and the rate of undulating, at a range of temperatures. Larvae maintained at (A) constant 15°C (■), (B) 13.3 - 16.7°C (▼), (C) 11.7 - 18.3°C (□) and (D) 10 - 20°C (△).

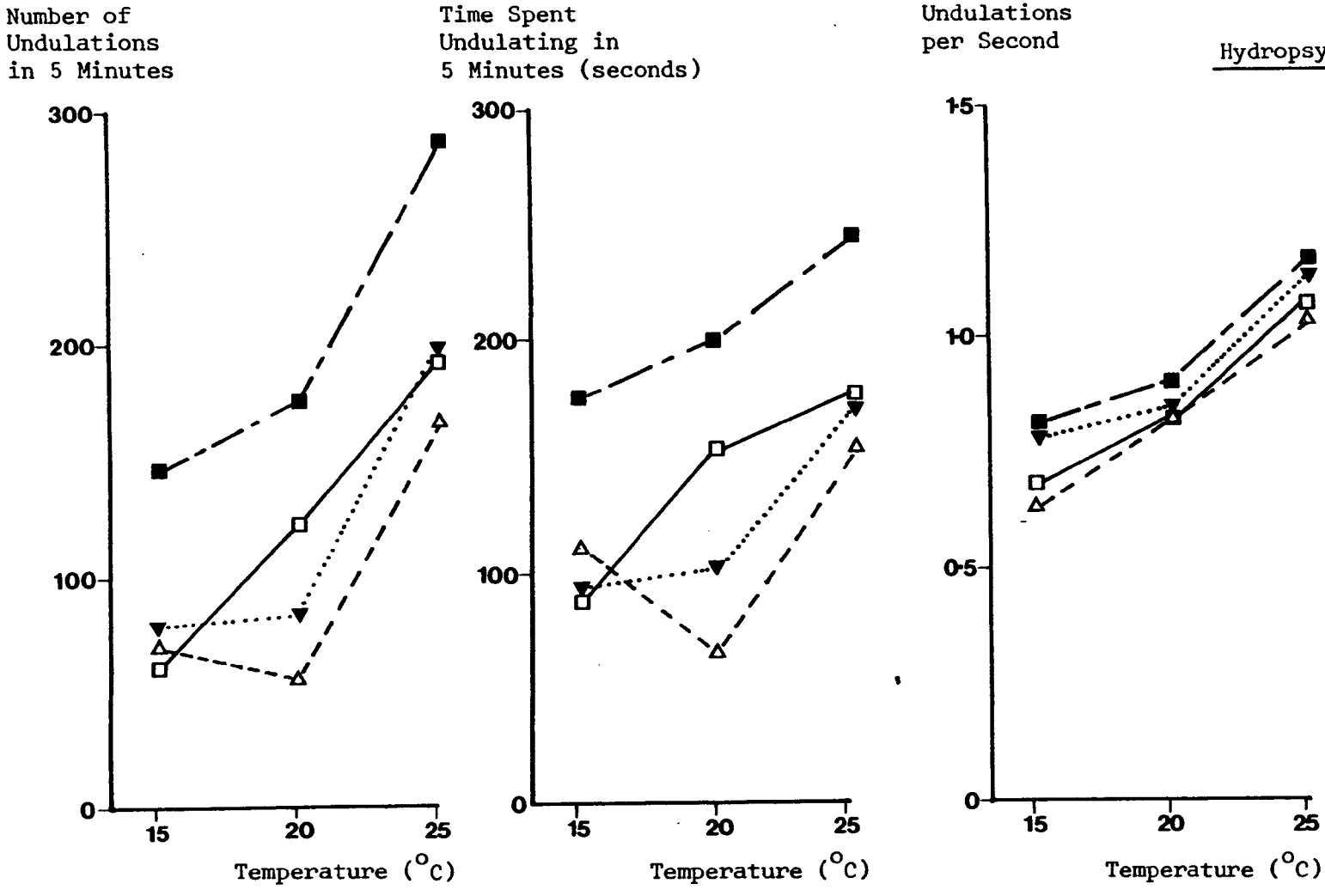


Fig. 7.4. Relationship between the ln weight specific respiration and ln dry weight for larvae of *Hydropsyche instabilis* maintained at daily temperature ranges of (a) constant 15°C, (b) 13.3 - 16.7°C, (c) 11.7 - 18.3°C and (d) 10 - 20°C.

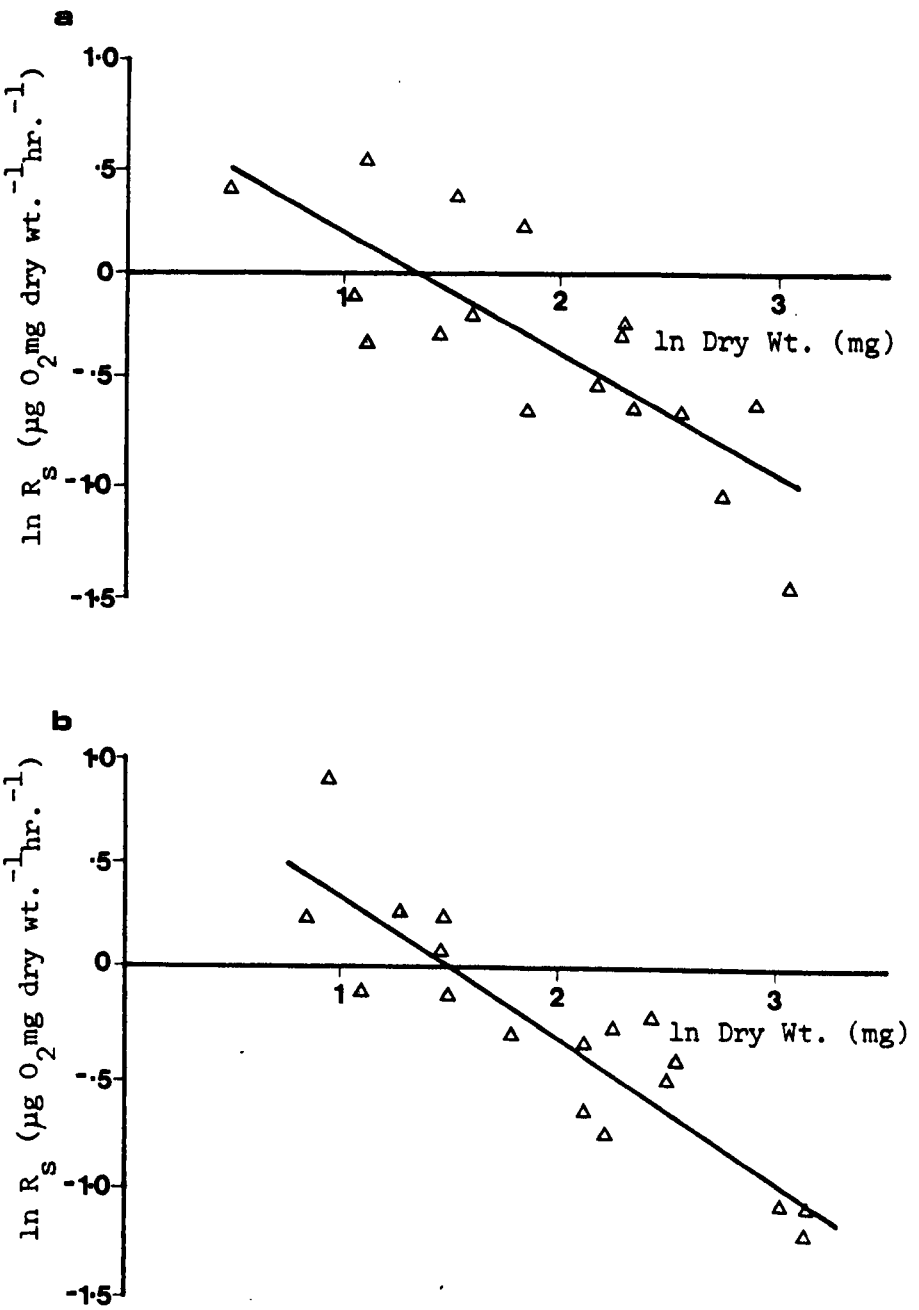
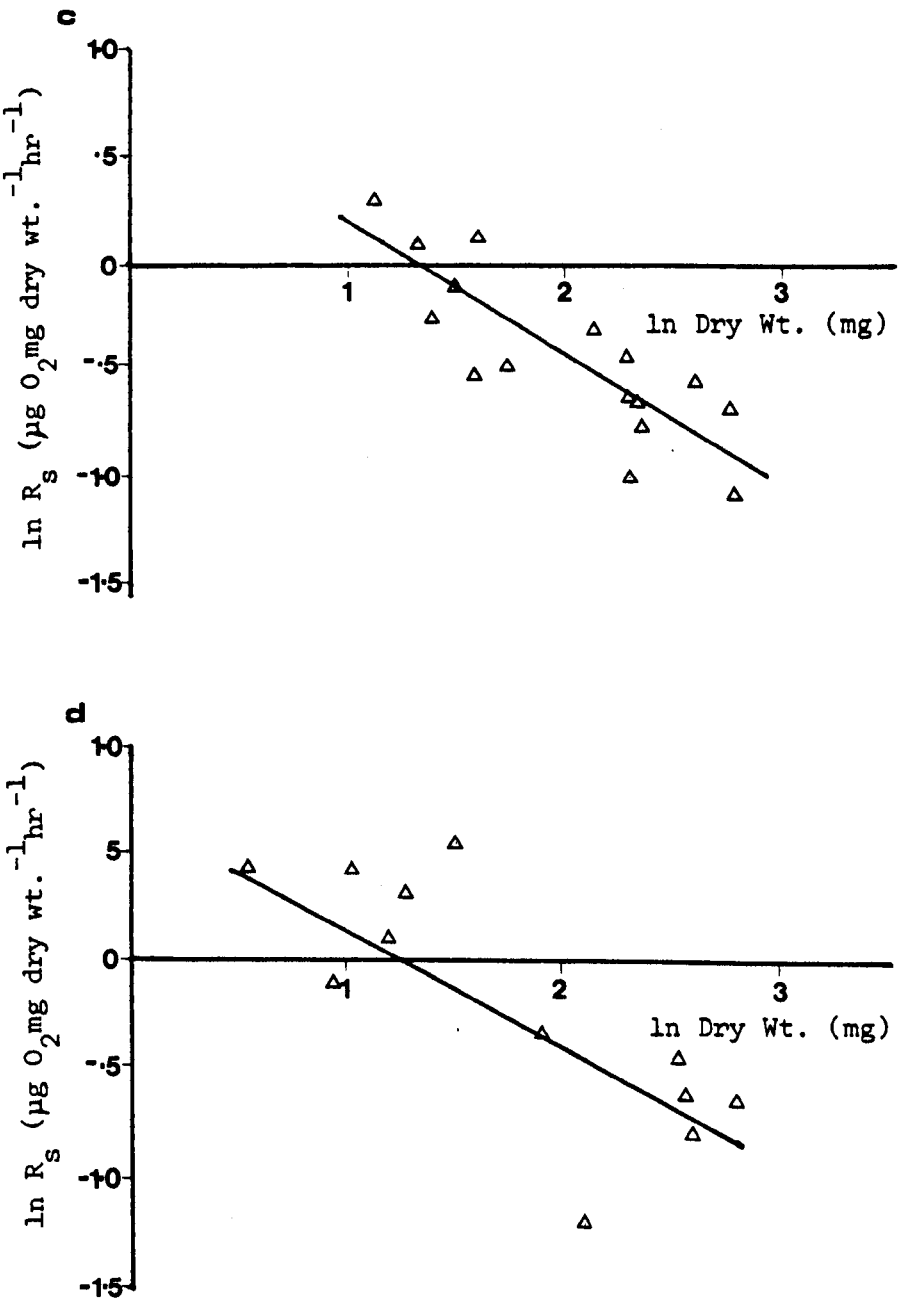


Fig. 7.4. (Cont.)


c
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ln R_s (µg O_2mg dry wt.$^{-1}$hr^{-1})
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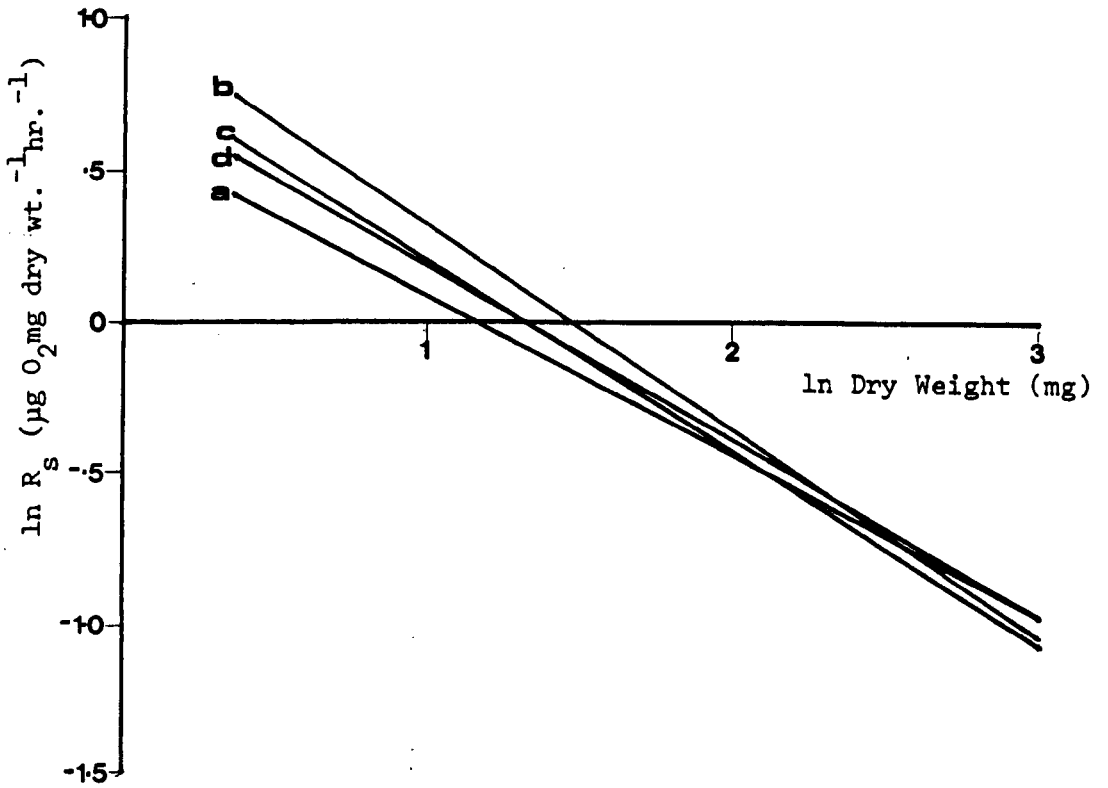
ln Dry Wt. (mg)
ln R_s (µg O_2mg dry wt.$^{-1}$hr^{-1})

Fig. 7.5. Least square regression lines obtained from the data in Fig. 7.4.a-d combined for comparative purposes.



The ln weight specific respiration of larvae of the final species, *P. cingulatus*, maintained at a constant 15^{o}C or at daily ranges of 13.75 <-> 16.25^{o}C, 12.5 <-> 17.5^{o}C, 11.25 <-> 18.75^{o}C and 10 <-> 20^{o}C, is plotted against the ln dry weight in Fig. 7.6a-e. A single plot of the five regression lines (Fig. 7.7) again indicates no significant differences between the data for the five temperature ranges.

7.4 Discussion

From these data for the three species investigated it appears that there is no significant difference in the oxygen consumption, measured at a constant 15^{o}C, following maintenance at temperature regimes of different daily amplitudes. However the undulation data for *H. contubernalis* does suggest that there is some difference in the metabolism of the larvae, those larvae maintained at the wider daily temperature range undulating for less time and more slowly.

This was only a preliminary limited investigation, and could not be extended further due to a lack of time, but would provide an interesting direction for further work. In particular it would be of interest to measure the oxygen consumption over a range of constant temperatures rather than just at a single temperature at the centre of the maintenance temperature range. More important would be to determine the oxygen consumption at different daily temperature ranges of larvae maintained at thermal regimes of different daily amplitude. An experiment was performed for *Hydropsyche angustipennis*, maintained at the same temperature conditions as used for *H. contubernalis* and *H. instabilis*, with the oxygen consumption being determined over a daily range of 10 to 20^{o}C using the flow through respirometer described in Chapter 3.2.5. Unfortunately the experiment failed due to a faulty electrode membrane and a lack of time prevented the experiment from being repeated.

Fig. 7.6. Relationship between the ln weight specific respiration and ln dry weight for larvae of *Potamophylax cingulatus* maintained at daily temperature ranges of (a) constant 15°C, (b) 13.75 - 16.25°C, (c) 12.5 - 17.5°C, (d) 11.25 - 18.75°C and (e) 10 - 20°C.

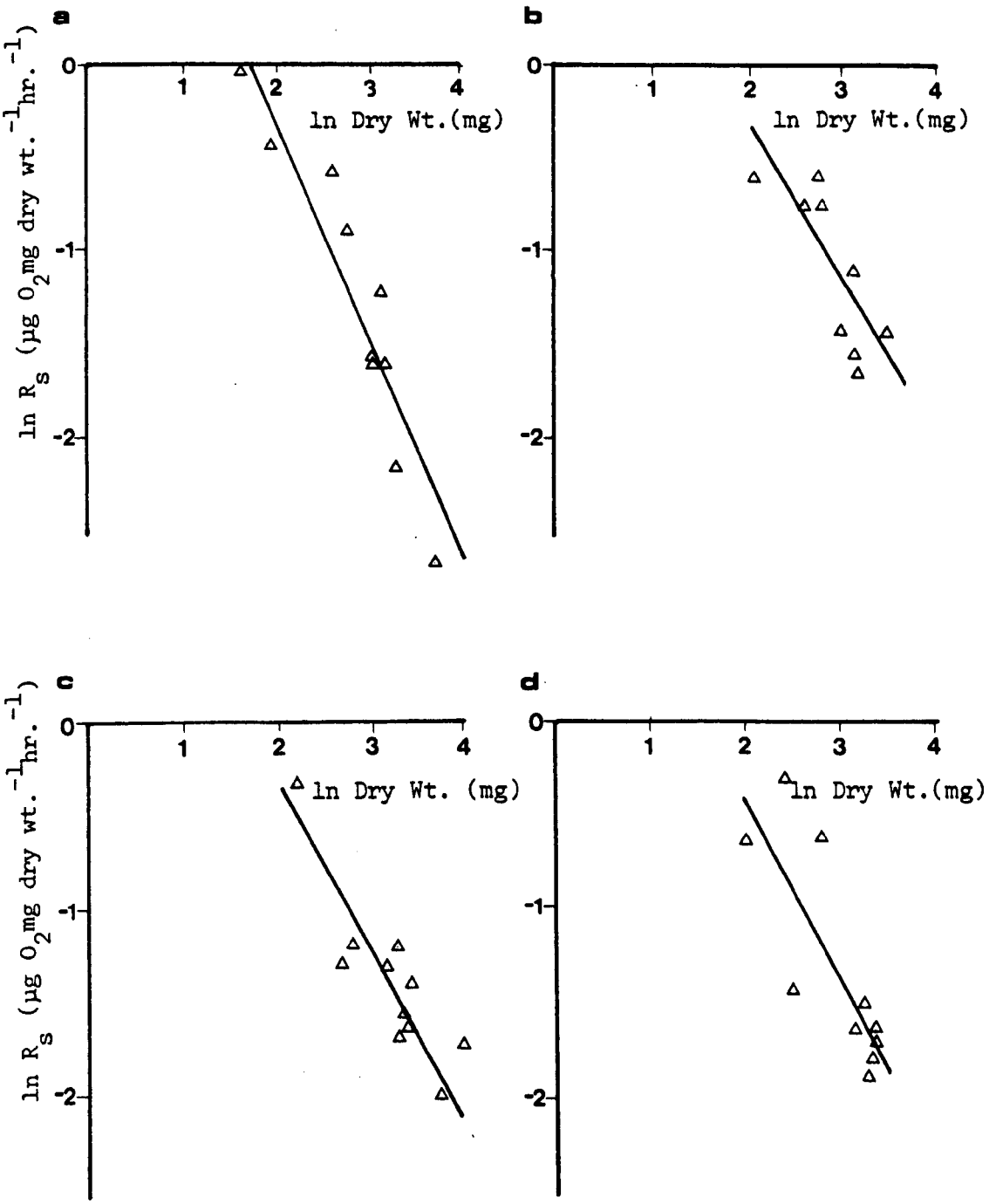


Fig. 7.6. (Cont)

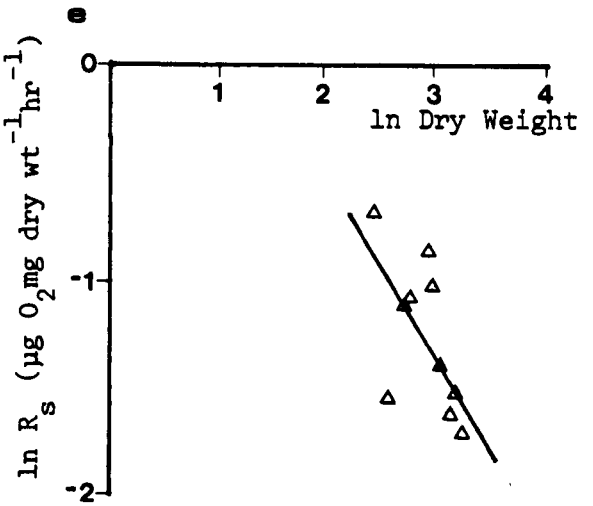
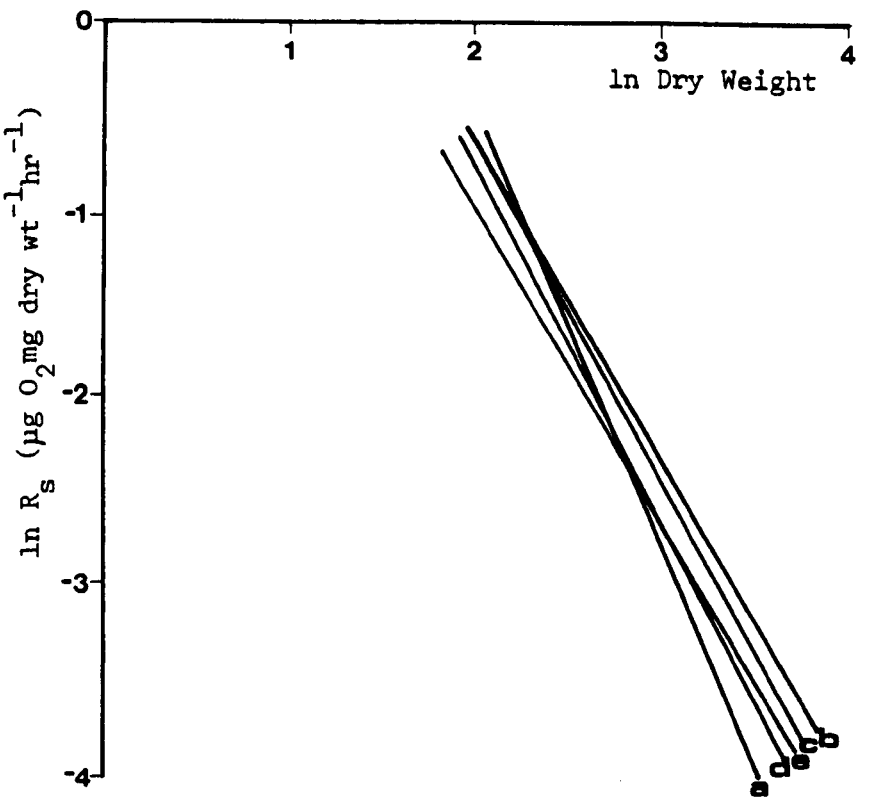


Fig. 7.7. Least square regression lines obtained from the data in Fig. 7.6a-e combined for comparative purposes.



CHAPTER 8 OBSERVATIONS ON GILL NUMBER WITH PARTICULAR REFERENCE TO DIFFERENT THERMAL CONDITIONS IN THE FIELD AND THE LABORATORY

8.1 Introduction

The occurence of gills on the body of aquatic insect larvae or nymphs is widespread, being present for example on mayflies, some stoneflies and caddisflies. In the Trichoptera the tracheal gills, when present, are filamentous and are arranged in four rows on each side of the body either singly or in tufts (Dodds and Hisaw, 1924). In addition there may also be retractable anal blood gills as in the hydropsychids and polycentropodids. The larvae lack functional spiracles, oxygen being acquired by cutaneous diffusion through the general body surface and the gills (Leader, 1971). The respiratory surface provided by the gills will therefore be important in relation to the oxygen concentration of the water or the oxygen consumption of the larvae.

It was noted by Dodds and Hisaw (1924) that there were considerable interspecific and slight intraspecific variations in the number of gills. They suggested that species from lakes and quieter areas of streams had a larger respiratory surface in proportion to the body size than the larvae living in streams. They also demonstrated that for cased larvae the number of filaments increased for larger species and during larval development. For hydropsychids no relationship was found between the size of the species and the number of gills.

Wichard (1974a and b) points out that the size of the respiratory surface of the tracheal gills depends upon both the size and the number of gills, and that the size is related to the larval size and the number determined by the environmental oxygen concentration. No morphological adaptations occur at the cellular level and the anatomical adaptations occur at the moult (Wichard, 1977). Wichard (1977) demonstrated that different populations of the same species may have different numbers of

gills, the gill number increasing in response to decreased environmental oxygen concentration. Preliminary work by Harrison (Badcock et al, 1987) demonstrated a difference in gill number for larvae of the limnephilid *Chaetopteryx villosa* (F) from adjacent streams with different thermal regimes. The aim of the work discussed in this Chapter was to investigate the influence of temperature on the gill number of a range of species. Data are presented for the relationship between gill number and dry weight and for the number of gills in larvae subjected to different thermal regimes both in the field and for larvae maintained at different temperatures in the laboratory. The relationship between gill number and weight specific respiration is also investigated.

All of the experiments were restricted to the determination of the number of gills, gill size not being measured. Data are presented for three cased species, *Potamophylax cingulatus*, *Anabolia nervosa* and *Sericostoma personatum*. No data were obtained for the hydropsychids due to the difficulty of counting the gills owing to their highly branched nature.

8.2 Methods and Results

8.2.1 Relationship between larval size and gill number

Method

The total number of gills were counted on 72 4th instar and 124 5th instar larvae of *P. cingulatus* collected from a single site on the Keele University campus. After counting the gills the head capsule width was determined, at the widest point, using a micrometer eyepiece, in order to check which instar the larva was. Finally the larvae were oven dried at 60^{o}C for 48 hours and their dry weights determined. The experiment was repeated for 5th instar *Sericostoma personatum* and *Anabolia nervosa*. For the latter species the number of gill sites was determined in addition to the gill number.

Fig. 8.1. Relationship between gill number and dry weight of 72 4th instar *Potamophylax cingulatus*.

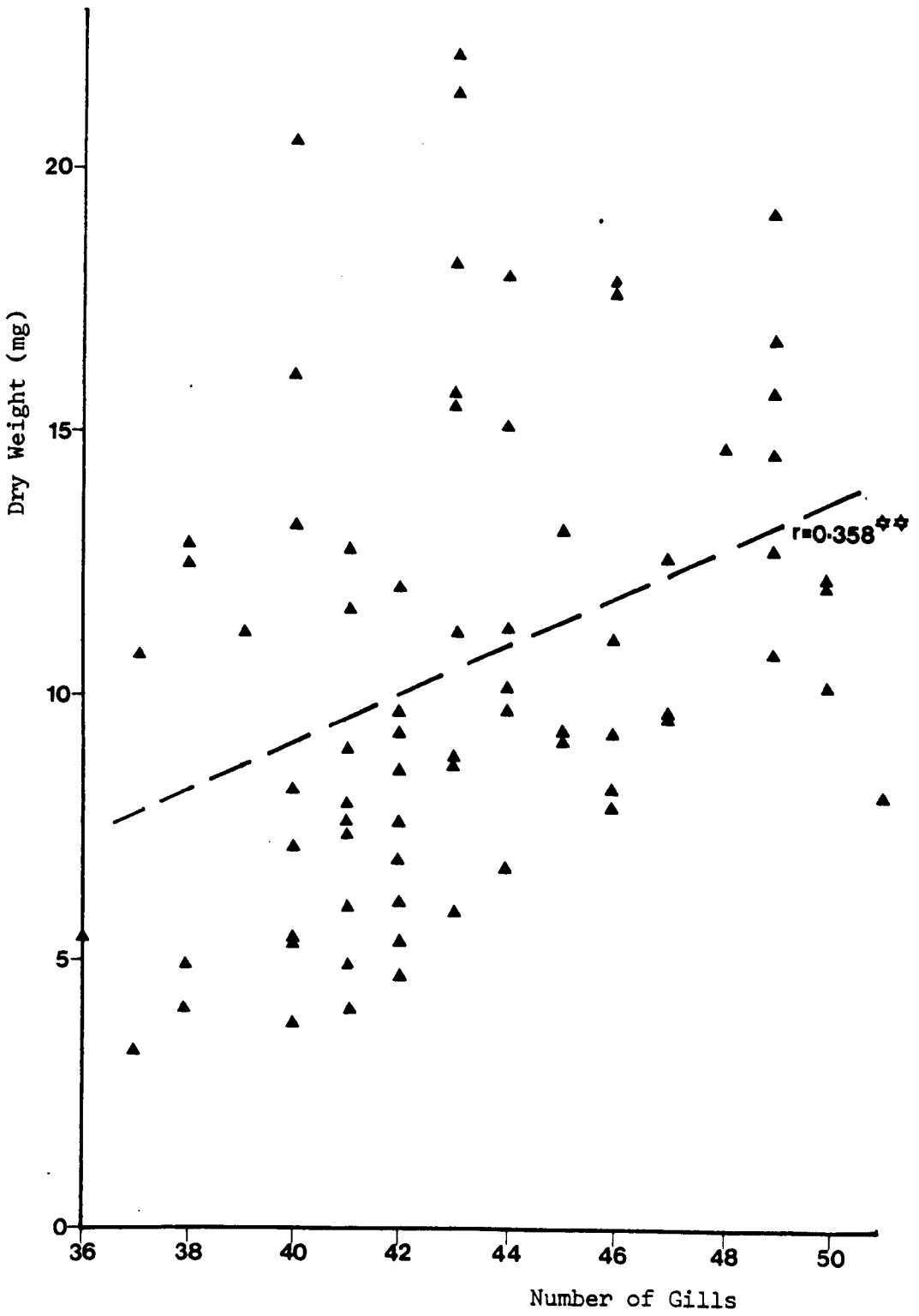


Fig. 8.2. Relationship between gill number and dry weight of 124 5th instar *Potamophylax cingulatus*.

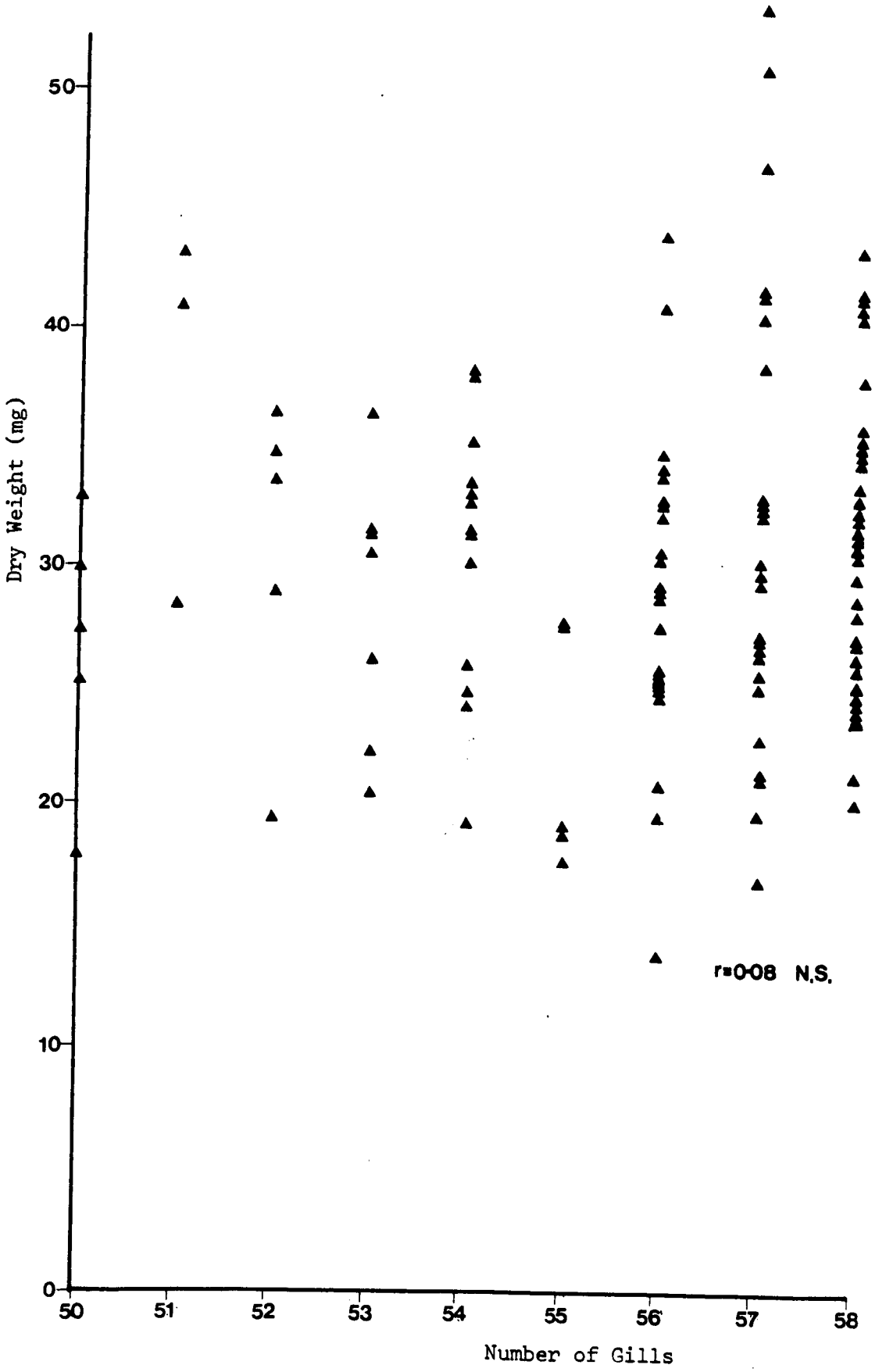


Fig. 8.3. Relationship between gill number and dry weight of 37 5th instar *Sericostoma personatum*.

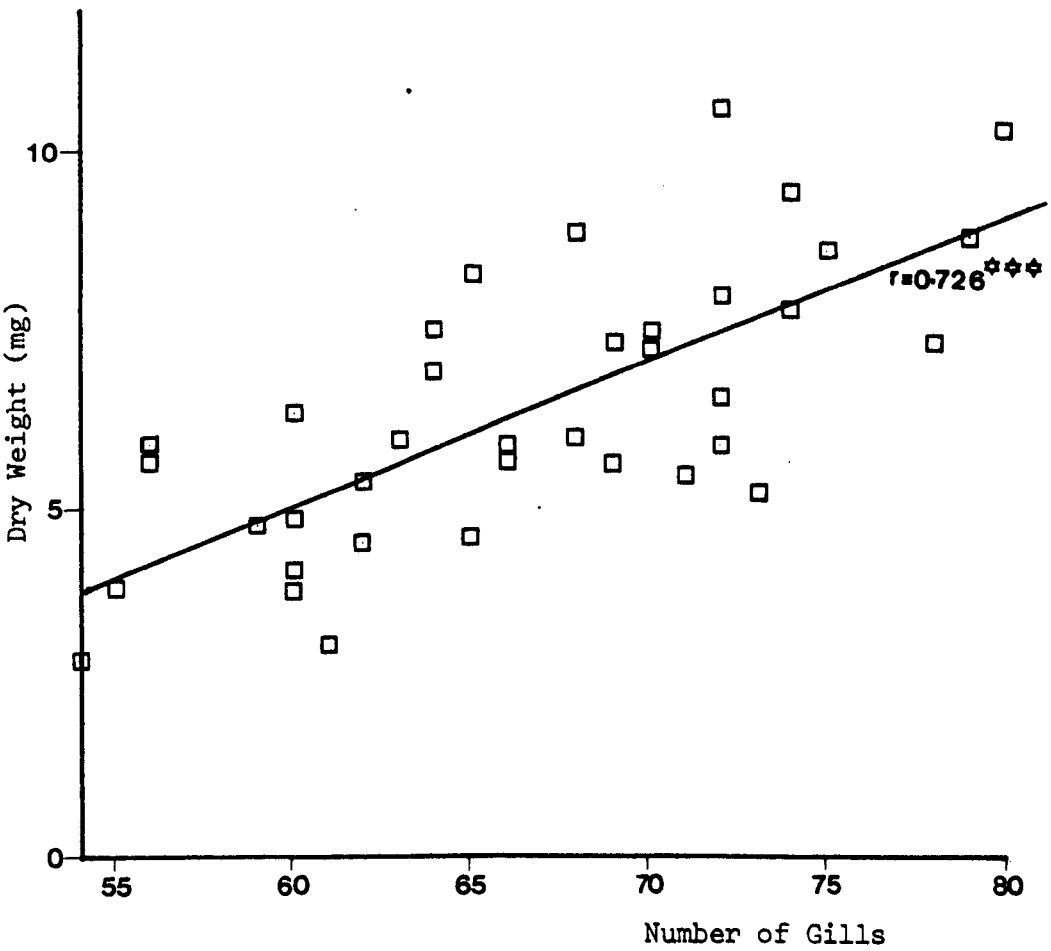
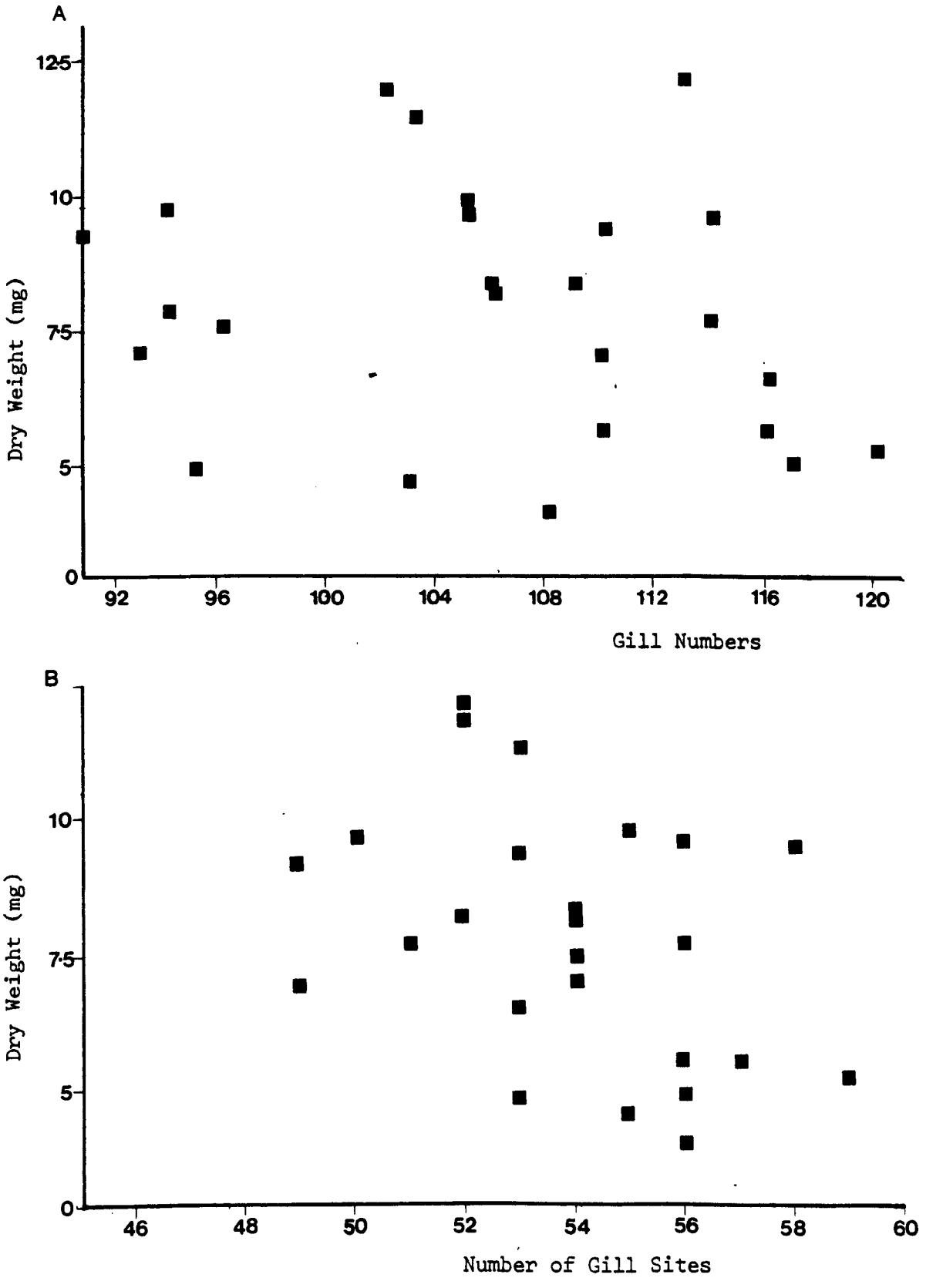


Fig. 8.4. Relationship between (A) gill number and (B) the number of gill sites, and dry weight of 25 5th instar *Anabolia nervosa*.



8.2.2 Results

The relationship between the dry weight, expressed in milligrams, and the number of gills for 4th and 5th instar larvae of *P. cingulatus* are presented in Figs. 8.1 and 8.2. A clear difference exists between the data for the 4th and 5th instars. Firstly there are considerably more gills on the 5th instar than on the 4th instar larvae. For the 5th instars larvae the mean is over 55 gills per larvae while for the 4th instars the mean is just over 43. The ranges for the two instars are 50 to 58 and 36 to 51 gills per individual respectively. Secondly, although the variation is considerable (larvae of very different weights having the same number of gills) there is a significant relationship between the number of gills and body weight in the 4th instar larvae, but not in the 5th instar larvae. For the 4th instars the increase in the number of gills is correlated with an increase in body weight. The final point of note from these data is the apparent maximum number of gills (58) for the 5th instar larvae, and the high proportion of the larvae having a gill number close to the maximum (approximately 68% of the 5th larvae having either 56, 57 or 58 gills).

The data for 5th instar *S. personatum*, shown in Fig. 8.3. differ from those for the previous species. The larvae have more gills than *P. cingulatus*, the mean value being over 68 and the range being 54 to 82. There is no concentration of larvae having an obvious maximum number of gills as was seen for the previous species. There is a significant positive relationship between the number of gills and the body weight in *S. personatum*.

The data for the final species studied, *Anabolia nervosa*, are presented in Fig. 8.4. This species has multiple gill filaments and therefore data is shown for both the total number of gills and the number of bunches of gills. The number of gills is considerably higher than that found for the previous two species. The mean value was 106 with a range from 94 up to 120. Interestingly the mean (53.9) and the maximum number of

gill bundles (60) correspond closely with the number of gills present on the other limnephilid studied *P. cingulatus*. No significant relationship is apparent from this data between the number of gills, or gill bundles, and the body weight.

8.2.3 Laboratory acclimation - method

Larvae of the desired instar were collected from a single site. The head capsule width of a sample of the larvae was determined to confirm which stage the larvae were at. The remaining larvae were divided into two groups and maintained at 6 and 18°C, under the conditions described in Chapter 2.2, for a number of weeks. At the end of the maintenance period the larvae were killed, their gill numbers counted and the head capsules measured in order to determine which instar the larva had reached.

8.2.4 Results

The number of gills, expressed as the mean and standard deviation, on 4th and 5th instar larvae of *P. cingulatus*, maintained at 6 and 18°C, are shown in Table 8.1.

Table 8.1 Number of gills, expressed as the mean and standard deviation, on 4th and 5th instar *P. cingulatus* maintained at 6 and 18°C. (All larvae collected as 4th instars)

Maintenance temperature		4th instar	5th instar
6°C	n	12	17
	$\bar{x}$	41.75	52.588
	σ_n	4.361	2.767
18°C	n	12	16
	$\bar{x}$	41.583	55.75
	σ_n	2.326	2.107

The larvae were collected as 4th instar larvae and during the period of maintenance some but not all developed into 5th instars. There is no significant difference in the gill numbers of the 4th instars maintained at different temperatures ($t_{(23)}$=0.082, N.S.) but there is a significant difference in the number of gills on those which had moulted to 5th instars ($t_{(31)}$=3.431, p=0.002)*, the larvae maintained at the warmer temperature having more gills than the larvae kept at the cooler temperature. The number of gills and the number of gill sites, both expressed as the mean and standard deviation, on 5th instar larvae of *Anabolia nervosa*, maintained at 6 and 18°C, are shown in Table 8.2.

Table 8.2 Number of gills and gill sites, expressed as the mean and standard deviation, on 5th instar *A. nervosa* maintained at 6 and 18°C. (Larvae collected as 4th instars)

		Larvae maintained at 6°C	Larvae maintained at 18°C
Number of gills	n	14	11
	$\bar{x}$	105.14	107.09
	σ_n	7.954	8.447
Number of gill sites	$\bar{x}$	53.64	54.18
	σ_n	2.467	2.622

The larvae were collected as 4th instar larvae, the gill number only being determined on larvae which had developed into 5th instars. (confirmed by measurement of the head capsule width). There is no significant difference in either the gill number ($t_{(23)}$=0.5878) or the number of gill sites ($t_{(23)}$=0.5246) although the values for both were slightly higher in the

* Calculated on arc sin transformed data on the percentage of the maximum gill number (58).

larvae maintained at the warmer temperature.

For the final species studied, *S. personatum*, the number of gills, expressed as the mean and standard deviation, on 5th instar larvae maintained at 6 and 18°C, are presented in Table 8.3.

Table 8.3 Number of gills, expressed as the mean and standard deviation, on 5th instar *S. personatum* maintained at 6 and 18°C. (Larvae collected as 4th instars)

	Larvae maintained at 6°C	Larvae maintained at 18°C
n	20	25
$\bar{x}$	64.4	70.76
σ_n	7.486	6.476

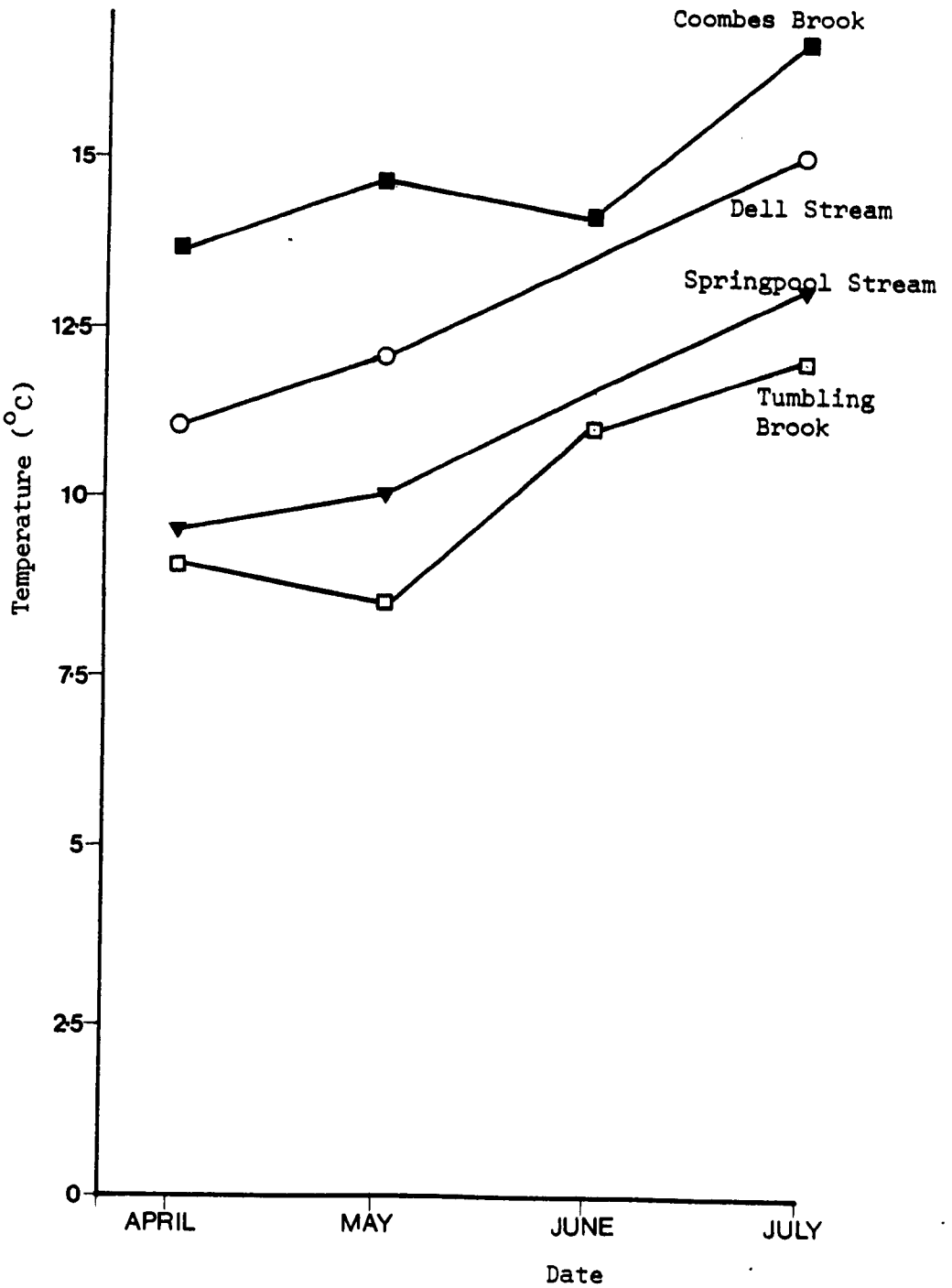
As with the previous species the larvae were collected as 4th instars and the gill numbers determined on larvae which had developed into 5th instars. Head capsule widths were not determined for this species but the differences in the gill number between the 4th and 5th instar larvae are sufficiently different for the instar designation to be correctly determined. There is a significant difference in the gill number ($t_{(43)}$=3.005, p=0.01), the larvae maintained at the warmer temperature developing more gills.

8.2.5 Investigation of the intraspecific variation in gill numbers in the field

Method

Larvae of *P. cingulatus* were collected from four sites (Tumbling Brook, Coombes Brook, Dell stream and Springpool stream - Details of locations in Table 2.1) and the number of gill filaments counted. The head stream, Tumbling Brook, is a tributary of the trout beck, Coombes Brook and

Fig. 8.5. Maximum water temperature for the four streams over the period April to July 1986.



has a more equable temperature than the main stream which is warmer and more extreme in summer (Fig. 8.5. and Harrison and Badcock (1981)). Of the two streams on the university campus the Dell stream has a warmer summer, and cooler winter, temperature than the Springpool stream (Fig. 8.5.) The difference in the summer temperature of these streams was of the order of 1-2^{o}C. Data are also presented for the number of gills on pupae collected from Coombes Brook and Tumbling Brook.

8.2.6 *Results*

The mean number of gill filaments on the 5th instar larvae collected from the four sites are shown in Table 8.4. Due to the lack of normality in the data, as a result of the maximum gill number (Chapter 8.2.2), statistical comparisons are made using an arcsin transformation of the data. An analysis of variance on the data is significant ($F_{3,179}$=25.43***) and individual t tests on each pair of streams are significant (Tumbling Brook/Springpool - t=3.239**, Tumbling Brook/Dell Stream - t=7.52***, Tumbling Brook/Coombes Brook - t=8.05***, Springpool/Dell Stream - t=3.854***, Springpool/Coombes Brook - t=4.582*** and Dell Stream/Coombes Brook - t=2.584*. The increase in the number of gills in larvae from the streams in the order Tumbling Brook, Springpool, Dell stream and Coombes Brook corresponds to increasing summer temperature in the same sequence.

Table 8.4. Number of gill filaments in 5th instar larvae of *P. cingulatus* in four streams. (Larvae collected in June 1985).

Stream	Sample Size	Mean no. of gill filaments larva^{-1}	Transformation* $\bar{x}$	σ_n
Tumbling Brook	29	51.6	69.8	± 7.22
Coombes Brook	22	57.6	85.4	± 5.57
Springpool	66	54.8	76.4	± 8.6
Dell	66	56.7	81.5	± 6.3

* Transformation = Arc sin transformation of the percentage of the maximum number of gill filaments.

A comparison of the mean number of gill filaments per larva on 4th instar *P. cingulatus* from the Springpool and Dell streams (Table 8.5) indicates a higher number of gills on the larvae from the Springpool Stream. An analysis of the data by the use of a t test indicates that the difference is significantly different ($p=0.05$). In contrast to the results obtained for the 5th instar larvae from these streams, in this case the larvae from the stream with the warmer summer temperature (Dell Stream) had fewer gill filaments.

Table 8.5 Number of gill filaments in 4th instar larvae of *P. cingulatus* in the Springpool and Dell streams. (Collected June 1985).

Stream	Sample Size	Mean no. gill filaments larva^{-1}	Standard deviation
Springpool	21	42.8	2.99
Dell	20	40.2	4.08

The number of gill filaments on pupae of *P. cingulatus*, from Tumbling Brook and Coombes Brook, are presented in Table 8.6. There is a considerable and significant difference in the number of gills on pupae from the two streams, those from the warmer stream (Coombes Brook) having more gill filaments (t_{26} = 6.01, p=0.001).

Table 8.6 Number of gill filaments in pupae of *P. cingulatus* in Tumbling and Coombes Brook. (Collected June 1985).

Stream	Sample Size	Mean number of gill filaments pupa^{-1}	Standard deviation
Tumbling Brook	14	48.4	4.77
Coombes Brook	14	57.1	2.50

Fig. 8.6. Relationship between the weight specific respiration and gill number in 5th instar *Anabolia nervosa*.

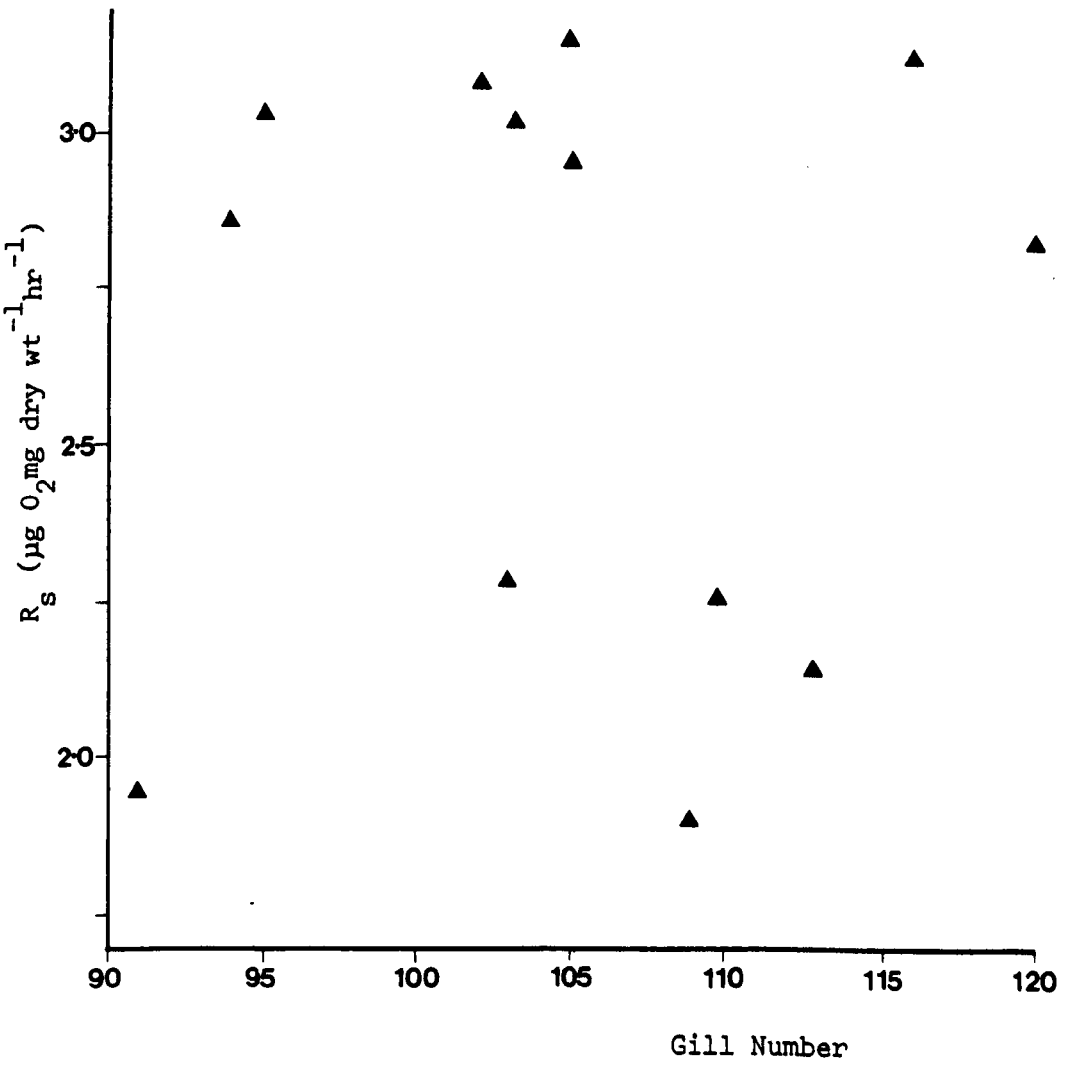
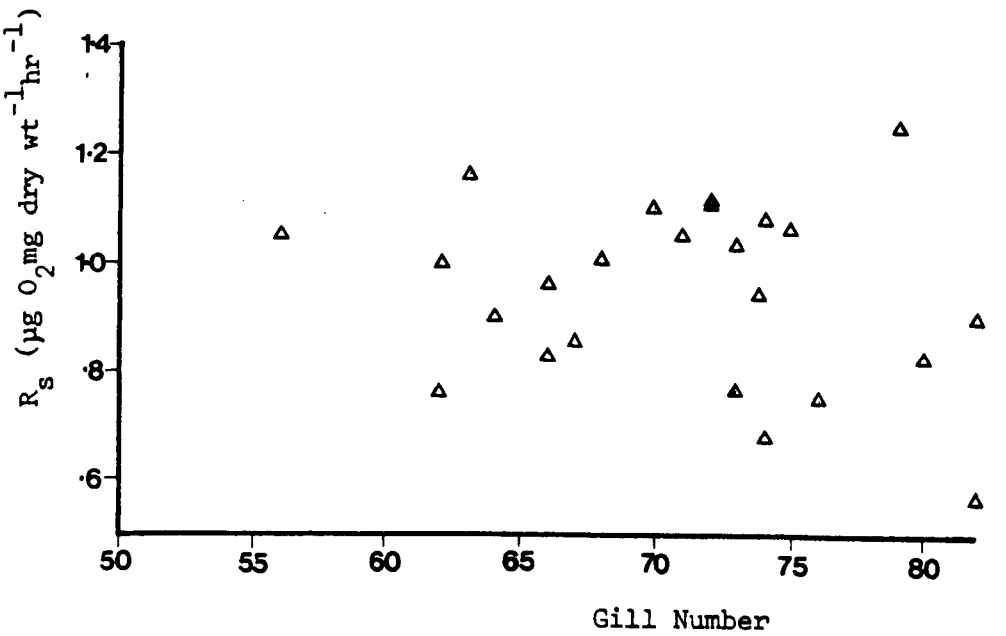


Fig. 8.7. Relationship between the weight specific respiration and gill number in 5th instar larvae of *Sericostoma personatum*.



8.2.7 *Relationship between the weight specific respiration and the gill number - Method*

The gill numbers were counted on 5th instar larvae of *A. nervosa* and *S. personatum* for which the weight specific respiration had been determined in previous experiments, (Chapter 5).

Results

The weight specific respiration of larvae of *A. nervosa* and *S. personatum* standarised to a body weight of 5mg, are plotted against the number of gill filaments on the larvae (Fig. 8.6 and 8.7). For both species there is no correlation between the weight specific respiration and the gill number.

8.3 *Discussion*

The data for the relationship between body weight and gill number indicates differences between the three species. There is a positive correlation between the gill number and body weight for 4th instar *P. cingulatus* (Fig. 8.1) and 5th instar *S. personatum* (Fig. 8.3) but no relationship between 5th instar *P. cingulatus* (Fig. 8.2) and 5th instar *A. nervosa* (Fig. 8.4). There is no obvious explanation for the difference between 4th and 5th instar *P. cingulatus* but the lack of an observed correlation in *A. nervosa* may be a result of the multiple gill filaments present on this species. The variation in the number of gills occurred in the species with single gill filaments by an increase in the number of gill sites further down the body. In no case did the single filaments become multiple filaments. For the species with multiple gill filaments, *S. personatum* and *A. nervosa*, the variation occurs by both a change in the number of sites from which gills arise and a change in the number of gills arising at each site.

The significance of the number of gills, and its relationship to body

weight, is difficult to determine. Presumably an increase in gill number, which increases the respiratory surface, will allow oxygen to be obtained more readily from the water. This may have the effect of reducing the energetic costs of respiration (for example by reducing the necessity for undulating the body) and therefore it is possible that an increased gill number allows enhanced growth. In 5th instar *P. cingulatus* and *A. nervosa* the gills may be sufficiently numerous to no longer impose a limit upon oxygen uptake and therefore have no influence upon the balance between respiratory costs and growth.

The results for maintenance of the larvae in the laboratory under different thermal conditions show that for *P. cingulatus* and *S. personatum* there is a significant difference in the number of gills (Tables 8.1 and 8.3). An increase in gill number occuring in the larvae maintained at the warmer temperature. No significant difference was found between cool and warm maintained larvae of *A. nervosa* (Table 8.2).

The lack of a significant difference in the data for 4th instar *P. cingulatus* (Table 8.1) confirms that the gill number can only change during the moult (Wichard, 1974B), these larvae not having moulted.

Obviously, although the number of gills are said to vary depending upon the maintenance temperature, it may in reality be a response to a difference in the oxygen content of the water. At $6^{o}C$ the fully aerated water would have contained 12.5mg l^{-1} oxygen while at $18^{o}C$ this would have fallen to 9.5mg l^{-1}.

The data for the larvae of *P. cingulatus* from four streams (Table 8.4) demonstrates the occurence of intraspecific differences in the gill number. Detailed stream temperature data were not obtained but throughout the summer the maximum water temperature was $5\text{-}8^{o}C$ warmer in Coombes Brook than in Tumbling Brook. The Dell stream had a maximum summer temperature $1\text{-}2^{o}C$ warmer than the Springpool Stream and these two streams had temperatures intermediate between those of Coombes Brook and Tumbling Brook (Fig. 8.5).

Oxygen determinations were not made due to the difficulty of obtaining meaningful information from spot samples.

The number of gills increase with increased water temperature (oxygen content of the water also possibly being involved), reaching virtually the maximum number of gills for this species (58) in Coombes Brook. The increase in gill number in warmer streams could allow the greater oxygen uptake required for the higher metabolic rate at warmer temperatures or would enable the oxygen to be obtained from warmer water containing less oxygen. It may be postulated that as the larvae from Coombes Brook had almost the maximum number of gills,this species might be at an increasing disadvantage in warmer habitats as the gill numbers could not be increased further.

The results for 4th instar *P. cingulatus* from the Springpool and Dell streams (Table 8.5) are the reverse of those found for 5th instars, the larvae from the cooler stream having marginally more gills. It is suspected that these larvae, collected in June, may have developed from 3rd to 4th instars during the winter when the Dell Stream is cooler than the Springpool Stream, after which they had overwintered as 4th instars.

The results for the number of gills on pupae from Tumbling and Coombes Brook (Table 8.6) are similar to those obtained for the larvae from these streams, the pupae from the warmer stream having considerably more gills.

As has already been suggested an increase in gill number could reduce the oxygen consumption of the larvae by enabling the necessary oxgygen to be obtained with a reduction in the undulatory activity. However the data, for *A. nervosa* and *S. personatum*, comparing the weight specific respiration of larvae with different numbers of gills (Figs. 8.6 and 8.7) provides no evidence for a relationship between the respiration and gill numbers.

8.4 *Conclusions*

A positive correlation was found between gill number and body weight in some but not all species. Differences were observed in the relationship for 4th and 5th instar larvae of the same species. The increase in gill numbers occurred by development at new sites (species with single filaments) or at new sites and by more gills per site (species with multiple gill filaments).

For two species *P. cingulatus* and *S. personatum*, the number of gills was shown to vary in larvae maintained at different temperatures. The individuals maintained at the warmer temperature were found to develop more gills. The change in gill numbers occurred at the moult. No significant difference was found for the number of gills in larvae of *A. nervosa* maintained at 6 and 18^{o}C. Pupae of *P. cingulatus* maintained at a warmer temperature had significantly more gills than those maintained at a cooler temperature.

Significant differences were found for the number of gills present on 5th instar larvae from four sites with differing thermal regimes. The gill number increased as the summer temperature of the stream increased. A comparison of the number of gills on 4th instar *P. cingulatus* from two sites demonstrated a significant difference, the number of gills in this case being higher in the larvae from the cooler stream. It is suggested that this anomaly may be a result of the larvae having developed to 4th instars during the winter when the temperature difference in the streams was reversed.

No relationship was observed between the respiration rate of the larvae and the number of gills present.

CHAPTER 9 FIELD ACCLIMATISATION

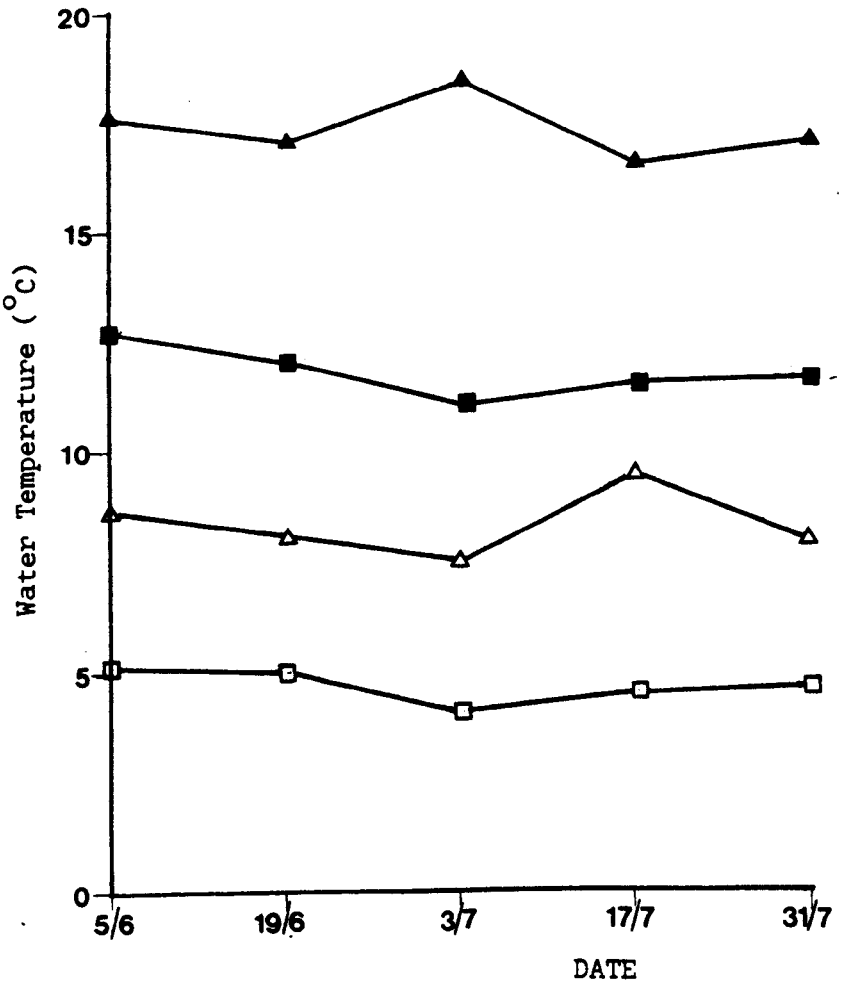
9.1 Introduction

For many years it has been noted that closely related species, and even animals of a single species, inhabit localities with extremely different temperatures (Krogh, 1914). Krogh suggested that it was unlikely that the respiratory rate would differ as much as would be ordinarily implied from the temperature difference.

Many studies have investigated the influence of water temperature on life cycle parameters such as adult emergence (Nebeker, 1971; Lillehammer, 1975) and growth rates (Markarian, 1980; Brittain, 1983; Elliott, 1987) but little work has been reported on interspecific or intraspecific differences in the metabolism of animals from different thermal regimes. In a number of hydropsychid species interspecific differences have been demonstrated in their metabolism which appeared to be related in part to water temperture (Edington and Hildrew, 1973; Philipson and Moorhouse, 1974; Hildrew and Edington, 1979). For larvae of the Dobsonfly, *Protohermes grandis*, Hayashi and Yoshida (1987) demonstrated seasonal differences in the respiration that were apparently related to the water temperature. Intraspecific variation in metabolism have been investigated by Berg (1953) and Harrison and Badcock (1981) for the limpet, *Ancylus fluviatilis* and the caddis larva, *Chaetopteryx villosa*, respectively. In both cases reverse acclimation was reported, larvae from the warmer habitat having a higher respiratory rate when measured at a common temperature.

Differences in water temperature occur as a consequence of a number of factors, some of which were discussed in Chapter 1. In this study the aim was to find sites with different thermal regimes whilst other factors remained as constant as possible. Ideally the sites should be close together, on the same water body and have a sufficiently large temperature difference to allow differences in metabolism to be discerned. Such sites

Fig. 9.1. Minimum and maximum water temperatures for Coombes Brook (▲△) and Tumbling Brook (■□). Determined using a minimum-maximum thermometer during June/July 1985.



are rare, perhaps occuring as the result of screening from insolation/frost (Edington, 1965), or due to differences in the inflow and outflow streams as a result of the influence of lakes or reservoirs.

The sites eventually used were not ideal but allowed a limited study to be made into the occurence, if any, of acclimatisation in the field in response to different thermal regimes for some species which were found to acclimate to temperature in the work described in Chapter 5.

9.2 Method

Two pairs of sites were used, Tumbling Brook and Coombes Brook and the Dell and Springpool streams (Map references - Table 2.1). In the first pair the spring fed Tumbling Brook is cooler in summer than Coombes Brook. Larvae were collected from the two streams just above the confluence of Tumbling Brook with Coombes Brook. In the second pair the Dell Stream is warmer in summer than the Springpool Stream.

Temperature data was obtained using minimum/maximum thermometers. For Coombes Brook/Tumbling Brook the temperatures were recorded for 8 weeks at fortnightly intervals and for the Dell and Springpool streams at weekly intervals for six weeks. At the end of the period of temperature data collection larvae were collected from the pairs of streams and maintained unfed for 12 hours at $10^{o}C$. The respiration was determined using the closed bottle method described in Chapter 4. The oxygen consumption was measured at $12^{o}C$ over a 24 hour period in 65ml glass bottles. At the end of the determination of the oxygen consumption the dry weights of the larvae were measured after oven drying at $60^{o}C$ for 48 hours.

9.3 Results

The temperature data for Coombes and Tumbling Brook, for the 8 weeks before the determination of the oxygen consumption, are presented in Fig. 9.1. During this time, the minimum and maximum temperature in both streams

Fig. 9.2. Comparison of the weight specific respiration, determined at 12°C, of *Potamophylax cingulatus* from Coombes Brook (□) and Tumbling Brook (■).

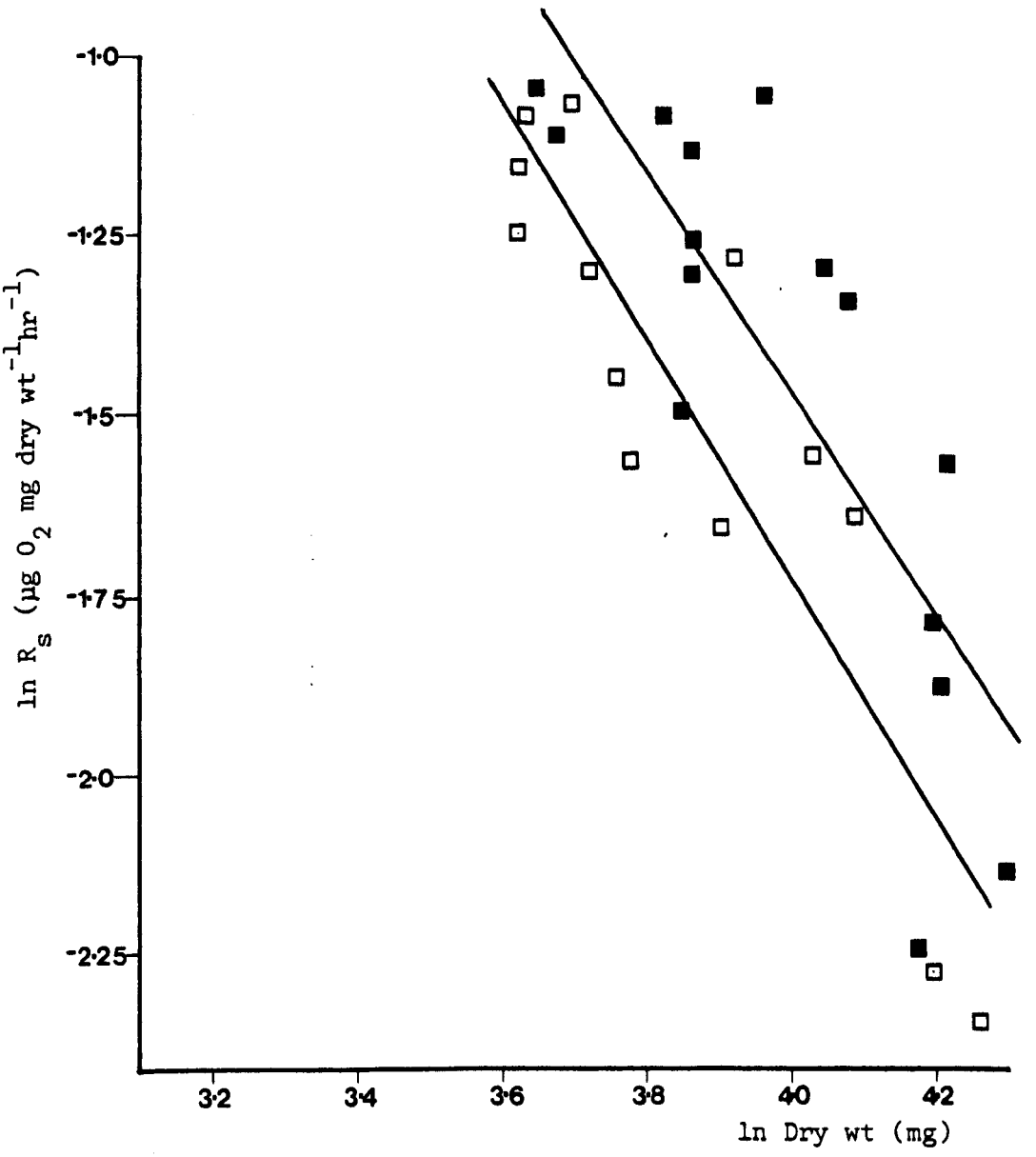


Fig. 9.3. Minimum and maximum water temperatures for the Spring pool (■) and Dell (▲) streams. Determined, using a minimum-maximum thermometer, during August/September 1986.

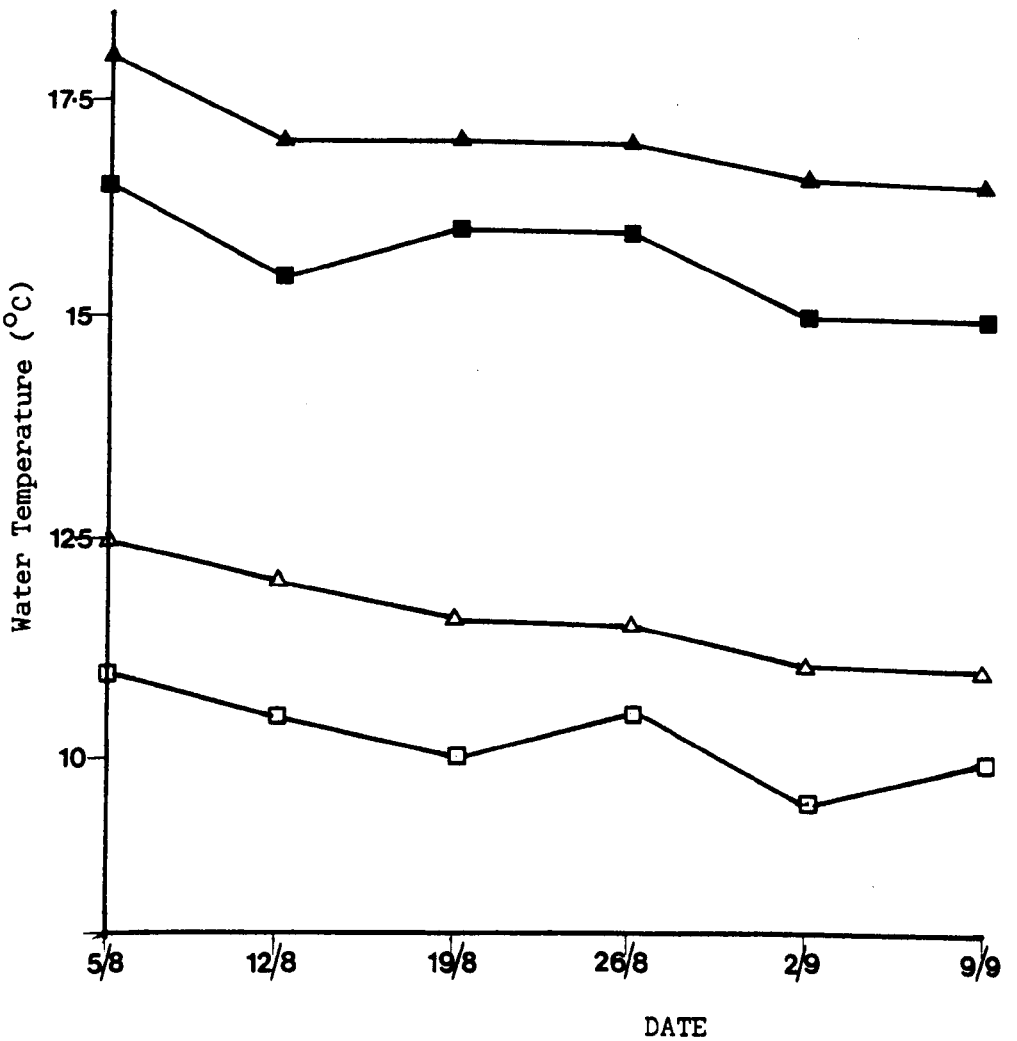
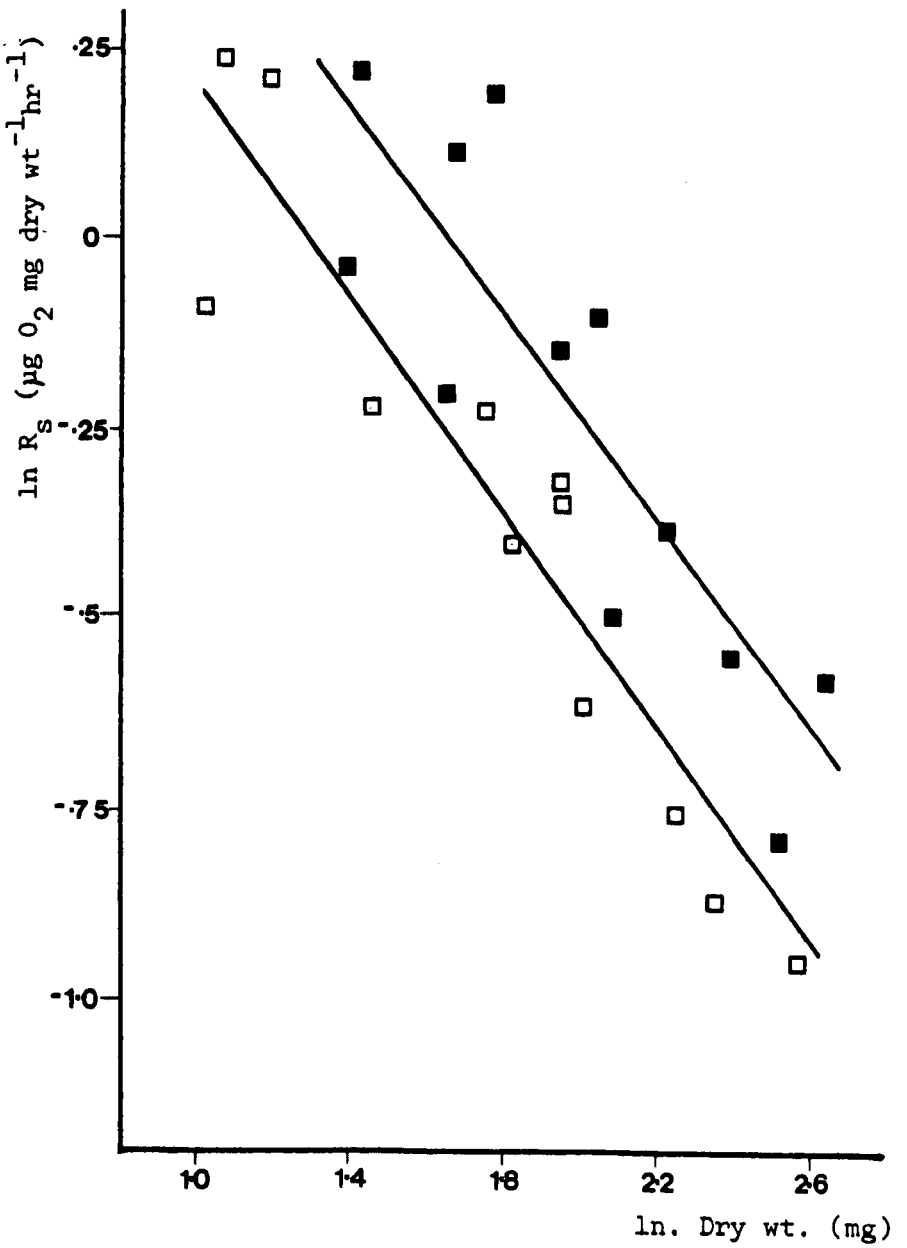


Fig. 9.4. Comparison of the weight specific respiration determined at 12°C of *Sericostoma personatum* larvae from the Springpool (■) and Dell streams (□).



remained fairly constant. The minimum temperature in Tumbling Brook was consistently 3-5^{o}C cooler than Coombes Brook and the maximum temperature between 5-7.5^{o}C cooler in Tumbling Brook compared with Coombes Brook. The ln weight specific respiration of 5th instar *Potamophylax cingulatus*, collected on the 31/7/85, is plotted against the ln dry weight in Fig. 9.2. Analysis of covariance demonstrates no significant differences in the slope of the two regression lines (F=0.033, N.S.) but a significant difference in the elevation of the lines ($F=8.66^{**}$). The larvae from the cooler stream (Tumbling Brook) have the higher weight specific respiration over the range of body weights tested.

The temperature data for the second pair of streams, Springpool and Dell Streams, are presented for the previous 6 weeks, in Fig. 9.3. As with the previous pair of streams the temperature in these two streams remained fairly constant, although declining slightly, over the period of data collection. The temperature difference between these two streams is less marked than that observed between Tumbling and Coombes Brook. For both the minimum and maximum temperature there is a difference of 1 to 1.5^{o}C between Springpool and the Dell streams, the former being cooler. The ln weight specific respiration at 12^{o}C of 5th instar *Sericostoma personatum*, collected on the 9/9/86, is plotted against the ln dry weight in Fig. 9.4. Again an analysis of covariance demonstrates no significant difference in the slope of the two regression lines (F=0.049, N.S.) and a significant difference in the elevation of the lines ($F=17.076^{***}$). As before the larvae from the cooler stream (Springpool) have the higher weight specific respiration over the range of body weights tested.

9.4 Discussion

From the limited data for these two species it can be seen that intraspecific differences occur in the respiration rate of larvae from different sites, those from the warmer streams having lower metabolic rates, indicating the occurence of acclimatisation to the different conditions. From the evidence of the ability to acclimate to temperature, which was demonstrated for these two species in Chapter 5, this could be a response to the difference in the temperature regime of the streams but is more likely to reflect differences in a number of factors including for example water flow rate and oxygen concentration.

This type of experiment was found to pose a number of difficulties, primarily that of finding sites with sufficiently different thermal regimes but with other factors remaining similar. Of the sites chosen the Springpool and Dell streams were alike in terms of width, depth and flow rate and had similar dissolved oxygen contents. Unfortunately the difference in the temperature of the streams, probably a result of the Springpool stream being springfed, was small. In contrast the second pair of streams, for which there was a large temperature difference, the springfed Tumbling Brook being summer cool, differ considerably in terms of width, depth, flow rate and dissolved oxygen. Possibly the best option would be to investigate seasonal variation in the metabolic rate within a single site, although again other factors will vary within a site at different times.

A second problem of this type of study is obtaining meaningful water temperature data. Ideally the temperature should be continously monitored with some type of thermograph but at many sites, especially those with public access, this is not feasible. The use of minimum/maximum thermometers can provide useful comparative data. Obviously the more frequently readings can be made the better but this can then become extremely time consuming especially if the sites are widespread.

Due to these problems this investigation was not extended further in this study but, as discussed in Chapter 10, this may offer an interesting direction for further investigation.

CHAPTER 10 GENERAL DISCUSSION

The data presented in the previous Chapters have been the result of an investigation into the ability of a range of species of caddis larvae to acclimate to temperature. The methods used for the measurement of respiration, including a description of a modification of the micro-Winkler method and the design of a novel flow through respirometer, were discussed in Chapter 3. The response to a number of factors, including oxygen concentration, body size, diel rhythms, container size and the duration of the experiment, which have implications for the design of the experiments and for the interpretation of the data obtained, were described in Chapter 4. The result of exposure of larvae to constant temperatures of 6 and 18^{o}C was investigated in terms of oxygen consumption, data being presented for six hydropsychid, three polycentropodid, two limnephilid and one sericostomatid species (Chapter 5). In addition, for a number of the species, body ventilation (Chapter 6) and differences in gill numbers were also studied (Chapter 8). Limited data were also presented on the metabolic response to maintenance at temperatures of different diel amplitude (Chapter 7) and evidence is presented for the occurence of acclimatisation in the field (Chapter 9).

No attempt was made to determine the mechanisms by which acclimation is achieved,nor was the time course over which acclimation occured investigated. These are discussed in Chapter 10.14 with reference to possibilities for further work.

10.2 The Measurement of Oxygen Consumption

Considerable work has been performed involving the measurement of the oxygen consumption of aquatic organisms. This is particularly true in recent years due to its relevance to energy flow studies and as a test parameter in ecophysiological studies (Scharf et al, 1981). For a variety

of reasons, including the size and mobility of the animals studied and the requirements of the experiment, the number of methods, or modifications of existing methods, for measuring oxygen consumption have proliferated. This continuous development of new methods probably reflects the impossibility of devising a single method which is widely applicable and has led to the creation of 'tailor made' systems, as in this study, suitable for particular experiments.

In making the decision to use the closed bottle method for the majority of the experiments, the benefits and disadvantages had to be balanced and contrasted with the other methods available. As discussed earlier (Chapter 3.3) the use of a Gilson Manometric respirometer was considered but initial tests confirmed the view of previous workers, for example Nagell (1975), that this method was totally unsuitable as a consequence of the disturbance caused by the shaking of the apparatus.

Despite the attractiveness of using a flow through system this method was not used for the majority of the experiments due to its complexity and the difficulty of obtaining sufficient replication. Wrona and Davies (1984) suggest that the complexity of flow through systems have precluded their wider use.

The closed bottle method has the advantage of simplicity and allows a high degree of replication. However, as discussed in Chapter 3.3, the method has a number of disadvantages, the implications of which must be considered in terms of the aims of the experiments. Unlike a study of the energetics of a species, where it is necessary to obtain a measurement of respiration which approximates as closely as possible to a 'natural rate', in this study, while closeness to the 'natural rate' is desirable, it was more important that consistent and comparable results were obtained for the groups of larvae maintained at the different temperatures, both within a species and between the species, and for each group at the different experimental temperatures.

Considering the first critiscism of the closed bottle method, the problem of decreasing oxygen and increasing metabolites during the course of the experiment (Kamler, 1969), these factors are going to be of more importance in bottles in which the oxygen consumption is greater. The response of aquatic animals to decreasing oxygen is well documented (Lampert, 1984) and can be divided into two categories, conformers and regulators. The experiment investigating the effect of decreasing oxygen concentration of the water upon the oxygen consumption of *Hydropsyche angustipennis* (Chapter 4.6) shows that this species is a conformer, the oxygen consumption decreasing in response to a decrease in the oxygen concentration of the water. Thus for this species a greater decrease in the oxygen concentration as a result of an increased oxygen consumption by the larva will cause a negative feedback response, the rate of oxygen consumption decreasing with time in the bottle. In comparing the metabolism of groups of larvae acclimated to different temperatures this factor can only diminish the difference between the two groups of larvae, but as the reduction in oxygen was restricted to less than 25% the effect will be negligable and can be ignored. The response of the other species to decreasing oxygen was not tested and, as Lampert (1984) stated, even closely related species may respond differently. If any of the species are regulators the decrease in oxygen will have no influence upon the rate of oxygen consumption and therefore the difference in the oxygen consumption of the groups of larvae acclimated to the different temperatures will be exaggerated in comparison with the results obtained for a species which is a conformer. However, again as the oxygen concentration is not allowed to fall too far, possible errors caused by this factor will be small and are ignored.

The greater increase in metabolites, produced by the larvae with the enhanced oxygen consumption, could cause a change in activity, leading to a change in oxygen consumption. No difference was observed in the locomotory

activity of the larvae acclimated to different temperatures, although this was not quantified, and so the build up of metabolites was not thought to cause a difficulty, although again any possible problem is minimised by restricting the extent to which the oxygen is allowed to decrease. This also diminishes the problem of the formation of oxygen gradients due to water stagnation, the second critiscism of the closed bottle method (Kamler, 1969).

Kamler also stated that inconsistent results are obtained in relation to the duration of the experiment. This was not confirmed by the work described in Chapter 4.10.3 - 4.10.7 which demonstrated no time related difference in the oxygen consumption of either cased or net-spinning larvae, although with the latter this was only true if the larvae were allowed to spin retreats in the bottles before the measurement of oxygen consumption began. However for other experimental reasons, mainly the diel variations in oxygen consumption (Chapter 4.4), it was considered desirable to maintain an approximately constant experimental period. As the size of the bottles was shown to have no influence upon oxygen consumption (Chapter 4.8) this factor could be varied so that the decrease in oxygen in the bottles at different temperatures was approximately the same.

Although the oxygen consumption was determined at each experimental temperature simultaneously for groups of larvae acclimated to different temperatures, different times (day/night) were used for determining the oxygen consumption at the four experimental temperatures. Two problems could be posed by this, the oxygen consumption could be influenced by light or by the time of day. Elliott (1970) demonstrated that light had a controlling influence upon activity, which might be expected to be reflected in differences in the oxygen consumption. The experiment discussed in Chapter 4.7 indicates that light had no influence upon the rate of respiration of *H. angustipennis*, but it is not known whether this is true for all of the species studied. A metabolic response to light in

any of the species would influence the form of the temperature-metabolism curve but will not affect the conclusions drawn at the same temperature for groups of larvae maintained at the different temperatures.

Diel rhythms have been demonstrated in the activity of caddis larvae (Elliott, 1969 and 1970), from which it would be expected that oxygen consumption would be enhanced during the day or night depending upon whether the species was day or night active. Measurement of the oxygen consumption over a number of daily cycles for two species, *H. angustipennis* and *P. cingulatus*, suggests the situation is more complex (Chapter 4.4). For both species the diel variation in oxygen consumption was demonstrated to be double peaked, reaching a maximum between 2400-0200 hours and 1400-1600 hours. Fortunately this has the advantage that comparisons are possible between oxygen consumptions determined during the day and night. They will differ slightly due to the different sizes of the two peaks, but not to the same extent as with a single peak. It is not known whether all of the species would have a similar pattern of diel variation in oxygen consumption and so again interspecific comparisons should be viewed with more caution than the intraspecific comparisons made between groups of larvae acclimated to different temperatures.

A further possible error may result from differences in the activity of larvae maintained at different temperatures when they are introduced to a single common temperature. This difference should be diminished by the period of exposure to a temperature intermediate between the acclimation temperatures for a period of 12 hours before the oxygen consumption determinations. A final complication which may make interspecific comparisons difficult is that the experimental factors may have different effects upon species. Possibly the most obvious factor could be flow rate. Species accustomed to different flow rates in the field may respond differently to exposure to still water in the closed bottle. However, in view of the work by Feldmeth (1970) the exposure to still water during the

maintenance period may negate this effect if acclimation to water flow occurs.

Due to the large differences in the numerical value of the weight specific respiration between the larvae of different species acclimated to 6 and 18°C, and the possible interspecific differences in the measurement of oxygen consumption due to the closed bottle method, the acclimation ability cannot be compared directly. Instead the ability to acclimate is stated in terms of the percentage decrease of the weight specific respiration of the 18°C acclimated larvae compared with the 6°C acclimated larvae. It is hoped that this provides a valid means of comparing the acclimation ability of different species, overcoming the interspecific differences that are inevitable in the measurement of the oxygen consumption.

10.3 Determination of the Oxygen Content of the Water

Having decided to use a closed bottle method it was necessary to determine the oxgyen content of the water. Initially a micro-Winkler method was used. The use of the Winkler method has been characterised by the development of a multitude of modifications aimed at improving the method (Carritt and Carpenter, 1966). In this study the major requirement was that a number of replicates could be performed simultaneously. The simple cuvette apparatus described in Chapter 3.2.2 fulfilled this requrirement, and from the tests made, it appeared to be both precise and accurate. The mixing procedure is readily standardised and thorough, and the means by which the reagents are added is suitable, constant quantities of the reagents being dispensed by the Finnpipette and the problem of loss of iodine by volatilization during transferance (Bryan et al, 1976) is eliminated. A major difficulty of the method was cleaning the cuvettes between each oxygen determination. This is important because residual manganese from a previous sample will produce excess iodine (Carritt and

Carpenter, 1966). The cuvettes were cleaned by rinsing a number of times, but the apparatus would be improved if it could be adapted so that the cuvettes were replaceable between each sample. Although in later experiments this method was replaced by the use of a Clark type oxygen electrode, which was found to be more rapid and convenient, the cuvette apparatus remains a useful technique to be used if a suitable oxygen electrode is not available.

10.4 The Flow-Through Respirometer

For many experiments the closed bottle method is totally unsuitable, for example, the study of diel variations in oxygen consumption and the response to varying conditions. This, and the disadvantages of the closed bottle method, has increasingly led to the development of designs for flow-through respirometers (Dries et al, 1977; Scharf et al, 1981; Wrona and Davies, 1984) but generally they fall into two categories:- 1) The oxygen consumption is continuously measured in a system with a small number of replicates (Dries et al, 1979; Scharf et al, 1981) or 2) samples are manually removed periodically, and injected into an electrode chamber, from a system with a greater number of replicates (Wrona and Davies, 1984). The development of the flow-through respirometer described in Chapter 3.2.5, which is capable of performing upto 16 replicates fully automatically, offers a number of benefits over previous designs and would allow the performance of experiments not feasible with existing designs. In addition the respirometer has the further advantage in that it can also be used in the closed mode.

The respirometer was designed and constructed too late to be widely used in this study but the experiments which were performed (Chapters 3.2.8-3.2.10, 4.4 and 4.6) demonstrated its potential. It would be of particular value in following acclimation over a period of days, although the duration of this period will be limited by the problem of bacterial

growth (Propp et al, 1982). This could be surpressed by the use of antibiotics, but although these have been used,possible side effects are poorly understood. Secondly the respirometer would be valuable in investigating the influence of fluctuating conditions, of for example temperature or oxygen, fluctuations having more relevance to the field situation than the constant experimental conditions normally used. Unfortunately with experiments involving fluctuating conditions the calculations become complex. Propp et al (1982) present an equation for determining the metabolic rate at any time after the onset of measurement, without the need to wait until the difference between input and output concentrations has become constant. However this equation is only strictly applicable for the situation where the metabolic rate remains constant, hence for the full potential of this respirometer in the flow through mode to be achieved further equations may need to be developed, or else the rate of change in a factor which would alter the metabolic rate must be sufficiently slow for the equation of Propp et al (1982) to provide an adequate approximation. There may also be a problem associated with the lag between a change in metabolism and its measurement, although Niimi (1978) provides an equation for correcting this.

Despite its benefits, the design for the flow-through system does have a number of disadvantages. Most notable is the mechanism by which the flow rate is produced. Firstly the flow rate has to be the same in each of the 16 animal chambers and secondly, transference of a sample of water to the electrode chamber causes a slight aberration in the flow rate through the animal chamber. In the existing design, this cannot be rectified, although a decrease in the electrode chamber size would diminish the second difficulty, particularly if a radiometer type oxygen electrode were used in place of the Clark type electrode, as this would allow the chamber dimension to be reduced to a greater extent.

The problem of the equal flow rate in the 16 chambers can only be

overcome by a complete alteration in the water delivery system, using a multichannel peristaltic pump in place of the existing system. This would pose practical difficulties due to the rotation of the apparatus and it would increase the complexity and cost of the apparatus.

The respirometer would be readily adaptable for use with animals of different size by varying the size of the animal chamber, and the number of replicates could be increased if required.

No evidence was found during the testing of the respirometer for significant diffusion of oxygen through the perspex wall of the animal chambers. Deoxygenation of the water in the water bath only resulted in a very slow decrease in the oxygen content of the water in the animal chambers. If gaseous diffusion were found to cause a difficulty, the animal chambers could be lined with glass tubing of a suitable diameter.

A further development of the apparatus would be to include impedance electrodes (Wrona and Davies, 1984) to record the activity of the animals. This could be arranged so that each of the electrodes on the outer edge of the animal chamber assembly makes contact with the impedance converter/amplifier/chart recorder circuit over a portion of the cycle.

10.5 Miscellaneous Factors Influencing Oxygen Consumption

The investigation into factors having an influence upon oxygen consumption have an intrinsic interest in addition to their relevance for the choice of the experimental protocol and for the interpretation of the results obtained.

The linear relationship between respiration rate, or weight specific respiration, and body weight, when both are expressed in a logarithmic form is well documented (Calow, 1975; Epp and Lewis, 1980; Sutcliffe, 1984; Laybourn-Parry and Tinson, 1985) but the results described in the experiments described in Chapter 4 and those obtained in Chapter 5 are notable for the low values found for the slope, (b), being in the range

0.12-0.26. Similar low values were obtained by Harrison and Badcock (1981) for two species of cased caddis larvae. The explanation for this is unclear but it may reflect differences in the physiologically active surface or weight of the larvae (Calow, 1975) which may vary as a proportion of the total body surface or weight during development. This was not investigated in any greater detail, as it did not directly impinge upon this study, but would be worth further study.

The size dependancy of metabolism is often ignored (Feldmeth, 1970b), which may be valid if the change in respiration rate is small in relation to the change in body weight (i.e. when the curve has levelled out). In this study, where in most experiments the body weight varied considerably, there may have been both inter- and intraspecific differences in the weight distribution of the groups of larvae. Intraspecific comparisons were made between the groups of larvae acclimated to the different temperatures by investigating the significance of differences in the slope and elevation of regression lines of ln weight specific respiration on ln dry weight, rather than by the use of mean values. Interspecific comparisons were made on data standardised to a common body weight.

It should be noted that the linear regression method used assumes that the body weight is known without error, and so strictly should not be used (Laws and Archie, 1981; Halfon, 1985), a model II regression method being applied instead (Ricker, 1973). However when the correlation between the two variables is high, as in this study, the results obtained by the use of the model I and II regression methods are similar (Laws and Archie, 1981) and therefore for ease of computation a model I regression method was used.

Little information was obtained regarding the effect of activity on oxygen consumption. The experiment described in Chapter 4.3 indicates that differences in the activity of the larvae resulted in different levels of oxygen consumption. A major difficulty of these experiments was the short time period over which activity occurs in relation to the time over which

it is feasible to measure oxygen consumption. It is difficult to see how this can be overcome, although the use of an anaesthetic may provide a measure of the 'standard' rate.

Little work has been published on the diel variation in oxygen consumption. Sigmon (1978) and Hart (1980) demonstrated no predictable variation in the oxygen consumption of a dipterous larva and a freshwater shrimp respectively. The double peaked diel variation in oxgyen consumption (reaching a maximum at 2400-0200 hours and 1400-1600 hours) demonstrated for *H. angustipennis* and *P. cingulatus* (Chapter 4.4), was well defined. This double peak contrasts with the single peak in activity which has been demonstrated for a number of caddis species (Lehmann, 1965; Elliott, 1969 and 1970). It is possible that this double peaked response may represent a means of avoiding fish predation, although this is speculative and it should be remembered that under field conditions, particularly the fluctuating temperatures, the response may be different. The development of the automatic flow through respirometer (Chapter 3.2.4-3.2.7) offers considerable potential for extending work into the relationship between oxgyen consumption and the time of day to further species, and of relating the variation in oxygen consumption to diel variations in activity.

The influence of temperature on oxygen consumption has been widely studied in a range of aquatic organisms (Pattee, 1955; Collardeau, 1961; Robinson et al, 1982; Laybourn-Parry and Tinson, 1985; Al-Dabbagh and Luka, 1986) and it is of central importance to this study. The data presented for *H. angustipennis*, for the influence of temperature, in the range 6-24^{o}C, on respiration (Chapter 4.5) indicates a 'typical' response. An increase in temperature causes an increase in oxygen consumption, with the rate of increase accelerating as the temperature increases. A plateau appears to be present between 12 and 18^{o}C, the Q_{10} being just over 1.2 for this temperature range, compared with a value exceeding 2.1 for the range 6 to 12^{o}C and 18 to 24^{o}C. It has previously been shown that the plateau

corresponds to the thermal regime normally encountered by the species in the field (Calow, 1975; Hildrew and Edington, 1979; Epp and Lewis, 1980; Harrison and Badcock, 1981). The possible significance of this is discussed in Chapter 10.8.

Many studies have been made of the relationship between oxygen consumption of aquatic invertebrates and the oxygen concentration of the medium (Konstantinov 1971; Mangum and Van Winkle, 1973; Nagell, 1974; Wynberg and Brown, 1986). Animals are classified into two groups, conformers and regulators, although many intermediate responses are observed between the two extremes. The data, presented in Chapter 4.6, for *H. angustipennis* indicates that this species was a conformer, the oxygen consumption decreasing as the oxygen concentration decreases. The rate of decrease was found to be greater once the concentration fell below 50 per cent air saturation (PAS). Under supersaturated conditions the oxygen consumption again decreased.

As the oxygen concentration decreased below 100PAS the rate of body undulations increased until the lowest oxygen concentration, when the number decreased. Thus, as the oxygen concentration decreases not only does the oxygen consumption drop but also the energy expenditure on ventilation increases.

Lampert (1984) points out that differences are frequently observed in the response to oxygen content in closely related species and therefore it would be useful to repeat the experiment for a range of species.

The investigation of oxygen attributable to the case epifauna (Chapter 4.9) indicates that this is of some significance, reaching 10-16% of the total oxygen consumption. The data for *Anabolia nervosa* suggests that the oxygen consumption is not influenced by the thermal history of the case, resulting in a slight underestimation in the difference in the oxygen consumption of groups of larvae acclimated to different temperatures.

The significance of light (Chapter 4.7), crowding and container size

(Chapter 4.8) and the duration of the experiment (Chapter 4.10.5.) were mainly of importance for the design of the experiment and have been discussed earlier. Container size would not be expected to influence oxygen consumption when the container posed no physical constraints upon the behaviour of the test animals. The significant difference in the oxygen consumption as a result of different animal densities (Chapter 4.8), the mean weight specific respiration increasing as the number of individuals per bottle increases, should be taken into account when comparing data produced from experiments using different numbers of animals per bottle.

The investigation of the relationship between oxygen consumption and the duration of the experiment for *H. angustipennis* (Chapter 4.10.5) demonstrates the importance of considering the behaviour of the animal under investigation. Time was shown to have no influence upon oxygen consumption if the larvae were introduced into the bottle 12 hours before the oxygen determinations were made. In contrast if the oxygen consumption was determined immediately after the introduction of the larvae the oxygen consumption was higher when measured over shorter time periods. This is thought to reflect increased activity associated with retreat building, during the initial period.

10.6 Temperature acclimation

The phenomenon of temperature acclimation has great significance, for both laboratory experiments and for understanding the influence of water temperature in the field.

Historically, although Kulkarni and Nagabhusanam (1978) suggests that there is irrefutable evidence that poikilothermic invertebrates undergo metabolic compensation in response to thermal variation, insects in general are said to have little ability to compensate for environmental temperature (Keister and Buck, 1974). However Harrison and Badcock (1981) and Hayashi and Yoshida (1987) have demonstrated the occurence of temperature

acclimation in two species of caddis larvae and seasonal acclimatisation in a Dobsonfly, respectively. The occurence of temperature acclimation in aquatic insects is widely assumed, frequently being taken into account to a greater or lesser degree in experimental procedures (e.g. Heiman and Knight, 1975). In view of its importance, it is surprising that so little work has been published relating to temperature acclimation, and the extent to which acclimation occurs is poorly understood.

Before considering the occurence of temperature acclimation it is perhaps valuable to consider the significance of a higher or lower respiration rate. An elevated respiration rate could be a reflection of a detrimental increase in maintenance costs, an increase in growth, an increase in activity or a consequence of the animals being in different physiological states (e.g. moulting or in the process of pupating). Within the context of these experimental conditions locomotion, although not quantified, did not appear to differ between the groups of larvae acclimated to different temperatures. However as shown by the data presented in Chapter 6 the ventilatory activity of the two groups of larvae did vary, the significance of which is discussed further in Chapter 10.10. As the larvae were unfed during the measurement of the oxygen consumption, growth can be disregarded and there was no reason to believe that the larvae were not in a similar physiological state. Therefore a decreased oxygen consumption, determined under these experimental conditions is indicative of those animals having an advantage as it represents a decrease in maintenance costs.

The choice of acclimation temperatures of 6 and 18^{o}C was aimed at providing temperatures which were sufficiently different for differences in metabolism of the larvae acclimated to different temperatures to be discerned even if the acclimation ability was small. In retrospect a less extreme upper temperature, perhaps 15^{o}C, may have been more suitable as maintenance of the larvae at the higher temperature posed some difficulties

for species normally restricted to cooler water (*Diplectrona felix* and *Plectrocnemia conspersa*). This is considered unlikely to have had any major influence upon the conclusions drawn from the experiments with these species, as the individuals which survived the maintenance period were the animals which were most likely to have compensated to the warmer temperature, yet no evidence was obtained of an ability to acclimate in either of these species.

10.7 Acclimation following maintenance at 6 and 18°C

The data presented in Chapter 5 provide evidence that temperature acclimation occurs in a number of the species studied, but such an ability to compensate is not universal. Of the six hydropsychid species tested four were shown to acclimate (*Hydropsyche instabilis*, *H. siltalai*, *H. pellucidula* and *H. angustipennis*) while for the remaining two species (*Diplectrona felix* and *H. contubernalis*) no evidence for an ability to acclimate was obtained. Only one of the polycentropodids tested (*Polycentropus flavomaculatus*) was shown to acclimate, again, no evidence being obtained for an ability to acclimate in two other species (*Plectrocnemia conspersa* and *Neureclipsis bimaculata*). All three of the cased caddis species tested (*Anabolia nervosa*, *Potamophylax cingulatus* and *Sericostoma personatum*) were shown to acclimate to temperature. In all cases where acclimation was shown to occur it was a partial, type III response (Precht, 1951), the larvae maintained at the warmer temperature having a lower respiration rate. For *P. cingulatus* this contrasts with the reverse acclimation reported for this species by Harrison and Badcock (1981). The authors of that study acknowledge that the data base was low and it is felt that more confidence can be placed on the data obtained in this present study.

Considerable interspecific differences were observed in the extent to which the larvae were able to compensate. The acclimation ability was

expressed as the percentage decrease in the weight specific respiration of the 18°C acclimated larvae compared with that of the 6°C acclimated larvae. For the four hydropsychid species in which an acclimation ability was demonstrated the extent to which it occured increased in the sequence *hydropsyche instabilis* (% decrease = 22.7), *H. pellucidula* (29.8), *H. siltalai* (36.6) and *H. angustipennis* (43.7). The corresponding values for the polycentropodid and the cased species were 24.3 for *Polycentropus flavomaculatus*, 33.5 for *Potamophylax cingulatus*, 35.1 for *Anabolia nervosa* and 40.9 for *Sericostoma personatum*.

The differences in the ability to acclimate were shown to be related to the field distributions of the species. Those species for which no evidence was found for an ability to acclimate (*Diplectrona felix*, *H. contubernalis*, *Plectrocnemia conspersa* and *Neureclipsis bimaculata* all have restricted distributions in habitats with relatively constant temperature regimes. An increasing ability to acclimate to temperature was shown to be associated with increasingly widespread distributions and occurrence at higher mean water temperatures. For example, *H. instabilis* with a relatively restricted distribution in cooler water acclimates to a small degree, while *H. angustipennis* with a much more widespread distribution in warmer conditions in the midlands and the south of Britain (Badcock, 1975) has the greatest acclimation ability of all the species tested.

A comparison of the acclimation ability of hydropsychids and polycentropodids with similar distributions (*H. instabilis/P. conspersa* and *H. siltalai/P. flavomaculatus*) suggests that the hydropsychids are able to acclimate to a greater extent, no evidence being found for *P. conspersa* to be capable of compensation and the extent of the acclimation ability being lower in *P. flavomaculatus* than in *H. siltalai*.

10.8 Variation in stream temperature and the possible relevance of interspecific differences in acclimation ability

It is useful to consider the ways in which stream temperature varies and discuss further its relevance to the results presented in Chapter 5. Stream temperatures have been sufficiently widely documented for generalisations to be made with reference to both the temporal and spatial differences which occur (Kamler, 1965; Edington, 1966; Crisp and LeCren, 1970; Boon and Shires, 1976). Ward and Stanford (1982) present an idealised description of the changes of stream temperatures with time, showing an annual variation, temperature reaching a maximum in summer, on to which diel variation is superimposed. Along a stream system the extent of the annual and diel temperature changes will vary.

The movement of individual caddis larvae is restricted (Erman, 1986), although there is likely to be some downstream movement due to larval drift (Elliott, 1971) therefore an individual animal will encounter a gradually increasing or decreasing stream temperature, depending upon the period over which development is occuring. A species which is capable of compensating its metabolism will to a greater or lesser extent be able to maintain a constant respiratory rate. During a period of increasing water temperature the compensatory effect due to acclimation will be reinforced by a decrease in weight specific respiration caused by the growth of the larvae. Under conditions of decreasing water temperature the decrease in oxygen consumption will be greatest in the species in which no acclimation ability was demonstrated, which as Wiley and Kohler (1984) state, could be detrimental if their metabolism decreased to the point where the insects become competitively inferior to others that are not similarly affected.

It would be expected that the species having the greater acclimation ability should be capable of maintaining a more equitable respiration rate in response to changes in stream temperature than species with a more limited acclimation ability. Thus it may be postulated that species having

a lower acclimation ability would be restricted to habitats having smaller seasonal temperature variations, over the life cycle of the species.

Spatial differences in temperature have been ascribed a major role in determining the downstream distribution of aquatic insects, with competitive displacement possibly truncating the range of temperatures that a species could otherwise occupy (Ward and Stanford, 1982). In general terms it may be said that mean water temperature increases in a downstream direction and the diel variation in the water temperature tends to decrease in a downstream direction due to the larger water volume (Langford, 1970). There are obvious exceptions to this pattern, for example springfed headstreams are summer cool and winter warm and have a small daily temperature range and shading from insolation or the presence of a lake or reservoir will alter the stream temperature.

The thermal equilibrium hypothesis (Vannote and Sweeney, 1980) provides a model by which the influence of spatial variations in stream temperature upon the distribution of species may be partially understood, the interaction between bioenergetic and developmental parameters for insects reared in optimum and nonoptimum thermal regimes being used to demonstrate that there is an optimum temperature at which growth and fecundity will be maximised. The model assumes a constant relationship between respiration rate and body weight. This is said to be a consequence of the occurence during periods of increasing temperature of a quasiequilibrium for metabolism, the increase in metabolism with an increase in temperature being counteracted by the decrease due to the increase in body size as the animal grows.

However larvae of a particular size will have a different weight specific respiration at different temperatures. The occurence of acclimation will reduce the temperature effect, eliminating it if the acclimation is complete. Thus, if acclimation is complete the model presented by Vannote and Sweeney (1981) remains valid and the animal does

have an optimum thermal regime. However in the absence of acclimation the position of the weight specific respiration - larval size curve will shift during the life cycle as the water temperature varies. If the other relationships remain the same there will only be an optimum thermal regime if there is no seasonal variation in water temperature. As the variation in water temperature increases a species will be exposed to increasingly non-optimum temperature conditions. Thus species not capable of temperature acclimation may be at a disadvantage as the habitat temperature becomes more seasonally variable.

The diel variations in temperature are too rapid for compensation to occur. Thus the most important aspect of the relationship between temperature and metabolism is the extent of the change in respiration over the daily temperature range encountered. The significance of the interspecific differences in the temperature-metabolism curve were discussed in Chapter 5.4.5. The extent of the information which could be obtained from the curves was limited, especially in terms of making intraspecific comparisons of groups of larvae acclimated to different temperatures. The limitation was largely a consequence of respiration being determined at only four experimental temperatures, much of the fine detail of the relationship was lost due to the $5^{o}C$ difference in temperature.

Temperature-metabolism curves were presented for five hydropsychid species. No zone of relative temperature independence was found in this study for *H. instabilis*, the increase in respiration being reasonably constant over the temperature range studied. For *H. siltalai* and *H. pellucidula* the results obtained were comparable with those produced by Hildrew and Edington (1979) for *H. pellucidula*. The respiration is relatively temperature independent over the range 5 to $15^{o}C$, after which there is an accelerating increase in the weight specific respiration. The range 5 to $15^{o}C$ covers much of the range of field temperatures encountered by these species. The results for *H. angustipennis* surprisingly showed

little indication of a plateau in the relationship, except possibly between 12°C and 18°C. It was suggested that this species has a different strategy for survival at warmer temperatures compared with the other species. However it should be remembered that the high ability to acclimate which was demonstrated for this species (Chapter 5.4.4) would help to maintain a constant metabolic rate over a range of temperature if measurements were made on animals acclimated to the experimental temperatures. The data presented for *D. felix* (Chapter 5.4.5) are unusual, the weight specific respiration increasing considerably between 5 and 10°C after which it declines at warmer temperatures. It is thought that this may reflect the onset of respiratory distress, at a temperature between 10 and 15°C, for this species normally restricted to cooler temperatures. It is suggested that the difference between this data and that obtained by Edington and Hildrew (1973), who found an increase in respiration upto 25°C, could be a consequence of the use of stirred respirometers in that study compared with unstirred respirometer bottles in this study.

The degree by which the weight specific respiration increases for an increase in temperature from 6 to 18°C, for larvae acclimated to 6 and 18°C respectively (Table 5.10), decreases in the order *H. instabilis*, *H. pellucidula* and *H. siltalai*, which corresponds to the order of increasing ecological distribution.

The temperature-metabolism curves of the two cased species for which sufficient data was available, *Potamophylax cingulatus* and *Seriocostoma personatum*, both show an increase in respiration as the temperature increases. For *P. cingulatus* a zone of relative temperature independence was found between 5 and 15°C, while for *S. personatum* the rate of increase in respiration remained constant over the full temperature range. As the distributions of these species are less well known than those of the hydropsychids it is difficult to relate this data to distribution.

The nature of the relationship between temperature and metabolism

requires further investigation, in particular the oxygen consumption needs to be determined at a greater range of temperatures, perhaps 10, in steps of 2°C, which would allow smaller differences in the temperature-metabolism curves to be discerned. As the occurence and nature of a plateau is of particular interest (Precht, 1973) it would be useful to investigate whether acclimation to different constant temperatures causes either a shift in the position or the width of the plateau. It would also be of interest to determine whether or not acclimation to diel ranges of differing amplitudes has any influence upon the temperature-metabolism curve. Interspecific differences in these responses would have relevance to the distribution of the species.

10.9 Interspecific differences in the weight specific respiration

The interspecific differences in the weight specific respiration were discussed in Chapter 5.4.3. For the hydropsychids the respiration rate was shown to increase in the sequence *H. siltalai*, *H. instabilis*, *H. pellucidula* and *H. angustipennis* (Fig. 5.14). The relative position of the respiration of *D. felix* varies depending on which experimental temperature was considered. At 5 and 20°C the weight specific respiration was shown to be lower than that obtained for the other species, while at 10°C the respiration rate exceeded the values obtained for the other species. The large increase in metabolic rate for this species as the temperature was increased from 5 to 10°C may explain the restriction of this species to cooler habitats, the efficiency of the species being reduced at warmer temperatures (Edington & Hildrew, 1973). It was suggested that the low value obtained for the metabolic rate at 20°C represented the onset of respiratory distress. The data obtained for *H. contubernalis*, at 5 and 20°C suggest that this species has a weight specific respiration similar to that obtained for *H. instabilis*.

The data obtained in this study for three species, *D. felix*, *H.*

instabilis and *H. pellucidula*, contrasted with results obtained for the same species by Hildrew and Edington (1979). In that study the weight specific respiration of *H. pellucidula* was found to be lower than that of *H. instabilis*, the reverse of what was found in this study. For *D. felix* Edington and Hildrew (1973) found an increase in weight specific respiration over the full temperature range, compared with the peak at 10^{o}C, and subsequent decline in metabolism, found in the present study. It was suggested in Chapter 5.4.3 that these discrepancies could be accounted for by the fact that stirred respirometers were used by Hildrew and Edington (1979), while in this study unstirred respirometers were used. Thus the differences in the relationship between the weight specific respiration in the two studies may reflect a differential response to water flow. As water current increases in a downstream direction (Hynes, 1970; Ledger, 1981) determination of the weight specific respiration under still water conditions may influence species from downstream to a greater extent, causing an increase in the measured weight specific respiration.

There was no obvious relationship between the weight specific respiration and the field distribution of the species. Intuitively it would be expected that species with a distribution in warmer conditions would have a lower respiration rate when determined at warmer experimental temperatures than species with a cooler water distribution. However no such relationship was observed, for example, two of the "warm" water species (*H. pellucidula* and *H. angustipennis*) had the highest weight specific respiration, and *H. instabilis*, a 'cool' water species, had a low weight specific respiration similar to that obtained for the 'warm' water species, *H. contubernalis*. This again may reflect interspecific differences in the response to other environmental factors, water flow being likely to be of major importance.

For the three polycentropodids there is again no obvious relationship between the weight specific respiration (Table 5.6) and the distribution of

the species. The weight specific respiration of these three species are comparable to, although slightly lower than, the respiration rates of the hydropsychids with comparable distribution. This may also be a reflection of the water current encountered in the field as the polycentropodids are generally found in regions of slower water flow than the hydropsychids (Hildrew and Edington (1981)). It was also speculated that the lack of tracheal gills in the polycentropodids may limit the rate at which oxygen can be obtained, imposing a limit on the respiration rate.

Few conclusions can be drawn for the cased caddis larvae studied as relatively little detailed information is available on the relationship between their distributions and field temperatures.

It would be useful to repeat these experiments in stirred respirometers in order to determine whether or not the anomaly between these data and those of Hildrew and Edington (1979) are caused by water movement in the respirometer.

10.10 Ventilatory behaviour following acclimation of 6 and 18°C

The ventilatory activity of larvae acclimated to the two constant temperatures were discussed in Chapter 6. Ventilation was shown to increase with an increase in temperature (Figs. 6.1-6.10) presumably a consequence of the increased oxygen consumption as the temperature increases. This was shown to occur by an increase in both the time spent undulating and the rate of undulations. For some, but not all, species a difference was demonstrated for the ventilatory activity of larvae acclimated to 6 and 18°C, larvae acclimated to the warmer temperature undulating for less time and more slowly when the two groups were compared at constant temperatures.

The relationship between the data for the body undulations and oxygen consumption, following acclimation to 6 and 18°C, was discussed in Chapter 6. For eight of the species the two sets of data were consistent, intraspecific differences in metabolism being mirrored by differences in

the ventilatory activity. These eight species include five (*H. instabilis*, *H. siltalai*, *H. pellucidula*, *H. angustipennis* and *P. cingulatus*) for which a difference was found between the respiration rate and the undulatory activity for larvae acclimated to the different temperatures, and three species (*D. felix*, *P. conspersa* and *N. bimaculata*) for which no difference was found in either the weight specific respiration or the undulatory activity following maintenance at different temperatures.

For the remaining two species for which data is available there was an apparent conflict between the respiratory and undulatory data. For *H. contubernalis*, a species in which no difference was found between the metabolism of the groups of larvae acclimated to the two temperatures (Fig. 5.6), there was a difference in the number, time and rate of undulations (Fig. 6.6). This suggests that the respiratory costs of the warm acclimated larvae are lower than the cool acclimated larvae and therefore although there was no difference in the weight specific respiration the warm acclimated larvae did have an advantage over the cool accimated larvae. For the final species, *P. flavomaculatus*, for which the warm acclimated larvae were shown to have a decreased weight specific respiration compared with the cool acclimated larvae, it would be expected that the warm acclimated larvae would undulate less as less oxygen is required. However, no difference was found for the undulatory activity of the two groups of larvae, indicating that a greater proportion of the oxygen is consumed by the process of body ventilation in the warm acclimated larvae.

Interspecific differences in the undulatory activity were also discussed with reference to the distribution of the species in Chapter 6. It was pointed out that the results obtained under these experimental conditions could bear little relationship to the field situation. Philipson and Moorhouse (1974) demonstrated that the undulatory behaviour decreased as the flow rate increased for three species, *H. siltalai*, *H. pellucidula* and *H. angustipennis*. At a water velocity of $10cms^{-1}$ the undulatory

behaviour was virtually eliminated when determined at 10°C and thus in the field body undulations may occur infrequently in the caseless caddis larvae. However, a relationship between the undulatory activity of the larvae and their distributions was suggested. The limited undulatory activity of *D. felix* and *P. conspersa* may prevent the penetration of these species into warmer water. Three of the species with widespread distributions (*H. pellucidula*, *H. siltalai* and *P. flavomaculatus*) had undulatory activities which remained constant over the temperature range 6-18°C, indicating that the energy costs of ventilation remain unchanged over a wide habitat temperature. The data for *H. angustipennis*, showing that the undulatory behaviour of warm acclimated larvae was minimal even at the warmer temperatures, are consistent with the distribution of this species in warmer habitats. The high undulatory activity of *H. contubernalis* and *P. flavomaculatus* is taken to indicate that the occurrence of these species at warmer sites is energetically expensive.

The ventilatory activity offers considerable potential for further study. It would be useful to determine the importance of ventilation under experimental conditions more relevant to the field conditions in order to deduce whether or not the intra and interspecific differences observed in this study have any relevance to them. Further studies into the ventilatory behaviour of the larvae would be aided if a means could be incorporated into the flow through respirometer for determining activity (Chapter 10.4). This would also allow the relationship between ventilation and respiration to be investigated further.

It would also be valuable to determine the occurrence of body undulations in the field, although this is likely to pose experimental difficulties, particularly for larvae spending much of their time under the shelter of stones.

Finally, the single experiment performed upon *H. angustipennis* (Chapter 6.3.4), which demonstrated that the influence of the maintenance

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of larvae at different temperatures varied with the length of the acclimation period, the undulatory activity decreasing as the time increases, suggests that this aspect of the ventilatory activity requires further investigation.

10.11 Gill numbers

The influence of temperature acclimation and field acclimatisation on the gill numbers of a number of cased caddis species was discussed in Chapter 8.

A positive correlation was found between gill number and body weight for two species, *S. personatum* and *P. cingulatus*, although for the latter this is true for 4th instar but not 5th instar larvae, many of the 5th instars having the maximum number of gills. For the third species studied, *A. nervosa*, there was no evidence for a significant relationship between the number of gills, or the number of gill bundles, and body weight. The increase in gill number occured by development at new sites further down the abdomen (species with single gill filaments) or at new sites and by more gills per bundle (species with multiple gill filaments).

The number of gills were shown to increase in individuals of *P. cingulatus* and *S. personatum* following maintenance at a warmer temperature for a period of time including a moult. Maintenance at different temperatures was not shown to alter the gill number of *A. nervosa*. Differences were demonstrated in the number of gills on pupae of *P. cingulatus* following maintenance at 6 and 18^{0}C during the development from 5th instar to pupa, individuals maintained at the warmer temperature again having more gills.

Significant differences were found for the gill number on 5th instar *P. cingulatus* from four field sites. The gill number was shown to increase as the summer temperature of the stream increased. A comparison of the gill number of 4th instar *P. cingulatus* from two streams was also shown to vary,

apparently being higher in the stream which was warmer in the winter when the larvae had developed from 3rd to 4th instars. Significantly more gills were shown to occur on pupae of *P. cingulatus* from the warmer of two streams.

No evidence was obtained for a relationship between the respiration rate of larvae of *A. nervosa* and *S. personatum* and the number of gills.

The relative importance of oxygen uptake through the general body surface and the tracheal gills is difficult to deduce and has not been studied. However it is reasonable to assume that an increase in gill number will allow oxygen to be obtained more readily. This could conceivably decrease the need to undulate the body, which in turn would decrease the oxygen consumption of the larvae, and thus could in part at least, explain the mechanism by which metabolic compensation occurs.

Further studies on gill numbers are required in order to determine whether the variation in gill number is a response to temperature, oxygen or a combination of the two factors. Useful information might be obtained by measuring the length of gills in addition to their number, and, if a means could be devised for quantifying the number/size of the gills, extending the work to the hydropsychids. No relationship was demonstrated between the gill number and the respiration rate of the larvae but the effect is likely to be small and may have been lost amongst the inherent variability of these kinds of measurements. Repetition of the experiments with more replicates may provide evidence for a relationship.

10.12 Acclimation to fluctuating temperatures

The majority of laboratory studies, including the greater part of this study, have evaluated thermal effects by the use of constant temperatures (Sweeney, 1984). Temperature variation is normally excluded from experimental design because of technical difficulties in producing suitable test environments, problems in interpreting the data obtained and the

association of constant temperatures with a controlled experiment (Beck, 1983).

Despite these difficulties it is important that, now considerable data have been published concerning the influence of constant temperature on various aspects of insect metabolism and life cycle parameters, more attention should be paid to responses to fluctuating temperatures. The data presented in Chapter 7 for *H. instabilis*, *H. contubernalis* and *P. cingulatus* provided no evidence of a difference in metabolism, measured at a constant temperature, for larvae maintained at fluctuating temperatures of differing amplitudes. A difference was observed in the undulatory activity of larvae of *H. contubernalis* maintained at different fluctuating temperatures, larvae acclimated to the wider daily temperature range undulating for less time and more slowly.

The thermogradient bar described in Chapter 2.2 provides a means by which animals could be maintained at temperatures of different diel ranges and the flow through respirometer described in Chapter 3.2.4 - 3.2.11 would allow the oxygen consumption to be determined under conditions of fluctuating temperature. If the difficulties involved in interpreting the data can be surmounted the respirometer could be used in the flow through mode, but if not, the closed mode could be used if the rate of change in the temperature was not excessive. Possible interspecific differences in the response of caddis larvae to maintenance at different diel temperature ranges may be related to their field distributions and requires further study.

10.13 Field acclimatisation

Having demonstrated the occurrence of temperature acclimation in the laboratory it was considered desirable to perform at least a limited amount of work concerning field acclimatisation. The data presented in Chapter 9, for *S. personatum* and *P. cingulatus*, demonstrated the occurence of

intraspecific differences in the metabolic rate of larvae collected from different sites, larvae from warmer streams having a lower weight specific respiration. In view of the ability of these species to compensate their metabolism in response to maintenance at different temperatures (Chapter 5) the field differences in respiration are in part likely to be a response to temperature, although factors including water flow and oxygen concentration are probably also involved.

The difficulties in performing such experiments, and possible ways of surmounting them, were discussed in Chapter 9.

This work requires extension to a greater range of species, and a comparison of the metabolic rates of larvae from different parts of their distribution may help to understand the relationship between metabolism and distribution.

10.14 Miscellaneous further work

In addition to the further work already discussed throughout this chapter three further avenues are suggested for the extension of this work. Firstly this study did not investigate the time course over which acclimation occurs. Interspecific differences in the rate at which acclimation occurs will influence the response of species to fluctuating temperatures and would be useful in determining the acclimation period required in other experiments.

Secondly, longer term studies could be made, following the development from eggs to adult at a range of temperatures, to investigate the differences between species with and without an ability to acclimate in terms of the life cycle parameters discussed by Vannote and Sweeney (1981).

Thirdly the work could be extended to include investigation into the inter-relationship between a number of factors, particularly the relationship between acclimation to temperature and acclimation to flow rate and oxygen concentration.

10.15 Conclusions

For the majority of the respiration experiments performed in this study the use of the closed bottle method was shown to be a suitable means of measuring the oxygen consumption of the caddis larvae studied, the limitations of the method not being critical in terms of the aims of these experiments.

The modified micro-Winkler apparatus designed provided an easily used method which overcomes a number of the problems associated with the micro-Winkler method. However, if available, an oxygen electrode is more convenient, and, as in much of this study, should be used.

The specially designed flow-through respirometer was shown to have advantages over previous designs, particularly in terms of its automatic nature but also due to its simplicity, the high degree of replication which is possible and its inexpensiveness. Although the respirometer was not widely used in this study it offers considerable potential for experiments over longer time periods.

A range of factors influencing oxygen consumption were investigated, initially in terms of their relevance to the experimental procedure used, but they also have an intrinsic interest.

A linear relationship was demonstrated between weight specific respiration and dry weight, when both were expressed in logarithmic forms. Differences in oxygen consumption were shown to be related to different levels of activity, 'standard', 'routine' and 'active' rates being identified. For two species, *Hydropsyche angustipennis* and *Potamophylax cingulatus* a double peaked diel variation in oxygen consumption was demonstrated, the peaks occuring at 2400-0200 and 1400-1600 hours. For *H. angustipennis* an increase in temperature was shown to cause an increase in oxygen consumption, with the rate of increase accelerating as the temperature increases but with a plateau occuring in the relationship between 12 and 18^{o}C. The oxygen consumption of *H. angustipennis* was shown

to decrease as the oxygen concentration decreases, indicating that this species was a conformer. Light, container size and the duration of the measurement of oxygen consumption had no influence upon oxygen consumption, although for the latter factor, this was only true for net-spinning larvae if they were allowed to spin a retreat before the measurement of the oxygen consumption began. The number of animals per bottle was shown to influence the oxygen consumption, the weight specific respiration increasing as the number of individuals per bottle increases.

For eight of the species studied an ability to acclimate to temperature was demonstrated (*Hydropsyche instabilis*, *H. siltalai*, *H. pellucidula*, *H. angustipennis*, *Polycentropus flavomaculatus*, *Anabolia nervosa*, *Potamophylax cingulatus* and *Sericostoma personatum*). No evidence for such an ability was obtained for the remaining four species studied (*Diplectrona felix*, *Hydropsyche contubernalis*, *Plectrocnemia conspersa* and *Neureclipsis bimaculata*). Within the species which were shown to acclimate the extent of the compensation varied in a way which was related to the distribution of the species, an increased ability to compensate being associated with an increased distribution. The hydropsychids were shown to have a greater ability to acclimate than polycentropodids with similar distributions.

Interspecific differences in the weight specific respiration were investigated, considerable variations occuring. No obvious relationship was observed between the weight specific respiration and the distribution of the species.

The undulatory activity of larvae maintained at different constant temperatures for four to five weeks was shown in some, but not all, species to vary, the larvae maintained at the warmer temperature undulating for less time and more slowly when compared at a constant temperature, with larvae maintained at the cooler temperature.

For five of the six species studied for which metabolic compensation

was demonstrated, a difference was also found in the undulatory activity. For three of the four species for which no metabolic compensation was demonstrated no difference was found either in the undulatory activity The inconsistency between the respiration and undulation data for the remaining two species, *Hydropsyche contubernalis* and *Polycentropus flavomaculatus*, is surprising and requires further study. It is suggested that there is a relationship between the undulatory activity and distribution, species having a minimal ability to undulate being restricted to cool water, species with widespread distributions having an undulatory behaviour which remains constant over a wide temperature range and species with warm water distributions (excluding *H. contubernalis*) apparently needing to undulate little even at warm temperatures.

For two of the three cased species studied a positive correlation was demonstrated between gill number and body weight, although for *Potamophylax cingulatus* this was shown to be true for 4th but not 5th instar larvae, where many individuals had the maximum number of gills. The number of gills was shown to increase in two species, *P. cingulatus* and *Sericostoma personatum*, following maintenance at the warmer of two temperatures. The change in gill number was shown to occur at the moult. A similar difference in gill number was found for pupae of *P. cingulatus*, again individuals maintained at the warmer temperature having more gills. The number of gills was also shown to vary within a species for individuals from different field sites for larvae and pupae of *P. cingulatus*, the number of gills increasing in response to increased stream temperature. For two species tested, *Anabolia nervosa* and *Sericostoma personatum* no relationship was demonstrated between the oxygen consumption of the larvae and the gill number.

Data presented for three species, *Hydropsyche instabilis*, *H. contubernalis* and *Potamophylax cingulatus* provided no evidence of a difference in metabolism following a period of maintenance at fluctuating

temperatures of differing amplitudes. However decreased undulatory activity was demonstrated in larvae of *H. contubernalis* maintained under conditions of greater temperature fluctuations, suggesting that the response to fluctuating temperatures requires further study.

The occurence of field acclimatisation was demonstrated in two species, *Sericostoma personatum* and *Potamophylax cingulatus*. Larvae collected from warmer sites were shown to have a lower weight specific respiration than individuals of the same species from a cooler site.

A considerable amount of further work is suggested by the results obtained in this study. These have been discussed throughout this chapter but three aspects of the work are of particular interest. These are to investigate the time course over which acclimation occurs, to study the influence of different maintenance temperatures over longer time periods and to extend the work to multifactorial investigations, particularly in order to determine to what extent the metabolic compensation is a response to temperature and to what extent it is a response to the change in oxygen content of the water as a result of the change in temperature.

SUMMARY

1. An ability to acclimate to temperature was demonstrated for eight of the species studied (*Hydropsyche instabilis*, *H. siltalai*, *H. pellucidula*, *H. angustipennis*, *Polycentropus flavomaculatus*, *Anabolia nervosa*, *Potamophylax cingulatus* and *Sericostoma personatum*). For the remaining four species studied no evidence for an ability to acclimate to temperature was obtained (*Diplectrona felix*, *Hydropsyche contubernalis*, *Plectrocnemia conspersa* and *Neureclipsis bimaculata*).
2. The extent of the compensation, increasing for the hydropsychids in the order *Diplectrona felix/Hydropsyche contubernalis*, *H. instabilis*, *H. pellucidula*, *H. siltalai*, and *H. angustipennis*, and for the polycentropodids, *Plectrocnemia conspersa/Neureclipsis bimaculata* and *Polycentropus flavomaculatus*, was shown to vary in a way which was related to the ecological distribution of the species, an increased ability to compensate being associated with an increased ecological distribution.
3. The hydropsychids were shown to have a greater ability to acclimate than polycentropodids with similar distributions.
4. A range of factors influencing oxygen consumption were investigated. The relationship between log weight specific respiration and log dry weight was shown to be linear. Differences in oxygen consumption were shown to be related to different levels of activity. For *Hydropsyche angustipennis* and *Potamophylax cingulatus* a double peaked diel variation in oxygen consumption was demonstrated. For *H. angustipennis* an increase in temperature was shown to cause an increase in oxygen consumption, while a decrease in oxygen concentration caused a decrease in oxygen consumption, indicating that this species is a conformer. The weight specific respiration was shown to increase as the number of individuals per bottle increases. Light, container size

and the duration of the measurement of oxygen consumption had no influence upon oxygen consumption, although for the latter, this was only true for net-spinning larvae if they were allowed to spin a retreat before the measurement of the oxygen consumption.

5. Considerable interspecific differences in the weight specific respiration were demonstrated. No obvious relationship was found between the weight specific respiration and the distribution of the species.
6. Field acclimatisation was demonstrated in two species of cased larvae, *Sericostoma personatum* and *Potamophylax cingulatus*, larvae from warmer sites having a lower weight specific respiration than individuals of the same species from a cooler site.
7. For some, but not all, species studied larvae maintained at a warmer temperature for 4-5 weeks were shown to undulate for less time and more slowly when compared, at a constant temperature, with larvae maintained at a cooler temperature.
8. For five of the six species studied for which metabolic compensation was demonstrated (*Hydropsyche instabilis*, *H. siltalai*, *H. pellucidula*, *H. angustipennis* and *Potamophylax cingulatus*) a difference was also found in the undulatory activity. Of the remaining four species, for which no metabolic compensation was demonstrated, no difference was found in the undulatory activity in three species (*Diplectrona felix*, *Plectrocnemia conspersa* and *Neureclipsis bimaculata*). The inconsistency between the respiration and undulation data for the remaining two species (*Hydropsyche contubernalis* and *Polycentropus flavomaculatus*) requires further investigation.
9. A relationship between undulatory activity and distribution was suggested, species with a minimal ability to undulate being restricted to cool water, species with widespread distributions having an undulatory behaviour which remains constant over a wide temperature

range and species with warm water distributions (excluding *H. contubernalis*) needing to undulate little even at warm temperatures.

10. For two of the three cased species studied a positive correlation was demonstrated between gill number and body weight, although for *Potamophylax cingulatus* this was true for 4th but not 5th instar larvae.
11. The number of gills was shown to increase in two species, *P. cingulatus* and *Sericostoma personatum* following maintenance at the warmer of two temperatures. The change in gill number occurred at the moult. The number of gills was also shown to increase in *P. cingulatus* pupae maintained at a warmer temperature during the period of development from 5th instar larvae.
12. Intraspecific differences were found in the number of gills on individuals from different field sites for larvae and pupae of *Potamophylax cingulatus*. The number of gills was shown to increase in response to increased stream temperature.
13. For two species, *Anabolia nervosa* and *Sericostoma personatum*, no relationship was demonstrated between the oxygen consumption of the larvae and the gill number.
14. Data presented for three species provided no evidence for a difference in metabolism following maintenance at fluctuating diel temperatures of differing amplitudes. However, decreased undulatory activity was demonstrated in larvae of *Hydropsyche contubernalis* maintained under conditions of greater temperature fluctuations.
15. A closed bottle method was used for the majority of the respiration experiments. A modified micro-Winkler apparatus was designed and shown to be a useful method, although an oxygen electrode was shown to be more convenient.
16. A flow-through respirometer was designed. This had advantages over previous designs in terms of its automatic operation, a high degree of replication, its simplicity and its inexpensiveness.

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