THE STRUCTURE AND FUNCTION OF THE CAUDAL LAMELLAE OF THE DAMSELFLY

Pyrrhosoma nymphula (SULZER) [ODONATA:ZYGOPTERA].

By

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A thesis submitted for the degree of

Doctor of Philosophy

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July 1995

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Acknowledgements

I would like to thank Professor R. S. Phillips for the use of facilities at the Department of Zoology and at the University Field Station, Rowardennan. I am also grateful to the following people who helped in connection with this project: Dr R. Tippett and staff of the Field station and the Department of Zoology for the provision of facilities and technical support; Lawrence Tetley and the staff of the electron microscope unit for advice and assistance with preparation of materials; Dr A. Taylor for useful advice regarding the physiological measurements; Dr A. Kiriki and the Institute of Freshwater Ecology for the loan of equipment; Dr N. B. Metcalfe for his help with the statistical analysis; Jill Farrell and Vanessa Warnock for help with typing; Sharon Swift for German translations and Dr C. E. Adams for his assistance and encouragement throughout.

I would also like to extend thanks and gratitude to my supervisors Dr K. H. Lockey, Dr M. H. Hansell and Prof. F. A. Huntingford for taking this project on and providing guidance and advice throughout.

SUMMARY

A characteristic of all zygopteran dragonfly larvae is the presence of three caudal lamellae attached to the last abdominal segment. The structure of these organs varies according to the species, and four basic forms have been recognised, laterally flattened, saccoid, reduced and triquetro quadrate. The most commonly found type is the laterally flattened lamella, which have been further subdivided into the simple unjointed forms and the constricted, nodate, subnodate and denodate jointed forms.

It is not certain what role these laterally flattened lamellae have. Superficially they resemble tracheal gills, and historically this has been presumed to be their main function. However, this has never been proven unequivocally for any species. Because of the confusion over the function of these organs there is clearly a need for a comprehensive study of the lamellae and their function, in a single species of zygoptera. The present study aimed to achieve this by investigating their ultrastructure, respiratory physiology, and ecological and behavioural importance of the lamellae of P. nymphula.

Previous experimental studies which have compared the oxygen consumption rates of larvae with against those without lamellae have reached conflicting conclusions. For example, some authors conclude they are important as gills and others that they are not. Interpretation and comparison of the results of these studies is problematic due to the variety of different techniques used and deficiencies in their experimental protocol.

Other functions have been suggested for laterally flattened lamellae including as sensory, locomotory, defensive and ion uptake organs. However, only limited investigations of the possible locomotory and defensive functions of lamellae have been carried out to date. These studies have found that in one species lamellae may be important for swimming and that in three further species they may function as attack deflecting organs. High frequencies of lamellar loss have been reported in wild larval populations of at least three species, and this has been related to injuries received during fights with conspecifics.

The ecological importance of the lamellae of P. nymphula was investigated by examining the frequency of lamellar injury in a wild population of larvae. The frequency of injury was then correlated with environmental factors and population parameters. Those that were found to be significantly correlated with lamellar injury were; larval size, sampling season and habitat type. It was concluded that lamellar injury in wild larvae of this species may be due either to interactions with predators or conspecifics. Further evidence for such a function comes from the study of the breaking joint which attaches the lamellae to the abdomen. This joint was found to be specially adapted to allow autotomy of the lamella with little damage to the larva. These results suggest that the lamellae of P. nymphula are used for defensive autotomy as has been suggested for other species of zygoptera.

Investigation of the respiratory morphology and physiology of the lamellae of P. nymphula confirmed that they do not function as respiratory gills. The lamellae had large surface areas and no tissues with high metabolic oxygen demand such as ion uptake or sensory organs. Both these characteristics suggested a respiratory gill function. However, the arrangement of the tracheoles within the hypodermis is critical for efficient gas exchange by tracheal gills. The arrangement of tracheoles within the lamellae of P. nymphula was not consistent with that required for a tracheal gill suggesting that this is not their main function. The results of the study of lamellar respiratory physiology also support this conclusion. The oxygen consumption rates of larvae with and without

lamellae under varying conditions of hypoxia did not differ significantly, showing that lamellar loss had no effect on larval respiration rates.

In addition, the lack of evidence of any ion uptake organs or concentrations of sensory organs suggest that these potential functions for the lamellae of P. nymphula must be rejected.

The lamellae of P. nymphula were found to be important as signals during aggressive encounters with conspecifics. Larvae with and without lamellae were matched in trials to determine which would gain and maintain occupancy of a perch. Larvae were matched to reduce the possibility of interference from other contests asymmetries such as size, fighting experience and familiarity with the area. In these contests, larvae with lamellae were found to be significantly better at occupying perches. However, lamellar loss did not affect the ability of larvae to initially occupy perches nor their non-lamellar related behaviour. Additional support for an aggressive posturing role for lamellae came from the investigation of their morphology. The lamellae had a large surface area and distinctive pattern both of which would increase their effectiveness as signals.

The present study therefore concluded that the main functions of the lamellae of P. nymphula were as signals or as attack deflectors during aggressive encounters with conspecifics or predators. The loss of lamellae can therefore be considered as a contest asymmetry. The consequences of lamellar loss are likely to be similar to those that result from the loss of contests due to other asymmetries and will lead to exclusion from resources. Such exclusion competition can in turn lead to reduced growth rates and potentially reduced breeding success.

The lamellae of P. nymphula are also likely to be important as a threat display or attack deflector during interactions with predators. However, further study of the long term survival and breeding success of affected larvae is necessary before this conclusion can be confirmed. The lamellae of P. nymphula do not however function as respiratory gills, ion uptake organs or sensory organs. The possibility that they also function as fins for swimming cannot be discounted at present.

These results suggest that in species of zygoptera which use displays involving the lamellae during aggressive intraspecific encounters these organs may also be used as signals or attack deflectors. However, many different forms of lamellae have been described amongst the zygoptera and caution needs to be exercised in generalising from the results presented here to lamellar function in other species. In zygopteran species in which intraspecific aggression does not occur, the lamellae may be adapted for other functions. These other functions would depend on selection pressures such as predation, environmental oxygen concentrations and life history strategies of larvae, but could include that of a respiratory a gill.

CHAPTER 1

1.1 General introduction.

Introduction to the Odonata.

The Odonata comprise one of the most ancient and widespread orders of the class Insecta (Corbet, 1980; Hennig, 1981). With few exceptions their life cycle consists of an aquatic larval stage and an aerial adult stage (Corbet, 1980). Taxonomically the Odonata are divided into two main groups, the Anisoptera and the Zygoptera and one relict group the Anisozygoptera. These groups all probably arose from a common zygopteran-like ancestor (Fraser, 1954; Hennig, 1981).

One conspicuous difference between zygopteran and anisopteran larvae is in their method of gaseous exchange (Corbet, 1962). The Odonata evolved from terrestrial air breathing ancestors and the larvae are therefore secondarily adapted to aquatic life (Clegg, 1986). A consequence of this is that the tracheal system of all odonate larvae is apneustic (gas filled and closed), except in the final instars of some aquatic species and in the semi-terrestrial species. (Corbet, 1960; Mill, 1974). All oxygen must enter these larvae by diffusion across the cuticle. This occurs over the entire body surface and also specialised respiratory gills (Mill, 1972, 1974). In species with tracheal gills, oxygen diffuses into a rich plexus of subcuticular tracheoles (Wichard and Komnick, 1974a).

The tracheal gills of the Anisoptera form an array of specialised plates in the rectal chamber (Tillyard, 1916). These plates form the branchial basket which is ventilated by pumping water into and out of the rectum (Tillyard, 1916; Chapter 3). The anisopteran rectum is multifunctional being an important site of ion uptake as well as a means of propulsion (Komnick, 1982; Kukulies, 1983).

Larval zygopterans have no branchial basket and are therefore generally accepted as having no rectal gas exchange, although some authors suggest that a little may occur at this site (Bodine, 1918; Miller, 1993; Tillyard, 1917a). In families such as the Polythoridae and Euphaeidae, gas exchange is by paired lateral gills on each abdominal segment (Norling, 1982). Whilst in the Amphipterygidae, gas exchange is carried out across the thin cuticle of the finely branching caudal tufts (Watson, 1966).

In the majority of the remaining families of the Zygoptera the laterally flattened caudal lamellae are presumed to be the main site of gas exchange (Chapman, 1980). The structure of these organs varies between species (MacNeill, 1960; Tillyard, 1917a+b). In most they are ferrular and rigid until about instar six, only later developing their familiar blade like shape and distinctive pigmentation (MacNeill, 1960; Tillyard, 1917b). They are connected to the last abdominal segment by a breaking joint from which they can be regenerated. (Child and Young, 1903; MacNeill, 1960; Tillyard, 1917a). The present study is of the laterally flattened lamellae of Pyrrhosoma nymphula which have been described by Corbet (1955), Gardner and MacNeill (1950) and Lucas (1900).

Alternative hypotheses of lamellar function.

Not all authors accept that caudal lamellae are respiratory gills and there has been some debate over other possible roles, which include locomotory (MacNeill, 1960; Robinson, et al., 1991), sensory (Norling, 1982), osmoregulatory (Eriksen, 1986) and behavioural (Johnson, 1991; Peckarsky, 1984; Rowe, 1980). Originally the respiratory function was inferred from observation rather than experiment, and has been difficult to prove. Whilst some authors have claimed to show that the lamellae are the main site of gas exchange (Eriksen, 1986; Harnisch, 1958; Koch, 1934; Pennack and McColl, 1944), others have failed to confirm this function (Patee, 1956; Thorpe, 1933; Zahner, 1960).

However, it is difficult to draw firm conclusions from the results of these studies as they were all limited by available facilities or their design.

A further difficulty with the interpretation of the function of the laterally flattened lamellae as respiratory gills arises when their autotomy is considered. A number of authors have reported that a significant proportion of wild caught zygopteran larvae have autotomised one or more lamellae (Corbet, 1950; Baker and Dixon, 1986; Robinson, et al., 1991). The affected larvae are able to survive this autotomy and regenerate the lamella at the next moult (Child and Young, 1903). Clearly if these organs were essential for gas exchange then their loss would result in the death of the larva and they therefore cannot be considered as the main site of gas exchange.

The lamellae are lost during fights with conspecifics predators (Baker and Dixon, 1986; Robinson et al., 1991) and also during the moult (Corbet, 1950). Lamellar autotomy affects larval distribution within the habitat and increases the risk of predation by conspecifics when larval densities are high (Robinson et al., 1991).

The functions of the laterally flattened lamellae are not clear and cannot easily be determined from the results of prior studies. The aims of the present study are therefore to resolve some of these difficulties in the interpretation of lamellar function. This was done by studying a variety of different aspects of the morphology, physiology and ecological importance of the lamellae of a single species of damselfly. Previous studies have not examined lamellar function using such a broad range of techniques. It was hoped that by using such an approach the function of the lamellae of at least one species of damselfly could be determined unequivocally.

The life cycle of Pyrrhosoma nymphula

The species chosen for this investigation was P. nymphula [Odonata: Zygoptera] (SULZER), the common or large red damselfly. This species is one of Europe's commonest and widespread species (Askew, 1988; Longfield, 1960; Lucas, 1900; Merritt, 1983). It is found in a wide range of habitats from acidic moorland bogs to swift flowing rivers (Askew, 1988; Corbet, 1957; Gardner and MacNeill, 1950; Lawton, 1970a; Longfield, 1960; Lucas, 1900,1930; Macan, 1964;1974). This species was selected for study as it is abundant and many studies have been carried out on its biology and life history.

P. nymphula is a *spring* species, as defined by Corbet (1954). Consequently most adults emerge synchronously at this time. Adults are on the wing from May to August and mating occurs over or near water. The eggs are laid below the surface of the water and in the stem of a plant, commonly Potamogeton sp. (Corbet, 1957, 1962; Gardner and MacNeill, 1950; Lawton 1971a; Macan, 1964,1974). In the U.K. P. nymphula commonly has a semivoltine life cycle, although some authors report life cycles of between one (Corbet, 1957) and three (Macan, 1964, 1974) years. Macan suggested that this variability in the duration of the life cycle was due mainly to food abundance and larval density.

Growth of P. nymphula larvae is synchronised resulting in well-defined age classes or emergence groups in the population. The smaller emergence group is usually referred to as the "junior" group and the larger the "senior" group (Corbet, 1957; Lawton, 1970a; Macan, 1964,1974). Larval growth occurs during the summer. During their first winter, the junior emergence group larvae burrow into the substratum presumably to avoid predation by the large emergence group larvae (Lawton, 1970a; Macan, 1974). By the end of their second summer of growth larvae will have reached the final or

penultimate instar. These larvae then diapause before emergence the following spring. In *spring* species of dragonfly many final instar larvae are found in the population during the winter (Corbet, 1957a; Lawton, 1970a; Macan, 1964,1974). However, some larvae in this "senior" emergence group are unable to reach the final instar before the winter diapause. In the spring, these larvae must grow and moult before emergence. This results in two or more emergence peaks of adults. The number of emergence peaks corresponding to the number of instars that the larvae have had to pass through prior to emergence (Corbet, 1957a, 1962; Lawton, 1970a; Macan, 1964,1974).

Aims of the present study.

The present study, which is aimed at determining the structure and function of the laterally flattened caudal lamellae of P. nymphula by using a wide range of techniques, falls into four main sections. The first was an investigation of the factors affecting lamellar loss in a wild population of larvae (Chapter 2). Previous studies, on other species of Zygoptera, have found that lamellar loss occurs to a significant extent in wild populations (Baker and Dixon, 1986; Robinson et al., 1991). The objective of this section of the present study was to establish the extent to which this occurred in a population of P. nymphula. To help to determine the underlying causes and effects of this loss, data on the general biology of the population and the habitat were also collected.

The second component of this study was to describe in detail the structure of these organs (Chapter 3). There have been no studies of lamellar structure since the general surveys carried out by MacNeill (1960) and Tillyard (1917a), and neither of these was at an ultrastructural level.

The third component of the present study was an investigation of lamellar respiratory physiology (Chapter 4). The protocol and design of previous investigations of this

function have been problematic (Chapter 4). This part of the present study therefore sought to establish unequivocally, using proven experimental techniques and protocol, whether or not lamellae have a role in increasing larval gas exchange under varying conditions of hypoxia and anoxia.

The final section of this study investigates the role of lamellae during intraspecific aggressive encounters (Chapter 5). Previous studies have implicated lamellae either as attack deflectors (Robinson, et al., 1991) or as signalling organs (Johnson, 1991). The aim of this section was to determine whether or not lamellar loss affected the ability of larvae to defend a resource, in this case a refuge, from an otherwise equally matched conspecific.

CHAPTER 2

THE OCCURRENCE AND EFFECTS OF CAUDAL LAMELLAR LOSS IN A POPULATION OF P. NYMPHULA.

2.1 Introduction.

2.1.1 Caudal lamellar loss in wild larval populations.

Lamellar loss

Generally when wild populations of larval zygopterans are studied it is the intact larvae with three fully developed caudal lamellae that are considered. However, a number of authors have reported a significant proportion of larvae in certain wild populations with some or all lamellae missing or regenerated, indicating prior loss or injury to these prominent organs (Baker and Dixon, 1986; Corbet, 1950; Gardner and MacNeill, 1950; Robinson et al., 1991).

The causes and consequences of lamellar loss have not been investigated previously in P. nymphula. The only studies specifically of lamellar loss in a wild population of zygopteran larvae are those by Baker and Dixon (1986) and Robinson et al., (1991). Baker and Dixon (1986) studied the frequency of lamellar injury in populations of Ischnura verticalis (Say) and Enallagma ebrium. larvae and found that it was related to the number of aggressive interactions between larvae, had a seasonal trend and was correlated both with larval size and the type of vegetation on which the larvae lived. They found that frequency of lamellar injury was highest in spring, when up to 40% of larvae collected had missing or regenerated lamellae. Robinson et al., (1991) found that 50.1% of Ischnura posita larvae they collected in the wild had at least one

lamella missing or regenerating and that in experimentally maintained populations the frequency of lamellar loss was density dependent. Although lamellae are clearly important during aggressive encounters between larvae, the studies by Baker and Dixon (1986) and Robinson et al., (1991) remain the only one to address what effects lamellar loss has on larvae in the wild. It is therefore important to determine whether these results can be verified in populations of other zygopteran species.

It is proposed here to determine the extent to which lamellar injury occurs in a wild population of P. nymphula larvae. Because there have been no previous studies of lamellar injury in P. nymphula this study will investigate firstly whether such injury occurs and secondly, how the pattern of lamellar injury relates to life cycle and habitat. The variables investigated are those that are known to be related to lamellar loss in other species (Baker and Dixon, 1986) and those that are predicted to be related to lamellar loss here. The three most likely sources of lamellar loss are 1) loss during an aggressive encounter with a conspecific (Baker and Dixon, 1986; Robinson et al., 1991), 2) loss to a predator (Robinson, 1990; Robinson et al., 1991), and 3) loss during moulting (Corbet, 1950; Robinson et al., 1991). The habitat variables investigated included basic water quality including temperature and oxygen levels. Although not directly relevant to this part of the present study, habitat oxygen levels were considered for their possible role in controlling the distribution of larvae. The effects of environmental oxygen gradients on larval distribution are discussed elsewhere (Chapter 4).

2.1.2. Loss during aggressive encounters.

Loss of lamellae during conflicts.

The larvae of some zygopteran species (including P. nymphula) are known to be aggressive in the presence of conspecifics (Chapter 5). Aggressive contests between larvae have been likened to "wars of attrition" since larvae may spend long periods apparently attempting to outlast opponents (Johnson, 1991).

It is during these aggressive encounters that the lamellae may be used either as threat displays or as attack deflectors (Baker, 1983; Johnson, 1991; Peckarsky, 1984; Robinson et al., 1991; Rowe, 1980). Numerous authors have described larval fights of a number of zygopteran species. In these, larvae fan and strike lamellae at antagonists (Baker, 1983; Harvey, 1985; Harvey and Corbet, 1986; Johnson, 1991; Robinson et al., 1991; Rowe, 1980). Frequently larvae will also strike at each other with their jaws, which can result in the loss or injury to lamellae and legs (Baker and Dixon, 1986; Baker, 1983; Harvey and Corbet, 1986; Robinson et al., 1991). Baker and Dixon (1986) showed that when pairs of I. verticalis or E. ebrium larvae were placed together the frequency of injury and lamellar loss was significantly correlated with the number of aggressive encounters between these larvae. Robinson et al., (1991) also found that lamellar removal was correlated with density dependent aggression in Ischnura posita larvae. In the field, Baker and Dixon (1986) found a significant correlation between lamellar injury and larval dispersal rates, season and larval size but not with larval density. They suggested that the frequency of injury to lamellae may be a useful index of the rate of aggressive interactions between larvae in wild zygopteran populations.

Baker and Dixon (1986) suggested that one reason they were unable to detect a correlation between density and the frequency of lamellar injury may be due to the complexity of the habitat. The lower the larval density or the more complex the habitat the fewer the number of encounters (Baker and Dixon, 1986; McPeek and Crowley, 1987). For example, Pierce et al., (1985) showed that as the density of Enallagma aspersum larvae increased then the rate of interactions between larvae increased. It is therefore important to consider both habitat type and complexity in relation to rates of lamellar loss and larval density.

Baker and Dixon (1986) also suggested that larval body size influences the frequency of lamellar loss because of its importance in determining the outcome of larval

aggressive encounters. Larval body size is one of the most important factors in determining the outcome of intraspecific conflicts (Johnson, 1991). During contests between larvae of greater than two instars difference, the smaller larvae will generally retreat without engaging the larger larva in a fight (Johnson, 1991; Harvey and Corbet, 1986). Though, Baker and Dixon (1986) showed that smaller larvae were more likely to sustain lamellar injury and they suggested that this was because small larvae lost conflicts.

P. nymphula has a semivoltine life cycle and well defined emergence groups with growth synchrony (Chapter 1). The size range of larvae within these cohorts can be small. If the frequency of lamellar injury is the result of injury during fighting and since fights are more likely to escalate when the size of opponents is similar (Chapter 5), then lamellar loss should be related to the size range of larval cohorts in P. nymphula. Possible lamellar loss due to attacks by larger conspecifics from older emergence groups will be considered under predation effects (2.1.3.).

Two further factors that may influence the frequency of lamellar loss are larval gender and the growth stage at which lamellae may become important as defence/threat organs. It has been suggested that in some species, changes in the shape and pattern of the lamellae are related to the onset of a behavioural function (Corbet, 1990; Johnson, 1991). For example, Corbet (1990) suggests that the change in shape of coenagrion sp. lamellae from lanceolate to lamellate may indicate a change in the behavioural function of these organs. Johnson (1991) suggests that the development of the pattern on the lamellae of Xanthocnemis zealandica larvae, described by Rowe (1985), is synchronised with the development of the ability of the eyes of larvae to see such displays which may indicate a behavioural function for these organs. Determining the developmental stage at which the frequency of lamellar injury becomes significant may help to explain the function of these organs.

Gender is thought to have little or no effect on the outcome of aggressive interactions between larvae or frequency of lamellar injury. However, Baker (1990) describes preliminary results of experiments with I. verticalis larvae which indicate differences in behaviour between male and female larvae. It is therefore possible that rates of lamellar injury may differ in the two sexes. Harvey and Corbet (1986) showed that short term breeding success of adult male P. nymphula larvae was related to adult and larval size, at least in the final larval instar; though large larval size may not be related to fecundity in female zygopteran larvae (Anholt, 1990; Corbet, 1990). Thus if size is more important for fitness for one sex then it is possible that the outcome of contests and frequencies of lamellar injury between male and female larvae may differ.

2.1.3. Loss to predators.

Causes of loss.

In addition to lamellar loss during intraspecific conflicts, lamellae can also be lost or injured when larvae are attacked by predators (Baker and Dixon, 1986). Again, the lamellae may be used as a threat display (Corbet, 1962) or for deflecting predatory attacks (Johnson, 1991; Johnson, et al., 1989; Robinson et al., 1991). Baker (1983) suggests that displays such as caudal striking may be a generalised threat response to similar or larger sized animals. Attacks by larger instars on smaller conspecifics are also included here as potential predation since larger larvae often consume smaller ones. A number of authors have reported a high incidence of cannibalism in some zygopteran species (Fischer, 1961; Johnson et al., 1985; Merril and Johnson, 1985; Robinson et al., 1991; Wissinger, 1988) which might help to regulate population density in some cases (Johnson, 1991; Van Buskirk, 1989).

Factors affecting the rate of loss

In larval dragonflies, rates of intra and interspecific predation depend on factors

such as density, substratum complexity, age, activity patterns and hunger levels (Johnson, 1991). Larvae can also detect different types of predator and modify their behaviour accordingly (Convey, 1988; Dione, et al.,, 1990; Dixon and Baker, 1986; Jeffries, 1990; Heads, 1985; McPeek, 1990; Pierce et al.,, 1985; Thomson, 1987; Wellborn and Robinson, 1987). The relationships between predator and prey both in zygopteran populations and in fresh water ecosystems as a whole are complex and not fully understood (Kerfoot and Sih, 1987). Consequently, it is considered beyond the scope of the present study to include a direct investigation of predation effects on the rate of lamellar loss in the study population. Instead, the types of predators found in the study site were identified, so that their potential as sources of lamellar loss could be estimated. If predation is important in the present context then the frequency of lamellar injury should be highest where potential predators are most abundant.

Adaptive significance of lamellar autotomy

If lamellae are used to deter or deflect predatory attacks then it is possible this is their main function. Caudal autotomy is a well known phenomenon in many animal groups and is defined as "the specialised loss of an organ in a predetermined way" (Arnold, 1988; Bellairs and Bryant, 1985). Autotomy as a defence against predation (including that from conspecifics) is found in many species including lizards (Cooper and Vitt, 1991; Vitt et al., 1977), stoneflies (Peckarsky, 1984) and crayfish (Robinson et al., 1970). In studies on lizards it has been found that the tail is lost in a predetermined way at a fracture point when it is seized by the predator or even before capture. The efficiency of these organs can be increased by their specialised patterns and colouration, movement or both (Cooper and Vitt, 1991). It is generally accepted that the large proportion of individuals surviving in a population with missing or regenerated tails reflects the effectiveness of caudal autotomy as a predator defence strategy (Arnold, 1984, 1989; Bellairs and Bryant, 1985).

Little investigation has been carried out on the use of caudal autotomy as a predator evasion strategy in the Zygoptera (Johnson, 1991(b); Johnson et al., 1989; Robinson, 1990). MacNeill (1960) for example, suggested that zygopteran caudal lamellae would not function well in this way as he felt that predators "would tend to eat the entire larva" (See also Baker and Dixon, 1986) and may even be "attracted by the increased area presented by the lamellae". Ironically this observation by MacNeill accurately describes the characteristics of an autotomous defence organ, that is to make the animal more prone to detection, whilst simultaneously increasing the probability of survival once detected. The costs of detection are outweighed by the benefits of increased probability of escape when attacked (Cooper and Vitt, 1991).

Antipredator defences can be predator-specific (Kerfoot and Sih, 1987). Thus the effectiveness of the lamellae as attack deflectors may depend on the type of predator. For example, Robinson (1990) described the results of experiments on the survival rate of E. civile larvae with and without lamellae in the presence of an actively foraging predator (Anax sp.) and a sit-and-wait predator (Ranatra fusca). These experiments showed that in the presence of a sit-and-wait predator there was an equal probability of capture for larvae with or without lamellae. In the presence of an actively foraging predator those with lamellae survived better. Robinson (1990) suggested that this was probably due to a more effective swimming escape response by larvae with lamellae. However, Convey (1988) found that swimming increased the vulnerability of attack on Coenagrion puella (Linn.) larvae by the predator Gasterosteus aculeatus, the stickleback. Convey suggests that swimming is the best escape from one type of predator (such as Anax sp.), but may increase the vulnerability to attack by other types of predators.

2.1.4. Loss during moulting.

Lamellae can be lost during moulting. Corbet (1950) for example, describes how larvae of P. nymphula kept in isolation autotomised their lamellae during the moult,

presumably because they were too large to pass through the constriction of the breaking joint (Chapter 3.). Although Robinson et al., (1991) reported that loss during moulting in Ischnura posita larvae was infrequent. If the frequency of lamellar injury is correlated with larval growth (and moulting) rates, then loss during the moult may explain any lamellar loss observed.

2.1.5. Predicted effects of lamellar loss on wild larvae.

Lamellar loss occurs with significant frequency in a wild population of at least one species of zygopteran larvae (Baker and Dixon, 1986; Robinson et al., 1991). If the lamellae have no vital function and their loss is simply due to a factor such as moulting then the effects on larvae will be minimal. In this case the frequency of lamellar loss should reflect larval growth rates. However, if the lamellae have a defensive, autotomous function then the loss of such prominent organs must have consequences for the survival and success of the affected larva. These consequences will depend on how the lamellae function and may be predictable.

Baker and Dixon (1986) showed that small larvae of Ischnura verticalis were more susceptible to lamellar injury because they were more likely to loose conflicts. Thus smaller P. nymphula larvae of the same or younger emergence group cohorts may show a higher frequency of lamellar injury. It is possible that there are further consequences of such lamellar loss including increased predation on larvae and reduced access to feeding sites (Johnson, 1991). If this is the case then larvae without lamellae may be limited in their distribution to poorer quality habitats. Dragonfly larvae are known to be able to detect different types of predators and to alter their use of the microhabitat, reducing predation risk (Dione et al., 1990; Dixon and Baker, 1986(b); McPeek, 1990; Wellbourne and Robinson, 1987). Robinson et al., (1991), for example, found that lamellar loss affected microhabitat distribution of Ischnura posita larvae.

If the lamellae are lost only during attacks by predators then the frequency of injury should be directly related to rates of predation. In the case of interspecific predation, lamellar loss should be related to the abundance and foraging strategies of potential predators. If lamellar injury is related to intraspecific predation then the frequency of loss should be highest when larvae of widely differing sizes are present in the same habitat (Johnson, 1991).

2.1.6. Aims.

The aims of the present study are as follows:-

1) To determine whether the finding of Baker and Dixon (1986) that lamellar loss occurs in a significant proportion of larvae in wild populations of I. verticalis and E. ebrium also applies to wild populations of P. nymphula.

2) To determine whether the frequency of lamellar loss was correlated with the variables described above (2.1.5.). The importance of gender in relation to lamellar loss was also investigated. Any correlations that were observed may be used to distinguish between possible functions of the lamellae.

3) Additionally, the seasonal fluctuations in basic parameters of water quality including pH, total ion content of the water (Conductivity), dissolved oxygen and temperature were be monitored. These data will be interpreted in terms of other possible lamellar functions.

2.2 Materials and methods

2.2.1. The study site

Site description

The site selected for study was the Ross Burn, near Rowardennan, Stirlingshire, Scotland (NGR NS 373 967) (Plate 2.1, Fig. 2.1). The burn flows North from a small mesotrophic lochan (Lochan Dubh) into Loch Lomond. The sample site was a pool approximately 3-4m wide, 0.5-1.5 metres deep and 25 metres long (Fig. 2.1). The flow in the pool is slow during the summer but fast during the winter months. The surrounding area is rough grazing to the east and deciduous woodland to the west. The banks of the pool are nearly vertical and undercut in places. The bottom is composed of silt, mud and deciduous leaf litter.

Site selection.

The study site was considered suitable for the following four reasons. 1) Preliminary sampling indicated it supported a significant population of larval P. nymphula and no other species of Zygoptera. There would therefore be no confusion in larval identification nor the possibility of interspecific competition. 2) Although small the site could be sampled easily at any depth from the bank. 3) Three different types of habitat were clearly delineated making collection of larvae from a particular habitat type possible 4) The site was never known to freeze or dry up because it was part of the outflow of a deep lochan and was therefore considered a reasonably stable habitat which could be sampled all year round.

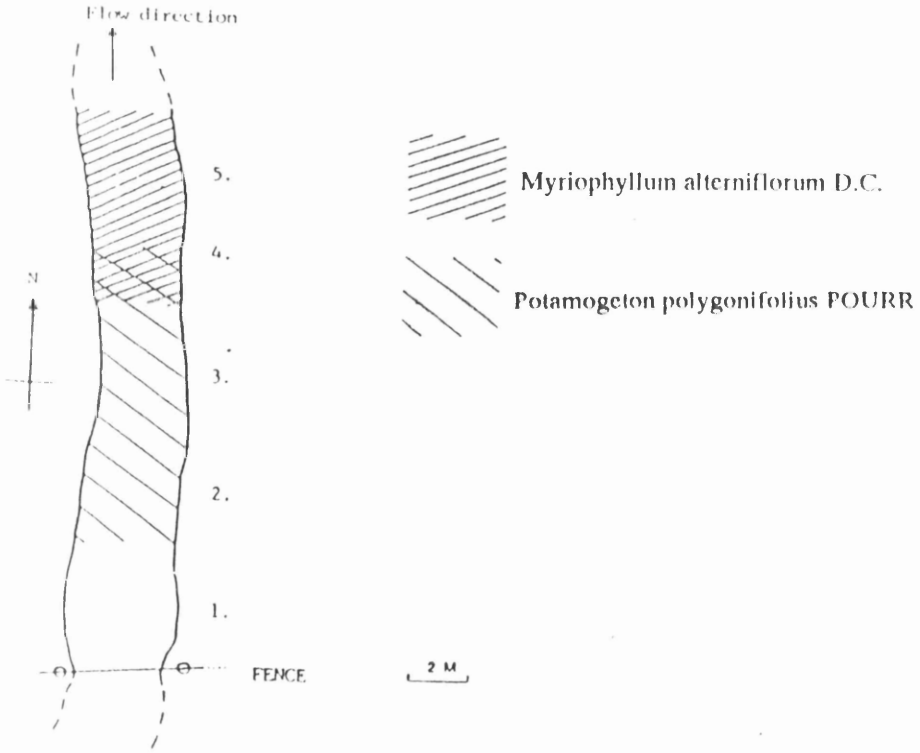
Sampling stations

Samples were collected from the bank, at five sampling stations (Fig. 2.1). The stations were selected to represent the main habitat types present. Station one, at the

Plate 2.1 The sampling site, Ross Burn (NGR:NS 373 967),Stirlingshire looking South.

Fig. 2.1 Plan of the sampling site, showing sampling stations 1, 2, 3, 4 and 5. Direction of flow and approximate extent of weed species are indicated.

Fig. 2.1


Flow direction
N
5.
4.
3.
2.
1.
FENCE
2 M
Myriophyllum alterniflorum D.C.
Potamogeton polygonifolius POURR

southern end of the site, consisted of sparse Potamogeton polygonifolius POURR with silt and leaf litter substrate. This station was subject to scouring by water flowing into the pool. Stations 2 and 3 consisted of denser P. polygonifolius with leaf litter and mud substrate. Stations 4 and 5 were dominated by P. polygonifolius and Myriophillum alterniflorum D.C. Site 3 was dominated by P. polygonifolius and 4 dominated by M. alterniflorum, both had a substrate of leaf litter and mud. Station 5 at the northern most end of the site consisted entirely of dense M. alterniflorum with mud and leaf litter substrate. Water flow was fastest at station 1 and slowed progressively to station 5. The pool finishes below station 5.

Environmental monitoring

Four indices of water quality were used to provide background information on the study site: dissolved O_2, pH, water temperature and conductivity. These were monitored monthly during both sampling periods (1984/85 and 1987/88). Spot measurements of dissolved O_2 and water temperature were taken on site using a "pHO_x" portable dissolved O_2-temperature meter. Readings were taken at station 3 at a depth of 10 cm below the water surface. Water samples were analysed for pH and conductivity. A Whatman pHA pH meter with Camlab injection flow reference half cell and Russel pH electrode was used for pH measurement and an "AGB" 70 conductivity meter to measure conductivity.

To determine the availability of oxygen in the microhabitat levels were measured on two occasions in core samples of water column and sediment using a Jenkins corer (Clegg, 1974). Using this method, undisturbed cores can be removed from the site for analysis. The technique requires removal of some substrate and for this reason sampling was kept to a minimum. For comparison, samples were collected when oxygen concentrations were likely to be at their most extreme. These were during the summer, when low flow and high water temperatures result in low O_2 concentrations and during

the autumn/winter when high flow and low water temperatures result in high dissolved O_2 concentrations (Jeffries and Mills, 1990). Six cores were collected on two occasions, on 20/6/88 and 4/10/88. Cores were collected from different areas of the pool working upstream to avoid disturbance from previous sampling. The cores were removed to an incubator, held at collection temperature, and O_2 levels were determined within 15-30 minutes of collection. Water samples were removed from measured levels in the water column using a glass syringe and immediately analysed at collection temperature using a "Strath Kelvin" polarographic oxygen sensor (P.O.S.) and meter. All glassware and equipment were maintained at the collection temperature of the sample in a constant temperature room to prevent temperature interference during oxygen determination.

2.2.2 Monitoring Of Larvae

Collection of larvae

Larvae were collected during two sampling programmes. The first consisted of three collections during the winter of 1984/85. The second involved monthly collection of larvae from October 1987 to November 1988 (to determine how lamellar loss relates to seasonal changes in size, sex and distribution of larvae). During this sampling period the sex of larvae and the presence of lamellar injury was also recorded. A larva with lamellar injury is defined here as having any lamellae missing, regenerating injured. This was done because of the relatively small numbers of larvae with all, one or two lamellae damaged. Larval gender was determined according to descriptions by Gardner and MacNeill (1950).

On both sampling occasions larvae were collected using a pond net of 25 cm^2 with a 1 mm mesh size. The net was used in a standard fashion to provide standard net sweeps (S.N.S.). These consisted of three sweeps approximately 1m in length parallel to the shore at each sampling station. The number of larvae in the sample divided by three gave the number of larvae per S.N.S. (Lawton, 1970a). Although this technique may not

provide a true estimate of actual larval density in the microhabitat, if used in a standard way it provides a relative measure of density for comparison between habitats and across seasons. Other techniques such as grab or core sampling were not considered suitable for this site. To obtain good quantitative results using such techniques and at the densities of larvae found would have required removing large amounts of substrate on each sampling visit. Recovery of the site would not have been possible between sampling days. In addition, during periods of high flow these methods would not be effective as material could be washed out during collection.

Larvae were sorted and identified on site. Larvae from each sub-sample were kept together and removed to the laboratory for confirmation of identification and measurement. Loss of lamellae during collection and prior to monitoring was a potential source of error as larvae kept at high densities may fight and remove lamellae from opponents (Baker and Dixon, 1986). In the present study this error was not considered to be a problem for two reasons. Firstly, the densities of larvae in the collection vessels were so high that larvae did not exhibit aggressive behaviour; instead, larvae formed dense clumps by clinging onto each other. Reduction in aggression at very high densities of larvae has been reported elsewhere (Baker, 1990). Secondly, no loose lamellae were observed in the sample containers after larval monitoring, suggesting that the collection method did not contribute towards lamellar loss. Following sorting and monitoring, and within four days, larvae were returned to their collection site.

Larval size

Larval size was measured using dimensions of the body rather than weight. A number of dimensions have been used by previous workers to assess larval size. The most accurate measurement of larval size can be determined from the different dimensions. The dimensions of the larvae assessed here were total body length, head width behind the eyes and head length (Fig. 2.2). In addition during the 1984/1985

Fig. 2.2 Diagram of a final (12 th) instar larva of P. nymphula Sulzer showing the dimensions measured during sampling programmes, a) head width behind eyes, b) head width across eyes, c) total body length, d) metathoracic wing pad length and e) head length (X 8).

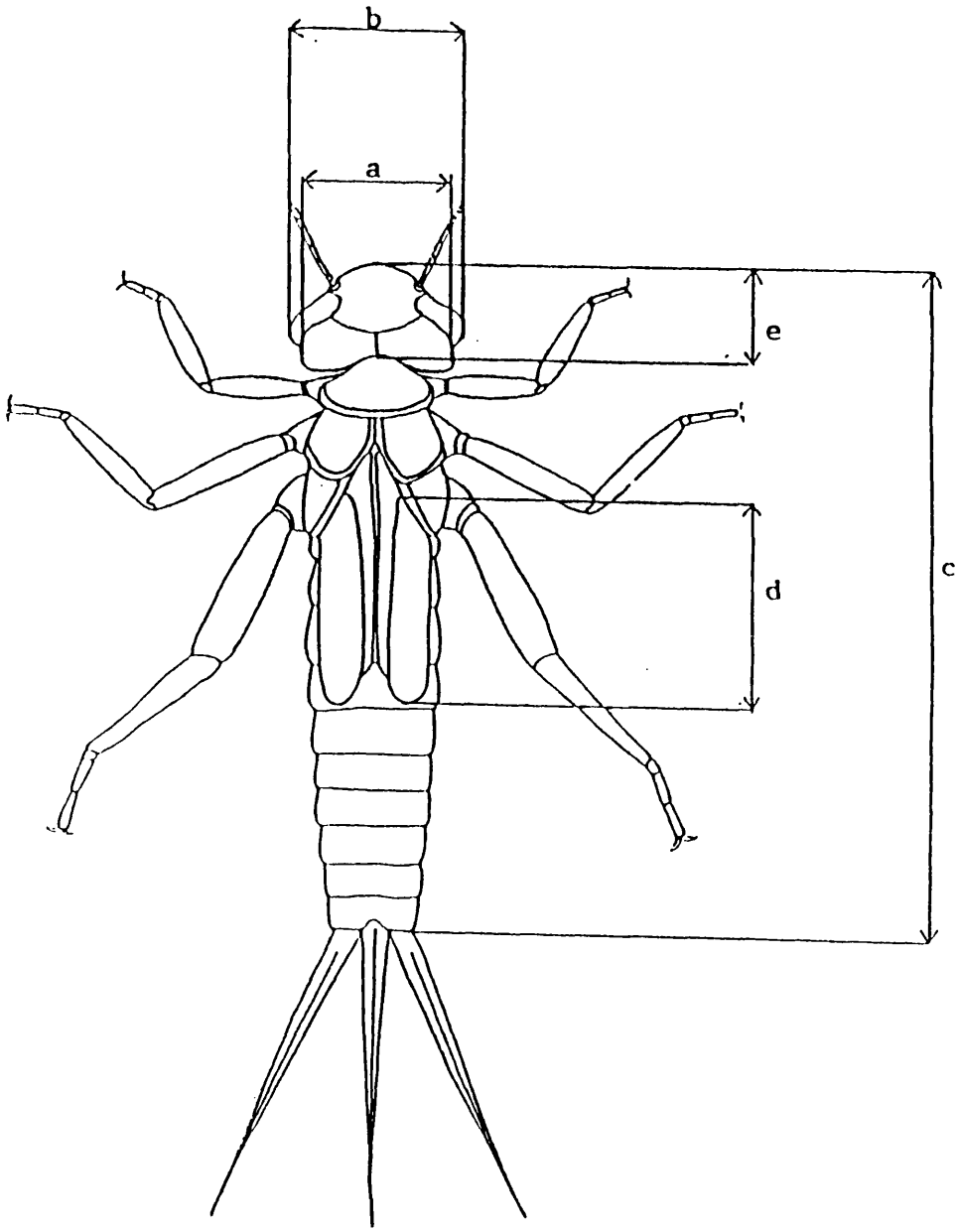


Fig 2.2

Fig 2.3 Diagram of a typical "junior" emergence group larva from samples collected during winter 1984/1985. Compare with Fig. 2.9 to show relative changes in proportion of organs (Approximately X 18).

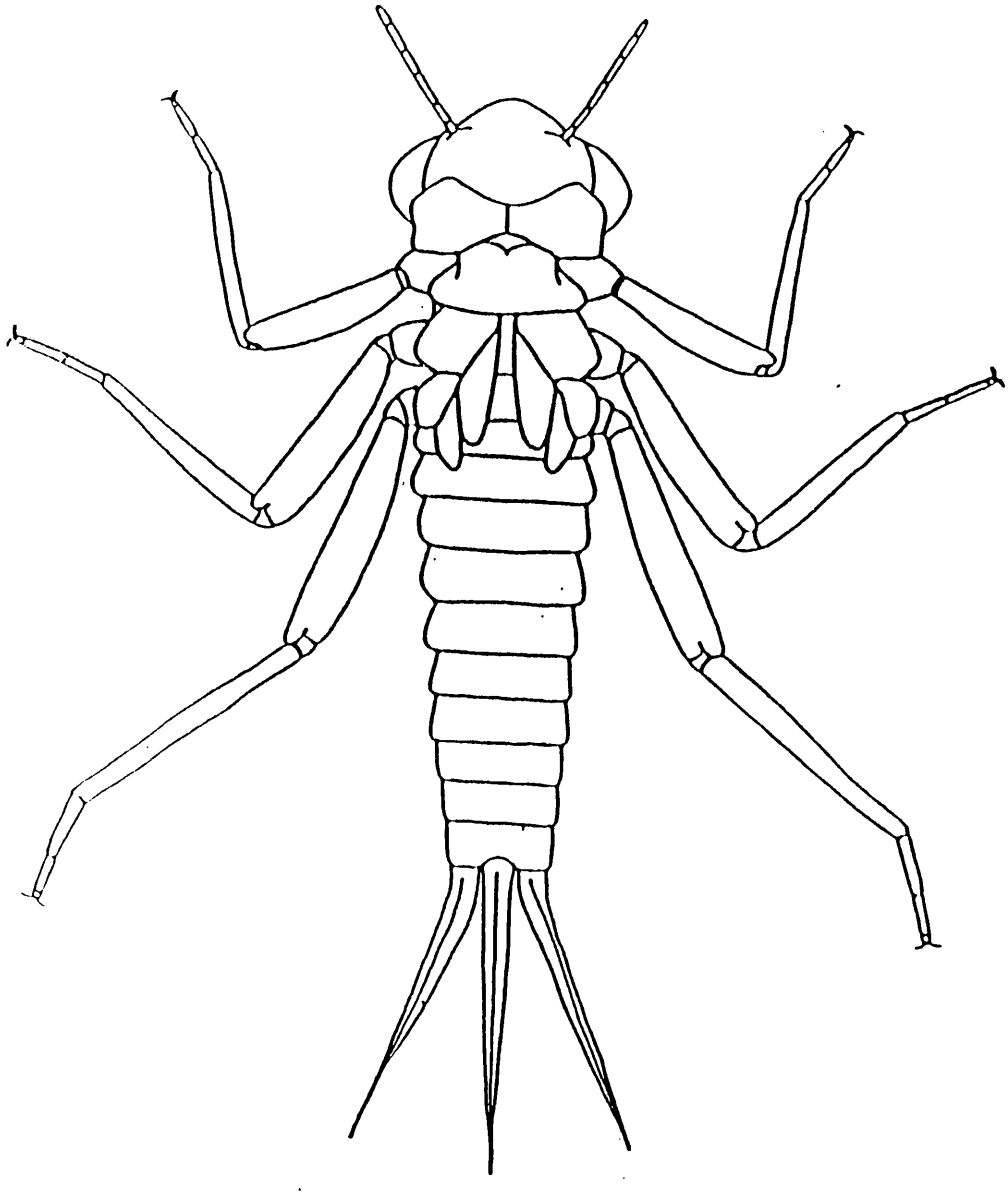


Fig 2.3

sampling, larval head width across the eyes and metathoracic wing pad lengths were also measured (Fig. 2.2). The variability in the relative proportions of these dimensions between large and small larvae can be seen by comparison of Fig. 2.2 and 2.3.

2.2.3.Statistical analyses

Analyses of the data were carried out using "Minitab" or "S.P.S.S." statistical packages on an I.B.M. P.C computer. Regression equations were calculated according to the least squares method (Sokal and Rohlf, 1969). Chisquare, and analysis of variance were carried out according to the methods of Sokal and Rohlf (1969) and Kruskal Wallis one-way analysis of variance according to Seigel and Castellan (1990).

2.3 Results.

2.3.1. The life cycle and growth of the study population.

The life cycle: 1984/1985 sampling.

The data on larval dimensions collected during the sampling programme of 1984/1985 are shown in Table 2.1. A typical senior emergence group larva from this sampling programme is shown in Fig. 2.2, and a smaller junior emergence group larva in Fig. 2.3. The year class cohorts were identified and separated into larger (senior) and smaller (junior) emergence group cohorts by eye using size frequency histograms. The mean total length of the larger, senior emergence group larvae did not increase significantly during sampling. The mean total length of the smaller, junior emergence group larvae increased from 3.08mm to 4.47mm (Table 2.1). Mean head width at eyes, behind the eyes, head length and metathoracic wing pad length of the two cohorts, changed only slightly between the two sampling dates (Table 2.1).

Head dimensions increase linearly with total size during development. Fig. 2.4 shows total length against head width at the eyes; head width increases linearly with increasing total length. However, there is a group of larvae (indicated in Fig. 2.4) with total length greater than 10mm and a head width across the eyes of between 3.25mm and 4.00 mm, which fall below the regression line. The head width of these animals is larger than would be expected for their size (Table 2.2, Fig. 2.4). These larvae may be undergoing metamorphosis during which the head increases in size relative to the body. Previous workers have described changes in the colouration but not the size of the head at this time (Corbet and Prosser, 1986; Harvey, 1985). The regression equations for all measured dimensions against total length are shown in Table 2.2. There is a strong correlation between the head dimensions measured and total length (Table 2.2).

Table 2.1 Summary of seasonal variation in the dimensions of P. nymphula larvae collected during 1984/1985 sampling. Lengths in mm. (S= senior, J= Junior emergence groups).

Sample date	Size group	N	Head length x	Head length S.E.	Head width x	Head width S.E.	Eyes width x	Eyes width S.E	Total length x	Total length S.E.	Mwp length x	Mwp length S.E
Oct 1984	>8mm S	30	1.75	0.025	3.05	0.085	3.41	0.076	11.52	0.238	5.04	0.199
	<8mm J	42	0.64	0.049	1.08	0.083	0.92	0.101	3.08	0.225	0.36	0.151
Nov 1984	>8mm S	9	1.66	0.069	2.85	0.057	3.45	0.161	12.33	0.408	5.13	0.348
	<8mm J	33	0.77	0.039	1.14	0.066	1.43	0.085	4.53	0.292	0.47	0.067
Feb 1985	>8mm S	31	1.64	0.034	2.75	0.061	3.42	0.084	11.52	0.253	4.74	0.255
	<8mm J	14	0.74	0.066	1.08	0.117	1.36	0.143	4.47	0.576	0.43	0.123

Mwp = mesothoracic wing pad length.
Eyes width = Head width across eyes.
Head width = Head width behind eyes.

Table 2.2 Correlation and regression between total larval length, head dimensions and mesothoracic wing pad length for larvae collected during 1984/1985 sampling.

Regression equation Total length =	R	Rsq	P <
1.18 + 3.35 X Head width	0.861	74.2%	0.001
0.11 + 3.49 X Eye width	0.900	81.1%	0.001
3.63 + 1.62 X Mpl	0.903	81.6%	0.001
0.38 + 7.01 X Head length	0.881	77.6%	0.001

Mpl = Length of mesothoracic wing pads.

Fig 2.4 Total length plotted against head width at eyes for P. nymphula larvae from 1984/1985 sample. Intermediate sized larvae are indicated (α) and metamorphosing larvae (β). Equation for regression line is shown in Table 2.2 ($r = 0.861$ $P<0.001$).

Fig 2.5 Total length plotted against metathoracic wing pad length for P. nymphula larvae collected during the winter of 1984/1985. Intermediate sized larvae are indicated (α) and metamorphosing larvae (β). Regression and correlation shown in Table 2.4. The wing pads are not present in larvae less than 3.1mm total length and increase in size disproportionately in comparison to head width (Fig 2.4).

Fig 2.4

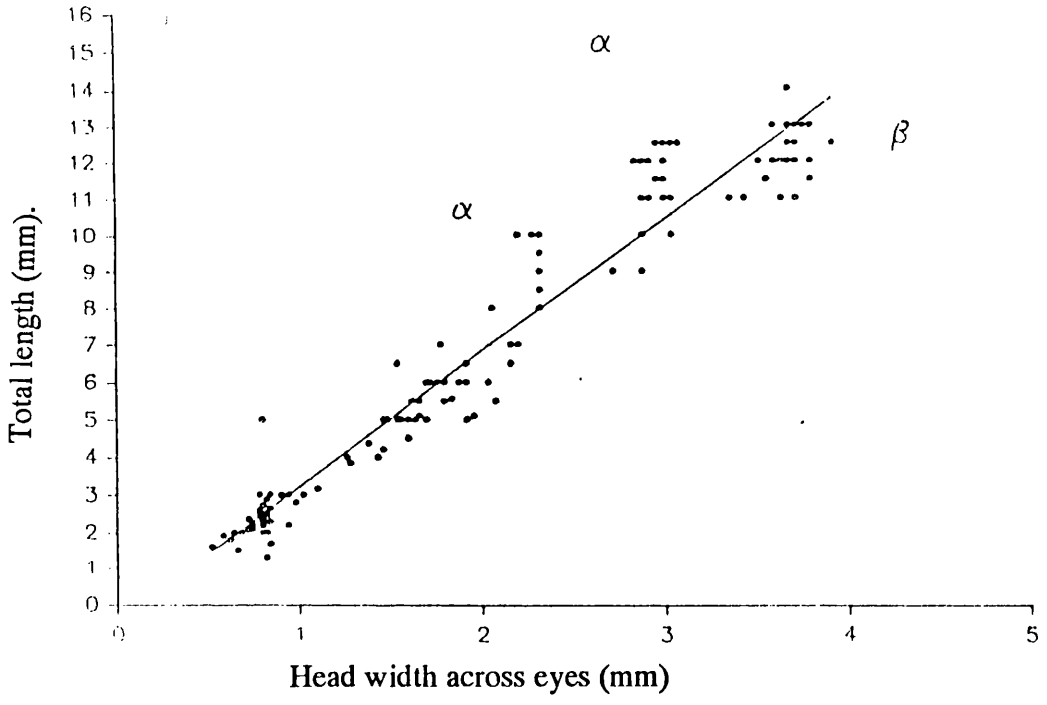

α
α
β
Total length (mm).
Head width across eyes (mm)

Fig 2.5

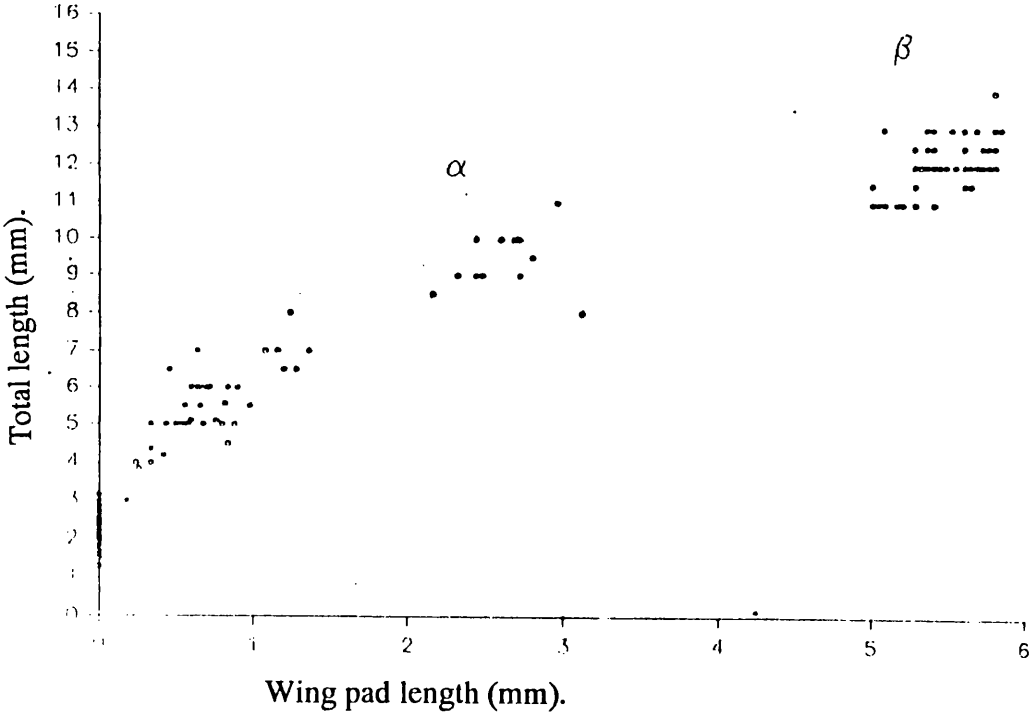

β
α
Total length (mm).
Wing pad length (mm).

Table 2.3 Summary of seasonal variation in dimensions of larvae collected during 1987/1988 sampling. Lengths in mm (S= senior, J= junior emergence groups).

Sample date	Size group		N	Head length x	Head length S.E.	Head width x	Head width S.E.	Total length x	Total length S.E.
10/11/87	>8mm	S	52	2.02	0.029	2.91	0.019	11.63	0.091
	<8mm	J	17	1.03	0.053	1.35	0.063	5.47	0.269
16/12/87	>8mm	S	15	1.64	0.057	2.93	0.022	12.25	0.176
11/ 1/88	>8mm	S	27	1.95	0.026	2.96	0.029	12.23	0.014
	<8mm	J	2	0.86	-	1.04	-	4.34	-
10/ 2/88	>8mm	S	29	1.89	0.029	2.85	0.028	11.78	0.149
	<8mm	J	1	1.28	-	1.52	-	5.80	-
18/ 3/88	>8mm	S	9	2.02	0.093	2.88	0.028	11.82	0.309
2/ 5/88	>8mm	S	19	1.86	0.045	2.84	0.043	12.47	0.184
	<8mm	J	37	0.95	0.043	1.30	0.061	4.95	0.194
13/ 6/88	1-9mm	S	140	0.92	0.019	1.27	0.027	5.09	0.118
2/ 8/88	3-10mm	S	165	1.34	0.017	1.93	0.028	7.72	0.109
12/ 9/88	>7mm	S	34	1.58	0.028	2.33	0.032	9.95	0.166
	<7mm	J	14	0.73	0.074	0.92	0.106	3.57	0.390
24/10/88	>8mm	S	10	1.98	0.038	2.94	0.032	11.83	0.159
	<8mm	J	14	0.61	0.254	0.75	0.032	2.78	0.126
29/11/88	>8mm	S	23	1.88	0.023	2.89	0.024	11.80	0.134
	5-8mm	I	2	1.34	-	1.54	-	6.30	-
	<5mm	J	15	0.52	0.016	0.67	0.022	2.54	0.032

Table 2.4 Correlation and regression between total length and head dimensions of larvae collected during 1988/1987 sampling (mm).

Regression equation Total length =	Rsq	P <
-0.570 + 6.31 X Head length	89.5%	0.001
-0.180 + 4.15 X Head width	97.0%	0.001

Fig 2.6 Size frequency histograms for total length of larvae collected in 1987/1988. Two distinct cohorts are evident throughout most of the year suggesting semivoltine development. Intermediate sized larvae that take one or three years to mature are indicted (a). Hatching occurs in July and emergence in May.

Fig 2.6

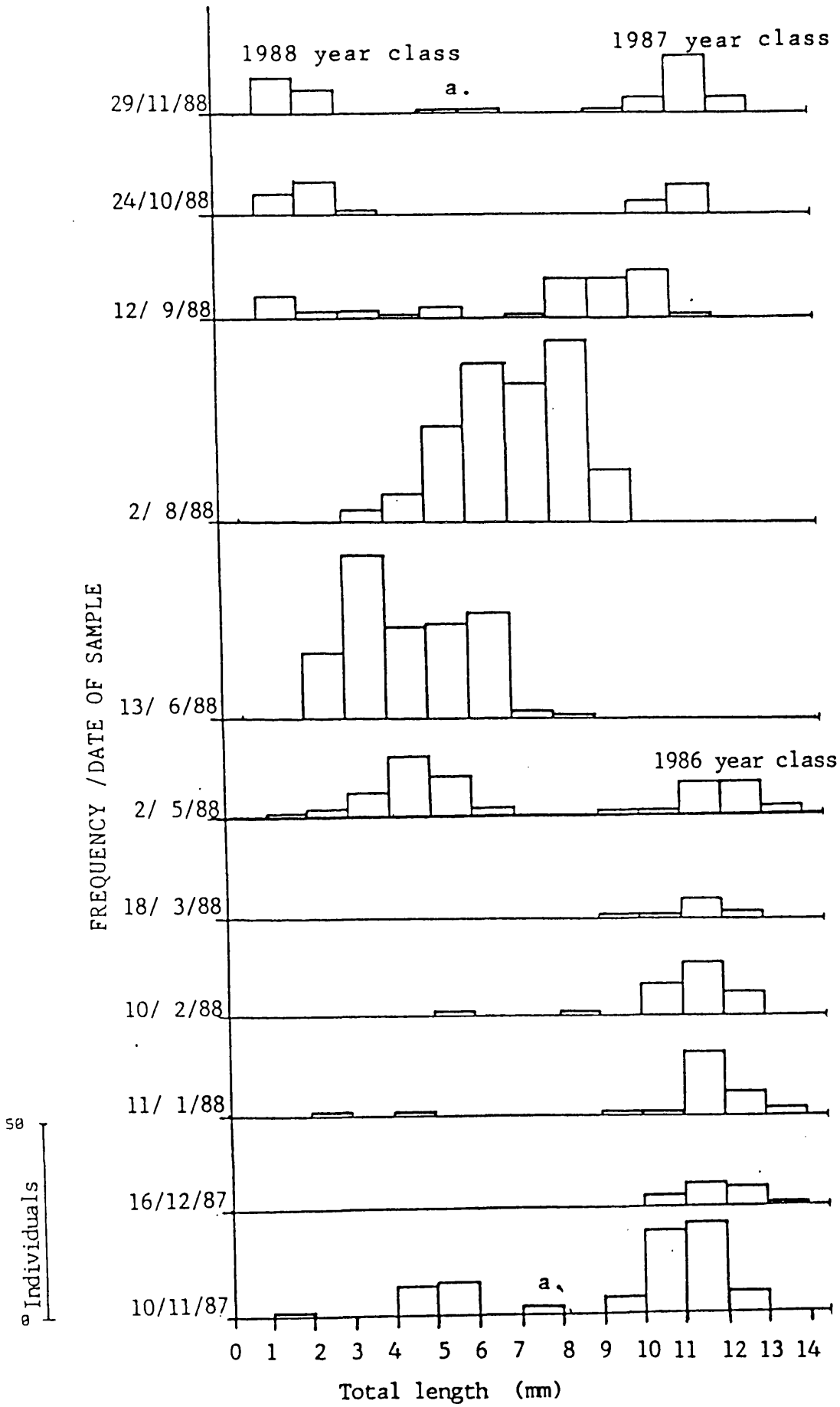
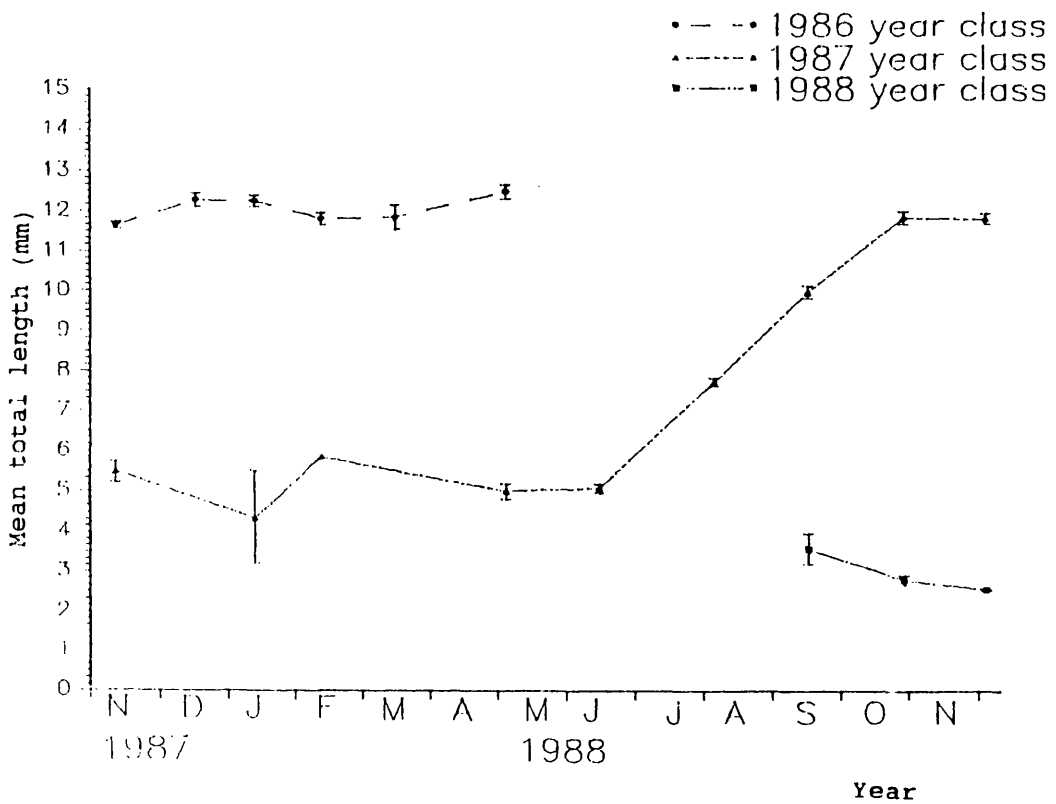

1988 year class
1987 year class
a.
29/11/88
24/10/88
12/ 9/88
2/ 8/88
13/ 6/88
1986 year class
2/ 5/88
18/ 3/88
10/ 2/88
11/ 1/88
16/12/87
10/11/87
a.
FREQUENCY /DATE OF SAMPLE
50
0
Individuals
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Total length (mm)

Fig 2.7 Seasonal variation in cohort size. Mean total length of P. nymphula larvae collected in 1987/1988 (+/- S.E.). The most rapid growth occurred from June to September, 1988 in the 1987 emergence group cohort.

Fig 2.7


1986 year class
1987 year class
1988 year class
Mean total length (mm)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
N
D
J
F
M
A
M
J
J
A
S
O
N
1987
1988
Year

Metathoracic wing pads did not appear until larvae were greater than 3mm total length (Fig. 2.5). Individuals of greater than 10 mm total length had disproportionately long metathoracic wing pads. As with the head width, these measurements fell below the regression line and can be explained by larvae entering metamorphosis. In this species, the wing pads undergo a disproportionate expansion in size in the final instar (Corbet and Prosser, 1986; Harvey, 1985).

Because of the correlation between the measured dimensions and total length (Table 2.2), the latter was assumed to be a sufficiently accurate indicator of larval size for subsequent analyses. Head dimensions are perhaps less reliable as a measure of total larval size, due to the disproportionate increase in size in the final instars. Similarly metathoracic wing pad length is not a reliable measurement of total larval size not only because of their disproportionate increase in size in the final instars, but also because they are absent from larvae of less than 3mm total length.

The presence of two emergence groups in the sampled population shows that development of most larvae at the sampling site was synchronised and semivoltine. The life cycle of larvae in the study population was similar to that described by other workers in different populations of P. nymphula including the presence of intermediate sized larvae (Corbet, 1957; Harvey, 1985; Lawton, 1970a; Macan, 1964, 1974). The occurrence of individuals of intermediate size, indicated in Fig. 2.4 and 2.5 also suggests that different rates of development occur. It was not possible to determine from these data whether these larvae were growing more slowly and taking three years to develop or growing more quickly and taking one year to reach emergence.

The life cycle during 1987/1988 sampling.

Data collected during the 1987/1988 sampling period are shown in Fig. 2.6 and Fig. 2.7 and Table 2.3. Larvae were separated into year classes by eye using size

frequency histograms (Fig. 2.6). Development of larvae was synchronised and for most of the year (September to May) two well defined cohorts were present in samples (Fig. 2.6). From June to August however, only one cohort of large emergence group larvae was found (Fig. 2.6). These results confirm that the majority of individuals show synchronised, semivoltine development. Again the presence of individuals of intermediate size between the senior and junior cohorts during November sampling (both in 1987 and 1988) suggests that a small number of larvae may have slower or faster development (Fig. 2.6). These results are consistent with those found by previous workers such as Corbet (1957a), Harvey (1985), Lawton (1970a) and Macan (1964, 1974). For both sampling occasions there was a significant correlation between head dimensions and total length (Table 2.4).

Development of emergence groups.

Three emergence groups were collected during the 1987/1988 sampling having hatched from the egg in 1986, 1987 and 1988 (Fig. 2.6)(These will be referred to as the 1986, 1987 and 1988 emergence groups). The 1986 emergence group larvae were sampled consistently from November 1987 to May 1988 (Fig. 2.6, Table 2.3). The mean total length of individuals of this emergence group increases slightly (from 11.63mm to 12.47mm) over this period (Table 2.3, Fig. 2.7). Head dimensions similarly increased in size over this period (Table 2.3). These results suggest that growth of this 1986 emergence group was negligible over the winter of 1987/1988. Emergence of the 1986 year class must have occurred during May 1988 as no individuals of this size were collected in subsequent samples in June and July (Fig. 2.6).

The 1987 emergence group larvae were present initially in samples collected during November 1987 (Fig. 2.6); these had a mean total length of 5.47mm (Table 2.3, Fig. 2.7). This year class was present in significant numbers in samples until May 1988 (Fig. 2.6, Table 2.3) when their mean total length was 4.95mm (Table 2.3). Absence of this smaller emergence group in winter samples has been reported by other workers

namely Corbet (1957), Lawton (1970a) and Macan (1974,1964). According to Lawton (1970a) these small junior emergence group larvae are usually missed in net samples collected at this time as they have burrowed into the substratum to avoid winter conditions and predation. This emergence group was present throughout the summer of 1988 to the end of sampling in November 1988 (Fig. 2.7, Table 2.3). Growth of this emergence group only occurs in the summer between June and October (Table 2.3, Fig. 2.7). For example, mean total length of larvae increases from 4.95 mm in May 1988 to 11.83 mm in October when growth slows . From June, 1988 this emergence group can be referred to as the senior emergence group, as it is the largest cohort present (Fig. 2.7, Table 2.3).

The 1988 emergence group larvae first appear in samples collected in September 1988 (Figs, 2.6 and 2.7) with a mean total length of 3.57mm (Table 2.3, Fig 2.7). These larvae hatched during July or August 1988 and did not appear in samples until September 1988, by which time they had attained a sufficient size to be caught in the net.

Larval density

Following Lawton (1970a), the number of larvae found per standard sweep of a pond net (S.N.S) was used as a relative measure of density. Table 2.5 shows the seasonal changes in larval density at each of the sampling stations. Total larval density was highest during June 1988 through to September 1988 reaching a peak during August of 18.22 larvae per S.N.S. (Fig. 2.8, Table 2.5). Total larval density was lowest during the winter with 1 larva per S.N.S. in December and 0.6 per S.N.S in March (Fig 2.8. Table 2.5).

Total larval density, however does not reflect the differing seasonal distribution of each of the age classes. During the winter (December 1987 to March 1988) for example, only the larger 1986 year class individuals were collected (Fig. 2.6). Thus total

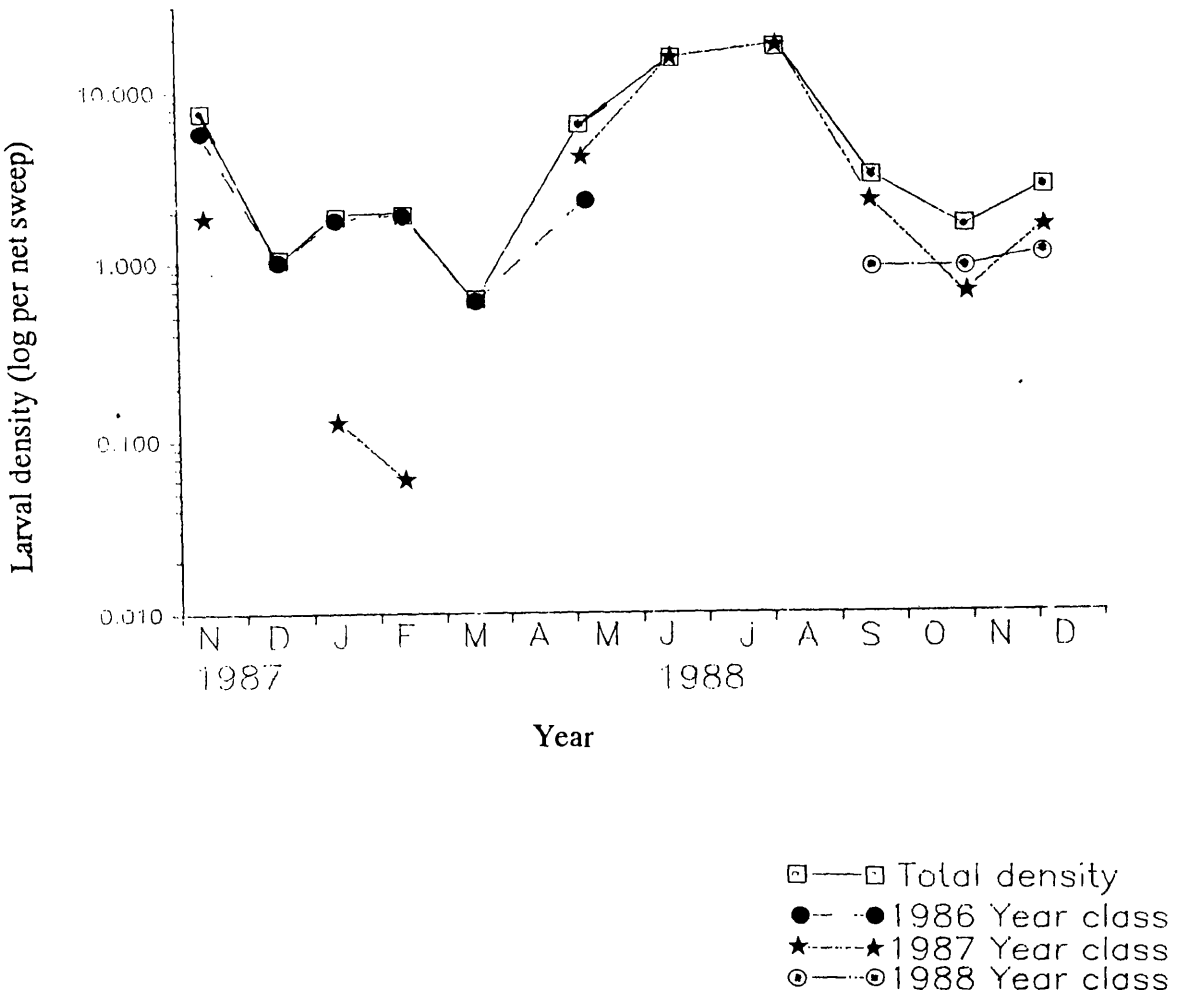
Table 2.5 Summary of seasonal variation in larval density (numbers per S.N.S.) at each sampling station during 1987/1988 sampling.

Date	Age class (year)	Individuals/Station 1	2	3	4	5	Age class totals	Total	Sweeps /station	Number per sweep /class	/total
10/11/87	S(86)	9	-	17	-	26	52	69	3(9)	5.78	7.67
	J(87)	4	-	9	-	4	17			1.89	
16/12/87	S(86)	2	0	5	3	5	15	15	3(15)	1.00	1.00
	J(87)	0	0	0	0	0	0			0.00	
11/ 1/88	S(86)	2	11	6	7	1	27	29	3(15)	1.80	1.93
	J(87)	1	1	0	0	0	2			0.13	
10/ 2/88	S(86)	2	6	10	7	4	29	30	3(15)	1.93	2.00
	J(87)	0	0	0	1	0	1			0.06	
18/ 3/88	S(86)	1	6	1	1	0	9	9	3(15)	0.60	0.60
	J(87)	0	0	0	0	0				0.00	
2/ 5/88	S(86)	5	-	5	-	10	20	58	3(9)	2.20	6.40
	J(87)	23	-	9	-	6	38			4.20	
13/ 6/88	S(86)	0	-	0	-	0	0	140	3(9)	0.00	15.50
	J(87)	8	-	73	-	59	140			15.50	
2/ 8/88	S(86)	0	-	0	-	0	0	164	3(9)	0.00	18.22
	J(87)	46	-	98	-	20	164			18.22	
12/ 9/88	S(87)a	10	4	5	4	11	34	48	3(15)	2.26	3.19
	J(88)b	2	1	1	7	3	14			0.93	
24/10/88	S(87)	0	0	10	0	0	10	24	3(15)	0.66	1.59
	J(88)	0	0	14	0	0	14			0.93	
29/11/88	S(87)	2	12	1	1	8	24	41	3(15)	1.60	2.73
	J(88)	4	0	1	1	11	17			1.13	

(-) = No sample collected
(a) = 1987 age class larvae now referred to as "senior"
(b) = 1988 age class larvae now referred to as "junior"

Fig 2.8 Seasonal variation in density (Log_{10} number of larvae per S.N.S.) of the different emergence groups of P. nymphula larvae during 1987/1988 sampling.

Fig 2.8


Larval density (log per net sweep)
10.000
1.000
0.100
0.010
N D J F M A M J J A S O N D
1987
1988
Year
Total density
1986 Year class
1987 Year class
1988 Year class

density at this time reflects density of 1986 year class larvae (Fig. 2.8). Similarly in summer (June to August 1988), when the highest densities of larvae were recorded (Table 2.5, Fig. 2.8), only 1987 year class larvae occurred in samples.

This reduction in numbers of larvae in emergence groups may not be due primarily to larval mortality. It is possible larvae are washed out of the sampling site in the stream "drift". This reduction in larval numbers will be referred to as loss rather than mortality.

Larval loss was estimated to be highest when the largest reduction in numbers occurred between sampling occasions. This is shown in the changes in abundance of each of the three different emergence groups (Fig. 2.8, Table 2.5). There were two drops in larval density recorded during the sampling programme. Firstly, during the winter of 1987 in the 1986 and 1987 emergence groups and secondly in the autumn of 1988 in the 1987 emergence group. This first reduction in numbers may have been due to sampling error as numbers of both the 1986 and 1987 emergence groups recovered the following spring. It is likely that these larvae were avoiding capture by overwintering in the substratum (as described earlier). These larvae only became "catchable" the following spring when they moved back into the weed. The second decline in numbers (in the 1987 emergence group) may be due to mortality rather than sampling error. This is because numbers did not recover in the following spring (Fig. 2.8). It was not possible to determine mortality rates of larvae post-hatching to their first winter from the present data.

Distribution

The densities of larvae at each of the sampling stations throughout the sampling programme are shown in Table 2.5. The absence of junior emergence group larvae during the winter of 1987/1988 (described earlier in this section) is the most marked change in recorded distribution.

Because of differences in the densities of the different emergence groups in the different habitats and across time, it was not possible to compare their densities directly. For example, it is clear that there was relatively few larvae in the mud/sparse potamogeton habitat (Table 2.5). However, by grouping the data together it was possible to use a Kruskal-Wallace one-way analysis of variance test (Siegel, 1956) to determine whether there was any preference for the three habitat types across the seasons. The four categories used for this test were as follows, 1) Senior emergence group larvae at station 1 during winter 2) Senior emergence group larvae at station 1 during summer 3) Senior (1986) emergence group larvae on Potamogeton polygonifolius (stations 2 and 3 combined) from November to May (including November 1988), 4) Senior (1986) age class on Myriophyllum alterniflorum (sites 4 and 5 combined) from November to May (including November 1988), 5) Senior (1987) emergence group larvae on P. polygonifolius (stations 2 and 3) from May to October and 6) Senior (1987) emergence group larvae on M. alterniflorum (stations 4 and 5) from May to October 1988. The result of this test showed no significant difference ($P>0.5$) in larval densities between the different weed types either within or between seasons (Table 2.6).

Gender

One of the aims of this study was to examine the effect of gender on larval size. Determination of larval gender was found to be most accurate for larvae over 6mm in length. This size limit was calculated from the number of larvae of indeterminate gender found in each cohort (following Lawton (1972))(Table 2.7). Female larvae of less than 6mm total length resemble male larvae and may be misidentified as males. Chi-square analysis was carried out on the number of larvae of each sex, in each cohort, over 6mm total length, for each sample, and for the sum of males and females found. Samples containing fewer than five individuals were not used. There was no significant difference in the proportions of males and females in each emergence group or in the population as a whole (Table 2.8).

Table 2.6 Kruskal Wallis analysis of variance on seasonal change in mean larval density in each habitat type during the 1987/1988 sampling. Median = median value of ranked densities.

Category (habitat type) and season	N observations (from Table 2.5)	Median	Average rank	Z value
Mud (Station 1) winter	7	0.67	11.6	-1.55
Mud (Station 1) Summer	7	2.17	18.5	0.64
Potamogeton (Station 2+3) Summer	6	1.42	15.7	-0.22
Potamogeton (Station 2+3) Winter	4	1.66	14.6	-0.43
Myriophyllum (Station 4+5) Summer	4	13.32	22.1	1.28
Myriophyllum (Station 4+5) Winter	4	4.16	18.9	0.54
Overall	32		16.5	

H= 4.10, d.f. = 5, P= 0.536

Table 2.7 Cohort sex ratios and mean sizes for larvae collected during 1987/1988 sampling.

Sample date	Size group	Cohort size	Males %	Males N	Females %	Females N	Unidentified %	Unidentified N
10/11/87	>8mm S	11.63	50	26	50	26	-	-
	<8mm J	5.47	70	12	18	3	12	2
16/12/87	>8mm S	12.25	27	4	73	11	-	-
11/ 1/88	>8mm S	12.23	37	10	63	17	-	-
	<8mm J	4.34	-	-	-	-	-	2
0/ 2/88	>8mm S	11.78	64	18	36	10	-	-
	<8mm J	5.80	-	-	-	-	-	-
18/ 3 88	>8mm S	11.60	33	3	67	6	-	-
2/ 5/88	>8mm S	12.48	47	9	53	10	-	-
	<8mm J	5.95	47	17	22	8	31	11
13/ 6/88	1-9mm S	5.05	27	37	25	35	48	68
2/ 8/88	3-10mm S	7.72	49	81	48	80	3	4
12/ 9/88	>7mm S	9.99	57	19	43	14	-	-
	<7mm J	3.57	40	6	7	1	53	8
24/10/88	>8mm S	11.84	70	7	30	3	-	-
	<8mm J	2.52	-	-	-	-	100	14
29/11/88	>8mm S	11.84	57	13	43	10	-	-
	<8mm J	2.54	-	-	-	-	100	21

Table 2.8 Mean total length (mm) and gender of larvae over 6mm total length collected during 1987/1988 sampling. S.E. = standard error of the mean.

Sample	Males			Female			Chi-square
date	Size	N	S.E	Size	N	S.E	P >
10/11/87	11.58	26	0.10	11.54	27	0.19	0.05
11/ 1/88	12.41	10	0.22	12.13	17	0.18	0.05
10/ 2/88	11.56	11	0.33	11.93	18	0.03	0.05
2/ 5/88	12.57	9	0.18	12.33	10	0.31	0.05
13/ 6/88	6.75	16	0.11	6.97	24	0.12	0.05
2/ 8/88	8.18	73	0.13	7.99	70	0.13	0.05
12/ 9/88	9.59	20	0.21	10.49	14	0.20	0.05
29/11/88	11.60	13	0.16	12.20	10	0.19	0.05

**Total= 178 Total= 190

Table 2.9 Analysis of variance; mean Total length of larvae over 6mm, larval gender and collection date. Mean values are shown in Table 2.7

Source of variation	Sum of squares	DF	Mean square	F	Significance of F
Main effects	1426.964	8	178.370	218.629	0.000
Sex	0.317	1	0.317	0.389	0.533
Date	1425.638	7	203.663	246.630	0.000
2-way interaction					
Sex and Date	11.988	7	1.713	2.099	0.043
Explained	1438.952	15	95.930	117.582	0.000
Residual	287.182	352	0.816		
Total	1726.134	367	4.703		

To determine whether or not there was a significant difference in size of male and female larvae throughout the sampling period, the mean total lengths of male and female larvae were compared for each emergence group and each month (using a two-way analysis of variance Table 2.9). There was no significant difference in mean total length of all males and females (F significant at 0.533, Table 2.9). There was a highly significant difference in the mean total length of males and females throughout the sampling period (F significant at 0.001, Table 2.9) due to larval growth (both male and female larvae are smaller in summer compared to winter (Table 2.8). When the size of males and females was compared across sampling periods there was a significant difference in size (F significant at 0.043, Table 2.9). However, this difference is likely to be an artifact as the mean difference in the sizes of males and females is not consistent with time, on occasion males are larger then females and vice versa (Table 2.8).

2.3.2. Frequency of lamellar injury in the sample population.

Sampling season and lamellar injury

The extent to which lamellar injury occurs in the sampled population is shown in Fig. 2.9 and table 2.10. The data are from all sampling sites combined, but each emergence group has been kept separate. The frequency of lamellar injury varies with both the age of the three emergence groups and with sampling season (Fig 2.9). In the 1986 emergence group lamellar injury increased from a frequency of between ten and twenty percent during the winter of 1987 to a peak of fifty percent prior to emergence in May 1988 (Fig. 2.9). In the 1987 emergence group the frequency of injury was forty seven percent in November and forty two percent the following May when the larvae next appeared in samples (Fig. 2.9). These larvae were absent during the winter of 1987. In the 1988 emergence group, injury occurred with a frequency of fourteen percent when the larvae were first recorded in samples in September 1988. Numbers of this group then rose to a peak of sixty four percent in October 1988 before falling to forty percent by November (1988) (Fig. 2.9, Table 2.10).

Table 2.10 Frequency of lamellar damage and mean total length (mm) of P. nymphula larvae with (L+) and without (L-) lamellar damage, collected from the Ross Burn sampling site during 1987/1988 sampling. C.V. = coeficient of variation and S.D. = Standard deviation of group means.

Sample date	Size group	Mean size	S.D.	C.V.	L+ Mean	L+ N	L+ S.E.	L- Mean	L- N	L- S.E.	% with damage
10/11/87	>8mm(S)	11.56	0.81	53	11.62	42	0.11	11.72	10	0.14	18
	<8mm(J)	5.47	1.11	17	5.37	9	0.42	5.28	6	0.20	47
16/12/87	>8mm(S)	12.25	0.68	15	12.21	14	0.18	12.88	1	-	7
11/ 1/88	>8mm(S)	12.23	0.72	27	12.21	20	0.13	12.30	7	0.40	26
	<8mm(J)				4.34	2	-	-	-	-	-
10/ 2/88	>8mm(S)	11.79	0.81	29	11.73	23	0.17	12.03	6	0.29	21
	<8mm(J)				5.80	1	-	-	-	-	-
18/ 3/88	>8mm(S)	11.82	0.93	9	11.60	5	0.43	12.10	4	0.47	44
2/ 5/88	>8mm(S)	12.47	0.78	20	12.52	10	0.16	12.42	10	0.32	50
	<8mm(J)	5.05	1.01	36	5.00	21	0.21	5.11	15	0.29	42
13/ 6/88	1-9mm(S)	5.06	1.40	140	4.84	81	0.15	5.35	59	0.18	42
2/ 8/88	3-10mm(S)	7.72	1.40	165	7.66	139	0.12	8.08	26	0.28	16
12/ 9/88	>7mm(S)	9.96	0.97	34	10.02	29	0.18	9.63	5	0.49	15
	<7mm(J)	3.57	1.46	14	3.50	12	0.41	4.00	2	1.60	14
24/10/88	>8mm(S)	11.84	0.50	10	11.90	9	0.16	11.28	1	-	10
	<8mm(J)	2.97	0.47	14	2.52	5	0.18	2.94	9	0.15	64
29/11/88	>8mm(S)	11.86	0.64	23	11.85	21	0.15	12.00	2	0.20	9
	<8mm(J)	2.99	1.31	17	2.44	9	0.10	2.70	6	0.12	40

Fishers exact test on cohort size and frequency of lamellar damage

	Fequency of damage in cohort	
	< 30%	>30%
Senior cohort	7	2
Junior cohort	1	4

Results: N= 14, S_1= 5, S_2= 6, X= 4, (P<0.01)

Fig. 2.9 Seasonal variation in the percentage of P. nymphula larvae in each emergence group with lamellar injury during the 1987/1988 sampling period.

Fig. 2.10 The frequency of larvae with lamellar injury (Arc-sine transformed) against larval density (Log_{10} number per S.N.S.).

Fig 2.9

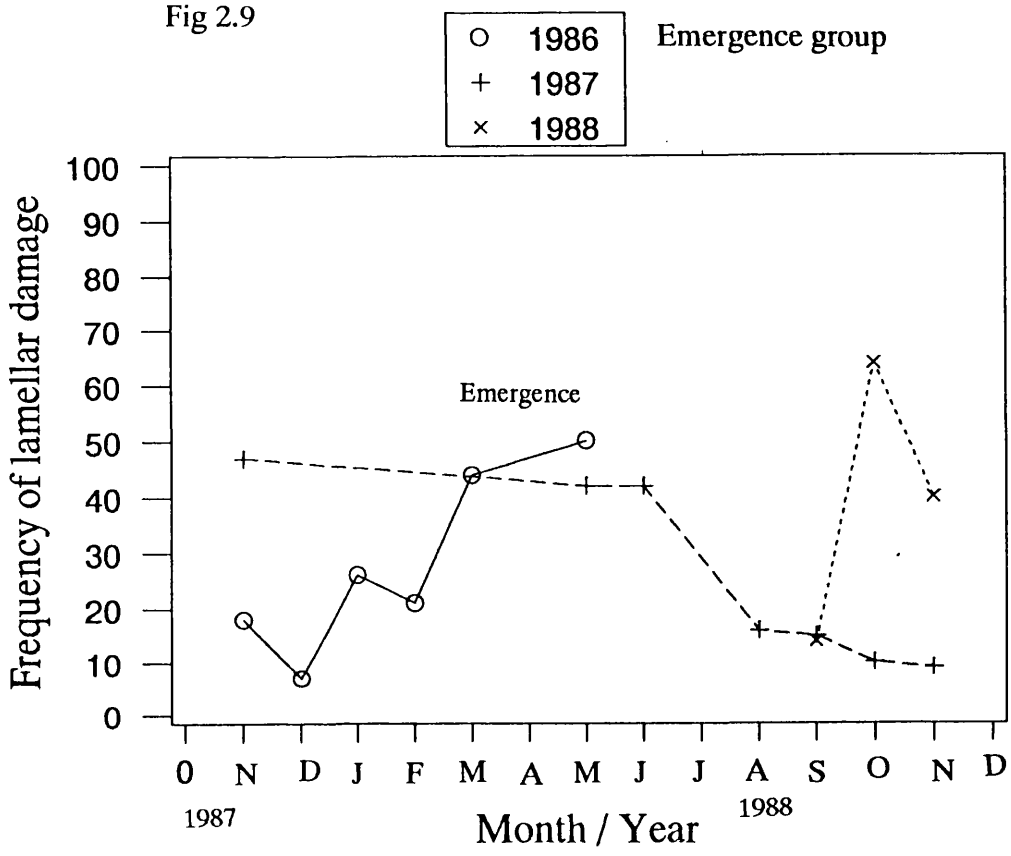


Fig 2.10

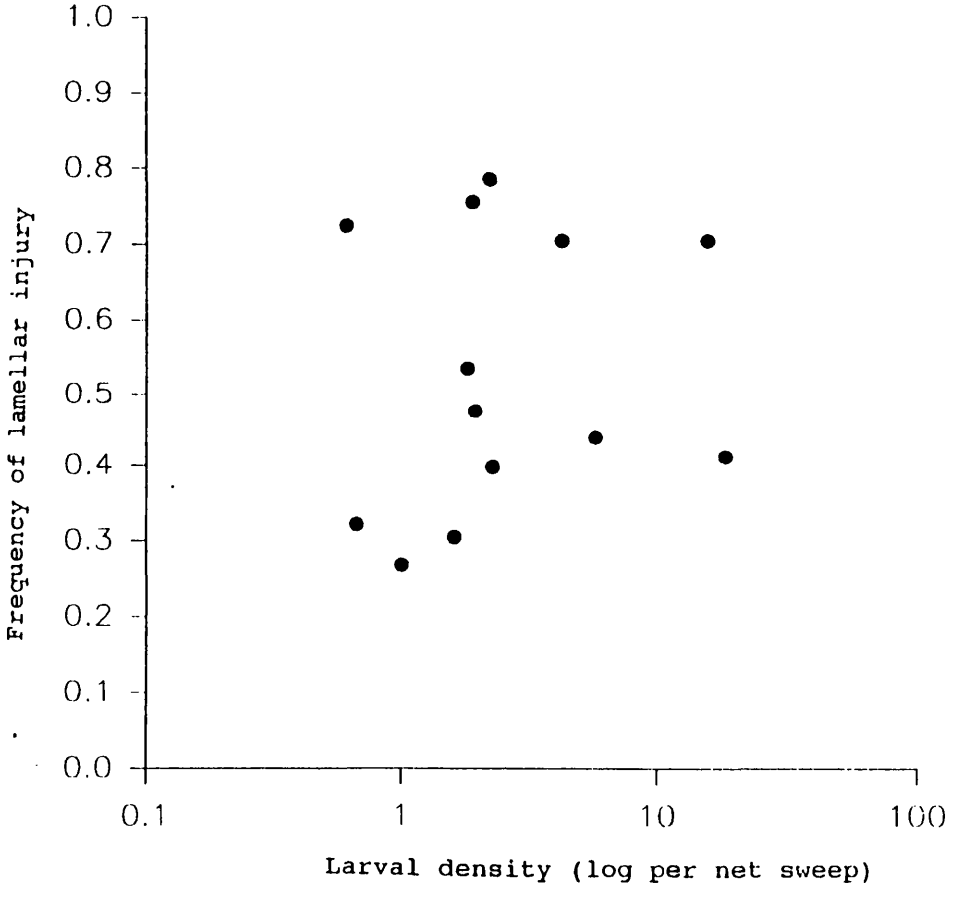
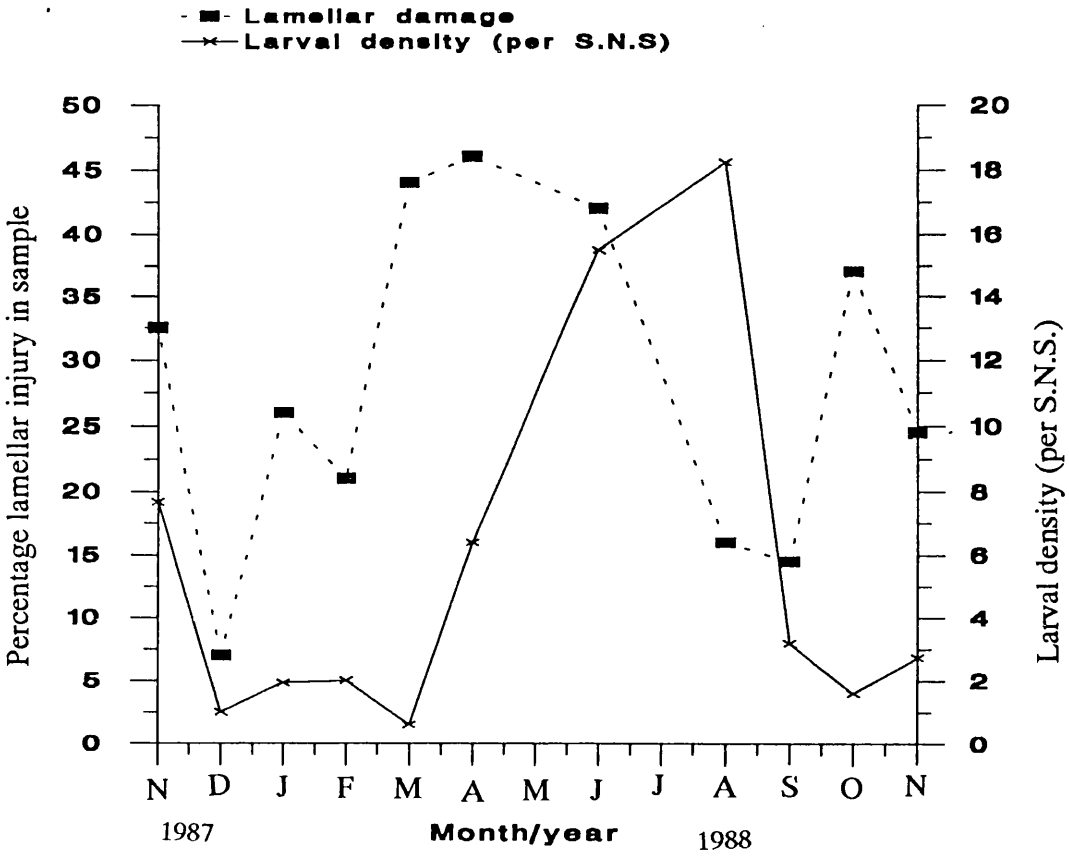


Fig. 2.11 Comparison of larval density and frequency of lamellar damage in each cohort throughout the year. This figure illustrates how the peak period of lamellar damage does not coincide with highest larval density.

Fig 2.11


Lamellar damage
Larval density (per S.N.S)
Percentage lamellar injury in sample
Larval density (per S.N.S.)
50
45
40
35
30
25
20
15
10
5
0
20
18
16
14
12
10
8
6
4
2
0
N D J F M A M J J A S O N
1987
Month/year
1988

Larval density and lamellar injury

To determine whether larval density was related to the frequency of lamellar injury, the percentage of larvae with injury in each emergence group was plotted against the mean larval density of that group and for each sampling occasion (Fig 2.10). However, there was no statistically significant correlation between these variables (Arcsine injury freq. = 0.5 + 0.063xlog density, Rsq =0% P=0.61) showing that frequency of lamellar loss was independent of larval density. This conclusion is confirmed when larval density is plotted against frequency of lamellar damage (Fig 2.11). In this figure it can be seen that the maximum larval density occurs out of phase with maximum lamellar damage. Larval density for the 1986 and 1987 emergence groups is highest during June and July whilst the frequency of injury is highest in April, May and October in these emergence groups. This comparison also confirms that the collection method does not contribute significantly to the rate of lamellar loss. If lamellar injury was related to density of larvae in the sample containers then injury should reflect the population density as the same number of containers were used on each sampling occasion and as population density increased so too did the number of larvae in the sampling containers. The increase in larval density did not result in an increase in the frequency of lamellar injury.

Larval gender and lamellar injury

A chisquare test was used to determine whether gender predisposed larvae to lamellar injury (Table 2.11). This comparison only used larvae in the senior emergence group for which sex could be determined accurately (2.1.3). These results show that with the exception of one occasion (larvae collected on 10/11/87, P,0.05) there was no statistically significant difference in the numbers of male and female larvae with lamellar injury. This result is likely to be an artifact of the small sample size on this occasion, as it did not recur in subsequent samples of this emergence group which would be expected if a real effect. When the sample size was large (during the summer sampling) there was

Table 2.11 Lamellar damage and gender of P. nymphula larvae over 6mm total length, collected during 1987/1988 from the Ross Burn. O= observed values and E= expected values for Chi-sq test.

Sample date	With damage				Without damage				Chi-sq	P<
	Males		Females		Males		Females			
	O	E	O	E	O	E	O	E		
10/11/87	8	5.0	2	5.0	18	21.0	24	21.0	4.46	0.05
16/12/87	0		1		4		10		-	-
11/ 1/88	2	2.6	5	4.4	8	7.4	12	7.4	0.29	0.95
10/ 2/88	4	3.9	2	2.1	14	14.1	8	7.9	0.02	0.95
18/ 3/88	3	1.3	1	2.7	0	1.7	5	3.3	-	-
2/ 5/88	4	4.3	5	4.7	5	4.7	5	5.3	0.06	0.95
13/ 6/88	15	17.5	19	16.5	22	19.5	16	18.5	1.36	0.95
2/ 8/88	14	13.0	12	12.9	67	67.9	68	67.1	0.15	0.95
12/ 9/88	2	2.9	3	2.1	17	16.1	11	11.9	0.75	0.95
24/10/88	1		0		6		3		-	-
29/11/88	1		1		12		9		-	-

no statistically significant difference in the number of males and females with lamellar injury.

Larval distribution and lamellar injury

The distribution of larvae with lamellar injury was investigated by determining the frequency of larvae with injury at each of the sampling sites. It was suggested that larvae within certain habitats may be more vulnerable to lamellar injury, or that larvae without lamellae may have a restricted distribution. The frequency of lamellar injury in each emergence group and from each habitat type during summer and winter are shown in Fig 2.12 and 2.13 respectively. The data from which these frequencies are calculated are shown in Table 2.12.

In summer (Fig 2.12) the proportion of senior (1987 and 1988) emergence group larvae with lamellae in both weed habitats (Potamogeton and myriophyllum) is similar at 71% and 70% respectively. Whilst in the mud habitat (Station 1) the frequency of intact larvae in the senior emergence group larvae was 78%. At this time the number of junior emergence group larvae sampled was too small to make comparisons. In winter (Fig 2.13, Table 2.12), the frequency of intact larvae in the senior emergence group was 61% in the mud habitat, 74% in the Potamogeton and 77% in the Myriophillum.

Larval emergence group and lamellar injury.

The frequency of lamellar loss may be related to the size range of larvae within emergence group cohorts. To test this, the amount of variation (Coefficient of Variation, Wardlaw, 1987) in total length within each emergence group cohort on each sampling occasion was correlated with the frequency of lamellar injury for that cohort (and sampling occasion) using a Spearman-Rank test (Siegel and Castellan, 1991) (Data in Table 2.10). The results of this test show that there was no significant correlation between variation in mean total length and frequency of lamellar injury within cohorts ($P=0.1$-0.05).

Table 2.12 Mean density of larvae with (L+) and without (L-) lamellae (number per S.N.S.), collected during 1987/1988 sampling.

Season/ age group		Habitat type								
		Mud			Potamogeton			Myriophyllum		
		N	Dens.	%	N	Dens.	%	N	Dens	%
Summer	S L+	50	5.55	78	130	10.83	71	72	6.00	70
	S L-	15	1.55	22	51	4.25	29	25	2.08	30
	J L+	2	0.22	-	1	0.08	-	6	0.50	-
	J L-	-	-	-	1	0.08	-	1	0.08	-
Winter	S L+	14	1.75	61	74	5.29	74	47	3.35	77
	S L-	9	1.13	39	26	1.85	26	14	1.00	33
	J L+	9	1.13	-	13	0.93	-	17	1.21	-
	J L-	11	1.38	-	26	1.42	-	5	0.35	-

L+ = with lamellae, L- without lamellae.
N = Total number sampled.
Dens = Number per S.N.S.
Winter = Samples collected from October to May
Summer = Samples collected during June to August.
% = % of larvae with damage during sampling period.

Fig. 2.12 Density of larvae (number per S.N.S.) with (L+) and without (L-) lamellae in each emergence group, according to habitat types during the summer (May to October) sampling (1988). Habitat type: Mud.= (Site 1) mainly mud and sparse Potamogeton sp., Pot. = (Sites 2+3) mainly Potamogeton sp., Myr = (Sites 4+5) mainly Myriophyllum sp. (See Fig. 2.1 for map of sampling site).

Fig. 2.13 Density of larvae (number per S.N.S.) with and without lamellae in each emergence group, according to habitat type during winter (October to May) sampling. Habitat type: Mud.= mainly mud and sparse Potamogeton sp., Pot. = mainly Potamogeton sp., Myr = mainly Myriophyllum sp. (See Fig. 2.1 for map of sampling site).

Fig 2.12

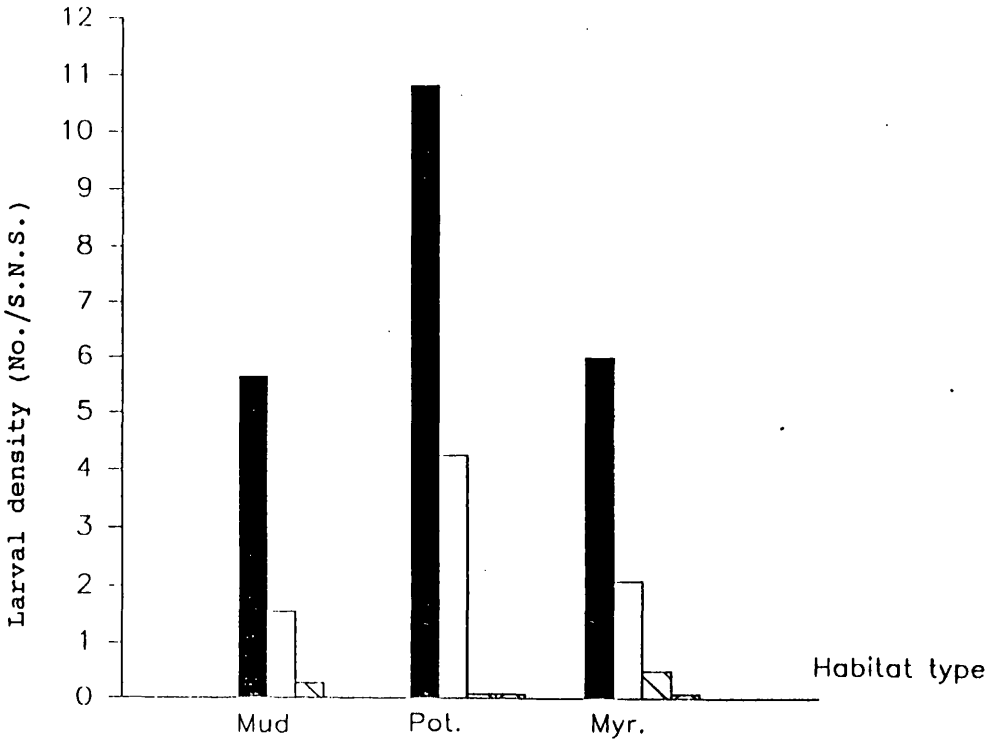
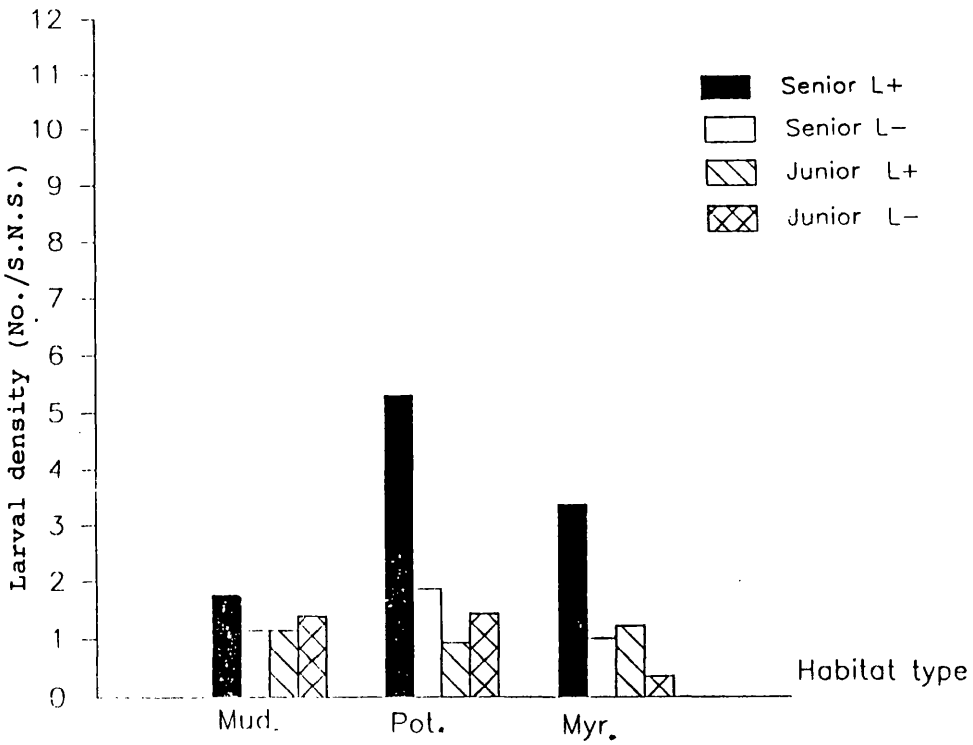


Fig. 2.13



However, the frequency of lamellar injury within cohorts, has a bimodal distribution. When emergence groups are larger they mainly have a frequency of lamellar injury of less than 30%, whilst those in the smaller emergence groups tended to have a frequency of injury greater than 30% (Table 2.10). The statistical significance of this observation was tested using Fishers exact test ($P<0.01$)(Siegel and Castellan, 1991)(Table 2.10). This result cannot be regarded as significant as the number of observations was small (N= 14), but suggests that the smaller emergence groups may suffer a greater incidence of lamellar injury than the larger cohorts.

Lamellar injury in the smaller emergence group may occur only when they are present in the same habitat as the larger emergence group larvae. The only occasions when two cohorts were found in the same sample were when samples were collected in May, September, October and November (of both years) and were therefore too infrequent to allow statistical comparison of the results. However, a visual examination shows that rates of lamellar injury in the smaller cohorts are indeed high at these times (Figs 2.6, 2.9).

Both these observations are consistent with the suggestion that lamellar injury is caused by larger conspecifics either through predation or during aggressive encounters. Although it is not possible to discount other factors which would selectively damage the lamellae of smaller larvae, such as predation by fish, as causes of lamellar loss.

2.3.3. Lamellar injury and larval size.

Lamellar injury and mean size of emergence group.

The results of the previous analyses showed that in the study population the frequency of lamellar injury was significant and varied according to larval age and

season. It was also suggested in the Introduction that lamellar injury may be related to larval size. Therefore any statistical comparison of the mean size (total length) of larvae with and without lamellae must take these factors into account. Such factors included the semivoltine life cycle of the study population which results in the presence of distinct emergence group cohorts. Habitat type must also be considered because previous authors have found that habitat influences larval growth rate in P. nymphula populations (Macan, 1964,1974). It was also suggested in 2.1.1. that larval gender may influence larval growth rates.

Comparison of the mean sizes of larvae with and without lamellae on each sampling occasion, and taking into account the other possible influences on larval growth described above, was done using a multiple analysis of variance (Sokal and Rohlf, 1969). Using this method the mean sizes of larvae with and without lamellae were compared within emergence group cohorts, then across seasons, between sexes and between habitat types. This type of analysis requires considerable data to increase the significance of a result, the data were therefore put into one of the following four groups:

1) Larvae grouped according to whether they were collected on Potamogeton (stations 2+3) Myriophyllum (stations 4 and 5) or mud/ sparse Potamogeton (station 1).

2) Larvae were grouped according to growth season as described earlier, either summer, larvae collected in June to September (growing) or winter, larvae collected from October to May (when no growth occurred). Larvae of the 1988 year class less than 8mm total length were omitted from analysis as this emergence group was not sampled consistently.

3) Following Baker and Dixon (1986), larvae with and larvae without lamellar injury were only included in analysis on those sampling occasions when there were greater than 5 larvae.

4) Larvae were grouped according to gender. This meant that a significant amount of data were lost to this analysis as larvae of less than 6mm total length could not be accurately assigned a sex. The addition of larval gender as a category therefore removed a substantial amount of otherwise useful data from the analysis.

The results of this analysis are shown in Table 2.12. No statistically significant difference was found between the mean size of male and female, nor male and female larvae with and without lamellae. It is thus possible to remove larval gender as a category from the analysis and repeat the analysis.

The results of this second analysis are shown in Table 2.14 and the mean total lengths of larvae with and without lamellae from the different habitat types for comparison in Table 2.15. The results of this analysis can be interpreted as follows:

a) Lamellar loss and larval size

During the whole sampling period the mean total length of larvae with lamellae was significantly greater (8.47mm +/- 0.15 S.E. N=426) than that of those without lamellae (7.80mm +/- 0.26 S.E. N=149) at P=0.039 (Table 2.15, 2.14).

b) Lamellar injury and larval size between seasons

No statistically significant differences were found when the mean size of larvae with and without lamellae was compared both within and between seasons (P=0.088, 2-way interactions Table 2.14). This suggests an equal probability of large and small larvae losing lamellae in summer and winter. However, as P (0.088) is almost significant it is not possible too discount entirely the possibility that the frequency of lamellar injury varies according to season and larval size.

Table 2.13 Analysis of variance; Total length of larvae by gender, season, sampling station and lamellar injury. Only larvae of known sex and greater than 6mm total length were used in this analysis, and grouped as described in the text (Season(summer or winter), Lamellae (with or without)).

Source of variation	Sum of squares	DF	Mean square	F	Significance of F
Main effects	1464.424	5	292.885	235.492	0.000
Sex	0.016	1	0.016	0.013	0.909
Date	1410.304	1	1410.304	1133.944	0.000
Station	2.156	2	1.078	0.867	0.421
Lamellae	2.705	1	2.705	2.175	0.141
2-way interactions	25.093	9	2.788	2.242	0.019
Sex and Date	0.572	1	0.572	0.460	0.498
Sex and Station	2.916	2	1.458	1.172	0.311
Sex and Lamellae	1.844	1	1.844	1.483	0.224
Date and Station	0.195	2	0.098	0.079	0.924
Date and Lamellae	6.867	1	6.867	5.522	0.019
Station and Lamellae	14.411	2	7.206	5.794	0.003
3-way interactions	20.760	7	2.966	2.385	0.021
Sex, Date and Station	4.399	2	2.199	1.768	0.172
Sex, Date and Lamellae	4.408	1	4.408	3.544	0.061
Sex, Station and Lamellae	0.659	2	0.330	0.265	0.767
Date, Station and Lamellae	9.318	2	4.659	3.746	0.024
4-way interaction					
Sex, Lamellae, Date and Station	0.967	2	0.484	0.389	0.678
Explained	1511.244	23	65.706	52.831	0.000
Residual	477.587	384	1.244		
Total	1988.831	407	4.887		

Table 2.14 Analysis of Variance, Total length of larvae by season, sampling station and lamellar injury. Using all larvae, regardless of gender and grouped according to season and lamellar injury.

Source of variation	Sum of squares	DF	Mean square	F	Significance of F
Main effects	3625.448	4	906.362	272.741	0.000
Date	3541.674	1	3541.674	1065.649	0.000
Lamellae	14.290	1	14.290	4.300	0.039
Station	8.166	2	4.083	1.228	0.294
2-way interactions	45.503	5	8.501	2.558	0.027
Date and Lamellae	9.715	1	9.715	2.923	0.088
Date and site	3.750	2	1.875	0.564	0.569
Station and lamellae	31.999	2	15.999	4.814	0.008
3-way interactions	10.364	2	5.182	1.559	0.211
Date, station and lamellae	10.364	2	5.182	1.559	0.211
Explained	3678.315	11	334.392	100.615	0.000
Residual	1871.125	563	3.323		
Total	5549.439	574	9.668		

Table 2.15 Mean total length of larvae (mm) with (L+) and without (L-) lamellae in each habitat type for the entire sampling period (1987/1988). See table 2.10 for analysis of variance.

Habitat type	L+			L-		
	Mean	N	S.E.	Mean	N	S.E.
Mud/Potamogeton (Station 1)	7.55	81	0.30	8.96	29	0.53
Potamogeton (Stations 2 and 3)	8.61	207	0.21	7.20	78	0.36
Myriofilum (Stations 4 and 5)	8.79	138	0.28	8.13	42	0.45
Total of overall means	8.47	426	0.15	7.80	149	0.26

c) Lamellar injury and larval size between habitat types

There was a statistically significant difference in the mean size of larvae with and without lamellae when compared within and between the three habitat types (regardless of sampling season), P=0.008 (2-way effects Table 2.14). The mean size of larvae from the six groups compared are shown in Table 2.15). These results show that in both of the weed types (Potamogeton and Myriophyllum) larvae with lamellae were larger than those without; in the mud and sparse Potamogeton the larvae without lamellae are larger than those with lamellae. The largest larvae of all were those without lamellae from the mud and sparse Potamogeton, and the smallest those from the Potamogeton without lamellae. The significance of these findings will be discussed later.

d) Larval size, lamellar loss, sampling season and habitat

When the mean size of larvae with and without lamellae was compared between and within sampling seasons and habitat types no significant difference in mean size was found.

e) Larval size, sampling season and habitat type

Over the whole sampling period there was no statistically significant difference in the mean total lengths of larvae from the three different habitat types (Table 2.14). The significant difference in the mean total length of larvae from the different seasons is due to changes in larval size due to growth from one season to another. There was no significant difference in the mean total length of larvae when compared according to season and habitat type, regardless of lamellar status (Table 2.14).

To summarise the results of this analysis, larvae with lamellae were found to be larger overall than larvae without lamellae. This was dependent on habitat type, larvae with lamellae being larger than those without lamellae in the weed habitat but in the mud habitat larvae with lamellae had a smaller mean size than those without lamellae.

Lamellar injury and larval size range within emergence groups.

In the above analysis, the mean size of larvae with lamellar injury was found to vary according to habitat type. It was suggested also that the size range of larvae within cohorts may be related to the frequency of lamellar injury.

The mean size difference of larvae with and without lamellar injury within emergence group cohorts on each sampling occasion are shown for the 1987 emergence group in the summer months in Fig 2.14. There is no consistent pattern of difference in mean size of the groups of larvae with and without lamellae during this time. Initially the mean size of both those with and those without lamellae is the same, larvae without lamellae then become larger until the September sample when larvae with lamellae are again larger (Fig 2.14). At the end of the summer there is only a slight size difference in favour of those without lamellae.

Another way to look at the size difference between larvae with and without lamellae, within cohorts is to examine the difference in mean size between the two groups on each sampling occasion (Fig 2.15). On the majority of sampling occasions larvae with lamellae were smaller (a negative size difference in this figure). Only on four occasions are those with lamellae on average larger. Thus although overall larvae with lamellae were larger, on the majority of sampling occasions those without lamellae were larger.

Lamellar injury, larval density and size difference.

It was also predicted that when the density of larvae was high then a greater number of smaller larvae would tend to lose lamellae through intraspecific predation and aggression. To determine whether this was so, the density of larvae was plotted against the size difference of larvae with and without lamellae within each emergence group (Fig. 2.16). When the mean size difference is large there should be a big difference in

Fig. 2.14 The mean size of larvae with and without lamellae in the 1987 emergence group during the six month sampling period between May and November 1988. All sampling stations combined (Error bars = standard error of the mean).

Fig 2.15 The difference in mean size of larvae with (L+) and without (L-) lamellae in each emergence group and on each sampling occasion in 1987 and 1988. A negative size difference indicates that larvae without lamellae are larger.

Fig 2.14

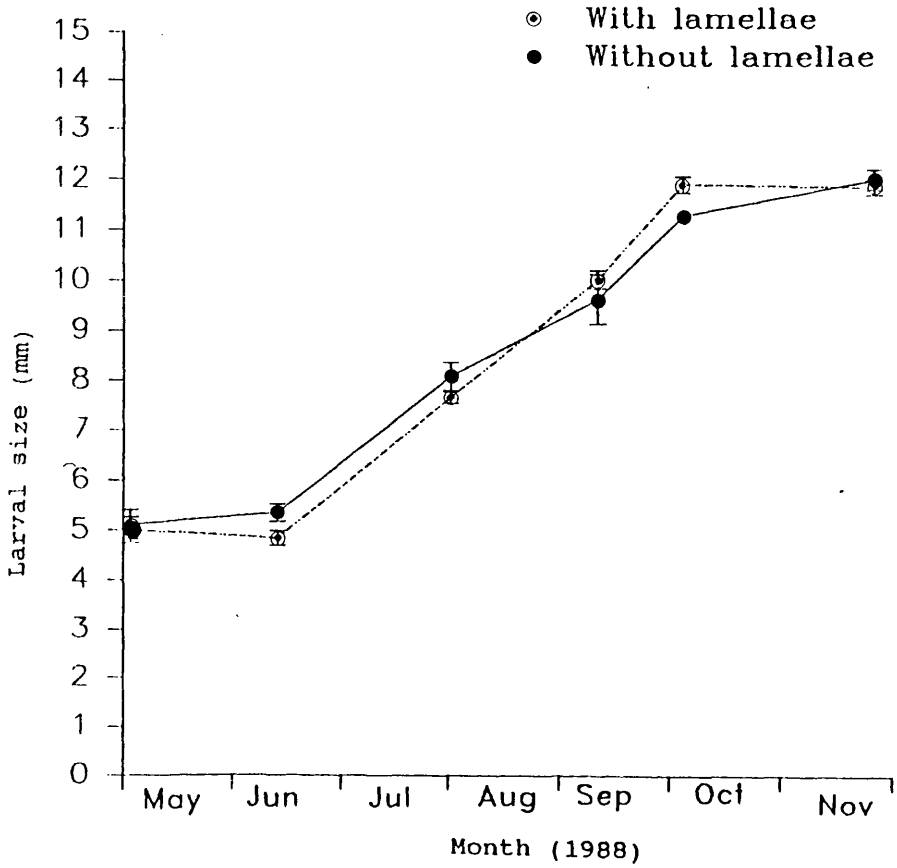


Fig 2.15

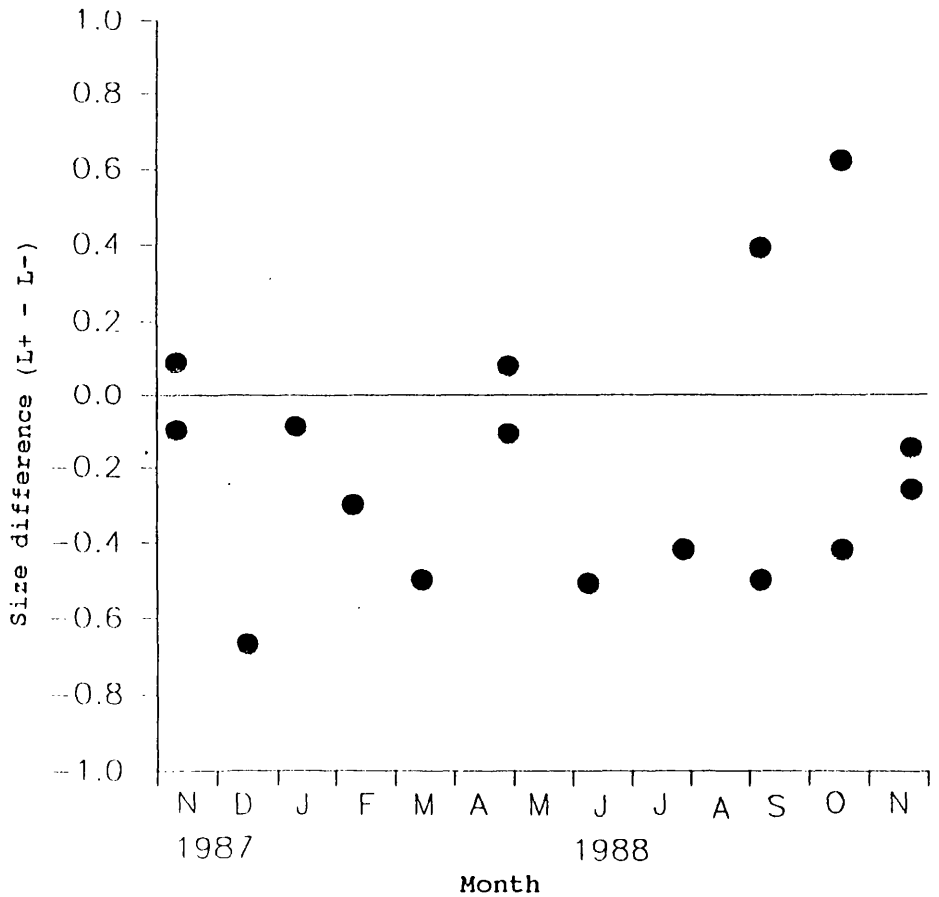
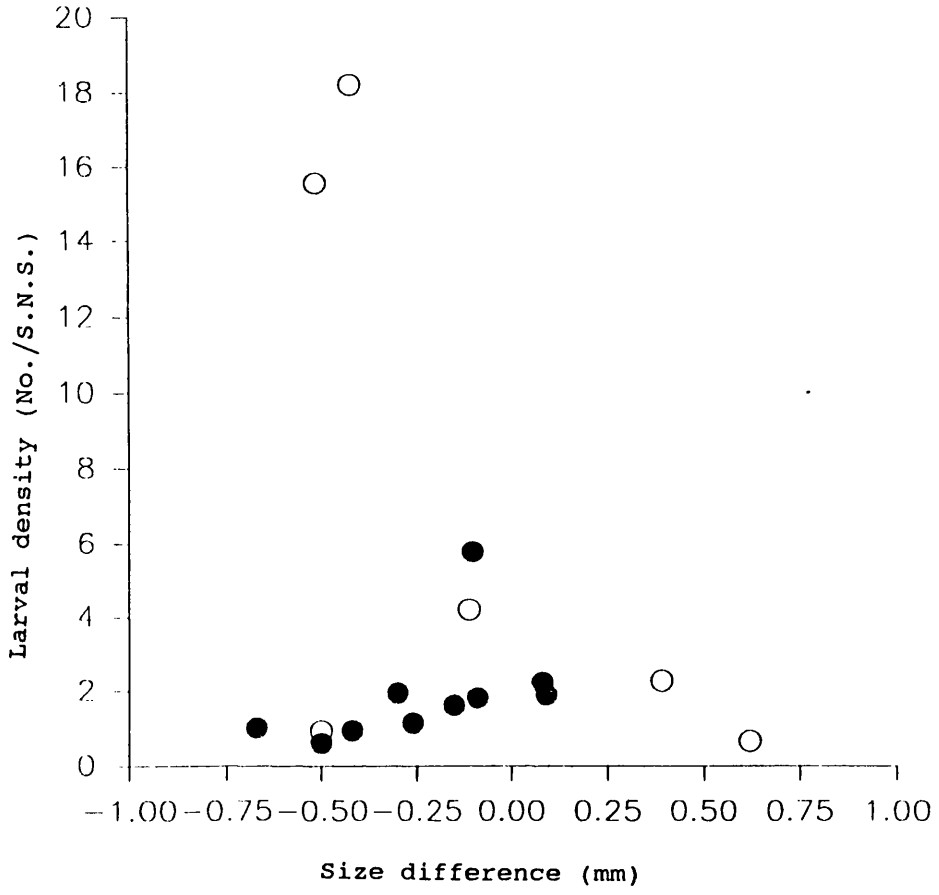


Fig 2.16 Density of larvae (number per S.N.S) on each sampling occasion against difference in mean size of larvae with (L+) and without (L-) lamellae in each emergence group. Summer (O) and winter (O) samples are indicated.

Fig 2.16


20
18
16
14
12
10
8
6
4
2
0
Larval density (No./S.N.S.)
−1.00 −0.75 −0.50 −0.25 0.00 0.25 0.50 0.75 1.00
Size difference (mm)

size between larvae with and without lamellae, there should also be a higher frequency of lamellar injury at this time. However, there was no statistically significant correlation between size difference and larval density.

2.3.4. Environmental monitoring.

Water quality

Spot surface readings of O_2 levels and temperatures from the 1984/85 and 1987/88 sampling programmes are shown in Fig. 2.16. Oxygen levels peaked twice in the year in October, November and December and also in April, May and June, and were lowest during December through to March and August/September. O_2 levels never fell below 8 mgO_2/l or rose above 12 mgO_2/l throughout the entire sampling period. It is unlikely that larvae would experience either respiratory or thermal stress within these ranges. Mean levels of pH and conductivity are shown in Table 2.16. These values are not outwith the expected environmental range for this species.

Microhabitat O_2 levels.

On two occasions during the 1987/88 sampling programme, core samples were collected from the sediment and water column (Fig. 2.17, Table 2.17). The samples were collected when O_2 levels were estimated to be at their most extreme that is during the summer when O_2 levels are low and the temperature is high, and during the winter when O_2 levels are high and temperatures low (Fig. 2.17).

In the samples collected during the summer, O_2 levels in the water column dropped from a surface value of between 6.0 and 7.0 mgO_2/l to approximately 0.5 mgO_2/l at the mud water interface (Table 2.17, Fig. ,,, 2.17), with the sharpest drop occurring between 7.0cm above the mud and the mud surface. Between 3.0 and 5.0 cm below the mud surface O_2 levels fall off rapidly to zero (Fig. 2.17) By contrast, in winter samples the level of O_2 remains at about 10.0 mg/L throughout the water column down

Table 2.16 Mean values of pH and conductivity for the Ross Burn during 1984/1985 and 1987/1988 sampling.

Sample years	pH			Conductivity (uS)		
	Mean	S.E.	N	Mean	S.E.	N
1984/1985	5.43	0.16	11	91.09	1.93	11
1987/1988	5.58	0.29	7	87.47	6.36	7

Table 2.17 Oxygen concentration at different depths from core samples taken during the summer and winter at station 3.

Sample date: 20/6/88 Temperature = 19°C

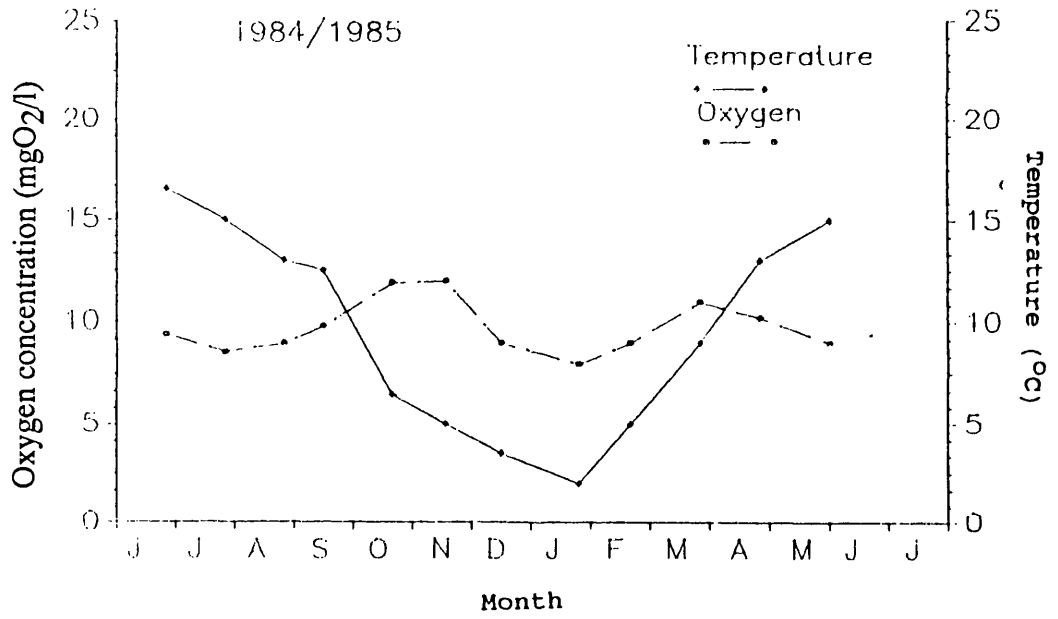
Core 1		Core 2		Core 3	
Depth cm	Oxygen mg/l	Depth cm	Oxygen mg/l	Depth cm	Oxygen mg/l
17.78	7.26	17.78	6.88	17.78	6.87
15.24	7.36	15.24	6.62	15.24	6.45
12.70	7.17	12.70	6.35	12.70	6.95
10.16	7.16	10.16	6.70	10.16	5.84
7.62	6.47	7.62	6.79	7.62	5.03
5.08	6.34	2.54	3.64	5.08	5.09
2.54	5.02	1.27	3.52	1.27	4.50
1.27	4.61	0	3.11	0	4.40
0	4.08	-1.27	0.32	-1.27	3.56
-1.27	1.14	-2.54	0.07	-2.54	0.009
-2.54	0.19	-3.81	0.009	-5.08	0
-5.08	0.083	*	*	*	*

Sample date: 4/10/88 Temperature = 9°C

Core 4		Core 5		Core 6	
Depth cm	Oxygen mg/l	Depth cm	Oxygen mg/l	Depth cm	Oxygen mg/l
17.00	9.70	20.00	10.39	16.50	8.49
15.00	9.74	18.00	10.27	13.00	8.43
10.00	9.43	16.00	9.70	9.00	9.12
6.00	9.45	12.00	9.70	5.00	8.77
5.00	9.81	7.50	10.04	3.00	9.72
2.00	9.35	2.00	10.04	2.00	9.93
1.00	7.04	1.00	9.90	1.00	9.70
0	7.18	0.50	9.70	0.50	9.47
-1.00	6.26	-0.50	7.50	0	8.89
-2.00	0.06	-1.00	4.80	-0.50	2.31
-3.00	0.023	-2.00	0.02	-1.00	0.023

Fig 2.17 Seasonal variation in oxygen levels and temperature in the Ross burn during the two sampling programmes. a) 1984/1985 b) 1987/1988.

Fig 2.17 a)


1984/1985
Temperature
Oxygen
Oxygen concentration (mgO2/l)
Temperature (°C)
25
20
15
10
5
0
J J A S O N D J F M A M J J
Month

b)

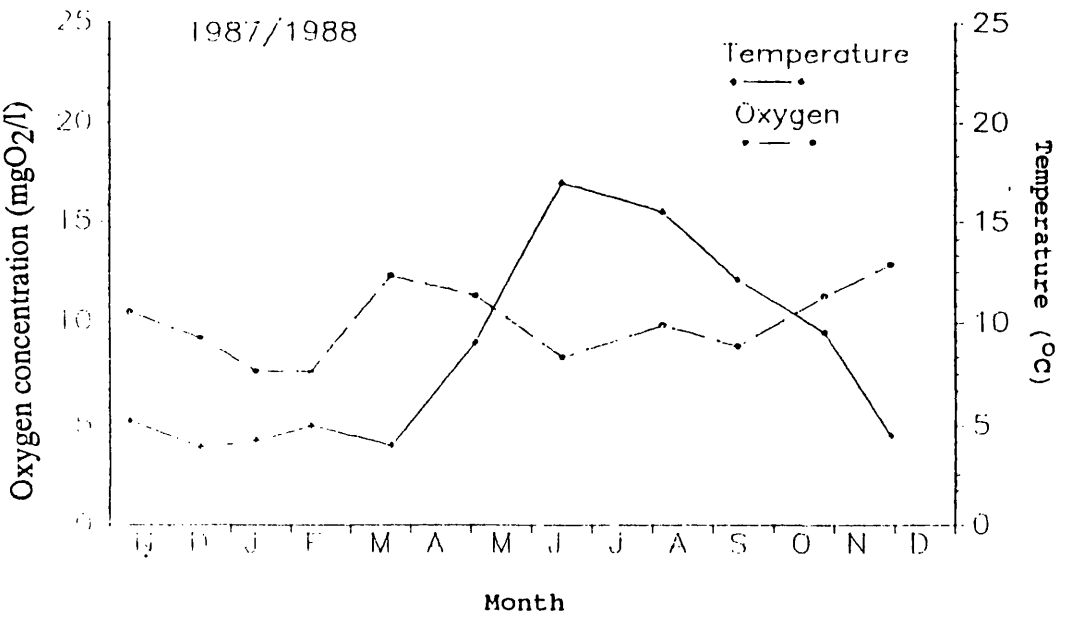
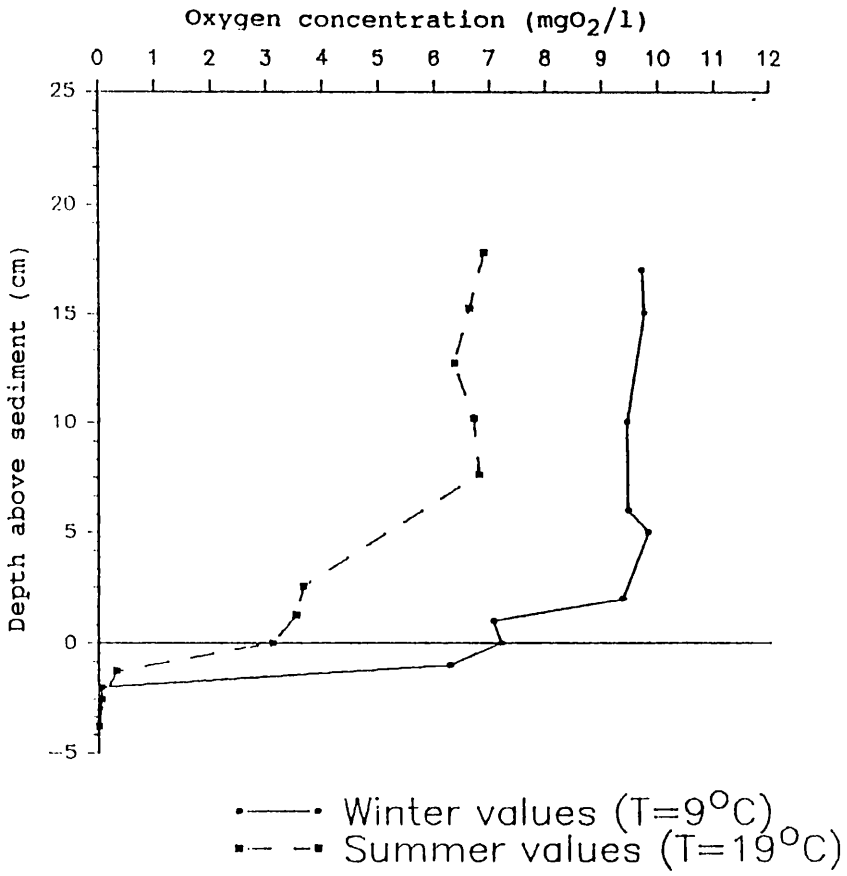

1987/1988
Temperature
Oxygen
Oxygen concentration (mgO2/l)
Temperature (°C)
25
20
15
10
5
0
J F M A M J J A S O N D
Month

Fig 2.18 Typical variations in microhabitat oxygen levels in the Ross burn during summer and winter (Table 2.15). Samples obtained using a Jenkins corer.

Fig. 2.18


Oxygen concentration (mgO_2/l)
0 1 2 3 4 5 6 7 8 9 10 11 12
25
20
15
10
5
0
-5
Depth above sediment (cm)
Winter values (T=9°C)
Summer values (T=19°C)

to 2.0 cm above the mud surface. Below the mud surface O_2 levels again fall off rapidly to zero at between 2.0 and 3.0 cm (Fig. 2.17, Table 2.17). These results are typical of the decline in oxygen levels in sediments (Revsbech, 1983).

More O_2 is available to larvae in winter than in summer throughout the water column but even in summer, levels of O_2 are greater than 5mg O_2/l. Thus it is unlikely that larvae living in the weeds, more than 5cm above the substrate will experience respiratory stress at any time of the year. Even at the mud surface and immediately beneath, O_2 levels are greater than 1 mgO_2/l in summer and greater than 6mgO_2/l in winter. Thus if P. nymphula larvae overwinter in this layer below the mud surface they would not normally be subject to respiratory stress. The respiration rates of P. nymphula larvae under conditions of anoxia are described in more detail in chapter 4.

2.4 Discussion

The main aim of the work described in this chapter was to examine the incidence of, and factors correlated with, lamellar injury in a wild population of P. nymphula larvae. The sources of lamellar injury that were suggested included predation, intraspecific aggression and loss during the moult (2.1.1).

2.4.1. The pattern of lamellar injury.

The most striking finding of the present study was the extent of lamellar injury in the study population. This confirms the findings of Baker and Dixon (1986) and Robinson et al. (1991) and shows that lamellar damage is a widespread phenomenon in wild zygopteran populations. The species studied to date: P. nymphula, I. verticallis and E. ebrium and Ischnura posita all belong to the Coenagrionidae and it is possible that in the Zygoptera the observed patterns of lamellar injury are restricted to this taxonomic group. Also significant was the finding that the frequency of lamellar injury varied according to larval size, sampling season and emergence group and larval size within habitat type.

Season and lamellar injury

The seasonal pattern of lamellar injury found in the study population differed from that reported by Baker and Dixon (1986) as follows (although comparison is difficult because of the semivoltine life cycle of P. nymphula). In the present study, the frequency of lamellar injury in the senior emergence groups was highest during spring and early summer, whilst in the junior emergence group it was highest in late summer and autumn. Baker and Dixon (1986) by contrast reported the highest rates of lamellar loss in I. verticallis and E. ebrium during late summer. In their study only one emergence group may have been considered. Baker and Dixon (1986) suggested that the main cause of lamellar injury and loss in these larvae was aggressive, intraspecific encounters. They

also suggested that the observed pattern of lamellar injury was related to larval dispersal during the spring and early summer, when aggressive encounters between larvae increased and consequently lamellar injury also.

Dispersal of overwintering larvae of the smaller junior emergence group is known to occur in P. nymphula populations. Larvae that have spent their first winter quiescent move into the weed in late spring and early summer (Corbet, 1957; Lawton, 1971a; Macan, 1964,1974). In the present study such dispersing larvae were found to have a high rate of lamellar injury at this time (Fig. 2.9), possibly due to increased aggression. As the summer of 1987 progressed the rate of lamellar injury in this emergence group fell off.

Larval density, habitat and lamellar injury

As in the study of Baker and Dixon (1986) there was no significant correlation between larval density and the frequency of lamellar loss. This is unexpected as a correlation between larval density and the number of aggressive encounters in some species of larval zygopterans is well documented (2.1.1). Robinson et al. (1991) for example, showed experimentally that lamellar injury was positively correlated with larval density but only in experimentally maintained populations where larval densities were artificially high. If lamellar injury is related to the number of aggressive encounters then it should also be correlated with larval density. The reason for this lack of correlation in both studies is probably due to the way larval density was measured in the wild (Baker and Dixon, 1986). In the present study larval density was expressed per unit sampling effort (Lawton, 1970a). Whilst this gives a reliable estimate for comparison between seasons for example, it does not necessarily give an accurate measure of true larval density because of the complexity of the habitat (Baker and Dixon, 1986; Lawton, 1970a). Larval distribution within the microhabitat may also vary according to larval hunger levels or the presence of predators (2.1.1). Thus without direct observation of

larvae it would not be possible to measure larval density accurately within the microhabitat.

Baker and Dixon (1986) suggested that when true larval density was high then larval encounters and lamellar loss would be more frequent and that this was thus a better estimate of true larval density than direct measurement. In the study population larval density was not found to be correlated with frequency of lamellar loss even between sampling stations (habitat types) and across seasons. If Baker and Dixon are correct then this result suggests that true larval density was not being measured accurately using the sweep net method. An alternative hypothesis may be that lamellar loss is not density dependent in this species.

A correlation was found between habitat type and the mean size of larvae with lamellar injury (2.3.3). Those larvae with intact lamellae which lived in the weed were significantly larger than those without lamellae. In contrast, in the mud habitat those larvae with lamellar injury were significantly larger than those with lamellae. The frequency of lamellar injury as described above was similar in all three habitats but in the weed small larvae tend to lose lamellae whilst in the mud large larvae loose lamellae. The significance of these findings is not clear but indicate that the causes of lamellar loss in the study population are complex and may vary according to habitat. For example, in the mud larger larvae may be more vulnerable to predation and thus lose lamellae more frequently whilst in the weed predation may be low whilst lamellar loss during contests with conspecifics may be greater.

Larval size and lamellar injury

Baker and Dixon (1986), reported that larvae without lamellae were smaller than those with them. This result is confirmed in the present study when all larvae are taken together. However, closer investigation of the data by emergence group shows that larvae with lamellae were not always larger than those without lamellae (Fig 2.12). In fact on

only four occasions during the summer sampling was the mean size of larvae with lamellae greater than that of those without lamellae (within emergence groups). This result suggests that the relationship between large larval size and lamellar injury may only be of importance during the summer and is consistent with the findings of Baker and Dixon (1986) that lamellar injury is related to the number of aggressive interactions between larvae. During the summer larvae are feeding and growing rapidly and there is the potential for more competition (2.1.1).

The reasons why larger larvae should be more susceptible to lamellar loss during the remainder of the year are not clear. Although this result was not statistically significant on all the remaining sampling occasions, on average larvae without lamellae were larger. When considered along with the finding that the risk of lamellar loss also varies according to larval size and habitat type, this result suggests that other factors such as predation may be responsible for some lamellar loss.

Because larval size was correlated with the frequency of lamellar injury it was expected that a significant correlation would also be found with the size range of larvae within emergence groups. However, no such correlation was found here, which suggests that interactions between larvae of the same emergence group do not result in a significant amount of lamellar injury. This result is not consistent with the suggestion that rates of lamellar injury are correlated with the number and type of aggressive encounters between larvae. One explanation for this could be that the analysis (2.3.3) included larvae from all sampling occasions. Whereas the crucial period for lamellar loss may only be during the summer months when larvae are growing, as described above. An insufficient number of samples were collected to allow analysis on the summer samples only.

Predation by large larvae of small ones was also predicted when both the senior and junior emergence groups were present in the habitat at the same time. In the study

population this occurred only in the spring and autumn samples. On these occasions there was a high frequency of lamellar injury found in the smaller (junior) emergence group. In addition analysis showed that smaller cohorts had a significantly higher frequency of lamellar injury than large cohorts (2.3.3). Intercohort predation by larger larvae of smaller larvae (as found by Robinson et al., 1991) may account for the lamellar injury in these smaller cohorts. However, because little is known of the location of these emergence groups within the microhabitat at these times it cannot be assumed that because larvae were collected in a sweep net sample they were in the same part of the microhabitat and interact in this way (2.1.1). It is possible other factors, that selectively cause the loss of lamellae in smaller emergence group, are responsible.

The lack of a statistically significant correlation between the size of injured larvae and larval gender suggests that the risks of losing lamellae are the same for both male and female larvae and also that both sexes grow at a similar rate. These results do not support the suggestion of Anholt (1990) and Baker (1990) that gender may influence larval behaviour and possibly growth in this species.

2.4.2 Causes of lamellar loss, and lamellar function.

It was suggested in the introduction that lamellae may be lost either during the moult, during aggressive encounters with conspecifics or during attacks by a predator when the lamellae may act as threat displays or as autotomous defence organs. The results of the present study are considered here in terms of these three possible causes of lamellar loss.

1) Lamellar loss and larval moulting

Loss of lamellae during moulting was reported in captive, isolated larvae of P. nymphula by Corbet (1950) and in captive populations of Ischnura posita (Robinson et al., (1991), and will be the source of some of the lamellar injury observed in the present study population. However, the highest frequencies of lamellar injury occurred during

spring and early summer in the larger (1987 and 1988) emergence groups and in the late autumn in the junior (1988) emergence group. Growth of these cohorts occurs only during summer. Wounds to lamellae will take longer to heal during the winter when growth is slow than during the summer when growth and regeneration is fast. This should result in a bias towards recording lamellar injury during the winter (Baker and Dixon, 1986). The fact that most lamellar injury is observed in spring and early summer when it should be hardest to detect suggests that actual rates of injury may be higher than those reported. Therefore the highest frequencies of lamellar injury did not occur when moulting loss of lamellae would be expected. Although, it is possible that lamellar injury in the senior emergence group during the spring may be due to moulting. Some larvae in this group are in the penultimate and antepenultimate instars at this time and have to grow rapidly and moult before emerging.

2) Lamellar injury and predation.

Predation, including that by larger conspecifics, was suggested here as a potential source of lamellar injury in the study population. If so then rates of lamellar injury should be highest when rates of predation on larvae are highest. The rates of lamellar injury would however vary according to whether the predation was interspecific or intraspecific.

Predation

It is difficult to determine when rates of predation of P. nymphula larvae are highest because of the many factors that influence rates of predation in fresh water invertebrate communities. Of major importance are the type of predator, its foraging strategy and the evasion response employed by the prey (Kerfoot and Sih, 1987). It is likely that lamellae are important for predator evasion. For example, Robinson (1990) describes the different effects of a sit and wait predator and a stalking predator on a laboratory population of Ischnura posita larvae both with and without lamellae. In his

experiment those with lamellae were able to avoid a stalking predator but not a sit and wait predator. Robinson suggested that the larvae had an effective swimming escape response from the actively foraging predator, whilst movement of the larvae made them more susceptible to predation by the sit and wait predator. There are many examples in the literature of the ability of zygopteran larvae to detect specific types of predator and modify their behaviour accordingly (Heads, 1985; Johnson, 1991; Wellborn and Robinson, 1987).

The main predators of zygopteran larvae are poikilotherms (Thomson, 1982), and consequently their predation rate will be related to water temperature and be highest during warm summer months (Baker and Dixon, 1986; Banks and Thomson, 1987). The potential predators of P. nymphula larvae that were found in the study site were trout (Salmo trutta), sticklebacks (Gasterosteus aculeatus), palmate newts (Triturus helveticus) and invertebrates including Cordulegaster boltonii (Odonata) and Notonecta sp (Hemiptera). Although the diets of these species were not examined, they are known to consume invertebrates of a similar size to P. nymphula larvae (Hynes, 1972).

The highest frequency of lamellar loss occurred in spring in the senior (1987) emergence group and autumn in the junior (1988) emergence groups. Neither of these peaks are correlated with high water temperatures which reached a maximum in August of that year (1988). Thus temperature-related predation of the type suggested is not likely to be a major cause of lamellar injury in the study population. Rates of lamellar injury in the senior emergence groups did reflect day length reaching a peak during the longest days in June. It is possible to speculate that larvae may be more susceptible to predation (including that by conspecifics) and consequent lamellar injury because longer day length allows a longer foraging time.

If loss of larvae from the sampling site was mainly due to predation then such reductions should have occurred when predation was highest. The largest reduction in

larval numbers was in senior (1987) emergence group larvae during late summer and autumn (1988) (risk from predation at this time may also increase due to seasonal reduction in the amount of vegetation). Thus if lamellae were lost during predator attacks, then the rate of lamellar injury should have reflected the rate of larval mortality. However, the peak period of lamellar injury occurred during spring and early summer in the senior (1987) emergence group. Whilst in the junior (1988) emergence group the peak rate of lamellar injury occurred during the late autumn when larval numbers were stable. These results therefore suggest that lamellar loss may not be predation rate dependent.

Intraspecific predation

A number of authors have shown that intraspecific predation of small larvae by large larvae can be a major source of mortality in wild populations (Fisher, 1961; Johnson et al., 1985; Merril and Johnson, 1984; Wissinger, 1987). In some species this type of predation is greatest if the size difference between coexisting cohorts is large (Wissinger, 1987). Adaptation such as spatial separation of cohorts may help to reduce this predation (Lawton, 1970a; Macan, 1964,1974).

Because of the semivoltine life history of P. nymphula larvae at the study site, there were two occasions when such predation may occur. Firstly when both senior and junior emergence groups are present in the same habitat at the same time and secondly, when the size range of larvae within an emergence group is greater than 2 instars. The results suggest that this type of predation may be occurring at the study site. Firstly, because when the size range of larvae within a cohort is large, rates of lamellar injury are high for example, during summer. Secondly, because when the two cohorts were present in samples at the same time then rates of lamellar loss in the junior cohort were also high. Although, for neither of these results were sufficient data collected for statistical analysis. Finally, the junior cohorts were also significantly more susceptible to higher rates of

lamellar injury overall than the senior ones. Thus it is possible to speculate that the observed lamellar injury in the junior cohorts is due to attacks by larger conspecifics.

3) Lamellar injury and intraspecific aggression.

Lamellar loss may occur as a result of aggressive encounters between larvae (other than predation by conspecifics) (2.1.1). It was predicted that if the lamellae of P. nymphula function as threat displays or autotomous defence organs then the rate of injury to and loss of these organs should be highest when rates of intraspecific aggression are high for example when larvae are competing for resources (2.1.1). The results reported here are consistent with lamellae being injured during intraspecific conflicts. For example, lamellar injury in the study population was correlated with large larval size and larval size within habitat types. The seasonal pattern of lamellar injury also indicated that lamellar loss, at least in the senior emergence groups may be related to larval dispersal. However, there were findings inconsistent with this hypothesis. For example, there was no correlation between cohort size range and lamellar injury and only during the summer and in the weed habitats were small larvae more likely to lose lamellae. Thus although intraspecific aggression may account for the lamellar loss during the summer, other factors such as predation may account for lamellar injury at other times of the year.

2.4.3. Life cycle

The life cycle of P. nymphula larvae from the Ross burn sampling site is similar to that recorded elsewhere in the U.K. for this species by Corbet (1957a), Harvey (1985), Lawton (1970a) and Macan (1964,1974). The majority of larvae take two years to reach emergence and there are two well defined cohorts in samples collected throughout most of the year. As in previous studies by Corbet (1957a), Lawton (1970a) and Macan (1964,1974), the newly hatched junior emergence group larvae were too small to be collected in samples until September. During their first winter of development these larvae were absent from samples. At this time they burrow into the mud between the roots of vegetation (Lawton, 1970a). Lawton (1970a) and Macan (1977), suggested that

this behaviour may allow larvae to avoid predation, especially by the larger instars, during the winter, and may also reduce the risk from freezing as the substrate, in comparison to the water column, is relatively well buffered thermally (Duffy and Liston, 1985).

Dispersal of the junior cohort larvae from their winter quiescence coincides with adult emergence of the senior cohort in May and June and may reduce predation by the senior cohort larvae on the junior cohort larvae (Macan, 1977). In their second year of development larvae grow rapidly and reach the final or penultimate instars by autumn. Senior cohort larvae diapause over the winter remaining on the weeds or on the substrate surface.

Larval growth

Growth of larval cohorts is highly synchronised for most of the year, although during their second summer of development there is a two to three-fold difference in size between the smallest and largest larvae in the senior cohort. Maximum growth of larvae occurs between May and October when the water temperature is above $11^{O}C$, which was also reported in this species by Lawton (1970a). Other species of zygopterans have also been shown to grow only above certain temperatures for example, Ischnura elegans (Banks and Thomson, 1987; Thomson, 1978), Enallagma boreale (Procter, 1973) and Coenagrion puella (Banks and Thomson, 1987).

In samples collected during the winter and autumn of both years of the sampling programme, some larvae intermediate in size between the senior and junior cohort were collected. Such intermediate sized larvae have been reported in populations of P. nymphula by previous workers such as Lawton (1971a) and Macan (1964,1974). Macan (1974), suggests that these larvae were either fast growers from the current years hatch, or slow growers from the previous years hatch. Larvae take three years to reach emergence if growing slowly or one year if growing quickly. Macan found that growth

rate was dependent on population density and habitat. When larvae were abundant competition for food would be greatest and growth would be reduced resulting in more larvae taking three years to reach emergence. Food shortage has been cited as a common cause of increased life cycle duration in larval odonates (Baker, 1982,1986; Johnson, et al., 1985, Lawton et al., 1980). Macan (1964, 1974) also showed in his study of a P. nymphula population that food availability varied according to habitat. Larvae that lived in the sedge Carex sp. which is regarded as a poor habitat took two or three years to reach emergence, whilst those in the weed Litorella sp. a rich habitat, took one or two years to reach emergence.

It is not certain whether the intermediate sized larvae found during winter sampling were slow or fast growers. It is unlikely that they were fast growers from the junior cohort because when they appear in samples these intermediate sized larvae are considerably larger than the junior cohort (1988) larvae. However, they do correspond in size to the smallest of the senior (1987) cohort larvae. Thus it is more likely that they are slow growers from the senior 1987) emergence cohort. Reduced growth rates may account for these intermediate sized larvae and the lack of synchrony within cohorts.

Failure to detect differences in the growth rate of larvae in the different habitat types of the study site may be due to the nature of the site. In contrast to the study sites of previous workers this site contained flowing water. This meant that food would be carried through the site in downstream drift (Hynes, 1972). If P. nymphula larvae intercept prey in the flow of water then it is likely that the prey would be evenly available throughout the study site. Consequently larval growth would be similar in the different weed types. Movement of larvae within the study site could also confuse comparison of larval growth rates between sampling stations. P. nymphula larvae tend not to move far after dispersal (Lawton, 1971). However, the water flow at the study site may have carried larvae downstream.

Mortality

Larval mortality was estimated to be highest during the late summer and autumn of their second year of development. Larval numbers reduced slightly throughout the winter indicating constant low mortality. Because of the limitations of the sampling technique it was not possible to determine the mortality rate of larvae less than one year old. Reduction in the number of senior cohort larvae at this time was probably due to mortality rather than to a habitat shift or movement away from the sampling stations. Lawton (1970a) and Macan (1964,1974) found little lateral movement of P. nymphula larvae within their sampling sites. Seasonal, density dependent mortality has been reported and may be common in dragonfly populations (Banks and Thomson, 1987), though Lawton (1970a) found no seasonal mortality pattern in his study of a population of P. nymphula. Mortality patterns are clearly variable between sites. The most likely cause of mortality in the study population is predation rather than starvation as starvation is considered to be an unlikely cause of death for dragonfly larvae (Baker, 1982; Lawton et al., 1980; Wissinger, 1987).

Larval sex ratios

The ratio of male to female larvae in this study was found to be 0.5 which indicates that there is no sex bias in the population. Other studies have found a significant sex bias in the population at or prior to emergence. Lawton (1972) for example, found more males than females in a population of P. nymphula. There are clearly differences in sex ratios between populations. There was also no statistically significant difference in the mean size of male and female larvae within emergence groups which indicates that growth rates of both sexes are the same at the study site. Therefore it is not necessary to differentiate between male and female larvae during analysis of the growth rates of larvae with and without lamellae.

2.4.4 Environmental conditions

Levels of oxygen throughout the year in the sampling site were high. However, microhabitat oxygen levels did decline to zero usually 1-2cm below the surface of the mud. These levels should not present respiratory problems for larvae in the water column however, larvae that overwinter in the mud may encounter oxygen stress below this depth. Lawton (1970a) found that junior emergence group larvae of P. nymphula overwintered in "oxygen rich" mud amongst the roots of the vegetation although he provided no values for these oxygen levels. In the present study the location of these overwintering larvae was not investigated. It is evident that if these larvae are within two to three cm of the mud surface then sufficient oxygen should be available. The oxygen requirements of these larvae may in any case be low. Duffy and Liston (1985) found that larvae of Enallagma boreale overwintering in the mud greatly reduced their respiration rates which is consistent with a quiescent species.

2.4.5. Conclusions.

The results of the present study confirm that lamellar injury is a widespread phenomenon in a third species of coenagrionid zygopteran. It is also confirmed that small larvae are generally more susceptible to lamellar injury than large larvae. Although this depends on the time of year and the type of habitat in which the larvae live. Failure to find a correlation between lamellar injury and larval density was possibly due to the method of estimating density. These results are compatible with the suggestion of Baker and Dixon (1986), that lamellae are injured and lost during aggressive encounters between larvae.

Because larvae grow only during the summer lamellar injury may be due to aggressive encounters with conspecifics only at this time. It is suggested that other factors such as predation during the remainder of the year may be responsible for lamellar injury, although it was not possible to estimate predation rates. These results are

harder to interpret than those of Baker and Dixon's (1986) study because of the semi-voltine life cycle of P. nymphula and the possibility of predation or interference of the larger emergence group on the smaller one. Predation though not investigated directly in the present study was not discounted as a source of lamellar injury. For example, it may be responsible for the lamellar injury of larvae in the mud habitat during winter when larvae are not growing. The pattern of lamellar injury is inconsistent with moulting as the main source of lamellar injury.

These results when considered in conjunction with those of Baker and Dixon (1986) and Robinson et al. (1991) strongly suggest that lamellar injury in P. nymphula is the result of interactions with conspecifics and predators. If so, further evidence for this may be found by investigating their morphology, physiology and function during aggressive encounters.

CHAPTER 3

THE STRUCTURE OF THE CAUDAL LAMELLAE OF Pyrrhosoma nymphula.

3.1 Introduction

In the previous chapter it was shown that in a wild population of P. nymphula lamellar damage occurred with a significant frequency. This damage may be the result of lamellae being deployed as attack deflectors or as threat displays (2.4) and suggests that autotomy may be an important function for these organs. At least four other functions have been suggested for the laterally flattened caudal lamellae. These include sensory, (Norling, 1982; Watson, 1966), locomotory (MacNeill, 1960), osmoregulatory (Eriksen, 1986) and respiratory (gill) (Eriksen 1986). Although the generally accepted function of these structures is that of a (gas exchange gill) (Chapman, 1980; Mill, 1974; Tillyard, 1917a). It is also possible that the lamellae have more than one function (Corbet, 1962).

Apart from the studies by Tillyard (1917a) and MacNeill (1960) there have been no detailed investigations of the morphology or ultrastructure of laterally flattened coenagrionid type caudal lamellae. The comprehensive study carried out by Tillyard (1917a) is still the only extensive account of the structure of these organs, but this work was limited by the technical facilities available at the time. On the other hand, the study by MacNeill (1960), was limited to an investigation of the general morphology and development of the lamellae and he did not investigate the ultrastructure of these organs. A more detailed investigation and description of lamellar morphology is essential to the understanding of their function. In this chapter the morphology of the lamellae of P. nymphula will be investigated with particular attention to their possible gas exchange role.

3.1.1 Lamellar types and structure.

Lamellar types.

All members of the sub order Zygoptera have three caudal lamellae which are attached to the tenth (terminal) abdominal segment, and which are the result of a hypertrophy of the fused epiprocts and paraprocts (MacNeill, 1960; Tillyard, 1917a; Watson, 1966). The fused paraprocts form the ventral, median lamella and the epiprocts the lateroventrally placed lateral lamellae (Tillyard, 1917a). In anisopteran dragonflies the epiprocts and paraprocts form the anal valve (Mill, 1974) or pyramid (Tillyard, 1916). The morphology of the lamellae can be used as a taxonomic character, but their loss and partial regrowth makes them unreliable unless fully formed (Tillyard, 1917a). Reviews of the historical accounts of the morphology of caudal lamellae and the origin of the terminology have been given by previous workers (for example, Bodine, 1918; Corbet, 1962; Tillyard, 1917a). The earliest descriptions of the typical laterally flattened caudal lamellae were given by Reaumur (1748) who referred to them as fins, and Roesel Von Rosenhof (1749) who called them "rudder feathers". Carus (1827) was the first worker to describe them as gills ("gill-like leaves") and from this date the term gill has been generally applied.

Three basic forms of lamellae have been described, namely saccoid, triquetro-quadrate and flattened (Tillyard, 1917a). Although MacNeill (1960), preferred to classify them as either simplex or duplex. The simplex and duplex types corresponding to Tillyard's (1917a) unjointed and jointed forms. Saccoid lamellae form tapered cylinders with a circular cross section (Norling, 1982; Tillyard, 1917a). There are two types, the constricted saccus, found for example in the Protoneuridae, and the simple saccus, found in the Epallagidae. The function of this type of lamella is not known. Tillyard (1917a) speculated that they may be an adaptation to a rheophilic life style; whilst Norling (1982) suggested that the simple saccoid lamellae found in the Epallagidae are primarily sensory with a secondary gas exchange role.

Triquetro-quadrate lamellae are found only in the Calopterygidae (Tillyard, 1917a). The two lateral lamellae are triangular whilst the median is quadrate in cross section. This arrangement is considered to be intermediate between the saccoid and laterally flattened lamellar types (Tillyard, 1917a).

Laterally flattened lamellae are the commonest type found and are usually used to illustrate the typical caudal lamella. Tillyard (1917a), described two types of flattened lamellae, dorsoventrally flattened and laterally flattened. The dorsoventrally flattened type are found only in Argiolestes sp. and are considered to be an adaptation to improve adhesion to the substratum in fast flowing water (Corbet, 1962; Tillyard, 19717a).

Laterally flattened lamellae are found in the remaining groups of Zygoptera including P. nymphula. Tillyard (1917a), classified laterally flattened lamellae into unjointed forms, found for example in Lestid larvae and the jointed forms found for example in the Agrionidae. Within these two broad groups many different forms are found (see Askew, 1988 and MacNeill, 1960). Accordingly Tillyard subdivided the jointed forms of lamellae into, α constricted, β nodate, τ subnodate and δ denodate vertical lamellae. A further characteristic feature of lamellae is their distinct pattern which takes the form of dark chevron bands or lines (MacNeill, 1960). These markings may be important for signalling to conspecifics (Johnson, 1991).

The lamellae of P. nymphula were classified by MacNeill, (1960) as (weakly) nodate and typical of the Coenagrionid type lamellae. Accounts of the general shape of the lamellae of final instar larvae of this species have been given by Askew (1988) Corbet (1955) Gardner and MacNeill (1950) Lucas (1900) and Merrit (1984), whilst lamellar development in this species has been described by Gardner and MacNeill (1950).

Lamellar ontogeny

It is possible that the function of lamellae change during larval development (Corbet, 1990; Johnson, 1991; MacNeill, 1960). Laterally flattened lamellae change in shape, colouration, and size during larval growth (MacNeill, 1960). In young larvae the lamellae are generally lanceolate and become flattened and blade like in older larvae (MacNeill, 1960; Tillyard, 1917b). The development of the lamellae in all species follows a similar pattern. From hatching to about instar six, lamellae are ferullar and rigid, the median lamella is usually quadrate in section and the lateral ones triangular. During development they become laterally flattened assuming their familiar blade like shape (MacNeill, 1960; Tillyard, 1917b). According to MacNeill (1960) two distinct patterns of growth are found in caudal lamellae, depending on whether the species has simplex or duplex lamellae. The simplex lamella grows by simple proportional expansion of the whole organ, whilst the duplex type grow by disproportionate expansion of the postnodal region (MacNeill, 1960). MacNeill (1960) suggested that this pattern of growth may be adaptive and increase gas exchange efficiency (3.1.3).

In many species dark pigmentation of the hypodermis has resulted in distinctive patterning (Tillyard, 1917). Johnson (1991) suggested that the development of a pattern on the lamellae of Xanthocnemis zealandica coincided with the development of the ability of larvae to detect this pattern. He suggested that at this stage of development the lamellae may become important for signalling to conspecifics. In chapter 2 of the present study it was found that rates of lamellar injury varied according to instar. Corbet (1990) suggested that in some species the change in shape of lamellae from ferrular to lamellate during larval development may be associated with a change in larval behaviour towards conspecifics.

3.1.2 Lamellar structure and function

Comparative morphology can be used as an aid to identifying the function of an organ. In the present study it is proposed to examine in detail the morphology of the

caudal lamellae of P. nymphula and to determine how this relates to their possible functions (sensory, locomotion, defence autotomy/threat display, osmoregulatory or gas exchange). The lamellar morphology of P. nymphula will be examined for characteristics which may provide evidence for any of these functions. Emphasis will be placed on investigating the respiratory morphology as this is their generally accepted function.

Sensory role

A sensory role was suggested for the modified lamellae found in the Amphipterygidae (Watson, 1966), and the saccoid lamellae of the Euphaeidae (Norling, 1982). This role was suggested because the lamellae of these species are covered by a dense layer of setae. In addition, and indicating acceptance that the lamellae are gas exchange gills also, both authors felt that a sensory function was possible in these species as they had "alternative" sites for gas exchange: the lateral abdominal gills. The use of caudal organs for predator detection occurs in other species of freshwater insect. In some stonefly (Plecoptera) species for example, the caudal organs may be used to detect predators either by mechanoreception or chemoreception, the mechanism by which this is achieved is not fully understood (Peckarsky, 1987). The laterally flattened lamellae of some zygopteran species could fulfil a similar role. If the lamellae of P. nymphula function in this way then they should have a significant number of sensory organs on their surfaces.

Locomotion

The possibility that laterally flattened lamellae may act as fins, rudders or "parachute brakes" has not been investigated. MacNeill (1960), who suggested the latter two functions, also discounted any locomotory function maintaining that zygopteran larvae rarely swim and that when they do it is a "laboured wriggling". MacNeill (1960) did not provide any experimental evidence to confirm this assertion and it is not clear how effective lamellae would be in such a role. Robinson (1990), suggests that in

Enallagma civile larvae, the lamellae may allow larvae to swim away from a foraging predator. If the lamellae of P. nymphula function primarily as fins or paddles then their structure should reflect this function.

Defence autotomy and threat display

The possible role that lamellae may have during aggressive encounters between conspecifics and predators is discussed elsewhere in the present study (Chapter 2, Chapter 5). Here only the morphological criteria for effective deflection or autotomy and threat/display organs will be reviewed.

Extensive study has been made of the function of autotomous organs in lizards and other species (Chapter 2.1; Cooper and Vitt, 1991). Effective autotomous organs deflect attacks away from the more vulnerable regions of the body. The deflective organs are either impervious to attack or can be sacrificed with little cost to the animal. If sacrificed then the organ is lost at a specialised fracture point and in a predetermined way to minimise damage (Arnold, 1984, 1989; Bellairs and Bryant, 1985). The structure and location of individual autotomous organs varies according to the species but all have a characteristic structure. That is, large area and distinctive patterning or colouration to make them more visible, the ability to be moved to attract attention and are attached to the animal by a joint which allows autotomy (Arnold, 1988; Bellairs and Bryant, 1985; Cooper and Vitt, 1991).

The morphological descriptions of lamellae provided by previous authors include characteristics that may be compatible with a defensive role. Firstly the markings, in species such as Ceragrion glabrum Burm the lamellae have distinctive dark chevron banding, whilst P. nymphula has a distinctive dark cross mark on its lamellae (MacNeill, 1960). MacNeill (1960) suggested that these patterns may act to camouflage the lamellae but could also increase their visualisation. Secondly, lamellar surface area, lamellae are accepted as having a large surface area for their bulk (3.1.3). During growth lamellae

shorten relative to body length, whilst vertical width has been shown to increase in some species and may indicate a changing function during growth. For example Corbet (1955), showed that lamellae of Coenagrion mercuriale(Charp) decreased from about 40% to 25% of total larval length from instars six to thirteen. The relationship between the surface area of the lamellae and that of the body during development has not been investigated in detail though Erickson (1986) estimated the surface area of the caudal lamellae of final instar larvae of Ischnura sp. to be approximately 60% of the total body surface area.

Thirdly, a good indication of an organ adapted for autotomy is the presence of a specialised fracture point which would allow the lamellae to be detached with little damage to the larva. Lamellae are connected to the last abdominal segment by a basal piece and are autotomised at this point of weakness (the breaking joint) (Tillyard, 1917a). According to MacNeill (1960) the breaking joint is then sealed by a sphincteral action to prevent loss of body fluids and water entering the tracheal system. Regeneration occurs from the surface of the breaking joint usually taking two to three moults to attain full size (Child and Young, 1903; MacNeill, 1960). The degree of regeneration depends on the delay between loss and the following moult (Child and Young, 1903). If the lamellae are damaged and are unable to be autotomised then the larvae will die, presumably through loss of body fluids (Eriksen, 1986). The function of this breaking joint has never been investigated.

Ion uptake

Ion uptake was suggested by Eriksen (1986) as another possible role for the lamellae. Ion uptake organs have been found in many species of fresh water insect and either resemble or are associated with respiratory gills. For example, the chloride epithelia of the anal "gills" in Aeshna sp. (Komnick, 1982; Kukulies, and Komnick, 1983) and the porous plates of mayfly nymphs (Filshie and Campbell, 1984). Eriksen

(1986) discounted this as a primary function of the lamellae, as their loss would render the larva unable to regulate its water balance, which would result in its death. Wichard and Komnick (1974a), have also shown that the main site of chloride ion uptake in Coenagrion sp. larvae is in the rectum. Laterally flattened lamellae have never been investigated for the presence of ion uptake organs or epithelia. The presence of such organs on lamellae would indicate that this is their main function.

Respiration

Most general texts on insect biology describe the caudal lamellae as respiratory gills. Because this is their generally accepted function investigation of their potential respiratory morphology will form the main part of this study. However, a respiratory gill function has never been clearly demonstrated for the lamellae of any zygopteran species by investigation of their ultrastructure, although the relative efficiency of lamellae as respiratory gills has been the subject of debate (Eriksen, 1986; MacNeill, 1960; Pennack and McColl, 1944). The evidence in favour of a respiratory function is not convincing. Some authors suggest that it is not their main function (Bodine, 1918; Tillyard, 1917a). Most support for the respiratory gill theory comes from physiological studies which are reviewed in chapter 4. These other experimental investigations do not provide unequivocal support for this theory. As so little study has been carried out on lamellae a review of the anatomical characteristics of gas exchange gills in other freshwater insect groups will be presented here. This will form the basis for a comparison with the structure of the lamellae of P. nymphula to determine their degree of respiratory adaptation.

3.1.3 Respiratory adaptation in aquatic insects.

Oxygen uptake in fresh water

One of the main problems facing animals that colonise fresh water is the change in availability of oxygen (Clegg, 1986; Davis, 1975; Gaufin, et al., 1974; Krogh, 1941;

Wiley and Kohler, 1984). In fresh water levels of oxygen can vary even within a habitat and seldom reach saturation values (Clegg, 1972). This variation in the oxygen content of the water is due to physical factors such as temperature, salinity, atmospheric pressure, degree of mixing and to biological factors, such as respiration and photosynthesis of plants in the water (Taylor, 1985; Wiley and Kohler, 1984). Of these factors, temperature has the greatest effect on the environmental oxygen levels experienced by aquatic insects (Mill, 1972; Wiley and Kohler, 1984).

Consequently, a wide range of respiratory adaptations are found in aquatic insects, these include, tracheal gills, siphons and respiratory pigments. Perhaps the commonest respiratory adaptation in the groups of fresh water insects with closed (apneustic) tracheal systems (including the Odonata), is the tracheal gill (Chapman, 1980; Krogh, 1941; Mill, 1972, 1974; Wigglesworth, 1983). In these species the spiracles are closed to the environment and are functionless, oxygen enters the body by diffusion across the gill and body cuticle (Mill, 1972, 1974; Wichard and Komnick, 1974a). These gills all have a similar, and characteristic structure which was first described by Krogh (1941) and more recently by Wichard and Komnick (1974a+b). If the structure of the lamellae of P. nymphula has similar or comparable characteristics then this will be a good indication of a respiratory function.

Gas exchange by tracheal gills

The tracheal gills of the fresh water insects which have been studied all have a characteristic and similar structure, that is thin cuticle, dense hypodermal tracheolation and a low metabolic oxygen demand (Dejours, 1975; Krogh, 1941; Mill, 1972, 1974). Oxygen enters the gill by diffusion across the cuticle into the underlying tracheolar plexus (Krogh, 1941; Mill, 1972; Wigglesworth, 1983). The tracheoles and associated tracheoblast cells are located within intracellular invaginations of the hypodermal cells (Wichard and Komnick 1974a; Wichard, 1973; Wigglesworth, 1983). Oxygen diffuses

from the tracheoles into the main tracheal trunks, and then to the respiring tissues (Krogh, 1941; Mill, 1972; Wigglesworth, 1983).

The amount of oxygen entering the tracheal system, depends upon the rate at which oxygen diffuses across the cuticle, into the tracheoles (Krogh, 1941; Mill, 1972, 1974). The relationship between the factors that influence this diffusion rate is shown in Krogh's diffusion equation (equation 3.1) (Dejours, 1975; Krogh, 1941; Mill, 1972, 1974; Taylor, 1985; Wichard and Komnick, 1974a).

$$\frac{dm}{dt} = -K F \frac{dp}{dx} \qquad \text{equation 3.1}$$

In this equation dm/dt the oxygen flux depends on 1) F the surface area of the gill 2) dp the difference in partial pressure over 3) the diffusion distance (dx) and 4) -K, Krogh's diffusion constant (Dejours, 1975; Wichard and Komnick, 1974a). This diffusion equation defines the physical limits within which adaptation of an insect's tracheal gill must occur. These limits have resulted in remarkable convergence in the structure of insect tracheal gills. In these gills functional efficiency is achieved by an increase in F or dp, and a decrease of dx in equation 3.1. The way in which these four adaptations have been used to improve the efficiency of insect tracheal gills are as follows:-

1) The constant K in equation 3.1, depends on the diffusion characteristics of the tissue, in this case the gill cuticle and hypodermis, and it is generally regarded as a constant (Dejours, 1975; Krogh,1941; Wichard and Komnick, 1974a). However, Beament (1961), showed that the lamellar cuticle of Coenagrion sp. (Odonata) had a higher permeability to water than that of the abdomen at relatively low temperatures. Beament suggested that this would result in a similar difference in permeability to oxygen. He related this to

differences in the structure of the waterproofing lipid layer of the cuticle, although it is not clear to what degree factors such as variable cuticle thickness may have influenced this result.

2) The oxygen flux depends directly on the surface area of the organism. The greater the surface area to volume ratio the greater the amount of oxygen that can enter the animal (Dejours, 1975; Krogh, 1941). Tracheal gills characteristically have a large surface area and a low volume (Mill, 1972; Wichard 1973; Wichard and Komnick, 1974a). The gill surface area of a particular species is related both to its oxygen requirements and to the range of oxygen concentrations found in its normal environment. For example Dodds and Hisaw (1924) showed that the gill surface area of several mayfly species was inversely proportional to the oxygen content of their environment, those species that lived in environments with normally high oxygen concentrations had smaller gills than those from environments with generally low oxygen concentrations. Corbet (1962) suggested a similar trend may exist with the size of lamellae in members of the Hawaiian genus megalagrion (Zygoptera). In the present study the oxygen content of the collection habitat was monitored for comparison with other studies (Chapter 2).

3) The efficiency of a tracheal gill is also increased by decreasing the diffusion distance for oxygen entering the gill, dx in equation 3.1 (Wichard and Komnick, 1974a). The oxygen flux in the gill is inversely proportional to the diffusion distance (Dejours, 1975; Krogh, 1941; Wichard and Komnick, 1974a). In tracheal gills this reduction is achieved in two ways, either by packing the tracheoles close to the cuticle or by reducing the cuticle thickness (Wichard and Komnick, 1974a). Because the oxygen must diffuse not only across the cuticle but also through the hypodermis and into the lumen of the tracheoles the efficiency of tracheal gills depends also on their ability to trap the oxygen once it has diffused across the cuticle. The arrangement of the tracheoles in a tracheal gill is therefore of critical importance. If the tracheoles in the hypodermis of the gill are spaced too far apart then oxygen diffusing in will be effectively lost to the dead spaces

between the tracheoles and efficiency will be reduced (Wichard and Komnick, 1974a; Wichard, 1973).

Wichard and Komnick (1974a) and Wichard (1973) showed that the arrangement of tracheoles in the tracheal gills of a range of insect species was similar and fell between two extreme types of organisation. These relied either on an excess or a minimum of tracheoles and is achieved during the moult when the tracheoles penetrate the hypodermis (Wichard, 1973). In the arrangement with high tracheolar densities, the tracheoles are densely packed and arranged randomly with the space between them never greater than twice their diameters. The diameter of the tracheoles varied between 0.2 and 1.0 μm and the diffusion distance was about equal to the thickness of the gill cuticle. In the arrangement using low tracheolar density, the tracheoles are evenly and regularly spaced, equal in size and have a similar diameter of about 0.2 μm. According to Wichard (1973) the diffusion catchment of the tracheoles is about equal to the distance between each tracheole and is constant throughout the gill. The diffusion distance with this arrangement is a function of the cuticle thickness and the radius of the diffusion catchment and is always greater than that found with the excess of tracheolar supply (Wichard, 1973; Wichard and Komnick, 1974a). If the arrangement of the tracheoles in the lamellae of P. nymphula is similar to either of the two types described above then this will be positive evidence showing these organs function as tracheal gills.

4) Finally, the difference in oxygen partial pressure between the tracheoles and the surrounding water (dp in equation 3.1) can be controlled to a certain extent by maintaining a high concentration of oxygen immediately above the cuticle. Oxygen flux is directly proportional to partial pressure difference (Dejours, 1975; Krogh, 1941; Mill, 1974). As oxygen diffuses into the gill, the water in its immediate vicinity becomes depleted (Feder and Booth, 1991; Wiley and Kohler, 1984). This layer is termed the boundary layer and can form an effective barrier to oxygen diffusion (Feder and Booth,

1991). Thus by controlling the flow of water over the gill and body surface, fresh water insects can maintain a high partial pressure gradient of oxygen between the surrounding water and the gill tissue. Ventilation of the body and respiratory surfaces has been recorded in many insect species including zygopteran larvae and is discussed further in Chapter 4.

Respiratory adaptation in the Odonata

The structure of the respiratory gills of some groups of the Odonata have been described by other authors. The most primitive gas exchange organ found in larval dragonflies is considered to be the lateral abdominal gill found in the Euphaeidae (Zygoptera) (Norling, 1982; Snodgrass, 1954). This form of respiration has been superseded in the Anisoptera by the branchial basket and in the Amphipterygidae (Zygoptera) by caudal tufts (Watson, 1966).

In anisopteran dragonflies, the external body cuticle is too thick to allow any significant gas exchange (Corbet, 1962) and oxygen is taken up over specialised plates in the hind gut (Greven and Rudolph, 1973; Mill, 1974; Saini, 1977; Tillyard, 1916). The rectum is multifunctional and along with gas exchange is used in locomotion, ion exchange and food storage (Greven and Rudolph, 1973; Komnick, 1983; Kukulies and Komnick, 1983; Moens, 1980; Wichard and Komnick, 1974c). The arrangement of these plates varies according to species (Tillyard, 1916) and each lamellar plate has two distinct regions of cuticle (Komnick, 1982) a region of thick cuticle which overlies the ion transporting epithelia, and a region of thin cuticle which overlies the gas exchange epithelium (Komnick, 1982; Saini, 1977). In the respiratory epithelium the tracheoles are very densely packed with variable diameters (Saini, 1977). The lamellar plates (within the rectum) are ventilated with fresh water by contractions of the abdomen which maintains a flow over the gills. Ventilation rate is inversely proportional to the oxygen content of the surrounding water (Hughes and Mill, 1966).

In three genera of Amphipterygidae (Odonata: Zygoptera) caudal tufts are considered to be the main site of gas exchange (Watson, 1966). The tufts, which are derived from the laminae sub anales, are attached to the last abdominal segment and are protected by reduced epiprocts and paraprocts and by plates derived from the cerci (Watson, 1966). The tufts form a series of repeatedly branching filaments, reaching a maximum length of 1 mm in the final instar larvae of Devadata sp. The total surface area of these tufts is about 5 mm^2 and the terminal twigs have a diameter of between 5 and 10 μm. In Pentaphlebia sp. and Rimanella sp. the surface area of the tufts is substantially greater (Watson, 1966). The cuticle of the tufts is about 2.5 μm thick at the base and 0.1-0.2 μm thick at the terminal filaments. The respiratory epithelium in these species contains a dense network of tracheoles which are variable in diameter. In larvae of Devadata sp. the reduced caudal lamellae, which are sparsely covered in setae, are considered to have a sensory or defensive role. Any gas exchange role of these organs is considered to be minimal due to the thick cuticle (between 10 and 15 μm thick) and reduced tracheation (Corbet, 1962; Watson, 1966).

In the Polythoridae and Epallagidae (Odonata : Zygoptera) lateral abdominal gills are used for gas exchange (Corbet, 1962; Norling, 1982; Wichard, 1979). One pair of these tapering filamentous tracheal gills is located laterally on each of the abdominal segments. These species also have three modified saccoid caudal lamellae. (Corbet, 1962; Norling, 1982; Wichard, 1979). Norling (1982) described the ultrastructure of both the caudal lamellae and the lateral abdominal gills in these families. In this study he showed that the tracheoles in the lateral abdominal gills run in parallel furrows along the gill and are of uniform diameter, evenly spaced and densely packed. The cuticle overlying the gills is between 3.6 and 4.7 μm thick. These observations led Norling (1982) to suggest that the primary function of these organs was gas exchange. He did not consider the caudal lamellae of these species, which are heavily sclerotised and sparsely tracheated, to be effective gas exchange organs (Watson, 1966; Wichard, 1979).

Gas exchange in the Zygoptera with laterally flattened lamellae

As described earlier it has generally been accepted that in species with laterally flattened caudal lamellae these organs are the main site of gas exchange. Morphological evidence in favour of this function is mainly inferred from general descriptions rather than experimental or detailed observations. For example, the term gill was first applied by Carus (1827) who first referred to the Lestid/Agrionid type lamellae as gill like leaves. This function was assigned purely because they "looked like gills" (Tillyard, 1917a). Laterally flattened lamellae on general inspection have some of the characteristics, described earlier, of respiratory gills such as a dense tracheal network, thin cuticle and a large surface area. However, assessment of the respiratory efficiency of these characteristics in laterally flattened lamellae has been through general rather than detailed observation. For example, the network of subcuticular tracheae found in the lamellae of many zygopteran species is assumed to indicate respiratory adaptation (MacNeill, 1960; Tillyard, 1917a). However, as Wichard and Komnick (1974a) showed it is the density and arrangement of the smaller subcuticular tracheoles that is of critical importance in determining the efficiency of a tracheal gill.

Another characteristic of a tracheal gill that lamellae are assumed to posses is a "reduced" metabolic rate (a lack of tissue with high metabolic oxygen demand). MacNeill (1960) describes the metabolism of laterally flattened lamellae as "feeble", implying it to be lower than that of the body. The metabolism of laterally flattened lamellae is investigated in more detail in chapter 4. However, there have been no studies that indicate that lamellae have a reduced metabolic rate. In the present study it is intended to determine whether there is any morphological evidence to indicate either elevated or reduced metabolism in these organs by investigating the occurrence and type of cell organelles found within their tissues.

The large surface area of lamellae is often cited as evidence of a respiratory gill function (Eriksen, 1986). Whilst these organs do clearly have a large surface area for their bulk the area of lamellae in relation to that of the body has only been investigated in one species of zygopteran (Eriksen, 1986). In this study Eriksen (1986) measured the area of the lamellae directly but only estimated the area of the body of final instar Ischnura sp. larvae. From these measurements and calculations Eriksen determined the area of the lamellae to be 60% of the total body area. This figure is clearly compatible with that required for a respiratory gill. However, because of deficiencies in the methods used by Eriksen (3.3) this figure greatly exaggerates their true surface area. Before a respiratory gill function is assigned to lamellae it is essential that an accurate measurement of their surface area in relation to that of the body is made. If the lamellae of P. nymphula for example are found to have a large surface area in relation to their volume then this would imply a respiratory gill function.

The diffusion distance for oxygen into laterally flattened lamellae has not previously been determined. This diffusion distance is of fundamental importance in assessing the respiratory efficiency of a tracheal gill. The only indication of diffusion distance from previous studies is that the cuticle of the lamellae of some species is reduced, which would be compatible with an enhanced respiratory efficiency (MacNeill, 1960; Tillyard, 1917a). MacNeill (1960) suggested that the pattern of growth found in the duplex type of lamellae (referred to as protrusive growth) resulted in the cuticle of the distal postnodal region becoming exceptionally thin and was thus an adaptation to improve their respiratory efficiency. In the present study it is proposed to determine the diffusion distance for oxygen into the different areas of the lamellae, and for comparison into the abdomen.

The morphological evidence which shows that laterally flattened lamellae are respiratory gills is not conclusive. The morphology of lamellae as described by other

authors is equally compatible with the other functions for lamellae suggested earlier. Thus a more detailed and accurate description of the morphology of the lamellae of one species of zygopteran will greatly assist the determination of their function in all species.

3.1.4. Aims

The aims of this part of the present study are to investigate the morphology of the lamellae of the common red damselfly Pyrrhosoma nymphula (Sulzer) and to relate this to their possible function. Five functions for laterally flattened lamellae have been suggested. 1) sensory, 2) locomotion 3) defensive/autotomy 4) ion uptake and 5) respiratory gill.

1) The sensory function will be investigated by determining the extent of sensory organs on the surface of the lamellae and the abdomen. The presence of high densities or unique sense organs on the lamellae would indicate a sensory function.

2) The locomotory function for the lamellae could not be investigated directly by examination of morphology. Determination of the morphological adaptation for swimming of such comparatively small structures was considered beyond the scope of the present study. However, the degree of adaptation of the lamellae to this function will be discussed in the light of the general descriptions provided.

3) The characteristics of a defensive autotomous organ were outlined in 3.1.2. These included large surface area, distinctive colouration a specialised fracture point and a "low value", that is containing no vital organs or functions. The characteristics of an efficient threat display organ in zygopterans are not known. However, zygopteran larvae rely mainly on sight for location of prey (Chapter 1) and an efficient threat display should have the characteristics described such as a large surface area and distinctive patterning.

Perhaps the most important characteristic of a defence autotomy organ is the presence of a specialised fracture point. Although it is known that zygopteran larvae are able to autotomise lamellae (Child and Young, 1903), the operation of this joint has not been studied in detail. In the present study the function of this joint was determined by investigating the structure of the joint in intact larvae, those with freshly autotomised lamellae and larvae in which the lamellae have been autotomised and allowed to heal.

4) Direct investigation of any ion uptake function in the lamellae was considered beyond the scope of the present study. However, the presence of ion uptake epithelia organs can be used as an indication of this function. These epithelia and organs have a characteristic and easily recognised structure which has been reviewed by Komnick (1983). The hypodermis and cuticle the lamellae of P. nymphula were examined for the presence of such structures.

5) The respiratory efficiency of the lamellae of P. nymphula was assessed by determining the extent to which their morphology is adapted to such a function. The characteristics of a respiratory tracheal gill that were investigated are described in 3.1.3. and included a large surface area, dense subcuticular network of tracheoles with a specialised packing arrangement, short diffusion distance across the cuticle and a lack of tissue with high metabolic oxygen demand. Because different areas of the lamellae may have different respiratory efficiencies (MacNeill, 1960), three different areas were investigated. These areas included the postnodal and prenodal regions of the lamellae and for comparison the cuticle on the last abdominal segment. The surface area of the lamellae was also determined in relation to that of the whole larva during different stages of growth.

3.2. Materials and methods.

3.2.1 Techniques

Morphology

The morphology of lamellae of P. nymphula larvae was investigated at three levels 1) The general morphology using light microscopy, 2) Scanning electron microscopy (S.E.M.) for surface detail and 3) Transmission electron microscopy (T.E.M.) for the ultrastructure.

1) General morphology by light microscopy.

The general morphology of the caudal lamellae was studied using a Watson binocular microscope. Freshly killed specimens were preserved in 40% alcohol. Whole mounts using dissected material were prepared using Bouins Fluid as a fixative.

Sections for examination using light microscopy were prepared according to standard histological procedures, which are detailed in appendix 1. Such techniques have been used previously in investigations of the ultrastructure of larval dragonflies, for example see Norling (1982) and Watson (1966). Specimens were fixed in Bouins fluid, dehydrated in a series of alcohols, sectioned in paraffin wax (5-7 μm), stained using Mallory triple stain and mounted using histomount.

2) Scanning electron microscopy (S.E.M.)

Preparation of material for S.E.M. followed standard procedures which are detailed in appendix 2. Specimens were fixed using gluteraldehyde and osmium tetroxide, dehydrated in a series of acetone dilutions then subjected to critical point drying and gold coating. Specimens were examined using a Phillips PSEM 500 scanning electron microscope.

2) Transmission electron microscopy (T.E.M.)

The techniques used in the preparation of material for the T.E.M. are outlined in appendix 3. Sections were either unstained or stained with lead citrate and uranyl acetate. Following fixation, specimens were mounted in Araldite resin and sectioned with a diamond knife. Sections were examined with an A.E.I. EM801 transmission electron microscope.

Surface area

The surface area of the lamellae and bodies of larvae were measured according to the methods of Chefurka and Pepper (1954), by this method the constants k and n in Meeh's formula ($S=kW^n$) are determined. In this formula S= surface area, W= weight and n and k = growth constants. Larvae were starved for at least 48 hours prior to weighing to allow the gut to clear (Lawton 1971a). Fresh specimens were air dried for 5 minutes at 20^{o}C and weighed to 0.1mg using a Stanton balance. The lamellae were then removed and weighed separately. Larvae were killed immediately after weighing and the cuticle removed. Fat and other tissue were removed from the cuticle before examination. Prior to mounting, the cuticle was fixed following the procedure outlined in appendix 1. To ensure that the cuticle was mounted flat, it was sandwiched between two glass slides in histomount and gently compressed for 24 hours. The surface area of the cuticle of each larva was measured by tracing the area onto paper using a projecting microscope. Areas were then measured using a digitiser interfaced to a B.B.C. model B microcomputer. The surface areas of the individual parts of the larval body were calculated from these measurements.

3.2.2 Material examined

Collection of larvae

P. nymphula larvae were collected from the Ross Burn sampling site downstream from the sampling stations describe in 2.2.1.

General morphology and tracheal supply

The general morphology and tracheal supply of the lamellae was investigated to provide a background for the more detailed study of their ultrastructure. Whole mounts and sections of two sizes of larvae were used. These were large larvae, between 10 and 13 mm in total length (instars 10 to 12), and small larvae of between 5 and 8 mm total length (instars 4-6). Total length refers (as defined in Chapter 2) to the length of the larva measured from the head to the end of the abdomen excluding the lamellae. Both longitudinal and transverse serial sections were made of larvae either with intact or autotomised lamellae. The abdominal and lamellar tracheal supply of P. nymphula was studied in the final instar larva only. The tracheal system of smaller larvae was not sufficiently developed to study in detail. Dark pigment present in the walls of the trachea allowed them to be traced without staining.

Ultrastructure of the lamellae

Examination of the ultrastructure of the lamellae was carried out for sensory and ion uptake organs on their surface, the amount of metabolism within the hypodermis and determination of their gas exchange efficiency. Gas exchange efficiency was determined by measuring the cuticle thickness of the pre and postnodal regions of the lamellae and for comparison the thickness of abdominal cuticle. In addition the density and arrangement of the tracheoles within the hypodermis of these three regions were also measured. Tracheolar density here refers to the number of tracheoles per 1 μm of hypodermis and measured linearly on T.E.M. micrographs. Larvae used in this investigation were of the same size categories as for that of the general structure. Material examined under the T.E.M. was only from final instar larvae.

Function of the breaking joint.

The function of the breaking joint was investigated in order to determine the degree of specialisation of the lamellae as autotomous organs. The material used for this study was from larvae of the final and penultimate instars. The breaking joints in three conditions of larvae were investigated. Those with intact lamellae. Those with freshly autotomised lamellae and those with "well healed" lamellar scars. The larvae of the final group were only investigated at least on week after autotomy of their lamellae. Lamellae were autotomised by gripping them lightly with forceps until the larva autotomised them. The three conditions of the breaking joint were investigated by sectioning for examination under the light microscope as described and by examining their surface structure using the S.E.M.

The surface area of larvae and their lamellae

The surface area of larvae was investigated to determine their efficiency as attack deflectors, threat displays and as respiratory organs. The surface area of larvae (including their lamellae) of 5mg to 63mg wet weight was measured.

3.3 Results

The results are presented in two parts, firstly a description of the general morphology and ultrastructure of the lamellae and secondly a more detailed description of their morphology in relation to function. The possible functions investigated were respiratory gill, defence organ, sensory organ, ion uptake organ and locomotory organ. The respiratory and defensive morphology was investigated in detail.

3.3.1 Morphology of the lamellae.

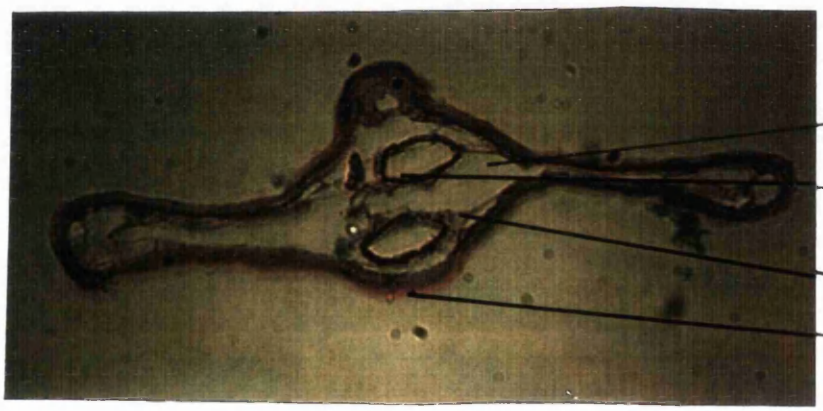
The caudal lamellae of P. nymphula are located on the last abdominal segment of the larva (Plate 3.1). The two lateral lamellae are held at an angle of about 45^{O} to each other and ventral to the median lamella and on the horizontal plane (Plate 3.1). The median lamella is usually raised at an angle of 45^{O} to the lateral ones (Plate 3.1). The normal colour of the lamellae is pale brown with a characteristic dark brown "X" mark two thirds along their length (Plate 3.1). The colour of the larvae from the Ross Burn varied from dark to pale brown, and typically specimens were coloured as in Plate 3.1. Frequently the surfaces of larva were encrusted with algae and occasionally mites. The size of the lamellae depends on the instar and their state of regeneration. The number of larvae with fully grown lamellae was variable (Chapter 2). In the final instar, the lamellae reach 6mm in length (Fig. 3.1), whilst in instar 5, the lamellae are no more than 3mm long (Fig. 3.2). The median lamella is always shorter and deeper than the lateral lamellae in this species (Plate 3.1).

The lamellae change shape from the tapering ferullar structure of the smaller larvae (Fig. 3.2) to the flattened blade-like shape of the final instar larva (Fig 3.1). During development, the lamellae grow dorsally and ventrally and become thin in cross

Plate 3.1 Final instar larva of P. nymphula (X 10). Note the three caudal lamellae attached to the last abdominal segment. The median dorsal lamella is raised horizontally at an angle of about 45^{O} to the pair of lateral lamellae. The dark "X" marking can be seen near the distal tip of the lamellae. The colour of this larva is typical of those collected during this study.

Plate 3.2 Transverse section through the prenodal region of a median lamella from an instar 4 larva of P. nymphula (X100). The lumen of the lamella is mainly filled by haemocoel (Hy), the tracheal trunks(T) occupy a relatively small proportion of the interior in comparison to that of the final instar larva (Plate 3.3). The tracheal trunks (T), are supported by internal laminae (La), which also direct the flow of haemolymph (Hy). The thickened mid rib (Mr) strengthens and supports the lamella. D= Dorsal V= Ventral.

Plate 3.3 Transverse section through the thickened mid rib (Mr) of a lateral lamella of P. nymphula (X100). The main tracheal trunks (T) occupy most of the mid rib compared to that of the instar 4 larvae (Plate 3.2). The area occupied by haemocoel (Hy) is limited compared to that of Fig. 3.2. Note the thickened endocuticle (End) and exocuticle (Exo) of the mid rib (Mr). D= Dorsal V= Ventral.


V
Hy
T
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La
Mr

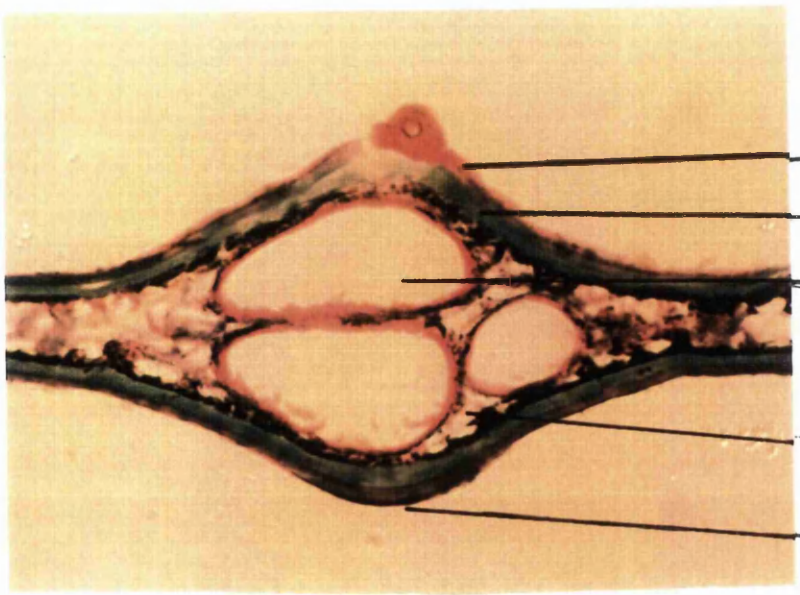

V
Exo
End
T
D
Hy
Mr

Plate 3.4 T.E.M. of a transverse section through the postnodal region of a lamella from a final instar larva of P. nymphula (Uranyl acetate and Lead citrate stain) (X4,690). Note the internal laminae (La), clear haemolymph (Hy) and haemocyte (Hc). The folding of the cuticle is the result of a widening of the cuticular lamellae at the ridges (?). The hypodermal layer (Hd) is sandwiched between the cuticle (Cu) and the basement membrane (Bm). The thickness of the hypodermis varies due to invaginations of the haemocoel. The tracheoles (Tr) are scattered randomly within the hypodermis.

Plate 3.5 T.E.M of a transverse section through a lateral lamella from a final instar larva of P. nymphula (unstained X76496). The section shows a typical cell-cell junction. Towards the cuticle (Cu) the zonula adherens (Za) of the junction can be seen. Moving along the junction towards the hypodermis (Hy) and basement membrane (Bm) there is a region of smooth septate desmosome (Ss) followed by pleated septate desmosome (Pd). The remainder of the cell junction is non-junctional (Nj). Rough endoplasmic reticulum (R.E.R) and mitochondria (M) are abundant in these cells.


La
Cu
Tr
Hc
Hd
Bm
Hy

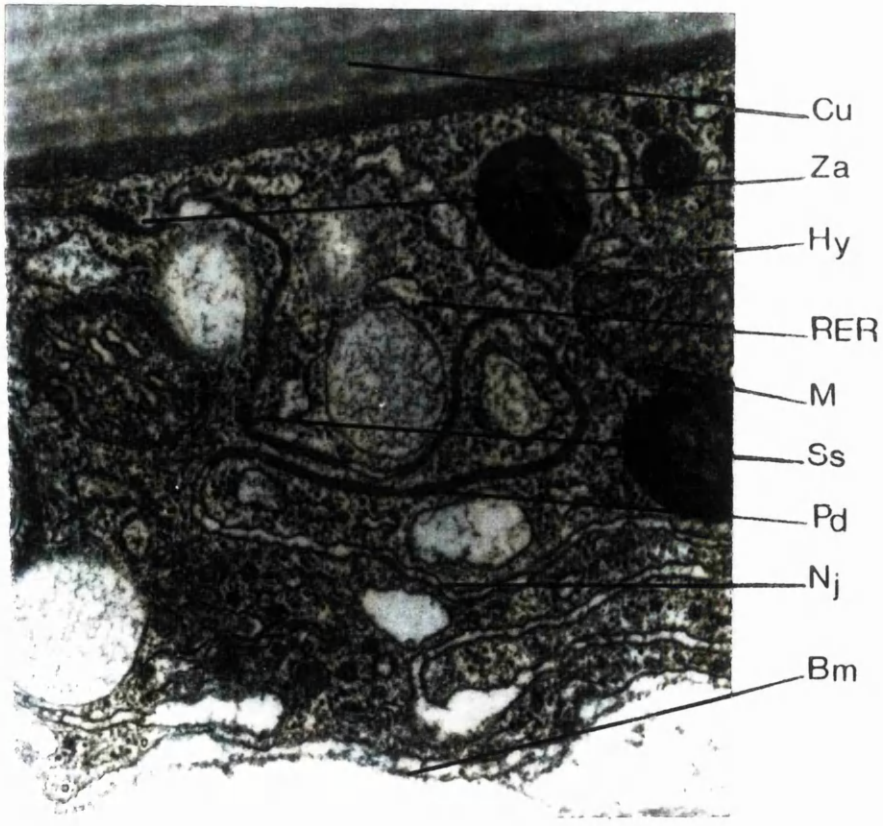

Cu
Za
Hy
RER
M
Ss
Pd
Nj
Bm

Fig 3.1 Diagram of a lateral lamella from a final instar larva of P. nymphula (X25). This lamella is laterally flattened to a greater extent than the lamella in Fig 3.2. The prenodal and postnodal regions are present in similar proportions to those of the instar 5 larva (Fig 3.2). The nodal point (Nd) is marked by the cessation of spiniform setae on the dorsal and ventral edges. Only piliform setae (Pi) are found on the edges of the postnodal (Po) area. The tracheal trunks (T) branch more frequently than those of the instar 5 lamella (Fig 3.2).

Fig 3.2 Diagram of a lateral lamella from an instar 5 larva of P. nymphula (X 40). This structure is more ferrular compared to that of the final instar larva (Fig 3.1). This figure shows the extent of the prenodal (Pr) and postnodal (Po) regions. The former lacks spiniform setae on its dorsal and ventral edges. The dorsal and ventral branching of the main tracheal trunks (T) is also shown.

Fig 3.1

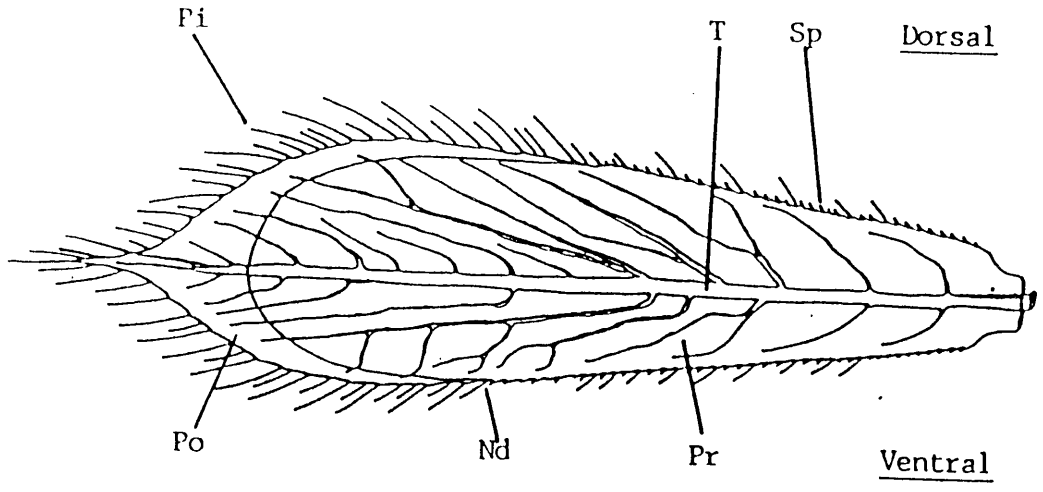
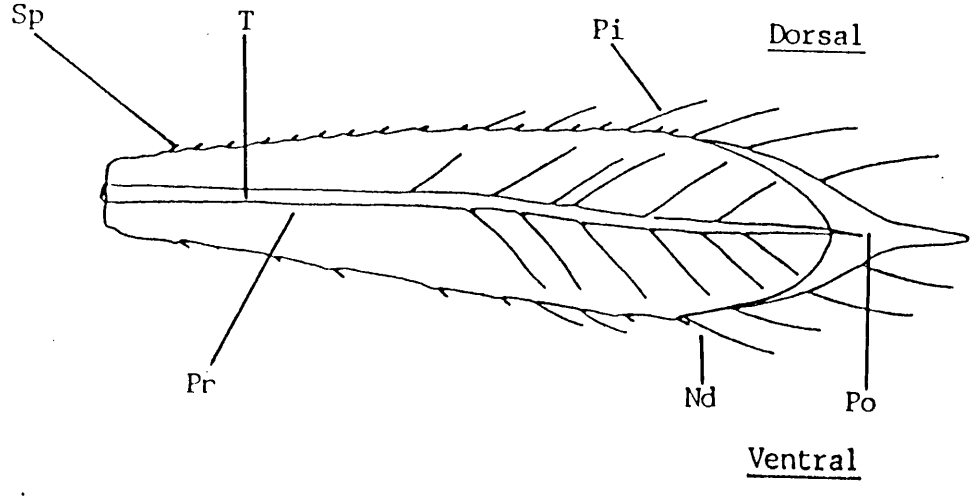


Fig 3.2



section. For example, in the instar 4 lamella (Plate 3.2) there is a relatively large haemocoel with small tracheae in comparison to the final instar lamella (Plate 3.3) in which the trachea fill the lumen of the mid rib and there is little haemocoel space.

The lumen of the lamella contains a supporting framework of laminae that divide it internally, into haemolymph channels (Plate 3.4). In the zygopteran species studied these internal laminae occur only in the lamellae (Calvert, 1915; Norling, 1982; Tillyard, 1917a; Watson, 1966). The fine structure of the laminae of P. nymphula is almost identical to that described in Coenagrion hastulatum by Norling (1982)(Plate 3.4). The laminae and microtubules are typical of epidermal tendons, tThe microtubules which are composed of tubulin are about 4nm in diameter and help to maintain the structural framework of the cells (Fawcett, 1981). According to Norling (1982) the laminae help to stabilise the outer structure of the lamellae.

The types of inter cellular junctions of the postnodal region of the lamellae differed from those of the prenodal and abdominal hypodermis (Plate 3.5). The cell junctions in the abdominal hypodermis are typical of those found in invertebrate epithelial cells (Lane and Skaer, 1980; Noirot and Noirot, 1980; Green and Bergquist, 1982; Staehelein, 1974) including the odonata (Saini, 1977; Kukulies and Komnick, 1983). The intercellular space is partitioned by regularly spaced septa which have a ladder like appearance in section (Plate 3.5). In the apical region, towards the cuticle, the zonula adherens or belt desmosome is evident. Proximally there is a region of smooth septate desmosome followed by the pleated septate desmosome (Plate 3.5). Smooth and pleated septate desmosomes are often interspersed in this way (Lane and Skaer, 1980). The remainder of the cellular boundary consists of non-junctional areas (Plate 3.5). In some of the sections, closed compartments can be seen (Plate 3.8). These are the extension of finger-like processes of a third cell forming a tricellular complex (Lane and Skaer, 1980). Tricellular complexes were more frequently observed in the hypodermis of

the postnodal region of the lamellae. The significance of this observation is not clear although tricellular complexes may improve the rigidity and strength of cell junctions (Lane and Skaer, 1980).

The haemolymph is separated from the hypodermis by the basement membrane (Plate 3.5). Haemolymph enters the lateral lamellae by a dorsal duct and leaves by a ventral duct in the breaking joint. According to Tillyard (1917a) the flow is reversed in the median lamella. In all three lamellae the flow of haemolymph follows the channels formed by the laminae (Tillyard, 1917a). The haemolymph is a clear fluid with infrequent blood cells or haemocytes (Plate 3.5).

3.3.2 Morphology and respiratory function.

The characteristics of a respiratory gill are described in 3.1. and include 1) reduced diffusion distance achieved by reducing cuticle thickness and having a good tracheal supply with tracheoles packed densely and close to the cuticle 2) Low metabolic activity (lack of large metabolic oxygen demand) and 3) Large surface area in relation to its respiring bulk.

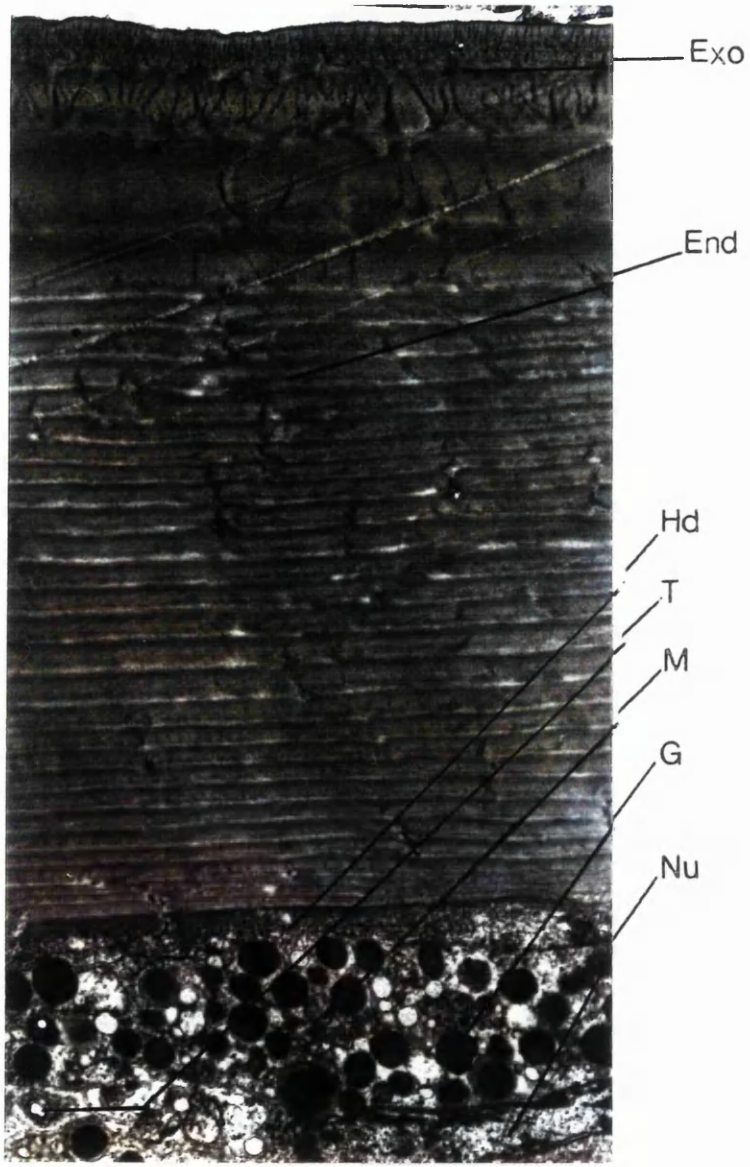
1) Diffusion distance

The diffusion distance for oxygen into the lamellae is critical to their effective functioning as a respiratory gill. This was investigated by examining the cuticle thickness, the tracheal system and the arrangement and packing of smaller subcuticular tracheoles.

The mean thickness of lamellar cuticle of a final instar larva varied from 2.34μm in the postnodal region (Plate 3.4) to 9.03μm in the prenodal region (Plate 3.6 Table 3.1). The mean thickness of abdominal cuticle was 12.52μm (Plate 3.7 Table 3.1).

Plate 3.6 T.E.M of a transverse section of the prenodal region of a lateral lamella from a final instar larva of P. nymphula (Uranyl acetate and Lead citrate stain)(X10868). The cuticle (Cu) in this region is relatively thick in comparison to that of the postnodal region and is not folded. Endocuticle (End), exocuticle (Exo) and epicuticle (Epi) can be differentiated. The cuticular laminae are laid down at a fixed angle resulting in the helicoidal appearance of the pore canals (Pc). The proportions of endocuticle (End) and exocuticle (Exo) are similar to that of the abdomen. A tracheole (Tr) is invaginated into the hypodermis (Hd).

Plate 3.7 T.E.M of a transverse section through the cuticle of the abdomen from a final instar larva of P. nymphula (Uranyl acetate and lead citrate stain)(X10032). Endocuticle (End) makes up about 75% of the cuticle with narrower cuticular lamellae than the exocuticle (Exo). Note the rounded cell nucleus (Nu) in the hypoderm (Hd) cell. The cytoplasm of this cell also contains mitochondria and granules (G).


Exo
End
Hd
T
M
G
Nu

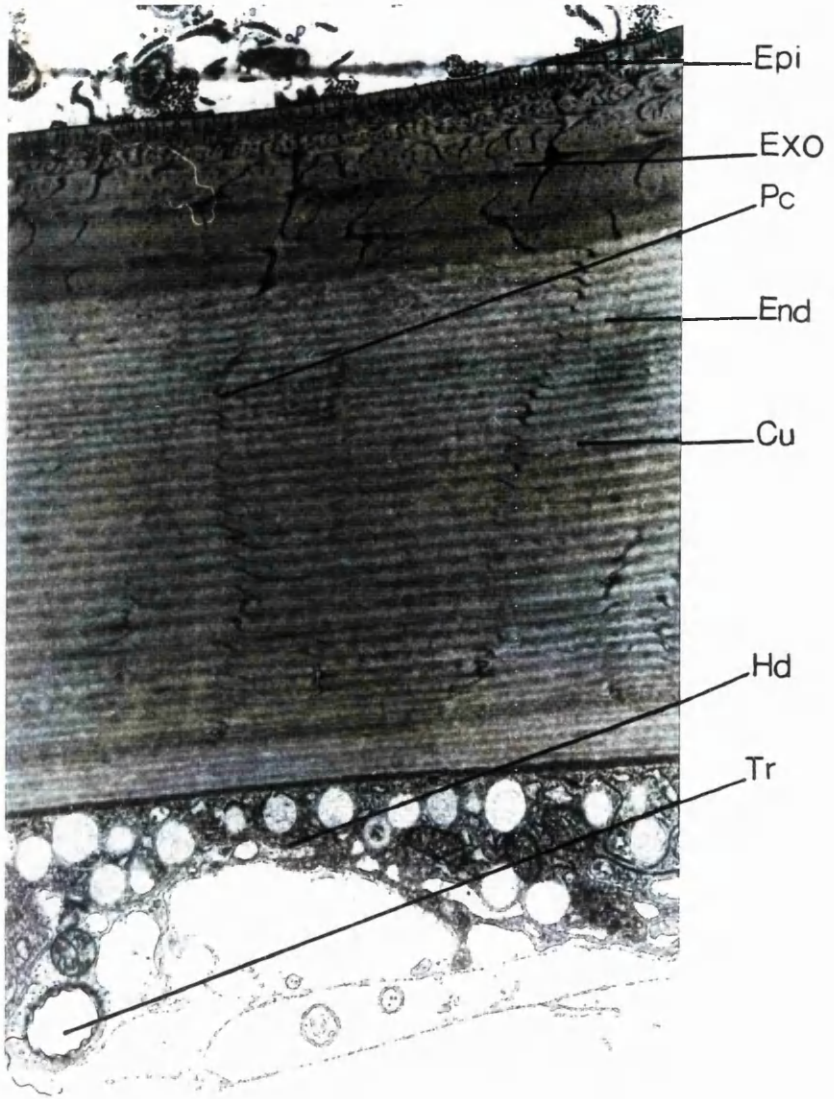

Epi
Exo
Pc
End
Cu
Hd
Tr

Plate 3.8 T.E.M. of a transverse section through the postnodal region of a median lamella of a final instar larva of P. nymphula (Uranyl acetate and lead citrate stain, X8,778). Note the closed compartments (Cc) of a tricellullar junction. The hypodermis (hd), between the basement membrane and the cuticle (Cu) is invaginated by the haemocoel and randomly spaced tracheoles (Tr).

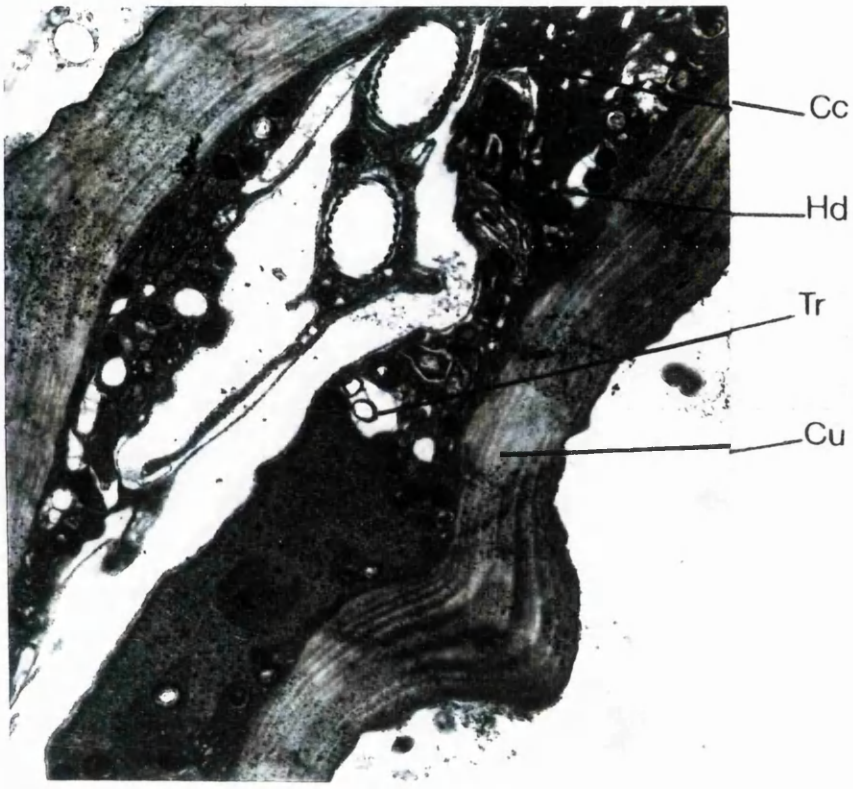

Cc
Hd
Tr
Cu

Table 3.1 Comparative morphology of insect tracheal gills. For comparison the cuticular thickness, tracheolar density and tracheolar arrangement in the caudal lamellae of *P. nymphula* are given. Values taken from the literature were from single specimens and no mean values could be calculated.

Taxa	Cuticle thickness (μm)	Tracheolar density No./um	Tracheolar arrangement
Trichoptera (abdominal gills)			
Limnephilus marmaratus[1]	0.5-1.0	0.88	regular
Molana angusta[2]	1.0	1.51	regular
Plecoptera (neck tufts)			
Perla marginata[3]	0.2-1.0	0.60	irregular
Taeniopteryx sp.[3]	0.2-1.0	3.00	"
Protonemoura aubertii[3]	0.2-1.0	1.80	"
Odonata			
Anisoptera (rectal gills)			
Aeshna sp.[4]	1.0	4.90	irregular
Aeschna cyanea[5]	0.1-0.2	0.87	"
Zygoptera			
Amphipterygidae (Caudal tufts)			
Rimanella arcana[6]	0.1-0.2	n/a	irregular
Pentaphlebia stahlii[6]	"	"	"
Devadatta argiolestes[6]	"	"	"
Euphaeidae (lateral abdominal gills)			
Epallage fatime[7]	3.0-4.0	0.63	irregular
" " [8]	1.0-2.5	0.77	irregular
Coenagrionidae			
Pyrrhosoma nymphula	mean (S.E/N)	mean (S.E/N)	
Lamellae prenodal	9.03 (0.26/11)	0.22 (0.06/ 5)	irregular
" postnodal	2.34 (0.25/19)	0.39 (0.09/14)	"
Abdomen	12.52 (0.19/ 6)	0.17 (0.18/ 5)	

References:-

1 Wichard (1973)
2 Wichard (1977)
3 Wichard and Komnick (1974a)
4 Saini (1977)
5 Wichard and Komnick (1974c)
6 Watson (1966)
7 Wichard (1979)
8 Norling (1982)

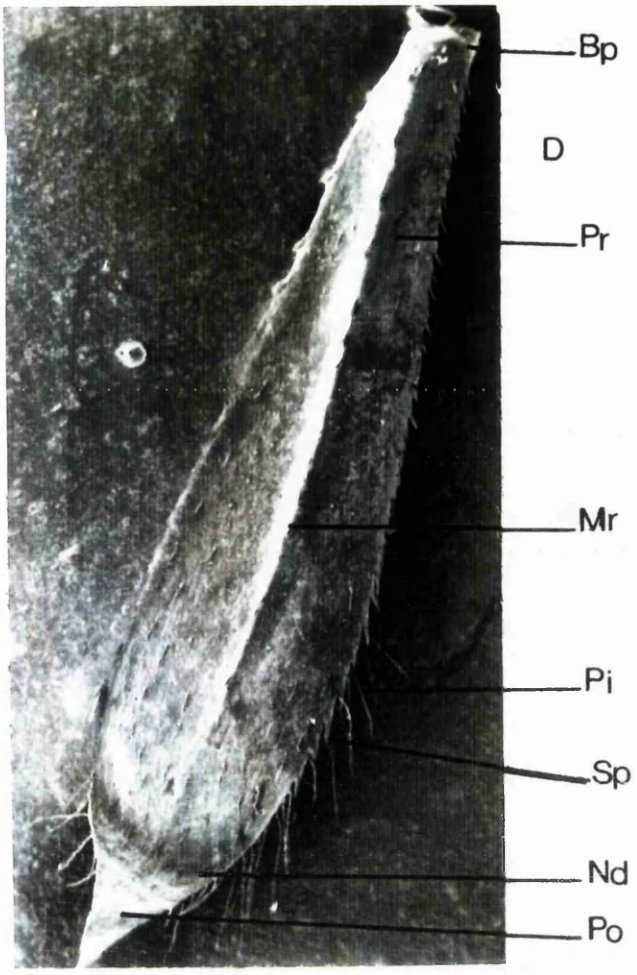
The structure of the cuticle of P. nymphula is typical of insect cuticle. There is a thin outer epicuticle over a thicker exocuticle and beneath this endocuticle (Plate 3.7). The microfibrils of the endocuticle and exocuticle are laid down at a fixed angle which results in the characteristic light and dark pattern of the cuticular lamellae (Plate 3.7) and the spiral pattern of the pore canals passing through the cuticle (Plate 3.7). The abdominal cuticle is uniform in composition and is about 75% endocuticle with the remainder consisting of exocuticle and a thin layer of epicuticle. Both endocuticle and exocuticle are present in constant proportions throughout (Plate 3.7). The exocuticle has wider bands or laminations than the endocuticle (Plate 3.7).

In the prenodal region of the lamellae (Plate 3.6) the cuticle is similar in structure to that of the abdomen both in the proportions of endocuticle and of exocuticle (Plate 3.7). In the postnodal region of the lamellae the cuticle is folded (Plate 3.8). The folds are produced by a thickening of the cuticular lamellae. Norling (1982) described a similar type of folding in the abdominal tracheal gills of larval Epallage fatime. These thickened ridges may increase the rigidity of the lamellae in a region of otherwise thin, flexible cuticle. Although the ridges increase the external surface area of the postnodal region they will also increase the diffusion distance for oxygen over the ridges.

MacNeill (1960), describes the lamellae of P. nymphula as weakly nodate and typical of the coenagrionid lamella. The node divides the lamella into proximal prenodal and distal postnodal regions. The extent of the two regions on the lamella of this species changes only slightly during growth. For example, in the fifth instar larval lamella (Plate 3.9, Fig. 3.2). the prenodal region makes up the largest area with the postnodal region restricted to the distal tip. In the final instar lamella (Plate 3.10, Fig. 3.1) the postnodal region is restricted to the distal tip of the lamella and accounts for a small proportion of the total area. The nodal point is marked firstly by changing cuticle from smooth prenodal to folded postnodal region cuticle and secondly by the cessation of spines on the dorsal and ventral edges (Plate 3.10).

Plate 3.9 S.E.M of a lateral lamella from an instar 5 larva of P.nymphula (X49). Spiniform (Sp) and piliform (Pi) setae are concentrated along the mid rib (Mr) and dorsal and ventral edges of the lamella. Along these edges the spiniform (Sp) and piliform (Pi) setae are interspersed until the nodal point (Nd), past which only piliform (Pi) seta e are found. The cessation of the spiniform setae marks the nodal point. The prenodal (Pr) and postnodal (Po) regions of the lamellae are indicated. The mid rib (Mr) runs from the basal piece (Bp) to the postnodal region curving ventrally (D= dorsal, V= ventral).

Plate 3.10 S.E.M of a the nodal region of a median lamella from a final instar larva of P. nymphula (X162). The change from the prenodal (Pr) to postnodal (Po) is marked at the nodal point (Np) by the absence of spiniform setae (Sp) on the dorsal and ventral edges and a change from smooth to folded cuticle. (Mr= mid rib, V= ventral, D= dorsal).


V
D
Bp
Pr
Mr
Pi
Sp
Nd
Po

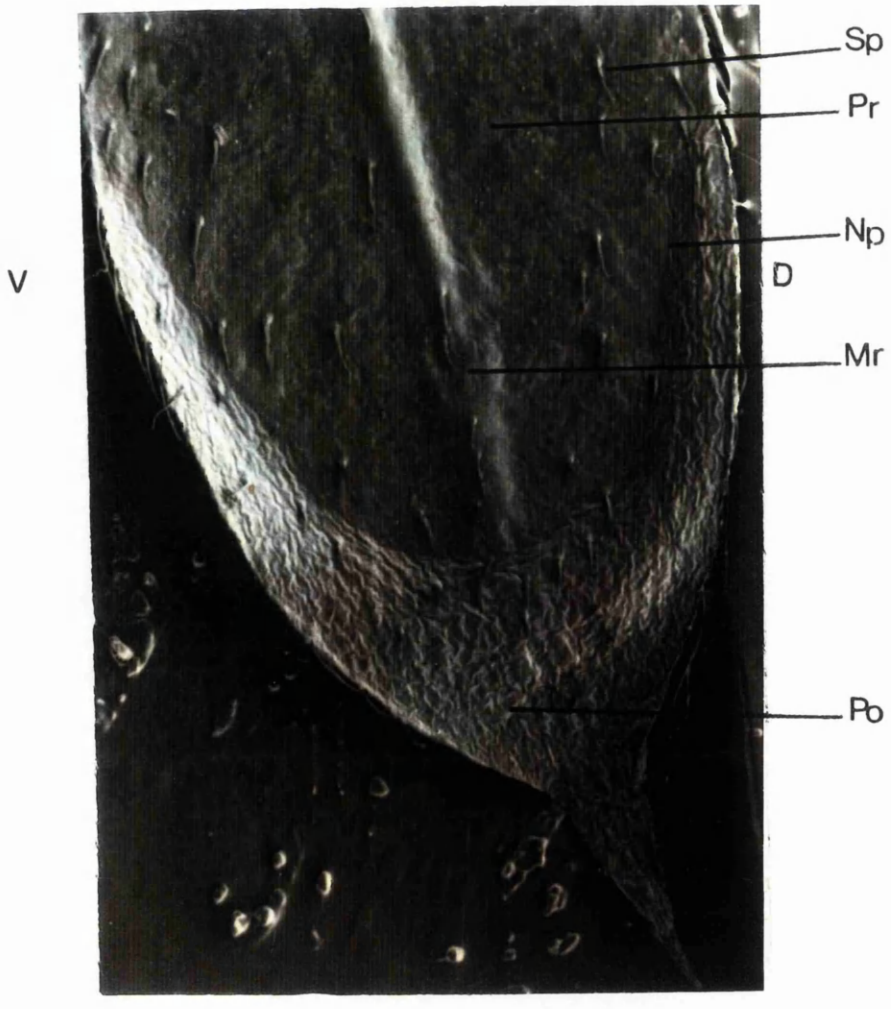
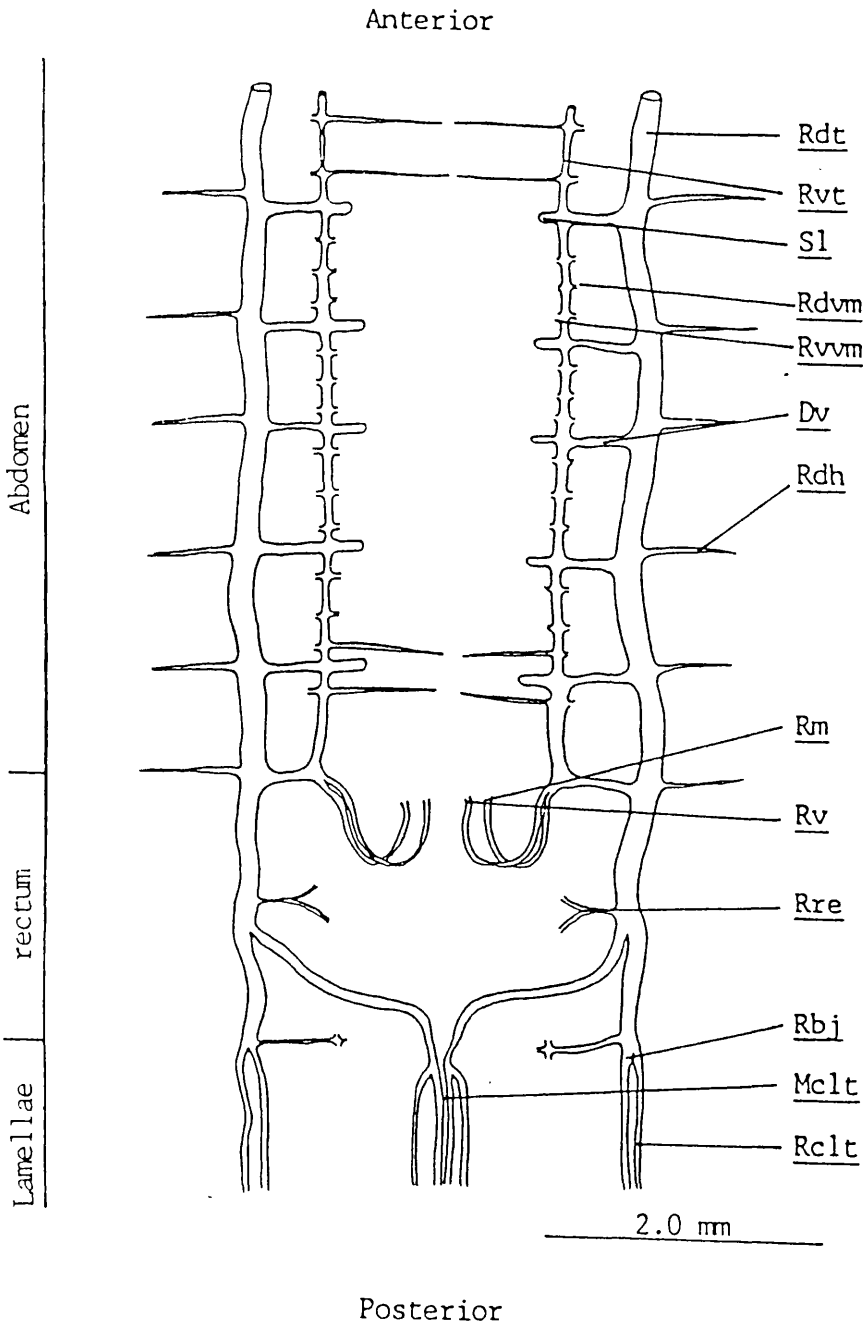

V
D
Sp
Pr
Np
Mr
Po

Fig 3.3 Diagram of the tracheal system of a final instar larva of P. nymphula from a dorsal dissection, only the tracheal branches of the right side of the animal have been labelled a similar nomenclature can be adopted for the left side. Key, Rdt = right dorsal tracheal trunk, Rvt = right ventral tracheal trunk, Sl = spiracle, Rdvm = Right branch of the dorsal tracheal trunk to mid-gut, Rvvm = Right branch of ventral tracheal trunk to mid-gut, Dv = Dorsal/ventral tracheal trunk link, Rdh = Right branch of dorsal tracheal trunk to heart, Rm = Right malphigian tracheal branch, Rv = Right visceral tracheal branch, Rre = Right rectal efferent tracheal branch, Rbj = Right breaking joint, Mclt = Median caudal lamellar trunk, Rclt = righ caudal lamellar trunk.

Fig. 3.3


Anterior
Abdomen
rectum
Lamellae
Rdt
Rvt
Sl
Rdvm
Rvvm
Dv
Rdh
Rm
Rv
Rre
Rbj
Mclt
Rclt
2.0 mm
Posterior

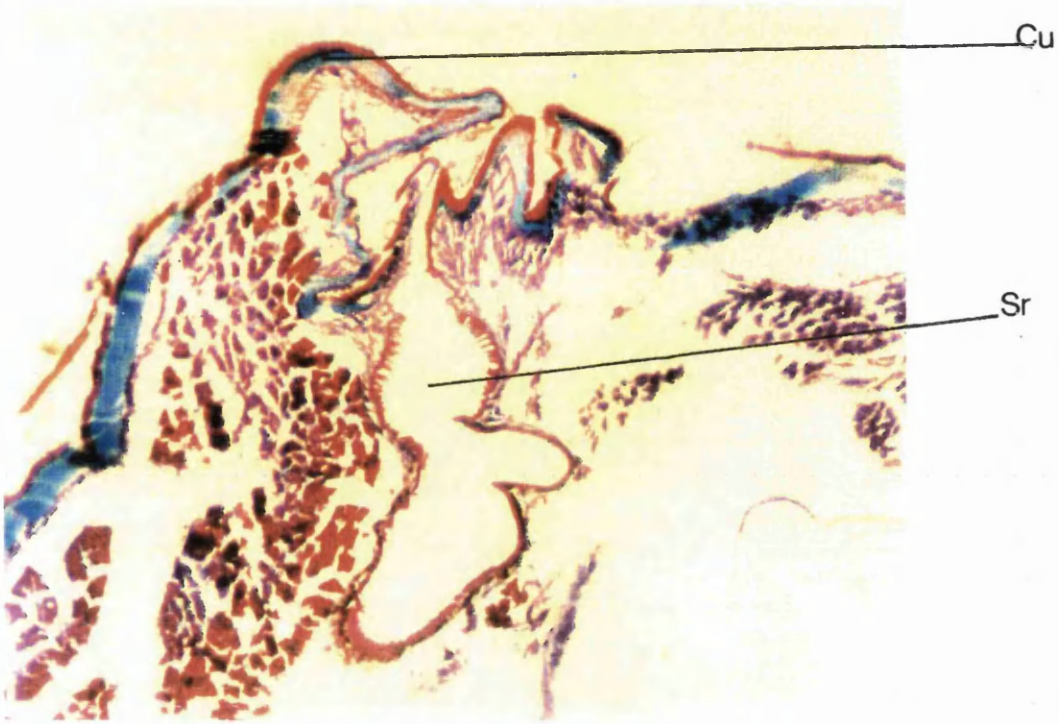
The tracheal system of the lamellae and abdomen of P. nymphula larvae has not previously been described. In the lamellae, the tracheal system usually consists of two main trunks in each of the lateral lamellae and four in the median lamella (Fig. 3.3). This arrangement is however flexible especially in the median lamella where two or three trunks may occur. The lamellar tracheal trunks arise from branches of the dorsal tracheal trunks of the abdomen (Fig. 3.3). One branch from each dorsal trunk enters each lateral lamellae and the remaining two branches (one from each dorsal trunk) enters the median lamella (Fig. 3.3). The tracheal trunks have the typical tracheal structure with spirally thickened cuticular intima.

In the abdomen there are four main tracheal trunks in this species, two dorsal and two lateral, which run the entire length of the abdomen (Fig. 3.3). The lateral trunks run to the functionless spiracles at the segment boundaries and dorsally and ventrally around the gut (Fig. 3.3). In P. nymphula the visceral trunks are reduced and the main tracheal supply to the gut is from branches of the lateral trunks. In final instar larvae the tracheal system is not closed, and one functional spiracle occurs between the prothoracic and mesothoracic segments (Plate 3.11). Functional spiracles have been recorded in the final instar larvae of other species of dragonfly, for example Anax sp. (Snodgrass, 1954). According to Corbet et al., (1960) and Snodgrass (1954), the spiracles allow the final instar larva to breath air at emergence and are not considered to be functional in water. The tracheal system of the larvae of P. nymphula is structurally similar to that of other examined zygopteran species such as Argia moesta, Argia talamanca and Thaumatoneura sp. (Cullen, 1918), Mecistogaster modestus, (Carrol, 1918), and Ischnura elegans (Launay and Razet, 1983).

In the lamellae, the main tracheal trunks branch dorsally and ventrally, with each division the main trunk is reduced in diameter. (Figs. 3.1 and 3.2). The angle of

Plate 3.11 Functional lateral spiracle (Sr) between the prothoracic and mesothoracic segments of a final instar larva of P. nymphula (X100). The endo and exocuticle (Cu) is indicated.

Plate 3.12 T.E.M through the hypodermis of the prenodal region of a lateral lamella from a final instar larva of P. nymphula (Unstained)(X62829). The tracheole (Tr) is located within a deep invagination of the hypodermal cell and is surrounded by a tracheoblast cell (Tb). In this cell rough endoplasmic reticulum (R.E.R) is abundant in the cytoplasm (Cu= cuticle, Bm= basement membrane).


Cu
Sr

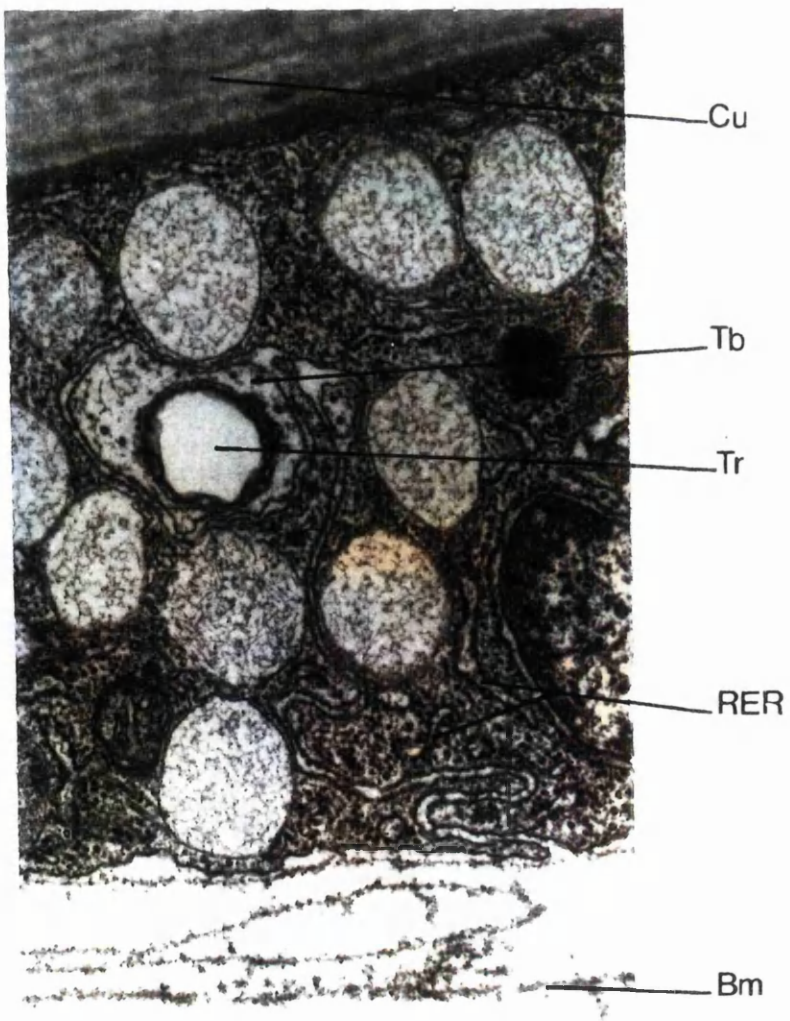
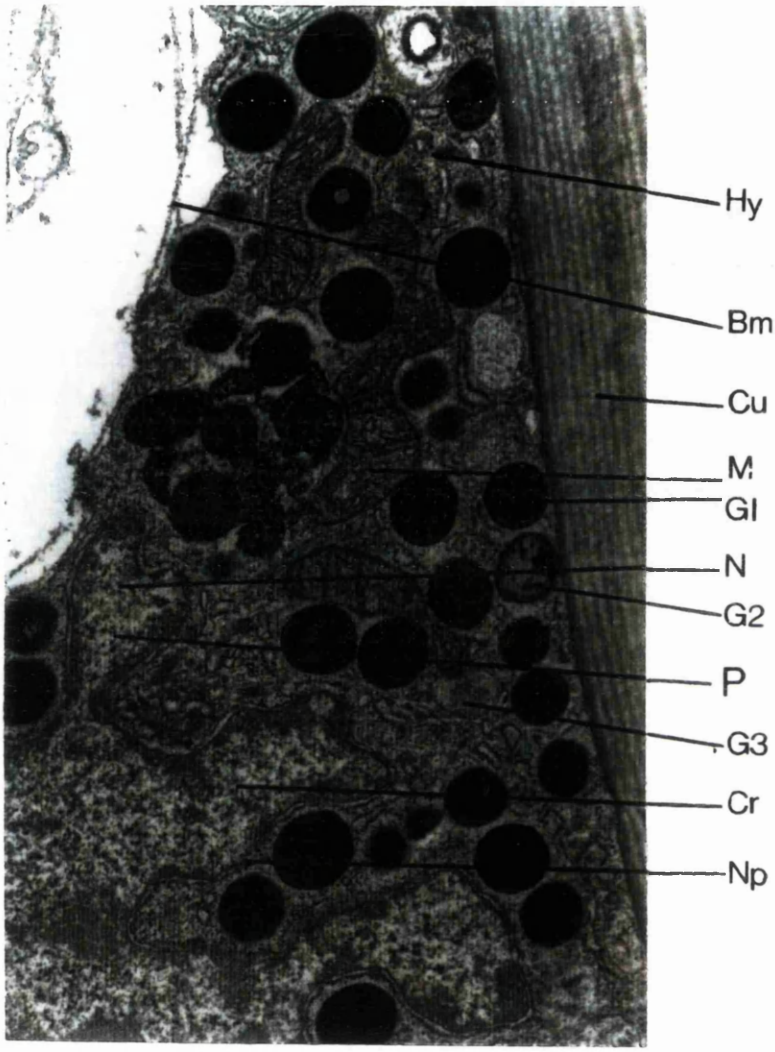

Cu
Tb
Tr
RER
Bm

Plate 3.13 T.E.M of a transverse section through the prenodal region of a lateral lamella from a final instar larva of P. nymphula (unstained)(X33509). The hypodermis (Hy) is located between the basement membrane (Bm) and cuticle (Cu). The large diffuse nucleus (N) of the hypoderm cell is drawn into processes (P) towards the cuticle, the nuclear pores (Np), chromatin (Cr) are also evident. The cytoplasm of the cell also contains mitochondria (M) and the dark staining granules, type 1(G1), 2 (G2) and 3 (G3).


Hy
Bm
Cu
M
GI
N
G2
P
G3
Cr
Np

branching depend on the state of regeneration of the lamellae, and can vary from 45^{O} in an intact lamellae to 30^{O} in a regenerated one (Gardner and MacNeill, 1950). There is a greater frequency of tracheal branching in the lamellae during development with relatively fewer branches in the earlier instars (Fig. 3.2) compared to final instar larvae (Fig. 3.1).

The tracheal trunks branch terminally to form a network of tracheoles. The tracheoles are located in parallel furrows in the hypodermis. Each tracheole is found with an associated tracheoblast cell (Plate 3.12). The density of tracheoles (measured linearly) varied within the lamellae, and was highest at 0.3 per μm of cuticle in the postnodal region with 0.25/μm in the prenodal region and 0.16/μm in the abdominal hypodermis (Table 3.1). In all specimens examined the tracheoles were arranged randomly and were of variable diameter (Plate 3.4, Plate 3.8).

2) Metabolic activity

The metabolic activity of the post and prenodal regions of the lamellae and the abdominal hypodermis was assessed by determining the extent and type of organelles present in the cells. A large amount of metabolic activity would be indicated by the presence of organelles such as mitochondria and Rough Endoplasmic Reticulum (R.E.R.).

In the lamellae metabolic activity is concentrated in the hypodermal layer. The haemocoel is fluid filled. The hypodermis consists of a layer of flattened epithelial cells between the cuticle and the basement membrane. In the abdomen its thickness varies from 1.6 to 2.5 μm (Plate 3.7). In the lamellae, hypodermal thickness shows some variability, especially in the postnodal region (Plate 3.4, Plate 3.8). This variability in the cuticle thickness is the result of deep invaginations of the haemocoel into the cell layer (Plate 3.4). The arrangement of the cells and the cell inclusions also varies between the different examined regions.

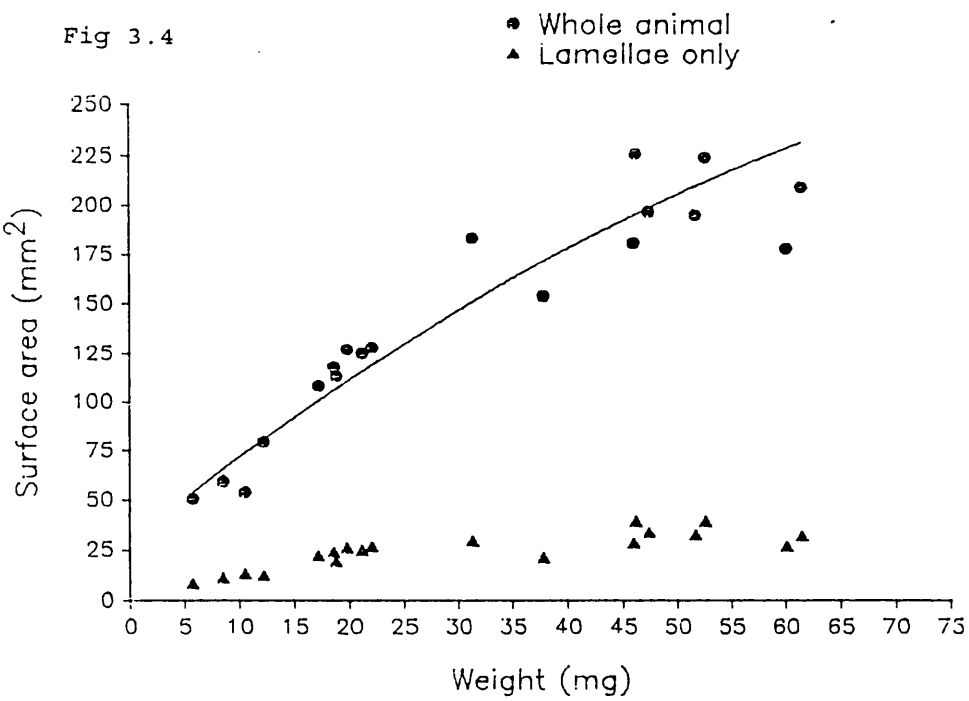
The largest inclusions in the hypodermal cells are the nuclei. The nuclei of the prenodal region are between 2 and 3 μm in thickness and are similar in size to those of the abdominal hypodermis (Plate 3.7). The shape of the nuclei differed between regions. In the lamellae the nuclei were more diffuse and had finger-like extensions directed towards the cuticle (Plate 3.4). Those of the abdomen were more rounded in section, lacked extensions and had more condensed chromatin (Plate 3.7). The nuclei of the lamellae had more diffuse chromatin and indistinct nuclear pores (Plate 3.4).

A major constituent of the cell contents was endoplasmic reticulum (E.R.) which is normally found as a continuous system of membrane-bound vesicles which are continuous with the nuclear membrane (Fawcett, 1981; Threadgold, 1976; Toner and Carr, 1971). Two types of E.R., rough E.R. (R.E.R) associated with protein synthesis and smooth E.R. (S.E.R) associated with lipid synthesis have been described (Fawcett 1981). E.R. is normally found in high densities in secretory cells and in low densities in cells with low metabolic activity (Fawcett, 1981). In the lamellae, and especially the prenodal region, R.E.R. occurs in higher concentrations (Plate 3.5) than in cells of the abdominal hypodermis (Plate 3.7). The R.E.R. in the lamellar hypodermis is in the form of elongated membrane bound vesicles which can be flattened or swollen (Plate 3.12). In some sections these vesicles are in the process of budding from the nuclear envelope (Plate 3.12) indicating that the cells of this region of the lamellae may be involved in protein synthesis.

A characteristic of all the examined hypodermal cells was the presence of numerous dark staining granules (Plate 3.13). The granules are uniform in size 0.2-0.3 μm in diameter and spherical. Three types occur which have been classed as type 1, 2 or 3 (Plate 3.13). Type 1 granules are dark staining and contain electron dense material. These are occasionally visible with a single unit membrane enclosing the organelles. The

Fig 3.4 surface area versus weight for larvae of P. nymphula ( ) total animal surface area and ( ) corresponding lamellar surface area. The solid line has formula S.A.= $16.07XW^{0.65}$. During growth, the lamellae increase in size disproportionally to the whole body of the larva.

Fig 3.4


Whole animal
Lamellae only
Surface area (mm^2)
250
225
200
175
150
125
100
75
50
25
0
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
Weight (mg)

the measured areas and weights, and arbitrarily chosen values of the constant n ranging from 0 to 0.1 for the lamella and from 0 to 3 for the whole animal. For each arbitrary value of n the standard deviation (σ) was calculated and expressed as a percentage of the average value of k (σ/k X 100). The value of n which best fitted the data was found by plotting this percentage (σ/k X100) against n. The optimum value of n occurs at the minimum percentage namely, 0.65 for the whole animal and 0.2 for the lamella. The corresponding value of k in Meeh's formula can then be calculated. The calculated surface area compares well with the actual surface area of the larvae (Fig. 3.4). This calculation produced equation 3.2 for the surface area of the whole animal and equation 3.3 for the lamellae alone.

$S.A. = 16.07 \times W^{0.65}$ equation 3.2.

$S.A. = 32.40 \times W^{0.2}$ equation 3.3.

$W^{0.65}$ for the whole animal indicates that this species conforms to the 2/3 (0.66) power rule (Chefurka and Pepper, 1954). While $W^{0.2}$ indicates that lamellae, in proportion to the whole body, increase in area at about 1/3 the rate expected.

The latter observation may be due to an experimental error which tended to give an overestimation of the body area of smaller larvae. This error arose because it was easier to measure the body areas of final instar larvae due to their larger size. With smaller larvae there would tend to be more rounding off of the edges of the body parts during measurement. However, it was equally easy to measure the area of the lamellae during all stages of development as they have a smooth edge. A small larva would therefore appear to have a smaller surface area of body in relation to that of it's lamellae. Thus, although the lamellae of smaller larvae make up 20% of their calculated area it is likely that the true area is much less and the percentage areas quoted for lamellae must be regarded as maximum values.

This method of determining surface area is more accurate than that used by Eriksen (1986). Eriksen (1986), calculated that the surface area of the lamellae of final instar Lestes disjunctus larvae was 60% of the total body surface area. This figure is more than three times that estimated for P. nymphula and is higher than expected even allowing for differences between species. The greatest error in Eriksen's (1986) estimate was to calculate the body area by assuming its shape approximated to a cylinder and using the appropriate mathematical formula although he measured the area of the lamellae directly.

An estimate of this error can be found by applying the technique used by Eriksen to larvae of P. nymphula of a known, measured area. For example, using two larvae, one of 40.5 mg, 12.8 mm total length and 2.5mm width and another of 10mg, 6.6mm total length and 1.5mm width. According to Eriksen's (1986) calculation the surface areas of these two larvae should be 98 mm^2 and 31 mm^2. However, by direct measurement the surface areas of these larvae were found to be 184 mm^2 and 73 mm^2, (of which the lamellae make up 28mm^2 and 11 mm^2 respectively). The actual surface area is thus overestimated by at least twice when calculated by Eriksens' method. Similarly the surface areas of the lamellae of these larvae accounted for 15% of total body area when measured directly but for 22.5% and 26.9% respectively when calculated using Eriksens method. Thus the values given by Eriksen (1986) for Lestes disjunctus clearly exaggerate the importance of the lamellar surface areas in that species.

3.3.3. Lamellar morphology and a defensive function.

The characteristics of effective threat displays for zygopteran larvae are not known, but large surface area and patterning to create maximum visual impact are considered to be important. The characteristics of an effective autotomous organ have been described in lizard species. In addition to a large surface area and distinctive pattern

to attract attacks, the organ must be expendable and have a specialised fracture point to allow the organ to be autotomised if seized (3.1.2).

Lamellae as a display organ.

Lamellae have characteristics that could be considered consistent with an effective display organ. They have a large surface area for their as described earlier (3.3.2) and a distinctive dark "X" mark located towards the distal portion of the lamellae (Plate 3.1). Both the large flat surface area and markings may increase the effectiveness of any display properties the lamellae may have.

Lamellae as autotomous organs

Lamellae also have characteristics that are consistent with an autotomous organ. In addition to their large surface area, pattern and "low value", already described, they also have a specialised fracture point. In addition to its structure, the function of the breaking joint was investigated by examination both before and after lamellar autotomy.

Structure of the breaking joint

Each lamella is attached to the last abdominal segment by a breaking joint. The breaking joint is oval in cross section and protected by the extended rim of the tenth abdominal segment (Plate 3.14). The lamella is fixed to the joint by the basal piece (Plate 3.9) which is a band of thickened cuticle, composed mainly of exocuticle, around the base of the organ (Plate 3.15). There is a similar band of cuticle on the abdominal side of the joint. The joint itself is filled by a plug of diffuse, alveolar tissue with a disc of dark staining tissue, possibly cuticle, separating it from the lamella. In P. nymphula the breaking joint is entirely filled by this plug except for the trachea and haemolymph ducts (Plates 3.14;3.15). Tillyard (1917a) described a tissue plug in the lamellae of Neosticta canescens Tillyard though in this species the plug does not entirely fill the lumen of the joint.

Plate 3.14 S.E.M of the area of the abdomen to which the lamellae adhere. (X93). The median and one lateral lamella have been autotomised at the disc shaped breaking joint leaving one lateral lamella intact. Scar tissue (..) can be seen formed on the dorsal (Dd) and ventral (Vd) haemolymph ducts and tracheal trunk endings (T) in the tissue plug (Tp).

Plate 3.15 Longitudinal section through the breaking joint of a lateral lamella from a final instar larva of P. nymphula, mallory triple stain (X200). The dorsal abdominal tracheal trunk (Ta) divides in the breaking joint to give two lamellar trunks (Tb). The cuticle of the abdomen and lamella is thickened on either side of the fracture point (Fp). The joint is filled by a plug of alveolar tissue (Tp). The internal laminae (La) and lamellar attachment muscle (Lm) can also be seen.


Dd
T
Vd
Tp

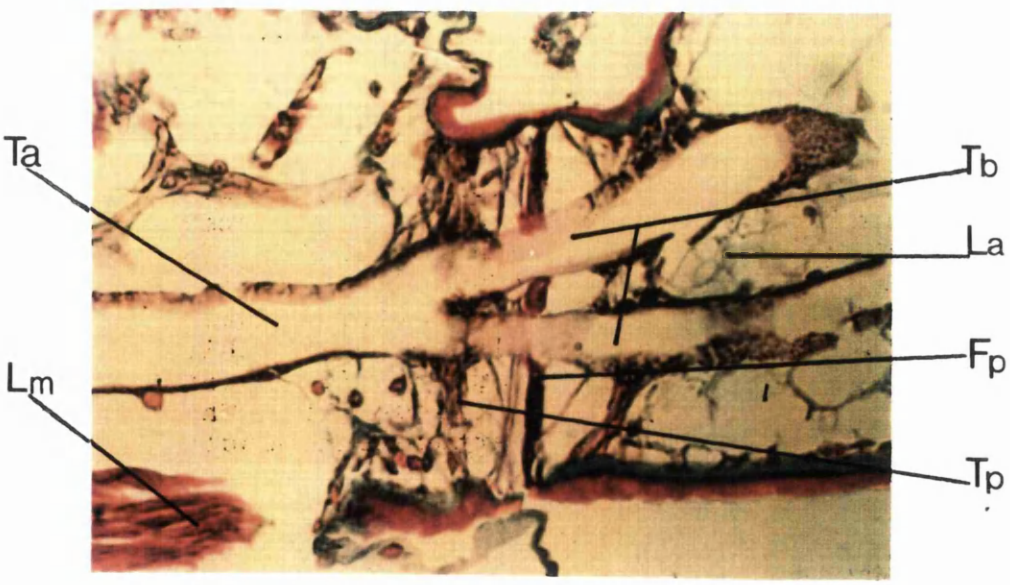

Ta
Tb
La
Fp
Lm
Tp

Plate 3.16 S.E.M. of the surface of a freshly autotomised breaking joint of a lateral lamella from a final instar larva of P. nymphula (X 462). The tissue plug (Tp) has been forced out of the constricted openings of the haemolymph duct (Dd) and tracheal trunks (T) which have receded into the abdomen. Note also the thick ring of cuticle (Cu) of the fracture point (Fp). The structure does not show evidence of contraction.

Plate 3.17 S.E.M of the surface of a lateral lamellar breaking joint following autotomy, from a final instar larva (X733). Scar tissue has formed over the tracheal trunk (T) and haemolymph duct endings (Dd, Vd).


T
Tp
Fp
Cu
Dd

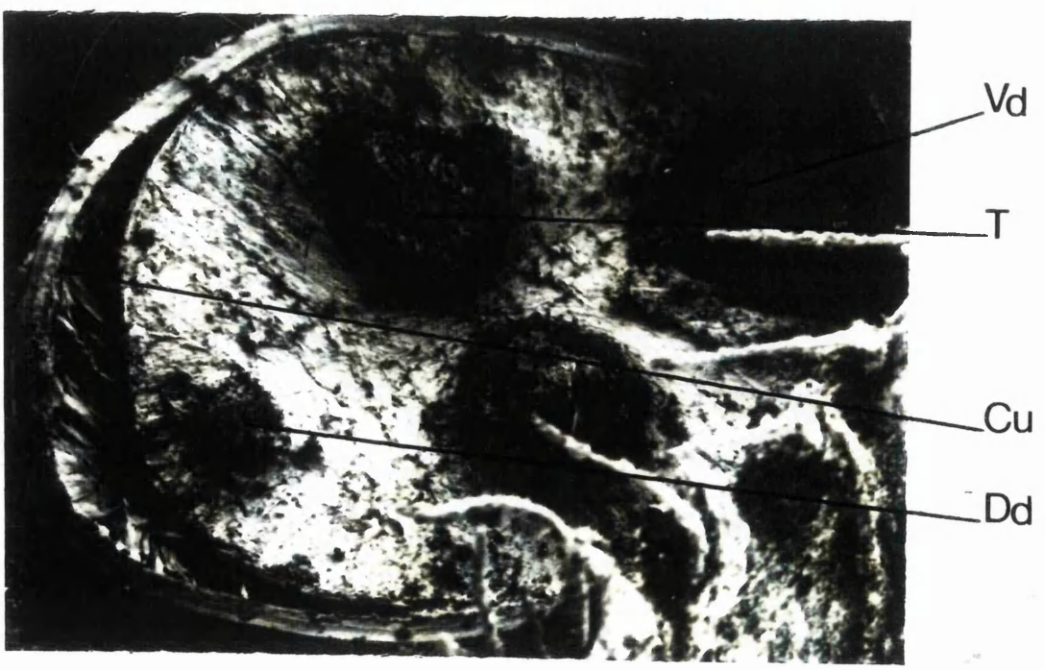
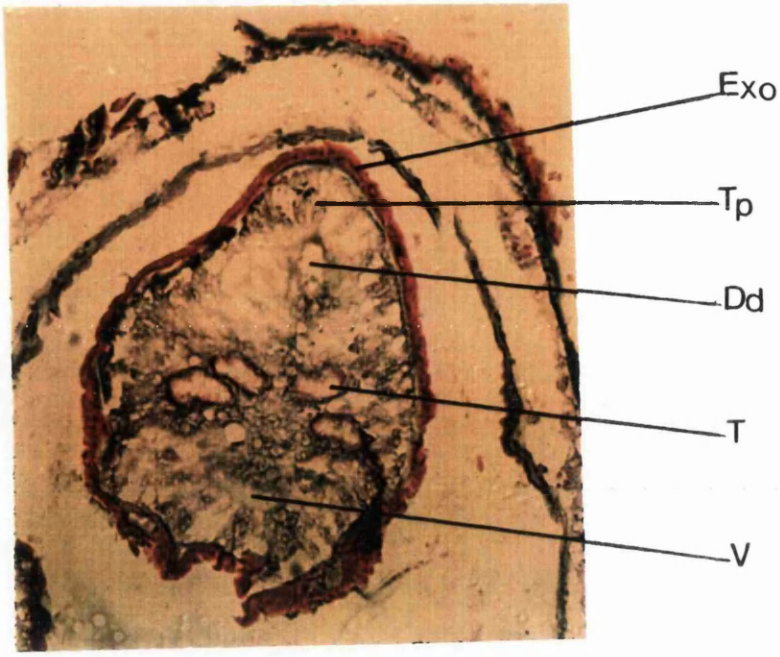
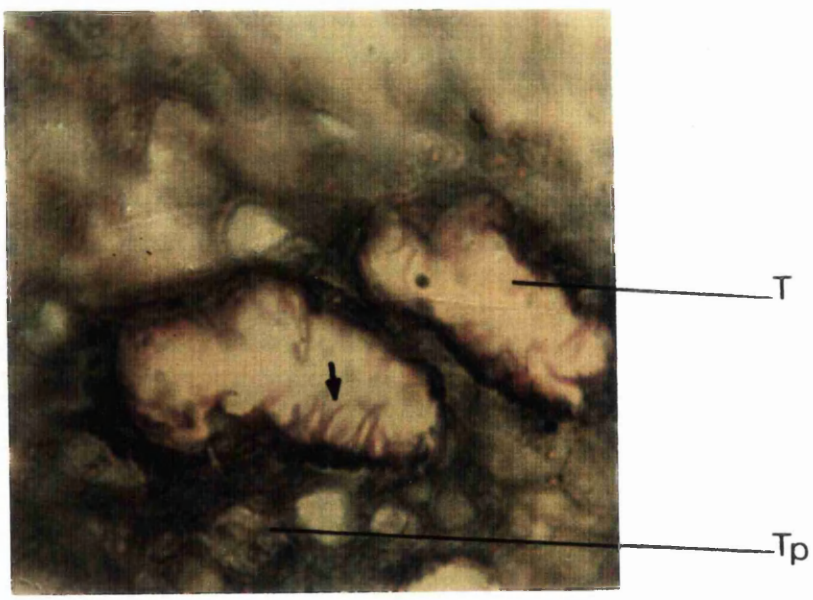

Vd
T
Cu
Dd

Plate 3.18 Transverse section through the fracture point of the breaking joint of a median lamella from a final instar larva of P. nymphula (Mallory triple stain)(X200). The joint fractures around this band of red staining exocuticle (Exo). The plug of diffuse alveolar tissue (Tp) contains the four tracheal trunks (T) and the dorsal (Dd) and ventral (Vd) haemolymph ducts. Just visible in the lumen of the tracheal trunks are the inwardly directed hydrofuge hairs.

Plate 3.19 Transverse section through the lamellar tracheal trunks (T) at the point of fracture of the breaking joint of a final instar larva of P. nymphula showing the inwardly directed hydrofuge hairs (?) (Mallory triple stain) (X1000).


Exo
Tp
Dd
T
V


T
Tp

Function of the breaking joint

Present observations suggest that the sealing of the breaking joint during autotomy is accomplished by passive rather than the muscular action suggested by MacNeill (1960) and Tillyard (1917a). Firstly there are no muscles visible in the joint itself. The only muscle visible in sections of the joint are those that attach the joint to the abdomen (Plate 3.15) and are used to fan and wave the lamella. Secondly, the surface of the joint after autotomy shows no sign of muscular contraction (Plate 3.16). The joint remains the same shape even one week after autotomy of the lamella (Plate 3.17).

The surface of the joint immediately after autotomy shows that the fracture occurs along the weak region of cuticle around the joint as described previously (Plate 3.16). Further, the remaining band of thickened cuticle around the joint would hinder any sphincteral contraction.

The tracheal trunks and the haemolymph ducts are blocked when the tissue plug is forced through the open end of the joint. During autotomy the fractured ends of the trunks and haemolymph ducts recede into the abdomen (Plate 3.16). The tracheal trunks are additionally protected from the influx of water by the presence of inwardly directed hairs in the lumen of the main trunks, these hairs may have hydrofuge properties (Plates 3.18 and 3.19). An external view of a freshly autotomised breaking joint shows the tissue plug intact and filling the exposed lumen of the joint and the sealed tracheal and haemolymph trunks (Plate 3.16). Eventually plugs of scar tissue form over the exposed trunks and ducts (Plate 3.17). This figure also illustrates the amount of debris that becomes attached to the exposed face of the breaking joint.

3.3.4. Lamellar morphology, locomotion, ion uptake, and sensory function

The function of the lamellae of P. nymphula as either ion uptake organs, fins, or sensory organs was not investigated directly in the present study. However, the efficiency

of the lamellae at these three functions can be assessed from the general morphological descriptions provided.

Ion exchange.

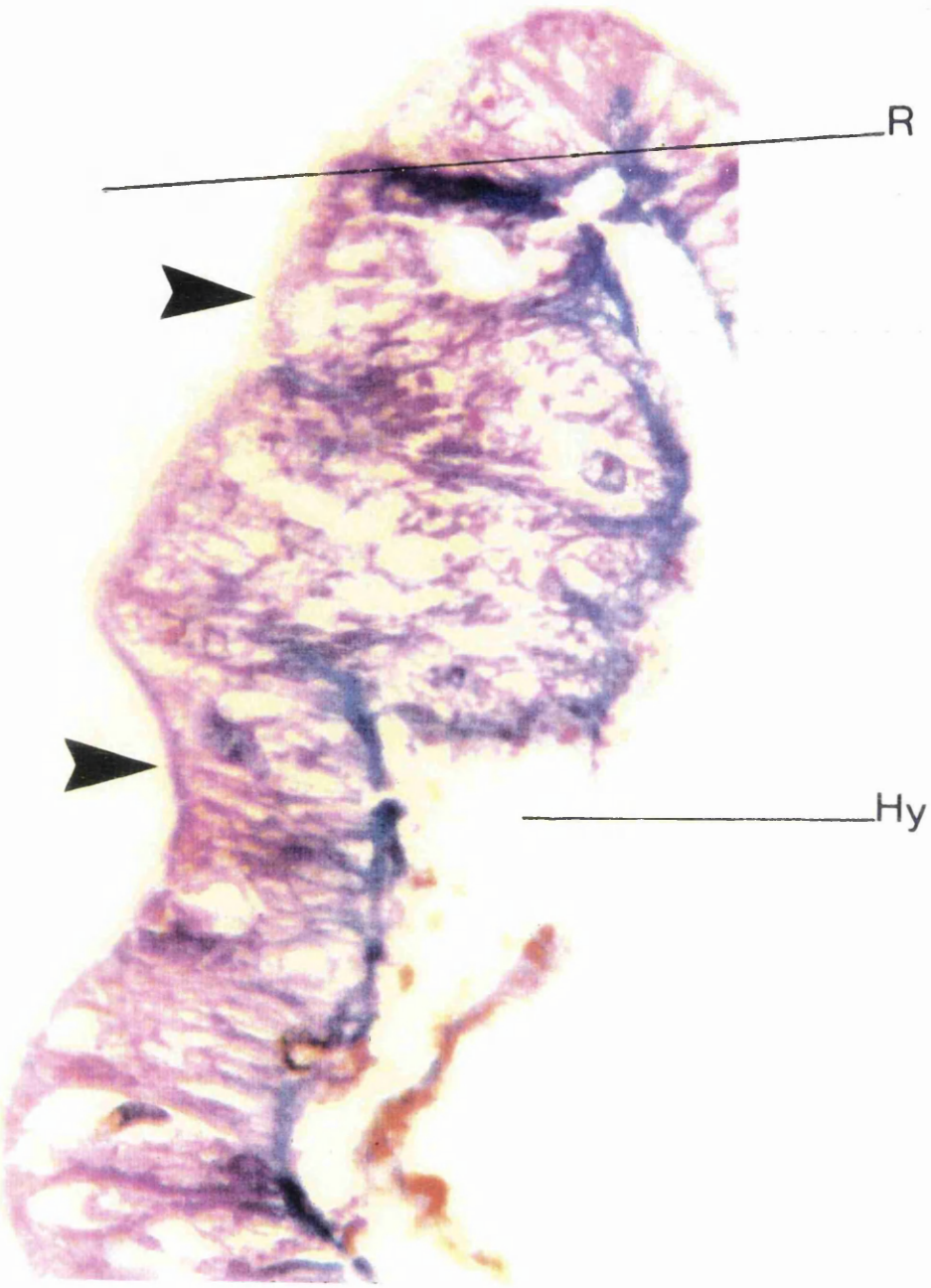
Ion uptake structures in fresh water insects whether epithelia or organs have a characteristic structure (Komnick, 1983). This includes a large surface area, thin cuticular covering, rich subcuticular tracheolation, highly folded plasma membrane and high concentrations of mitochondria (Komnick, 1983). The lamellar material of P. nymphula investigated contained normal amounts of mitochondrial material and had no folded plasma membrane. As described earlier the densities of mitochondria in lamellar and abdominal hypodermis were similar. Investigation of the surface of the lamellae showed no specialised organs that could be involved in ion uptake.

Ion uptake organs have been studied in other species of zygopteran (Coenagrionidae)(Wichard and Komnick, 1974a) in which the main site of chloride ion uptake was found to be across a specialised thickened hypodermis of the hind gut. Examination of the hind gut of P. nymphula shows a similar thickened epithelium present (Plate 3.20). However, as no experimental investigation of the metabolism of this area was carried out it cannot be concluded that this hypodermal region is a site of Chloride ion uptake.

Sensory function

The sensory efficiency of the lamellae was assessed by determining the abundance of sensory organs present. Four types of organs were found on the lamellar surface whose function can be interpreted as sensory. These were piliform and spiniform setae, hairs (very thin piliform setae) and "sensory pits". These sense organs were connected to unmyelinated nerve fibres which ran through the haemocoel attached to the basement membrane. These were typical of invertebrate nerve cells (Toner and Carr, 1971).

Plate 3.20 Transverse section through the rectum of a final instar larva of P.nymphula. (X200). Showing thickened epithelial cells (..), Rectum (R) and Haemocoel (Hy).


R
Hy

The thicker spiniform setae are interspersed with the longer, thinner, piliform setae (Plate 3.21) along the dorsal and ventral edges of the lamella (Plates 3.21, 3.9 and 3.10). The spiniform setae on the faces of the lamellae are much less robust than those along the mid rib and edges. Only piliform setae are present past the nodal point on the dorsal and ventral edges (Plate 3.9 and 3.10). The remaining spiniform and piliform setae were found along the thickened mid-rib. The mid rib provides support for the lamella and encloses the main tracheal trunks, it runs along the centre of the lamellae curving ventrally towards the distal tip in a way described by Gardner and MacNeill (1950)(Plate 3.9). The cuticle of the mid rib is thickened in comparison to the faces of the lamellae to provide the strengthening (Plate 3.3). The remaining surface of the lamella is almost entirely smooth cuticle.

The external structure of the spiniform setae is similar to that of the sensory spines described on the larvae of the anisopteran Anax sp. (Pill and Mill, 1978, 1981) and various zygopteran larvae (MacNeill, 1960). According to Pill and Mill (1978), each spine has one central nerve fibre and acts as a mechanoreceptor. Watson (1966), described similar sensory setae on the saccoid lamellae of Epallage fatime and suggested that they may be sensory. The lamellae of P. nymphula by comparison have relatively few setae and may not have a major sensory role.

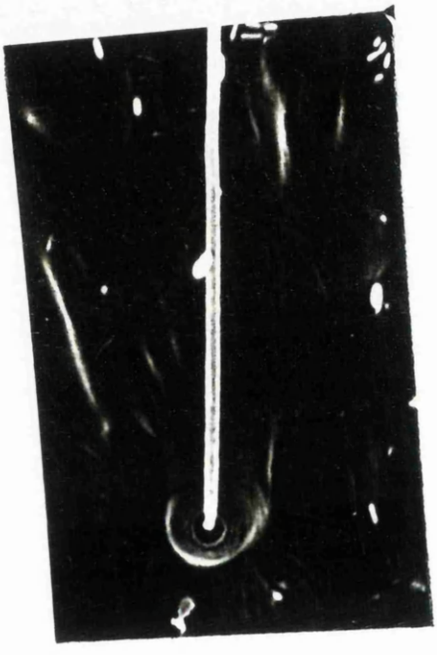
Two other structures which are interpreted as having a sensory function were found on the lamellar surfaces namely "fine piliform setae" (Plate 3.22) and "pits" (Plate 3.23). The fine piliform setae are similar in structure to those on the larvae of Epallage fatime which Norling (1984) suggested may be sensory. The pits which contain a dome (Plate 3.23) have not been described before and may be sensory. Both types of organ were distributed sparsely on the body as well as the lamellae.

Plate 3.21 S.E.M of the dorsal edge of a lateral lamella from a final instar larva of P. nymphula (X816), showing the spiniform (Sp) and piliform (Pi) setae interspersed. The spiniform setae on the faces of the lamella are less robust than those of the edges.

Plate 3.22 S.E.M of a sensory hair (fine piliform seta) on a median lamella from a final instar larva of P. nymphula (X 1920).

Plate 3.23 S.E.M of a sensory pit (?) on the postnodal region of a median lamella from a final instar larva of P. nymphula. (X 1920).


SP
Pi



Locomotion

The results of the present study showed that the lamellae are rigid structures with no internal muscles that would be capable of flexing or controlling their shape. Movement and positioning of the lamellae is carried out entirely by the abdominal musculature (Plate 3.15). It is not known whether the shape of the lamellae is consistent with that of an efficient paddle or fin. However, the presence of the breaking joint suggests these organs are not efficient as fins because if too much lateral strain is put on them the lamellae may be broken off at the breaking joint.

3.4 Discussion

3.4.1 General description.

The description of the gross morphology of the caudal lamellae of P. nymphula here is similar to that given by other authors such as Gardner and MacNeill (1950), Corbet (1955), Lucas (1900) and Merritt (1983). This structure is typical of Coenagrionid lamellae which are lanceolate in small larvae and laterally flattened in large larvae. Growth of these organs is non-protrusive (MacNeill, 1960) and the area of the postnodal and prenodal regions remains approximately equal in proportion throughout development. According to MacNeill (1960) the caudal lamellae of this species are "weakly nodate", duplex structures though Merritt (1983) using Tillyards (1917a) classification considered them to be denodate. This divergence of opinion arises from the difficulty of identifying a nodal point. The present study shows that the nodal point is present and that it is marked clearly by a change in the cuticle and in the arrangement of setae on the lamellar surface. The lamellae of this species should be regarded as δ subnodate, vertical according to Tillyard's classification (1917a).

3.4.2. Lamellar function

The results of the present study can be interpreted in terms of the five functions proposed for lamellae (chapter 1). These were 1) respiration, 2) locomotion, 3) ion uptake, 4) defence and 5) sensory.

1) The respiratory function

The characteristic features of a gas exchange gill were outlined in 3.1.3. and included 1) a large surface area, 2) a low metabolic oxygen demand and 3) a short diffusion path for oxygen from the environment to respiring tissues. Insect tracheal gills have additional respiratory adaptations to reduce the diffusion path for oxygen entering

the tracheal system such as a specialised arrangement and packing of the subcuticular tracheoles and reduced cuticle thickness (Wichard, 1973; Wichard and Komnick, 1974a).

The caudal lamellae of P. nymphula were found to make up from 15% to 20% of the total surface area of the larva but only 4% of the total weight of the larva, depending on the instar. Thus in relation to their weight the lamellae of this species do have a large surface area. However large surface area alone may not be a good indicator of respiratory function. For example, Norling (1982) showed that in larval Epallage fatime the lateral abdominal gills and lamellae were of approximately equal surface area. In this species the saccoid lamellae have no respiratory role and may instead function as defensive organs (Norling, 1982).

Though many authors state that a large surface area is a requirement for a respiratory gill (Dejours, 1975; Mill, 1972,1974), what constitutes a "large surface area" is uncertain as few workers have measured the area of tracheal gills in relation to that of the body. In the Zygoptera only one study has been carried out, on the surface area of the lamellae of Lestes disjunctus (Eriksen, 1986). Eriksen calculated that the lamellae of this species occupied a constant 60% of the surface area of the animal throughout growth. Although due to technique deficiencies in Eriksen's study the true figure for the surface area of the lamellae of this species is likely to be half that quoted by him. Even so the lamellae of P. nymphula occupy a smaller proportion of the surface area than those of Lestes disjunctus.

Environmental oxygen concentration has been shown to influence tracheal gill area both between (Dodds and Hisaw, 1924) and within (Wichard, 1977) species. Dodds and Hisaw (1924) showed that mayfly species from well oxygenated environments had a smaller area of gill than species from environments with consistently low oxygen concentrations. Corbet (1962) suggested a similar trend might exist in the lamellae of the

Hawaiian genus Megalagrion (Odonata). The oxygen content of the environment may affect gill area even within a species. According to Wichard (1977) individuals of the trichopteran species Molanna angustata Curt. from environments with consistently high oxygen levels had a significantly smaller surface area of gill than individuals of the same species from poorly oxygenated habitats. Wichard (1977) suggested that this morphological adaptation was in response to differences in flow rate and hence oxygen concentration in the habitats of the different sub-populations investigated.

The present study (Chapter 2) found that the environment in which the study animals were collected was well oxygenated throughout the year suggesting that gills may not be required in any case in the study population. If so then in this species the whole body wall acts as a gas exchange gill. There are no data available for comparison of the environmental oxygen concentrations and lamellar areas for other zygopteran species. Larvae of P. nymphula or other species collected from habitats with consistently low oxygen levels may have a larger surface area of lamellae, which may indicate a respiratory gill function. Although the available data on the distribution of P. nymphula suggests that this species prefers cooler, well oxygenated habitats and consequently may not require respiratory gills. In the Northern latitudes of its range in Europe this species inhabits standing or slow flowing water but in the southern part of its range, it is found in faster flowing water and at higher cooler altitudes where oxygen levels will be greater (Askew, 1988). It is therefore possible that other zygopteran species that inhabit less well oxygenated habitats may use their lamellae as tracheal gills.

A further aspect of lamellar surface area that suggests they may not be respiratory gills is the way their surface area changes in relation to that of the body surface area during growth. As insects grow, the surface area to volume ratio decreases and oxygen diffusion to the tissues becomes more difficult for the larger instars (Thorpe, 1950). Consequently some form of gill becomes necessary and in many species of fresh water invertebrate respiratory gills are found only in the later instars and pupal stages.

These gills can increase both in complexity and number with age. In Epallage fatime Charp (Odonata) for example, the filamentous lateral gills increase in number and in the proportion of the total surface area during development (Norling, 1982).

If the caudal lamellae of P. nymphula are similarly important as gills then they should follow a similar pattern of development and make up an increasing proportion of the total surface area as the larva grows. The lamellae of P. nymphula however, constitute a constant if not reducing proportion of the total body surface area as they grow. Because of the surface area effect the respiratory efficiency of these lamellae will decrease at a time when their efficiency should be increasing. This is confirmed further by the presence of a functional spiracle in final instar larvae. This is an adaptation to allow larvae to breath air when out of the water and prior to emergence, although under conditions of oxygen stress it is possible that this spiracle can be used for air breathing. Zahner (1959) showed that under hypoxic conditions that calopteryx larvae would move to the water surface and expose their abdomen and thorax to the air, which indicates an ability to breathe air.

Tissue that has a high metabolic rate and hence high oxygen demand characteristically has a high density of mitochondria (Chapter 3.1). The type of metabolic activity of tissue can be determined from the type and arrangement of cell organelles such as endoplasmic reticulum and secretory granules. Tracheal gills typically have few mitochondria. The hypodermis of the caudal lamellae of P. nymphula contained few mitochondria or cell inclusions that indicate significant metabolic activity. The arrangement and type of organelles suggests that the metabolism of the lamellar hypodermis is no greater than that of the abdominal hypodermis. Though there is no indication that the lamellae have the "feeble metabolism" suggested by MacNeill (1960). The metabolic oxygen demand of the lamellae of P. nymphula is therefore consistent with that required for a respiratory gill.

3) Oxygen diffusion distance.

The oxygen flux across a tracheal gill depends directly on the diffusion distance from the environment to the tracheoles. In tracheal gills the oxygen flux is enhanced by a characteristic efficient packing of the tracheoles and a reduction of cuticle thickness (3.1). Table 3.1 summarises available data on the cuticle thickness and tracheolar arrangement of the tracheal gills of a number of fresh water insect larvae for comparison. These data are taken from published work from various authors and because of the limited data available statistical comparison between species could not be carried out. A comparison of the data in table 3.1 with those derived for P. nymphula shows:-

a) The structure of the cuticle of the lamellae of P. nymphula is similar to that found in other aquatic insect larvae (Credland, 1983; Norling, 1982). However, in comparison to the tracheal gills of other fresh water insect species the lamellar cuticle of P. nymphula is thicker and as a result is less efficient at gas exchange. For example, the cuticle of the tracheal gills of other species is between 0.1 to 1.0 μm thick. Exceptionally, the tracheal gill cuticle of a species of Euphaeidae was 2.5μm thick (Table 3.1). However, the lamellar cuticle of P. nymphula was always greater than 2.5μm thick and as much as 9.04μm thick in the prenodal region. The cuticle of the lamellae is therefore much thicker than would be expected for a tracheal gill.

b) The tracheoles in the lamellae of P. nymphula are similar in structure to those of other species (Table 3.1). However, the arrangement and packing these tracheoles is substantially different. In these other species described the tracheoles are either densely packed with a random arrangement or evenly spaced with an ordered arrangement and all within the hypodermis and immediately below the cuticle. The lamellae of P. nymphula have at a maximum 0.39 tracheoles per micron with an irregular, random arrangement (Table 3.1). In comparison to the tracheal gills of the other species described, both the arrangement and density of the tracheoles would make the lamellae of P. nymphula inefficient as respiratory gills.

However tracheolar arrangement is known to be flexible (Wigglesworth, 1983). Though not demonstrated in the Odonata Louden (1989) found that in meal worm larvae tracheal hypertrophy could occur in response to lowered environmental oxygen concentrations. Harnisch (1958), recorded an unusually rich development of subcuticular tracheoles in the abdominal hypodermis of Coenagrion sp. (Zygoptera) larvae following the removal of the lamellae, though this may have occurred in response to the injury. It is possible that the tracheal systems of P. nymphula larvae from habitats with lower oxygen concentrations than those encountered by the study population could show similar modifications. Comparison of the lamellar morphologies of larvae from these different subpopulations would be needed to determine whether these organs were adapted for gas exchange in some habitats.

Comparison with the tracheal gills of other fresh water insect larvae suggests the lamellae of P. nymphula are not efficient as gas exchange structures. However, a respiratory role for the laterally flattened caudal lamellae found in other species of Zygoptera cannot be entirely discounted. It is possible that morphological adaptation of the tracheal system and tracheal gills, reported in other insect species (Dodds and Hisaw, 1954; Harnisch, 1958; Louden, 1989; Wichard, 1977; Wigglesworth, 1983), also occurs in the Zygoptera. Such morphological adaptation could occur in P. nymphula larvae living in habitats with consistently low oxygen levels but the scale of the changes in morphology required to make the lamellae of P. nymphula efficient gills are so great as to make this unlikely.

If the lamellae are not gills and P. nymphula larvae have no other obvious gas exchange surfaces the question must arise as to how larvae obtain sufficient oxygen for their respiratory needs from the environment. In Krogh's diffusion equation (equation 3.1) it is shown that respiratory adaptation can occur at a morphological or physiological

level. The results of this chapter of the present study therefore suggest that P. nymphula should show a physiological or behavioural response to low oxygen levels as they have no morphological adaptation to withstand respiratory stress. This will be investigated further in the following chapter.

2) Ion uptake function

Organs involved in chloride ion uptake are frequently found associated with respiratory gills and may have a similar morphology. These epithelias typically have a thin cuticle a large surface area a dense supply of subcuticular tracheoles, but unlike tracheal gills have a high metabolic oxygen demand (Komnick and Schmitz, 1977; Kukulies and Komnick, 1983). Eriksen (1986) suggested ion exchange as a possible function for the lamellae of Lestes disjunctus. However, larvae can survive lamellar loss which suggests that any ion uptake function these organs have is secondary (Eriksen, 1986). In the present study no ion exchange tissues were identified in the caudal lamellae of P. nymphula. The rectal location of the ion uptake apparatus of zygopteran larvae has been demonstrated in coenagrionid (Odonata:Zygoptera) larvae by Wichard and Komnick (1974b) and it is likely that the ion exchange epithelia in P. nymphula is similarly located in the rectum. More detailed analysis of this region would be required to confirm this.

3) Sensory role.

In the Polythoridae (Odonata:Zygoptera) the lamellae have a dense covering of setae and a reduced respiratory function which led Norling (1982) to suggest a sensory role for these organs. Similarly, Watson (1966) suggested that the reduced, hairy, lamellae of Pentaphlebia (Zygoptera) larvae may also be primarily sensory. If the degree of hairiness is related to a sensory role then the lamellae of P. nymphula which have relatively few setae must be regarded as poor sensory organs.

4) Locomotion.

MacNeill (1960) suggested that the laterally flattened lamellae of zygopteran larvae did not aid movements such as swimming because of their rigidity, breaking joint and membranous postnodal region. The results of the present study confirm that the lamellae of P. nymphula with their central mid-rib are rigid structures which are not capable of flexing except towards the distal tip where the cuticle is reduced. Furthermore, there are no significant muscles within the lamellae and the restricted range of lamellar movements available to the larva, such as fanning, are effected by the muscles that attach the breaking joint to the abdomen. As suggested by MacNeill (1960), the breaking joint may make the lamellae ineffective swimming organs because of the ease by which they could be autotomised when stress is placed on them during swimming. However, as described earlier Robinson (1990) suggested that the lamellae of E. civile may increase the probability of escape from a predators (2.1.2). A locomotory function for the lamellae of P. nymphula cannot be discounted at this stage.

5) Defence and threat displays

The use of lamellae by larvae during aggressive interactions with conspecifics or predators is discussed in Chapters 2 and 5. In this chapter only the structural adaptations of the lamellae are considered. Some elements of the morphology of the lamellae are compatible with a defensive function. The possible defensive adaptations of the lamellae of P. nymphula were described earlier and included a large surface area, distinctive pattern, low replacement cost and most importantly a specialised fracture point (3.1.4). If lamellae act as a threat display then their area and patterning will enhance this function.

Firstly the surface area, the lamellae of P. nymphula have a disproportionately large area for their bulk. If attacks by predators or conspecifics are directed at the body in proportion to surface area then the lamellae would receive disproportionately more attacks. However, attacks are not made with equal distribution over the whole surface of the

animal. The response of a larva to a predator is to direct its lamellae towards the attacker (Chapters 2 and 5) thus decreasing the probability of damage to the body and further increasing the chance of damage to the lamellae.

A further aspect of the surface area of these lamellae which suggests adaptation for defence is their shape. The overall shape of the lamellae is that of a flat plate which presents the surface area in the most visible way possible. A similar surface area could be presented much less obtrusively using many small cylindrical branches such as is found in the anal tufts of the Amphipterygidae (2.1.3). This suggests that the shape and visual impact of the lamellae is more important than simply the area alone.

Secondly, the distinctive patterning of the lamellae of P. nymphula may also aid in attracting attacks. In this species the lamellae have a characteristic dark cross mark. Other species have dark chevron bands (Enallagma cyathegerum (Charp))(MacNeill, 1960). Because of the light and dark contrast these patterns create, the lamellae may be made more visible compared to the relatively uniformly coloured body and so attract more attacks.

Thirdly, no important metabolic or sensory functions were implied by the anatomy of the lamellae of P. nymphula. In addition, because the lamellae make up only 4% of the bulk of the animal, their loss would require relatively little expenditure of energy to be made up. Full regrowth of the lamellae may take place over a number of moults further spreading the energetic cost of their regrowth.

Fourthly, the most convincing morphological evidence in favour of an autotomous function for the lamellae of P. nymphula is the presence of the specialised breaking joint. Though the general structure of this joint has been described before in some other species (Tillyard, 1917), the results of the present study show conclusively adaptation for autotomy. This joint clearly limits damage to the body of the larva during

an attack by a predator or conspecific. The thickened bands of cuticle around the joint allow the lamellae to be lost with no damage to the surrounding cuticle. The tissue plug prevents body fluids escaping and the hydrofuge hairs within the trachaea prevent water entering the tracheal system. If the lamellae are not autotomised at this joint the larva will die due to loss or dilution of body fluids (Eriksen, 1986). The way in which this joint functions is significant because it represents the only clear specialisation found on the lamellae of this species.

3.4.3. Conclusion.

The results of this section of the present study are significant for two reasons. Firstly, they show that although the lamellae of P. nymphula superficially resemble tracheal gills their ultrastructure indicates otherwise. Important respiratory adaptations that were missing from the lamellae studied were thin cuticle and a specialised arrangement of the subcuticular tracheoles. This still leaves open the question of how larvae are able to take up oxygen from the environment if they have no specialised tracheal gills. It is suggested here that larvae respond to respiratory stress at a behavioural or physiological level (3.4.2). However, although the morphology of the lamellae of P. nymphula indicates otherwise it is still important to determine whether the lamellae of this species have any significant respiratory role, even a relatively minor one. Consequently this will be investigated in more detail in the following chapter (Chapter 4).

The second important finding of this section of the present study is that the lamellae of this species may have a defensive function. This is inferred mainly because of the specialised function of the breaking joint but also because of their large surface area and their lack of tissues and organs implying significant metabolism. When considered in conjunction with the results of chapter 2, the evidence in favour of a defensive/threat display role for the lamellae is reinforced further. In chapter (2) it was shown that the rate

of lamellar loss in the study population was significant and correlated with larval size and habitat type. Baker and Dixon (1986) and Robinson et al., (1991) have shown that lamellar loss occurs in three species of larval zygopterans and that it was related to the frequency of intraspecific larval interactions. Because this pattern has now been found in an additional species (P. nymphula; Chapter 2) and because the lamellae of P. nymphula show morphological adaptation for autotomy further investigation of the defensive/threat role for these organs is merited.

CHAPTER 4

THE RESPIRATORY PHYSIOLOGY OF THE CAUDAL LAMELLAE OF P. nymphula.

4.1 Introduction.

4.1.1. Lamellae as respiratory gills.

The results reported so far suggest that the lamellae of P. nymphula do not function as respiratory organs. In the previous chapter, it was shown that their morphology differs markedly from that expected of an insect tracheal gill. In the field, a high percentage of larvae were found surviving with missing lamellae, the frequency of lamellar loss being correlated to physiological and environmental variables (Chapter 2). These findings are inconsistent with the work of authors who conclude that lamellae have an important role in larval respiratory gas exchange. The respiratory role of lamellae has never been convincingly demonstrated by experiment for any zygopteran. Much of the confusion regarding lamellar function is due to the way this role was originally assigned. The term "gill" was first applied to lamellae because their morphology superficially resembled that of a tracheal gill (3.1), their respiratory function being assumed rather than demonstrated experimentally. Since then, this role has been generally accepted (Corbet, 1962; Johnson, 1991; Tillyard, 1917a).

Evidence for a respiratory function.

The studies that have shown lamellae to be important as respiratory gills include those by Eriksen (1986), Harnisch (1958), Koch (1934), Pennack and McColl (1944) and Zahner (1959). For example, Koch (1934), used the rate of CO_2 diffusion into the tracheal system as an indicator of the oxygen flux of the lamellae in Agrion (Coenagrion) pulchellum larvae. Larvae were placed in water supersaturated with CO_2 and the rate of

evolution of gas bubbles from an opening in the tracheal system observed. In experiments with intact larvae, the rate of evolution of gas bubbles was 32-45% greater than those experiments using larvae without lamellae. Koch suggested that this indicated that larvae with lamellae had more permeable cuticles (due to the lamellae) and hence greater respiratory efficiency than those without lamellae. However, diffusion of CO_2 is not usually restricted to the tracheal system as this gas has a relatively higher solubility coefficient in body tissues and water than oxygen (Dejours, 1975; Krogh, 1941; Taylor, 1985). The diffusion rate and paths of CO_2 and oxygen in larvae are not therefore comparable.

Most studies of lamellar respiratory function have used the oxygen consumption rate of larvae as an indication of respiratory efficiency. Harnisch (1958), measuring oxygen consumption rates, determined that the lamellae of Agrion (Coenagrion) sp. larvae were responsible for 38.5% of oxygen uptake. Whilst Zahner (1959), also measuring oxygen consumption rate, reported that without lamellae the ability of Calopteryx sp. larvae to take up oxygen was reduced by as much as 30%.

Perhaps the most widely quoted study showing that lamellae act as respiratory gills was that by Pennack and McColl (1944), who used a large closed bottle respirometer to measure the oxygen consumption rates of groups of 20 Enallagma sp. larvae either with or without lamellae. In these experiments the oxygen concentration of the water in the jars was measured before larvae were sealed into them. The oxygen concentration of the water was measured again once "most of the larvae had died". Pennack and McColl (1944) then calculated from this group rate of oxygen consumption the average oxygen consumption rate of the "individual" larvae. From their results they concluded that those larvae without lamellae died at a higher oxygen concentration than those with lamellae.

More recently, Eriksen (1986), used a closed bottle respirometer to measure the oxygen consumption rate of individual Lestes disjunctus larvae, either with or without lamellae, under varying environmental oxygen concentrations and temperatures. Eriksen also measured larval respiratory ventilation rate and anoxia survival. To date, these experiments are the best controlled of all the investigations of lamellar function. Eriksen described a statistically significant difference in the rate of oxygen uptake between larvae with and without lamellae at temperatures greater than 10^{o}C and under hypoxic conditions. He concluded that the lamellae of this species accounted for 60% of larval oxygen uptake. However, technical deficiencies in these experiments, which are discussed later, make these results questionable.

Evidence against a respiratory function.

However, a number of studies have found that lamellae have no respiratory function (see Patee (1956) and Thorpe (1932)). Thorpe (1932) used the unicellular Polytoma, which congregates in areas of oxygen saturation, to determine where oxygen depletion occurred around the bodies of Agrion (Coenagrion) sp. larvae. Thorpe found that the anterior region of the abdomen was the main site of oxygen uptake, followed in importance by the thorax and then the lamellae. Patee (1956), measuring oxygen consumption rate of Calopteryx sp. larvae in closed bottle respirometers, found that those without lamellae had a higher oxygen consumption rate than those with.

Lack of consensus between authors has occurred for several reasons (technique deficiencies, the collection of insufficient data for statistical analysis and the use of inappropriate analyses). Interpretation of the results of Patee (1956) and Zahner (1959) is hindered by the fact that neither author identified the species of Calopteryx used in their experiments. It is therefore possible that the differing results are simply due to interspecific differences. The Calopteryx larvae studied must have belonged to one of the

three European species. The lamellae of these species have a similar morphology and are a taxonomic character of the genus (Askew, 1988). Failure to identify the species used in experiments has occurred in a number of other studies (Harnisch 1958; Pennack and McColl, 1944).

In none of the studies prior to that of Eriksen (1986) were sufficient data collected to allow full statistical analysis of results. Even the study by Eriksen (1986) is weakened by the use of inappropriate analyses such as "eye" fitting of regression lines that are later used to determine the relative respiratory efficiency of lamellae. Further inaccurate estimations were used in his study for the calculation of lamellar surface area (3.1).

The technical deficiencies found in these previous studies are primarily due to the facilities available at the time. However, even the more recent ones lack standardisation of techniques and the use of controls. For example, Eriksen (1986) did not adjust respiratory rate measurements to allow for differences in larval weights. This would certainly affect the results of these experiments as he used larvae of widely varying sizes (6-14 mg dry weight)(4.1.2.). Eriksen was also able to test for and detect differences in the rate of oxygen uptake of larvae with and without lamellae under conditions of up to 200% oxygen saturation. Such unstable conditions in sealed respirometers stress experimental larvae and make measurement of oxygen levels inaccurate and produce unreliable results.

Different but equally serious technical deficiencies are found in other studies. For example, in the study by Pennack and McColl (1944), the lamellae were removed from experimental larvae by amputation rather than autotomy. Larvae will shed or autotomise lamellae when these organs are restrained, resulting in minimal damage to the animal

(Chapter 3). However, Eriksen (1986) and Child and Young (1903) demonstrated that removal of lamellae by amputation rather than by autotomy caused rapid death of larvae (8 hours). Because Pennack and McColl (1944) stopped their experiments and measured dissolved oxygen in their respirometers when "most larvae had died", the premature death of larvae without lamellae due to such wounding would clearly affect their results. The most notable effect would be that larvae without lamellae would apparently die of asphyxia at relatively high oxygen levels when in fact they were dying due to the wounding.

Experimental designs

Although the results of these studies are questionable, they do provide a basis for the design of more conclusive experiments. Further experiments must include procedures which will accomplish the following:- 1) Measure accurately the differences in oxygen uptake between larvae with and without lamellae. 2) Provide sufficient, accurate data for statistical analyses. 3) Control for or account for all significant environmental and physiological variables that may influence larval respiratory rates.

Such experimental protocols have been widely adopted for the study of the respiration rates of a range of aquatic invertebrates (Gnaiger, 1983), but not previously for the investigation of zygopteran lamellar function. It is therefore important to consider all the factors that may influence larval respiratory rates before designing suitable experimental apparatus and procedures.

4.1.2. Factors affecting larval respiration rates.

Larval respiration rates are affected by both physiological and environmental factors which must be carefully controlled during any such experimental studies. The

most important environmental factors are oxygen concentration, temperature and, because of its effect on the partial pressure of oxygen in water (pO_2), atmospheric pressure (Jeffries and Mills, 1990). Physiological factors include the status of the animal with respect to activity, size (growth stage), reproductive condition, nutritional status and diet, stress and temperature acclimatisation (Duncan and Klekowski, 1975; Kiester and Buck, 1974). Because larvae are used in the present study, the effects of reproductive condition on respiration rates will be ignored.

Physiological factors

The oxygen consumption rate (O.C.R.) of an animal depends on its weight specific respiration rate (S.O.R. in $mgO_2/g/h$) and its weight (Keister and Buck, 1974). This relationship is usually described by the formula, $O.C.R. = aW^b$. In this formula a is weight specific respiration, W, larval weight and b, the regression coefficient of a line fitted to the log of respiration rate and larval weight data for that species (Keister and Buck, 1974). Weight specific oxygen consumption rate (S.O.R.) does not increase directly with weight but rather decreases (Duncan and Klekowski, 1975; Keister and Buck, 1}74). Thus although a large larva uses more oxygen than a small larva because of its greater weight, the large larva uses less oxygen per unit weight than a small larva. This relationship has been found to hold for all animals and across taxonomic groupings (Reuger et al., 1969). Failure to account for the differences in larval respiration rates due to weight could lead to errors in the interpretation of larval respiratory rate data. Eriksen (1986), for example could detect no correlation between larval weight and respiration rate which suggests experimental error in experimental technique or design.

Activity is known to increase metabolic rate and consequently oxygen consumption in both endothermic and ectothermic animals (Keister and Buck, 1974). Increased respiratory rates of aquatic invertebrates due to stress in unsuitable

experimental environments is a well documented source of error in respirometry experiments. Stress may increase larval respiration rates (Gnaiger, 1983; O'Connor et al., 1985). Umezewa (1986) for example, showed that the O.C.R. of the angel fish was raised by isolation-induced stress which, could be reduced by visual contact with conspecifics (the group effect). There is some evidence to suggest that the group effect may be found in fresh water insects, although opposite in effect to that found in angel fish. For example, Jana and Pal (1982) showed that the individual respiratory rates of a range of aquatic invertebrates, including Chironomid sp., increased as density of individuals increased. In previous studies of lamellar respiratory function the group effect has hitherto been ignored. In some cases one or more and even up to 20 larvae were placed in single respirometers, with an unknown effect on individual respiration rates (Eriksen, 1986; Pennak and McColl, 1944). Many species of larval zygopterans, including P.nymphula, are known to be aggressive towards conspecifics (Chapter 5). For larvae of such an aggressive species, the presence of other larvae in the same respirometer, or even the ability to see other larvae, may increase stress and hence respiration rates.

Environmental factors

The main environmental factors that affect larval respiration rates are temperature and environmental oxygen concentration (Davis, 1975; Gnaiger, 1983)). The relationship between respiratory rate and temperature in ectothermic animals, including zygopteran larvae, has been extensively studied (e.g. Duffy and Liston, 1985; Jana and Pal, 1982; Philipson and Moorhouse, 1976; Wiley and Kohler, 1984). As temperature rises, so the respiratory rate of the animal increases logarithmically up to a temperature tolerance limit (Duncan and Jana and Pal, 1982; Klekowski, 1975). At low temperatures larvae may enter a torpor. For example, Enallagma boreale (Zygoptera) larvae are able to survive freezing conditions in winter by entering a state of torpor (Duffy and Liston, 1985). The body fluid of this species is prevented from freezing by

antifreeze agents such as polyhydroxyl alcohols or by thermal hysteresis proteins which prevent the seeding of ice crystals within the tissues (Duman et al., 1982). Increases in water temperature can also affect the respiratory rate of fresh water insects indirectly by reducing the oxygen content of the water and thus reducing respiration rate (Chapter 3).

However, it is perhaps environmental oxygen concentration that is the most important factor in determining the respiration rate of aquatic insects. In water concentrations of oxygen are much lower and more variable than in air and are frequently limiting to respiration (Mill, 1972, 1974).

4.1.3. Responses to Hypoxia and Anoxia in aquatic insects.

Hypoxia and oxy-conformers

The terms oxy-conformer and oxy-regulator can be used to describe the response of an aquatic insect to increasing hypoxia. Whether conformers or regulators, declining oxygen concentration ultimately affects larval metabolic rate (Hochachka, 1987; Mangum and Van Winkle, 1973; Mill, 1972; Patee and Rougier, 1969; Wiley and Kohler, 1984; Yeager and Ultsch, 1989).

The respiratory rate of oxy-conforming species is linked directly to environmental oxygen levels. As the oxygen concentration of the surrounding water falls, so their respiration rates decrease in proportion (Mangum and Van Winkle, 1973; Hochachka, 1987). For example, Fox et al., (1937) showed that as environmental oxygen concentration fell the respiration rate of small Baetis sp larvae decreased in proportion indicating that they were oxy-conformers (though large Baetis sp. larvae were found to be oxy-regulators).

Hypoxia and oxy-regulators.

Oxy-regulating species by contrast are able to maintain their respiratory rates over a limited range of environmental oxygen concentration, down to a critical point whereupon they become oxy-conformers (Hochachka, 1987; Mill, 1972; Wiley and Kohler, 1984; Williams et al., 1987). For example, the mayfly Leptophlebia marginata maintains its respiratory rate at 2.1 $mgO_2/g/hr$ at an oxygen concentration of 9.0 mgO_2/l; as the environmental oxygen concentration falls to 4.0 mgO_2/l (the oxygen critical point for this species) its respiratory rate drops to 2.0 $mgO_2/g/h$. However, below 4.0 mgO_2/l the respiratory rate falls off rapidly to 0.7 $mgO_2/g/h$ at 2.0 mgO_2/l (Fox, et al., 1937).

The distinction between oxy-conformers and oxy-regulators is not always clear. For example, Yeager and Ultsch (1989) state that a basic characteristic of organisms, even oxy-conformers, is the ability to maintain some degree of regulation under changing ambient conditions. Whilst Mangum and Van winkle (1973) concluded that no species could be categorised as strict oxy-conformer or oxy-regulator and some limited degree of regulation would always be expected.

The ability of species to regulate their respiratory rates varies widely and with few exceptions is linked to the normal range of environmental oxygen concentrations experienced by that species (Mangum and Van winkle, 1973; Nagel, 1973; Wiley and Kohler, 1984; Williams et al., 1987). Animals that are adapted to live in lentic environments tend to be oxy-conforming and rely on morphological adaptation to survive hypoxia. If oxy-regulating, these species tend to have lower oxygen critical points than species from well oxygenated environments. Species adapted to life in well oxygenated, lotic environments tend to be oxy-regulators and rely on ventilation or movement to avoid hypoxia rather than morphological adaptation (Davis, 1975; Knight and Gaufin, 1984; Mill, 1974).

Susceptibility to hypoxia varies considerably between species. For example, Chironomous plumosus (Diptera) can withstand short periods of anoxia (Walsh, 1950), whilst other species including many plecopterans may succumb to even a moderate reduction in environmental oxygen levels (Gaufin and Gaufin, 1961; Reuger, et al., 1969). The distribution of many freshwater insect species, within the microhabitat, is also known to be limited by oxygen levels (Kovolak, 1976; Rahel and Kolar, 1990; Williams, et al., 1987). Although the environmental oxygen concentration selected by a species may be for an optimal rather than maximal respiratory rate (Gamble, 1969; Williams et al., 1987). The range of oxygen concentration found in the normal environment of a species can be used as an indication of the tolerance that the species has to low oxygen concentrations.

Determination of the regulatory ability of a species relies on the measurement of its oxygen critical point. Nagel (1973) defined the oxygen critical point of an oxy-regulating species as "that oxygen concentration where respiratory rate becomes dependent on environmental oxygen concentration". Previous studies of lamellar respiratory function have not made use of critical points as a means of comparing the respiratory rates of larvae with and without lamellae. If lamellae do function as gills, then the critical point for larvae without lamellae should occur at a higher environmental oxygen concentration than those with lamellae. If critical points are to be used for comparison then the way the critical point is determined is important.

Critical points are usually estimated visually from graph plots of respiratory rate against environmental dissolved oxygen concentration data for a species. However, there have been attempts made to predict oxygen critical points more objectively using mathematical models (Mangum and Van Winkle, 1973; Yeager and Ultsch, 1989).

Mangum and Van winkle (1973) determined the regulatory abilities of a range of freshwater invertebrates using a log and a second degree polynomial transformation of respiratory rate and environmental oxygen concentration data. Although these models do not predict critical points they provide a more objective method of determining regulatory ability of larvae than eye fitting of curves. The critical points may then be estimated from the lines fitted to the data. Another method of determining critical points is to use a two-segment line fitting method (Yeager and Ultsch, 1989). By this method, the critical point is determined at the intersection of the regression lines fitted to the data in the zone of regulation and zone of conformity (Yeager and Ultsch, 1989).

There is some evidence that zygopteran larvae are able to oxy-regulate under hypoxic conditions. For example, Lawton (1971a) reported that intact P. nymphula larvae were able to maintain their oxygen consumption rate down to an environmental oxygen concentration of 50% oxygen saturation (at 10^{o}C). However, Eriksen (1986) found that the ability of Lestes disjunctus larvae to regulate their oxygen consumption was relatively poor suggesting that this species tends towards oxy-conformity.

If P. nymphula larvae regulate respiratory rates, then the way they do this may be important in determining whether lamellae function as gills. Freshwater insects regulate their oxygen uptake by morphological adaptation such as gills (Chapter 3) or by behavioural adaptation. These behavioural adaptations or regulatory responses include ventilatory movements and movement to regions of higher oxygen concentrations. Ventilatory responses are generally triggered by oxygen depletion of an area immediately surrounding the animal, the boundary layer. This layer can present a significant barrier to the diffusion of oxygen into the animal. For example, Feder and Booth (1991) showed experimentally in salamanders that even in the fastest flows of water tested the boundary layer could be responsible for one third of the total resistance

to oxygen diffusion into these animals. In more sedentary species of freshwater insect, encrustation of the body and gill surface by algae and other organisms can increase the effective depth of the boundary layer (Norling, 1982; Wright, 1943).

A number of authors have reported what they consider to be ventilatory behaviour in various species of Zygoptera (Corbet, 1962; Eriksen, 1986; Pennack and McColl, 1944; Robert, 1958; Zahner, 1959). These movements include caudal swinging, in which the abdomen and lamellae are waved gently (Corbet, 1962; Eriksen, 1986; Harvey, 1985 Norling, 1982; Patee, 1956; Reuger, et al., 1969; Zahner, 1959), pull-downs, in which the whole body of the larva is rapidly pulled onto the substrate and back (Eriksen, 1986) and whole body movements such as "shivering", in which the body is vibrated (Eriksen, 1986, Zahner, 1959). Eriksen (1986) found that ventilatory movements of the lamellae of Lestes disjunctus, increased with decreasing oxygen concentration, down to a cut off point at 3.0 mgO_2/l. At this point lamellar waving ceased and total body movements (pull-downs) increased. Larvae without lamellae under hypoxic conditions carried out more pull downs than those with lamellae. However, it is not certain that these ventilatory responses are in fact related to respiratory stress. In some zygopteran species behaviours such as some forms of the "caudal swing" and "pull downs" have been interpreted as aggressive and part of agonistic interlarval displays (See chapter 5.1; Johnson, 1991; Rowe, 1985).

Freshwater insects, including zygopteran larvae can adjust their position and orientation to maximise water flow and environmental oxygen concentration (Rahel and Kohler, 1990). For example, under hypoxic conditions, Calopteryx sp. larvae showed a strong behavioural response to hypoxia by orientating their bodies in the water flow to maximise the ventilatory effect of the current. Under conditions of prolonged hypoxia, the larva would then expose its abdomen, head and thorax to the air at the water surface.

Larvae without lamellae exposed only the tip of the abdomen to the air (Zahner, 1959). It seems likely that swimming and "whole body movements" in response to hypoxia enable the larva to reach areas of higher environmental oxygen concentration (Eriksen, 1986; Krogh, 1941; Zahner, 1959). This type of response may be widespread in freshwater invertebrate species (Kovolack, 1976; Rahel and Kolar, 1990).

Metabolic responses to anoxia and hypoxia.

Another factor that may be important in determining the role of lamellae under hypoxic and anoxic conditions is the extent to which the larva relies on anaerobic respiration. Under normoxic conditions and mild hypoxia, larval metabolism is aerobic. However, as environmental oxygen levels fall below a certain limit, metabolism may switch wholly or partly to anaerobic respiration (Hochachka, 1987; Mangum and Van Winkle, 1973). The extent to which anaerobiosis occurs depends on the species and its energy requirements (Mangum and Van Winkle, 1973; Redecker and Zebe, 1988).

Long or short term anaerobiosis allows some freshwater insects to survive anoxia. For example, Redecker and Zebe (1988) demonstrated long term anaerobic metabolism in larvae of Chironomous thumni (Diptera) and short term, low anaerobic capacity in larval Culex pipiens (Diptera). The latter species lives in consistently anoxic environments, the former in well oxygenated environments. Short term anaerobic metabolism has also been found in species of Trichoptera, Ephemeroptera and Plecoptera (Weiderholm, 1984).

Some species rely on shutting down aerobic respiration completely to survive periods of anoxia (anaerobic shutdown). This response can greatly extend tolerance to hypoxic or anoxic conditions. For example, in the bivalve mussel Mytilus sp. anaerobic shutdown can extend the tolerance of anoxic conditions by 20 times or in some species of

brine shrimp by as much as 60 times (Hochachka, 1986, 1987; Keister and Buck, 1974). In Enallagma boreale (Zygoptera) larvae, anaerobic shutdown is known to occur in response to anoxic conditions encountered during winter (Duffy and Liston, 1985). If an animal relies on metabolic shutdown or anaerobiosis to survive anoxic or hypoxic conditions, then dependence on respiratory gills for oxygen uptake under such conditions may be limited. However, investigation of the type of metabolism found in P. nymphula larvae is beyond the scope of the present study.

4.1.5 Aims.

It can be concluded that many factors can affect the respiratory rates of larval zygopterans, regardless of the presence of lamellae. It is also likely that larvae have both metabolic and behavioural responses to hypoxia, such as anaerobic respiration and ventilation, that may negate the need for respiratory gills. However, in all previous studies of lamellar function many of these factors were not considered or were poorly controlled for. The results of these studies, although useful as general observations, must therefore be considered as unreliable.

Studies of the metabolic and behavioural responses of larvae to hypoxic conditions are likely to be important in determining the extent to which larvae rely on lamellae as respiratory gills. However, what is clearly required as a first step is a properly controlled investigation of the effects of lamellar loss on the ability of larvae to regulate oxygen uptake. Therefore, the main aim of this part of the present study is to determine how important the lamellae of P. nymphula are as respiratory gills under different levels of hypoxia. Before this can be done it is essential to develop a methodology for accurately measuring larval respiration rates. The experimental protocol used must control for all the factors described that can influence larval respiration rates and must produce sufficient data to allow statistical analysis of results.

By comparing the respiratory rates of larvae with and without lamellae under varying conditions of hypoxia it was proposed to assess the usefulness of several models for the estimation of oxygen critical points. A number of different conditions have been used to describe the status of an animal during measurements of its respiration rate (Duncan and Klekowski, 1975). In the present study, it was the "relative metabolism" of larvae that was measured. Relative metabolism can be defined as the resting or steady state metabolism, with the animal making "spontaneous rather than directed movements" (Fry, 1957).

Because ultimately the presence of efficient gills should allow larvae to endure periods of hypoxia, the survival of larvae with and without lamellae under hypoxic conditions was used as a guide to the efficiency of lamellae as gills under hypoxic conditions.

4.2 Materials and methods.

The materials and methods are presented in three sections, firstly, those concerning the survival of larvae with and without lamellae under hypoxic conditions, secondly those related to measurement of larval respiration rates using a flow through respirometer and lastly, those to do with measurement of larval respiration rates using the closed bottle respirometer.

4.2.1. Survival under hypoxia

Apparatus

Larvae were placed in two airtight plastic aquaria each containing 10 litres of filtered Loch Lomond water and plastic gauze to act as a substratum.

The apparatus was kept in a constant temperature room (+/- 0.5^{o}C). The oxygen level in the experimental chamber was controlled by bubbling a mixture of oxygen-free nitrogen (O.F.N.) and air into the water. An airlock allowed excess gas to escape. Oxygen levels in the chambers were monitored using a "Strathkelvin" oxygen meter and with a polarographic oxygen sensor. The advantage of this experimental arrangement over those described by previous workers (Eriksen, 1986; Pennack and McColl, 1944) is that the large volume of water used diluted larval metabolic waste and prevented the oxygen concentration from varying rapidly from the set levels.

Selection and maintenance of larvae

For each run of an experiment, two fresh groups of final or penultimate instar larvae were collected from the Ross Burn sampling site, downstream from station 5 (2.2). Larvae without lamellae were either collected in that state or, where necessary,

were induced to autotomise their lamellae by restraining the larva by the lamellae with forceps until the lamellae were autotomised. Autotomy was carried out two weeks prior to the experiments.

Larvae were maintained at the experimental temperature in oxygen saturated water for one week before the start of the experiments. An excess of bloodworms (Chironomidae) were supplied as food up to 48 hours before the start of the experiment after which larvae were starved to allow their gut to clear (Duffy and Liston, 1985; Lawton, 1971a). Larvae showing signs of moulting or metamorphosis were not used. Two sets of 20 larvae with lamellae (L+) and 20 larvae without lamellae (L-) were kept at $10^{O}C$ and two sets of 15 L+ and 15 L- larvae at $18^{O}C$. To ensure that any deaths observed in the experimental chambers were due to hypoxia rather than lamellar ablation a control group of 15 L+ and 15 L- larvae was kept in identical apparatus but under normoxic conditions.

Experimental procedure

In these experiments the oxygen level of the water in the experimental chambers was lowered from saturation to the test level, whilst that of the control chamber remained at saturation. The number of larvae surviving in each chamber was recorded at intervals from the start of the experiment. Larvae were recorded as "dead" when they no longer clung to the substrate and lay on their backs on the floor of the aquaria. Such larvae had their abdomens bent slightly ventrally and their legs closed on the thorax. This posture was adopted by all larvae when they succumbed to hypoxia and was considered to be a reliable indication of death or near death. During the runs at $10^{o}C$ the oxygen concentration was reduced to between 0.5 and 1.2 mgO_2/l over 12 hours and maintained at that level for 4 days. During the first run at $18^{O}C$ the oxygen concentration was reduced to between 0.2 and 1.0 mgO_2/l over three hours. During the

second run of the experiment, the oxygen concentration was reduced to the test level over a period of 15 hours to minimise stress to animals. The oxygen concentration at the test levels was found to fluctuate by +/- 0.8 mg/l.

4.2.2. Larval respiration using the flow through respirometer.

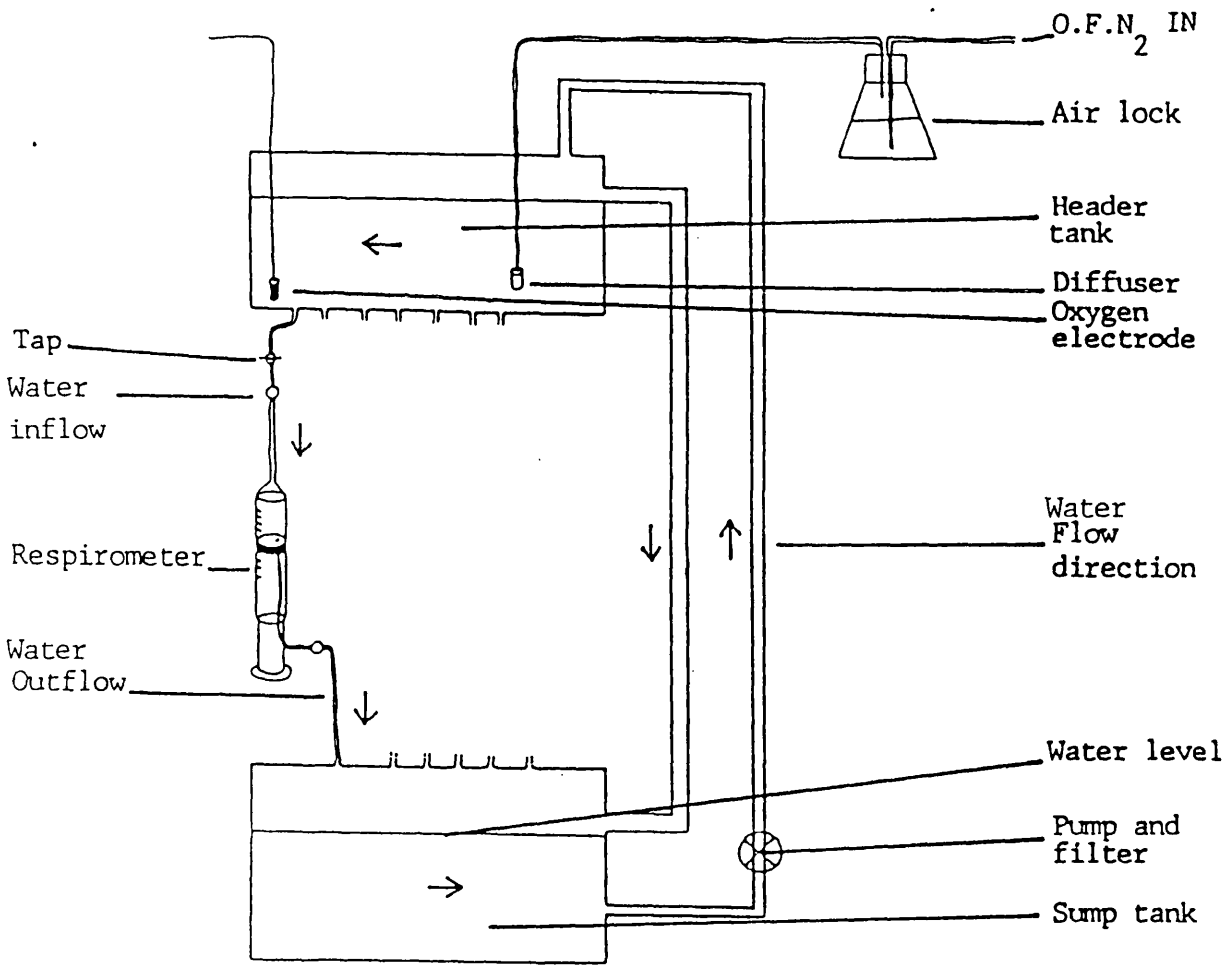
Apparatus

Flow-through respirometers have frequently been used to measure the respiration rates of dragonflies and other fresh water invertebrates (Corea et al., 1983; Forstner, 1983; Gnaiger, 1983; Nagel, 1987). For use in the present study, a flow-through respirometer was designed to determine the respiratory rates of individual L- and L+ P. nymphula larvae under varying environmental oxygen concentrations and temperatures. The design was based on those used by previous authors and took into account environmental and internal factors which may influence larval respiration (4.1). For example, temperature was controlled by placing the whole apparatus into a temperature controlled room. Daylength was set at ambient. The possible effects of stress, activity and diet/hunger on larval respiration rates were minimised and controlled as far as possible by standardising the handling of larvae as described (4.1).

The respirometer chambers consisted of 6 X $50cm^3$ "B.D. PLASTIPAK" plastic syringes. Water entered through the mouth of the syringe and left through a hole drilled in the plunger (Fig. 4.1). The respirometers were connected to a 25 Litre header tank and sump tank using plastic tubing. The respirometer flow rates were adjusted using glass taps fitted between each respirometer and the header tank (Fig. 4.1.). The water level in the header tank was maintained at a constant level by recirculating water from the sump tank using an "Eheim" aquarium pump. Excess water in the header tank returned via an overflow to the sump. This system provided a constant pressure of water

Fig. 4.1 Diagram of Flow-through respirometer. The respirometer is gravity fed with recirculated water from a header tank. Arrows show direction of water flow. Oxygen levels in the water are controlled by adding O.F.N. (Oxygen-free Nitrogen).

Figure 4.1


O.F.N2 IN
Air lock
Header tank
Diffuser
Oxygen electrode
Tap
Water inflow
Respirometer
Water Outflow
Water Flow direction
Water level
Pump and filter
Sump tank

flowing into each of the respirometers. The pump incorporated a glass wool and activated charcoal filter to remove metabolic waste products. The whole system was made airtight and filled with filtered Loch Lomond water (pH 6.2, Cond. 80μs). Bacterial growth in the respirometers was minimised by flushing the respirometers with a solution of "Milton" sterilising fluid between experiments. The respirometers were isolated from the water flow, filled with the Milton solution and allowed to stand overnight. Prior to the experiments the Milton was flushed out using changes of tap and then loch water before reconnection to the respirometer.

Dissolved oxygen concentrations were controlled by using a mixture of O.F.N. and air. The oxygen concentration of the whole system was monitored approximately using a "pHOX" dissolved oxygen meter and oxygen electrode. During experiments, the oxygen concentration of water flowing into and out of the respirometer was measured by removing 3mls samples for analysis using the micro-Winkler technique (described by Fox and Wingfield (1938) with later modifications of Carpenter (1965), Golterman (1983) and Hetherington and Hilton (1982)). The Winkler technique has been used by previous workers in studies of the respiration rate of P. nymphula larvae and has high accuracy comparable with techniques such as Cartesian diver, Gilson and Warburg methods (Lawton and Richards, 1969). The respirometers and tanks were kept in a constant temperature room (+/- 0.5^{o}C). The respirometers were kept under low light levels and under ambient photoperiod.

Maintenance and selection of larvae

Experimental animals were collected and maintained under the same conditions as the hypoxia survival experiments 4.2.1. Larvae showing signs of metamorphosis or moulting were not used. Larvae were acclimatised to experimental temperatures for at least 24 hours prior to the experiment.

Three size ranges of larvae were used. These had fresh weights of 0 to 18.0 mg, 18.0 to 30.0mg and greater than 30.0 mg. Fresh weight was determined after each measurement by air drying larvae to a constant weight (five minutes on blotting paper) and weighing to 0.1mg using a "Stanton" balance.

Experimental procedures

Larval oxygen consumption rates were measured by placing one larva into each respirometer along with a piece of plastic gauze which was used as a substratum. One respirometer was used as a reference blank and contained only loch water and a piece of plastic gauze and was treated identically to the experimental chambers. The larvae were allowed to settle for at least one hour before measurement commenced. Respiration rates were only measured during daylight to reduce stress to experimental animals although Lawton (1971a) found no diel cycle in the respiratory rate of P. nymphula larvae. The flow rates of water in each of the respirometers was measured using a stopwatch and Measuring Cylinder after the completion of the experiment.

In the literature a variety of units have been used to express the oxygen consumption rate (O.C.R.) of animals. These vary greatly according to the techniques used and include volume, mass and molar concentration and partial pressure of oxygen (Duffy and Liston, 1985; Golubkov, 1986; Mangum and Van Winkle, 1973; Mill, 1974; Reuger, et al., 1968). This lack of standardisation makes the comparison between species and results difficult. The recommended standard unit for the expression of O.C.R., which will be adopted in the present study, is molar oxygen consumption rate (M.O.R.) (mols of oxygen per larva per unit time (e.g. molO_2/larva/h))(Gnaiger, 1983). The weight specific oxygen consumption rate (S.O.R.) will refer here to the molar rate of oxygen consumption per unit fresh weight of animal (e.g. molO_2/g/h).

The use of fresh weight rather than dry weight of animal is considered adequate and it has the advantage that it does not require the sacrifice of the experimental animal (Keister and Buck, 1974).

As it was difficult to control accurately the oxygen concentration in the whole apparatus, the oxygen concentration of the water was set approximately to within one of the following four ranges of concentration using an oxygen meter. Accurate determination of the oxygen concentration in the respirometers was then carried out as detailed above.

1) 100% saturation (between 8.0 and 9.45 mgO_2/l),
2) 75% saturation (between 6.0 and 7.9 mgO_2/l)
3) 50% saturation (between 3.5 and 5.9mgO_2/l)
4) 25% saturation (between 0.4 and 3.4mgO_2/l).

The rate of oxygen consumption of larvae was measured by subtracting the oxygen concentration at the outflow from that of the inflow. The oxygen consumption of the control was then subtracted from this value to discount possible effects of bacterial respiration and to give the final result which was calculated according to the methods of Gnaiger and Forstner (1983).

4.2.3. Larval respiration rates using the closed bottle respirometer

Apparatus

The oxygen consumption rates of P. nymphula larvae both with and without lamellae were also measured using "closed bottle" respirometers. Closed bottle respirometers have been used successfully in a number of studies of freshwater insect

respiration rates (Green, 1977; O'Connor et al., 1985; Williams et al., 1987). However, closed bottle respirometry is a less desirable technique to use than flow-through respirometry as the measurements are more prone to interference from factors such as bacterial respiration, accumulation of metabolic waste, over depletion of oxygen and uneven mixing of respirometer contents. However, if the experimental conditions are controlled, these interferences can be minimised (Gnaiger and Forstner, 1983).

Maintenance and collection of larvae

Experimental animals were collected and maintained as in the preceding experiments (4.2.1; 4.2.2). However, because these experiments were carried out in a closed bottle respirometer larvae could not be acclimatised to the experimental temperature and oxygen concentrations in the respirometers prior to the start of the experiment. Instead the isolated individual larvae were give a 12 hour period of acclimatisation under experimental conditions prior to the start of the experiment in a plastic aquarium.

The same fresh-weight ranges of larvae were used as in the previous experiment (4.2.2). These were:

1) 0 to 18mg, mean= 8.35mg (S.E.= 0.37, N=77)
2) 18 to 30mg, mean= 22.7mg (S.E.= 0.26, N=77)
3) >30mg, mean= 40.32mg (S.E.=0.43, N=120).

Experimental procedures

The procedure for measuring larval respiratory rates using the closed bottle respirometers was as follows. One larva was placed in each respirometer. The respirometer was then flushed twice gently with filtered Loch Lomond water at the

desired oxygen concentration and temperature. The respirometers were then sealed and incubated for 1 hour to allow larvae to settle. 3 ml of water was then drawn off through the stopper using a syringe and the oxygen concentration determined. The respirometers were then incubated at the experimental temperature and at the end of the experiment the contents of the respirometer were gently mixed by inverting. A further 3 ml of water was then drawn off for oxygen determination. The duration of each experiment and the volume of the respirometer used depended on the size of the larva and the starting oxygen concentration.

The range of oxygen concentrations under which animals were tested were the same as those used in the flow through respirometer (4.2.2). Animals were tested at only one temperature (18^{o}C). This was because it was felt that differences in respiration rate between L+ and L- larvae would be most evident at higher temperatures and low oxygen concentrations (4.1).

To prevent stress to the experimental animals due to over depletion of oxygen in the chambers and the build up of waste products it is recommended that the oxygen concentration in the experimental chambers does not drop by more than 20% of its initial value during the experiment. This was accomplished in two ways, firstly by shortening the duration of the experiments to allow an oxygen depletion of less than 20% of the initial value and secondly by adjusting the amount of water in the syringe according to the size of the animal and the oxygen concentration of the water at the start of the experiment. For example, if a large larva was being used then oxygen consumption would be rapid therefore the syringe would be filled with more water to compensate, or if low oxygen concentrations were being used the syringe could be filled with more water. However, when the respiratory rates of some large larvae were measured at low oxygen concentrations a depletion of 20% of the starting oxygen

concentration occurred within the one hour settling period. In some cases therefore a depletion in excess of 20% had to be allowed. Limiting the duration of the respirometer runs to a depletion of 20% the initial oxygen concentration also limits the build up of metabolic waste products. To ensure an even oxygen concentration in the respirometers the water was thoroughly mixed by inverting the respirometer gently before measurements were taken. Bacterial respiration was compensated for by running a blank control along with each respirometer. This control contained water and a piece of gauze as substratum and was treated identically to respirometers containing larvae. The oxygen consumption of the bacteria (within the chamber) was then subtracted from the final oxygen consumption rate of each respirometer (O'Connor et al., 1985). Larval stress was also minimised by keeping light levels low and having the substratum for larvae to settle on

In order to calculate accurately the volume of water in each of the respirometers during each experimental run, the whole filled respirometer was weighed immediately before the final oxygen determination. The dry weight of the syringe, stopper, substratum and larva were then subtracted from the syringe final weight to give the weight of only the water in the respirometer. This weight of water could then be converted to its volume from a table of weight/volume constants for water at that temperature and atmospheric pressure (Weast, 1989).

The respirometers were filled with filtered Loch Lomond water at the desired oxygen concentration and temperature. Oxygen concentration was adjusted by bubbling a mixture of air and O.F.N. through water in an airtight 10 L tank at the experimental temperature, as in experiments 1 and 2. Aliquots of 3 cm^3 were drawn off for oxygen determination through the stopper using a syringe and needle. The oxygen concentration of the water was determined in this experiment using a "Strathkelvin"

oxygen meter and microcathode polarographic oxygen sensor mounted in a "Strathkelvin MC100 microcell" (accuracy +/- 0.02 mg/l O_2). Temperature was controlled by placing the respirometers in an incubator at the experimental temperature. The experimental temperature of 18°C was approximately equal to the highest temperature recorded at the sampling site (Chapter 2).

4.2.4. Statistical analysis

Statistical analysis was carried out using "Minitab" and "S.P.S.S." statistical programmes on an I.B.M. P.C. computer. The S.O.R. and fresh weight data from the flow through respirometer were transformed using the Log_{10} function and regressions calculated according to the least squares method (Snedecor and Cochran, 1962; Ryan et al.,1985). The regressions of Log_{10} S.O.R. on log_{10} fresh weight for L- and L+ larvae at each of the experimental oxygen concentrations (approximately 100%, 75% 50% and 25% saturation) were then compared using analysis of covariance (Snedecor and Cochran, 1962). The mean S.O.R. of L- and L+ larvae were also compared using a two sample t-test (Snedecor and Cochran, 1962).

Calculation of oxygen critical points.

In order to test the ability of P. nymphula larvae to regulate their oxygen consumption under conditions of hypoxia, the data collected during the closed bottle respirometry experiments were tested against the three models described (4.1). These were the log, polynomial and critical point determination methods (Mangum and VanWinkle, 1973; Yeager and Ultsch, 1989). The program used to calculate critical points is given in Yeager and Ultsch (1989) and was run on an I.B.M. PC.

4.3 Results

4.3.1 Survival during Hypoxia

At 10^{O}C there were no deaths of larvae after 4 days at the reduced oxygen concentration. However, both L+ and L- larvae increased body movements, such as swimming to the surface, as the oxygen concentration was reduced. These movements became more frequent as the oxygen concentration fell below 5.0 mgO_2/l. When the oxygen concentration was reduced further to 0.5 mgO_2/l both L- and L+ larvae became inactive and clung tightly to the substratum. The experiment was terminated after 5 days.

In the first run of the experiment at 18^{o}C, 40% of the L+ larvae were recorded as "dead" after 9 hours of the experiment (Fig. 4.2). This was approximately 6 hours after the oxygen concentration was reduced to the test level. After 24 hours (20 hours of anoxia) all the L+ larvae were dead whilst 40% of the L- larvae were dead (Fig. 4.2). When the oxygen level was returned to saturation, all of the L- larvae recovered whereas none of the L+ larvae recovered (Fig. 4.2).

In the second run of this experiment, the oxygen level was reduced more slowly over 15 hours and a different pattern was observed. Onset of death in L+ larvae was rapid and occurred after 6 hours at the reduced oxygen level (20 hours from the start of the experiment) and 5 hours later all L+ larvae were dead. The experiment was terminated when all L+ larvae had bee recorded as dead. L- larvae were recorded as dead after 15 hours of the experiment whilst oxygen levels were still high (50-10% saturation). The number of L- larvae recorded dead increased to 50% by the end of the experiment (Fig 4.3). In neither set of experiments did any of the control larvae die indicating that larval death was due to hypoxia rather than lamellar autotomy.

Fig. 4.2 Results of the first run of the hypoxia survival experiment at $18^{0}C$. The number of larvae with and without lamellae surviving is shown along with the dissolved oxygen concentration (% Saturation). In this experiment, the oxygen concentration was lowered over 5 hours. There were no mortalities in the control group.

Fig. 4.3 Results of second run of the hypoxia survival experiment at $18^{0}C$. The number of larvae with and without lamellae surviving and the experimental oxygen concentration are shown. In this experiment the oxygen concentration was lowered over 15 hours. There were no deaths reported in the control group of larvae.

Fig. 4.2

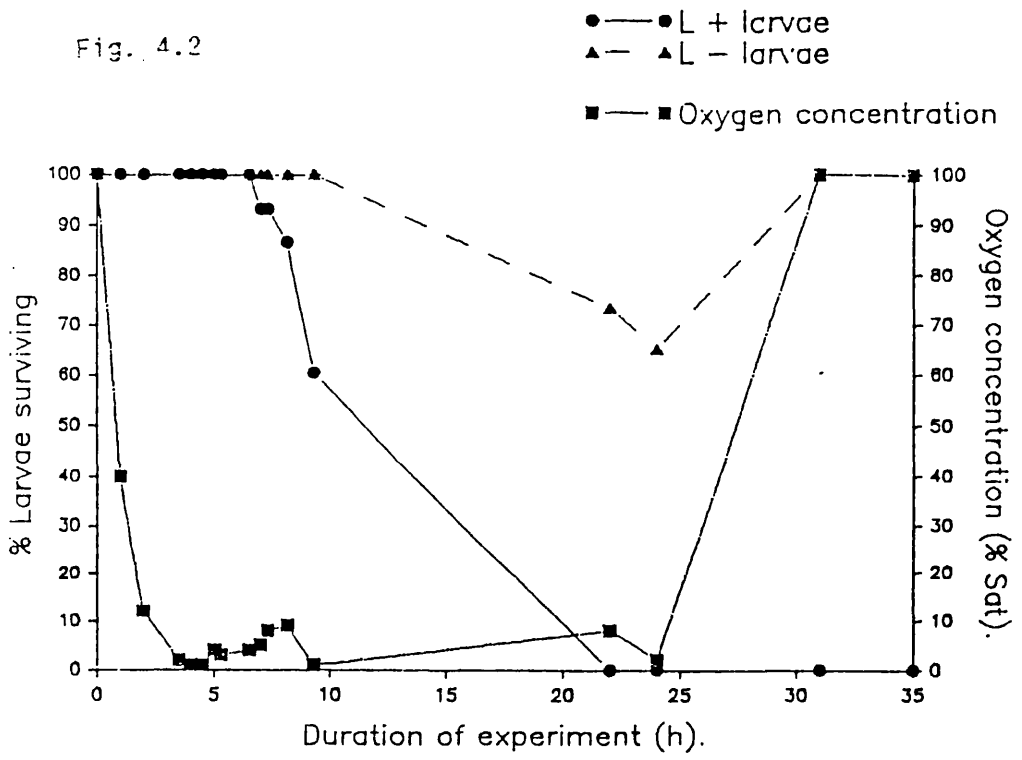
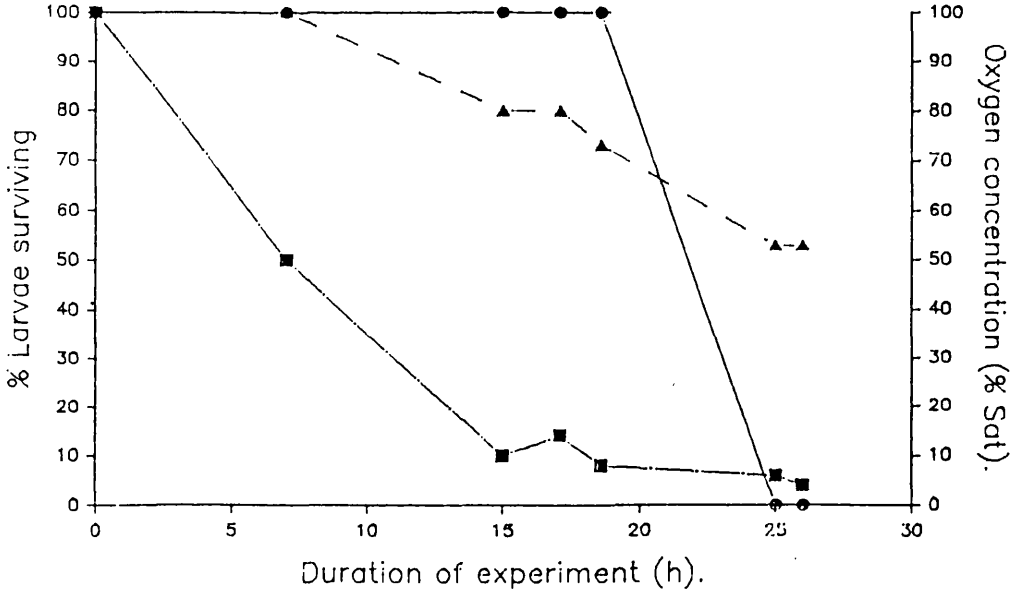

L + larvae
L − larvae
Oxygen concentration
% Larvae surviving
Oxygen concentration (% Sat).
Duration of experiment (h).
0
10
20
30
40
50
60
70
80
90
100
0
5
10
15
20
25
30
35

Fig. 4.3


% Larvae surviving
Oxygen concentration (% Sat).
Duration of experiment (h).
0
10
20
30
40
50
60
70
80
90
100
0
5
10
15
20
25
30

These experiments did not yield conclusive results as the rate of death of L- compared to L+ larvae could not be tested statistically. However, the aim of the experiments was to determine whether L+ larvae were able to withstand anoxia better than L- larvae. This is clearly not the case as there was no clear pattern of susceptibility observed and L- larvae show no indication of being more susceptible to anoxia than L+ larvae. This suggests lamellar loss has no effect on the survival of larvae under hypoxic conditions. The behavioural response of larvae to mild hypoxia involved an increase in larval activity as larvae attempted to reach areas of higher oxygen concentration. Under conditions of severe hypoxia these movements ceased.

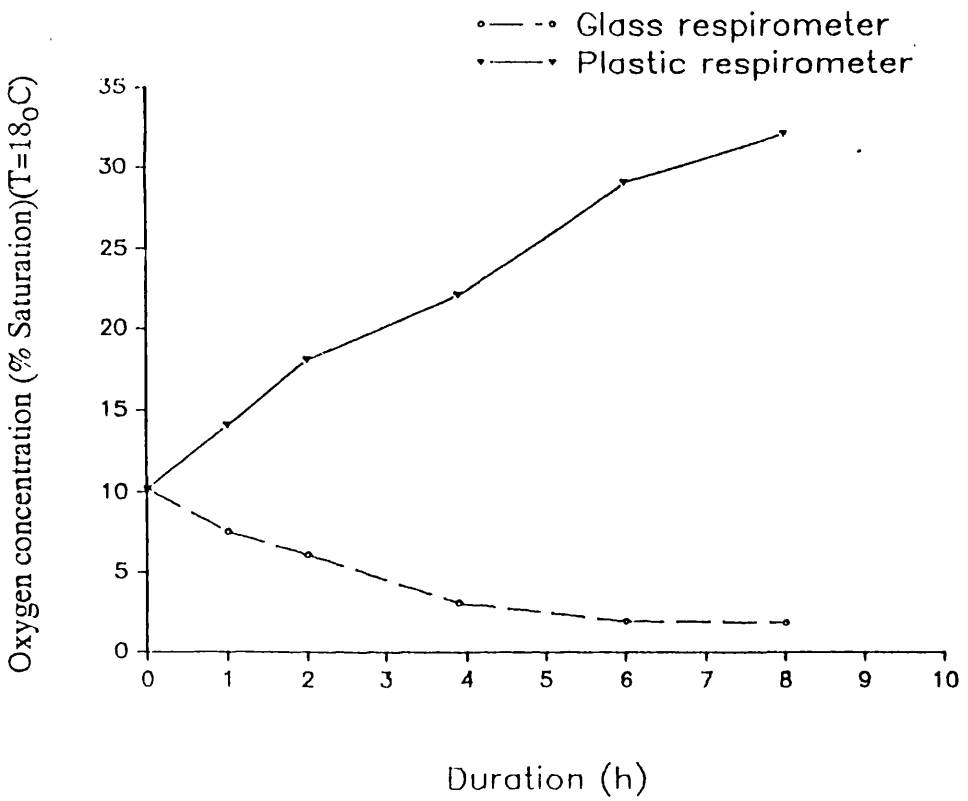
4.3.2 Larval respiration rates using a flow-through respirometer.

Using the flow-through respirometer described above (Fig 4.1), the respiration rates of L+ and L- larvae of greater than 30mg wet weight were measured at $5^{O}C$, $10^{O}C$ and $15^{O}C$. The results for each set of experiments are reported in Appendix 4. In Figures A.4.1-3. molar oxygen consumption rates (M.O.R.) of larvae are shown plotted against environmental oxygen concentration (D.O.). In Figures A.4.4-6, log weight specific respiration rates (S.O.R.) of larvae are shown plotted against log larval fresh weight. Presentation of the data in this way allows direct comparison with subsequent analyses.

The results of these experiments were considered unreliable for two reasons. The first was the wide range of respiration rates recorded for larvae of similar weights and at similar D.O.'s. These results would be expected to be fairly similar. Secondly, there was no statistically significant correlation between S.O.R. and larval fresh weight (A.4.4-6). If the experimental results were reliable there should be a good correlation between Log fresh weight and Log S.O.R. Finally, a large number of negative respiration rates were recorded. These negative respiration rates are not shown in the figures (A.4). Negative respiration rates imply a net output of oxygen by larvae during the experiments. The

Fig. 4.4 A test to determine the source of oxygen entering the flow-through respirometers. The change in oxygen concentration of glass and plastic respirometers is shown with time. The respirometers were filled with 30ml of filtered Loch Lomond water and incubated at $18^{O}C$. The drop in oxygen concentration in the glass respirometer is the result of bacterial respiration. The increase in oxygen concentration in the plastic respirometer is due to oxygen entering the chamber.

Fig. 4.4


Glass respirometer
Plastic respirometer
Oxygen concentration (% Saturation)(T=18°C)
35
30
25
20
15
10
5
0
0
1
2
3
4
5
6
7
8
9
10
Duration (h)

source of these errors was traced to the plastic syringes used as respirometers. Tests of the rate of oxygen depletion occurring in loch water in sealed plastic and glass respirometer syringes were carried out (Fig 4.4). Both respirometers were filled with the same water and maintained at a constant $18^{O}C$ in the dark. The increase in oxygen concentration in the plastic respirometer indicates entry of oxygen. This error accounted for the "negative" respiration rates recorded at high temperatures under anoxic conditions as more oxygen was able to enter the chamber than was consumed by the larvae inside. It would also account for the unexpected variability in the data collected. The use of plastic respirometers was thus abandoned in favour of glass respirometers. However, the glass respirometers could not be incorporated in the flow through respirometer apparatus and therefore had to be used as closed bottle respirometers.

4.3.3 Larval respiration rates using the closed-bottle respirometer.

Lamellar loss and larval M.O.R.'s

For all size-groups of larvae there was little change in mean molar oxygen consumption rate (M.O.R.) from a mean environmental oxygen concentration (D.O.) of 9.08 mgO_2/l (saturation) down to a mean D.O. of between 1.5 and 2.0mgO_2/l (Figs. 4.5, 4.6, 4.7, Table 4.1). In each figure a line has been used to join up the mean M.O.R. of larvae with and without lamellae within the four ranges of D.O. used. For example, at a mean environmental D.O. of 8.89mgO_2/l, the mean M.O.R. of intact larvae greater than 30mg was 2.58 $\mu molO_2/h$, and at mean D.O's of 6.8, 4.4, and 1.6 mgO_2/l mean M.O.R's were 3.15, 3.55 and 2.0 mgO_2/h (Fig 4.5, Table 4.1). In all cases larvae were able to maintain or slightly increase M.O.R. down to a mean D.O. of around 4 mgO_2/l. The slight increase in M.O.R. reflects the increase in larval activity with falling D.O. However, below about 4 mgO_2/l larval M.O.R. drops off significantly for all sizes of larvae as activity stops (Figs 4.5, 4.6, 4.7, Table 4.1).

Table 4.1 Mean respiration rate of larvae with (L+) and without (L-) larvae according to weight category and mean experimental oxygen concentration. Respiration rate in μmols/larva/h, weight in mg, oxygen concentration in mgO^2/l.

Weight range (mg)	Without lamellae Resp. Rate	S.E	Oxygen Conc.	S.E.	N	With lamellae Resp. Rate	S.E.	Oxygen Conc.	S.E.	N
0-18	10.88	2.34	8.75	0.11	5	7.64	1.51	8.73	0.06	13
"	11.51	1.45	6.97	0.07	5	8.48	1.66	6.84	0.05	13
"	15.68	3.56	5.01	0.05	5	10.62	2.52	4.86	0.09	14
"	1.73	0.03	1.75	0.70	2	2.51	0.39	1.67	0.15	20
18-30	2.97	0.45	8.71	0.03	8	2.58	0.39	8.69	0.03	5
"	2.71	0.34	6.71	0.19	8	2.56	0.43	6.58	0.16	5
"	3.73	1.02	4.54	0.13	8	2.32	0.48	4.33	0.04	5
"	2.07	0.27	1.72	0.08	22	2.40	0.40	1.70	0.07	16
>30	3.94	1.29	8.69	0.13	10	3.51	0.44	8.89	0.13	6
"	3.09	0.30	6.68	0.13	9	3.15	0.23	6.81	0.17	6
"	3.50	0.48	4.57	0.09	10	3.55	0.33	4.48	0.12	6
"	2.11	0.23	1.66	0.10	42	2.00	0.18	1.60	0.10	31

Fig. 4.5 Molar oxygen consumption rate (M.O.R.)and environmental dissolved oxygen concentration (D.O.) for larvae with (L+) and larvae without (L-) lamellae and greater than 30mg fresh weight at 18^{O}C. The two lines join up the mean respiration rates for L+ and L- larvae within each of the four D.O. categories (approximateley 100%, 75%, 50% and 25% oxygen saturation (See 4.2.2. for fuller explanation of categories used)). Error bars = Standard error of the mean.

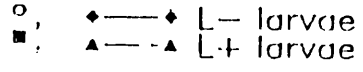


Fig. 4.6 Molar oxygen consumption rate (M.O.R.)and environmental dissolved oxygen concentration (D.O.) for larvae with (L+) and larvae without (L-) lamellae and between 18 and 30mg fresh weight at 18^{O}C. The two lines join up the mean respiration rates for L+ and L- larvae within each of the four D.O. categories (approximateley 100%, 75%, 50% and 25% oxygen saturation (See 4.2.2. for fuller explanation of categories used)). Error bars = Standard error of the mean.

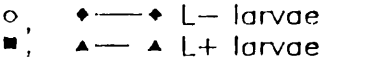


Fig. 4.5

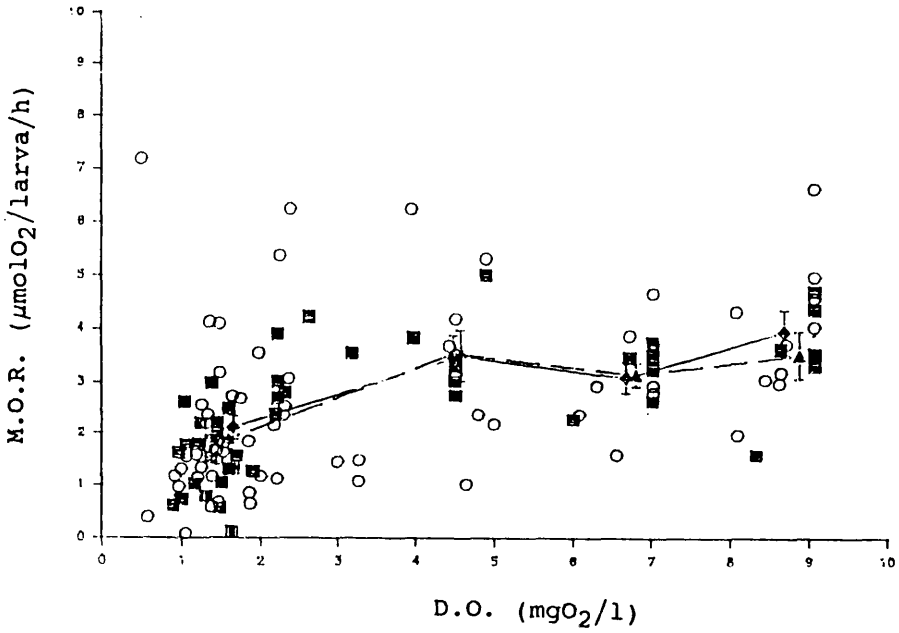


Fig. 4.6

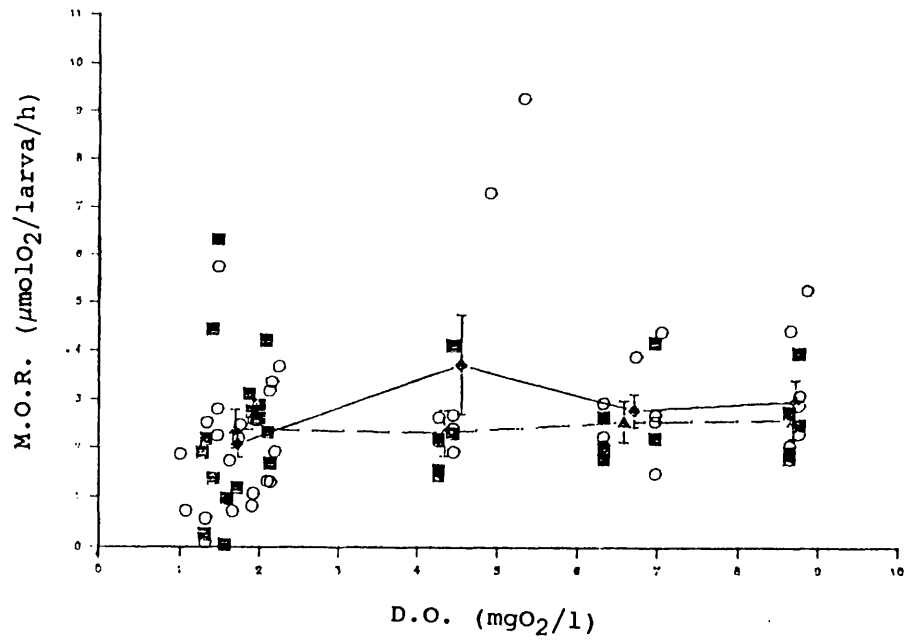


Fig. 4.7 Molar oxygen consumption rate (M.O.R.) and environmental dissolved oxygen concentration (D.O.) for L+ and L- P. nymphula larvae of less than 18mg fresh weight at 18°C. The two lines join up the mean respiration rates for L+ and L- larvae within each of the four D.O. categories (approximateley 100%, 75%, 50% and 25% oxygen saturation (See 4.2.2. for fuller explanation of categories used)). Error bars = Standard error of the mean.

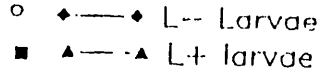
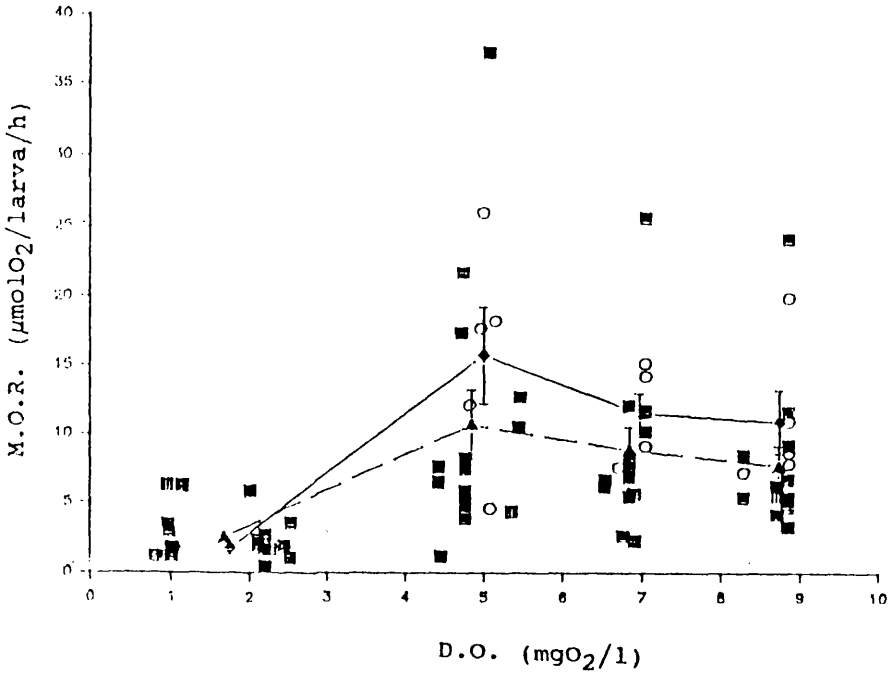


Fig. 4.7


M.O.R. ($\mu molO_2$/larva/h)
D.O. (mgO_2/l)
0
5
10
15
20
25
30
35
40
0
1
2
3
4
5
6
7
8
9
10

Visual comparison of the lines joining up the mean M.O.R. of each size group of larvae does not suggest clear differences between the respiration rates of L- and L+ larvae. Nor does visual estimation allow any more than a subjective estimate of the oxygen critical point of L- and L+ larvae (between 3 and 5 mgO_2/l). Therefore this M.O.R./D.O. comparison cannot be used to determine the respiratory efficiency of lamellae.

Oxygen critical points of L- and L+ larvae.

The use of oxygen critical points to determine the respiratory efficiency of larvae with and without lamellae was discussed earlier (4.1). Three models are used here to estimate critical points. These are the Log and second degree polynomial models used by Mangum and VanWinkle (1973) and the two segment line model used by Yeager and Ultsch (1989) and Nickerson et al., (1989) (4.1.1).

To compare the goodness of fit of the log and second degree polynomial models to M.O.R./D.O. data, the correlation coefficient R-squared was used to determine which best explained the variation in M.O.R.

$$D.O. = 1.26+0.53(M.O.R)-0.028(M.O.R.)^2 \quad \text{equation 4.1}$$

$$Log_{10} D.O. = 2.12 + 0.49(Log_{10} M.O.R.) \quad \text{equation 4.2}$$

Of the two models, the Log_{10} model (equation 4.2) was found to fit the data best ($R^2 = 27.6\%$); for the polynomial function $R^2 = 25.6\%$. However, these correlation coefficients are low and neither model could be said to fit the data well.

Using the methods of Yeager and Ultsch (1989), the M.O.R. /D.O. data for larvae with and without lamellae and for each different size group was sorted into

ascending order using the abscissa as the primary key and the ordinate as the secondary key. The critical point was then calculated as the point of intersection of the two "best fit" regression lines that divide the data into the two sets. The regression formulae, and midpoint (critical point) approximations are shown in Table 4.2. The calculated regression for larvae with and without lamellae are plotted along with the data in Appendix 5. However, for only two experimental groups of larvae, L- larvae of <18mg and L+ larvae of >30mg, does the model give a fit resembling that of a "classic" oxy-regulator as described by Yeager and Ultsch (1989) (App. 5.).

Of these three models, the log-model fits the data best. However, none of the models really provides a good description of the data. These methods cannot therefore be used to calculate oxygen critical points for this species and consequently cannot be used to test the respiratory efficiency of lamellae. The results do indicate that under these experimental conditions P. nymphula larvae are not "classical" oxy-regulators.

Weight-specific oxygen consumption (S.O.R.) and lamellar loss.

To allow for the different respiration rates of larvae of different fresh weights, Larval S.O.R. was also used here to compare the respiratory rates of L- and L+ larvae.

For this comparison, log S.O.R. ($mgO_2/g/h$) for L- and L+ larvae is plotted against Log larval fresh weight (Figs, 4.8, 4.9, 4.10,4.11). This was done for larvae tested within each of the four ranges of oxygen concentration used (4.2.1). A regression line was then fitted to each set of points. The significance of fit of this line indicates good correlation between S.O.R. and larval fresh weight, the negative slopes of the lines reflects the expected reduction in weight specific respiration rate with the increase in larval fresh weight (Table 4.3). To determine whether there was any statistically significant difference in the respiration rates of L- and L+ larvae the slope and intercept

Table 4.2 Regression formulae for respiration rate data of P. nymphula larvae with (L+) and without (L+) lamellae. Lines above the mid point correspond to the "zone of regulation" and those below the mid point to the "zone of conformity". The mid point is the calculated oxygen critical point of each of the larval groups, the values for a and b refer to the regression formula given. Calculated according to Yeager and Ultsch (1989).

Larval size	lamellar status	Regression (M.O.R.[1] = a + b(D.O.[2])) above mid point a	above mid point b	below mid point a	below mid point b	Mid point mg/l
< 18 mg	L+	16.40	1.03	1.80	0.50	5.04
	L-	11.10	0.01	-6.30	4.90	4.59
18-30mg	L+	-26.30	21.86	1.90	0.08	1.57
	L-	1.84	0.11	9.12	0.76	4.67
> 30 mg	L+	1.02	0.36	3.27	0.02	2.20
	L-	7.98	-7.39	1.70	0.25	1.05

[1] M.O.R ($\mu molO_2/g/h$)

[2] D.O. (mgO_2/l)

Table 4.3 Correlation and regression of log Specific Oxygen Consumption rate (S.O.R.) on log fresh weight. Note the inverse relationship between respiration rate and wet weight indicating reduction in S.O.R. with increase in size. The regression lines and data are shown plotted in Figs 4.8-4.11.

Lamellae	Oxygen[1] conc. mg/l	Regression[2] formula Y= C + AX	Rsq %	P
L+	>7.9	3.32-0.569X	53.4	<<0.001
L-	>7.9	3.37-0.559X	42.2	<<0.001
L+	5.9-7.9	3.37-0.605X	47.1	<<0.001
L-	5.9-7.9	3.52-0.709X	59.6	<<0.001
L+	3.3-5.9	3.57-0.758X	48.4	<<0.001
L-	3.3-5.9	3.62-0.744X	38.6	= 0.001
L+	0.0-3.3	2.56-0.241X	2.4	= 0.111
L-	0.0-3.3	2.20+0.009X	0.0	= 0.969

1 Oxygen concentration range at start of experiment.

2 Regression formula = Log S.O.R.((μmols/g/h) X100)=C+A(Log_{10}Weight (mg)).

Table 4.4 Results of analysis of covariance on the regresions of log Specific Oxygen Consumption rate (S.O.R.) on log fresh weight for larvae with (L+) and larvae without (L-) lamellae. Regression formulae are shown in Table 4.3 and lines in Figs 4.8-4.11.

conc. mg/l	elevation F	Sig F	D.F	slope F	Sig F	D.F
7.9 -9.08	0.93	0.34	1, 44	0.00	0.95	1, 43
5.9-7.9	0.08	0.77	1, 43	0.57	0.32	1, 42
3.3-5.9	0.62	0.43	1, 45	0.00	0.95	1, 44

Fig. 4.8 Log_{10} specific oxygen consumption rate (S.O.R.(X10)) of larvae with (L+) and without (L-) lamellae against Log_{10} fresh weight (mg) at a dissolved oxygen concentration of between 7.9 and 9.08 mgO_2/l. Least squares regression lines are fitted to the data, formulae in Table 4.3. L- larvae solid line, L+ larvae dashed line.

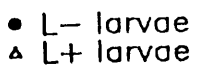


Fig. 4.9 Log_{10} specific oxygen consumption rate (S.O.R.(X10)) of larvae with (L+) and larvae without (L-) lamellae against Log_{10} fresh weight (mg) at a dissolved oxygen concentration of between 5.9 and 7.9 mgO_2/l. Least squares regression lines fitted to the data from formulae in Table 4.3. L- larvae solid line, L+ larvae dashed line.

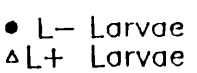


Fig. 4.8

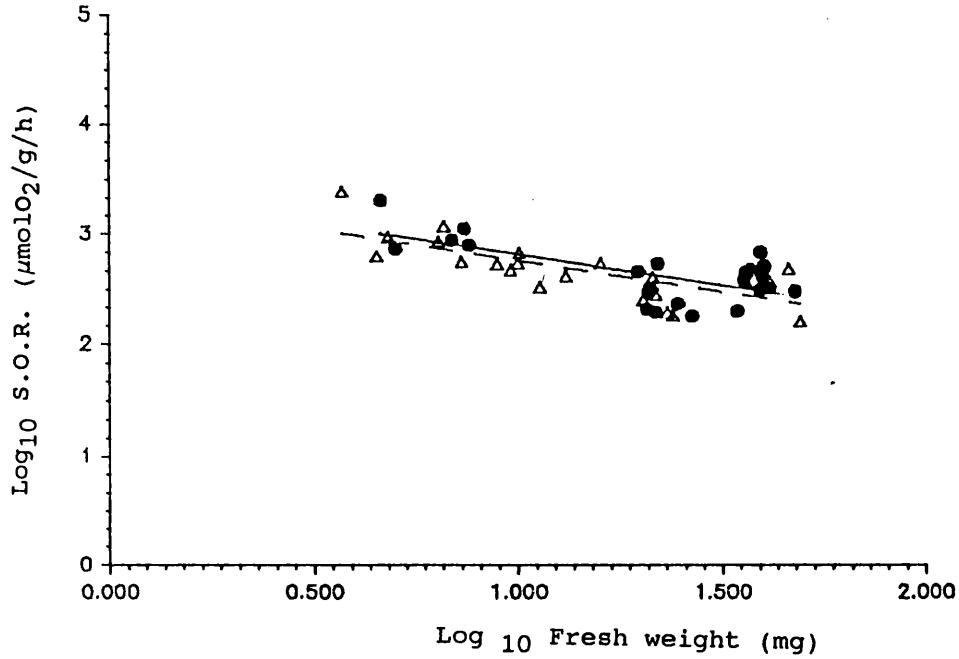

Log10 S.O.R. (μmolO2/g/h)
5
4
3
2
1
0
0.000
0.500
1.000
1.500
2.000
Log 10 Fresh weight (mg)

Fig 4.9

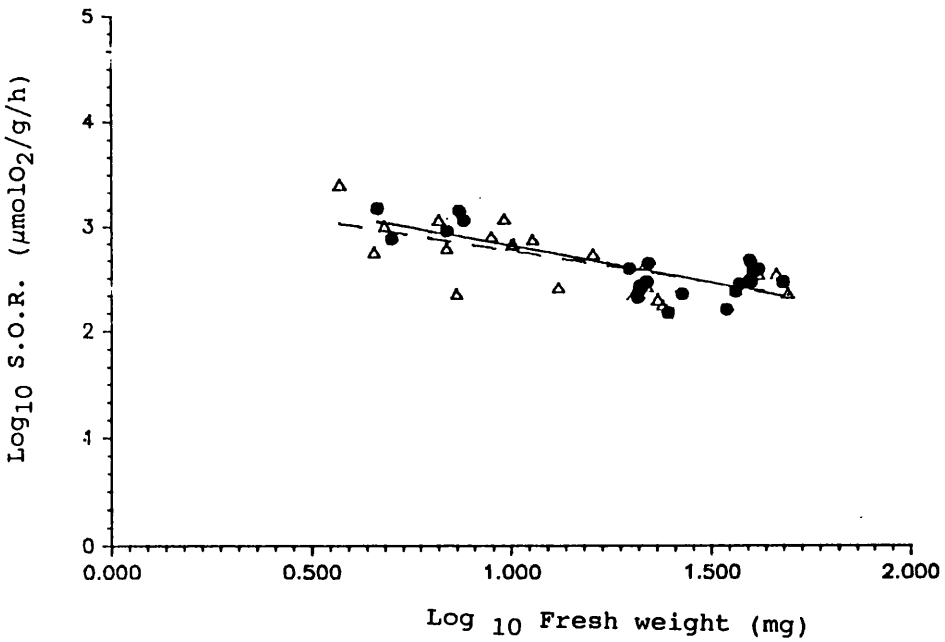

Log10 S.O.R. (μmolO2/g/h)
5
4
3
2
1
0
0.000
0.500
1.000
1.500
2.000
Log 10 Fresh weight (mg)

Fig. 4.10 Log_{10} specific oxygen consumption rate (S.O.R.(X10)) of larvae with (L+) and larvae without (L-) lamellae against Log_{10} fresh weight (mg) at a dissolved oxygen concentration of between 3.3 and 5.9 mgO_2/l. Least squares regressions are fitted to the data using formulae in Table 4.3. L- larvae solid line, L+ larvae dashed line.

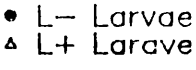


Fig. 4.11 Log_{10} specific oxygen consumption rate (S.O.R.) of larvae with (L+) and larvae without (L-) lamellae against Log_{10} fresh weight (mg) at a dissolved oxygen concentration of between 0.4 and 3.3 mgO_2/l. Least squares regression lines fitted to the data using formulae in Table 4.3. L- larvae solid line, L+ larvae dashed line.

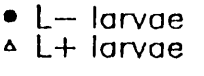


Fig. 4.10

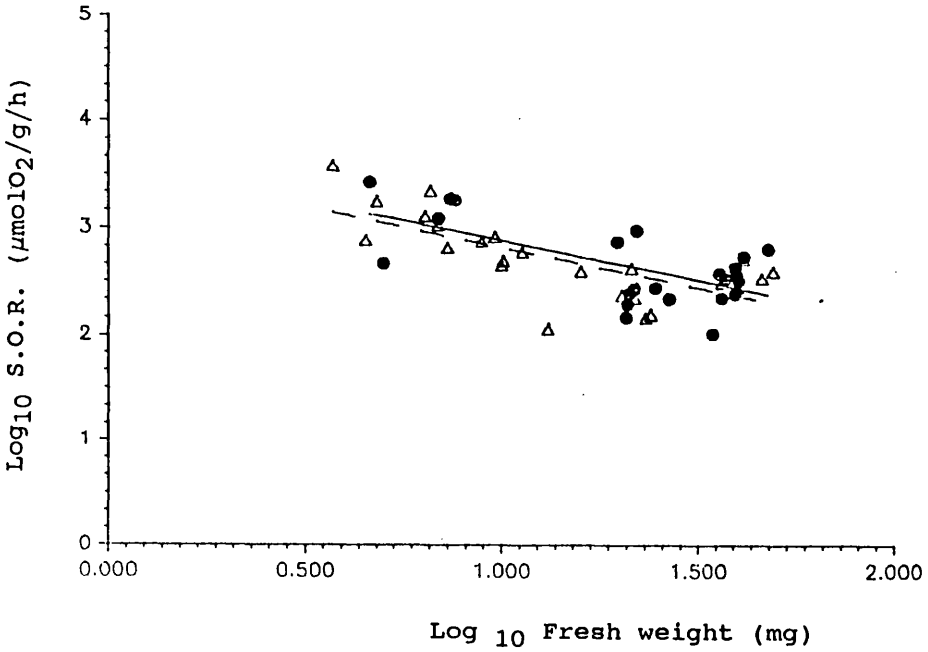
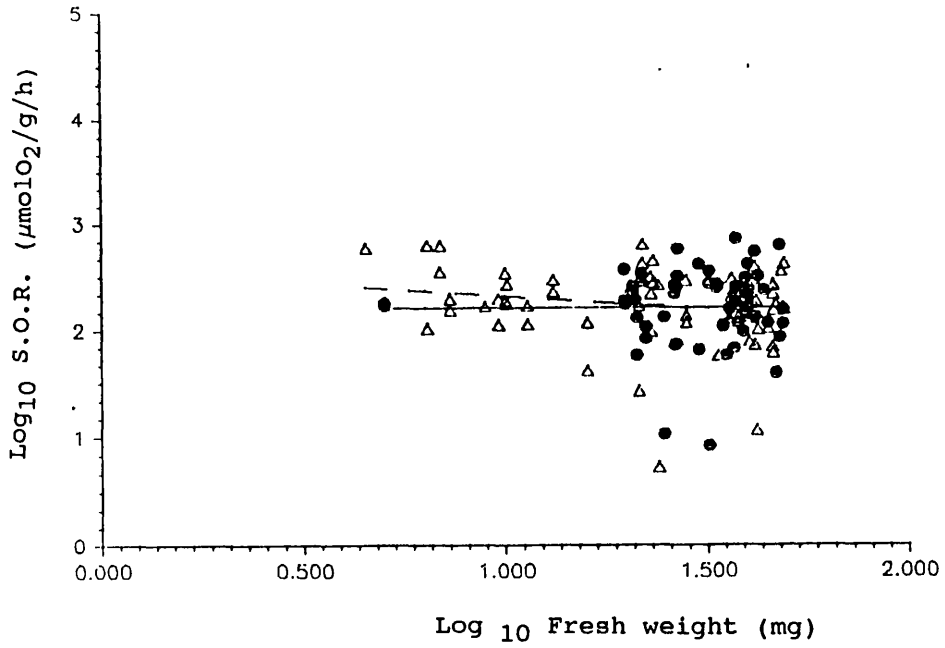


Fig. 4.11



of the two lines were compared using analysis of covariance (Table 4.4). This analysis has the advantage over the paired t-test used by Eriksen (1986) in that it allows compensation for the difference in S,O.R.'s of small and large larvae.

For example, Fig. 4.11 shows the specific oxygen consumption rate (S.O.R.) of both L+ and L- larvae against larval fresh weight at an oxygen concentration of between 7.9 and 9.08 mgO_2/l (100% saturation). The log_{10} transformation is used as it gives the most significant correlation (Table 4.4). However, no statistically significant correlations were found between the S.O.R.'s of L- and L+ larvae at this dissolved oxygen concentration (Table 4.4). The covariance analysis was repeated for L- and L+ larvae at dissolved oxygen concentrations of 5.9 to 7.9 mgO_2/l and 3.3 to 5.9mgO_2/l and again no statistically significant differences in the respiratory rates of L+ and L- larvae were found (Table 4.4).

No statistically significant correlation was found between the Log_{10} S.O.R. and Log fresh weight of L- or L+ larvae at dissolved oxygen concentrations of less than 3.3 mgO_2/l. Therefore analysis of covariance could not be used to compare the respiratory rates of these two groups of larvae. This lack of a statistically significant correlation at low D.O. was due to the difficulty in measuring the respiratory rates of small larvae at low oxygen concentrations. Thus at this oxygen concentration relatively few data were collected for small larvae compared to that for large larvae resulting in a skewed data distribution and poor correlation between S.O.R. and fresh weight (Table, 4.3, Fig 4.11).

Thus instead of using analysis of covariance on the data from these larvae, the difference in respiration rates of only the large (L+ and L-) larvae at dissolved oxygen concentrations of less than 3.3 mgO_2/l were compared using a students T-test (Sokal and Rohlf, 1969). As long as one size group of larvae was used there is no need to

compensate for the differences in fresh weight of large and small larvae. No statistically significant differences in respiration rates were found between the mean S.O.R. of L- and L+ larvae of the size groups 18-30mg ($p=0.51$, $DF=27$, $T=0.66$) and greater than 30 mg ($p=0.33$, $DF=116$, $T=-0.98$).

4.4. Discussion

4.4.1. Lamellae and larval respiration rates.

The main objective of these experiments was to determine whether lamellar loss had a significant effect on the rate of larval oxygen uptake. To test this, two sets of experiments were carried out. Firstly, those using the flow-through respirometer (4.2.2), and secondly those using the closed bottle respirometers (4.2.3). The results of the closed bottle respirometer experiments clearly show that lamellar removal has no effect on the ability of larvae to take up oxygen. This was true for all sizes of larvae tested and at all dissolved oxygen concentrations (D.O.), down to a mean of 1.7mgO_2/l (4.3.3.). The only exception to this was larvae of less than 18mg fresh weight for which insufficient data were collected at the lowest D.O. concentrations.

The conclusion that lamellar loss does not affect the ability of larvae to take up oxygen is further confirmed by the results of the hypoxia survival experiments (4.3.1.). These results showed that larvae without lamellae were not more susceptible to hypoxia then those with lamellae. If anything larvae with lamellae appeared to be more susceptible to hypoxia than those without (4.3.1). Had lamellae been essential as respiratory organs then it would have been expected that those without lamellae would have died at significantly higher oxygen concentrations.

The fact that larvae were only tested at one temperature and that it was not possible to collect data from small larvae at low D.O.'s should not affect this conclusion. It could be argued that lamellae may be as important as gills at other temperatures or only in small larvae at low D.O.'s. However, both these suggestions are unlikely. As far as temperature is concerned, larval respiration rates decrease as temperature is reduced

(Lawton, 1971a). Therefore as temperature is reduced so is the need for a respiratory gill. Respiratory oxygen demand would increase with temperature and this combined with reduced oxygen levels may increase respiratory stress. However, the water temperature used in the present study during experiments was the highest recorded in the habitat in which the larvae were collected. This can be regarded as near the environmental limit for this population (2.2). Thus larvae from this population at least would not normally encounter such high water temperatures and low oxygen levels.

Concerning larval size and lamellar loss, it is also unlikely that the lamellae of small larvae would be important as respiratory gills when the lamellae of larger larvae are not (under similar conditions of hypoxia). This is because the diffusion rate of oxygen into the tissues across the body wall will be more rapid than in large larvae, due to the shorter diffusion distances (3.1). It is only as larvae grow that diffusion distances increase and some form of respiratory adaptation is required. In many species of fresh water insect gills only appear once larvae have attained a particular size (3.1).

4.4.2. Assessment of experimental results and data analysis.

Further aims of the present study were, to determine a suitable experimental protocol for such experiments and, to determine which methods of analysis were most suitable for this type of data.

Experimental protocol.

The design of the flow-through respirometers was considered suitable for investigation of larval respiratory rates. Failure of the equipment to produce reliable results was due to unsuitable materials rather than the design. There is no reason why this

design should not function more reliably if glass or similar materials are used rather than PVC. Ultimately, the use of glass closed bottle respirometers produced satisfactory results.

Data analysis.

The methods of data analysis used in previous studies of lamellar respiratory function have resulted in questionable conclusions (4.1). Most of these studies have carried out visual comparison of data, and statistical tests of significance were rarely used. Here, more objective methods of data analysis were investigated to determine which is most suitable for this type of data.

In the past, Molar Oxygen consumption rate (M.O.R.) has been used for the comparison of respiratory rates of larvae with and without lamellae. In doing so, these analyses assume that there is no variation in larval respiration rates due to larval weight. In the present study, M.O.R. was used to estimate oxygen critical points (4.3.3.). It was suggested that if lamellae were important as respiratory gills then the critical point of those larvae with lamellae should be lower than that of those without (4.1.3.). An initial visual estimate of the oxygen critical point of this species puts it at between 3.0 and 4.0 mgO_2/l at 18^oC, slightly lower but close to Lawton's (1971a) figure of 5.0 mgO_2/l for this species.

Visual estimation of critical points is not sufficiently accurate for the comparison of the respiratory efficiency of larvae with and without lamellae. To provide a more objective estimate of the oxygen critical points for comparative analysis, three models were used. These were the log, polynomial and "2-segment line fitting" (4.1.3.). The results of these analyses proved inconclusive. While it is clear that larvae are able to regulate their oxygen consumption, the models did not satisfactorily explain the degree

of variation in the data (4.1.3.). Consequently, they cannot be used to estimate critical points for comparison of larvae with and with lamellae. Further modification of these models to take into account the behavioural and metabolic responses of different species would make these techniques more applicable.

It is perhaps not surprising that this type of analysis did not prove useful in the present study. If the distribution of M.O.R. data for larvae is examined visually it can be seen that larvae are not "classical" oxygen regulators (4.3.3.). That is as D.O. falls respiratory rate apparently increases. It is most likely that this rise in respiratory rate is due to the way larvae respond to falling D.O. As D.O. falls larvae increase activity as they try to escape hypoxia (4.3.3). Although if hypoxia becomes more severe respiration rates eventually fall off as activity ceases (4.3.3.). Both larvae with and without lamellae exhibited this response. Although not monitored directly, larvae without lamellae did not appear to start this escape behaviour at higher D.O. levels than larvae with lamellae as would be predicted if the lamellae functioned as gills.

Previous studies of the respiration rates of aquatic invertebrates indicate that the response of P. nymphula larvae to falling D.O. is not exceptional. For example, Williams et al., (1987) and Mangum and Van Winkle (1973) surveyed the responses of a wide range of aquatic invertebrates and demonstrated that in fact few responded in the "classical" way with clearly defined zones of conformity and regulation and clearly defined critical points.

These results show that P. nymphula larvae have a strong behavioural escape response to falling oxygen levels similar to that described in other species of freshwater invertebrate (Wiley and Kohler, 1984). Consequently the use of available models for predicting critical points is not recommended.

The other approach used here to for the analysis of the data was to adjust the results for larval fresh weights (S.O.R.). These adjusted respiration rates were then compared according to the larval size and oxygen ranges and lamellar status as described in the methods (4.2.2.). This approach controls for the differences in larval respiration rate due to larval size variation. In addition, because only larval respiration rates from within an "oxygen range" were compared there was no interference due to increases in larval activity as D.O. fell. It is assumed that all larvae will react in a similar way under similar conditions. This approach although not novel in the investigation of aquatic animal respiratory rates has not hitherto been used for the investigation of lamellar respiratory efficiency. Comparison of this S.O.R. against D.O. data was carried out using covariance analysis. In this analysis, the slope and intercept of lines fitted to the log S.O.R. and log fresh weight data is then compared (4.3.3.). The results show clearly that for large and medium sized larvae lamellar removal had no significant effect on larval respiration rates. The significant correlation of the S.O.R. on fresh weight data (Table 4.3) indicates that most of the variation in the data is due to larval fresh weight. It was not possible to use this approach for the data from larvae of less than 18mg fresh weight as in sufficient data were collected (4.3.3.). This type of analysis proved useful for the data from the present study.

4.4.3. Lamellae and respiratory adaptation in the Zygoptera.

The results of the present study are compatible with the results of previous studies that found lamellae did not act as respiratory gills. Pateé (1956) for example, found that Calopteryx sp. larvae without lamellae had higher respiratory rates than those with lamellae in oxygen saturated water. Thorpe (1932), concluded that the most important site for gas exchange in larval Agrion sp. was the anterior of the abdomen followed by the lamellae and then the thorax. However, the results of the present study

are at odds with those of authors such as Eriksen (1986), Harnisch (1958), Koch (1934), Pennack and McColl (1944) and Zahner (1959) who all concluded that lamellae were important as respiratory gills (4.1).

The technical deficiencies of these previous studies aside, one important reason for the different conclusions reached may simply be interspecific differences, especially considering the wide range of lamellar structure found in amongst the different species (MacNeill, 1960). It is unlikely that such different forms have a similar function. However, some authors have suggested that lamellar types with similar characteristics have the same respiratory function. MacNeill (1960) for example, speculated that respiratory adaptation occurred in zygopteran species with "duplex" lamellae (by an expansion in area and a reduction in cuticle thickness of the postnodal region)(2.1). The "Simplex" type lamellae not being adapted for respiration.

Such generalisations are clearly not applicable. For example, Harnisch (1958) and Koch (1934) suggested that the duplex lamellae of Agrion sp. larvae were important as respiratory gills whilst Thorpe (1932) found that in Agrion the abdomen was the primary site of oxygen uptake. In other species of Coenagriidae with duplex lamellae such as Enallagma sp., the lamellae have been shown to be important as respiratory gills (Pennack and McColl, 1944), whilst in P. nymphula (Coenagriidae) which has weakly duplex lamellae, they do not function as respiratory gills. According to MacNeill (1960) simplex lamellae are not used as respiratory gills, but in Lestes disjunctus (Lestidae) (which has simplex lamellae) Eriksen (1986) found them to be important both for larval survival and for regulation of oxygen uptake under hypoxic conditions (4.1). In the Agriidae, which have simplex lamellae, Zahner (1959) found the lamellae of one species of Calopteryx to be important as gills. Whereas Pateé (1956) showed that the lamellae of Calopteryx were not important as gills at least at relatively high oxygen concentrations.

Broad generalisations regarding lamellar function are therefore not helpful. In attempting to determine the function of such an organ it must be remembered that even similarly shaped structures in similar species can have differing functions. For example, Williams et al., (1987) investigated case function in caddis larvae and showed that respiratory adaptation occurred in some species but not in others, even when the structure of the case was similar. Williams et al., suggested that the adaptation of the case to either a respiratory or defensive role depended on the evolution of that species. However, they were only able to reach this conclusion after a detailed study of the habitat preference and respiratory requirements of a large number of species. It is clearly not possible to allocate lamellar respiratory function solely on the basis of general morphology or even on taxonomic grouping.

4.3.4. Habitat, life cycle and respiratory adaptation of lamellae.

The present study investigated only P. nymphula. However, because of the wide range of habitats occupied by zygopteran larvae, it is possible that the lamellae of other species are adapted as respiratory gills. If this is the case then this adaptation may be related to particular habitats and life histories. It is unlikely that such respiratory adaptation would follow taxonomic groupings as this has not been the case in all other fresh water invertebrates studied (Mangum and Van Winkle, 1973; Williams et al.,, 1987). An example of adaptation to a specific type of habitat is respiratory ventilation. Ventilatory behaviour tends to occur in species adapted to life in habitats with consistently high oxygen levels. Such species cannot withstand long periods of anoxia and are susceptible to even moderate falls in oxygen level (Wiley and Kohler, 1984).

P. nymphula larvae clearly rely on ventilation rather than gills to avoid hypoxia, suggesting adaptation for well oxygenated habitats. Larvae use the whole body wall for gas exchange. The results of the present study seem to confirm this. In the habitat in which larvae were collected, oxygen levels in the water column never fell below 7.0 mgO_2/l. This is well above the oxygen critical point for this species. Distribution records also suggest a preference for well oxygenated habitats. Askew (1988) for example, reports that in the northerly and mountainous parts of its range P. nymphula is found in lochans, peat pools, ponds, ditches and sluggish streams. These habitats in the cooler north and at high latitudes have higher oxygen levels than similar habitats in the more southerly or lower latitude parts of its distribution. In these southerly regions P. nymphula is found more in swiftly flowing streams where oxygen levels will be higher than standing water.

The results of previous studies of lamellar respiratory efficiency can also be considered in relation to the habitat preference of the species. For example, the pond dwelling species Lestes disjunctus, Enallagma civile and E. cyathigerum would be expected to use their lamellae as respiratory gills (Eriksen, 1986; Pennack and McColl (1941). Indeed, all these studies report the lamellae of these species to be important as respiratory gills. Whereas species such as P. nymphula and Calopteryx sp. (Patee, 1958) both of which are adapted to relatively well oxygenated habitats do not do so. Although Zahner (1959) found the lamellae of Calopteryx sp. to be important as respiratory gills.

However, at present, the existence of such trends must remain speculation, mainly because of the technical deficiencies and contradictory results of these previous studies and also because of the lack of data on the microhabitat preferences of the species concerned. In addition, other factors such as food availability, predation and developmental stage may influence the distribution of larvae within a habitat and limit

the ways they can respond and adapt to hypoxia (Wiley and Kohler, 1984). For example, the adoption of particular feeding or predator avoidance strategies may be incompatible with some types of respiratory regulation.

Developmental stage and respiratory adaptation

In most previous studies of lamellar respiratory efficiency, only large final instar larvae have been used. However, there are at least two critical periods during development when larvae of P. nymphula may be unavoidably exposed to hypoxia. The first is during periods of prolonged ice cover in ponds and lakes. During such periods, hypoxic conditions can develop in the benthic/hyporheic zone and spread upwards in the lake (Rahel and Kolar, 1990). Other species of fresh water invertebrate migrate or move to more favourable conditions (Rahel and Kolar, 1990). It is not known how P. nymphula larvae respond under such conditions, but larvae do increase movement. This suggests larvae are attempting to find regions of higher D.O. Larvae may also be able to respond metabolically by switching to anaerobic metabolism. The extent to which this occurs is not known but is an important consideration. P. nymphula larvae are tolerant to low environmental oxygen concentrations at least at low temperature (4.3.1). In the present study at 10^{o}C and severe hypoxic/anoxic conditions larvae survived for periods in excess of four days. There was no indication that longer exposures would have resulted in larvae death. Death due to asphyxiation was recorded only after prolonged exposure to severe hypoxia at temperatures corresponding to the maximum found within the collection habitat during summer (18^{o}C), when ice cover and access to the surface is not a problem.

The second important time when larvae may be exposed to hypoxia is during their first year of growth when they overwinter in the hyporheic zone of the stream (2.3). Again this period should not present larvae with problems. Firstly, because oxygen levels

were high in this zone for a significant depth (2.3) and secondly, because larval respiration rates will be low due to low temperatures. Oxygen levels in this hyporheic zone of streams can be much higher than that recorded in chapter 2. Some authors have reported oxygen levels as high as 45-70% saturation at depths of 30cm below the substratum, although levels vary between sites (Williams, 1984). This suggests that at other similar sites hypoxia will not be a problem for these larvae at this time.

Predator avoidance and feeding strategies.

The presence of predators in a habitat and a species predator avoidance strategy may limit the way larvae can adapt to hypoxia. In many species of Zygoptera, predator evasion is primarily by crypsis (Corbet, 1962). Effective crypsis requires reduction and restriction of movement, species that rely on crypsis as a predator defence are usually solitary and ambush their prey (Macan, 1977; Sih, 1987). Thus any type of respiratory ventilation that requires vigorous and continuous movement of water over gills or the body would not be compatible with such a cryptic life style. This has not been investigated in the Zygoptera. However, Rahel and Kolar (1990) studied the response of stoneflies to both hypoxia and predation. In this study, larvae were subjected to a gradient of hypoxia which was strongest on the substrate and weakest near the surface. Normally under such conditions, larvae would swim to the surface to avoid the hypoxia. However, in the presence of a predator the larvae remained in conditions of greater hypoxia. Thus species evolved in the presence of a predator will be limited in the way they can respond to hypoxia. Although there are clearly exceptions to this such as species of anisopteran dragonflies that are able to use both crypsis to avoid predation and respiratory ventilation to improve gas exchange efficiency. In these species the tracheal gills are concealed within the hind gut and external ventilatory movements are slight (Corbet, 1962).

4.4.5. Conclusion.

The results of the present study clearly imply that the lamellae of P. nymphula are not effective as respiratory gills. The absence of lamellae does not influence larval survival under hypoxia, nor does it significantly affect larval oxygen uptake rates under these conditions. The evidence available on the distribution and habitat preference of this species suggests that it is adapted to relatively well oxygenated habitats. Consequently, this species would be more likely to exhibit respiratory adaptation in the form of ventilation rather than to possess gills (4.1). Respiratory adaptation is related to factors other than environmental oxygen level, such as predator avoidance and feeding strategy. Thus the possibility that the lamellae of other species of Zygoptera are adapted as respiratory gills cannot be discounted. It is important that further study be carried out on a wide range of zygopteran species, firstly on how larval distribution and behaviour is affected by changes in oxygen concentration and secondly on the effects of lamellar loss on respiration rates.

CHAPTER 5

THE ROLE OF LAMELLAE DURING AGGRESSIVE ENCOUNTERS BETWEEN LARVAE

5.1 Introduction.

5.1.1. The possible behavioural roles of lamellae.

The results of the previous two chapters of the present study showed that the lamellae of P. nymphula do not function as respiratory organs. The investigations were primarily concerned with the respiratory morphology and physiology of the lamellae and whilst ruling out a respiratory gill function do not indicate anything of their true role. However, the results of the field survey (Chapter 2) showed that in a wild population of P. nymphula, lamellar loss was correlated with environmental and physiological factors such as larval size and habitat type. Lamellar loss has been reported in wild populations of other species of damselfly and linked to the frequency of aggressive interactions between larvae (Baker and Dixon, 1986; Robinson, 1991). The lamellae of P. nymphula also appear to be adapted for autotomy, possessing a specialised breaking joint (Chapter 3). These findings suggest that, rather than functioning as respiratory gills the lamellae of P. nymphula may be adapted for defensive autotomy and could also be important during conflicts between larvae either as attack deflectors or as a threat display.

A defensive autotomy role for lamellae has been demonstrated in Ischnura posita larvae by Robinson et al., (1991), who found that the frequency of lamellar loss in experimental populations of Ischnura posita was density dependent and increased the vulnerability of larvae to cannibalism. Robinson et al., (1991) also suggested that lamellar loss increased predation rates on larvae by reducing the ability of larvae to swim. It is likely that the lamellar loss observed in the wild population of P. nymphula

examined in the present study was also caused by intraspecific aggression or predation. Some authors have suggested that lamellae may be important as threat displays during aggressive intraspecific encounters, but this has never directly investigated (Corbet, 1962; Johnson, 1991). An investigation of the role of lamellae during intraspecific aggressive encounters is therefore essential in determining their function. Although it is also likely that lamellae are important during encounters with predators (Chapter 2), such an investigation was beyond the resources available for the present study.

5.1.2. Lamellar function during larval conflicts.

Conflicts between larvae.

There have been no direct investigations of the role of lamellae in determining the outcome of aggressive encounters between larvae. However, the results of a number of studies of the behaviour of larvae during such encounters suggests that lamellae may have an important role in determining their outcomes (Johnson, 1991). The larvae of many species of Zygoptera (including P. nymphula) are aggressive towards conspecifics when defending a resource such as food or a refuge (Harvey and Corbet, 1986; Johnson, 1991), although it is not certain whether larvae of all species are truly territorial or simply aggressive towards conspecifics (Corbet, 1990; Johnson, 1991)(Some species of Zygoptera such as Enallagma cyathegerum and Lestes sponsa are not aggressive to conspecifics in the larval stages (Baker, 1980; Johnson, 1991)). These contests have been likened to the "war of attrition" type of conflict described by Maynard-Smith (1974) with competing larvae attempting to outlast each other (Johnson, 1991). However, Harvey and Corbet (1986) suggest that a better interpretation of these contests is as "asymmetric wars of attrition" (Hammerstein an Parker, 1982), as conflicts can escalate and result in injury to legs and lamellae.

The behaviour of larvae during aggressive encounters has been documented in a number of species (Johnson, 1991). For example Rowe (1983; 1985), describes the interactions between larvae of three New Zealand species of Zygoptera. In Xanthocnemis zealandica, "The advancing animal (usually the intruder) moves slowly towards its opponent with its lamellae spread and swinging continuously" (Rowe, 1983). In P. nymphula, the intruding larva will try to displace the occupant by advancing and signalling using a range of displays including a "ritualised waving of the lamellae" (Harvey and Corbet, 1986).

Waving, fanning and striking the lamellae at opponents is a major component of aggressive displays in all the species with aggressive larvae that have been studied (Baker, 1983; Harvey and Corbet, 1986; Johnson, 1991; Rowe, 1980; 1985). The terminology used to describe displays varies according to the species and the authors, but a number of similar, possibly homologous, behaviours have been described (Johnson, 1991; Rowe, 1985). For example, the straight raising of the abdomen, called *raised* in P. nymphula (Harvey and Corbet, 1986), *abdomen raised* in Xanthocnemis (Rowe, 1983) and *weak head down* in Austrolestes (Sant and New, 1989) are all similar in form. Other behaviours involving the lamellae include *slow wave*, a high amplitude low frequency waving of the abdomen and lamellae and *lateral display*, the bending of the abdomen and lamellae through 90^{o} (Harvey and Corbet, 1986).

Opinion is divided as to the function of at least one of these displays, the *static caudal swing* (SCS). This display has been observed in a number of species both during conflicts and at other times (Harvey and Corbet, 1986; Johnson, 1991). In P. nymphula (and other species) this has been interpreted as a respiratory adaptation (Harvey and Corbet, 1986; Harvey, 1985; Johnson, 1991). Harvey (1985) for example, recorded this behaviour more frequently at $20^{o}C$ than at $2^{o}C$. He suggested this was due to the lack of

oxygen at the higher temperature. Rowe (1985) however, interpreted SCS as part of territorial behaviour in X. zealandica as it was only observed in larvae when they were on vertical stems. Whilst in Ischnura verticalis it has been related to feeding and feeding related respiration (Johnson, 1991).

In P. nymphula these contests can escalate and lamellae are often moved rapidly towards opponents in a behaviour termed *lamellar swipe* (Harvey and Corbet, 1986). This behaviour may simply be a response to a threat from a similar sized or larger object and has been observed in a number of species in response to a spider (Corbet, 1962), forceps and fingers (Harvey, 1985; Rowe, 1985). Injury during contests is inflicted by *labial strikes*, with larvae seizing the legs or lamellae of opponents in the jaws of their labium (Johnson, 1991). The frequency of lamellar injury has been used as an index of aggressive interactions in wild population (Baker and Dixon, 1986). In experimental studies the rate of lamellar injury has been related to density dependent aggression in Ischnura posita (Robinson et al., 1991), although in wild populations of P. nymphula (Chapter 2), Ischnura verticalis and Enallagma ebrium (Baker and Dixon, 1986) the frequency of lamellar injury was not related to larval density.

5.1.3. The outcome of larval encounters.

Contest asymmetries

Other contest asymmetries such as body size, weapons and information about earlier encounters with an opponent are known to be important in the assessment of 'resource holding power' (RHP) during aggressive contests between animals (Archer, 1988). In larval zygopterans, larval size, hunger levels, prior experience and occupancy of perches and also prior experience in contests have all been found to affect the outcome of encounters (Johnson, 1991; Harvey and Corbet, 1986). Of these, larval size is perhaps the most important factor. In most species large larvae tend to win encounters, especially

when the size difference between larvae is greater than two instars (Baker, 1983; Crowley et al., 1988; Johnson, 1991), although Harvey and Corbet (1986) found that larval size did not affect contest outcome between P. nymphula larvae. This result may have been due to the fact that they used only final instar larvae with a small size range.

Hunger level and prior experience of aggressive conflicts were found to be contest asymmetries in I. cervula larvae (Baker, 1983). Baker (1983) found that well fed larvae and larvae with prior experience as winners of conflicts were more likely to win encounters, whilst Harvey and Corbet (1986) showed that P. nymphula larvae with prior ownership of a perch (occupant) were significantly more likely to win encounters. However, in some other zygopterans prior occupancy has no effect on contest outcome (Baker, 1981; 1983).

The outcome of aggressive contests between larval zygopterans would therefore appear to depend on prior asymmetries, with contests settled "quickly, and by convention" (Harvey and Corbet, 1986). It would be expected that during contests larvae asses each others RHP relative to their own. The larva with the lower RHP would then retreat. Escalation of contests being reserved for occasions where larvae are equally matched (Archer, 1988; Harvey and Corbet, 1986). However, it is not always possible to predict the outcome of an encounter by the presence of a single asymmetry. Harvey and Corbet (1986) for example, in a study of territorial interactions of P. nymphula larvae found that on 22% of occasions intruder larvae won encounters. In their study, prior occupancy of a perch was an asymmetry and should have ensured that the occupier won the encounter. On these occasions therefore, other asymmetries had a much stronger effect in determining outcome.

The potential costs of lamellar loss.

If lamellae are important in determining the outcome of encounters between larvae then the consequences of their loss may be greater than simply the loss of the contest. Larvae without lamellae may be excluded from a resource such as food or predator refuge. Such intraspecific competition has been referred to as exclusion (or interference) competition and can affect growth rates and possibly even reproduction (Johnson, 1991; Harvey and Corbet, 1986).

Exclusion competition has been recorded in a range of aquatic insects (Wiley and Kohler, 1984) including the Zygoptera (Convey, 1988; Baker, 1981; Harvey and Corbet, 1986; Johnson 1991) although in some species such as Lestes disjunctus [Odonata: Zygoptera] no exclusion competition has been recorded (Baker, 1980; Johnson, 1991). Exclusion competition tends to occur when population densities are high and can result in reduced growth rates among smaller, excluded larvae (Baker, 1986; Banks and Thomson, 1987; Gribben and Thomson, 1990; Pierce and Crowley, 1985). For example, Gribben and Thomson (1990) showed that exclusion competition by larval Ischnura elegans resulted in reduced growth rates among smaller, excluded larvae. Crowley et al., (1988) found that exclusion competition resulted in reduced feeding and higher predation rates and inhibited dispersal in small larvae of Tetragneuria cynosura.

Reduced feeding rates are known to result in slow growth in a number of zygopteran species (Lawton et al., 1980; Pickup et al., 1984; Crowley et al., 1988). Reduced growth rates in P. nymphula populations have been recorded in both the present and previous studies (Lawton, 1970; 1980; Macan, 1964;1974). Under normal field conditions in the U.K. larvae have a semivoltine life cycle. However, in years when food is scarce or larval densities high then larvae may take three or more years to reach

emergence (Chapters 1; 2). Ultimately, food shortage in P.nymphula may lead to smaller sized adults at emergence and a reduction in the short term mating success of males and presumably the fecundity of females (Harvey and Corbet, 1986). Exclusion of larvae from refuges may also increase predation on excluded larvae (Convey, 1988; Johnson, 1991; Wellborn and Robinson, 1987). For example, Convey (1988) showed that large Coenagrion puella and Ischnura elegans larvae excluded smaller larvae from predator refuges.

Lamellae may therefore be important during encounters as display organs. The results of Chapter 2 of the present study and those of other authors (Baker and Dixon, 1986; Robinson et al., 1991) suggest they may also be important as attack deflectors (Baker, 1983; Johnson, 1991; Peckarsky, 1984; Rowe, 1980). However, this study will be restricted to an investigation of their possible role as threat displays during contests. It is predicted here that lamellar loss will result in an asymmetry in contests between larvae. If so, then lamellae may be involved in two ways during contests, either to signal information about larval RHP or to transmit information on larval intentions during the contests (Archer, 1988; Harvey and Corbet, 1986).

5.1.3. Aims.

The aims of this section of the present study were therefore to investigate whether lamellae were important in determining the outcome of aggressive encounters between larvae. Two aspects of this problem were investigated:

1) An investigation of the effects of lamellar removal on the ability of final instar P. nymphula larvae to occupy and retain a resource. This was carried out by determining which of two equally matched larvae, one with and one without lamellae could retain occupancy of a refuge. Because this study was on the effects of lamellar removal on

conflict outcome, all other known asymmetries such as prior experience of opponents and experimental chambers, hunger levels and prior occupancy of perches were controlled for (5.1.2.).

2) An investigation of the effects of lamellar removal on the behaviour of larvae during aggressive encounters with conspecifics. During experiment (1) described above careful note was made of the behavioural displays and position of experimental larvae, using time series sampling, to allow comparison of the behaviour of those with lamellae with that of those without.

5.2 Materials and methods.

5.2.1 Ownership of perches

Apparatus

The apparatus used to investigate perch ownership consisted of 10 circular opaque plastic bowls 14 cm in diameter and 7cm deep. A piece of black plastic drinking straw (3 cm long by 0.5 cm diameter) was fixed vertically in the centre of each as a perch for one larva only (Fig. 5.1). This straw was the resource over which larvae would fight and provided a refuge rather than a feeding territory as food was in excess and evenly spread throughout the container. Larval zygopterans are able to select perches that provide the best camouflage (Moum and Baker, 1988). In this case the dark straw was the only suitable camouflage in the opaque white container.

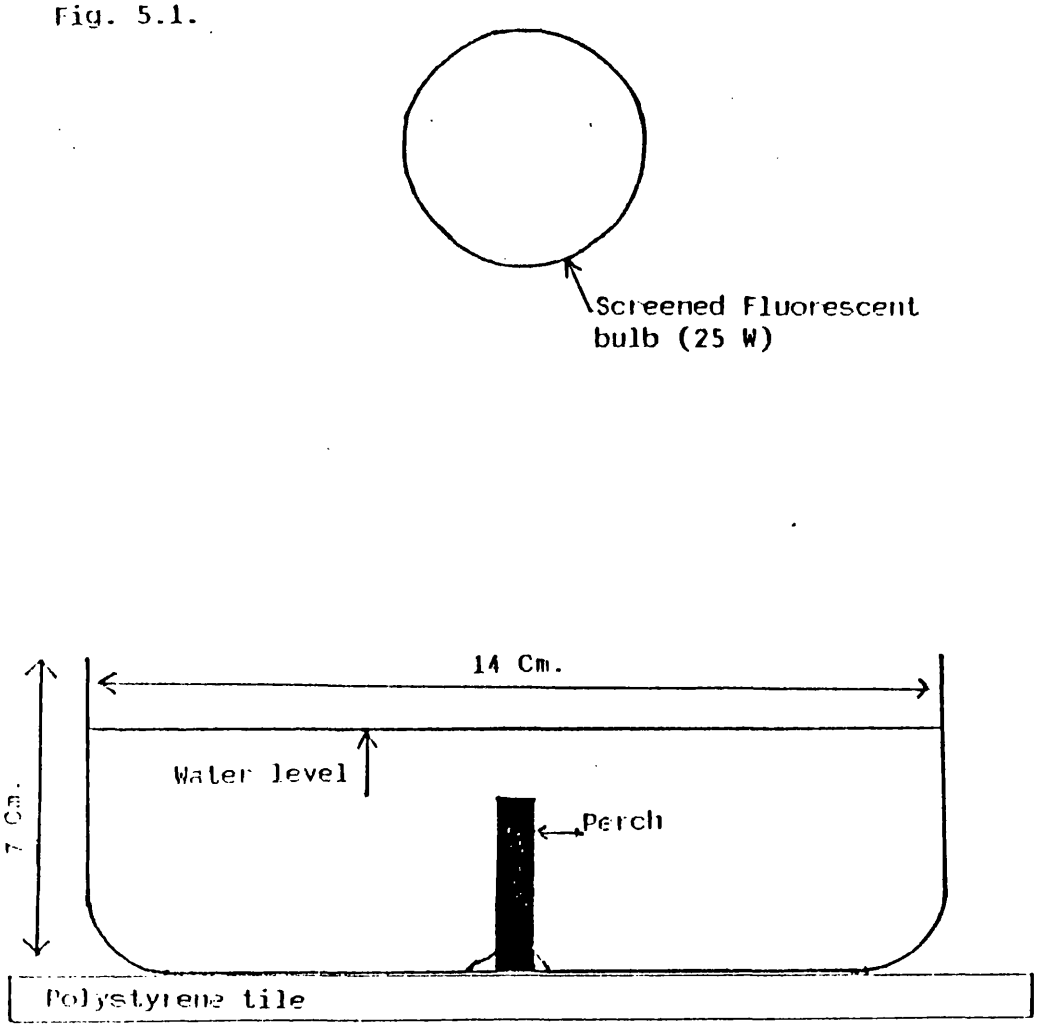
The containers were filled with Loch Lomond water to a depth of 6cm. The temperature throughout the experiments was maintained at 21(+/- 2)^{o}C. Light levels were kept low using a shaded 25 W fluorescent bulb with a 12 hour L/D photoperiod. The light was arranged to give overhead illumination of each chamber. To minimise interference due to noise or movement by the observer during monitoring of larvae the containers were arranged under a screen in a darkened room and observations carried out from above the light. Noise and vibration disturbance were further reduced by insulating the bowls from the bench top using a polystyrene tile.

Experimental larvae

Larvae were collected downstream from the Ross Burn sampling site during summer and autumn of 1991 (2.1.2), and kept in a 25 L aquarium at 15-20^{o}C. Coils of plastic "Netlon" provided a substratum. Larvae were fed excess food and kept under a

Fig. 5.1 Diagram of experimental chamber. The chambers were 14cm in diameter and 7 cm deep. A piece of black drinking straw 0.5 cm wide and 3 cm long provided a perch for larvae. Chambers were filled to a depth of 6 cm. Light was provided by the screened fluorescent bulb (approximately 50 cm above the containers). Observations were carried out from above and to one side of the bulb. The whole apparatus was screened using black PVC and set up in a darkened room.

Fig. 5.1.


Screened Fluorescent
bulb (25 W)
14 Cm.
Water level
7 Cm.
Perch
Polystyrene tile

photoperiod of 12h light/dark (Harvey and Corbet, 1986). Larvae were fed large zooplankton such as cladocera, and chaoboros larvae collected from the Lochan Dubh (2.1.2.).

Larvae used during experiments were either in the final or in the penultimate instar and either with or without lamellae. Where necessary, lamellae were removed by autotomy at least one week prior to the start of experiments. Head width and total body length were used to match the size of larvae (2.2). Larvae were used only once for these experiments and then returned to the sample site.

Experimental procedure

During experiments it was essential to minimise any interference by other asymmetries on the outcome of contests. The asymmetries during intraspecific conflicts have been discussed already (5.1.2.) and include larval size, hunger levels, prior experience and perch occupancy. These were controlled for in four ways a) Any effects of larval size were controlled by using larvae with the same head width and body total length (Chapter 2). b) Larval hunger levels were standardised by providing excess, evenly distributed food throughout the experiment. c) Larvae were both introduced to the experimental chamber simultaneously from chambers identical to the experimental one so neither had prior experience of the container. This would have the effect of making both larvae occupants before the start of the experiment. Larvae were only used once therefore had no prior experience of the containers. d) The history of experimental larvae with respect to prior experience in conflicts was unknown. However, by using randomly selected larvae from an experimental "pool", and isolating them for 48 hours prior to the experiment, the effects of any prior experience were minimised.

Experimental trials were initiated by isolating two size-matched larvae (one with and one without lamellae) into separate containers, identical to the experimental ones, for 48 hours before the start of the experiment. These larvae were maintained under identical environmental conditions to those found during the experiment.

To start a series of observations, the two larvae were introduced to the experimental chambers simultaneously. This was done by mixing the two larvae together in one container and pouring the contents into the experimental chamber, this minimises handling stress and ensures both larvae enter the chamber together. By introducing larvae to the chamber simultaneously there was an equal probability of those with and those without lamellae becoming the initial perch occupant. Experiments were started at the same time of day, usually between 8 and 9 am. Larvae were left to settle for three hours before the first observation. Larvae were monitored during each day of the experiment at 9am, 12pm and 5pm. These observations were carried out until there had been no change in the perch occupant during three consecutive sets of observations. In all sixty replicates were carried out with eight pairs of larvae run simultaneously. In addition to each set of eight pairs of experimental larvae run, two sets of control chambers were run. Control chambers and larvae were treated identically to experimental larvae except that there was only one larva per chamber.

Observations were standardised as follows to monitor three aspects of larval behaviour. These were:

1) Perch occupancy. To determine whether larvae with or without lamellae were gaining occupancy of the perch the lamellar status of the occupant was recorded.

2) Location. To determine whether there was any difference in the behaviour of larvae regarding their position in the containers, the location of both larvae and their lamellar status was recorded. Position was recorded according to their location on a "clockface" ie directly away from the observer, 12, to the left 9 etc.

3) Larval behaviour during encounters.

Prior to recording the displays used by larvae during encounters it was necessary to compile a reference list of those carried out by P. nymphula larvae. The list provided here is based on the extensive descriptions of the behaviour of P. nymphula larvae during conflicts given by Harvey (1985) and Harvey and Corbet (1986). The additional descriptions of displays given here are of the positioning of the lamellae and abdomen during conflicts.

This behavioural repertoire was determined by video recording larvae for 3 hour periods, in flat sided, perspex aquaria (20X8X8 cm). A single piece of drinking straw provided a perch for larvae. This aquarium was filled with Loch Lomond water (2.4.2.) and maintained under the experimental conditions described above (5.2.1.). Larvae used for the determination of behavioural repertoire were collected and maintained as described in 5.1.1. Larvae were introduced into the experimental chamber as described in the previous section (5.2.1). A continuous video recording of the larvae was then made for three hours. The video tapes were reviewed and the number, duration and type of behaviours recorded.

The display shown by each larva during the experimental observations was then recorded at each monitoring occasion (5.2.1.). The frequency and type of display shown was then used to determine whether there were any behavioural differences between larvae with and without lamellae during these experiments.

5.3. Results

5.3.1 Ownership of perches

Lamellar status and perch occupancy

The lamellar status of the perch occupant was recorded after each time interval. The frequencies of perch occupancy for all trials combined, according to lamellar status, are shown in Fig. 5.2. On a number of occasions either both larvae were off the perch or both on, this situation being referred to as "neither alone" on the perch.

Initially, occupancy of the perches was divided evenly between those larvae with lamellae, those without and the "neither alone" category. This suggests that initial occupancy of the perches is random and that lamellar status conferred no advantage here (Fig. 5.2, Table 5.1). As the experiments progressed, the number of times larvae with lamellae were observed occupying the perch increased, with a consequent decrease in the number of times larvae without lamellae were occupants or that "neither alone" was observed (Fig 5.2).

The numbers of larvae with and without lamellae at each observation period were compared using a Chisquare test. The results of these tests show a significant difference in frequencies of larvae with and without lamellae occupying perches on all but the first observations on day one (Table 5.1). On all these occasions there are more larvae with lamellae on perches than those without. Therefore, larvae with lamellae are more likely to gain occupancy of the perch.

Control larvae

In the absence of another larva, perches were all eventually occupied by larvae indicating a preference for perches over the container. Initially, in the control group of

Table 5.1. Frequency of perch occupancy by larvae according to lamellar status and time. Chisquare tests exclude "neither alone" observations. In all cases D.F. = 1.

Day	Frequency						Chisq (L-/L+)
	L- On perch		L+ on perch		Neither		
	N	%	N	%	N	%	X^2 [P].
Day 1							
AM	3	38	3	38	2	25	0.00 [>0.999]
12	13	23	25	45	18	32	7.75 [0.01-0.001]
PM	14	26	28	52	12	22	9.33 [0.01-0.001]
Day 2							
AM	12	26	27	57	8	17	11.53 [<0.001]
12	14	29	26	54	8	17	7.20 [0.01-0.001]
PM	14	29	27	56	7	15	8.24 [0.01-0.001]
Day 3							
AM	11	27	29	71	1	3	16.20 [<0.001]
12	11	28	26	65	3	8	12.16 [<0.001]
PM	8	24	21	62	5	15	11.66 [<0.001]

L- = Larvae without lamellae
L+ = Larvae with lamellae
Niether = Both larvae either on or off the perch (neither alone on).
AM = observations carried out @ 9 am
12 = Observations carried out around noon
PM = Observations carried out @ 6 pm

Table 5.2. The number and type of change in perch occupancy during experiments.

Day	Change in ownership of perch					
	(L+)To (L-)	(L-)To (L+)	ON TO OFF	2 ON/OFFTO L+ ON	L- ON	NO CHANGE
Day 1						
AM						
12	1	3	3	-	-	25
PM	1	4	2	6	-	33
Day 2						
AM	1	-	3	4	3	40
12	2	2	2	2	1	36
PM	2	1	1	2	2	38
Day 3						
AM	1	1	2	6	2	35
12	1	5	2	1	-	34
PM	0	2	2	1	-	29
Tots	9	18	17	22	8	270

L- = Larvae without lamellae
L+ = Larvae with lamellae
ON TO OFF = Change from single larva occupant to neither occupant
2 ON/OFF TO.. = Change from neither larva alone on the perch to either L- on or L+ on, as specified.
NO CHANGE = No change in occupancy since the last observation.
AM = observations carried out @ 9 am
12 = Observations carried out around noon
PM = Observations carried out @ 6 pm

Fig. 5.2 Frequency of perch occupancy by the three categories of larvae during experiments with two larvae in containers. The number of disputed perches and those occupied by larvae without lamellae decreases during the experiment. The number of larvae with lamellae occupying perches increases during this time. A= AM samples, 12= midday samples, P= PM samples.

Fig. 5.2

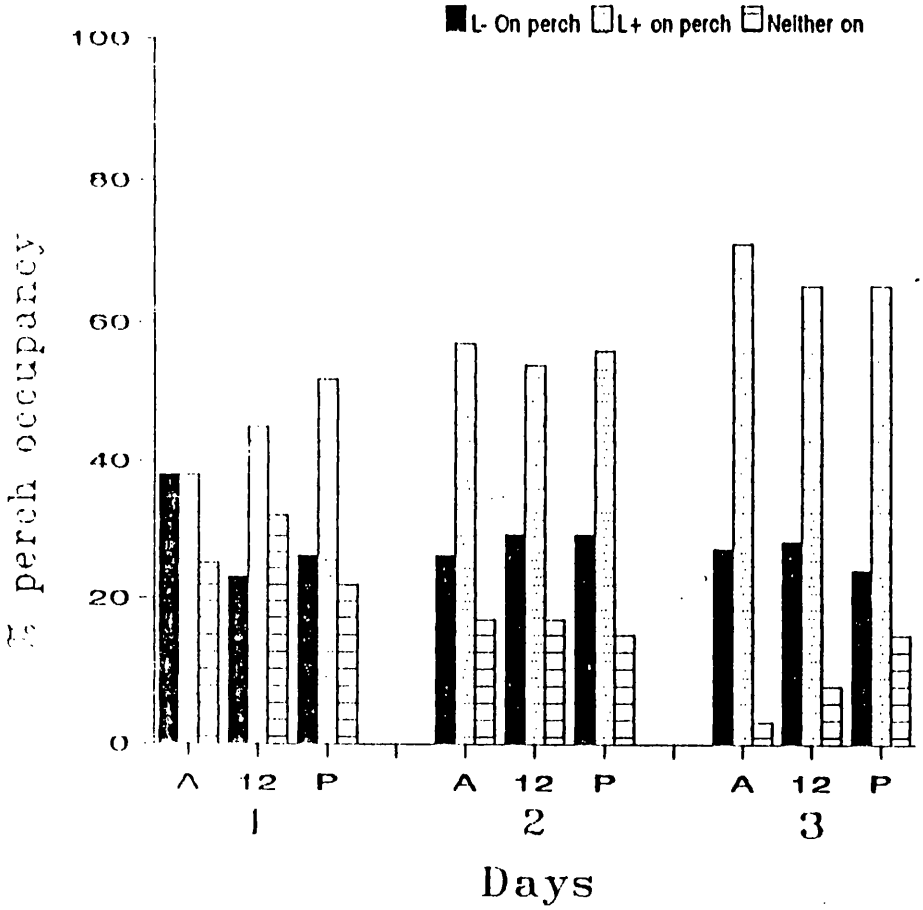
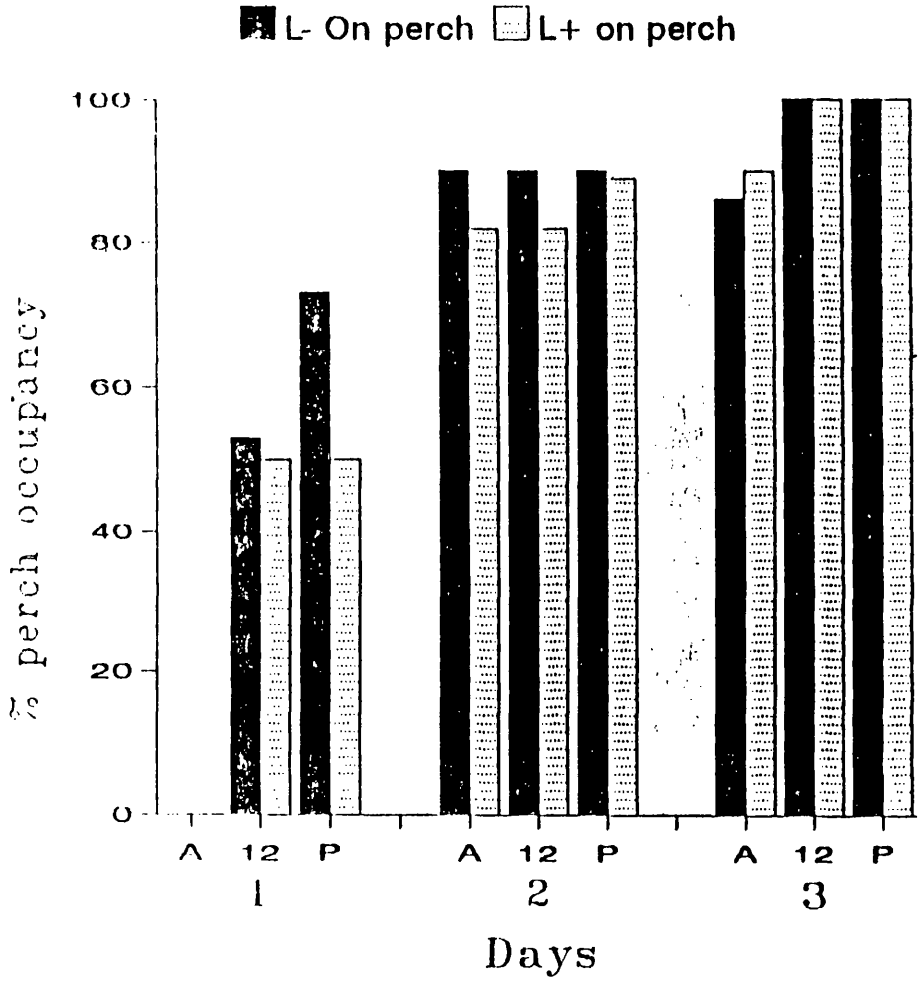

L- On perch
L+ on perch
Neither on
100
80
60
40
20
0
% perch occupancy
A
12
P
1
2
3
Days

Fig. 5.3 Frequency of perch occupancy by control larvae (larva alone in container). Within three days all perches are occupied by larvae. Larvae with and without lamellae have a clear preference for perches. A= AM samples, 12= midday samples, P= PM samples.

Fig. 5.3


L- On perch
L+ on perch
% perch occupancy
100
80
60
40
20
0
A
12
P
1
2
3
Days

larvae, few perches were occupied immediately (Fig, 5.3). By noon of the first day more than 50% of the perches were occupied and in equal proportions by larvae with and without lamellae. By the end of the second day more than 80% of perches were occupied, and by the end of the experiment all were occupied (Fig. 5.3). Compared to experimental larvae, control larvae were therefore slower at occupying perches.

The pattern of larval displacement.

Thus larvae with lamellae are more likely to be perch occupants. The number and type of change in ownership of the perches can be used to determine how this arises. There are two possibilities: either larvae with lamellae occupied vacant perches more rapidly, or they are able to displace those without lamellae. The number and type of changes in occupancy are shown in Table 5.2.

Of the changes in occupancy which resulted in a single larva occupant on the perch, 40 resulted in a larva with lamellae on the perch whilst 17 resulted in a larva without lamella as occupant (Chisquare= 18.56, P=<<0.001). Thus larvae with lamellae were gaining and retaining occupancy of perches by displacing larvae without lamellae rather than simply occupying vacant perches.

The number of changes of ownership for any single pair of larvae, before final occupancy was settled, was low. In most cases there was only one observed change in ownership. On one occasion there were four changes of ownership, on three occasions three changes and on two occasions two changes of ownership. Thus, once a larva was challenged and lost occupancy it was unlikely to reoccupy the perch. However, due to the nature of the sampling regime it was possible that more changes of ownership occurred between the monitoring times and these would have been missed.

5.3.2. Larval behaviour during experiments.

The behavioural repertoire.

Prior to recording larval behaviour during experiments it was necessary to compile a reference list of acts and their definitions. This list was made using 3 hour video tapes of encounters between larvae in experimental chambers. The list used in the present study is based on that provided by Harvey (1985) and Harvey and Corbet (1986) for P. nymphula larvae during conflicts. Only those behaviours observed during the subsequent experiments are described in this list which is therefore not comprehensive (Appendix 6).

Perch occupancy, lamellae and larval behaviour

The total number of times a behaviour was observed during monitoring is listed according to lamellar status of larvae in Table 5.3. From this list it can be seen that larvae were observed to be resting in most observations. The next most frequent behaviours observed were *head up* and *abdomen raise*. Experimental larvae exhibited active behaviours more frequently than the control larvae (Chisquare = 49.2, df=1, $P<<0.001$). Larvae with lamellae in containers with two larvae were observed to perform 153 acts compared to 69 for larvae without lamellae. This appears to indicate that larvae with lamellae display more frequently than those without. However, this conclusion does not take into account the effects of perch occupancy as a contest asymmetry. This will be considered in the following section.

By contrast, the control larvae displayed relatively few active behaviours, 13 for larvae with lamellae and 11 for larvae without lamellae. The greater frequency of acts shown by experimental larvae compared to control larvae is due to the presence of the other larva in the container. Behaviour shown only in the presence of another animal was

Table 5.3 The frequency and type of behaviour shown by experimental and control larvae. Totals exclude records of *resting* behaviours. The definitions and descriptions of the behaviours listed are given in Appendix 8.

Behaviour	Observation group			
	Experimental		Control	
	L+	L-	L+	L-
Rest	209	293	111	118
Head up	44	29	5	6
Abdomen raise	15	20	0	2
Rest and fan	31	-	3	-
Head up fan	22	-	2	-
Swimming	6	7	1	1
Lateral display	12	2	0	-
Fight	4	4	0	0
Raise and fan	7	-	0	-
Tip raise	3	3	0	0
Static caudal swing	3	2	2	0
Grooming	2	1	0	-
Feeding	8	9	0	2
Lamellar display	1	-	0	0
Slow wave	1	-	0	0
Totals	159	77	13	11

L+ = with lamellae, L- = without lamellae,

Table 5.4. Frequency of displays by larvae with and without lamellae either on or off the perch. Data corrected for displays shown only by larvae with lamellae. Totals in brackets for larvae with lamellae are those including the behaviour "rest and fan".

Day	Category							
	Without lamellae				With lamellae			
	ON Perch		OFF Perch		ON perch		OFFperch	
	Agg	N/Agg	Agg	N/Agg	Agg	N/Agg	Agg	N/Agg
Day 1								
AM	1	4	0	4	3	0	0	6
12	6	9	2	26	11	15	1	16
PM	4	11	1	29	8	20	1	13
Day 2								
AM	6	6	2	27	4	27	-	12
12	4	10	-	29	8	20	-	15
PM	2	12	1	26	8	21	-	15
Day 3								
AM	2	9	-	26	12	14	1	10
12	-	11	1	24	7	18	-	11
PM	1	7	-	21	3	17	-	8
Total	26	79	7	212	64	151	3	106
					(85)	(125)	(5)	(103)

L- = Larvae without lamellae
L+ = Larvae with lamellae
ON = On perch
OFF = Off perch
Agg = Displaying aggressive behaviour
N/Agg = Note displaying aggressive behaviour

AM = observations carried out @ 9 am
12 = Observations carried out around noon
PM = Observations carried out @ 6 pm

considered potentially aggressive. Of the acts observed (excluding resting) 7 were shown by both control and experimental larvae leaving 8 only shown when in the presence of other larvae. These included, *slow wave, lamellar display, grooming, tip raise, fighting* and *lateral display. Feeding* was also observed only in experimental larvae but was not considered aggressive. Some behaviours such as *static caudal swing* (SCS), *head up fan, rest and fan* and *abdomen raise* were considered aggressive as they were observed infrequently in control larvae.

In order to determine whether perch ownership or lamellar status affected the frequency with which larvae displayed it was necessary to reclassify some of the data. This is because recording of some of these behaviours is clearly biased towards larvae with lamellae. For example, larvae without lamellae can never exhibit behaviours such as *rest and fan, head up and fan* and *raise and fan* as they have no lamellae. Therefore, *rest and fan* must be reclassified as *rest, head up and fan* as *head up* and *raise and fan* as *raise*. Such reclassification reduces the overall total of observations of aggressive behaviours carried out by larvae with lamellae on the perches to 125 acts (compared to 77 acts for larvae without lamellae on perches).

However, perch occupancy is a conflict asymmetry. Harvey and Corbet (1986) found that P. nymphula larvae occupying perches displayed more than larvae off perches. The result described above which suggests larvae with lamellae display more could simply be due to the fact that they occupy perches more often (5.3.1.). To test this, the number of aggressive behaviour shown by larvae on the perch either with or without lamellae can be compared. Similarly, the frequency of acts displayed by larvae either with or without lamellae and off the perch can be compared.

Three analyses were carried out on the data totals given in Table 5.4. Firstly the frequency of displays shown by larvae on and off the perch regardless of lamellar status were compared using a Chisquare test. The number of times larvae on perches exhibited active behaviours was 90 in 320 observations and those off the perches 10 in 328 observations (Table 5.4)(Chisquare(1 df) = 80.78 P=<<0.001). Thus regardless of lamellar status, larvae on perches displayed behavioural acts more frequently (28% of occasions) than those off the perch (3% of occasions).

To determine whether this effect was due to perch occupancy or lamellar status, the frequency of aggressive displays by occupant larvae with lamellae was compared with that of occupant larvae without lamellae. Larvae with lamellae (and occupant) exhibited behaviours on 85 of 210 observations whereas larvae without lamellae (and occupant) exhibited behaviours on 26 of 105 observations (Fig. 5.4)(Chisquare(1df)= 3.78, P= >0.05). Therefore there was no significant difference in the number of behavioural acts shown by larvae with or without lamellae and on the perch.

The third analysis carried out was to determine whether there was a statistically significant difference in the frequency of displays carried out by larvae with and without lamellae off the perch. In this case larvae off the perch and with lamellae displayed on 5 of 108 observations and those without lamellae 7 in 219 observations (Table 5.4) (Chisquare (1df)= 0.371, P=>>0.05).

Thus although overall larvae with lamellae displayed behavioural acts more frequently than those without lamellae (Table 5.3, 5.4), this was due to their more frequent occupancy of perches rather than possession of lamellae. Loss of lamellae does not influence the overall frequency of behaviours observed during encounters.

If the above analyses are carried out including the records for all displays involving the lamellae (including *rest and fan*), then the results of these analyses become statistically significant (Table 5.4). For larvae on the perch with versus without lamellae, Chisq=7.75 DF=1. P=0.01-0.001, and for larvae off the perch with versus without lamellae Chisq=0.42 DF=1, P>>0.46. Thus the absence of lamellae must restrict the ability of larvae to advertise their presence.

Lamellar status and larval location.

The relative positions of larvae within experimental chambers was recorded to determine whether this was affected by lamellar loss (Table 5.5). The presence of the intruder larva in the same quadrant of the container as the occupant was presumed here to represent a challenge to the occupant. Harvey and Corbet (1986) found that contests between larvae were initiated at about 4cm distance. The design of these containers meant that larvae were almost always within this distance of each other and could only avoid such a challenge by hiding behind the perch.

To determine whether lamellar status influenced the frequency of challenges to the occupant, the number of times larvae with and without lamellae were challenging or not challenging the occupant was compared (Table 5.5) (Chisq (df=1) = 2.189, P=>>0.05). Thus there is no significant difference in the frequency of challenges to the occupant by larvae with or without lamellae.

5.4. Discussion.

5.4.1 Lamellae and the outcome of contests.

Perch defence.

The results of the present study confirm that the caudal lamellae of P. nymphula are important as part of the threat display performed during intraspecific contests over a resource. Such a function has been inferred by previous authors but never demonstrated (Baker and Dixon, 1986; Corbet, 1962; Johnson, 1991). Loss of lamellae resulted in a statistically significant reduction in the ability of larvae to defend the perch (5.3.1.).

The inability of larvae without lamellae to defend perches was not due to lamellar loss affecting their ability to move to perches. Robinson et al., (1991) for example, showed that lamellar loss impaired swimming in Ischnura posita and consequently reduced their ability to avoid predators. In the present study, inability of larvae to swim to a perch could have affected initial perch occupancy, as larvae without lamellae would be at a disadvantage. However, this was not found to be the case as vacant perches were initially occupied in similar numbers by both types of larva (5.3.1.). Once displaced from perches larvae without lamellae proved unable to reoccupy them. These larvae were displaced usually with only one observed change of ownership although on one occasion four changes were observed. Initial occupancy of perches was faster in containers with two larvae than in the controls (see Fig. 5.2, Fig. 5.3). This suggests that in the presence of another larva competition for the refuge is greater.

5.4.2. Larval behaviour during contests.

Lamellar status and behaviour

The loss of lamellae did not significantly alter the aggressive behaviour of experimental or control larvae. Although larvae with lamellae displayed more, this was

because they occupied perches more frequently. The results of the present study and those of previous authors (Harvey and Corbet (1986) have found that in P. nymphula perch occupancy was an asymmetry in larval conflicts and consequently occupants display more frequently than intruders.

Nor did lamellar loss affect the frequency of challenges to the perch occupant as throughout experiments there was no significant difference in the number of times larvae with or without lamellae were recorded in the same quadrant as the perch occupant (5.3.2.).

The role of lamellae during contests.

It is not clear from the results of the present study how lamellar loss reduces the ability of larvae to defend or displace another larva from a perch. It was suggested (5.1.2.) that if lamellae were important as signals during contests then they may transfer two types of information, either on resource holding power (RHP) or on larval intentions during fights (Harvey and Corbet, 1986).

Removal of lamellae would result in a reduction in the area of larva visible to an opponent (Robinson et al., 1991). Size is considered to be one of the most important asymmetries in conflicts between larvae (5.1.2.). Thus if larvae use size to asses RHP then lamellar removal would certainly reduce the effectiveness of such displays. It is also possible that lamellae and lamellar related behaviours are used as "badges of status" to advertise RHP or presence prior to contests (Huntingford and Turner, 1987). An example of such a display may be *static caudal swing* (SCS). There has been some debate over the function of this display as it is observed in a number of species and has been interpreted as respiratory (Harvey, 1985), feeding related (Johnson, 1991) and a territorial display (Johnson, 1991). Rowe (1985) also suggested that SCS in

Xanthocnemis zealandica was part of a territorial display as it was only shown by larvae on vertical stems. In the present study this display was shown by control and experimental larvae both with and without lamellae infrequently, even although oxygen levels in the experimental containers were likely to be high.

Lamellae could also be important to signal intention during a conflict (5.1.3.). If so then loss would result in an inability to send signals or lead to signals being misinterpreted. Harvey and Corbet (1986) found a significant difference in the frequency of lamellar-related behavioural displays during occupant won contests between P. nymphula larvae. They suggested that these displays, which included *slow wave, lateral display* and *lamellae swipe* could be seen as transmitting information during the contest. If so loss of lamellae would restrict or confuse information transfer. The results of the present study found no difference in the frequency of non lamellar related behaviours between larvae with and without lamellae, indicating that following lamellar loss larvae do not adjust their behaviour. If signals are given and misinterpreted due to lamellar loss then larvae could "mistake their roles" during the conflict. Harvey and Corbet (1986) suggested that such role misinterpretation occurred during intruder-won conflicts between P. nymphula larvae with intruders behaving more like occupants. Thus even although occupants had the advantage they could still lose encounters.

How the results of the present study relate to larval behaviour in the wild is not clear. Important considerations must be the context of the encounter and how the various contest asymmetries, described earlier (5.1.2), interact. The experimental conditions used in the present study were unnatural in a number of ways such as the matching of only two larvae of identical size in a closed container. In the wild it is likely that encounters would arise involving two or more larvae of varying size, experience and hunger levels and where defeated larvae are able to escape the occupant. It would not be possible to

predict for example, that a larger hungry larva without lamellae would be able to defeat a smaller well fed larva occupying a perch.

The relative importance of these contest asymmetries in wild populations of zygopteran larvae is not known, although in P. nymphula the effects of size asymmetry during conflicts may be limited. The semivoltine life cycle of P. nymphula results in larvae growing in well defined emergence group cohorts with a narrow size range of individuals (Chapter 2). The smaller and larger cohorts are spatially separated for most of the year so that at this time larvae of each emergence group are likely only to encounter larvae of similar size (Chapter 2). The results of the study by Harvey and Corbet (1986) may confirm this as they could not find size asymmetry effects during staged encounters between final instar larvae of P. nymphula. Thus in wild populations contest asymmetries such as lamellar loss, hunger levels and experience may be more important than size in P. nymphula.

5.4.2. The adaptive significance of lamellar loss

The lamellae of P. nymphula are therefore important in determining the outcome of conflicts between larvae. Because their loss results in an asymmetry during conflicts, the effects of their loss on larvae would be similar to those caused by other asymmetries (such as size, hunger levels, experience and occupancy of resource, and experience as a winner) (5.1.2.). The defeated larva would then be excluded from a resource such as food or refuge (5.1.2). In the present study, the resource defended was a refuge (5.2.2.). If larvae are excluded from food then this will result in slow growth, smaller size at the moult and possibly reduced breeding success whilst larvae excluded from a predator refuge will suffer increased predation (Robinson et al., 1991).

5.4.4. Conclusion

The results of the present study demonstrate that the lamellae of P. nymphula are important in determining the outcome of conflicts between larvae over perches. It is not clear how this is caused but it may be due to the loss of either information on larval RHP or of larval intention during the conflict. Lamellar loss does not significantly influence the frequency of non-lamellar related behaviour of larvae. The only contest asymmetry found in the present study was that of perch occupancy. The potential costs of lamellar loss to larvae are reduced access to food, increased predation and possibly reduced breeding success as an adult.

The three laterally flattened lamellae of zygopteran larvae are prominent structures yet their function is still uncertain. In the past it has been generally accepted that they function as respiratory gills (Chapter 1). However, this has never been demonstrated unequivocally and several other functions have been put forward which are not mutually exclusive. These include: ion uptake (Eriksen, 1986), locomotion (MacNeill, 1960; Robinson et al., 1991), sensory (Norling, 1982; Watson, 1966) and attack deflection (Baker and Dixon, 1986; Robinson et al., 1991). The aim of the present study has been to determine which of these possible functions is served by the lamellae of P. nymphula. To distinguish between these hypotheses the investigations carried out here were directed at lamellar respiratory morphology and physiology, the importance of lamellae during aggressive interactions with conspecifics and the frequency of lamellar loss in a wild population of P. nymphula.

The results of the present study allowed rejection of at least three of these functions for the lamellae of P. nymphula. These include those of respiratory gills, ion uptake organs and sensory organs. Ion uptake organs are found in association with respiratory gills in a range of freshwater insect species (Komnick, 1988; Komnick and Schmitz, 1?87; Chapter 3). However, there was no evidence of such specialised organs or epithelia on the lamellae of P. nymphula (3.4.2.). In addition, the fact that these organs are lost with such frequency in the wild (2.3.1.) excludes this as a function, as loss of the main site of ion uptake would lead to the death of the larva. The most likely site of ion uptake in this species is in fact the rectum, where thick columnar epithelial cells are found (3.4.2.). These cells are similar in structure to those found in the specialised ion uptake epithelia present in the rectum of other species of Coenagrionidae (Komnick, 1974). However, further study of the function of this epithelia is required in P. nymphula

larvae before this can be verified.

It is also unlikely that the lamellae of P. nymphula have a sensory role. Four potential sense organs were found on the surface of the lamellae. However, none of these were unique to the lamellae and all were found in similar densities on the abdomen and thorax (3.3.4). The lamellae of this species cannot be considered to be uniquely adapted as sensory organs.

Until now the most widely accepted lamellar function has been that they act as respiratory gills, so particular attention was given to this possibility in the present study. Two approaches were used here, firstly, an investigation of their respiratory morphology and ultrastructure (Chapter 3), and secondly, a study of their respiratory physiology under varying conditions of hypoxia (Chapter 4).

The morphological features indicative of a respiratory gill are: large surface area, short diffusion distance for oxygen from the environment into the tracheal system and few mitochondria indicative of low metabolic rate (Chapter 3.1.). The results showed that although the surface area and oxygen consumption rate of the lamellae appeared to be compatible with that required for a respiratory gill, this was unlikely to be their main function. For example, in comparison to the whole animal, the surface area of the lamellae was large (15-20%) compared to their weight (4%), whilst examination of their ultrastructure revealed no concentrations of cell organeles such as mitochondria, which would indicate high metabolic oxygen demand. However, the area of the lamellae was found to reduce in proportion to that of the body as the animals grew. This is the reverse of what would be expected if lamellae acted as the main respiratory organ (3.4.2.). Thus although lamellae have a large surface area, the way in which they develop suggests a decreasing respiratory importance with age.

More convincing evidence against lamellae functioning as tracheal gills came

from the study of the density and arrangement of the tracheoles within the lamellar hypodermis. The diffusion distance for oxygen across the cuticle and into the tracheoles is of critical importance when considering the efficiency of a tracheal gill. Because of the physical constraints on the way this diffusion distance can be reduced, the tracheal gills of the different groups of freshwater insect have evolved convergently. That is, they have a thin cuticle with either regularly spaced tracheoles with a relatively large and constant diameter or randomly spaced tracheoles that are densely packed but with varying diameters (Wichard and Komnick, 1974a). The thickness of the lamellar cuticle, the density of subcuticular tracheoles and the tracheolar arrangement was measured in P. nymphula and compared both with that of the abdomen and with that found in the tracheal gills of other freshwater insects (3.3.2.). This comparison revealed that the lamellae of P. nymphula would be inefficient as gas exchange gills because of their relatively thick cuticle and the arrangement and diameter of the tracheoles (3.3.2). Thus the lamellae seem not to be adapted for respiration even though aspects of their general morphology are compatible with such a function.

The investigation of respiratory physiology also failed to provide evidence that the lamellae of P. nymphula function as respiratory gills (Chapter 4). The respiration rates of a wide size range of larvae both with and without lamellae did not differ significantly, even at low environmental oxygen concentrations (4.3.1.). The response of larvae to hypoxia and anoxia was behavioural, with larvae attempting to swim to regions of higher oxygen concentration. This resulted in increased larval respiration rates as the environmental oxygen concentration decreased (4.4.3.). In summary the results of the investigation of lamellar morphology and respiratory physiology clearly demonstrate that the lamellae of P. nymphula are not adapted for gas exchange.

A locomotory function for lamellae has been suggested by a number of authors, but remains unconfirmed in P. nymphula larvae (3.4.2.). Robinson et al., (1991) for

example, found that lamellar removal in Ischnura posita larvae affected the willingness of larvae to swim from a predator. The presence of the specialised breaking joint (3.3.4.) may however preclude this function as it could allow autotomy during vigorous swimming. Some species of insect do have organs that autotomise but can be used for vigorous movement (such as the wings of termites used in dispersal flights); it is possible lamellar autotomy is controlled in a similar way in zygopteran larvae (Johnson, 1969). Further investigation of the swimming ability of larvae with and without lamellae is required to determine whether they are important in this respect.

The most significant positive result of the present study was the finding that the lamellae of P. nymphula are important in determining the outcome of intraspecific conflicts between larvae (Chapter 5). Larvae without lamellae were less able to defend perches or to displace resident larvae with lamellae. In contests a pair of otherwise equally matched larvae one with and one without lamellae, were allowed to fight over a perch (5.3.1.). Even after a period of one day, larvae with lamellae were significantly more likely to be the perch occupant than those without lamellae.

These results suggest that the lamellae of P. nymphula are used larvae to send signals (on resource holding power or intentions) to opponents during conflicts (5.1). This interpretation of lamellar function could also explain the large surface area and colouration of the lamellae since these traits would enhance the effect of such signals (3.3.2). Loss of lamellae reduces the visual impact of and thus reduces the ability of the affected larva to displace others from, or to defend a resource. Lamellar loss or injury can therefore generate through a contest, asymmetry during larval conflicts (Corbet and Harvey, 1986), the relative importance of lamellar loss, compared to other conflict asymmetries, such as size, hunger levels, perch occupancy and experience remains to be determined. The costs of lamellar loss include exclusion from resources such as feeding territories or refuges (5.3.1.). It is likely that the effects of total lamellar loss are important in the wild as in both the present study (2.3.1.) and those by other authors

(Baker and Dixon, 1986; Robinson et al., 1991) showed significant frequencies of lamellar damage in wild populations.

In addition to a signalling function it is also likely that the lamellae of P. nymphula function as autotomous defence organs (Chapter 2). These organs deflect attacks from the body and may be shed at the specialised breaking joint when seized by an opponent or predator, thus allowing escape (Vitt et al., 1977). The evidence that the lamellae of P. nymphula may function in this way comes from both the field survey (Chapter 2) and the investigation of lamellar morphology (Chapter 3). Previous investigations by other authors have related the frequency of lamellar damage in wild and captive populations of other zygopteran species to the frequency of aggressive encounters between larvae (Baker and Dixon, 1986; Robinson et al., 1991). The results of the present study found that a significant number of larvae in the wild had some or all of their lamellae missing or partially regenerated (Chapter 2). The frequency of lamellar loss in this wild population was correlated with larval size, distribution and sampling season (2.3.1.). Other authors have reported similar results in other species of zygopteran and found lamellar loss to be correlated with density dependent aggression (Baker and Dixon, 1986; Robinson et al., 1991). It is likely that the lamellar injury observed in the study population has the same cause. Additional evidence for a defence autotomy function comes from the study of lamellar morphology (Chapter 3), in which the structure and operation of the breaking joint was investigated. The joint was found to have traits typical of breaking joints by preventing body fluids from escaping and water from entering the tracheal system.

These results suggest that lamellae are used as attack deflecting organs. However, no experimental studies have yet demonstrated that lamellar loss increases larval survival. Further investigation of the survival and long term breeding success of larvae with and without lamellae is essential before this conclusion can be confirmed.

Investigations of caudal autotomy in other animal groups, such as lizards (Vitt et al., 1977), have provided models of the energetics of this phenomenon that could be tested on zygopteran populations.

These results are significant, as they demonstrate clearly for the first time that the lamellae of at least one species of zygopteran, function as signalling organs rather than respiratory gills. Whether this will prove to be the case for all species of zygopteran with laterally flattened lamellae is not clear. The larvae of P. nymphula are aggressive towards conspecifics and their lamellae are adapted as signalling organs. The same is likely to be the case for species that display aggressive behaviour to conspecifics using similar displays of the lamellae. However a number of zygopteran species such as Enallagma cyathegerum (Baker, 1980) and Lestes sponsa are not aggressive towards conspecifics (Johnson, 1991). In these non-aggressive species the lamellae may be adapted for other functions.

If lamellae are adapted to functions other than display organs then these will depend on selection pressures such as predation, development rates, activity levels and feeding strategies. The way a larva responds to predators for example could put constraints on how they cope with hypoxia. Sih (1987), for example, described the ways in which fresh water invertebrates respond to predation, these include; crypsis, reduction in movement, the use of stressful or ephemeral habitats and evasion of capture once detected by unpalatability, evasive movements, weapons, group living or an unwieldy shape to prevent consumption. In the Odonata predator avoidance is primarily by crypsis (Corbet, 1962) or the use of refuges (Wellborn and Robinson, 1987). However effective crypsis requires reduction in movement (Macan, 1977; Sih, 1987) and this is incompatible with some types of respiratory adaptation such as rapid ventilation of the body or movement to areas of higher oxygen concentration (3.4.1). Thus cryptic species would be expected to increase their respiratory efficiency by adaptations such as gills and metabolic adaptation rather than ventilation (3.4.). There are exceptions to this, such as

the larval anisopterans that are cryptic and have rectal gills and ventilation. In these species the gills are concealed within the hind-gut and therefore ventilation can be carried out with little movement.

The study of trichopteran respiratory adaptation and predator evasion by Williams et al., (1987) provides an indication of how complex their evolution can be. Williams et al., (1987) found a significant trend in the respiratory adaptation of the case of trichopteran larvae. They suggested that in these species adaptation of the case from a defensive one to a respiratory one occurred in response to colonisation of lentic environments from the ancestral lotic ones. Similar trends may be occur in the Zygoptera depending on whether species evolved in habitats with predators at high environmental oxygen concentrations. Thus although the lamellae of P. nymphula have no respiratory adaptation it is possible that species from different habitats with, for example, different oxygen levels and predation pressures, will have lamellae that are adapted as respiratory gills.

APPENDIX 1

Histological methods:- Light microscopy.

General procedure

1. Fix in Bouin's Fluid (Drury and Wallington, 1967) for 24 hrs.
2. Rinse in 70% alcohol (3 changes over 24 hrs).
3. Dehydrate through alcohol sequence to 100% ethanol ("AnalaR" grade of purity).
4. Clear with Histoclear.
5. Block in parrafin wax.
6. Section at 5 to 7 um.
7. Stain with Mallory's triple stain.

Mallory triple stain.

1. Histoclear (5 mins).
2. Absolute alcohol (5 mins).
3. Alcohol series (3 min changes) from absolute to Distilled water (rinse)
4. Mallory A (4 mins) and blot.
5. Transfer to Mallory B (15-18) mins and blot.
6. Transfer to 70% alcohol (15 secs)
7. Absolute alcohol (15 secs).
8. Histoclear (5 mins)
9. Mount in Histomount.

APPENDIX 2

Histological methods:- Scanning Electron Microscopy.

General procedure

1. Primary fixation using Gluteraldehyde/Cacodylate buffer.
2. Secondary fixation using Osmium tetroxide.
3. Dehydration through acetone sequence to AnalaR Acetone (30,50,70.90%)
4. Dry in critical point dryer (liquid CO2).
5. Gold coat.
6. Examine with S.E.M. 0.2M Sodium Cacodylate solution A 0.2M solution of sodium cacodylate buffered to pH 7.2 was prepared as follows:- 0.2M Sodium Cacodylate soln (pH 7.2):- 4.28g/100ml(0.2M HCL)

Fixing:- 25ml 0.2M Cacodylate soln

1ml 0.1% Calcium chloride.

22ml Distilled water

2ml 25% Gluteraldehyde

Rinsing:- 25ml 0.2M Cacodylate

25ml Distilled water

1g Sucrose

APPENDIX 3

Histological methods:- Scanning Electron Microscopy.

General procedure

1. Primary fixation using Gluteraldehyde/Cacodylate buffer (Protein stabilizer).
2. Secondary fixation using Osmium tetroxide (lipid stabilization.
3. Fix with heavy metal salt (Uranyl acetate)
4. Dehydrate through acetone sequence to "AnalaR" acetone (30,50,70.90%)
5. Embed in Epoxy resin.
6. Section using ultramicrotome (0.5 to 2.0 um/60 to 100 um.
7. Stain using Uranyl acetate and lead citrate.
8. Examine with Transmision Electron Microscope.

Primary and secondary fixation as for S.E.M. procedures, Appendix 2.

Appendix 4

Results from the closed bottle respirometry experiments.

Fig. A.4.1. Molar oxygen consumption rate (M.O.R.) and experimental dissolved oxygen concentration (D.O.) for Larvae with (●) and larvae without (▲) lamellae over 30mg fresh weight at $15^{O}C$.

Fig. A.4.2. Molar oxygen consumption rate (M.O.R.) and experimental dissolved oxygen concentration (D.O.) for larvae with (●) and Larvae without (▲) lamellae over 30mg fresh weight at $10^{O}C$.

Fig. A.4.3 Molar oxygen consumption rate (M.O.R.) and experimental dissolved oxygen concentration (D.O.) for larvae with (●) and larvae without (▲) lamellae over 30mg fresh weight at $5^{O}C$.

Fig. A.4.1.

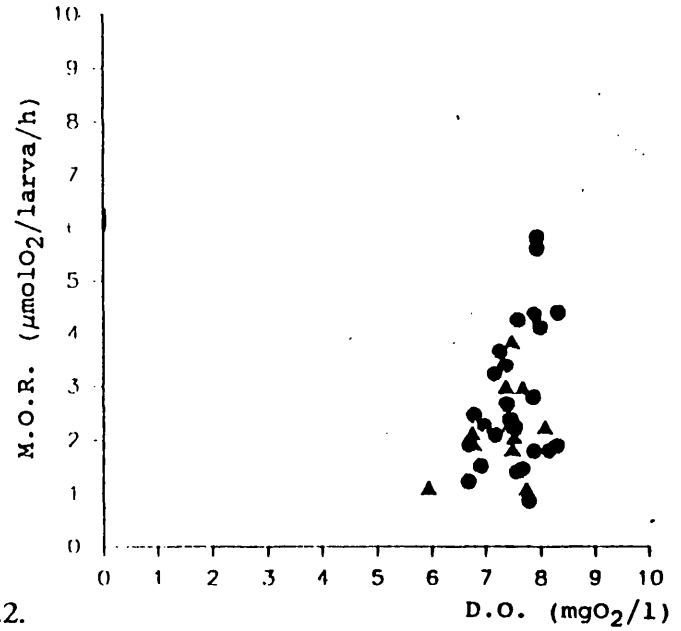


Fig. A.4.2.

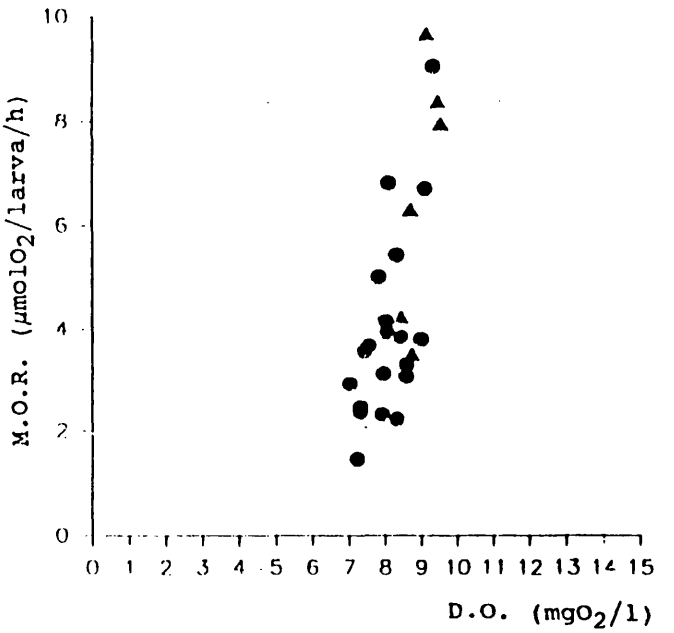


Fig. A.4.3

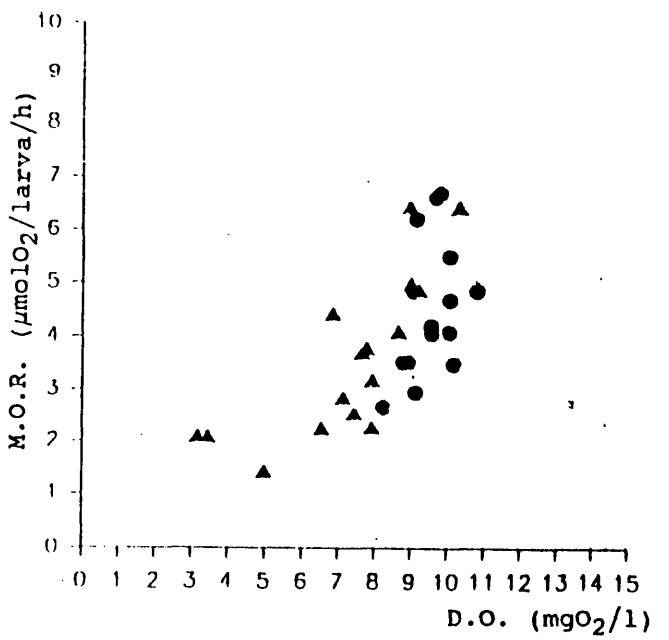


Fig. A.4.4. Log_{10} specific oxygen consumption rate (S.O.R.) of larvae with (●) and larvae without (▲) lamellae against Log_{10} fresh weight at a dissolved oxygen concentration of between 6.0 and 9.0 mgO_2/l and at a temperature of $15^{o}C$.

Fig. A.4.5 Log_{10} specific oxygen consumption rate (S.O.R.) of larvae with (●) and larvae without (▲) lamellae against Log_{10} fresh weight at a dissolved oxygen concentration of between 6.0 and 9.0 mgO_2/l and at a temperature of $10^{o}C$.

Fig. A.4.6 Log_{10} specific oxygen consumption rate (S.O.R.) of larvae with (●) and larvae without (▲) lamellae against Log_{10} fresh weight at a dissolved oxygen concentration of between 6.0 and 9.0 mgO_2/l and at a temperature of $5^{o}C$.

Fig. A.4.4.

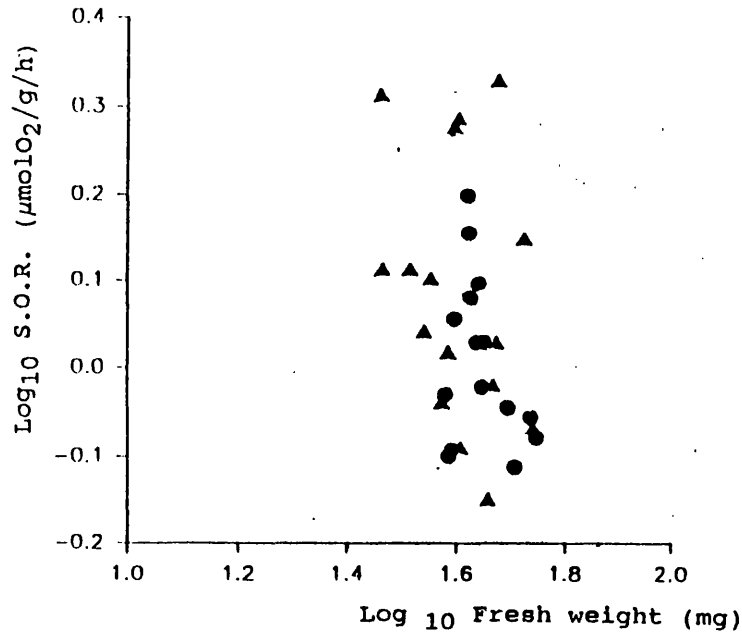


Fig. A.4.5

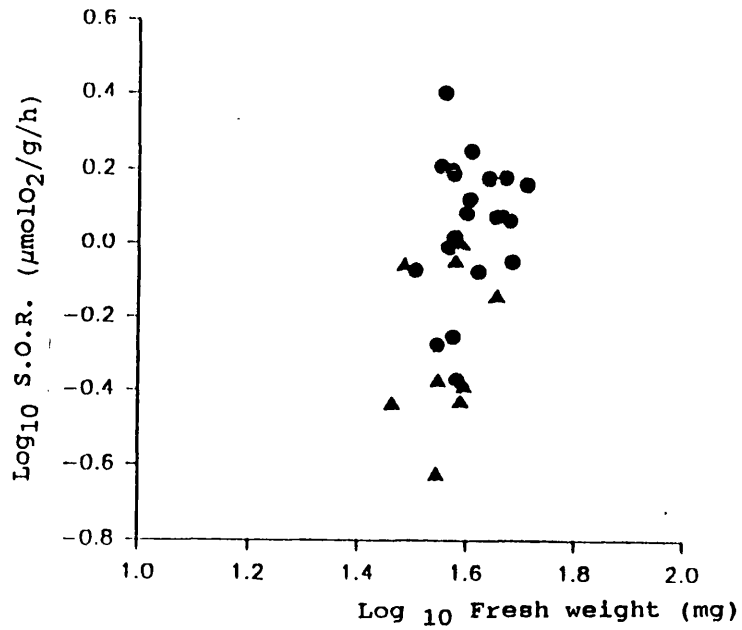


Fig. A.4.6

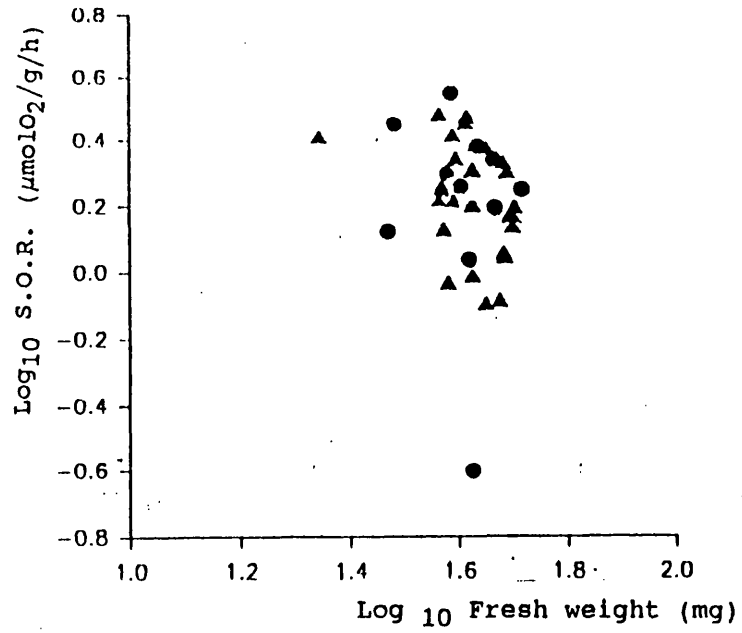


Fig. A.4.4. Log_{10} specific oxygen consumption rate (S.O.R.) of larvae with (●) and larvae without (▲) lamellae against Log_{10} fresh weight at a dissolved oxygen concentration of between 6.0 and 9.0 mgO_2/l and at a temperature of $15^{o}C$.

Fig. A.4.5 Log_{10} specific oxygen consumption rate (S.O.R.) of larvae with (●) and larvae without (▲) lamellae against Log_{10} fresh weight at a dissolved oxygen concentration of between 6.0 and 9.0 mgO_2/l and at a temperature of $10^{o}C$.

Fig. A.4.6 Log_{10} specific oxygen consumption rate (S.O.R.) of larvae with (●) and larvae without (▲) lamellae against Log_{10} fresh weight at a dissolved oxygen concentration of between 6.0 and 9.0 mgO_2/l and at a temperature of $5^{o}C$.

Fig. A.4.4.

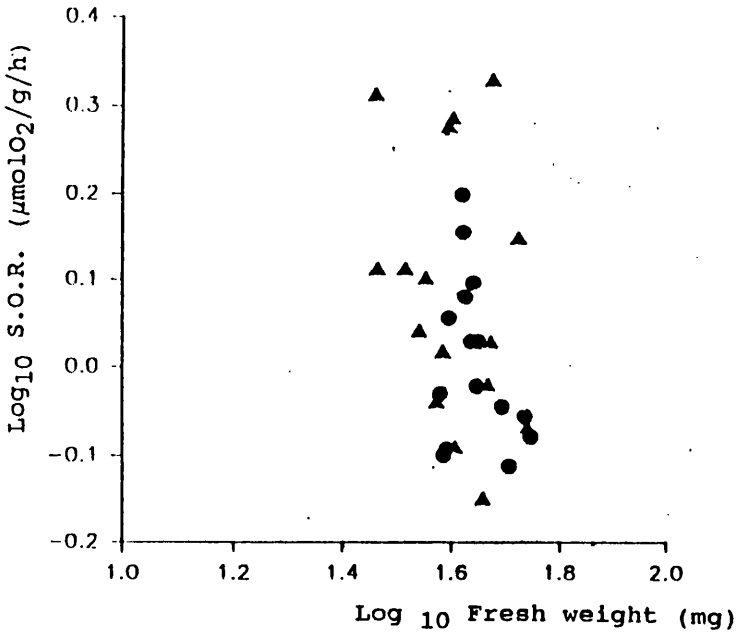


Fig. A.4.5

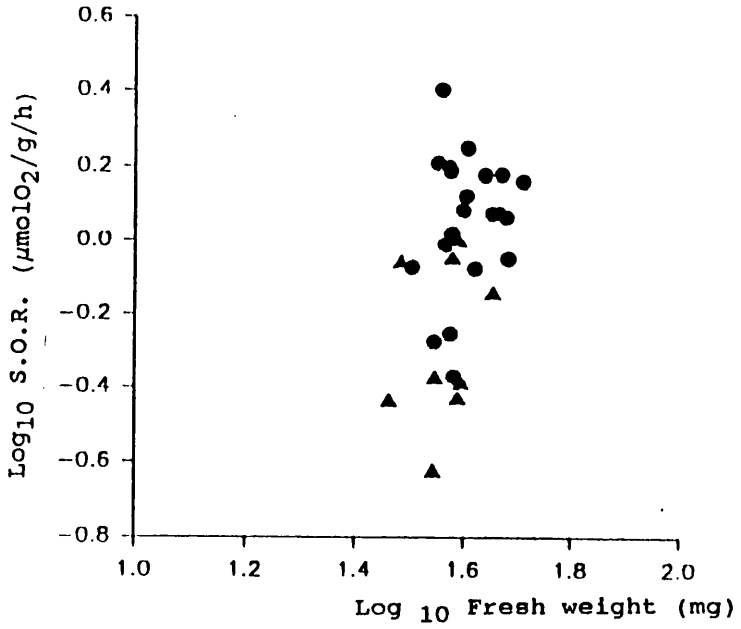
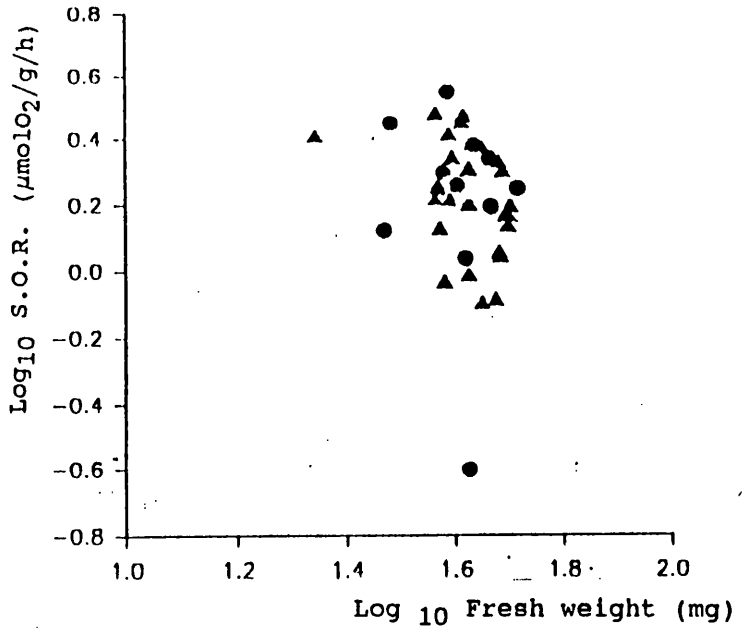


Fig. A.4.6



Appendix 5

Results of critical point analysis on Molar oxygen consumption rates (M.O.R.) using the technique of Yeager and Ultsch (1989).

Fig. A.5.1. Molar oxygen consumption rate (M.O.R.) and dissolved oxygen concentration (D.O.) for larvae with(L+, ●) and larvae without (L-, O) lamellae of less than 18mg fresh weight at 18^{O}C. Regression lines fitted from formulae in Table 4.2. These lines show respiration rates for larvae with (L+) and without (L-) lamellae calculated according to the two-segment line fitting method.

Fig. A.5.2. Molar oxygen consumption rate (M.O.R.) and dissolved oxygen concentration (D.O.) for larvae with (L+, ●) and larvae without (L-, O) lamellae between 18 and 30mg fresh weight at 18^{O}C. Regression lines fitted from formulae in Table 4.2. These lines show respiration rates for larvae with (L+) and without (L-) lamellae calculated according to the two-segment line fitting method.

Fig. A.5.1.

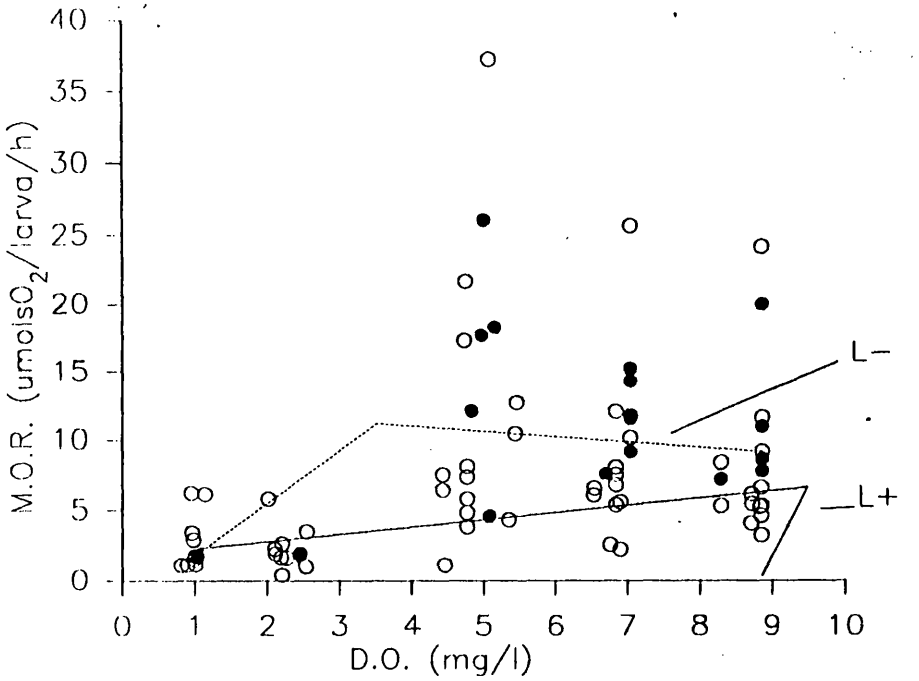


Fig. A.5.2.

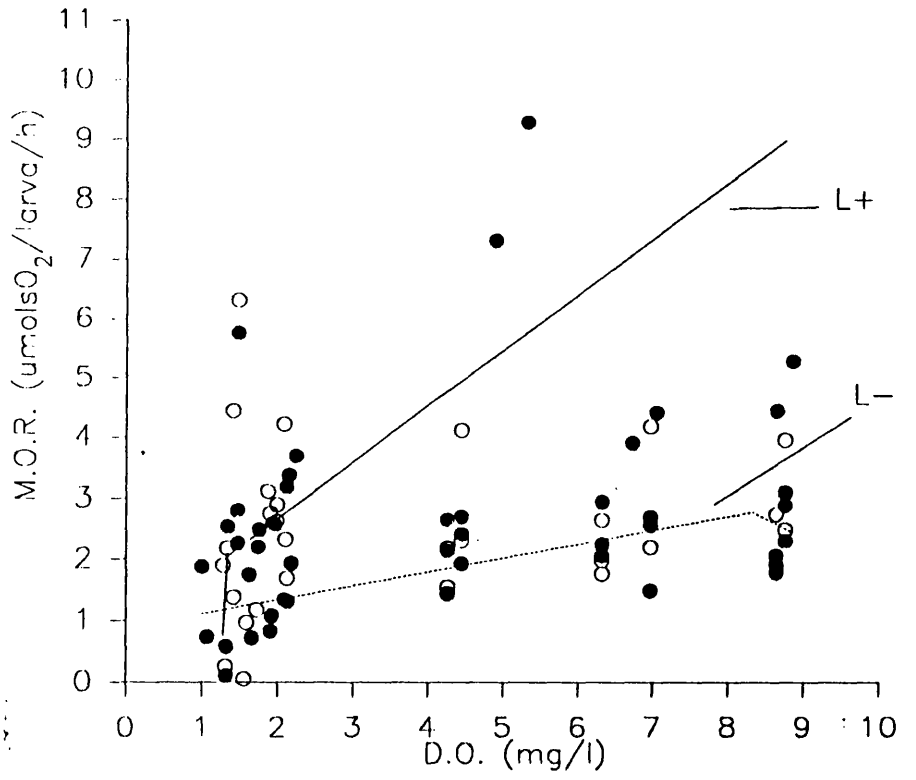
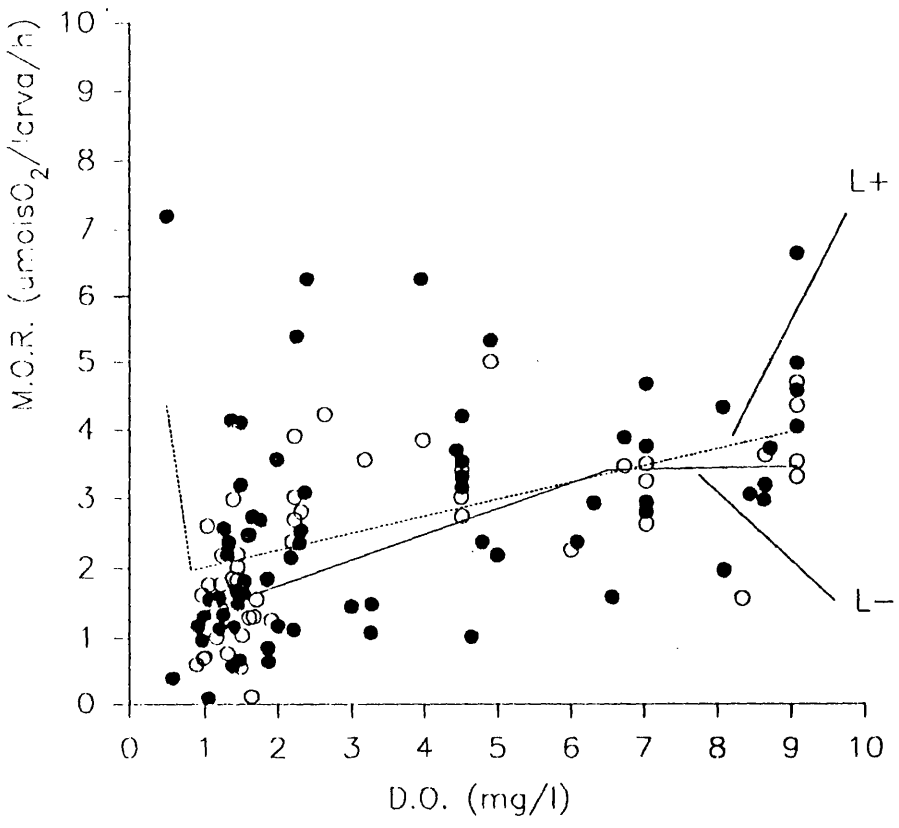


Fig. A.5.3. Molar oxygen consumption rate (M.O.R.) and dissolved oxygen concentration (D.O.) for larvae with (L+, ●) and larvae without (L-, O) P. nymphula larvae greater than 30mg fresh weight at 18°C. Regression lines fitted from formulae in table 4.2. These lines show respiration rates for larvae with (L+) and without (L-) lamellae calculated according to the two-segment line fitting method.

Fig. A.5.3.



Appendix 6

Repertoire of behaviour recorded in P. nymphula

The following list of categories and descriptions was used as a reference for recoding the displays of larvae in experimental trials over perch ownership. The list was compiled from video observations of contests between larvae. The nomenclature follows as far as possible that used by Harvey, (1985) and Harvey and Corbet (1986), where full descriptions of the behavioural repertoire of P. nymphula larvae are provided. Because of particular interest in the lamellae in the present study some of the descriptions have been adapted or added to where necessary. The main additions are descriptions of lamellar positioning during encounters.

1. *Resting.* : The same as the description used by Harvey and Corbet (1986). The head thorax and abdomen of the larva is held close to the perch. The resting position of lamellae is very slightly apart.

2. *Rest and Fan*: *Rest* (1) combined with fanning and spreading of the lamellae.

3. *Head up*: Head and thorax raised from the perch, abdomen either as in resting position or with the tip raised. The degree to which head and abdomen are elevated varied. This is possibly the same display as "the *raised* observed by Harvey and Corbet (1986) in which the body is held raised from the support. It is possible that the vertical orientation of the perch made the larvae raise only their heads.

4. *Head up and Fan*: Body held as in (3) and lamellae spread and fanned.

5. *Abdomen raised*: Head and thorax held raised and parallel to the perch or inclined slightly towards it. The abdomen held straight and elevated at an angle of greater than about 20^0 to the perch. The abdomen may also be extended. This is possibly the same behaviour as *raised* described by Harvey and Corbet (1986) but is more like the *Weak head down* display of Austrolestes psyche (Sant and New (1989)) or the *Abdomen raised* of Xanthocnemis zealandica (Rowe, 1985).

6. *Abdomen raise and Fan*: Body as in *Abdomen raised* (5) but lamellae fanned.

7. *Lateral display*: Abdomen swung round at an angle of 90^o to the body, lamellae slightly fanned. The same as *lateral display* described by Harvey and Corbet (1986). Similar also to the *Abdomen bend* display described in Xanthocnemis zealandica (Rowe, 1986).

8. *Tip raise*: Body in resting position, tip of abdomen only, bent upwards at an angle of between 45^o and 90^o. Similar to *Abdomen raise* but only the tip of the abdomen angled.

9. *Tip raise and fan*: Body as in *Tip raise* (8) but lamellae fanned.

10. *Static Caudal Swing* (SCS): Head and thorax as in the *resting* position. Abdomen extended and rigid and swung from side to side with low amplitude and high frequency wave. Lamellae fanned slightly. This act is described by Harvey and Corbet (1986) (but considered respiratory). Similar to the *rigid abdomen wave* of Coenagrion resolutum (Baker, 1981) and the *Static caudal swing* of Xanthocnemis zealandica (Rowe, 1985).

10. *Slow wave*: Head and thorax held slightly raised from the perch. A high amplitude, low frequency wave of the abdomen and lamellae. The same as *slow wave* described by Harvey and Corbet (1986). This display may incorporate other elements as on some occasions a distinctly sinuous movement of the body was observed, like the *S-bend* display observed in larval Xanthocnemis zealandica (Rowe, 1985).

11 *Lamellar display*: Body resting but lamellae fanned or raised and lowered individually. Similar to the *Semaphore* and *Caudal lamellar spread/close* observed in Xanthocnemis zealandica (Rowe, 1985).

12. *Swim*: Larva swimming

13. *Feed*: larva orientating towards and capturing prey.

14. *Fight*: Larva aggressively in contact with each other, using labial strikes as described by Harvey and Corbet (1986).

15. *Groom*: Larvae grooming body with legs.

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