Studies on the ecological energetics of damselfly larvae (Odonata: Zygoptera)

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Studies on the Ecological Energetics of Damselfly Larvae (Odonata: Zygoptera)

by

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"A lake ---- forms a little world within itself - a microcosm within which all the elemental forces are at work and the play of life goes on in full, but on so small a scale as to bring it easily within the mental grasp."

Stephen A. Forbes 1887.

"He ---- went and fought the dragon-flies of Paradise and vanquished them."

J. R. R. Tolkein.

"The Adventures of Tom Bombadil: 3, Errantry."

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SUMMARY

The study was the first to obtain a complete annual energy budget for a natural population of invertebrate carnivores. Larval populations of the large red damselfly Pyrrhosoma nymphula (Sulz.) (Odonata : Zygoptera) were studied at Brasside, adjacent to Durham City. Energy utilisation by individual larvae during complete development was estimated in two ponds designated B (surface area 93m^2) and F (surface area 13m^2), whilst total larval population energy flow was estimated only in pond B.

Mean monthly pond temperatures (measured using a sucrose inversion technique) ranged between 15°C in July to 2-3°C in December and January.

Faecal pellet analysis showed that Pyrrhosoma took a wide range of prey types. However, chironomid larvae made up 75 percent of the energy consumed. Throughout larval development, Pyrrhosoma obtained 85 percent of its energy from browsers, and 15 percent from other carnivore populations.

A restricted spring oviposition, and well synchronised larval growth allowed accurate estimation of growth rates (P) from field measurements. Exuvium production (Ev) was measured in the laboratory.

Percentage assimilation ($\frac{C-F}{C} \times 100$; estimated in the laboratory) decreased from 95 percent in small larvae to just over 85 percent in final instars. Percentage assimilation was unaffected by temperature and feeding rate; prey type also had a relatively small effect.

Respiration rates (R) were estimated at 5, 10 and 16°C using a Winkler technique, and a Cartesian Diver apparatus at 16°C for very small larvae. Respiration rates increased markedly during metamorphosis,

were relatively unaffected by decreased oxygen tension down to 50 percent saturation, and did not differ between the sexes. Pyrrhosoma showed no metabolic acclimatisation to temperature: between 5 and 10°C, Q_{10} was 2.20, whilst between 10 and 16°C, Q_{10} increased to 3.12.

Field consumption rates (C) were measured from estimates of gut contents and gut clearance times. Larval consumption between June 1967 and April 1968 estimated from P+R+Ev+F was 10.1 percent higher in pond B and 4.3 percent lower in pond F than the estimates based on gut clearance times. Close agreement of these independent estimates of C provided a useful check on the accuracy of many of the methods used during the study. Field feeding rates were never more than 70 percent and were usually less than 50 percent of potential maximum feeding rates for the same size of larvae at the same temperature.

Total consumption from hatching to emergence amounted to 189 calories per larva in pond B and 185 calories per larva in pond F. Of this, equal amounts (42 - 43 percent) were used for growth and respiration: 2.4 percent was lost as exuvia and 12.2 percent as faeces.

Annual population energy flow was measured for two consecutive years (July 1966 - June 1967 and July 1967 - June 1968) in pond B. Results were as follows:

	1966-67	1967-68
Mean biomass (K_{cals} per m^2)	0.93	1.42
R	3.17	3.67
P	3.94	3.59
Ev	0.50	0.30
F	0.86	0.99
C	8.47	8.55

All as K_{cals} per m^2 per annum.

Population growth efficiencies were high. Gross population growth efficiency ($\frac{P}{C}$ x 100) amounted to 46.5 percent and 42.0 percent in the two years. Net population growth efficiencies ($\frac{P}{A}$ x 100) were 51.8 and 47.5 percent respectively.

Annual population mortality losses in the two years of study amounted to 3.40 and 1.66 K_{cals} per m^2 per annum. Surviving biomass at the end of each study year was 0.39 and 0.29 K_{cals} per m^2 . Annual emergence amounted to 0.15 and 1.64 K_{cals} per m^2 per annum. These large differences were a result of very different population levels and mortality rates in the two years. Annual population energy flow, however, particularly population consumption, was remarkably stable in both years. It is suggested that this could have important consequences for attempts to understand the functional stability of population and ecosystems based on a study of population numbers alone.

Energy utilisation by the browsers at the base of the food chain "supporting" the Pyrrhosoma population was approximately 5 percent of net primary production within the pond. It was concluded that Pyrrhosoma had a minimal effect on the pattern of energy flow within the study pond.

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Chapter 1

INTRODUCTION

In formulating the laws of thermodynamics Kelvin suggested a possible exception in the case of living organisms. He was of course wrong and in applying these laws to animals, plants and their environment, ecologists have made use of a most powerful and useful concept. Ecologists, unlike physiologists, were comparatively slow in applying energy measurements to the complex systems studied by them. Paradoxically it is possible that this very complexity hindered the application of an approach which clarifies, simplifies and, above all, quantifies interactions within ecological systems. In an attempt to understand these complex interactions ecologists must measure and describe not only the structure but also the function of ecosystems (Odum 1959, 1962). Rates of energy transfer are a fundamental measure of ecosystem function.

Attempts have been made to formally identify the pathways of energy flow through biological ecosystems with the components of Gibbs free energy equation e.g. Brody (1945), Patten (1959) and Phillipson (1966) quoting Wiegert (1964). Wiegert (1968) has since convincingly argued that energy utilisation by individual organisms, by populations or by units of ecological organisation above the population level can be completely described by equations based on the first law of thermodynamics, without recourse to Gibbs free energy equation and the second law.

Biologically, it is convenient to consider energy exchange within a system under conditions of constant pressure. The energy exchange within an open system (i.e. one in which there is both matter and energy exchange)

may then be formally stated according to the first law of thermodynamics as:-

$$\Delta H_s = (H_1 - H_2) + (Q_1 - Q_2) + (W_1 - W_2) \qquad 1$$

where ΔH_s = overall change in enthalpy of the system or the change in heat content at constant pressure and temperature.

H_1 = enthalpy content of matter entering system

H_2 = enthalpy content of matter leaving system

Q_1 = heat energy entering system

Q_2 = heat energy leaving system

W_1 = work done on system by environment

W_2 = work output of system

Work exchange $(W_1 - W_2)$ is negligible in biological systems so that equation 1 may be rewritten as:-

$$\Delta H_s = (H_1 - H_2) + (Q_1 - Q_2) \qquad 2$$

The equation normally used to describe ecological energy transformations may be stated as follows. (The symbols are those recommended for use during the International Biological Programme and are taken from Ricker (1968). Definitions of these symbols are presented in Appendix 1 of the present work.)

$$C = P + R + F + U \qquad 3$$

or

$$P = C - (F + U) - R \qquad 4$$

The components of equation 4 may be equated with the thermodynamic

functions in 2 as follows:-

Production (P) is clearly equivalent to ΔH_s.

Consumption (C) is equivalent to H_1.

Faeces and Urine (F + U) energy losses are equivalent to H_2.

Q_1, the absorbtion of thermal heat energy, may be temporarily important in some organisms e.g. lizards or Lepidoptera, but on a long term basis is reradiated and can be considered as being equal to zero.

R, respiratory heat loss, in equation 4, is equivalent to Q_2 in equation 2.

Energy exchange within a biological system is clearly satisfactorily described by equations based on the first law of thermodynamics.

The historical derivation of the balanced energy equation for organisms may be outlined as follows. Ivlev (1939a, 1945) following Terroine and Wurmser (1922) first used the equation in the form:-

$$Q = Q' + Q_R + Q_t + Q_v + Q_w$$

where Q = the quantity of energy that an organism gets in its food

Q' = the energy used for growth

Q_R = the energy in the unutilized part of the food (faeces and excreta)

Q_t = the energy of primary heat

Q_v = the energy of external work

Q_w = the energy of internal work

Subsequent investigations (particularly Winberg 1956) have shown the concept of "primary heat" (Q_t) to be erroneous. The equation

therefore simplifies to:-

$$Q = Q' + Q_R + Q_V + Q_W$$

Q_V and Q_W may be combined to represent total metabolic work and therefore represents energy dissipated as heat. Davies (1964) and Davis and Warren (1968) summarise the main categories for which this maintenance energy is utilised, incorporated into a scheme of total energy flow through an organism.

Ideally, metabolic heat loss from an organism should be measured by direct calorimetry. Physiologists attempted this for poikilotherms as long ago as 1911 (e.g. Hill 1911, working on frogs, newts, earthworms and snakes). Recently Davies (1966) has applied the technique to Carassius, the gold fish, and Calvet and Prat (1963) have reviewed the literature. Ecological energetics work, however, has relied entirely on indirect calorimetric measurements of metabolic heat loss i.e. the measurement of respiratory rate.

The equation used by Ivlev, therefore, simplifies further to the basic

$$C = P + R + (F + U)$$

or $$Q = Q' + (Q_V + Qw) + Q_R$$

Although the conversion of oxygen intake or carbon dioxide output to metabolic heat loss has proved to be very satisfactory, it is not without error. Anaerobic respiration is an obvious example that has been assumed to be negligable in ecological energetics work. Wiegert (1968) has also pointed out two further sources of error in a growing

animal.

Some of the oxygen may be incorporated into the organic matter synthesised during growth: alternatively, oxygen may be released during this process. In the first case, oxygen uptake measurements will overestimate the energy released in the oxidation of foodstuffs and in the latter case the heat energy released will be underestimated.

Secondly, not all the energy released by oxidation of foodstuffs is dissipated as heat in the growing animal. During growth a portion of this energy is conserved as an increase in the chemical potential energy of the material synthesised. Therefore, in measuring both growth and respiration, part of the energy transfer is measured twice. Direct calorimetric measurement of heat loss does not give rise to this error.

The error arising from the "storage", in growth, of some of the potential energy released by oxidation of foodstuffs will be to some extent compensated for by a further error not discussed by Wiegert resulting from indirect rather than direct calorimetry. Digestion of macromolecules involves a small heat release (about 16.4 g calories per gram) which, since there is no process by which to couple this energy to do useful work, results in transfer of the heat to the environment (Morowitz 1968). This process remains entirely undetected by respiratory measurements of heat loss, but will clearly be measured by direct calorimetry.

The errors arising from the use of indirect calorimetry to measure metabolic heat losses are small and to some extent cancel out. For ecological purposes, the method appears to be entirely satisfactory, at

least until improvement in other ecological measurements (particularly population sampling) and in extrapolating from laboratory measurements to the field, justifies greater refinement.

Building on the pioneer work of Ivlev (1939a, b, 1945), Transeau (1926) and above all Lindeman (1942), the study of ecological energetics may be conveniently divided into several broad categories. (Both Engelmann 1966 and Phillipson 1966 have recently provided extensive reviews of most of the literature up to this date. Emphasis has therefore been placed on studies not dealt with by them.) The period between 1950 and the early 1960's may be termed the "whole ecosystem" era, and as such typified by the studies of Odum and Odum (1955), Odum (1957) and Teal (1957, 1962). The works of Sitaromaiah (1967) on total energy flow through a tropical fresh water community and of Tilly (1968) on a temperate spring provide later examples of this approach. Kozlovsky (1968) has reviewed a number of whole ecolsystem studies in his analysis of various energy transfer efficiencies within ecosystems.

Since the early 1960's most ecological energetics work has been concerned with a second approach; the study of "key species". Typical examples are provided by Engelmann (1961), Mann (1965), Phillipson (1962, 1963) and Wiegert (1964, 1965). More recent studies are those of Fitzpatrick (1968) on the aquatic isopod Asellus, Healey (1967) on the collembolan Onychiurus, Qasrawi (1966) on the grasshopper Chorthippus, Saito (1965) on the isopod Ligidium, Saito (1967) on the diplopod Japonaria and Woodland (1967) on the crayfish Cherax.

The "key species" approach concentrates on the measurement of energy

flow through populations of single species, chosen (usually on subjective grounds) because they appear to be the most "important" (key) organisms within the ecosystem being studied. The "key species" approach has a number of advantages over the whole ecosystem approach, not least because it permits greater precision of measurement. Although comparison of energy flow data between single key species populations are of great interest in themselves, the ultimate aim of key species studies must be to understand the interaction of each species "in context" (that is within the whole ecosystem) and therefore to build up a picture of total community energy flow. No ecosystem has yet been studied in sufficient detail for this latter aim to be achieved.

During the late 1950's and early 1960's, a separate approach to ecological energetics problems also developed, based entirely on laboratory experiments and laboratory ecosystems e.g. Richman (1958) and Slobodkin (1959, 1964). Recently, Brocksen et al. (1968) have carried out extensive experiments using artifical stream communities. Although it is sometimes difficult to extrapolate from the laboratory to more complex field situations, the approach has much to commend it in that most of the variables are under direct experimental control.

Despite the increasingly large number of studies on ecological energetics, many more studies are required on which to base future methodological and conceptual developments. The present study was a logical extension of earlier key species work and attempted to provide a complete and detailed study of total annual energy flow through an invertebrate carnivor population. Previously, only partial field energy budgets

have been obtained for invertebrate carnivores e.g. Itô (1964) on the spider Lycosa, Paine (1965) on the mollusc Navanax and Phillipson (1960a, b, 1962, 1963) on phalangids. Alternatively, all work has been carried out entirely in the laboratory e.g. Berezina (1959) for the dragonfly larva Aeshna, Brocksen et al. (1968) on the stonefly larva Acroneuria and Fischer (1966, 1967a) on larvae of the damselfly Lestes.

Most ecological energetics studies were based on only one years field data. Very few attempted to measure population energy flow for longer than one year. Those that have included Qasrawi (1966) for the grasshopper Chorthippus, Saito (1967) for the diplopod Japonaria and Wiegert (1964, 1965) for Philaenus (a spittlebug) and Melanoplus (a grasshopper). In order to understand the functional stability of ecosystems and their component species populations, it is clearly of great importance to extend ecological energetics measurements over periods greater than one year. Therefore, in the present study, population energy flow measurements were made for two consecutive years.

Despite the fact that nearly all earlier ecological energetics studies of whole ecosystems were of aquatic habitats, later key species studies have concentrated on terrestrial systems. Only those of Fitzpatrick (1968) on the isopod Asellus, Kuenzler (1961) on the mollusc Modiolus, Mann (1965) on several fish species in the River Thames and Woodland (1967) on the crayfish Cherax have been on aquatic poikilotherms. (It is interesting to observe, however, that all the laboratory experimental studies referred to above e.g. Slobodkin, Richman and Brocksen et al. have been on aquatic systems.) The need to provide energetics data

on field populations of aquatic invertebrates for comparison with the terrestrial work is obvious and the present study was designed to correct, at least partially, the imbalance between aquatic and terrestrial key species work.

To achieve the above mentioned objectives, dragonfly (Odonata) larvae were considered to be extremely suitable experimental animals. As a group, they have a number of advantages. Their general biology, particularly in temperate regions, is well known (see Corbet et al. 1960 and Corbet 1962) ensuring a sound basis on which ecological energetics data could be superimposed. The number of species occurring in Britain is small (44) and the identification of the adjults and all but the smallest larvae easy (Gardner 1954, Longfield 1949).

The species chosen for study was the large red damselfly Pyrrhosoma nymphula (Sulz) (Odonata: Zygoptera). Pyrrhosoma nymphula is one of the three commonest Odonata of the British Isles, if not the commonest (Corbet et al. 1960, Lucas 1930) and occurred in large numbers at several localities near Durham City. It could therefore be reasonably regarded as a key species. The general biology and life history of Pyrrhosoma nymphula has been the subject of a number of studies by Corbet (1952, 1957b), Gardner and MacNeill (1950) and Macan (1964).

Several workers have commented on the apparent importance of Odonata larvae in aquatic ecosystems (Kennedy 1950, Needham 1949, Wright 1943, 1946); such opinions were largely subjective. Thus an additional aim of the present study was the elucidation of the importance and functional role of Odonata larvae in aquatic habitats, particularly in terms of their

contribution to total community energy flow. Although Pyrrhosoma nymphula was not the only species of Odonata larvae present in the study ponds (see Appendix 2), it was numerically the most abundant and it was felt that conclusions based on a study of Pyrrhosoma nymphula would provide some indication of the ecological importance of Odonata larvae in the ponds.

Energy budgets for individual Pyrrhosoma nymphula larvae from hatching to emergence were estimated in two separate ponds but the population energy budget was measured in only one of these. Growth (P) was estimated from field measurements. Respiration (R) and Exuvium production (Ev) were estimated on the basis of laboratory studies. Summing P + R + Ev and using laboratory measurements of percentage assimilation gave an estimate of food consumption (C) in the field. This was checked by an independent estimate of field feeding rate based on a combination of laboratory and field measurements. Comparison of the two estimates of consumption gave a check on the accuracy of the methods employed in measuring all the components of the energy budget. Detailed studies of larval food and the position occupied in the pond food web were also carried out.

During the work, information was obtained that was more relevant to general odonatan biology than to ecolgical energetics. The emphasis of the study was on the latter rather than the former and, although aspects of general dragonfly biology have been discussed, they have not been dealt with in great detail.

Each chapter contains a detailed discussion of its contents and consequently, the final chapter of discussion offered (chapter 15) is relatively short.

Chapter 2

PYRRHOSOMA NYMPHULA

2.1 GENERAL

Pyrrhosoma nymphula (Sulz.) is the only member of the genus to occur in the British Isles and it has therefore been referred to throughout the study by its generic name only.

The life history of Pyrrhosoma has been studied in detail by both Corbet (1957b) and Macan (1964). According to Corbet (1957b, 1962) it is a typical spring emergence species with a well synchronised adult emergence taking place in May and June. Typically the life history takes two years to complete. Nondiapause eggs are laid shortly after emergence and hatch without delay in July. The larvae grow rapidly until November and spend their first winter in about the sixth instar: they resume growth in April and enter the final instar the following October. After spending the second winter as final instars they enter metamorphosis in March and April and, shortly after, emerge. The populations in the present study showed this typical life history.

Throughout the study, the terms year class and junior and senior age class have been used. Since larvae take two years to complete development, two generations or year classes are present in the pond at any one time. Year classes are defined by the year in which the larvae hatched e.g. the 1966 year class hatched in July 1966 from eggs laid by the 1964 year class. In their first year of life, larvae are referred to as the junior age class and in their second year as the senior age class.

2.2 IDENTIFICATION

Pyrrhosoma is one of the easiest zygopteran larvae to identify, although newly hatched individuals present some difficulty. Corbet (1957b) believed that identification was possible only from instar 4, but with practice it was found to be quite feasible to identify both instar 2 (the first free living stage) and instar 3 with certainty. This was facilitated by the restricted range of other species present in the study ponds.

Identification of Instar 2 Larvae

For the purpose of the present study, it was sufficient to be able to distinguish newly hatched Pyrrhosoma larvae from other members of the family Coenagriidae present in the study ponds i.e. Coenargrion puella L., Enallagma cyathigerum (Charp.) and Ishnura elegans (van der. Lind.); the newly hatched larvae of which proved indistinguishable. In view of the latter situation it was considered adequate to determine a means of separating Pyrrhosoma from the others by using Coenagrion puella as a standard.

Plate 1 shows instar 2 larvae of Pyrrhosoma and Coenagrion hatched in the laboratory from eggs laid in the field by identified females: the magnification of the two photographs is identical.

Table 1 shows larval sizes as measured by a micrometer eye-piece.

	Length (mm) without lamellae	Length of lamellae (mm)	Total length (mm)	Head width (mm)
Pyrrhosoma	1.24 (1.29 ± 0.07)	0.96	2.20	0.400 (0.400 ± 0.009)
Coenagrion	1.40 (1.46 ± 0.09)	1.19	2.59	0.379 (0.380 ± 0.005)

Table 1 Measurements of newly hatched instar 2 larvae shown in plate 1. Also shown in brackets are means (± 2 S.E.) for 20 instar 2 larvae of each species.

The sizes of these two larvae were typical of a long series measured in July 1966. At this stage, Pyrrhosoma showed a tendency, very marked in later life, to be shorter, more "dumpy" than Coenagrion with a proportionally wider head and shorter body and lamellae. Though not measured, the legs were observed to be proportionally shorter.

Two other features distinguish Pyrrhosoma in instar 2.

i) The caudal lamellae are colourless throughout their length in Pyrrhosoma whilst those of Coenagrion bear bands of pigment about half way down.

ii) Pyrrhosoma larvae have two obvious spines at the back of the head a short distance behind the eyes. These post ocular spines are absent in Coenagrion.

Identification of Instar 3 Larvae

The general body shape, particularly in the squaring up of the head,

resembles that of the larger instars, so that the larvae are readily identifiable on head and body shape alone. Plate 2 shows an instar 3 Pyrrhosoma larvae with an enlarged view of the head of the same individual. This particular animal measured 1.68 mm excluding the lamellae and had a head width of 0.536 mm which was demonstrated to be typical of instar 3 larvae (mean length 1.66 ± 0.05, mean head width 0.524 ± 0.016). Other points used in recognition were:-

i) The post ocular spines which increased to three on each side.

ii) The lamellae which were in general colourless, though in some individuals examined traces of dark pigment were present in a band slightly less than half way from the base of the lamellae.

Later Instars (Instar 4 onwards)

The dark pigmentation which characterises the lamellae of all larger Pyrrhosoma larvae was strongly developed in the 4th instar, when the head shape took on the completely square shape seen in later stages. From this stage, appearance changed little and identification was easy in all stages.

2.3 SIZE AND WEIGHT IN PYRRHOSOMA

Throughout the study, length or weight were used as measured of larval size. A comparatively small larval population set a definite limit on the number of experiments where dry weight could be measured directly, since this involved killing the larvae. Relationships between length, wet weight and dry weight were therefore determined and used to

convert all subsequent measurements in terms of length or wet weight into dry weights.

Standard Weighing And Measuring Procedures Used Throughout The Study

The presence of food in the gut had no effect on length, but had an appreciable effect on both wet and dry weights. Length measurements were made at any time during or after feeding but all weighings, either on larvae collected from the field or at the completion of an experiment, were only made after sufficient time had elapsed for the guts to be cleared. The time taken for complete gut clearance depended on larval size: less than 12 hours was sufficient for small instars but over 24 hours was required by final instars at 15°C (see chapter 12).

Length Measurements

All length measurements were made from the anterior margin of the head to the tip of the last abdominal segment and did not include the caudal lamellae. Larvae below 5 mm long were measured in a drop of water on a well slide using a micrometer eye-piece (1 division = 0.052mm). Larvae above 5 mm long were placed in water in a watch glass and measured with callipers under a low-power binocular microscope.

Weight Measurements

Three different balances were used:-

i) A Cahn Electrobalance: model M-10 Cahn Instrument Company, Paramount, California (range 0.001 to 100 mg). The majority of wet and dry weight determinations were made with this instrument.

ii) A Mettler Balance: model H 16, Mettler, Zurich (minimum detectable weight 0.05 mg), was used for wet weight measurements of larger larvae.

iii) An Electromicrobalance: model EMB-1, Research and Industrial Instruments Company, London (range 0.001 to 200 mg), was employed in some dry weight measurements and a few wet weight determinations.

In making wet weight determinations, larvae were removed from the water, blotted on filter paper to remove excess surface moisture and weighed in an open pan. Even very small larvae appeared to suffer no ill effects from this treatment when returned to the water. As in most wet weight determinations of aquatic animals, Pyrrhosoma larvae continued to lose water steadily by evaporation during the actual weighing, but by carefully standardising the procedure this introduced a negligible error and a more elaborate procedure (e.g. Sugden 1967) was not considered necessary.

For dry weights, larvae were dried in a vacuum oven at 60°C until a constant weight was achieved. They were stored in a desiccator over anhydrous calcium chloride and self indicating silica gel., until they were weighed.

The Relationship Between Length And Wet Weight

i) All Instars Excluding Final Instars

The relationship between length (mm) and wet weight (mg) based on data from 233 Pyrrhosoma larvae ranging in size from newly hatched to penultimate instars is shown in figure 1. All larvae were measured

and weighed as soon as possible after collection from the field.

The relationship is a straight line on a log: log plot and the regression has been calculated from the product moment correlation coefficient. For the purpose of this calculation, wet weight values were multiplied by 100 to eliminate negative logs. The equation of the regression is:-

$$\underline{y = 2.852x + 0.475}$$

$$\underline{r = 0.9966}$$

where $y = \log (\text{wet weight} \times 100)$

$x = \log \text{length}$

ii) Final Instars

Data were analysed separately for final instars in order to give a more detailed picture of weight changes in this interesting and important stage in the life cycle.

The relationship between length (mm) and mean wet weight (mg) of final instars is shown in figure 2. The means are based on 199 larvae measured during the two winters 1966-67 and 1967-68; collections and measurements were made in every month between October, when Pyrrhosoma enters the final instar, and April, prior to entering metamorphosis. Data on larvae that had entered metamorphosis are included in section 2.6 below.

The results show a steady increase in wet weight with increasing length, no distinction being made in the graph between the sexes which were equally represented in each size category. However, in four size

classes, there was enough data to show a statistically significant difference in wet weights between the sexes, female larvae being slightly heavier on average than males of the same length. The analysis was carried out using Fischer's t test and the results are summarised in table 2.

The data show that female final instars are slightly heavier than males of a similar length. This difference is not due to the presence of eggs, for like most Odonata, no maturation of eggs appears to take place whilst Pyrrhosoma is still in the final instar (Corbet 1962). The difference may be related, however, to the greater size differential shown by mature adults.

Length (mm)	n	Mean wet weight (mg)	I.S.E.	p	
13.00♂ 13.00♀	26 26	46.14 49.43	0.667 0.680	0.01-0.001	Highly significant
13.50♂ 13.50♀	12 11	49.24 52.75	0.999 1.553	0.1-0.05	Not significant
13.75♂ 13.75♀	12 11	49.37 52.67	1.013 0.606	0.05-0.02	significant
14.00♂ 14.00♀	14 18	52.47 56.99	1.304 1.506	0.01-0.001	Highly significant

Table 2 Comparison between mean wet weights of male and female final instar Pyrrhosoma.

In absolute terms, the wet weight difference between the sexes is

slight and in calculations of wet weights of final instars in the field could be effectively ignored.

In order to convert length measurements of final instars into mean wet weight values a regression was calculated for the first seven points shown in figure 2 (larvae up to 13.75 mm long). The equation is:-

$$y = 4.482x - 10.545$$

$$r = 0.847$$

where y = mean wet weight (mg)

x = mean length (mm)

The wet weights of larger larvae, for which the relationship between length and wet weights was not linear, were read directly from the graph.

The Relationship Between Length and Dry Weight

Data from final instars was analysed separately from all other instars.

i) All Instars Excluding Final Instars

Figure 1 shows the relationship between length (mm) and dry weight (mg) based on data from 199 Pyrrhosoma ranging in size from newly hatched to penultimate instars. All larvae were measured as soon as possible after collection.

Two regressions were fitted to the data. For the purpose of the regression calculations, the dry weight values were multiplied by 100 to eliminate negative logs. The equations of the regressions are:-

For larvae $<$ 2.75 mm in length (n = 63)

$$y = 3.002x - 0.306$$

$$r = 0.9683$$

For larvae $>$ 2.75 mm in length (n = 136)

$$y = 2.734x - 0.184$$

$$r = 0.9917$$

Where y = log (dry weight x 100)

x = log length

ii) Final Instars

Figure 2 shows the relationship between length (mm) and mean dry weight (mg) in the final instar. The means are based on 79 larvae measured during the two winters 1966-67 and 1967-68, collection and measurements being made in every month from October to April inclusive, with the exception of November and January. As in the data for wet weights, no metamorphosing larvae have been included in this analysis but are considered in section 2.6 below.

No attempt to show any sexual difference in dry weights was made, the number of samples in each size category being insufficient. However, the sexes are approximately equally represented in each size group and any error introduced in assuming a similar mean dry weight for males and females will be negligible.

To convert length measurements of final instars into mean dry weight values, a regression was calculated for the nine points shown in figure 2. The equation for the line is:-

$$y = 2.904x - 29.845$$

$$r = 0.3799$$

where y = mean dry weight (mg)

x = mean length (mm)

2.4 INSTAR NUMBER IN PYRRHOSOMA NYMPHULA

The Pronymph

It seems probable that all Zygoptera larvae will ultimately be shown to possess a pronymph larval stage (Corbet et al. 1960) but it has not yet been reported in Pyrrhosoma. In most Odonata it is of extremely short duration and at the most lasts only for a matter of hours (Corbet 1962), although in Pyrrhosoma it probably lasts for only a few seconds (Gardner and MacNeill 1950). During the present study, a pronymph stage was not observed despite careful daily examination of eggs hatching in the laboratory. Since pronymphs do not feed and are of such short duration, they can be effectively ignored in energy budget calculations.

The pronymph is considered a true larval stage and in the present study, following Corbet (1962), it has been designated as instar 1. Not all authors have followed this procedure, both Balfour -Browne (1909) and Kormondy (1959) for example calling the first active feeding stage to which the pronymph moults instar 1. On the basis of the classification used here, this stage is termed instar 2.

Determination of Instar Number

A large series of head width measurements was available from monthly

population samples taken between July 1966 and May 1968. This information was used in the determination of the average number of instars shown by Pyrrhosoma during its development.

Head width measurements were made within 48 hours of collection (using a micrometer eye-piece). Figure 3a shows the results expressed in the form of the head width: frequency histogram. Size categories of 0.05 mm were used for small larvae (head widths between 0.35 and 1.20 mm) and 0.10 mm size categories for larger larvae (head widths > 1.20 mm). Larger size categories were used initially but without the very fine division of head widths, instar recognition was found to be impossible. This is probably why Chutter (1961), using comparatively large 0.3 mm head width categories, failed to distinguish most of the instars of the South African damselfly Pseudagrion.

In figure 3a, eleven modes corresponding to eleven instars can be distinguished. More exact analysis of the data could have been carried out using probability paper, but the additional information so gained was not thought to justify the labour involved. Instead, a check on the interpretation of the results was made by plotting the log of the 11 modal head width values taken from the head width: frequency graph, against the instar number to which they probably correspond (see figure 3b). A linear relationship clearly holds thereby supporting the interpretation that each mode corresponds to a separate instar with head width increasing by a constant factor at each moult. In many Arthropod, size increases by a factor of 1.26 at each moult (the well known Brooks-Przibram Growth Factor) and the mean constant increase in size between

the modal head widths designated in figure 3a was found to be 1.25. Several authors obtained data on the Brooks-Przibram growth factor in Odonata: both Calvert (1929) and Kormondy (1959) review the literature. All the reported values lie close to the figure of 1.25 found in the present study. From the evidence it is reasonable to suggest that typically Pyrrhosoma passes through 11 instars after moulting from the pronymph and therefore has twelve larval stages in all.

Normally, Odonata possess between 9 and 14 instars (Corbet 1962) and clearly the situation in Pyrrhosoma was similar to that found in other species. An individual male Pyrrhosoma, which developed from egg to imago in the laboratory, passed through 12 instars (Gardner and MacNeill 1950), identical to the number found in the present study. However, Backhoff (1910), in Calvert (1929) reported only 9 instars in Pyrrhosoma and although this probably did not include the pronymph, it suggests that instar number may vary. A similar conclusion was drawn by Calvert (1934) for instar number and the accompanying changes in structure in Anax junius and by Kormondy (1959) who found variation in instar number in Tetragoneuria spp.

Application of Instar Number

The number of moults is important in the determination of the energy lost with the exuvia. The data on instar number was therefore utilised in the appropriate section on exuvium production (see chapter 11).

Occasionally instar number was used as a convenient description of the approximate development stage under consideration, but all experimental and population data were collected using weight or length measure-

ments of larvae. The obvious exception to this was instar 12, the final instar, where larvae with a head width below 3.5 mm were never found and were therefore quite distinct from the penultimate instar 11, the largest head width in this instar being 3.3 mm. Throughout the study, final instars were identified and referred to as such, this being the only case where instar number was used to indicate a definite developmental stage reached by the larvae.

2.5 FINAL INSTAR DIAPAUSE

Corbet (1956, 1957b, 1962) considered Pyrrhosoma to be a typical spring emergence dragonfly and as such it might be expected to possess a diapause in the final instar. Typically diapause results in a considerable reduction in metabolic rate on a unit weight basis (e.g. Clarke 1967, Keister and Buck 1964) and it was clearly important to determine the duration of the diapause stage in Pyrrhosoma and to elucidate its effects on metabolic rate. Consequently, final instar respiration rates, maximum feeding rates and percentage assimilation were measured monthly from October on entering the final instar until March or April prior to entering normal metamorphosis in the field. These experiments are reported in chapters 9, 10 and 12. Experiments to determine the duration and the nature of the diapause are reported in chapter 13.

2.6 METAMORPHOSIS

Pyrrhosoma passes through a series of clearly recognisable stages during metamorphosis; these have not been described previously and are

reported here. Observations were made on the morphological changes shown by over 30 larvae in the laboratory during metamorphosis and it was from these data that the three stages were distinguished. They are as follows:-

Stage 1 No Metamorphosis

a) Wing cases overlap.

b) Eyes blackish on both upper and lower surface.

c) Labium with muscle bands clearly visible.

d) Upper surface of abdomen brown.

Stage 2 Early Metamorphosis

a) Wing cases swollen and parallel.

b) Lower surface of eyes usually greyish.

Otherwise as stage 1.

Stage 3 Completed Metamorphosis

a) Wing cases swollen and parallel.

b) Eyes - both upper and lower surface pale grey-brown or yellow-brown.

c) Labium completely clear with muscle atrophied and adult mouthparts visible at base.

d) Upper surface of abdomen frequently with reddish coloration.

Emergence follows fairly soon after entering stage 3 metamorphosis. The stages form a continuous series but, since intermediate stages were passed through rapidly, the majority of larvae could be assigned to a definite stage quite easily.

Weight Changes During Metamorphosis

Table 3 shows the mean wet and dry weights for stage 2 and stage 3

metamorphosis larvae. As with the wet weights for non-metamorphosing final instars, the tendency for females to be slightly heavier is obvious, though only in one case is the difference significant. Consequently, the data from both sexes in each stage have been pooled: this is unlikely to introduce any appreciable error in application to field conditions. The pooled data are presented in table 4.

A significant increase in wet weight and a decrease in dry weight is apparent between stages 2 and 3. Atrophy of the labial muscles prevents feeding in stage 3 metamorphosis (feeding continues until the end of stage 2), whilst the respiration rate during metamorphosis is exceptionally high (see chapter 10) so that a decrease in dry weight between stages 2 and 3 might be expected. The increase in wet weight is presumably due to the intake of water.

Meta-morphic stage	Wet or dry weight	Sex	n	Weight (mg)	1 S.E. (mg)	t	p
2	wet	♂ ♀	31 30	58.49 62.78	1.36 1.35	2.244	0.05-0.02
2	dry	♂ ♀	13 11	14.36 15.18	0.56 0.40	1.200	Not significant
3	wet	♂ ♀	16 14	63.28 67.03	1.48 2.02	0.418	Not significant
3	dry	♂ ♀	16 14	13.36 13.91	0.25 0.45	1.063	Not significant

Table 3 Mean wet and dry weights in stage 2 and stage 3 metamorphosing final instars of Pyrrhosoma; sexes analysed separately.

	Meta-morphic stage	n	Weight (mg)	1 S.E. (mg)	t	p
wet weight	2	61	60.60	0.99	2.793	0.01-0.001
	3	30	65.03	1.24		
dry weight	2	24	14.73	0.36	2.542	0.02-0.01
	3	30	13.62	0.06		

Table 4 Mean wet and dry weights in stage 2 and stage 3 metamorphosing final instars: both sexes pooled.

At the low temperatures prevailing in the pond in spring, larvae spent up to two months in metamorphosis (see chapter 6). Since this was an appreciable length of time, the possible effects of metamorphosis on respiration, assimilation and feeding were examined. Results of these experiments are discussed in chapters 9, 10 and 12.

Plate 1. Newly hatched (instar 2) larvae of *Pyrrhosoma nymphula* (upper) and *Coenagrion puella* (lower). X55 approx.

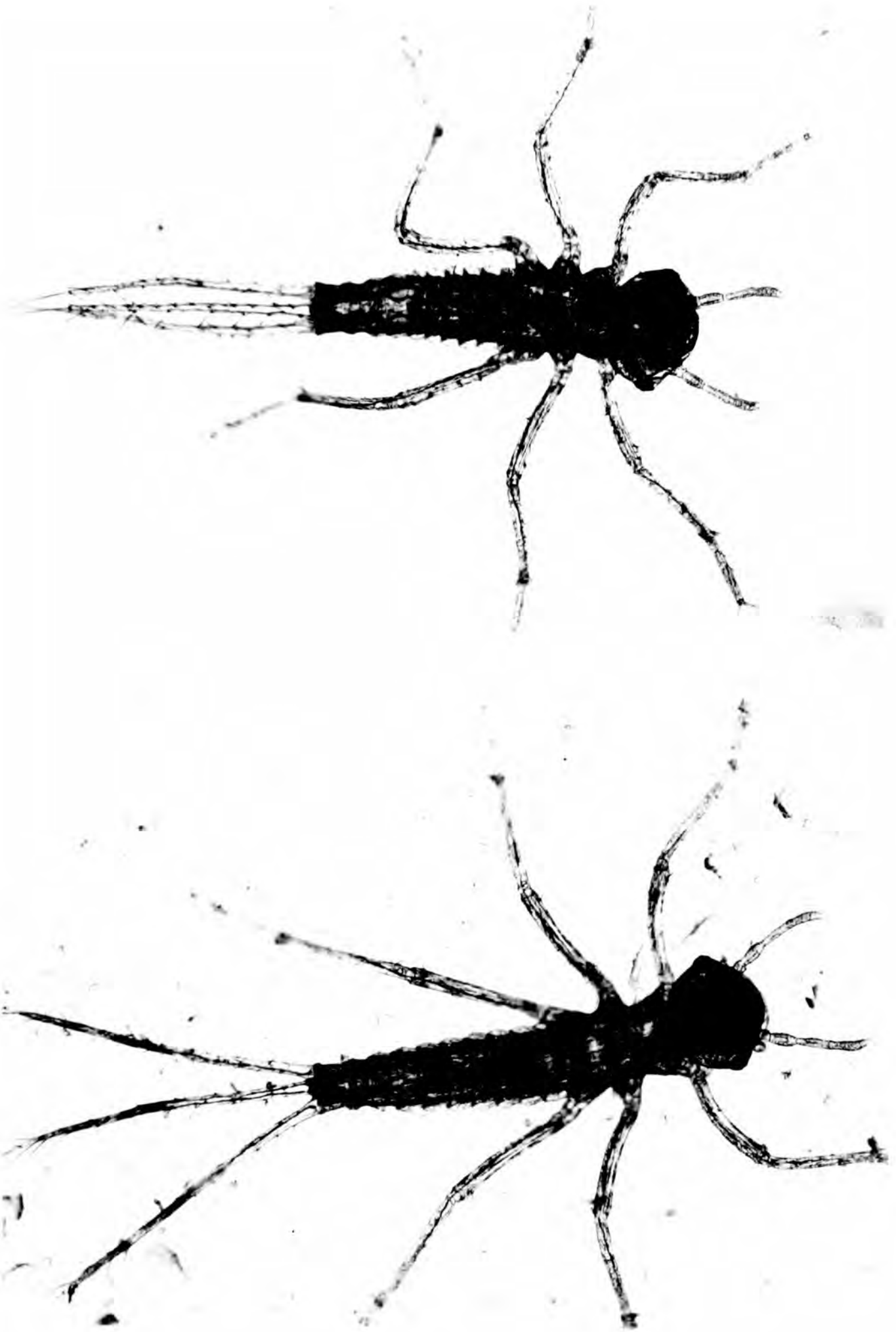
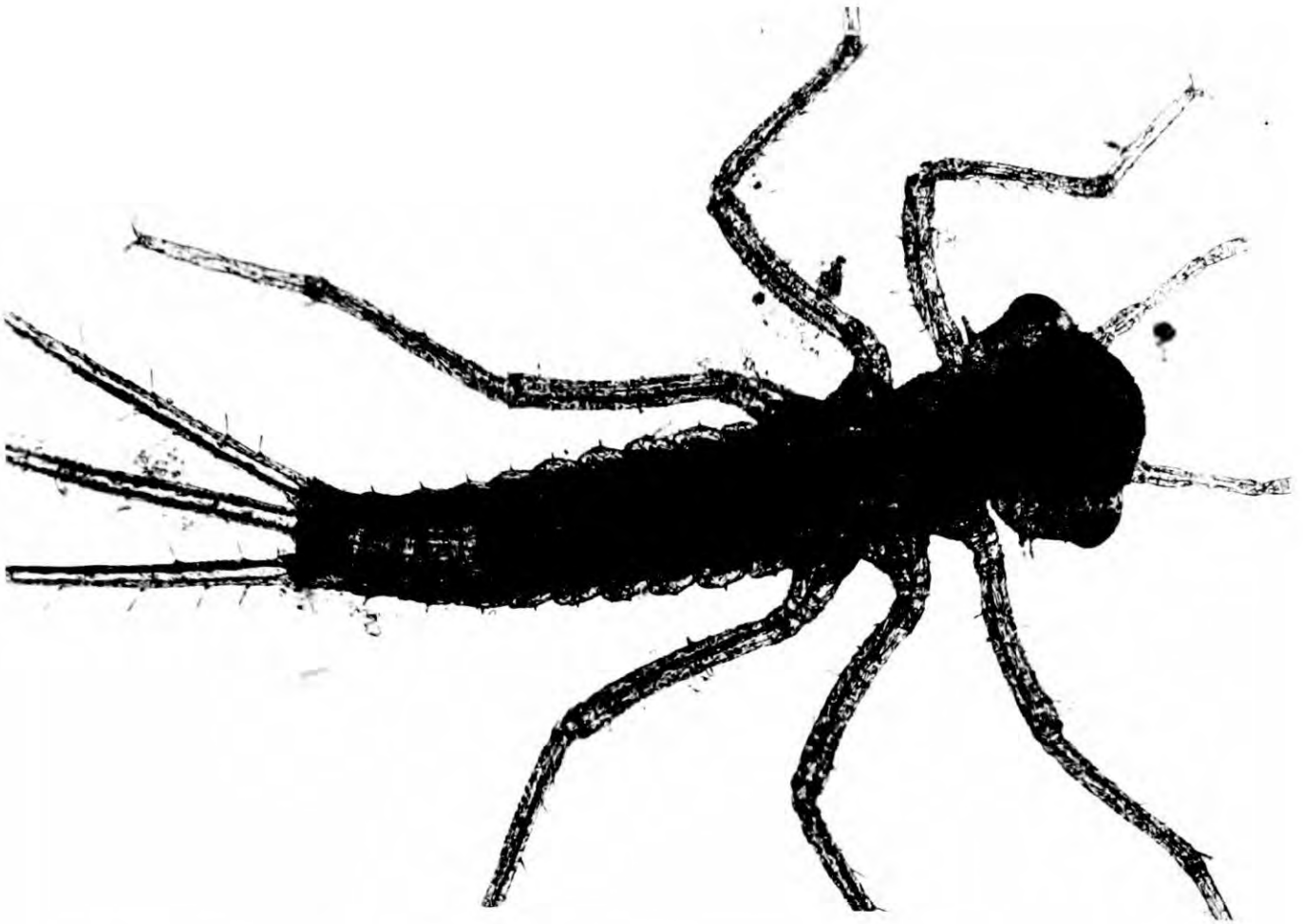


Plate 2. Instar 3 *Pyrrhosoma nymphula* larva, with an enlarged view of the head of the same individual. Whole larva X 60, enlarged view of head X 115 approx.



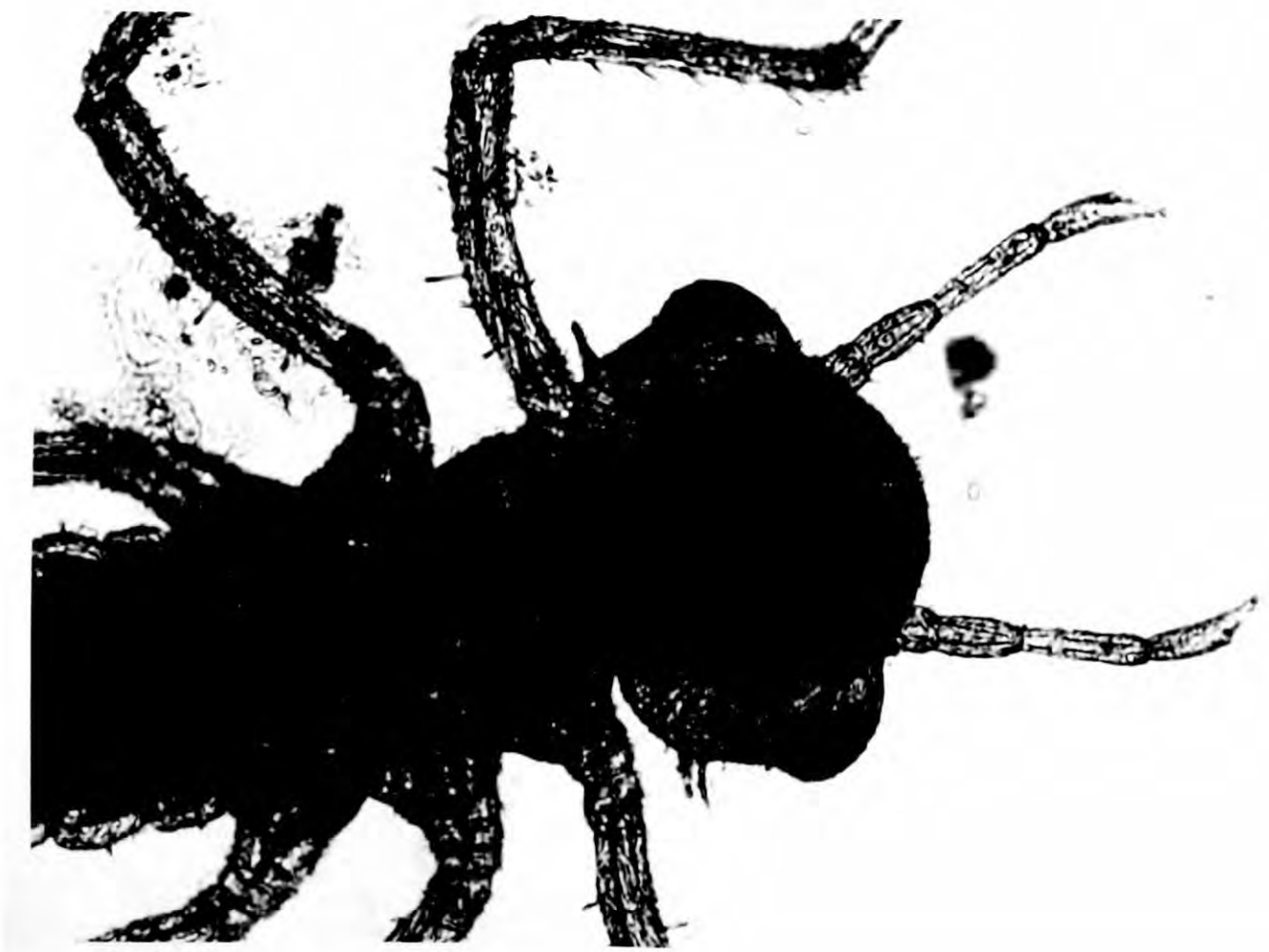


Fig. 1. Length: wet weight and length: dry weight relationships in Pyrrhosoma larvae, excluding final instars. The calculated regressions are presented in section 2.3.

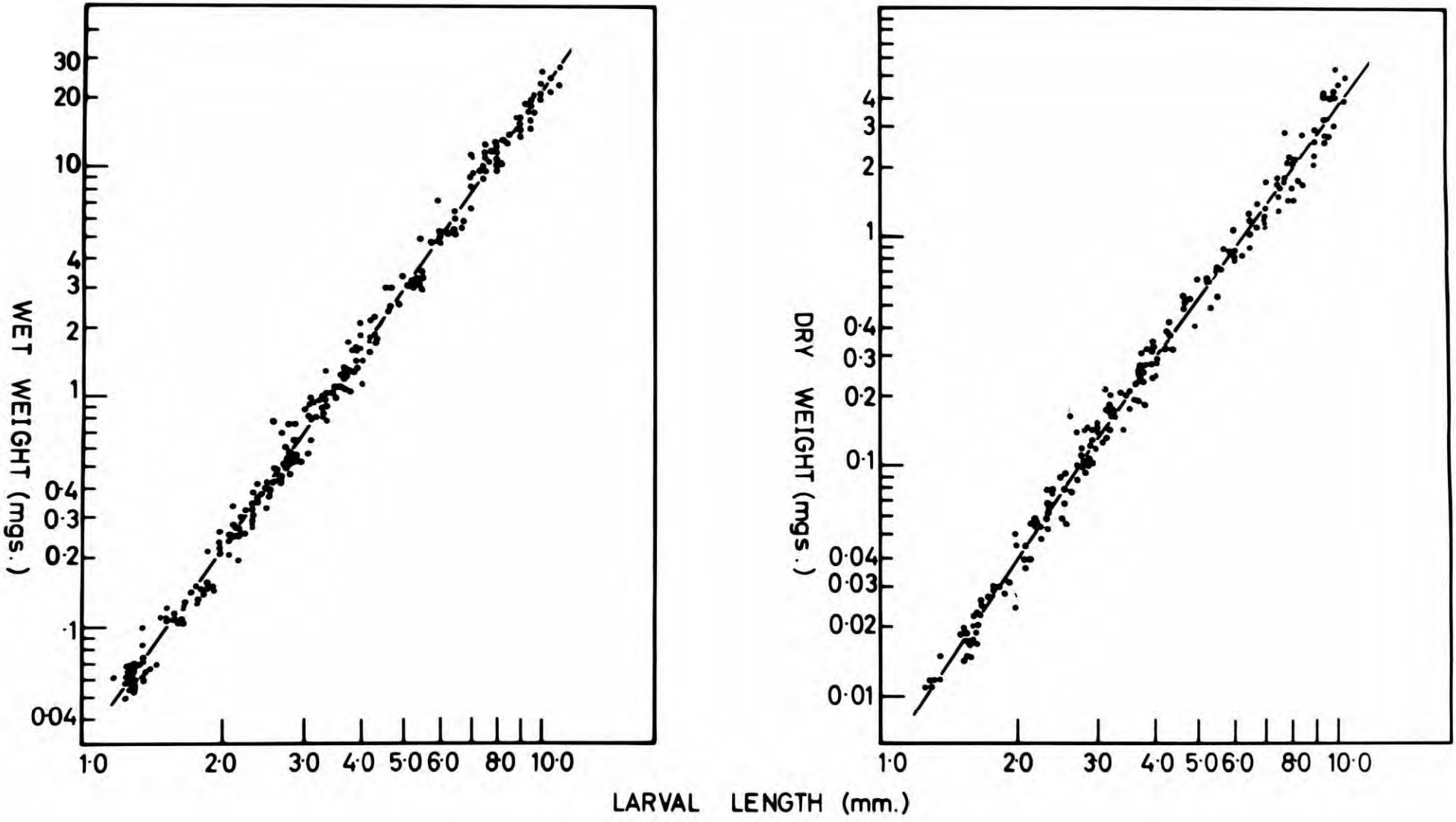

WET WEIGHT (mgs.)
30
20
10
4
3
2
1
0·4
0·3
0·2
·1
0·04
1·0
2·0
3·0
4·0
5·0
6·0
8·0
10·0
DRY WEIGHT (mgs.)
4
3
2
1
0·4
0·3
0·2
0·1
0·04
0·03
0·02
0·01
1·0
2·0
3·0
4·0
5·0
6·0
8·0
10·0
LARVAL LENGTH (mm.)

Fig. 2. Length: wet weight and length: dry weight relationships in final instar Pyrrhosoma larvae. The graphs show mean weights ± 2 standard errors, except for the last point in the upper graph, where the range of results is shown. The calculated regressions are presented in section 2.3.

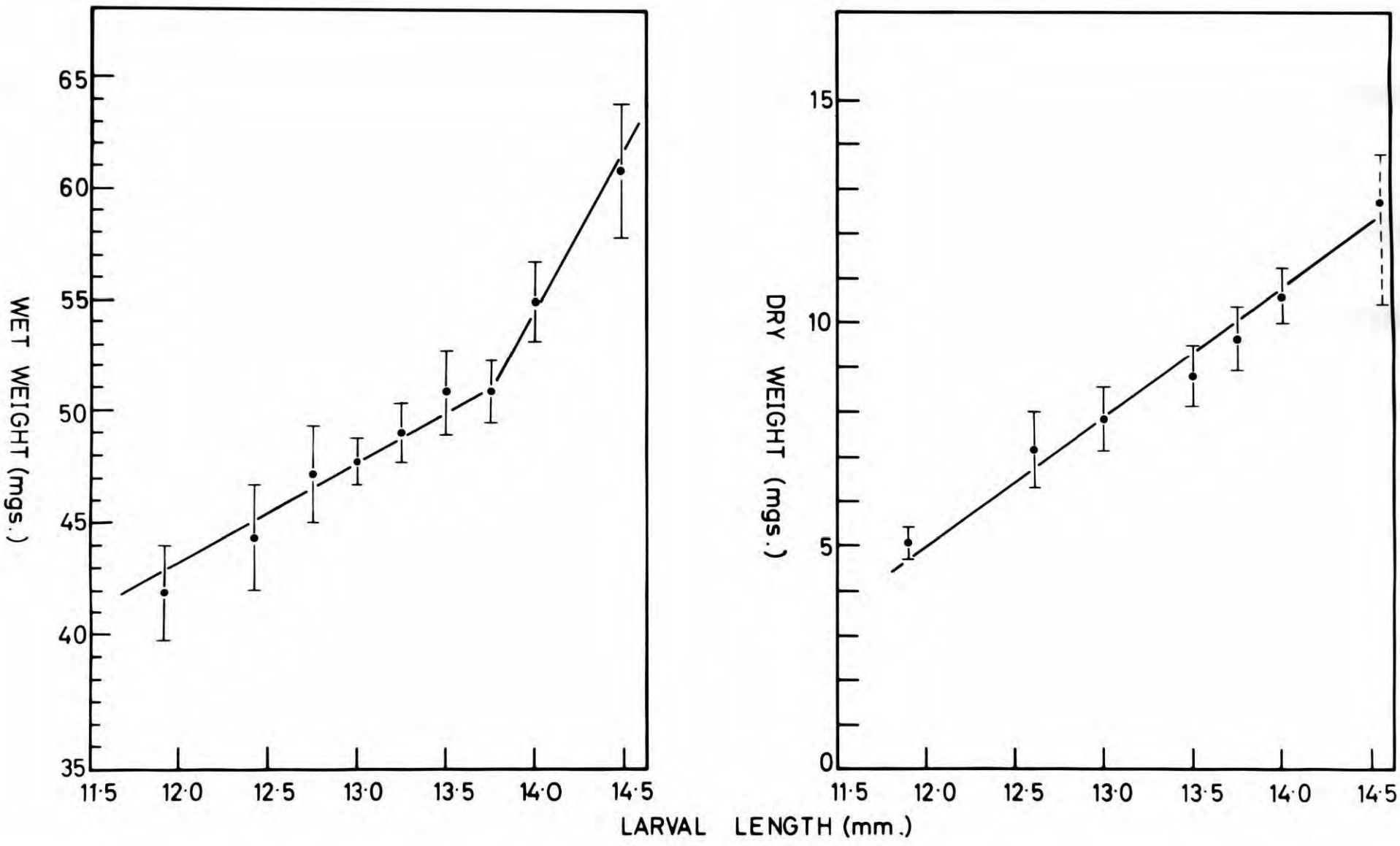
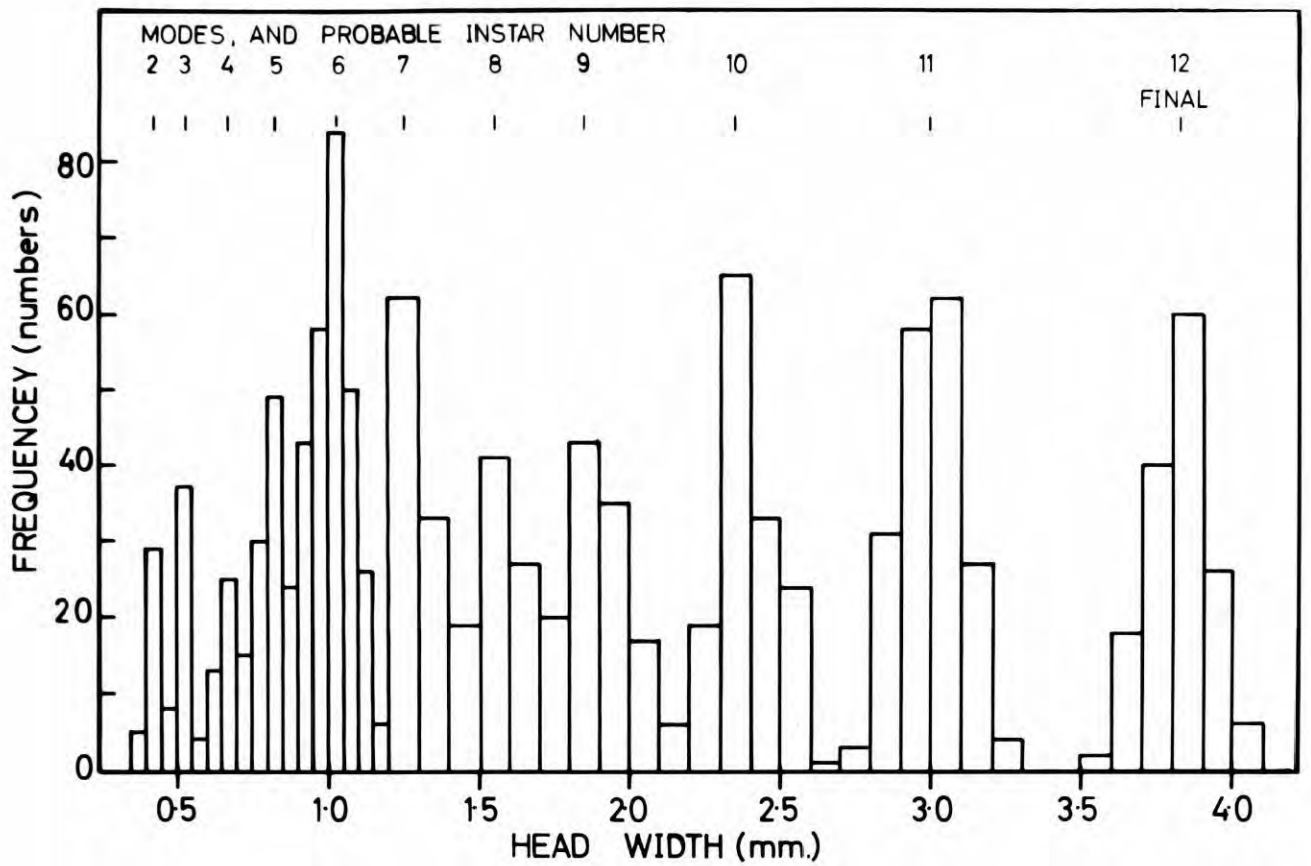
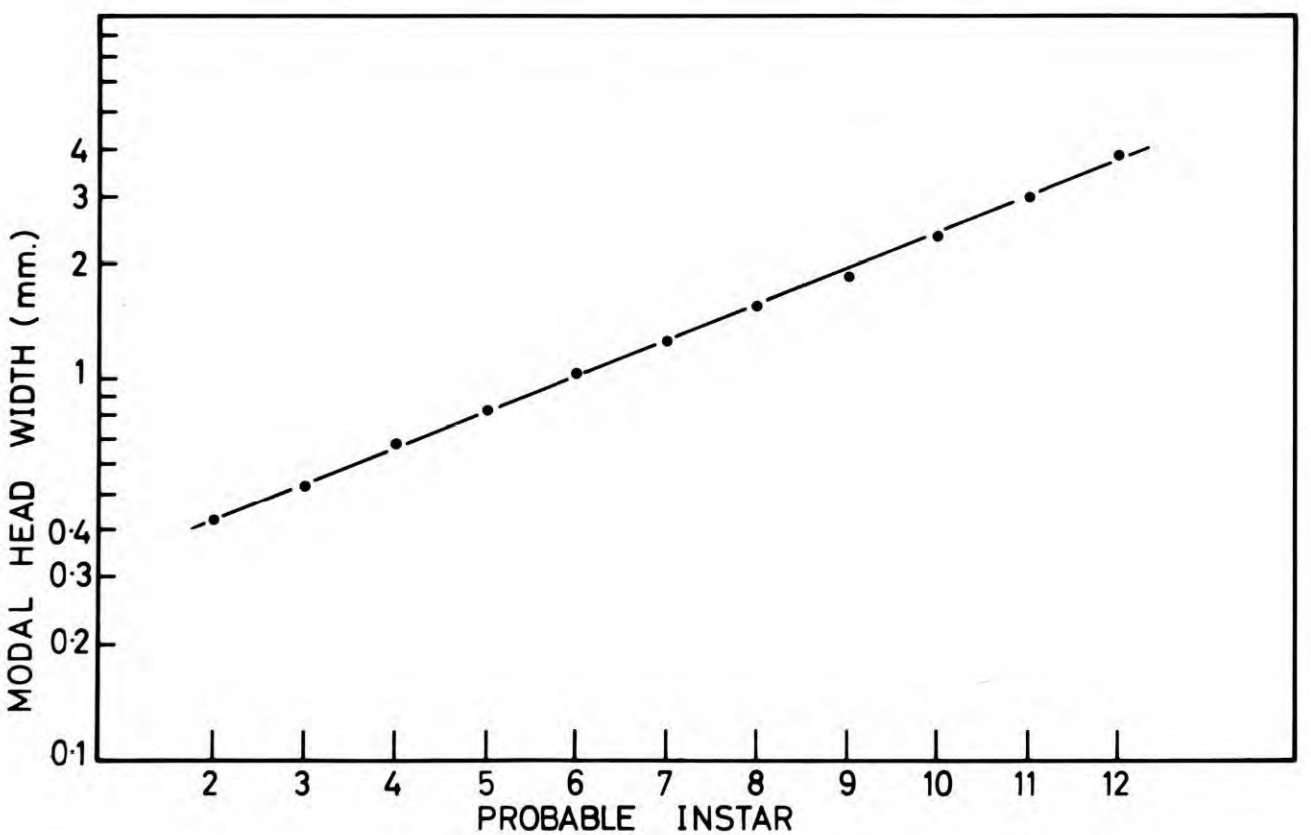

WET WEIGHT (mgs.)
65
60
55
50
45
40
35
11·5
12·0
12·5
13·0
13·5
14·0
14·5
DRY WEIGHT (mgs.)
15
10
5
0
LARVAL LENGTH (mm.)

Fig. 3a. Head width: frequency histogram for Pyrrhosoma larvae collected from the field. Modes and probable instar numbers distinguished.

Fig. 3b. Modal head widths from fig. 3a, on a log. scale, plotted against probable instar number.


MODES, AND PROBABLE INSTAR NUMBER
2 3 4 5 6 7 8 9 10 11 12
FINAL
FREQUENCEY (numbers)
80
60
40
20
0
0·5 1·0 1·5 2·0 2·5 3·0 3·5 4·0
HEAD WIDTH (mm.)


MODAL HEAD WIDTH (mm.)
4
3
2
1
0·4
0·3
0·2
0·1
2 3 4 5 6 7 8 9 10 11 12
PROBABLE INSTAR

CHAPTER 3

BOMB CALORIMETRY

Methods

Calorific values (Heats of Combustion ΔE or Change in Internal Energy) were determined in a Phillipson Oxygen Microbomb Calorimeter (Phillipson 1964) manufactured by Gentry-Wiegert Instruments Inc., Aiken, South Carolina. The procedure followed was similar to that outlined by Phillipson (1964) and in the operating instructions provided with the bomb calorimeter (Wiegert and Gentry September 1965).

All material was prepared by drying in a vacuum oven at 60°C and then stored in a desiccator over anhydrous calcium chloride and self-indicating silica gel. until required. Pellets were weighed to 0.01 mg on an Electromicrobalance model EMB-1 (Research and Industrial Instruments Co. London.).

Results

a) Calibration

22 initial benzoic acid calibration burnings were carried out by R. Dutton and the present author. After completing the calorific value determinations of the materials listed in table 5, a further 15 calibration burnings were carried out by P.J. Bolton. The results of these two calibrations were as follows:-

R. Dutton and J.H. Lawton

0.5659 mV/100 cals SD 0.020 mV

P.J. Bolton

0.5658 mV/100 cals SD 0.014 mV

It is clear that the calibrations of the bomb remained constant during this time.

b) Experimental Material

The results are presented in table 5. All data are in gcal per g. dry weight and include ash. Percentage ash values calculated from unburned material left in the bomb are also given, from which ash free figures may be calculated. The ash contents were all below 50 per cent and there was therefore no necessity to apply corrections for endothermic reactions (Paine 1966).

Most of the samples show a variation within the 3 - 4 per cent usually regarded as acceptable (e.g. Golley 1961). A few samples show greater variability, probably due to poor homogenisation of the material, which was particularly difficult with final instar samples.

The results show that there was no consistent difference in the calorific value per mg of male and female larvae and the data from both sexes were therefore pooled.

Non-metamorphosing final instar larvae in December, February and April (samples 9 - 14 in table 5) appeared to have slightly higher calorific values per mg than October final instars and small larvae (samples 1 - 13). Calorific values per mg also appeared to change during metamorphosis (samples 15 - 18). Mean calories per mg were therefore calculated for the following:-

i) Larvae between hatching and October final instars

ii) Non-metamorphosing final instars (December - April)

iii) Stage 2 metamorphic larvae

iv) Stage 3 metamorphic larvae

The results are shown in table 6.

The only other data presented in table 5 that were combined were the reproductive and non-reproductive Daphnia > 1.5 mm long (prey species samples 21 and 22). The results are also shown in table 6.

In all subsequent energy budget calculations, calorific values per mg were taken from table 6, unless they were for material that had not been pooled, when the individual results presented in table 5 were utilised.

The calorific value of one species, Cloeon dipterum (L.), was taken from Clennel (1967), who obtained a value of 5454 cals per g dry weight. Clennel's material was collected from the same ponds as in the present study and showed no variation with instar.

Discussion

Scott (1965) pointed out that the heat of combustion ΔE, as measured in the bomb calorimeter, at constant volume and temperature, differs slightly from the maximum energy released in complete degredation of the same material by another organism (the change in Enthalpy ΔH), since biological reactions take place at approximately constant pressure and temperature. The difference however is usually small and within 3 - 4 percent variation observed between samples. In accordance with general practise, correction for this difference has not been made.

There are few previous published data on calorific values of Odonata. King and Ball (1967) obtained a figure of 5028 cal per g dry weight for mixed insect carnivores which included Gomphidae, Cordulegasteridae and Libellulidae (Anisoptera) and Coenagriidae and Agrioniidae (Zygoptera), but also included insects other than Odonata e.g. Plecoptera and Sialis. Davis and Warren (in Ricker 1968), give figures of 4905 cal per g dry weight (range 4026 - 5191) for Coenagriidae (Zygoptera). Their figures are for animals of unknown size and metamorphic conditions collected in the spring, but are reasonably close to those obtained for Pyrrhosoma. Fischer (1967a) measured the calorific value of Lestes sponsa larvae four times during development in the laboratory. Values were calculated from fat, protein and carbohydrate analysis and not by bomb calorimetry. Unlike Pyrrhosoma, values changed markedly through larval life viz:-

Newly hatched 4400 cal per g dry weight

4 days old 4700 cal per g dry weight

10 days old 4900 cal per g dry weight

final instars prior to emergence (36 days old)

4500 cal per g dry weight

They are also appreciably lower than in Pyrrhosoma. Whether these differences are real or are due to the different methods used is not known. Nor is it possible to say whether the change in calorific value per g with instar in Lestes is typical of Odonata or whether a comparatively constant value as seen in Pyrrhosoma is more usual. Between insect groups there is much variation. Wiegert (1965) found that

colorific values per g increased with increasing instar number in Philaenus. Qaswari (1966) also showed an increasing calorific value per g with increasing instar number in the grasshopper Chorthippus but there was an unexpected drop in calorific value per g in instar 3 followed by a further increase with size after instar 3. Clennel (1967) could find no change with instar number in Cloeon dipterum larvae. Obviously the situation must be examined separately for each species.

The calorific values obtained for prey species (samples 21 - 25 table 5) were similar to those obtained by other workers (see Cummins 1967). The calorific value of faeces were all slightly less than those of the prey from which they were derived, whilst the ash contents were higher. Field faeces showed a very low calorific value per mg, a result discussed in chapters 8 and 9.

Table 5

Calorific value determinations

Number	Material	Number of samples	Mean cal/g dry wt.	range	% ash
	Pyrrhosoma				
	Size categories. Lengths without caudal lamellae (in mm)				
1	1.00 - 2.49 mm	1	5157.7	-	5.64
2	2.50 - 5.49 mm	5	5116.4	5001.6 - 5189.6	5.19
3	5.50 - 8.49 mm ♂	4	5103.1	5031.3 - 5205.9	6.31
4	5.50 - 8.49 mm ♀	5	5039.9	4980.7 - 5114.4	4.81
5	8.50 - penultimate instar ♂	5	5226.0	5094.5 - 5314.2	4.67
6	8.50 - penultimate instar ♀	4	5163.7	5123.6 - 5225.7	4.82
7	final instar Oct. ♂	4	5175.2	4837.2 - 5687.4	5.76
8	final instar Oct. ♀	5	5037.5	4866.8 - 5212.9	6.29
9	final instar Dec. ♂	4	5246.1	5158.7 - 5291.8	4.30
10	final instar Dec. ♀	4	5212.7	5112.8 - 5300.0	5.91
11	final instar Feb. ♂	4	5218.7	5120.6 - 5335.6	5.01
12	final instar Feb. ♀	4	5302.8	5195.2 - 5393.3	4.85
13	final instar premet. April ♂	4	5307.9	5262.7 - 5389.3	3.80
14	final instar premet. April ♀	4	5334.6	5224.0 - 5431.5	4.61

Number	Material	Number of samples	Mean cal/g dry wt.	range	% ash
	Metamorphosing final instars				
15	stage 2 ♂	3	5515.5	5470.2 - 5589.5	3.06
16	stage 2 ♀	3	5376.2	5344.0 - 5419.8	2.76
17	stage 3 ♂	5	5294.0	5135.2 - 5416.8	44.16
18	stage 3 ♀	5	5290.3	5241.1 - 5336.9	4.70
	Exuvia: Pyrrhosoma				
19	All instars excluding finals	4	4536.1	4386.9 - 4631.6	7.16
20	final instar exuvia	5	3836.5	3705.2 - 3983.5	17.75
	Prey Species				
21	Daphnia obtusa > 1.5 mm. rep.	5	5130.5	5051.5 - 5270.8	6.43
22	Daphnia obtusa > 1.5 mm. non-rep.	4	5079.9	4979.1 - 5138.6	6.99
23	Daphnia obtusa < 1.5 mm.	4	4830.5	4818.3 - 4843.8	8.49
24	Asellus aquaticus	3	3440.4	3397.4 - 3481.7	15.80
25	Chironomid sp.	4	5516.0	5389.1 - 5650.3	10.44
	Pyrrhosoma faeces				
26	From Daphnia prey	5	4657.9	4540.8 - 4755.3	11.21
27	From Asellus prey	2	2565.4	2510.4 - 2620.3	34.06
28	From Chironomid prey	2	4568.5	4516.6 - 4620.3	15.95
29	From Cloeon prey	2	5006.6	4969.2 - 5043.9	4.53
30	Faeces from larvae collected in field	3	2794.4	2754.9 - 2872.1	31.92

Material	Number of samples	Mean cal/g dry wt.	Range where n < 10	1 S.E.
Newly hatched larvae to October final instars Pyrrhosoma (both sexes): samples 1 - 8 table 5.	33	5124.8	-	25.9
Post October final instars, excluding metamorphosing larvae (both sexes): samples 9 - 14 table 5.	24	5270.5	-	18.2
Stage 2 metamorphosis (both sexes): samples 15 and 16 table 5	6	5445.9	5344.0 - 5589.5	-
Stage 3 metamorphosis (both sexes): samples 17 and 18 table 5	10	5292.1	-	24.9
Reproductive and non-reproductive Daphnia > 1.5 mm long: samples 21 and 22 table 5.	9	5108.0	4979.1 - 5270.8	-

Table 6. Combined calorific values from table 5.

Chapter 4

THE STUDY AREA

4.1 DESCRIPTION OF PONDS

Two ponds at Brasside, two and a half miles (4.0 Km) north east of Durham City, Co. Durham, were chosen for study (Normal National Grid Reference NZ (45) 291:451). The locality which stands approximately 200 ft (61 m) above sea level, lies on the Laminated Clays of the old submerged valley of the River Wear (Maling 1955). The clays have been extensively worked and the abandoned workings have flooded to form a series of ponds and marshes designated as an S.S.S.I. by the Nature Conservancy.

The study area which contained the two study ponds, covered an area of 2-3 acres (1 ha) at the southern end of the clay workings. According to Durham Rural District Council records, workings were started on this section soon after 1939 and were abandoned on 4th January 1949. In addition to the two study ponds, five other similar small water bodies, a large pond and a marshy complex of small pools have formed in the hollows of the workings. Figure 4 shows a map of the study area, with the main ponds and marshy areas. The surrounding vegetation, largely Nardus grassland growing on the acid clay soil, supported a few scattered bushes of hawthorn (Crataegus monogyna Jacquin), dog rose (Rosa Canina L.), willow (Salix sp.) and bramble (Rubus sp.) with isolated groups of oak trees (Quercus sp.). The clay workings exposed underlying base rich soils and a varied fen flora occurred in the lowest

marshy hollows. Cattle and horses grazed freely over the whole area.

The two ponds designated B and F in figures 4 were the ones chosen for detailed study: both may be termed ponds on the basis of the habitat classification of Elton and Miller (1954). The larvae in these ponds were sampled regularly to provide the necessary data on growth, mortality and other population parameters. Measurements were made of the mean temperatures prevailing in the study ponds during the period of time in which the population studies were made. With a few exceptions, all the larvae removed from ponds B and F were returned within three days of collecting. The other ponds within the study area were used as a source of larvae for experimental work. In this way, an adequate supply of experimental animals was assured, without depleting the populations in the two study ponds. Though the larval populations were isolated, they formed part of one gene pool during adult life and there was no reason to suspect that the experimental larvae differed in any way from the larvae followed in the population studies.

Both pond B and pond F were connected via narrow shallow channels to adjacent water bodies. For numerous reasons, however, these were the only two ponds suitable for detailed study. The other water bodies in the study area either carried too small a Pyrrhosoma larval population, showed great seasonal fluctuations in area and depth, were difficult to sample because of size, or were already being studied by other workers. In practice, it was considered justifiable to treat each larval population as isolated for Pyrrhosoma larvae show very little movement parallel to the shore (Macan 1964), and immigration and emigra-

tion between the study ponds and the adjacent water bodies to which they were connected was probably negligible.

Pond B

Pond B was chosen as the main study pond. It was 17½ m long and 5-6 m wide for most of its length with a surface area of 93 m^2 and a maximum depth of just over 1 m. A shallow layer of mud and detritus overlying clay formed the pond bottom. A photograph of the pond is shown in plate 3 and the morphology of the basin in figure 5. Depth contours were taken on 29/6/67 when the water level was quite low. Water level readings were taken at an arbitrarily chosen permanent reference point whenever the pond was visited; fluctuations were not great and are shown in figure 6.

Figure 5 shows that the aquatic plants formed three distinct areas or vegetation types. Their relationship with depth is obvious.

i) The north side of the pond supported a thick growth of Juncus effusus (L.).

ii) Apart from two isolated clumps, J. effusus was absent from the south side of the pond where the main vegetation consisted of Juncus articulatus (L.) with Eleocharis palustris (L.) and some Potamogeton natans (L.).

iii) Potamogeton natans alone, or, in the more shallow parts, Potamogeton and Eleocharis covered the centre of the pond. In figure 5, the area supporting Potamogeton only has been differentiated form that also supporting a sparse growth of Eleocharis. These two areas

were found to be very similar as far as Pyrrhosoma population density was concerned and were treated as one vegetation type in the population studies.

Plants not referable to any particular vegetation type were, isolated parches of Alisma plantago-aquatica (L.) and small clumps of Carex nigra (L.), Juncus inflexus (L.) and Myosotis caespitosa Schultz. The floating aquatic liverwort Ricciocarpus natans (L.) was present throughout the study period, initially in very small quantities. It started to increase slowly during the winter of 1966-67 but during August and September 1967 increased very rapidly to cover quite large areas. After declining during the winter of 1967-68, it then increased again until the end of the study and gradually replaced Potamogeton on the surface of the open water. This was the only visible change in the aquatic vegetation pattern during the study period. Because Ricciocarpus was drifted by the wind, it was not possible to show its distribution in figure 5.

The areas of the three vegetation types listed above were found to be as follows:-

Juncus effusus zone	=	22.3 m^2
Potamogeton/Eleocharis zone	=	49.2 m^2
Juncus articulatus with Eleocharis and Potamogeton zone	=	21.6 m^2
Total	=	93.1 m^2

Pond F

Pond F was studied less intensively than pond B. It was roughly

rectangular and quite small measuring only 4 m by 3 m with a surface area of 13.2 m^2 and a maximum depth of just over 80 cm. A photograph of pond F is shown in plate 3 and the morphology of the basin in figure 5: depth contours were taken on 29/6/37. Pond F varied less than pond B in depth and detailed fluctuations were not recorded. The bottom of the pond was covered with a thick layer of organic detritus overlying the clay.

The pattern of vegetation in pond F was simple. Potamogeton natans dominated the whole pond, the steep sides confining the emergent vegetation to a narrow zone round the edge. Where present, this consisted of Juncus effusus (and some J. inflexus) with Alisma plantago-aquatica and Eleocharis palustris. Figure 5 shows the distribution of the vegetation.

A shallow intermittently flowing ditch drained into pond F on the north side and out on the south side. One corner of pond F (see figures 4 and 5) connected to another similar sized pond through a narrow detritus choked channel.

Previous Work in the Study Area

Only four previous studies had been carried out within the study area of which three provided information about the energy flow through the herbivore and decomposer trophic levels of the small ponds.

Morphy (1966) was the first person to work on the ponds and provided information about the population dynamics and general ecology of the pulmonates Limnaea stagnalis (L.) and Planorbis planorbis (L.) in pond A. MacEwan (1967) extended this work and examined some of the components of

the energy flow through Planorbis planorbis populations. Further energetics information was provided by Clennel (1967) for Cloeon dipterum L. (Ephemeroptera) and by Fitzpatrick (1968) for Asellus aquaticus L. (Isopoda), both in pond C.

Reference will be made to these works where appropriate.

4.2 POND TEMPERATURE

Introduction

Mean pond temperature, over time intervals varying between a fortnight and one month, were measured using a sucrose inversion method. A similar technique for measuring water temperature appears to have been used by Schmitz (1954), (quoted in Macan 1963), but the methods employed in the present study were slight modifications of those described by Berthet (1960).

The inversion of sucrose to glucose and fructose is irreversible and, at a constant pH, the rate of inversion is proportional to the temperature. Since sucrose and the final glucose/fructose mixture rotate polarised light to different degrees, the amount of inversion at any time during the reaction can be measured using a polarimeter. If the relationship between inversion rate and temperature is determined in the laboratory at a constant pH, this information can then be used to convert inversion measurements made on solutions that have been placed in the field into mean field temperatures. It should be noted that the rate of inversion is not linear with temperature, higher temperatures being proportionally more important than lower temperatures. Therefore,

the final mean calculated from a solution which has been subject to fluctuating temperatures is an exponential mean and is larger than the equivalent arithmetic mean. However, the difference between the two, at the maximum temperature prevailing in the ponds for any length of time, is only about 1°C and for most of the year is considerably less. No attempt was made to correct for this difference.

Method

The "Rapid Inversion Solution" described by Berthet was employed. Two solutions are made up which, when mixed together in equal parts, give a solution of pH 1.21.

i) Buffer

3.730 g KCl plus 33.9 ml N HCl diluted to 500 ml with water.

ii) Sucrose Solution

400 g of Gurr's Bacteriological Saccharose dissolved in 260 ml of water and 10 ml of 35 percent formaldehyde added as an anti-biotic. The solution is filtered. The two solutions may be stored separately for several months and were mixed together as required.

For determining field temperature, 12 ml of the combined solutions were placed in small individually numbered screw-top bottles and the tops greased to prevent entry of water. These bottles were transported in vacuum flasks containing a freezing mixture of hydrated calcium chloride and ice at a temperature of -20°C and, if required, the bottles were stored in the laboratory in a deep freeze. These precautions ensured that inversion occurred only whilst the bottles were in the

study ponds.

A polarimeter manufactured by Bellingham and Stanley (London) with an accuracy of 0.01° was used to measure the inversion of the solutions. 10 ml samples were employed and the contents of each tube measured individually. Bottles were rapidly thawed in warm water and brought to room temperature before measurements were made. It was found that thawed, but cold, solutions showed a rotation which was as much as 1° smaller than their value after reaching room temperature, this change being the reverse of that expected if noticeable inversion was proceeding whilst they were warming up. Because of this, care was taken to warm up each tube to room temperature before taking a reading. For each tube, two readings were made and the mean calculated.

For each batch of solution, i.e. for each field determination, a constant C' (see sample calculation) was determined by placing tubes from the batch in 5, 10 and $15^{\circ}C$ constant temperature rooms: (3 tubes in all). This was the only point where the method differed from that described by Berthet who used the same mean constant for all solutions. This slight change was thought to be necessary when variability was found in the pH and initial rotation values of the solutions despite efforts to eliminate it. In this way, the differences between batches of solutions were compensated for.

During the winter the tubes were left in the study ponds for one month, but during the summer when pond temperatures were higher and inversion more rapid they were changed at more frequent intervals.

Distribution of Tubes in the Study Ponds

Tubes were placed in the study ponds in pairs six inches (15 cm) and one foot six inches (46 cm) below the surface on rods pushed vertically into the pond bottom. Three pairs were set up in pond B and two pairs in pond F distributed as shown in figure 5. It was assumed that this distribution of tubes was adequate to give a reliable estimate of the mean temperature of the main water mass in each pond. Without more specific information than is at present available about the microhabitats occupied by Pyrrhosoma larvae in the field, it was felt that a more detailed study of pond temperature was not justified and consequently all subsequent energy budget calculations have been made on the basis of the mean temperature for the entire pond basins.

Calculation of Results

The equations used in the calculations are those derived, and justified at length, by Berthet (1960). His notation was followed exactly. The equations used were:-

$$\text{Log } K'T = \frac{C' - a'}{T} \qquad 1$$

where:-

K'T is the Inversion Constant at a given pH and temperature T.

T is the absolute temperature.

C' and a' are constants.

Since a' is independent of pH, the value of 5,854 for this constant derived by Berthet, was employed in the calculations.

C' varies with pH and was the constant calculated for each batch of solution. Values obtained were always slightly higher than the figure of 18.99053 given by Berthet.

Equation 1 can be written

$$T = \frac{5,854}{C' - \text{Log } K'T} \qquad 2$$

In this form it is used to calculate field temperature T in degrees absolute.

C' is calculated from the following equation, applied to solutions from the constant temperature rooms.

$$C' = \frac{5854}{T} + \text{Log} \left(\frac{1}{t} \text{ Log } \frac{\alpha_o - \beta_o}{\alpha - \beta_o} \right) \qquad 3$$

where:-

t is time (in days) in the constant temperature room.

T is temperature (absolute) of the constant temperature room.

α_o is the initial rotation of the solution before inversion.

β_o is the value of the rotation at complete inversion: it is a constant given by Berthet as -9.17°.

α is the rotation of the solution after time t in the constant temperature room.

The Inversion Constant K'T for the field tubes is given by

$$K'T = \frac{1}{t} \text{ Log } \frac{\alpha_o - \beta_o}{\alpha - \beta_o} \qquad 4$$

where:-

t is the time in the field.

α_o and β_o are as above

α is the rotation of the solution after being in the field for time t.

Using equations 2 3 and 4 above, the following is a typical calculation of field temperature.

Specimen Calculation

A. Duration of Measurement

i) Tubes placed in field 1100 h 9/1/68.

ii) Tubes removed from field 1100 h 6/2/68, time t =28 days.

iii) Calibration tubes placed in constant temperature rooms 1200 h 9/1/68.

iv) Calibration tubes removed from constant temperature rooms

a) 15°C constant temperature room 1100 h 20/1/68

b) 5 and 10°C constant temperature rooms 0900 h 6/2/68

Time t in 15°C constant temperature room = $10\ ^{22}/_{24}$ days

Time t in 5 and 10°C constant temperature rooms = $27\ ^{21}/_{24}$ days

B. Polarimeter Readings; Pond B

Tube	Vegetation type and depth below surface	α values
1	Juncus 15cm below surface	22.00°
2	Juncus 46cm below surface	19.45°
3	Potamogeton 15 cm below surface	22.48°
4	Potamogeton 46 cm below surface	19.40°
5	Potamogeton and Eleocharis 15cm below surface	22.40°
6	Potamogeton and Eleocharis 46cm below surface	19.07°
	Total	124.80°
	Mean α	20.80°

Constant temperature rooms:- $5^{\circ}C$ $\alpha = 11.09^{\circ}$
$10^{\circ}C$ $\alpha = -1.80^{\circ}$
$15^{\circ}C$ $\alpha = 0.17^{\circ}$

Initial Rotation value $\alpha_o = 48.52^{\circ}$

C. Calculation of C'

$$C' = \frac{5854}{T} + \text{Log} \left(\frac{1}{t} \text{Log} \frac{\alpha_o - \beta_o}{\alpha - \beta_o} \right)$$

For $5^{\circ}C$ constant temperature room

$$C' = \frac{5854}{278} + \text{Log} \left(\frac{24}{669} \text{Log} \frac{48.52 + 9.17}{11.09 + 9.17} \right)$$

$$C' = 19.2722$$

A similar calculation for $10^{\circ}C$ and $15^{\circ}C$ constant temperature rooms gives C' values of 19.1958 and 19.1883 respectively.

Mean C' = 19.2188

D. Mean Temperature of Pond B

$$K'T = \frac{1}{t} \text{Log} \frac{\alpha_o - \beta_o}{\alpha - \beta_o}$$

$$K'T = \frac{1}{28} \text{Log} \frac{48.52 + 9.17}{20.80 + 9.17}$$

$$K'T = 0.01016$$

hence Log 'KT = $\bar{2}.0068$ or -1.9932 and therefore

$$T = \frac{5854}{C' - \text{Log } K'T} = \frac{5854}{19.2722 + 1.9932}$$

$T = 275.98^{\circ}$ absolute and

$T - 273 = 2.98^{\circ}C$

The mean temperature of the pond between 9/1/68 and 6/2/68 was therefore 2.98°C

Mean date was 23/1/68.

Results

Temperature measurements were made in pond B from 14 September 1966 until 16 June 1968. Two early measurements made in July and August 1966 were both unsuccessful, due to entry of water into the tubes in July and leaving the tubes in the pond too long in August, so that complete inversion occurred. These were the only difficulties encountered and neither occurred again. Temperature measurements for pond F were obtained for a shorter period from 28 February 1967 until 3 June 1968.

Figure 7 shows the seasonal changes in pond temperature. Each point represents the mean temperature calculated for the whole pond basin at the mean date i.e. the date halfway between introducing and removing the tubes from the pond. A curve has been drawn to represent the probable temperature change between the points and from the area under this curve mean monthly temperatures in both ponds were obtained. The results are presented in table 7. Also shown in table 7 are the mean monthly air temperatures, recorded at Durham University Observatory: the Observatory stands at just over 300 feet (91 m) above sea level two and a half miles (4 km) south west of the study area.

Both ponds follow the mean air temperature closely, pond B particularly being within 1°C of the mean air temperature in 15 of the 20 monthly estimates. The temperature in pond F was consistently below

that in pond B and was usually slightly below the mean air temperature. The difference between the pond temperature and the mean air temperature in the same month are shown in the last two columns of table 7.

For energy budget calculations to be made over the desired period (July 1966 - June 1968 inclusive in pond B and March 1967 - May 1968 inclusive in pond F) the available temperature data were satisfactory for F but not for B, where no estimates of mean pond temperature were available for July, August and September 1966, and June 1968. However, reliable estimates of the pond temperature in these four months can be made with very little error from the mean air temperature over the same period. This was carried out as follows. During the months May to August (1967 and 1968) pond B was on average 0.94°C warmer than the mean air temperature, whilst from September to November (1966 and 1967) it averaged 0.15°C warmer. The mean pond temperature in July and August 1966 and June 1968 were therefore found by adding 0.94°C to the mean monthly air temperature and in September 1966 by adding 0.15°C viz:

July 1966	14.1 + 0.94°C = 15.04°C
August 1966	13.5 + 0.94°C = 14.44°C
September 1966	11.8 + 0.15°C = 11.95°C
June 1968	13.4 + 0.94°C = 14.34°C

These estimated pond B temperatures were then utilised together with the actual measurements for all other months in subsequent energy budget calculations.

Month	Year	Mean monthly air temperature at observatory °C	Mean monthly pond temperature by sucrose inversion method °C		Pond temperature minus observatory temperature	
			Pond B	Pond F	Pond B	Pond F
July	1966	14.1	-	-	-	-
Aug.		13.5	-	-	-	-
Sep.		11.8	-	-	-	-
Oct.		9.1	9.55	-	+0.45	-
Nov.		5.3	6.72	-	+1.42	-
Dec.		3.8	3.61	-	-0.19	-
Jan.	1967	3.2	2.82	-	-0.38	-
Feb.		5.0	4.91	-	-0.09	-
Mar.		6.5	6.17	5.62	-0.33	-0.88
April		7.3	6.44	6.07	-0.86	-1.23
May		8.7	9.93	8.88	+1.23	+0.18
June		12.9	13.52	12.49	+0.62	-0.41
July		14.9	15.30	14.15	+0.40	-0.75
Aug.		14.7	14.72	14.09	+0.02	-0.61
Sep.		12.5	12.31	11.41	-0.19	-1.09
Oct.		9.4	8.35	7.91	-1.05	-1.49
Nov.		4.7	4.83	4.06	+0.13	-0.64
Dec.		3.7	2.86	2.66	-0.84	-1.04
Jan.	1968	3.4	2.84	2.96	-0.56	-0.44
Feb.		1.3	3.17	2.82	+1.87	+1.52
Mar.		6.2	5.58	4.42	-0.62	-1.78
April		7.2	6.99	5.68	-0.21	-1.52
May		8.1	10.53	8.89	+2.43	+0.79
June		13.4	-	-		

Table 7. Mean monthly pond temperatures measured by the sucrose inversion method and mean monthly air temperatures at Durham University Observatory $2\frac{1}{2}$ miles(4 km)south west of the ponds.

Discussion

Other temperature dependent, irreversible chemical reactions have been used to measure field temperatures in ecological studies e.g. Edington (1966), but this appears to be the first time that the sucrose inversion method developed by Berthet (1960) for terrestrial work has been applied to aquatic habitats. The method appears to be highly satisfactory, particularly since expensive or complex field recording equipment is not required. It is simple to use and, with care, gives accurate and consistent results.

Measurements were made in order to calculate energy budgets using mean monthly temperatures. The data obtained were satisfactory for this purpose and a more detailed analysis of the temperature changes and stratification patterns within the ponds, though of undoubted interest, was not felt to be necessary and could probably not have been completed in the time available. A number of simple conclusions can however be made about the temperature regimes in the ponds. It is clear that the ponds followed the mean air temperature closely and, in this respect, resembled other, small shallow water bodies with a large surface area to volume ratio (Macan 1963: Welch 1952). Other factors clearly influenced temperature as well. The consistently lower temperature in pond F may be related to its greater exposure to the prevailing winds than pond B and also to its greater depth in relation to surface area. Though not analysed in detail, it was apparent that both ponds usually showed a temperature difference between the surface water (tubes at 6 inches) and deeper water (tubes at 1 foot 6 inches). The surface water was

warmer than the deeper water in summer and colder in winter, with periods of uniform temperature with depth in early spring (February - March) and Autumn (October). These temperature differences were not great, but were quite stable, perhaps due to the thick plant growth which tended to prevent mixing of the water (Welch 1952).

A number of authors have studied the effects of temperature on Odonata larvae e.g. Macan and Maudsley (1966), Fischer (1958). Wright (1943) reviews the earlier literature. The temperatures prevailing in the study ponds were probably never high nor low enough to be a direct cause of mortality in Pyrrhosoma, nor were they low enough to prevent feeding (see chapter 12).

A few energy flow studies have utilised approximations to the mean annual temperatures prevailing in the study area e.g. Phillipson and Watson (1965). The majority of workers however, have used some measure of the mean monthly temperatures e.g. Englemann (1961), Menhinick (1967), Saito (1965, 1967), Small (1967) etc., so that although use of the mean monthly temperatures in the study ponds in the present study was probably as accurate as in any work yet carried out, it does present a number of problems. For example, Golley and Gentry (1964) considered the possible effects on energy flow of variations in microclimate within different parts of the habitat occupied by the ant Pogonomyrmex, (though their actual measurements fall short of their theoretical approach). In common with nearly all other published work, however, microclimate data for the microhabitat (see Luff 1966) occupied by Pyrrhosoma was not available, and indeed, such information would have been exceptionally difficult to

obtain particularly since the plant zones occupied by the larvae changed seasonally (see chapter 5). Despite such difficulties it is felt that the possible effects on energy flow of variation in micro-climate within the habitat occupied by a species might repay further investigation. Probably more important and equally neglected in ecological energetics studies are problems of metabolic acclimatisation. However, since Pyrrhosoma appears to be typical of those invertebrates that do not show metabolic acclimatisation (see chapter 10), these do not appear to arise in the present study, though it is still possible that field temperatures varying about a mean may have quite different metabolic effects than the same mean held constant in the laboratory, even without the added problems of acclimatisation. A great deal of basic work remains to be done in this field.

Clearly the influence of temperature on energy flow could be extremely complex, but without a great deal more information than is at present available, the methods employed in the present study are probably as accurate as can be achieved at this time.

4.3 THE SAMPLING GRIDS

The two study ponds were divided into a number of equally sized subdivisions by means of marker posts on the banks.

Pond B

The south side of the pond was designated as side 1 and the north as side 2, and the pond divided across from side 1 to side 2 into three zones, as shown in figure 5. The length of shore on each side in each

zone was approximately 5m. A smaller area between zone 3 and the narrow channel at the top of the pond was designated zone 4 (not labelled in figure 5). Zones 1-3 were then further subdivided into five 1 m wide strips running across the pond and numbered 1-5 in each zone: zone 4 was divided into two such strips.

Each of the seventeen 1 m strips included the three vegetation types described in section 4.1 i.e. Juncus articulatus with Eleocharis and Potamogeton on side 1, Potamogeton in the centre and Juncus effusus on side 2.

The surface area of each zone was:-

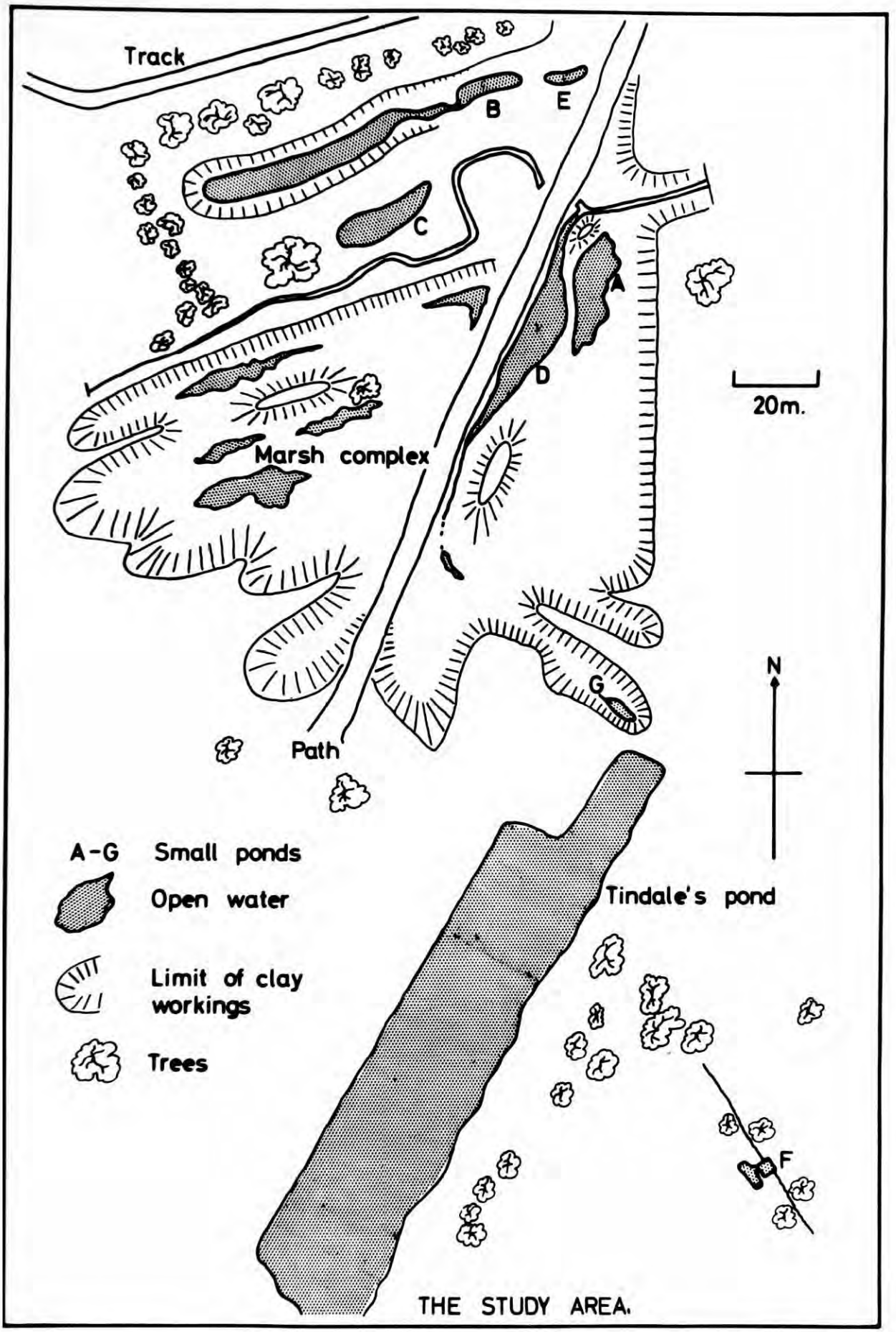
Zone 1	22.1	m^2
Zone 2	27.6	m^2
Zone 3	28.5	m^2
Zone 4	14.9	m^2
Total	93.1	m^2

Pond F

The sampling grid in F was much simpler than in B. The four sides were each divided into three equal parts as shown in figure 5 dividing the pond into nine approximately equal sized rectangles, only one of which had no shore line.

Plate 3. The study ponds in June 1966. Upper photograph: pond B, looking west with zone 1 nearest the camera and zone 4 furthest away. Lower photograph: pond F, looking north from zone 6.

Fig. 4. The study area at Brasside, with the main small ponds (A - G) and marshy areas distinguished.



THE STUDY AREA.

Fig. 5. The two study ponds (B and F) showing morphology of their basins and vegetation types (section 4.1), the positions of the temperature tubes (section 4.2) and the sampling grids (section 4.3).

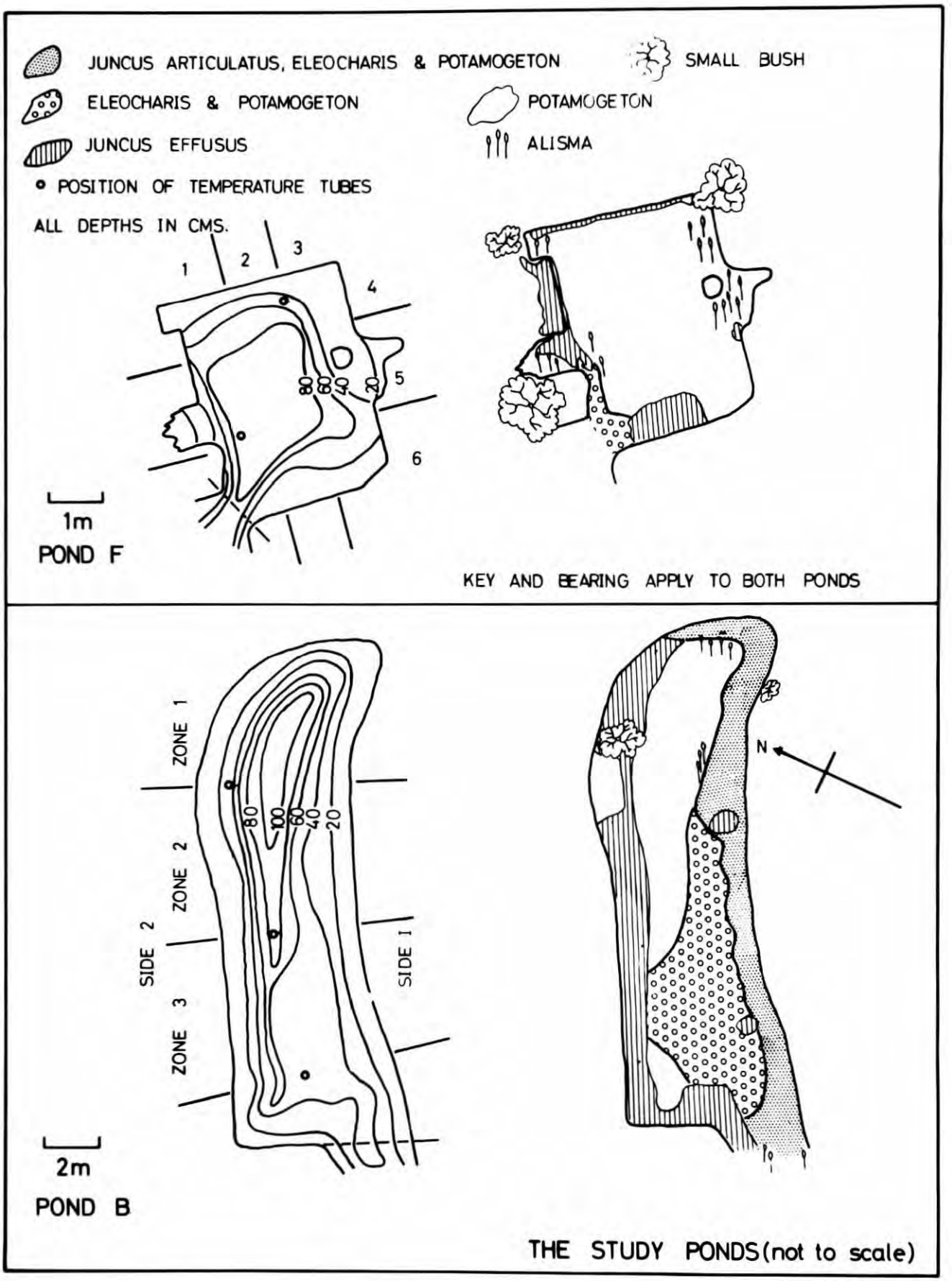

JUNCUS ARTICULATUS, ELEOCHARIS & POTAMOGETON
SMALL BUSH
ELEOCHARIS & POTAMOGETON
POTAMOGETON
JUNCUS EFFUSUS
ALISMA
POSITION OF TEMPERATURE TUBES
ALL DEPTHS IN CMS.
1
2
3
4
5
6
80
60
40
20
1m
POND F
KEY AND BEARING APPLY TO BOTH PONDS
ZONE 1
ZONE 2
ZONE 3
SIDE 2
SIDE 1
80
100
60
40
20
N
2m
POND B
THE STUDY PONDS (not to scale)

Fig. 6. Variation in depth of pond B over one year, at a permanent reference point.

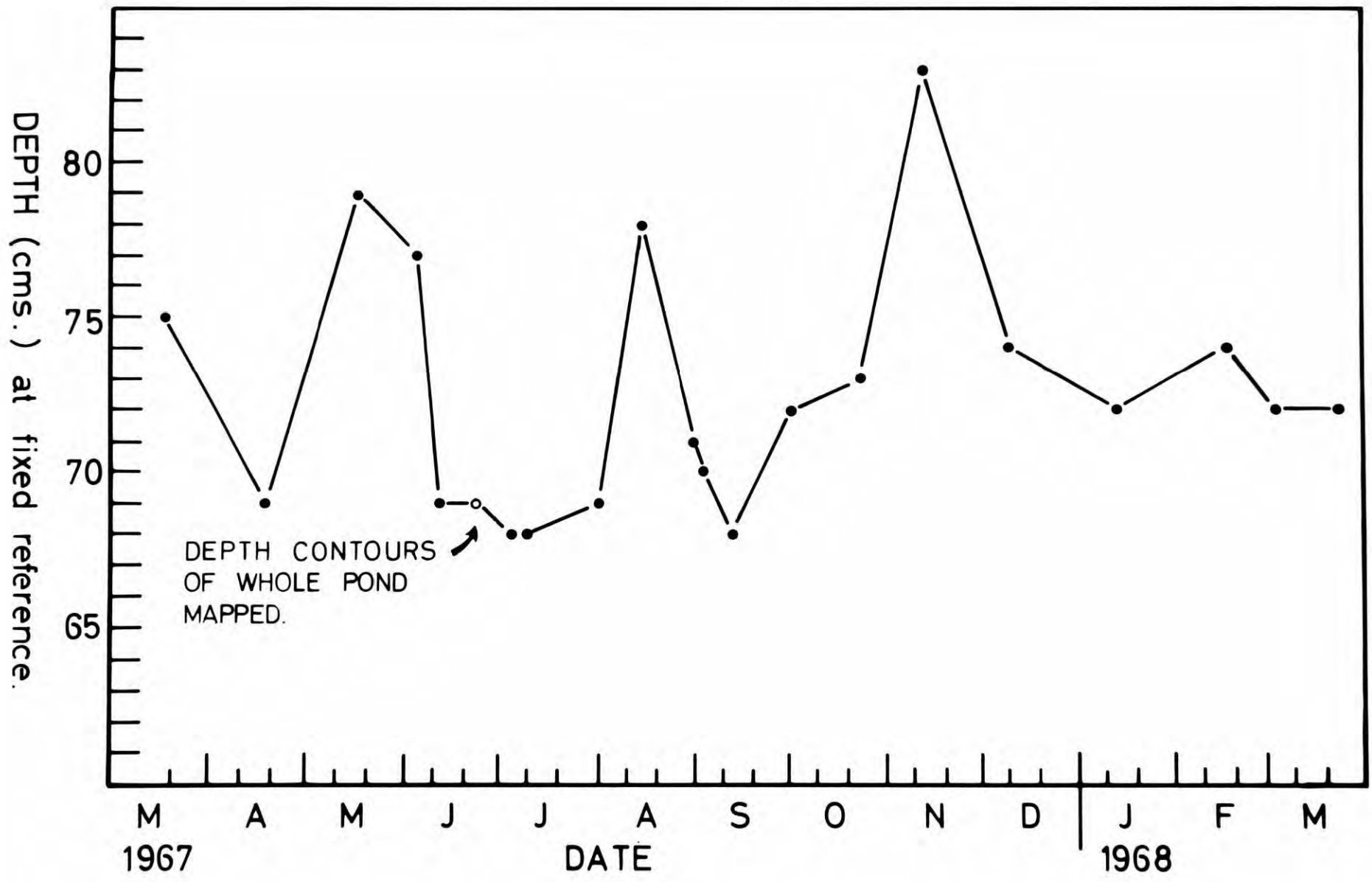
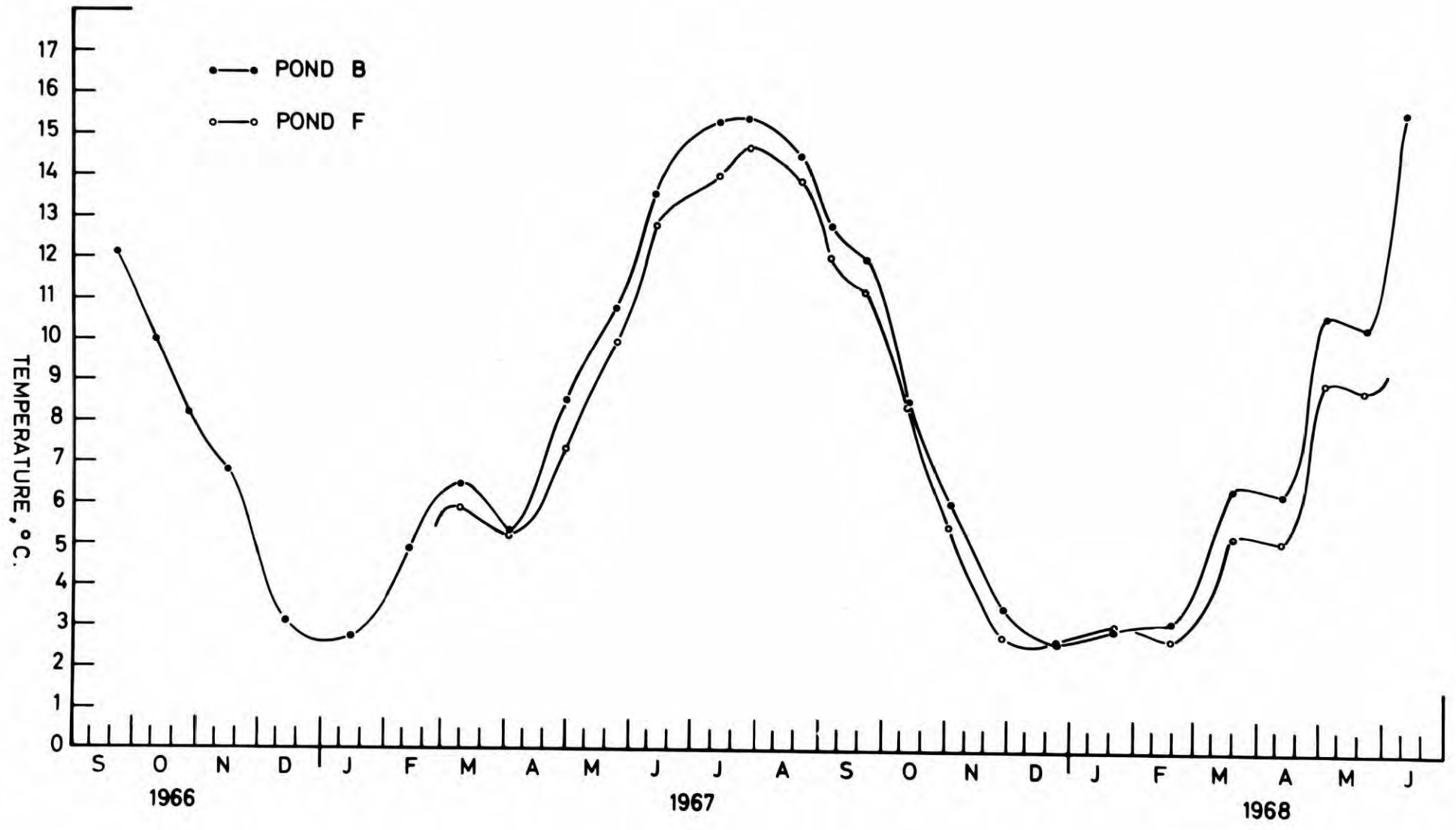

DEPTH (cms.) at fixed reference.
80
75
70
65
DEPTH CONTOURS
OF WHOLE POND
MAPPED.
M
A
M
J
J
A
S
O
N
D
J
F
M
1967
DATE
1968

Fig. 7. Seasonal changes in the mean water temperature in ponds B and F, measured using a sucrose inversion technique.


POND B
POND F
TEMPERATURE, °C.
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
S O N D J F M A M J J A S O N D J F M A M J
1966
1967
1968

Chapter 5

POPULATION STUDIES PART 1

(Numbers per m^2, mortality and changes in larval distribution)

Introduction to Population Studies

The larval populations in ponds B and F were sampled at approximately monthly intervals, except during metamorphosis and emergence when more frequent observations were made. From these samples information was obtained on a number of topics e.g. metamorphosis, emergence and oviposition (chapter 6), larval growth (chapter 7) faeces analysis (chapter 8) and field feeding (chapter 12).

The present chapter deals with pond B only and is concerned with numbers per m^2, mortality and changes in larval distribution with season.

5.1 METHODS

5.1a The Standard Net Sweep

Because of the small size of the ponds, a quantitative sampler which removed areas of vegetation would rapidly have destroyed the habitat; consequently, (in both B and F) all samples were taken with a pond net used in a standard way. A meter strip was measured and marked on the edge of the pond opposite the point where the sample was to be taken. The net was then carefully placed in the water and swept three times (across, back and across) parallel to the bank along the same path over the measured meter, care being taken to keep the speed of movement constant. This consituted one standard net sweep (S.N.S.) A

net with a mouth 25 x 30 cm and a 1mm mesh as used throughout the study. Experiments to test the ability of small larvae to escape through the net are described in section 5.1b.

The contents of each S.N.S. were immediately emptied into large white trays and carefully sorted. The numbers of each year class in each S.N.S. were recorded (from this information numbers per m^2, and, ultimately, mortality rates were calculated). The larvae were then placed in individual tubes of clean water for transport to the laboratory where they were measured for growth rate estimates. The faeces which they produced were either used to estimate field feeding rate or were dissected to provide information on the main food species. Finally the larvae were fed and returned to the pond at the sampling point where they were obtained, always within three days and usually within forty-eight hours of collection. Corbet (1957a) was able to return Anax larvae within twenty four hours but, because of the additional information required, this was not possible in the present study. It seems unlikely that the population was appreciably affected by this sampling procedure. Mortality of larvae brought into the laboratory was virtually non-existent during this time and they appeared to suffer no other ill-effects.

5.1b Ability of Small Larvae to Escape Through the Net

Appreciable numbers of small larvae escaping through the meshes of the net would have affected the mortality, biomass and growth rate estimates. Consequently, laboratory experiments were carried out to examine this source of error.

A known number of small Pyrrhosoma larvae were placed in an open ended glass cylinder 7 x 3 cm which was suspended vertically in a large beaker of water. A piece of net of 1mm mesh identical to that used in the field was stretched across the bottom of the cylinder; larvae passing through this net were collected in the beaker.

Larvae were placed in the cylinder and left for one hour after which the number passing through the net of their own accord was recorded. Since this did not simulate use of the net in the field, the larvae were replaced in the cylinder which was then drawn in and out of the water five times; the numbers passing through the net were again noted. The experiment was repeated several times with larvae of different sizes. The results are presented in table 8 and show that under simulated field use only a small proportion of instar 4 larvae were able to pass through the net; no instar 5 larvae were able to do so. (Note that the experiment with instar 5 was replicated and gave the same result.) However, an appreciable number of instar 2 and 3 were able to escape. (It is interesting to note that more instar 2 larvae escaped passively through the net than passed through in simulated field use.) From these data, it was clear that the junior age class could not be sampled effectively with the particular net size used until the great majority of them had entered at least instar 4; in the field this was usually by early September. Consequently, the first standard net sweeps for growth, mortality and biomass estimates with the junior age class were not made until after this date. The possibility of using a finer net (i.e. with a mesh $<$ 1mm) was considered. This would have permitted S.N.S. data to be obtained

throughout development, including the smallest larvae. However, those used became so choked with detritus and algae that this was abandoned in favour of the 1 mm mesh which, for the majority of the life cycle, gave satisfactory results.

A number of authors have reported similar experiments on the ability of a variety of insect species to avoid capture by escaping through various mesh sizes e.g. Crisp (1959), Jonasson (1955), Macan (1958b). It is clear that results vary widely depending on the species, particularly for example on whether the head width or general body size limits the ability of the larvae to escape. In Pyrrhosoma the latter seems more important. It is nevertheless clear that experiments of this type should always be carried out in a sampling programme involving the use of a pond net to capture animals for census purposes.

Mean length of larvae (mm)	Mean head width (mm)	Instar	n	% passing through net in 1 hour	% passing through net in 5 dips
1.30	0.40	2	18	11.1	66.7
1.65	0.53	3	26	46.2	15.4
2.23	0.73	4	24	0	4.2
2.80	0.85	5	15	0	0
2.80	0.85	5	15	0	0

Table 8 Results of experiments to test the ability of small Pyrrhosoma larvae to pass through a pond net of 1 mm mesh either of their own accord or in simulated use of the net.

5.1c Conversion of S.N.S. to Numbers per m^2

The pond net gave no information on absolute population figures so that a conversion factor was required to convert S.N.S. data to numbers per m^2. The conversion factor was obtained by taking quantitative samples and standard net sweeps in close proximity and relating the two. To ensure that the taking of quantitative samples did not disturb the results from the adjacent S.N.S. and vice versa, the quantitative sampler was first pushed carefully into the substrate to seal off an area of the pond (see below). The adjacent S. N.S. was then taken before the contents of the quantitative sampler were determined.

Quantitative samples were taken with a cylinder 40 cm deep enclosing an area of 0.142 m^2. The bottom rim carried three long spikes which were pushed firmly into the mud thereby sealing the bottom edge but leaving the upper end above the water surface . The contents of the cylinder were then systematically dredged out using a pond net with a width of 25 cm (i.e. just over half the cylinder diameter). One dredge consisted of a thorough cover of the entire cylinder several times round and across with the net and at least five dredgings were made in each sample, after which they were continued until three consecutive ones produced no further zygopteran larvae of any species.

Conversion factor data were obtained once from 2 August to 4 August 1967 and applied to all S.N.S. data in pond B. The quantitative samples and associated standard net sweeps were made on side 1 where the vegetation was almost vertical. On side 2, the tangled aerial stems of Juncus effusus were trapped under the cylinder edge so that collection

of larvae from within the cylinder was made exceptionally difficult and attempts were eventually abandoned. The larger areas of relatively level bottom on side 1 also made the placing of the cylinder much easier. The water was too deep to use the cylinder in the middle Potamogeton zone: however, the number of larvae here was usually small and, though the structure of the vegetation was rather different to that at the sides of the pond, the same conversion factor was used.

The conversion factor was obtained from the senior age class (larvae hatched in 1966), which were just over a year old and about half to three quarters grown at this time, and then applied to all samples regardless of larval size.

Results are presented in table 9 and 10. The mean number of larvae per S.N.S. was 3.5 and the mean number per quantitative sample 6.1. Since the area of the quantitative sampler was 0.142 m^2 there was $\frac{6.1}{0.142}$ or 42.95 Pyrrhosoma per m^2.

The conversion factor to convert numbers per S.N.S. to numbers per m^2 is then given by

$$\frac{42.95}{3.5} = 12.3$$

Numbers per m^2 were calculated by multiplying numbers per S.N.S. by 12.3.

Obtaining population data for small, weed dwellings aquatic organisms is generally agreed to be difficult (Southwood 1966 p 158) and though the conversion factor method employed in the present study leaves much to be desired, it was probably the best method available

Standard Net Sweeps			
S.N.S.	Date	Zone	Number of *Pyrrhosoma* in each S.N.S.
1	2.8.67	1	6
2	2.8.67	1	1
3	2.8.67	1	1
4	2.8.67	2	8
5	3.8.67	2	6
6	3.8.67	3	4
7	2.8.67	3	1
8	4.8.67	3	1
9	4.8.67	4	4
10	4.8.67	4	3
Total = 35 Mean number per S.N.S. = 3.5			

Table 9 Number of *Pyrrhosoma* larvae captured per S.N.S. on side 1, for use in conversion factor estimates.

Quantitative Sampler			
Sample	Date	Zone	Number of *Pyrrhosoma*/ sample
1	2.8.67	1	2
2	2.8.67	1	3
3	2.8.67	2	14
4	2.8.67	2	3
5	3.8.67	22	5
6	3.8.67	3	11
7	2.8.67	3	2
8	4.8.67	3	9
9	4.8.67	4	6
10	4.8.67	4	6
Total = 61 Mean number per sample = 6.1			

Table 10 Number of *Pyrrhosoma* larvae captured in the quantitative sampler (area 0.142 m^2) on side 1, for use in conversion factor estimates.

due to the limits imposed on sampling by the habitat. A larger pond would have permitted the use of a grab or other sampler removing areas of vegetation but, even if a suitable large pond had been available, it might have presented other problems not experienced in the present study. A check on the method employed was possible from a study of the number of larvae emerging from pond B and also from population figures for Pyrrhosoma obtained by Macan (1964). Both suggest that the conversion factor used gave a reasonably accurate picture of the absolute changes in population density of Pyrrhosoma. (The number of larvae emerging for the pond is discussed in chapter 6, section 6.1c; Macan (1964) is discussed in the present chapter, section 5.3.)

5.1d Attempted Mark-Release-Recapture Estimates

An attempt to obtain absolute population figures by mark-release-recapture was made between 21/3/66 and 28/3/66. A total of 245 final instars were marked with cellulose dope on the fore-femur but, of these, only two were recaptured. Cellulose dope did not bond to the wet cuticle and attempts to dry the larvae by keeping them out of the water invariably resulted in their death, though Corbet (1957a) found that this was successful in the larger Anax. Consequently, attempts were made to dry the cuticle with filter paper. This was satisfactory in the laboratory, but obviously attempts to dry the cuticle in this way under more difficult field conditions were less successful and it appears that most of the marks were quickly lost when the larvae were returned to the pond.

Other marking techniques were tried but were not sufficiently

satisfactory to warrent field trials and the experiments were abandoned.

5.1e Number and Distribution of Samples and Correction for Areas of Vegetation types

At approximately monthly intervals, three S.N.S. were taken in each of the tree vegetation types (chapter 4, sections 4.1 and 4.3) - a total of nine samples. One of the fixed five 1 meter strips in each of the zones 1 - 3 were chosen at random and the nine samples for that month taken across the pond in these three 1 meter strips. (Every four months zone 4 was sampled instead of a sample being taken in one of the other three zones.) Suppose strips 3, 1 and 4 had been selected at random in zones 1, 2 and 3 respectively, then the nine samples would have been distributed as follows:-

Vegetation Type	Zone	1m strip sampled
SIDE 1 *Juncus articulatus* with *Eleocharis* and *Potamogeton*	1 2 3	3 1 4
MIDDLE OF POND *Potamogeton* (and *Potamogeton* with sparse *Eleocharis*)	1 2 3	3 1 4
SIDE 2 *Juncus effusus*	1 2 3	3 1 4

Once a strip within a zone had been sampled, it was not resampled until the remaining strips within that zone had also been utilised.

In this way several months elapsed between samples in the same part of the pond and habitat disturbance was reduced to a minimum.

Each of the three vegetation types differed in area but the same number of samples (3) were taken in each. Consequently, movements of larvae from one vegetation type to another would give rise to changing numbers caught per S.N.S. without there being any real change in the total number of larvae in the whole pond. Therefore, in converting from S.N.S. data to absolute population figures, the S.N.S. data were corrected for area before multiplying by the conversion factor of 12.3 to obtain numbers per m^2. The areas of the three vegetation types were presented in chapter 4, section 4.1. The following is a typical calculation based on the sample data presented in table 11.

Part of pond	Vegetation type	Zone	1 meter strip	Number of 1966 year class captured in each S.N.S.
Side 1	Juncus articulatus with Eleocharis and Potamogeton	1 2 3	3 2 2	7 23 3
Middle	Potamogeton and some sparse Eleocharis	1 2 3	3 2 2	49 34 3
Side 2	Juncus effusus	1 2 3	3 2 2	26 23 21

Table 11 Typical samples used in the calculation of population figures, October 1966.

Samples 4/10/66, 1966 year class

Total number caught in each vegetation type multiplied by its area was:-

Side 1	33 x 21.6	=	712.8
Middle	86 x 49.2	=	4231.2
Side 2	70 x 22.3	=	1561.0
		TOTAL	6505.0

The sum of the above (6505.0) divided by 3, the number of samples in each vegetation type, and by 93.1, the area of the whole pond in square meters, and multiplied by 12.3 the conversion factor gives the mean number of 1966 year class larvae per m^2.

$$\frac{6506.0 \times 12.3}{3 \times 93.1} = \underline{286.5 \text{ larvae per } m^2}$$

To obtain the total population density on this date the figure of 286.5 larvae per m^2 was added to the result of a similar calculation for the 1965 year class, which was also present.

A similar correction for the areas of the three vegetation types was necessary in the calculations on changes in larval distribution with season (see section 5.2a). Before calculating the percentage of the total Pyrrhosoma population in each of the three vegetation types, the results for the middle Potamogeton and Potamogeton and Eleocharis were doubled since this had an area approximately twice that of the other vegetation types. (A more precise correction of the type carried out for the estimation of numbers per m^2 was not considered necessary.)

5.1f Catches of Junior Age Class Larvae in Winter

Once sufficient data had been obtained, it became apparent that the winter net catches were reflecting something other than mortality in the junior age class. Table 12 shows the total number of larvae caught in the nine S.N.S. taken per month. Data from the three year classes studied in pond B are presented separately so that equivalent life cycle stages can be compared across the table. (A year class is defined by the year in which the larvae hatched - see chapter 2, section 2.1).

The number of junior age class larvae in the 1966, and 1967 year classes caught in the nine S.N.S. were high in the first autumn, fell sharply in November but increased again the following June before any recruitment in the total population occurred. It is clear that these changes did not reflect mortality and recruitment but were probably due to changes in the availability of larvae to the net.

During the winters of 1966-67 and 1967-68, a large number of collection were made in an attempt to locate these small larvae. Despite an intensive search of all parts of the pond, they were only found in the shallow water at the very base of the plant stems on sides 1 and 2. In order to catch the larvae, the corners of the net had to be pushed firmly into the bottom of the pond so that a normal sample (S.N.S.) was impossible. The small number of junior age class larvae captured in the normal S.N.S. at this time were probably isolated individuals which for some reason had moved rather higher up the vegetation. All

the larvae taken from the base of the plants amongst the rootstocks were associated with a fine, brown, oxygen rich, organic detritus. In deeper water (more than 30-40 cm) black, anoxic detritus prevailed and larvae were never found.

Month	YEAR CLASSES 1965	1966	1967	Reference points in life cycle
Sept		1966 -	1967 114	July hatch
Oct		189	54	
Nov		32	3	First winter as junior
Dec		5	-	age class
Jan	1966	1967 8	1968 -	
Feb		1	2	
Mar		14	5	
April		2	5	
May		1	4	
June		55	56	Larvae become senior
July		114	31	age class following
Aug	study started	92	study ended	hatch of next
Sept	1966	118	1968	generation
Oct	95	41		
Nov	39	30		Entry to final instar.
Dec	(16)*	-		Second winter as senior age class
Jan	1967 12	1968 -		
Feb	18	34		
March	4	19		
April	4	27		Metamorphosis followed by emergence

Table 12 Total number of Pyrrhosoma larvae caught in 9 S.N.S. in each month arranged so that equivalent life cycle stages can be compared across the table.
* sample taken November 28th 1966.

It is clear that because of this behaviour, reliable population estimates could not be made for the junior age class between November and May using the methods employed in the present study. Consequently, the S.N.S. data obtained for the junior age class during this time were omitted from the mortality rate and biomass calculations. (Data obtained for the senior age class between November and May were not, of course, subject to this error and S.N.S. data were used to calculate population changes for the senior age class during this time.)

5.2 RESULTS

5.2a Changes in Seasonal Distribution of Pyrrhosoma Larvae in Pond B

The number of larvae caught each month in each of the three vegetation types were calculated as percentage of the total monthly catch in 9 standard net sweeps (corrected for vegetation area - see section 5.1e). Data from the three year classes (1965, 1966 and 1967) were very similar and were therefore pooled and a mean percentage calculated for each vegetation type each month. In this way the percentage of the total larval Pyrrhosoma population in each of the three vegetation types each month was obtained for a typical development cycle. The results are presented in figure 8.

It is clear that marked changes occured in the distribution of the larvae which may be attributed both to movements between vegetation types and also to differential mortality between vegetation types. For the purposes of the present discussion they are treated entirely as larval movements.

The first S.N.S. samples were taken in September, one to two months after the hatch in July (see section 5.1b). However, samples were taken with a fine p ankton net for growth estimates in July and August and showed that the majority of the larvae occurred in the middle Potamogeton zone at this time. They were therefore still close to the oviposition sites in the Potamogeton.

By September, the junior age class were still present in the Potamogeton, though slightly less than half were already on side 1 in the Juncus articulatus and side 2 in the Juncus effusus.

During October and November, the Potamogeton died down and the larvae moved out so that the middle of the pond was deserted by December. The first winter was spent in the Juncus effusus on side 2 and the Juncus articulatus on side 1. The exact location of the larvae at the base of the plant stems at this timehhas been discussed in section 5.1f.

In the spring following the first winter, the proportion of larvae increased markedly on side 2 which then remained the most important area of the pond. This was accompanied by a decline on side 1. The Potamogeton was also reoccupied by a small number of larvae which disappeared again the following winter.

It is clear that for about six months (January - May) each year the Potamogeton was virtually devoid of any Pyrrhosoma larvae of either size class. It is also apparent that the two size classes were usually well separated in the pond. There were very few senior age class larvae in the Potamogeton (about 10 percent) when the junior age class

hatched; the two age classes overwintered in very different areas of the pond and the large larvae then emerged at about the time when the junior age class spread into the Juncus effusus. This differential distribution with age probably helped to reduce cannibalism by the senior age class on the junior age class (chapter 8).

The unequal distribution of larvae in different parts of the pond raised the question of whether the population data should have been calculated separately for the three vegetation types. Since Potamogeton was relatively unimportant for most of the year, it may have been possible, for example, to eliminate this from the sampling programme and to have taken more samples in the other two areas, (Southwood 1966 p. 17). However, from an ecological energetics point of view, it was obviously more logical to treat the entire pond as a single energy fixing and utilising system of which Pyrrhosoma was an integral part. Final population figures were therefore calculated for the whole pond as the mean number of larvae per square meter of pond surface.

5.2b Mean Number per m^2 and Mortality

Population figures for pond B are presented in table 13 and graphically in figure 9. The first population samples were taken on 4/10/66 and the last on 8/7/68: the 1966 year class was therefore followed through from shortly after the hatch to emergence, whilst the 1965 year class was followed during its second year when it was the senior age class and the 1967 year class in its first year when it was the junior age class. The eggs in Pyrrhosoma hatch in July and,

Sample date and year class	Days from 1st July	Calculated number per square meter
1965 year class		
4/10/66	461	104.7
2/11/66	490	44.2
28/11/66	516	20.3
21/ 1/67	570	11.7
20/ 2/67	600	6.8
18/ 3/67	626	3.9
18/ 4/67	657	3.8
1966 year class		
4/10/66	96	286.5
12/ 6/67	347	55.4
10/ 7/67	375	117.7
1/ 8/67	397	101.7
4/ 9/67	431	138.0
2/10/67	459	48.3
14/11/67	502	35.2
9/2/ 68	599	36.6
25/ 3/68	633	18.4
22/ 4/68	661	26.3
1967 year class		
4/9/67	66	157.7
2/10/67	94	77.4
11/ 6/68	346	55.8
8/ 7/68	373	30.2

Table 13 Population figures in pond B expressed as the mean number of *Pyrrhosoma* larvae per m^2. Data from table 13 have been plotted in fig 9.

for convenience, all sample dates have been calculated as days from July 1st in the year in which the larvae hatched.

The reasons for the lack of data on junior age class larvae between November and May were given in section 5.1f. Data were unavailable for senior age class larvae in December 1966 and 1967 and in January 1968 because thick ice prevented adequate sampling.

Fig 9 shows the number of larvae per m^2 on a log scale plotted against the data of sampling, for the three year classes. Despite scatter, the points clearly fall on straight lines, suggesting constant mortality rates in the three year classes. Three regressions were calculated.

1965 year class $y = -0.0062x + 4.63$

$r = -0.84$

1966 year class $y = -0.0018x + 2.59$

$r = -0.80$

1967 year class $y = -0.0012x + 2.10$

$r = -0.66$

where $y = \log$ (mean number per m^2)

x = days from 1st July in year of hatch.

Although the mortality rate within each year class appears to be constant, there are obvious differences between year classes. Mortality in the 1965 year class was 99.5 percent per annum but only 77.7 percent per annum in the 1966 year class. It may have been slightly lower

again (nearer 70 percent) in the 1967 year class but the small number of sample points made exact estimation for this group impossible. However, mortality rates in the 1966 and 1967 year classes were clearly very similar and were much lower than in the 1965 year class.

The larval density of each year class also varied. There were approximately three times as many junior age class larvae shortly after the hatch in the 1966 year class as in the 1967 year class. Also, the number of senior age class larvae in the 1965 year class was initially much higher than in the 1966 year class, but because of the differential mortality rates, the number of final instars emerging in the 1965 year class was only about one-tenth that of the 1966 year class.

The population data alone indicated that the impact of Pyrrhosoma in the pond ecosystem varied from year to year. This is discussed further in chapter 14.

5.2c Causes of Mortality

Non Predatory Mortality

Dead, apparently undamaged larvae were taken fairly often in the population samples. Although the cause of death was unknown, starvation, bacterial, or virus infection are possiblities.

In the laboratory, virtually all mortality took place at the moult, usually shortly after completion, but sometimes immediately prior to, or in the process of moulting. Again the cause is obscure though it is clear that since the moult is a time of physiological stress, some increased mortality might be expected in animals of reduced vitality

(Clark et al. 1967).

It is unlikely that temperature was ever a direct cause of death. Larvae survived in the laboratory for several days at 0°C and 25°C without suffering any mortality and, on no occasion, was the main water mass in the pond observed to reach these extremes of temperature (see chapter 4, section 4.2).

Predatory Mortality

Casual observations were made in pond B on a number of species that were known, or suspected, to prey on Pyrrhosoma. These observations are summarised in table 14. Column one lists those species that were seen to capture Pyrrhosoma in the sorting tray in the field and the number of observations is given in column two. The caddis larvae were something of a surprise and it is not known whether the Pyrrhosoma captured were healthy, already dead or dying.

Column three shows the results of laboratory feeding experiments carried out with two newts, Dytiscus marginalis adults, Aeshna cyanea larvae, other Zygoptera (Coenagrion puella and Ishnura elegans), Pyrrhosoma itself and Chlorohydra viridissima. In all cases, Pyrrhosoma of a suitable size were offered as the only source of food for a period of 2-3 days, after which alternative prey were provided. Only Aeshna and Chlorohydra readily took Pyrrhosoma. None of the other species took Pyrrhosoma but all fed actively when presented with alternative foods - Asellus (Triturus palustris), earthworms (Dytiscus marginalis) and Daphnia (the remainder).

Potential predator	Seen to capture Pyrrhosoma in field (in sorting trays)	Number of observations of field capture	Observed to take Pyrrhosoma in laboratory feeding experiment	Size of Pyrrhosoma taken in laboratory or field	Zygopteran remains in faeces analysed	Number of observations of Zygoptera in faeces and number of pellets examined
Triturus palustris great-crested newt	NO	-	NO	-	No, only Asellus	0 in 10
T. vulgaris smooth newt	NO	-	NO	-	-	-
Dytiscid larva	YES	2	-	Final instar and small larva	-	-
Dytiscus marginalis adult	NO	-	NO	-	-	-
Notonecta spp.	YES	2	-	Final instar and small larva	-	-
Trichoptera larvae (Limnephilidae)	YES	3	-	Final instar and small larva	-	-
Aeshna cyanea	NO	-	YES	Final instars	-	-
Other Zygoptera	NO	-	NO	-	-	-
Pyrrhosoma nymphula (cannibalism)	YES	1	NO	newly moulted final instar	YES	2 in several 100
Chlorohydra viridissima	NO	-	YES	instar 2	-	-

Table 14. Observations on predators of Pyrrhosoma see text for further details

Triturus palustris faeces were analysed and found to contain Asellus remains only. The results of Pyrrhosoma faeces analysis are reported in detail in chapter 8. Possible cannibalism was noted only twice in many hundred of faecal analyses (see table 26, chapter 8).

It is clear from these fragmentary observations that Pyrrhosoma had a number of predators and that it was not a top carnivore in pond B.

The extent or effect of this predation on Pyrrhosoma in the field is unknown. However, it is possible that part, at least, of the very heavy mortality observed in the 1965 year class in the winter of 1966-67 may be attributed to predation by Aeshna cyanea. A number of penultimate and final instar Aeshna cyanea larvae were present in pond B in the winter of 1966-67 and were most frequently taken under the Juncus on side 2 where final instar Pyrrhosoma were also most common. Aeshna cyanea larvae however, were entirely absent in the following winter (1967-68) when Pyrrhosoma mortality was much lower.

5.3 DISCUSSION

The reasons for chosing the particular sample methods used were discussed earlier. The size of the habitat and the failure of mark-release-recapture methods left no other practical choice.

A number of authors have observed changes in the distribution of Odonata larvae with season similar to those in the present study. Macan (1964) provided data for Pyrrhosoma. Although exact comparison with other studies are difficult because the nature of the various ponds and their vegetation types clearly differed, general comparisons can

be made. Newly hatched larvae tend to be concentrated near to the oviposition sites (Kormondy 1959, Macan 1964), but usually disperse quite quickly. This was found to be the case in the present study. Movements often occur to avoid unfavourable conditions, for example before winter (Corbet 1957a) and toward suitable emergence sites during metamorphosis. It is possible that some of the changes in larval distribution are also related to food supply, larvae moving away from areas of low to areas of high prey density and as much may be termed Aggregative Responses (Hassel 1966).

Both Corbet (1957b) and Macan (1964) reported the "disappearance" of small Pyrrhosoma larvae in winter, Macan suggesting that the larvae "retreat toward the bases of the plant stems at that season". It was initially hoped that by sampling in a small pond, where a more thorough coverage was possible, that this difficulty might be overcome. The present study however simply confirmed this behaviour pattern and provided information on the exact area in which the larvae occurred. Macan also suggested that the senior age class showed a similar winter "disappearance" but this was not noticed in the present study. This was probably because a large number of the larvae studied by Macan took three years to complete development so that in their second winter they were still not in the final instar, and probably showed behaviour patterns which resembled the smaller first winter larvae.

The disappearance of the junior age class in winter in pond 3 gave rise to a period of approximately seven months (November to May) when no reliable population estimates could be made for junior age class larvae. This situation was highly undesirable but unavoidable

and any future population work on Pyrrhosoma should be designed to deal with population changes during this time.

Changes in activity or behaviour, either seasonally or diurnally, e.g. the winter distribution of small larvae, are bound to influence population samples taken with a standard net sweep. However, there was no evidence for any other changes likely to influence the number of larvae caught by the net (other than actual changes in the number of larvae themselves) and application of a constant conversion factor to obtain numbers per m^2 was probably justified. Macan (1964) demonstrated a variable ratio between quantitative samples and net catches for Pyrrhosoma but included both summer and winter catches. Macan's figures were further complicated by the three year life cycle of some of the larvae and are therefore not strictly comparable with the present work.

There are few published figures of larval population density in Odonata. Berg and Peterson (1956) used an Ekman bottom sampler in their study of Lake Gribsø to obtain population figures for the entire community, including Odonata. Cordulia aenia (L.) was the commonest anisopteran with a maximum density of just over 200 per m^2 at a depth of 1.5 m, and 75 per m^2 at 0.5 m depth. Five species of zygopteran were present including Pyrrhosoma; these were grouped and mean zygopteran densities of 12 per m^2 at 0.5 m depth and 25 per m^2 at 1.5 m depth were obtained. This is much lower than was found in pond B though the total dragonfly density in Lake Gribsø including Cordulia was probably

greater. Other low figures have been published. Dineen (1953) obtained only 10 Libellulidae (Anisoptera) per m^2 in a Minnesota pond. Unfortunately he did not sample the Coenagriidae which were "common". Mann (1964) found only 0.07 Agrion splendens (Harr) per m^2 in the River Thames but this very low figure is presumably associated with a habitat that is not particularly favourable for most Odonata in Britain. Ball and Hayne (1952) presented data for all zygopteran larvae in the littoral of Three Sisters Lake. Species were not determined. Peak numbers per m^2 occurred between July and October (presumably after oviposition and hatching) and were very similar to the figures obtained for Pyrrhosoma in the present study, ranging between just over 400 per m^2 in July and August 1941 to just over 100 per m^2 in August and September 1939. The work of Macan (1964) in Hodson's Tarn is the most detailed published information on larval Odonata populations. Estimates of numbers per m^2 were available for Pyrrhosoma, Enallagma cyathigerum (Charp) and Lestes sponsa (Hans.). Population densities for Pyrrhosoma alone were much larger than were obtained in the present study with, for example, over 1,400 newly hatched larvae per m^2 in both Carex and Litorella and 450 second summer larvae per m^2 in Carex. Clearly the density of larval Odonata populations varies widely, as might be expected. From the few published figures available, the numbers found in pond B for Pyrrhosoma would appear to be intermediate between the very high figures found by Macan in Hodson's Tarn and the lower zygopteran figures published by Berg and Peterson in Lake Gribsø.

It was clear that the population densities of the year classes i.e. the initial numbers per m^2, varied from year to year in pond B. This was further complicated by differential mortality rates between the year classes. However, variation in the initial size of the year classes can probably be attributed to the number of eggs laid in the pond (Macan 1964). Prevailing climatic conditions during oviposition, the area of suitable vegetation available for oviposition and territory size in the male may all influence the ultimate size of the new generation.

The only published estimates for larval mortality rates in Odonata are those of Macan (1964 and 1966) in Hodson's Tarn, though because of the three year life cycle shown by some Pyrrhosoma larvae in the tarn, care in comparison of the results is necessary. Pyrrhosoma mortality rates within each year class in Hodson's Tarn were constant, exactly as found in the present study. From Macan (1966a p445 table 7) it is possible to calculate mortality rates varying between 85 percent per annum and 68 percent per annum; a mean of approximately 75 percent per annum is given by Macan (1964). These figures are very similar to those obtained for the 1966 and 1967 year classes in pond B. Clearly both in the amount of mortality and in the constant rate throughout larval life, Macan's data for Pyrrhosoma resembles that obtained for Pyrrhosoma in the present study.

Despite the obvious limitations of the data and methods employed, it is believed that the final population model set up for Pyrrhosoma in pond B is reasonably accurate and is the best yet available for a larval Odonata population. An additional check on the accuracy of the

figures is provided in the section dealing with emergence (chapter 6, section 6.1c).

5.4 POPULATION FIGURES USED IN SUBSEQUENT ENERGY BUDGET CALCULATIONS

For energy flow calculations, a population model assuming constant mortality within a year class was utilised. Population figures were calculated on the first of each month from July 1966 until July 1968, using the three regression equations given in section 5.2b (p72) for the 1965, 1966 and 1967 year classes. All energy flow figures were calculated on the basis of these smoothed monthly population estimates which are presented in table 15.

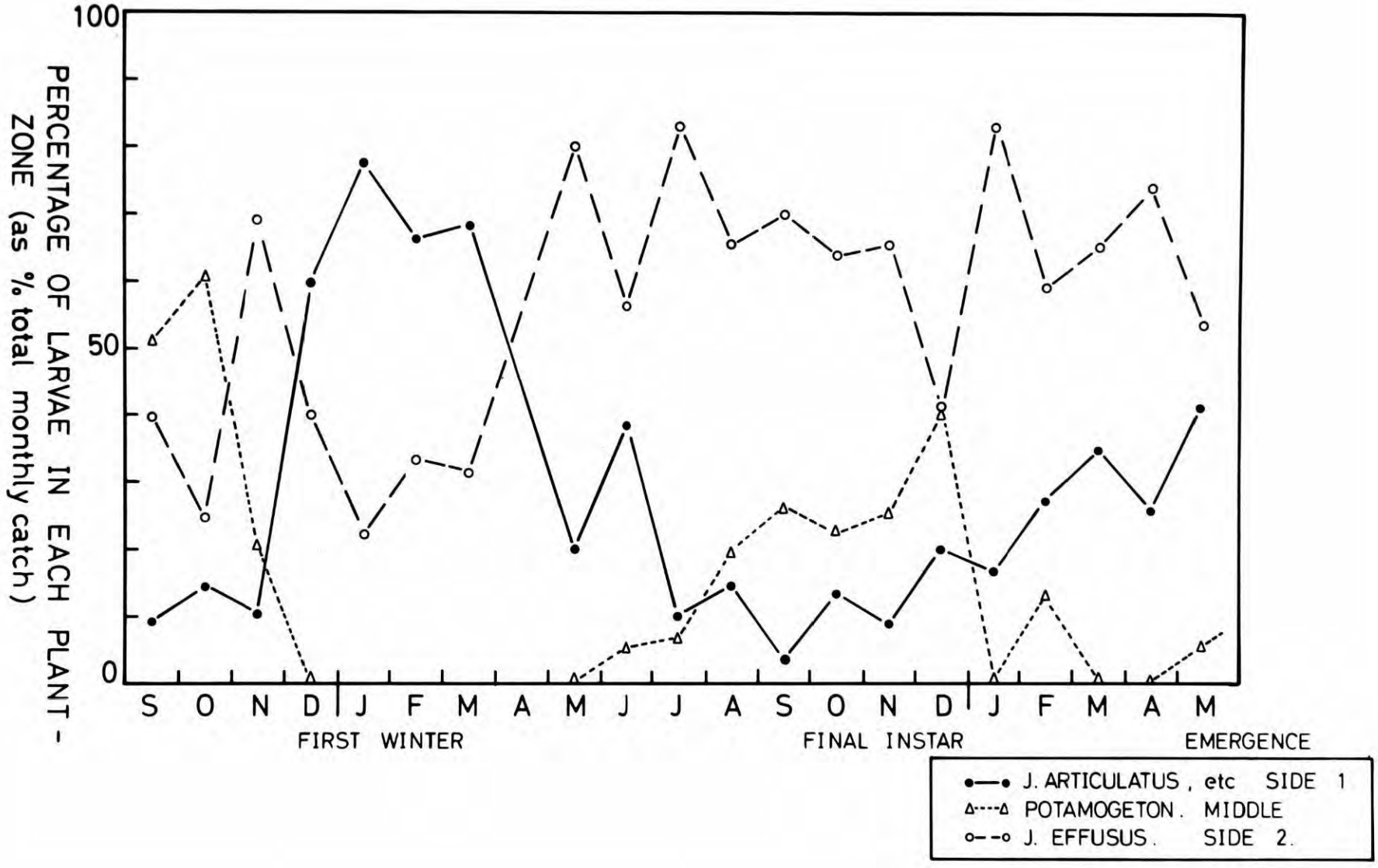
In table 15, the calculated final instar figures for 1 June are bracketed since by this date approximately 50 percent of the larvae had actually emerged (see chapter 6). Numbers in July for the junior age class are calculated at the mean hatching date (see chapter 6) and not on the 1st of the month.

	Number per square meter on the 1st of the month in each year class.		
	1965 year class	1966 year class	1967 year class
1966			
July	226.4	381.6*1	
Aug.	145.3	344.3	
Sept.	93.3	303.1	
Oct.	60.8	267.8	
Nov.	39.0	235.7	
Dec.	25.4	208.4	
1967			
Jan.	16.3	183.3	
Feb.	10.5	161.4	
Mar.	7.0	143.8	
April	4.5	126.6	
May	2.9	111.8	
June	(1.9)	98.5	
July		87.0	119.6 *2
Aug.		76.6	114.0
Sept.		67.4	104.5
Oct.		59.6	96.1
Nov.		52.5	88.1
Dec.		46.4	81.0
1968			
Jan.		40.8	74.2
Feb.		35.9	68.0
Mar.		32.0	62.9
April		28.2	57.7
May		24.9	53.0
June		(22.1)	48.6
July			44.7

Table 15 Calculated population figures (numbers per m^2 on the first of each month in the three year classes studied. These figures are the ones utilised in subsequent energy budget calculations.

*1 and *2 numbers per m^2 on the average hatching date (July 7th 1966 and July 15th 1967).

Fig. 8. Seasonal changes in the distribution of Pyrrhosoma larvae throughout development in pond B, based on the percentage of larvae in each of the three vegetation types. Pooled data from three year classes.


100
50
0
PERCENTAGE OF LARVAE IN EACH PLANT-ZONE (as % total monthly catch)
S O N D J F M A M J J A S O N D J F M A M
FIRST WINTER
FINAL INSTAR
EMERGENCE
J. ARTICULATUS , etc SIDE 1
POTAMOGETON . MIDDLE
J. EFFUSUS . SIDE 2.

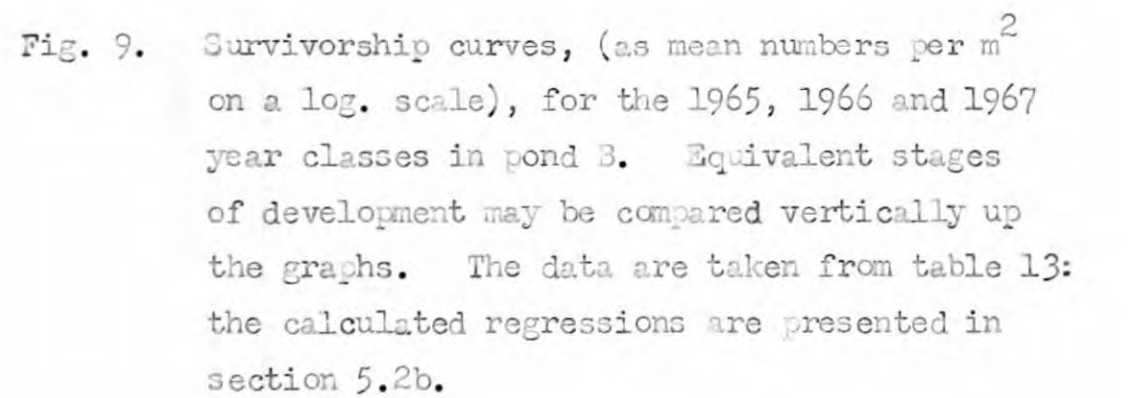
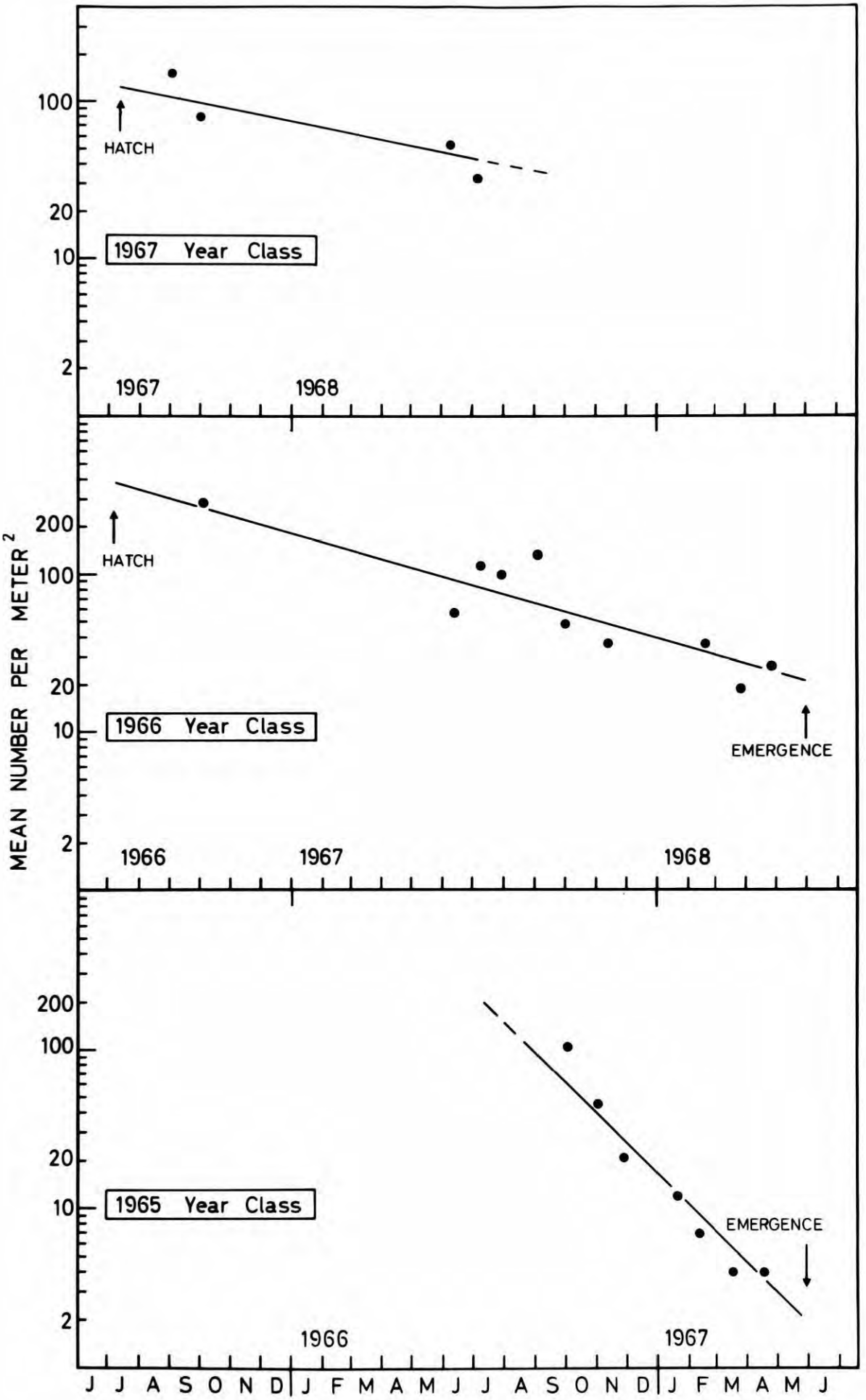


Fig. 9. Survivorship curves, (as mean numbers per m^2 on a log. scale), for the 1965, 1966 and 1967 year classes in pond B. Equivalent stages of development may be compared vertically up the graphs. The data are taken from table 13: the calculated regressions are presented in section 5.2b.


MEAN NUMBER PER METER 2
100
20
10
2
HATCH
1967 Year Class
1967
1968
200
100
20
10
2
HATCH
1966 Year Class
EMERGENCE
1966
1967
1968
200
100
20
10
2
1965 Year Class
EMERGENCE
1966
1967
J J A S O N D J F M A M J J A S O N D J F M A M J

Chapter 6

POPULATION STUDIES PART 2

(Metamorphosis, Emergence and Reproduction. Observation on Sex Ratio.)

This chapter includes observations that were essential for a detailed calculation of energy flow through Pyrrhosoma populations. The problems dealt with are among the most interesting and complex encountered in the general biology of Odonata. Some of the data obtained in the present study are relevant to these general problems, for example, the effects of photoperiod and temperature on metamorphosis and emergence, or the sex ratio of Odonata larvae. However, whilst recognising their importance, discussion on these general aspects of odonatan biology has been omitted or dealt with only very briefly. Only the data essential to the main problem has been presented.

6.1 METAMORPHOSIS, EMERGENCE AND OVIPOSITION

6.1a Metamorphosis

Patterns of metamorphosis were studied in pond B in 1967 (1965 year class) and ponds B and F in 1968 (1966 year class). Samples were taken more frequently than in the normal monthly population study but the sampling points were fixed on the usual random basis in the sampling grids. From April onwards, sampled larvae were assigned to specific stages of metamorphosis in the field and returned to the pond immediately. The three metamorphosis stages described in chapter 2 (section 2.6) were

utilised throughout.

Figure 10 shows the percentage of the senior age class in each stage of metamorphosis from March until the start of emergence in May. Sampling ceased after emergence began.

The patterns were similar in all cases. No larvae entered metamorphosis before 20 March and by the end of March only 20-30 percent of the larvae were in stage 2 metamorphosis. During April more larvae entered this stage so that by the end of April, at least 70 percent had entered stage 2. In May, events proceeded more rapidly, the majority of larvae (90 percent) being in stage 2 by 10 May and the first larvae entered stage 3 between 10 May and 15 May. In 1968 events in pond F suddenly slowed down after 15 May but in pond B in 1967 and 1968 they continued rapidly with over 50 percent of the larvae entering stage 3 metamorphosis shortly after 20 May. The main emergence started in pond B on 26th May 1967 and 23 May 1968, but not until 28 May 1968 in pond F.

For the purpose of energy budget calculations, the different stages of metamorphosis were important. Table 16 shows the significant dates during metamorphosis.

6.1b Emergence

Emergence was studied by collecting exuvia, which if carried out carefully can provide information on a number of aspects of larval life (Corbet 1962). Pyrrhosoma exuvia were usually found low down among the Juncus and Eleocharis stems about 10-20 cm above the water surface. Occasionally they were found much higher and very rarely in other sites

Year Class	Year of Emergence	Pond	Dates by which Specif ied Points were Reached									Days from start of emergence to E.M.50 point.	Total days in emergence
			Metamorphosis		Emergence			Adult Reproductive Sequence					
			50% enter stage 2	50% enter stage 3	1st day of emergence	50% emergence E.M. 50	Final day of emergence	First ♂ return to pond	First ♀ return to pond	Fairing and laying commenced	Maximum oviposition activity	Last date of oviposition	
1964	1966	B	-	-	16/5	-	about 9/6	"Present a few days before laying		2/6	5-6/6	after 9/6	-	-
1965	1967	B	10/4	25/5	26/5	1/6	14/6	7/6	11/6	12/6	13-15/6	before 28/6	7	20
1966	1968	B	17/4	23/5	23/5	30/5	16/6	-	-	-	-	-	8	25
1965	1967	F	-	-	25/5	29/5	14/6	8/6	11/6	12/6	13-15/6	before 28/6	6	21
1966	1968	F	20/4	about 25/5	28/5	1/6	-	-	-	-	-	-	-	-

Table 16 Summary of main dates in metamorphosis, emergence and reproduction cycles of Pyrrhosoma in ponds B and F. See text for details.

e.g. on protruding Potamogeton leaves close to the bank. The exuvia were easily recognised, retaining the characteristic shape of the larva and were considerably more robust than those of other Coenagriidae in the ponds. They could be sexed easily.

From observation, the majority of larvae emerged before mid-day, a fact noted by Lucus (1930) and Corbet (1952). Consequentally, all exuvium collections were made in the afternoon mostly between 16.00 and 19.00 h (B.S.T.). In order to facilitate collections, the thickest Juncus effusus clumps in B and F were either cut down or thinned a few days before emergence started. The large size of pond B meant that only zones 2 and 3 were searched; whereas the whole of pond F was covered.

The date of first emergence was studied in Pond B in 1966 (1964 year class) and pond F in 1968 (1966 year class). In both cases the ponds were visited each day for one week before the expected emergence date. When the first exuvia were noted, detailed studies ceased. More detailed studies were made in ponds B and F in 1967 (1965 year class) and pond B in 1968 (1966 year class). In these cases, exhaustive collections of exuvia were made daily within the areas defined so that total emergence figures were obtained over the complete emergence cycles. Observations were continued for several days following the discovery of the last exuvia to ensure that emergence was actually finished.

Fig 11 shows the results of the detailed daily emergence studies; table 16 shows dates of significance in the emergence cycles.

The total number of larvae emerging each day in the detailed emergence studies were:-

Pond B 1967 (zones 2 and 3)	70 ♂	68 ♀	6 unsexed; TOTAL 144
Pond B 1968 (zones 2 and 3)	406 ♂	383 ♀	25 unsexed; TOTAL 814
Pond F 1967 (whole pond)	75 ♂	74 ♀	14 unsexed; TOTAL 163

(When the sex was undetermined, the exuvium was either lost or damaged before it could be sexed.)

Fig 11 shows that a small number of larvae emerged in pond B between 16/5/68 and 22/5/68; this clearly represents an early emergence by an small group of larvae (19 in a total of 814 or about 2 percent) out of phase with the rest of the population. The phenomenon was not apparent in either B or F in 1967 nor in Hodson's Tarn (Macan 1964). Since the emergence in B in 1968 was extremely large, it is possible that this phenomenon is typical of Pyrrhosoma but was over looked in the other cases because of the smaller numbers involved. Alternatively and perhaps more likely, these larvae may represent a small percentage of the population in which emergence had been affected by the sampling programme, particularly by being brought into the laboratory in February and March. They were therefore omitted from the data on emergence, which was assumed to have started on 23/5/68 in pond B.

A useful statistic in comparing the amount of synchronisation shown by a population at emergence is the number of days elapsing between the start of emergence and the date by which 50 percent of the emergence has taken place - the E.M.50 point (Corbet 1962). The dates by which 50

percent of the larvae had emerged are shown in table 16, together with the number of days from the start of emergence to the E.M.50 point. This was reached after 6-8 days emergence in Pyrrhosoma in a total emergence cycle of 20-25 days.

6.1c Use of Emergence Data to Check Population Figures in pond B

A complete collection of exuvia was made within a defined area of pond B (zones 2 and 3) and it was therefore possible to calculate the number of adults emerging per m^2 of pond surface and hence obtain a completely independent check on the population data presented in chapter 5.

From the regression equations given in section 5.2b, the number of final instars per m^2 at the start of the annual emergence was calculated.
Calculated number of 1965 year class per m^2 on 26/5/67 = 2.1
Calculated number of 1966 year class per m^2 on 23/5/68 = 22.7
Then, the total area of zones 2 and 3 was 56.1 m^2 (see chapter 4, section 4.3).
Total emergence 26/5/67 to 14/6/67 from zones 2 and 3 = 144 or 2.6 per m^2.
Total emergence 16/5/68 to 16/6/68 from zones 2 and 3 = 814 or 14.5 per m^2.

Therefore in 1967 2.6 adults emerged per m^2 compared with an estimated number of final instars present at the start of emergence of 2.1 per m^2. The number emerging was therefore 23.8 percent more than the estimated number present. In 1968, 14.5 adults emerged per m^2 compared with an estimated number of final instars present at the start of emergence of 22.7 per m^2. The number emerging was therefore 34.1 percent less than the estimated number present.

Errors of ±30 percent are not infrequent in population studies. Also the two estimated of population density based on the emergence data lie as close to the calculated regression lines shown in fig 9 as many of the sample points on which the lines are based. The task of finding exuvia was difficult and only part (just over half) of the pond was searched. In view of these facts, the agreement between the estimates based on the mortality rate regressions from standard net sweep data, and those based on exuvium counts is considered reasonable.

Because the regression lines were based on a series of points, the population of final instars per m^2 at emergence was calculated from the regression equations and not from the exuvium counts.

6.1d Oviposition and Hatching

To determine the data on which Pyrrhosoma returned to the pond after emergence and the approximate peak egg laying period, observations were made on pond B in 1966 and ponds B and F in 1967.

Preliminary laboratory studies on the effects of temperature on egg development were carried out. Weekly samples were taken in the study ponds with a fine plankton net, near the expected hatching dates to determine the date on which instar 2 larvae first appeared in the pond.

Tables 16 summarises the dates on which adult Pyrrhosoma returned to the ponds, paired and layed eggs, in 1966 and 1967.

In both years, the first adults to return to the ponds were males approximately two weeks after the start of emergence. Peak oviposition

activity occurred three weeks after the start of emergence and, in 1967, two to three weeks after the period of peak emergence. Adults were present at the study ponds for a period of about three weeks, whilst all oviposition was restricted to a period of about two weeks.

In July 1966, pairs in tandem were observed ovipositing in the leaves and stems of Potamogeton and Ranunculus flammula L. in the marsh complex. The plants containing the eggs were collected immediately after laying and transported to the laboratory where they were placed in small tanks of filtered pond water in 10, 15 and 20°C constant temperature rooms. Except in the 10°C C.T.R., the eggs experienced natural photoperiod.

From a total of 13, only 5 batches of eggs were successfully hatched. None hatched at 10°C either because the temperature was too low or because the eggs experienced continuous dull illumination only. 2 batches hatched and 3 failed to hatch at 15°C, and 3 hatched and 2 failed to hatch at 20°C. The eggs were examined every day and newly hatched larvae were removed as they appeared. The data obtained are shown in table 17.

Development time at any one temperature was remarkably constant and the effect of temperature pronounced. Very few studies have been made, under controlled conditions, of the duration of the egg stage in Odonata. The results were summarised by Corbet (1962) and are very similar to those obtained for Pyrrhosoma in the present study.

On the basis of these laboratory data, weekly samples with a fine

Temperature	Days from laying to first hatch	Number of days for which hatching continued	Days from laying when maximum number hatched
15°C	44	2	44
15°C	43	2	44
20°C	21	3	21
20°C	21	4	23
20°C	22	6	23

Table 17 Effect of temperature on egg development time in Pyrrhosoma.

plankton net were started approximately three weeks after oviposition was first observed, thereby permitting determination of the hatching date in the field.

Pond B 1966 No newly hatched larvae were present on 30/6/66, 24 days after maximum oviposition activity, but a week later, on 7/7/66, newly hatched instar 2 larvae were abundant in the Potamogeton.

Ponds B and F 1967 No newly hatched larvae were present on 30/6/67 or 6/7/67 but they were abundant on 15/7/67 in the Potamogeton in both ponds.

The dates on which instar 2 larvae were first found were just over 30 days after the maximum oviposition activity in the two ponds, suggesting a surface water temperature of between 15 and 20°C over this period. This agrees closely with the sucrose inversion measurements of pond

temperature made in 1967; (chapter 4).

6.2 DISCUSSION ON METAMORPHOSIS, EMERGENCE AND REPRODUCTION STUDIES

Corbet (1957b) observed the incidence of metamorphosis in Pyrrhosoma larvae from three habitats, but did not distinguish between the metamorphosis stages. None of the populations studied showed any metamorphosis before 1 March, but by the end of that month between 60 and 80 percent of the larvae had entered metamorphosis and the entire final instar population had done so by the end of April. The incidence of metamorphosis was obviously slightly in advance of that at Brasside, but the general picture was very similar.

Corbet (1957a) studied the metamorphosis stages passed through by Anax larvae in a manner similar to the present study. The total time spent in metamorphosis was approximately 45 days, rather less than the time in Pyrrhosoma. Corbet also showed that within the population there was a small percentage of larvae that entered metamorphosis late, at a time when the majority of the population were ready to emerge. In all probability, these were precocious one year old larvae that moulted to the final instar in spring, though slowly growing two year old larvae might produce a similar effect. During the present study, larval growth was extremely well synchronised (see chapter 7); late, poorly synchronised metamorphosis by a small group of Pyrrhosoma larvae did not occur. This had important consequences for the synchronisation of emergence, oviposition and the hatch of the new generation.

In Anax imperator, the "classical" spring emergence species, 50 percent of the annual emergence occurred in the first 3 days of a 50 day emergence period (Corbet 1957a). In Pyrrhosoma, the $E.M._{50}$ points occurred 6 to 8 days after the start of emergence, in emergence periods of just over 20 days. Emergence in Pyrrhosoma was therefore less explosive than in Anax: this is confirmed by Macan's (1964) data on the emergence of Pyrrhosoma in Hodson's Tarn, and also by Corbet (1952). It is particularly noticable that the emergence curves obtained by Macan (1964) and in the present study, though still positively skewed are much less so than the extremely skewed distributions found in Anax. Nevertheless, compared with a typical summer emergence species such as Aeshna cyanea, with an $E.M._{50}$ point reached after 25 days (S.A. Corbet 1959), emergence in Pyrrhosoma is still extremely well synchronised.

In one respect however, emergence of Pyrrhosoma at Brasside was better synchronised than in Anax. Characteristic of the Anax emergence curve (and also present in the spring emergence species Tetragoneuria cynosura (Say) studied by Kormondy 1959) was a long tail in which a small number of larvae continued to emerge for several weeks after the main emergence was over, and which sometimes gave rise to a second very small emergence peak. Some populations of Pyrrhosoma obviously showed this phenomenon too (Corbet 1952, 1957b), but it was conspicuously absent in the Pyrrhosoma populations studied at Brasside. The tail and second small emergence peak appear to be produced by larvae that moult to the final instar in spring, enter metamorphosis late (see above) and

emerge essentially as summer species (Corbet 1962). Since larvae showing this late moult to the final instar were absent from the populations in ponds B and F, the absence of the tail at emergence is readily explicable.

Corbet (1952) showed that the pattern of oviposition in Pyrrhosoma, although displaced by approximately two weeks, followed that of the emergence curve very closely. A similar maturation period between emergence and oviposition of approximately two weeks was confirmed in the present study. However, because of the lack of a tail on the emergence curve, the period over which mature adults were present at Brasside appears to have been shorter than in the Pyrrhosoma population studied by Corbet (1952).

Preliminary laboratory studies showed that egg hatching in Pyrrhosoma was itself well synchronised.

It is clear from the above that larval recruitment in the study ponds took place over a comparatively restricted period of probably not more than three weeks. The majority of young larvae probably hatched within an even shorter period. This had important consequences for the study of growth rates in the field which, because this remarkable synchronisation persisted throughout larval life, could be estimated with very great accuracy.

6.3 SEX RATIO

Pyrrhosoma larvae could be sexed from about instar 8 or 9 (length 6-7 mm); before this, from about instar 5 or 6 (length c.3 mm), signs

of the gonapophyses are present on the 9th abdominal segment but these all resemble those of the male. Drawings of the gonapophyses and cercoids of male and female Pyrrhosoma larvae are shown by Gardner and MacNeill (1950).

Sex ratio data were collected before it became apparent that there was little or no difference between the energy balance of the sexes. The slight tendency for female final instars to be heavier than males was discussed in chapter 2.

The results, obtained from population samples in both ponds B and F, are presented in table 18. Only those samples where $n > 20$ have been included. There is a clear, if slight, excess of males in the populations of both ponds, in three different year classes, with only occasional samples showing an excess of females. A number of observations suggested that occasionally groups of larvae of virtually all one sex were found in parts of the ponds: no detailed studies of this phenomenon were made but it might repay further investigation.

Once it became apparent that the sexes did not differ in their energy requirements, the sex ratio data were not required in subsequent energy budget calculations. They are presented because this appears to be the first time that a larval Odonata population has been regularly sampled in this way to provide information on the sex ratio, though a number of workers (e.g. Corbet 1967a, Kormondy 1959) have obtained ratios at emergence from exuvial collections of Anisoptera. It is also the first detailed data to be obtained for Zygoptera.

In all Anisoptera studied, there appears to be a slight excess of

females, with males forming between 41 and 49 percent of the population (Corbet 1962). Johnson (1963) has discussed the significance of this imbalance. In a preliminary investigation of Pyrrhosoma, Corbet (1952, 1962) found an excess of males at emergence, a result confirmed in the present study. If Pyrrhosoma is typical of the Zygoptera as a whole, then the sex ratio imbalance would appear to be the reverse of that found in the Anisoptera.

One possibility not examined in the discussion on Odonata sex ratios is the suggestion by Klomp (1964) that in many insects, the homogametic sex is generally at an advantage when the population is high and intra-specific competition increases. In Odonata, the homogametic sex appears to be the female and it would be interesting to examine sex ratios within one species at widely varying larval densities.

	Number of ♂	Number of ♀	Total	% ♀♀ in population
1964 year class pond B				
21/3/66 - 28/3/66	126	119	245	48.6
1965 year class pond B				
27/ 7/66	21	29	50	58.0
31/ 7/66	19	16	35	45.6
1/ 9/66	10	15	25	60.0
2/ 9/66	19	22	41	53.7
4/10/66	19	20	39	51.3
2/11/66	20	8	28	28.6
28/11/66	12	10	22	45.5
20/ 2/67	12	8	20	40.0
Emergence	70	68	138	49.3
1966 year class pond B				
1/ 8/67	27	25	54	46.3
4/ 9/67	32	23	55	41.8
2/10/67	22	19	41	46.3
13/11/67	14	21	35	60.0
12/12/67	17	10	27	37.0
12/12/67	19	16	35	45.7
15/ 1/68	16	12	28	42.9
9/ 2/68	19	18	37	48.7
25/ 3/68	13	15	28	53.6
22/4/68	15	18	33	54.6
14/ 5/68	20	12	32	37.5
Emergence	406	383	789	48.5
1965 year class pond F				
Emergence only	75	74	149	49.7
1966 year class pond F				
2/ 8/67	29	29	58	50.0
5/ 9/67	37	22	59	37.3
3/10/67	26	23	49	46.9
16/10/67	14	8	22	36.4
11/11/67	19	14	33	42.4
18/11/67	12	9	21	42.9
16/ 1/68	15	16	31	51.6
18/ 2/68	13	17	30	56.7
26/ 3/68	20	14	34	41.2
22/ 4/68	14	15	29	51.7

Table 18 Sex ratio of Pyrrhosoma in ponds B and F.

Fig. 10. The pattern of metamorphosis in final instar Pyrrhosoma larvae in pond B (1967 and 1968) and pond F (1968). Metamorphosis stages are described in chapter 2, section 2.6.

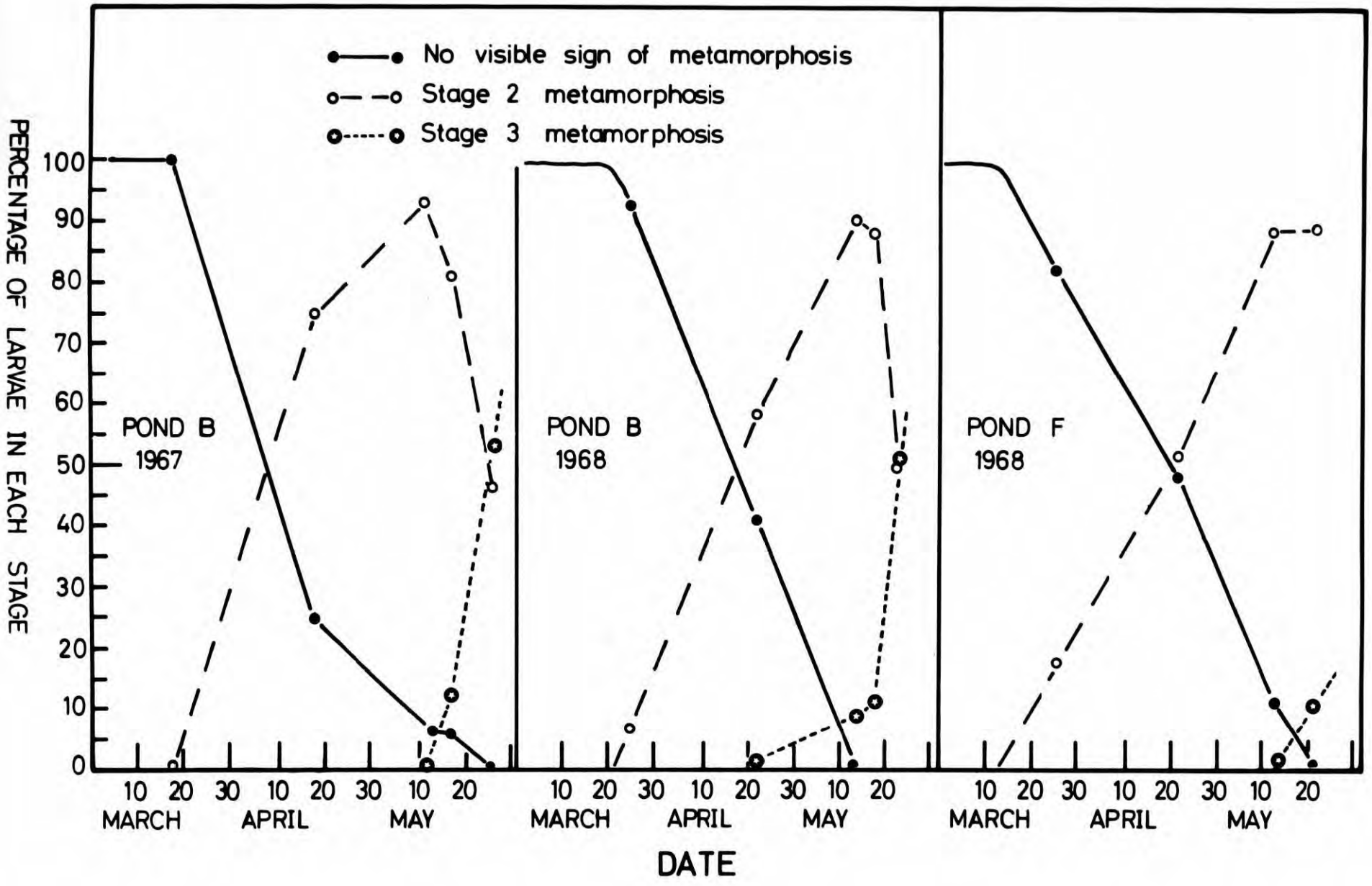
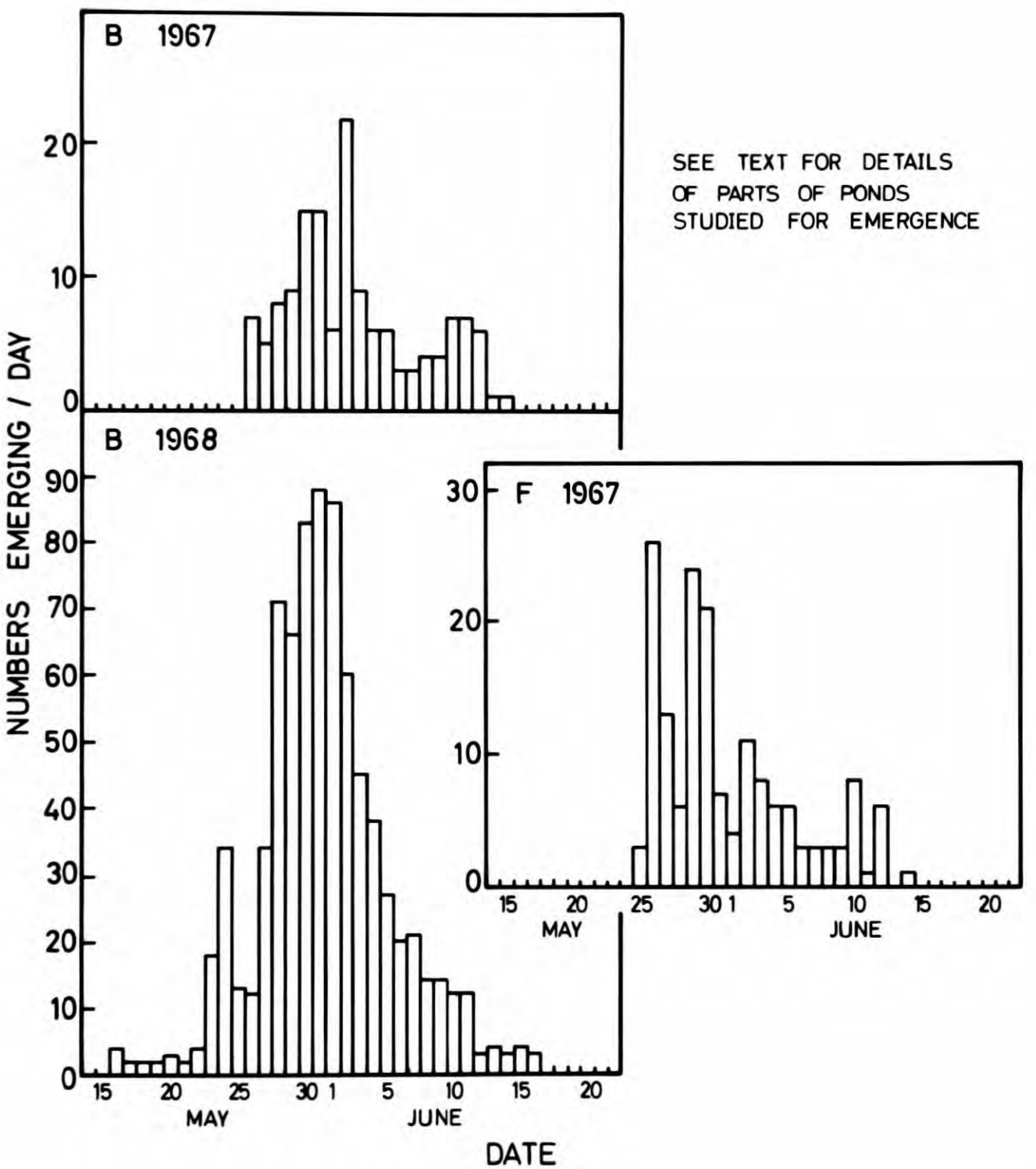

No visible sign of metamorphosis
Stage 2 metamorphosis
Stage 3 metamorphosis
PERCENTAGE OF LARVAE IN EACH STAGE
100
90
80
70
60
50
40
30
20
10
0
POND B
1967
POND B
1968
POND F
1968
10 20 30 10 20 30 10 20
MARCH APRIL MAY
DATE

Fig. 11. The pattern of daily emergence in pond B (1967 and 1968) and pond F (1967). The figures are for total daily emergence of Pyrrhosoma in pond F, and daily emergence from zones 2 and 3 in pond B, based on counts of exuvia.


B 1967
B 1968
F 1967
NUMBERS EMERGING / DAY
DATE
MAY
JUNE
SEE TEXT FOR DETAILS
OF PARTS OF PONDS
STUDIED FOR EMERGENCE

Chapter 7

GROWTH (P)

7.1 FIELD GROWTH CURVES

7.1a Methods

The well synchronised emergence, followed by an equally restricted oviposition period in Pyrrhosoma meant that the annual hatch occurred within a comparatively short space of time. This resulted in a well defined cohort, or year class, easily recognisable until the larvae emerged two years later. A life history of this type provided exceptional opportunity to measure growth rates in the field.

Larvae were transported to the laboratory and measured as soon as possible after collection, following the procedure outlined in chapter 2, section 2.3. The exception was in April and May when final instars were measured in the field with callipers and returned to the pond immediately.

Pond B

The samples used in obtaining population estimates were also used to provide growth data. Nine samples were available each month, three in each of the three vegetation types. Where the number of larvae captured per S.N.S. was high, only the first 10 from each sample were brought back to the laboratory to be measured.

During the winter, very few small larvae were usually obtained in the nine sweeps (see chapter 5, table 12) and additional random sweeps were taken: these extra samples were used for growth estimate only and were never utilised in the population date. Newly hatched larvae in July and August were able to pass through the 1mm mesh of the net used for Standard Net Sweeps (See chapter 5, Section 5.1b). Consequentially, until September, growth of the newly hatched junior age class was obtimated from samples taken with a fine plankton net.

Growth data were obtained in pond B from July 1966 until July 1968. The 1966 year class was therefore followed for the whole of their development; the 1965 year class during its second year and the 1967 year class from hatching until the end of their first year.

PondF

The methods used were as described for pond B. Three samples were taken each month, the sampling points being selected at random from the nine rectangles into which F was divided by the sampling grid.

Growth data in pond F were obtained between February 1967 and May 1968. The 1966 year class were therefore followed from the end of their first winter until emergence and the 1967 year class from hatching in July until the end of February 1968. By com-

bining the end of the 1967 year class data with the beginning of the 1966 year class data, a complete growth curve was obtained for larvae in pond F, which provided a useful comparison with data from pond B.

7.1b Results

Figures 12 and 13 show the growth curves obtained in ponds B and F where the mean lengths of the larvae in mm are plotted against the data of collection. All means are based on samples of at least 10 larvae and the majority of points are based on samples of 20-40 larvae each: ±2 standard errors of the mean are shown. Smoothed curves have been fitted by eye through the points and require little explanation except during the first winter (November - May) in each year class, the period when the junior age class larvae were most difficult to obtain (see chapter 5, section 5.1f).

In pond F, small larvae were more readily available during the winter than in pond B and the data obtained for the 1967 year class showed that no detectable growth occurred during the winter, at least until the end of February (see figure 13). The 1966 year class showed signs of a very slight increase in length in March and April but this did not become significant until May. The less satisfactory data for pond B (figure 12) nevertheless suggested a similar growth pattern during the first winter; though

the sample points shown in figure 12 tended to be more scattered. A number of samples were also available where n 10 and which were therefore not shown in figure 12; by combining these with the sample points in figure 12 and by assuming that no growth occurred in the small larvae between November and March, a large sample was available from which the mean larval length over the first winter could be calculated. This was carried out separately for the two winters 1966-67 and 1967-68; the results were as follows.

2.11.66 to 18.3.67 Mean length 3.43 mm ± 0.16 (n = 74)

14.11.67 to 25.3.68 Mean length 3.44 mm ± 0.20 (n = 69)

(± two standard errors shown)

The mean lengths in the two winters in pond B were clearly identical and both were very similar to the mean sizes of larvae in pond F over the same period (approx. 3.4 mm from the curve drawn by eye to the data on F). Between the dates given the growth curve was drawn using the two calculated means, which fit well with the points on either side of this period.

Although the junior age class appeared to show no detectable growth during the winter, the senior age class (consisting entirely of final instars - see below) did increase slightly in length over the same period.

The small standard errors on most of the data show how well growth was synchronised throughout life. No evidence was found for isolated larvae completing development in one year, or extending development over three years and no larvae were found that had mouted to the final instar in spring prior to emergence.

Moult to Final Instar

The percentage of the senior age class in the final instar is shown in table 19. The data were again obtained from the monthly population samples.

Date of sample	n	Percentage of senior age class in final instar
Pond B 1965 year class		
1/9/66	41	0
4/10/66	40	85
2/11/66	28	100
Pond B 1966 year class		
4/9/67	55	0
2/10/67	41	78
14/11/67	35	100
Pond F 1966 year class		
5/9/67	59	0
3/10/67	49	76
11/11/67	33	100

Table 19 Percentage of senior age class in final instar.

The well synchronised growth of the larvae is reflected in the moult to the final instar, over 75 percent doing so within one month (September). The whole cohort in each year had entered the final instar by November and no penultimate instars were taken despite extensive sampling for the rest of the winter.

7.2 GROWTH EXPRESSED AS WET WEIGHT, DRY WEIGHT AND CALORIFIC VALUES

From the smoothed growth curves, the mean lengths of the larvae on the 1st of each month were obtained. Using the equations given in chapter 2, section 2.3, these were converted to mean wet weights (which were used to calculate population respiration rates) and to mean dry weights: finally, the dry weights were converted to calories from the information given in chapter 3. These data are presented in tables 20-23, which require explanation on a number of points.

In the column headings, the "1ist of the month" has been used for convenience because, in most cases, it does indeed denote this. However, at the hatch of the junior age class in July it denotes the mean length, wet weight etc. on the date of hatching (7/7/66 and 15/7/67). During metamorphosis in April and May, divisions shorter than one month were necessary and the "1st of the month" denotes the weight etc. at the start of these shorter time intervals.

Different regressions were used to calculate final and non-final instar weights. Therefore the smoothed mean population length could not be used to calculate mean larval weights in October, when only part of the senior age class were in the final instar (see table 19). In October,

mean lengths of final and non-final instars (the latter were mostly penultimate instars) were both recalculated separately from the population data, and used to calculate mean weights of both groups using the appropriate regression. The separately calculated wet and dry weights were then pooled according to the percentage of final instars in the population.

Calculation of data during metamorphosis was more complicated. Mean lengths were not calculated, since the relationship between length and wet weight was not known for metamorphosing larvae. Mean wet weights were not required since respiratory data was obtained for larvae in different stages of metamorphosis and could be applied directly to field populations (chapter 10).

Growth during metamorphosis was calculated as follows:- The dates on which 50 percent of the larvae entered each stage of metamorphosis and by which 50 percent of the emergence had taken place were noted from the information presented in chapter 6 (table 16). These represented the dates at which average members of the population entered each successive stage. The mean dry weights of stage 2 and stage 3 metamorphosing larvae (see chapter 2, section 2.6) were then entered in the tables at the last date in which 50 percent of the population were in that stage. This was an approximation: ideally the mean weights should have been entered mid-way through the development of each metamorphic stage in the field, but information on this point was lacking. For example, it is clear that in the case of stage 2 metamorphosis the date half-way between 50 percent entering

Month	Smoothed length on 1st of month (mm)	Wet weight on 1st of month (mg)	Dry weight on 1st of month (mg)	Mean calories/ individual on 1st of month	g. calories growth during month
July 1966	1.30	0.063	0.011	0.056	0.110
August 1966	1.87	0.178	0.032	0.166	0.255
Sept 1966	2.55	0.431	0.082	0.421	0.421
Oct 1966	3.25	0.861	0.164	0.842	0.133
Nov 1966 to Feb 1967	3.43	1.003	0.190	0.975	0.000
March 1967	3.43	1.003	0.190	0.975	0.056
April 1967	3.50	1.064	0.201	1.031	0.454
May 1967	4.00	1.557	0.289	1.485	0.757
June 1967	4.65	2.392	0.438	2.242	2.259
July 1967	6.00	4.949	0.878	4.501	5.042
August 1967	7.90	10.84	1.862	9.542	8.144
Sept 1967	9.90	20.64	3.451	17.686	10.171
Oct 1967 penultimate instar	11.14	28.90	4.766	27.857	9.233
final instar	12.16	43.96	4.469		
Nov 1967	12.70	46.38	7.037	37.090	2.295
Dec 1967	12.85	47.05	7.473	39.385	0.766
Jan 1968	12.90	47.27	7.618	40.151	1.530
Feb 1968	13.00	47.72	7.908	41.681	2.296
March 1968	13.15	48.39	8.344	43.977	9.184
April 1968	13.75	51.08	10.087	53.161	13.529
17th April 1968 - 50% population enter stage 2 metamorphosis.			-	-	-
1st May 1968			-	66.690	13.529
23rd May 1968 - 50% enter stage 3 metamorphosis.			14.73	80.218	-8.140
30th May 1968 - 50% emerge			13.62	72.078	-

Table 20. Growth expressed as wet and dry weights and calorific value. 1966 year class pond B. For details see text.

Month	Smoothed length on 1st of month (mm)	Wet weight on 1st of month (mg)	Dry weight on 1st of month (mg)	Mean calories/ individual on 1st of month	g. calories growth during month
July 1966	5.75	4.382	0.782	4.006	5.311
August 1966	7.83	10.57	1.818	9.317	8.128
Sept 1966	9.85	20.33	3.404	17.445	7.248
Oct 1966					
Penultimate instar	11.04	28.17	4.650	24.693	7.804
Final instar	11.90	42.79	4.714		
Nov 1966	12.40	45.03	6.166	32.497	3.827
Dec 1966	12.65	46.15	6.892	36.324	3.827
Jan 1967	12.90	47.27	7.618	40.151	3.826
Feb 1967	13.15	48.39	8.344	43.977	8.419
March 1967	13.70	50.86	9.941	52.396	3.061
April 1967	13.90	53.00	10.522	55.457	12.381
10th April 1967 - 50% of population enter stage 2 metamorphosis.			-	-	-
1st May 1967			-	67.837	12.382
25th May 1967 - 50% enter stage 3 metamorphosis.			14.73	80.218	-8.140
1st June 1967 - 50% emerge			13.62	72.078	-

Table 21 Growth expressed as wet and dry weights and calorific values. 1965 year class pond B. For details see text.

stage 2 and 50 percent entering stage 3, was not the mid development point because development proceeded more rapidly towards the end as pond temperatures increased.

G. cals. per individual were estimated on 1st May. This may be illustrated using the data for the 1966 year class in pond B (see Table 20). The calorific values per individual on 1/4/68 and 23/5/68 were 53.161 and 80.218 calories respectively, so that growth between these dates amounted to 27.057 calories. It was assumed that growth rates between 1/4/68 and 1/5/68 and between 1/5/68 and 23/5/68 were equal.

	Smoothed length on 1st of month (mm)	Wet weight on 1st of month (mg)	Dry weight on 1st of month (mg)	Mean calories/ individual on 1st of month	g. calories growth during month
July 1967	1.30	0.063	0.011	0.056	0.069
August 1967	1.70	0.136	0.024	0.125	0.272
Sept 1967	2.50	0.407	0.078	0.397	0.445
Oct 1967	3.25	0.861	0.164	0.842	0.142
Nov 1967 to Feb 1968	3.44	1.013	0.192	0.984	0.000
March 1968	3.44	1.013	0.192	0.984	0.063
April 1968	3.52	1.080	0.204	1.047	0.292
May 1968	3.85	1.396	0.261	1.338	0.970
June 1968	4.70	2.465	0.450	2.308	4.283
July 1968	6.90	7.367	1.286	6.592	-

Table 22 Growth expressed as wet and dry weights and calorific values. 1967 year class pond B. For details see text.

This was not unreasonable since the length measurements indicated continuous growth during this time and the slightly shorter period from 1/5/68 to 23/5/68 made allowance for more rapid growth due to rising temperatures. In this particular example, 13.529 (27.057 ÷ 2) calories were put on in each period by each individual. Hence, an estimate of the calorific value per individual on 1/5/68 is 53.161 + 13.529 cal or 66.690 cal per individual. This estimate agrees closely with one of 65.3 calories per individual made directly from the mean length on 1/5/68 and using the normal length: dry weight regression for non-metamorphosing final instars. The more complex calculation was employed since it utilised data for metamorphosing larvae only, but it appears that either method would have given similar results. A similar procedure for calculating the calorific values/individual on 1st May was employed for all other senior age classes.

Month	Smoothed length on 1st of month (mm)	Wet weight on 1st of month (mg)	Dry weight on 1st of month (mg)	Mean calories/ individual on 1st of month	g. calories growth during month
July 1967	1.30	0.063	0.011	0.056	0.069
August 1967	1.70	0.136	0.024	0.125	0.249
Sept 1967	2.45	0.385	0.073	0.374	0.333
Oct 1967	3.05	0.718	0.138	0.707	0.245
Nov 1967 to March 1968	3.40	0.979	0.186	0.952	0.000
(1966 and 1967 year classes joined at this point)					
April 1967	3.40	0.979	0.186	0.952	0.162
May 1967	3.60	1.153	0.217	1.114	0.582
June 1967	4.20	1.788	0.331	1.696	3.556
July 1967	6.35	5.817	1.025	5.253	8.794
August 1967	9.10	16.23	2.741	14.047	6.903
Sept 1967	10.53	24.62	4.088	20.950	9.711
Oct 1967					
Penultimate instar	11.33	30.36	4.997		
Final instar	12.39	44.96	6.137	30.661	6.429
Nov 1967	12.70	46.38	7.037	37.090	5.509
Dec 1967	13.06	47.99	8.083	42.599	2.450
Jan 1968	13.22	48.71	8.547	45.049	0.612
Feb 1968	13.26	48.89	8.664	45.661	1.377
March 1968	13.35	49.29	8.925	47.038	3.062
April 1968	13.55	50.19	9.506	50.100	15.059
20th April 1968 - 50% of population entered stage 2 metamorphosis.			-	-	-
1st May 1968			-	65.159	15.059
25th May 1968 - 50% entered stage 3 metamorphosis.			14.73	80.218	-8.140
1st June 1968 - 50% emerge			13.62	72.078	-

Table 23 Growth expressed as wet and dry weights and calorific values; composite growth curve in pond F from 1966 and 1967 year classes. For details see text.

In stage 3 metamorphosis, the larvae lose weight and their calorific value falls. Growth in this stage was therefore negative.

7.3 DISCUSSION

Slight differences existed between equivalent monthly growth increments in the two ponds and in different years, probably reflecting differences between the habitats in prey availability and temperature, but the growth patterns were very similar.

Macan (1964) made detailed comparisons between growth studies of Pyrrhosoma in Hodson's Tarn (English Lake District) and those of Corbet (1957b) in Hampshire: larvae with a two year life cycle in Hodson's Tarn showed only small differences in their growth curves when compared with Corbet's data and both are essentially similar to the Brasside growth curves. There are obvious minor seasonal differences, as might be expected from the geographical separation of the three sites, but the general pattern of the typical two year life cycle in Pyrrhosoma is now well established. It appears that the Brasside populations showed none of the variations noted in the other studies and, consequently, demonstrate typical development in its simplest form. This was particularly fortunate for the purposes of this study. The high degree of uniformity in the growth of the Brasside larvae may be due to two main factors. On the one hand, their well synchronised growth can probably be attributed to summer temperatures that were too low for very rapid, univoltine development of the type suggested by Corbet (1957b) for larvae in a more southern population. The rapid one year growth observed by Corbet was undoubtedly responsible for part

of the spring moult to the final instar. On the other hand, population densities in Brasside were never as high as those observed by Macan (1964), so that no part of the population was at a great disadvantage in competing for suitable vantage points from which an adequate food supply for normal development could be obtained. The high population density noted by Macan (1964) probably resulted first of all in part of the population growing slowly and again moulting to the final instar in spring and, in extreme cases, when populations were exceptionally high, to part of the year class extending development to three years.

Finally, synchronization seems to be partially self-perpetuating. Lack of any "tail" on the emergence cycle caused by late emergence has already been referred to for the Brasside populations (see chapter 6) and consequentally, the year classes at Brasside were probably better synchronised on hatching than those studied by either Macan or Corbet. This would naturally increase the chances of remaining well synchronised throughout larval life.

Growth in Pyrrhosoma may be compared with that of the classical spring emergence species Anax imperator. Leach (Corbet 1957a). The life cycle similarly take two years to complete, but Pyrrhosoma starts to grow earlier and finishes later in the year than Anax. One consequence of this is that in all populations so far studied, Pyrrhosoma did not start to moult to the final instar until at least September. In Brasside, about 75 per cent had entered the final instar by 1st October and 100 percent by 1st November. In other Pyrrhosoma populations

(see Corbet 1957b figure 3) events were even later and the moult continued until December. In Anax, 5 percent of the larvae entered the final instar in mid July, 75 percent by the end of August and almost all by the end of September (Corbet 1957a), which places the moult approximately one or even two months ahead of that in Pyrrhosoma. This could have important consequences when the nature of the diapause in the two species is considered and is discussed in chapter 13.

Regular measurements of a recognisable cohort have been used by a number of workers to study growth of insect larvae in the field. Among the first was Moon (1939) for Ephemeroptera. Apart from the work of Corbet (1957a and b) and Macan (1964) already referred to, field growth rates in Odonata have been measured using similar methods to the present work in several other studies e.g. Buchholtz (1951) for Agrion splendens (Harris), Corbet (in Corbet et al. 1960) for Sympetrum striolatum (Charp.) Chutter (1961) for Pseudagrion salisburyense (Ris) and Lutz (1968) for Lestes eurinus Say. Probably because they greatly simplify calculation, several ecological energetics studies have also taken advantage of non-Odonata species with recognisable cohorts, growing in a synchronised manner, similar to Pyrrhosoma e.g. Mann (1965), for fish in the river Thames, Saito (1965, 1967) for Ligidium and Japonaria, Smalley (1960) for Orchelimum and Wiegert (1965) for Melanoplus. Field growth estimates used to compute population production only have been used by Boysen Jensen (1919), Allen (1951) and many others.

The possible errors which may arise when measuring growth rates of insect larvae in the field are well known. Macan (1958a) and Corbet

(1957a) point out that a protracted hatch, spread out over several samples will reduce the apparent growth rate of the population based on sample means. To avoid such "dilution" effects with Plecoptera and Ephemeroptera larvae, Elliot (1967) used modes rather than means. Differential mortality of large or small individuals between samples could also introduce error, as would failure to sample all sizes of larvae equally (Hynes 1941) e.g. by the escape of smaller larvae through the net. In view of the precautions taken in sampling small larvae and the well synchronised emergence and oviposition periods (see chapter 6), these errors were not applicable in the present study.

In converting from mean length to mean weight, an additional error arises (Tesch, in Ricker 1968). In a given age group, of average length L and average weight W, the value of W is greater than the average weight of the individual larvae whose lengths are exactly L. The amount of this difference increases with the amount of variability in length within the cohort and, in the present study where the larvae are highly synchronised, can probably be ignored. (A similar error will be present for example whenever the relationship between larval size and an experimental variable is logarithmic, as with respiratory rate. As in the case of the length: weight relationships, the synchronisation of the population was assumed to be such that the error was negligible.)

It is pertinent to ask what factors limit the growth rate in Pyrrhosoma. A two year life cycle appears to be typical of many British Odonata (Corbet et al. 1960 p. 81 - 82), but it is particularly

significant that in the data which they present most of the Zygoptera are univoltine and it is the larger Anisoptera which are usually semivoltine. Of the Zygoptera, Lestes sponsa shows exceptionally rapid development: larvae in pond B (personal observations, unpublished) grew from 0.04 mg dry weight on hatching in mid April to 6 - 10 mg dry weight at emergence in mid July, whilst Pyrrhosoma larvae in the junior age class increased in weight from 0.2 mg dry weight to 1 mg dry weight in the same three months. This presents particularly forcefully the difference between two species of Zygoptera in the same pond at the same time. Calvert (1929) and Hodgkin and Watson (1958) showed that Odonata larvae differed markedly in their genetic ability to respond to favourable conditions for growth, and Pyrrhosoma is probably typical of those species unable to grow very rapidly even under the most favourable conditions.

Very little appears to be known about the genetically controlled physiological differences between fast and slow growing Odonata larvae. Calvert (1929) could find no relationship between length of larval life and size, but included a wide range of species from diverse habitats when it is probably only valid to compare larvae from similar habitats.

More rapidly growing species might be expected to have higher maximum feeding rates, achieved by larger gut capacity and rapid gut clearance times. High assimilation efficiency and high gross and net growth efficiencies (K_1 and K_2 of Ivlev 1945) or low respiratory rates per unit weight would also be advantageous.

Finally, the ability the detect and capture prey will probably be

greater in rapidly developing species; Lestes, for example, has evolved prey detection and capture mechanisms more like those of the advanced Aeshnidae than other Zygoptera, particularly in the primary use of the eyes rather than the antennae in prey detection (Ando 1957, Richard 1960 a, b, Corbet 1962).

In Pyrrhosoma and species with a similar life cycle, it appears that the evolution of rapid growth rates has not been a feature of their evolutionary history. Emphasis instead has been placed on synchronisation of the emergence in spring. Synchronisation in Anax is remarkable; in Pyrrhosoma emergence is less well synchronised but it is nevertheless still a highly evolved seasonal phenomenon and presumably has high survival value. The advantages of a spring synchronised emergence have been discussed at length by Corbet (1957a, 1962) and Macan (1958a) Once present, it sets definite limits on all other aspects of larval growth and development, because for it to be achieved the majority of larvae must be in the final instar before winter inhibits growth. A diapause then serves to increase synchronisation and the population metamorphose and emerge in the spring as soon as conditions become favourable. (Corbet 1957a, 1957b, 1962 etc.). The new generation hatching in July must, therefore, either complete development in four months in order to reach the final instar by the first winter or grow more slowly and reach this point a year later. Intermediate growth rates, giving rise to summer emergence and a loss of synchronisation would probably be selected against. Extremely rapid growth is a specialisation shown by very few Odonata, so that in most habitats,

slow growth and two year development become inevitable if spring synchronisation is to be maintained.

Fig. 12. Larval growth curves in pond B (mean larval length ± 2 standard errors).

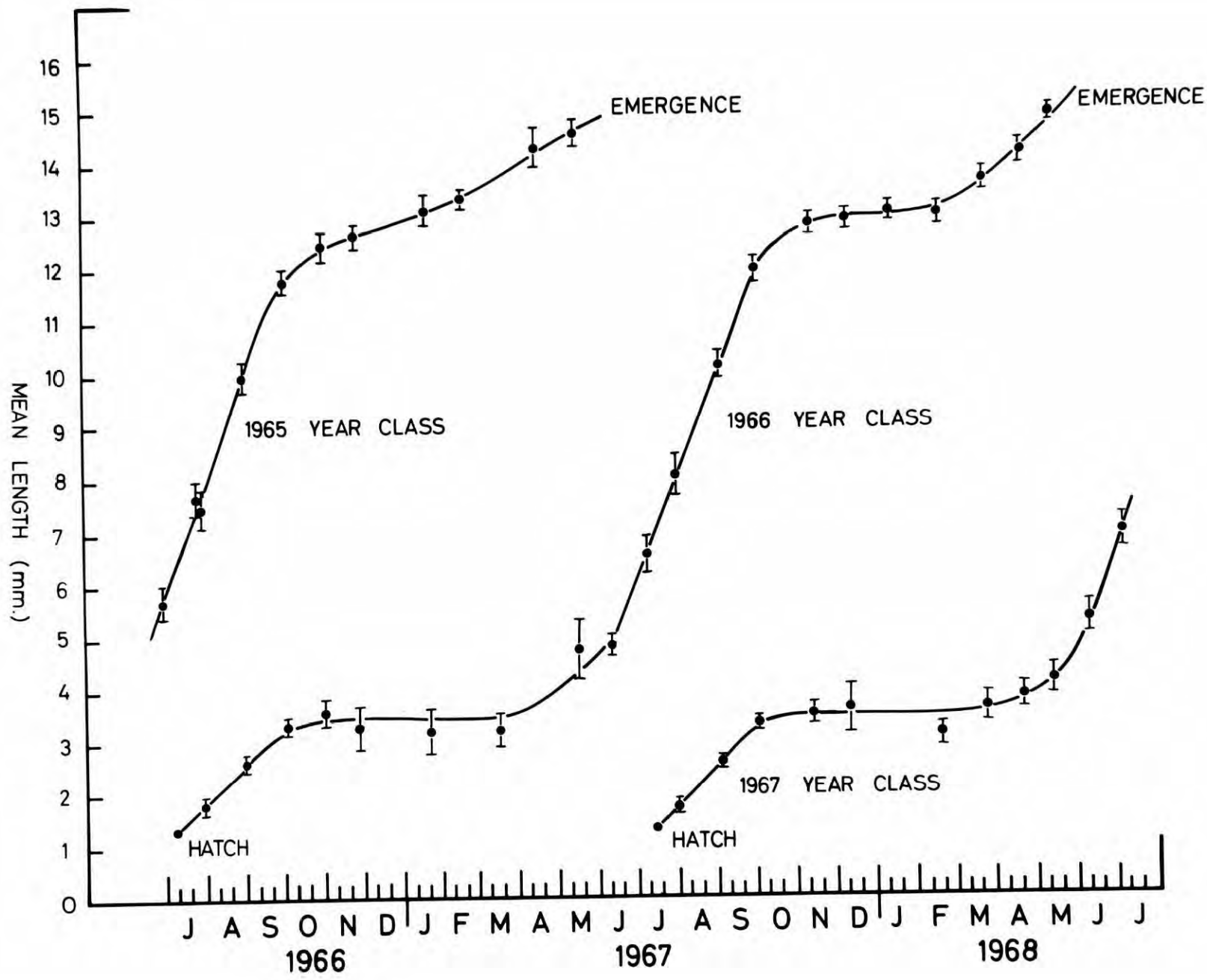
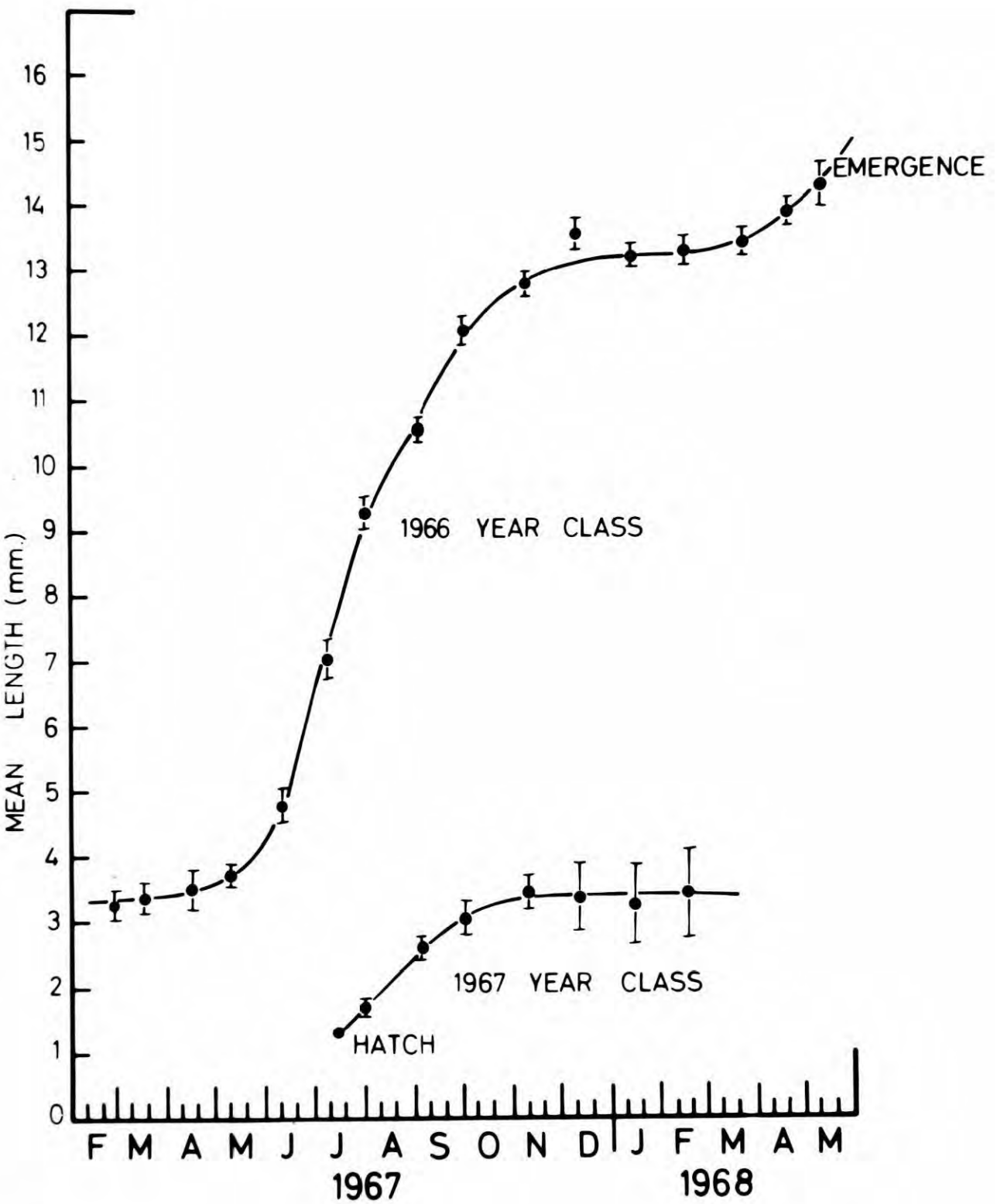

MEAN LENGTH (mm.)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
EMERGENCE
1965 YEAR CLASS
HATCH
EMERGENCE
1966 YEAR CLASS
1967 YEAR CLASS
HATCH
J A S O N D J F M A M J J A S O N D J F M A M J J
1966
1967
1968

Fig. 13. Larval growth curves in pond F (mean larval length ± 2 standard errors.).


MEAN LENGTH (mm.)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
EMERGENCE
1966 YEAR CLASS
1967 YEAR CLASS
HATCH
F M A M J J A S O N D J F M A M
1967
1968

Chapter 8

THE PREY SPECIES OF PYRRHOSOMA

Information on the main prey species taken by Pyrrhosoma was obtained by faecal pellet analysis: since virtually all prey in Odonata is consumed whole, this was considered to be a reliable method. For very small Pyrrhosoma larvae, faeces analysis was not possible and a number of food preference experiments were carried out. From a knowledge of the size of the prey organisms taken and the food of the prey species, it was possible to build up an accurate picture of the trophic position of Pyrrhosoma in the pond food web for most of larval life.

8.1 FOOD PREFERENCE EXPERIMENTS WITH SMALL LARVAE

Food preference experiments were carried out with laboratory hatched instar 2 larvae. Less detailed observations were made on instar 3 larvae.

Larvae that had been starved for 24 hours were placed on a well slide in a drop of water containing a selection of potential prey organisms and examined under a x 25 binocular microscope. Each larva was left in the well until "interest" in the prey had apparently ceased or for a maximum of 15 minutes. Three categories were recorded:-

1) Larvae showed interest by turning and directing antennae towards prey, sometimes accompanied by a movement of the labial palps.

ii) Attempted capture by ejection of labium.

iii) Successful capture and ingestion of prey.

Two "food preference cultures" were used for instar 2 larvae.

i) This contained a variety of infusoria obtained from old hay cultures

to which pond water had been added.

Table 24 shows the species present (ranked in order of abundance) and their mean size in mm.

Species	Abundance rank	Mean size (mm) measured with a micrometer eye-piece (diameter or length and width)
Paramecium caudatum	Abundant	0.23 x 0.04
Euglena sp.	Abundant	0.08 x 0.04
Stylonychia sp.	Common	0.22 x 0.07
Amoeba sp.	Present but not common	0.25
A rotifer, possibly Diurella sp.	Present but not common	0.20 x 0.09

Table 24 Species of infusoria present in first "food preference culture"

ii) This contained the same Paramecium sp. as the first (this was for comparative purposes) and two Cladocera as shown in table 25.

Species	Abundance rank	Mean size (mm) measured with a micrometer eye-piece (maximum length or length and width)
Paramecium caudatum	Abundant	0.23 x 0.04
Chydorus sp.	Common	.36
Newborn Daphnia obtusa	Common	0.80

Table 24 Species present in second "food preference culture."

Results: first "food preference culture", instar 2.

A total of 10 newly hatched Pyrrhosoma larvae captured or attempted to capture a total of 52 prey items as follows:-

Paramecium	25	(48.1 per cent)
Stylonychia	14	(26.9 per cent)
Rotifer	11	(21.2 per cent)
Euglena	1	(1.9 per cent)
Amoeba	1	(1.9 per cent)

When this is compared with the rough abundance rank, it is clear that Paramecium, Stylonychia and rotifers were readily eaten but that Amoeba and Euglena were rarely attacked. Euglena was probably at the lower end of the detectable size range and Amoeba moved too slowly. It is interesting that the single Amoeba captured was immediately rejected and resulted in violent cleaning movements of the labium as if highly distasteful.

The observations on prey in which the larvae showed interest confirmed the above results. Very few showed interest in Euglena and Amoeba but all showed a strong response to the other three types.

Results: second "food preference culture", instar 2.

A total of 10 newly hatched Pyrrhosoma larvae captured or attempted to capture 63 prey items as follows:-

Daphnia obtusa	26	(41.3 per cent)
Chydorus	33	(52.4) per cent)
Paramecium	4	(6.4 per cent)

The response to Cladocera was dramatic and larvae largely ignored the Paramecium. However, successful captures were rare. Daphnia invariably escaped but some larvae managed to remove the antennae, which they ate. Chydorus was frequently held but the carapace was too strong to pierce.

Results: instar 3

Observations similar to those made on instar 2 larvae showed that instar 3 larvae were able to capture and eat both small Daphnia and Chydorus successfully. Paramecium was rarely taken.

Conclusions

Newly hatched larvae clearly took any moving small prey of a suitable size, the lower limit probably being about the size of Euglena (0.08 mm long) whilst at the upper limit they attempted to capture surprisingly large Cladocera (0.3 - 0.8 mm long).

Following the moult to instar 3, the ability to capture small Cladocera improved and it is probable that dependence on protozoa and rotifers declined. In the field, small Cladocera and similar sized organisms probably become increasingly important from instar 3 onwards.

A dependence on protozoa and protozoan sized metazoa in instar 2 Odonata larvae followed by a shift to larger metazoa in instar 3 or 4 has been noted by many workers e.g. Balfour - Browne (1909), Gardner (1950), Kormondy (1959) and Krull (1928).

8.2 FAECES ANALYSIS IN LARGER LARVAE

8.2a General Methods

Data were obtained in pond B only: the faeces produced by senior

and junior age classes taken in the monthly population samples and in occasional additional samples made for this purpose were collected between July 1966 and June 1967 and stored in 70 percent alcohol until required for analysis. The faeces produced by the junior age class between July and November were too small to be analysed satisfactorily. Pellets from each larva were collected and stored separately. Odonata larvae produce compact faeces enclosed in a strong peritrophic membrane, making them very convenient to handle and store without loss of material.

For analysis, faeces were placed in a drop of alcohol on a side squared at 1 mm intervals and dissected under a binocular microscope with fine needles. The separated remains were placed under a cover slip, the contents of each 1 mm square examined under x 60 magnification and the number of specimens in the pellet recorded. The remains of prey species were also measured with a micrometer eye-piece, from which data on the approximate sizes of the prey being consumed were obtained.

Comparisons were made between the number of individuals of each kind of prey captured each month, regardless of the size of the prey. A further analysis was made in which the dry weight of each prey type consumed was examined. In order to eliminate minor monthly fluctuations and to illustrate more clearly changes in the proportion of each type of prey consumed on a dry weight basis at different stages in the life history, three broad life cycle divisions were recognised viz:-

i) Small larvae (November - May)

ii) Growing larvae (June - October)

iii) Final instars (November - May)

8.2b Prey Identification and Estimation of Numbers

A reference collection of all potential prey species to be found in pond B was made, from which specimens of cuticle, limbs, jaws and head capsules were prepared. For the commoner species, "reference faeces" were also produced by feeding them to starved Pyrrhosoma larvae.

The criteria by which all prey items were identified and the 15 categories into which they were classified were as follows:-

Crustacea

i) Simocephalus vetulus - portions of transparent carapace showing linear striations: also typical mandibles, tarsal claws and limbs.

ii) Copepoda - occasionally nearly whole bodies were present but usually these were reduced to fragments particularly of limb segments and small stout chaetae.

iii) Ostracoda - valves from bivalve carapace usually present intact.

iv) Chydorus spp. - usually present intact. C. sphaericus identified from remains but other species present also.

v) Asellus aquaticus - large pieces of transparent, slightly brownish exoskeleton, lacking chitin, were highly characteristic. Limb fragments also.

Insect Larvae

vi) Chironomidae (except Tanypodinae) - characteristic head capsules usually present intact. Proleg claws, tail tufts and jaws also present. (Several species were involved but could not be identified - see Macan 1963 p. 15).

vii) Tanypodinae - differ from other Chironomidae in having head capsules without a well sceleretised hypostomium but usually with a sceleretised glossa in roughly the same position as the hypostomium but of quite a different shape. Retractile antennae also characteristic (Bryce 1960).

viii) Ceratopogonidae - extremely long narrow head capsules, without obvious antennae, hypostomium or glossa. Also pieces of clear cuticle bearing fine longitudinal striations.

ix) Chaoborus sp. - extremely complex distinctive mandibles resembling "elk antlers" and a characteristic "fan" of setae from anal region.

x) Dixa sp. - heavily chitinised cuticle with many thick black bristles of characteristic form. Also a chitinised "pad" bearing short spines from anal region.

xi) Cloeon dipterum - large, rather complex mandibles and cuticle with distinct markings were characteristic: also fragments of large compound eyes and numerous small segments from 'tails' usually present.

xii) Dytiscidae - cuticle again with characteristic markings: also present were unsegmented long tail processes and pointed sickle shaped mandibles.

xiii) Zygoptera - labium and short setae on limb fragments (see MacNeill 1967).

xiv) Hydracarina - always virtually intact.

xv) Oligochaeta - characteristic short chaetae present in large numbers. These were transparent, usually slightly S shaped and carried a

small bulge half way down length: also sometimes forked at one end.

A small number of prey items, usually chitinised and including limb fragments, could not be identified. These have been included in the analysis as unidentified arthropod remains.

Quatifying the number of prey items present in each pellet was relatively easy for most of the prey types. In the case of the larger species, it was usually obvious that only one was present from the number of mandibles or other distinctive remains. Other types were quantified from counts of head capsules (vi, vii, viii), whole bodies or valves (iii) iv, xiv) and mandibles (i, ix). Badly broken up copepod remains and oligochaetes could not be quantified and were recorded as single occurrences: they were therefore slightly unerestimated. Remains from one large prey item e.g. Cloeon often appeared in two faecal pellets from one larvae, but were only counted once.

The chief problem in the analysis of Pyrrhosoma faeces was not the identification of remains, nor in quantifying those found, but in being certain that no prey species were being overlooked because they did not leave recognisable remains or being badly underestimated because the remains were very difficult to find. For very small larvae, both these errors are likely to increase. However, reference to the faunal list for pond B (appendix 2) and knowledge of the type of faecal remains produced by all potential prey species, particularly those poorly represented or absent in the faecal pellet analysis, suggested that neither of these factors was a serious source of error.

8.2c Estimation of Size and Dry Weight of Prey Species

Chironomidae

The lengths of all undamaged head capsules found in the faeces were measured with a micrometer eye-piece from the tip of the hypostomium (or glossa in Tanypodinae) to the rear edge of the capsule and the mean lengths calculated separately for the three life cycle divisions of Pyrrhosoma.

Samples of chironomids from pond B were taken and their head capsule lengths measured. Larvae were killed by momentarily dipping them into boiling water to facilitate measurement. Larvae in which the head capsule length was within ±2 standard errors of the three sizes found for the faeces head capsuled were dried in a vacuum oven at 60°C for dry weight determination: the remaining chironomids were discarded. In this way, the mean dry weights of three sizes of chironomid, equivalent to the three sizes taken by Pyrrhosoma, were obtained.

Ostracoda, Chydorus spp. and Hydracarina

The majority of specimens appeared intact in the faeces and sizes were measured directly. There appeared to be no difference between the sizes captured in the three life cycle divisions (probably because they were much smaller than the chironomids and easily caught by all sizes of larvae) and only one mean was calculated for each species.

Samples of these three prey types were taken from pond B and all individuals within ±2 standard errors of those measured in the faeces were taken for dry weight determinations.

Simocephalus and Copepoda

It was not possible to measure the faeces remains in a way that pro-

vided a satisfactory relationship between the prey consumed and its initial size. Instead, random samples were made of all parts of the pond and the average dry weight of a "typical individual" found for both prey types. This was not particularly satisfactory, but provided a figure that was of the right order of magnitude. It will be seen from the results that the dry weights of these two prey items were considerably less than those of individual chironomids, so that errors in determining their weights would be small compared with this difference.

Other Prey Species.

Most of these were relatively unimportant numerically and were assigned approximate weights on the basis of their size in comparison with the other more important prey species.

8.2d Results

The total number of individuals of each prey type found each month in the faeces are presented in table 26. It will be seen that the number of prey items found in the faeces of the junior age group between November and June was small, largely because of the difficulty experienced infinding sufficient small larvae at this time (see chapter 5). Consequently, in analysing the percentage contribution (on a numerical basis) of each prey type, it was necessary to group the data from several months in order to obtain a large enough sample. The months where this was done are clear from table 27, which shows the number of specimens of each prey type expressed as a percentage of the total number of prey items for each month or group of months.

Month	Number of Larvae in sample	Total number of prey items identified	Crustacea					Insect Larvae							Others		
			Simocephalus vetulus	Copepoda	Ostracoda	Chydorus spp.	Asellus aquaticus	Chironomidae except Tanypodinae	Tanypodinae	Ceratopogonidae	Chaoborus sp.	Dixa sp.	Cloeon dipterum	Dytisidae	Zygoptera	Unidentified arthropods	Hydracarina	Oligachaeta
Nov	10	20		4		4		6										6
Dec	12	19	2	8		2		3	1							1		2
Jan	10	14		7		3		1								1		2
Feb	9	11	1	5				2										3
March	4	6		2												1		3
April	9	15	1	5		1		3						1				4
May	30	46	6	22	1	2		11	1									3
June	13	25	6	4		10		1						1		1	2	
July	32	123	12	5	10	39		44			1		1			3	6	2
Aug	38	135	20	13	12	14		60		1	3		1	2		2	7	
Sept	47	171	21	11	65	3	4	46	10		2	1	2	1		3	2	
Oct	41	231	23	2	155			33	5	1	1		4	1	1	3	2	
Nov	48	312	13	20	221	1	2	42	1	2	1		1	2	1	3	2	
Dec	17	53	12	8	22		2	1	1				1	1		2		3
Jan	13	70	2	6	53			2	2		3					2		
Feb	19	185	1	11	163			7			1		2					
March	8	22	2	4	5			9					1			1		
April	63	342	14	17	141			126	10	2	2		4	5		2	19	
May	29	111	34	10	13			31	15		1		1	2		1	3	

Table 26 Numbers of each kind of prey item found in the food of Pyrrhosoma each month from November (four months after hatching) until May, prior to emergence.

Month	Crustacea						Insect Larvae									Others		
	Entomostraca						Diptera						Others					
	Simocephalus	Copepoda	Ostracoda	Chydorus	Total Entomostraca	Asellus	Chironomidae except Tanypodinae	Tanypodinae	Ceratopogonidae	Chaoborus	Dixa		Cloeon	Dytiscidae	Zygoptera	Unidentified arthropods	Hydracarina	Oligochaeta
Nov-Jan	3.8	35.9		16.9	56.6		18.9	1.9								3.8		18.9
Feb-April	6.3	37.5		3.1	46.9		15.6							3.1		3.1		31.3
May-June	15.7	37.1	11.4	17.1	71.3		17.2	1.4						1.4		1.4	2.9	4.3
July	9.8	4.1	8.1	31.7	53.7		35.8			0.8			0.8			2.4	4.9	1.6
Aug	14.8	9.6	8.8	10.4	43.6		44.4		0.7	2.2			0.7	1.5		1.5	5.2	
Sept	12.3	6.4	38.0	1.8	58.5	2.3	26.9	5.8		1.2	0.6		1.2	0.6		1.8	1.2	
Oct	10.0	0.9	67.1		78.0		14.3	2.2	0.4	0.4			1.7	0.4	0.4	1.3	0.9	
Nov	4.2	6.4	70.8	0.3	81.7	0.6	13.5	0.3	0.6	0.3			0.3	0.6	0.3	1.0	0.6	
Dec	22.6	15.1	41.5		79.2	3.8	1.9	1.9					1.9	1.9		3.8		5.7
Jan	2.9	8.6	75.7		87.2		2.9	2.9		4.3						2.8		
Feb	0.5	5.9	88.1		94.5		3.8			0.5			1.1					
March	9.1	18.2	22.7		50.0		40.9						4.6			4.6		
April	4.1	5.0	41.2		50.3		36.8	2.9	0.6	0.6			1.2	1.5		0.6	5.6	
May	30.6	9.0	11.7		51.3		27.9	13.5		0.9			0.9	1.8		0.9	2.7	

Table 27 Percentage of each kind of prey in food of Pyrrhosoma. Analysis based on number of each prey type and no account has been made of prey size.

Prey	Size measured in faeces	n	Mean size (mm)	1.S.E.
Chironomids (taken in Nov-May by small larvae)	Head capsule length	13	0.23	0.015
Chironomids (taken in June-Oct by growing larvae)	Head capsule length	125	0.29	0.010
Chironomids (taken in Nov-May by final instars)	Head capsule length	99	0.38	0.017
Ostracoda (all faeces)	Total length	50	0.50	0.021
Chydorus (all faeces)	Maximum diameter	18	0.32	0.008
Hydracarina (all faeces)	Total body length	15	0.48	0.003

Table 28 Mean sizes of prey species measured in faeces.

Prey type	Size of *Pyrrhosoma* taking prey	Estimated mean dry weight of prey (mg)	Method of estimating prey weight
Chironomids	Small larvae Nov-May	0.50	From sizes given in table 28 and weights of similar sized prey from field
Chironomids	Growing larvae June-Oct	0.65	
Chironomids	Final instars Nov-May	1.96	
Ostracoda	All sizes of larvae	0.014	
Hydracarina	All sizes of larvae	0.010	
Chydorus	All sizes of larvae	0.002	
Simocephalus	All sizes of larvae	0.042	Random samples of field pops.
Copepoda	All sizes of larvae	0.045	
Oligochaeta	All sizes of larvae	0.010	Estimated weights on basis of approximate sizes
All other arthropods	Small larvae Nov-May	0.50	
All other arthropods	Growing larvae and final instars June-Oct; Nov-May	0.75	

Table 29 Mean dry weights (mg) of prey species taken by *Pyrrhosoma*.

Main food category of prey	Prey type	Percentage by dry weight in diet of *Pyrrhosoma*		
		Small larvae Nov-May	Growing larvae June-Oct	Final instars Nov-May
1. Browsers	Chironomids except Tanypodinae	70.4	70.1	74.7
	Cloeon	0	2.9	2.2
	Oligochaeta	2.0	0	0
	Chydorus	0.2	0.2	0
	Ostracoda	0.1	1.5	1.5
	Simocephalus	2.0	2.6	0.9
	Asellus	0	1.5	1.0
	Total % Browsers in each life cycle division	74.7	78.8	80.3
2. Carnivores	*Chaoborus*	0	2.6	1.5
	Hydracarina	0	0.2	0
	Dytiscidae	4.1	4.2	1.3
	Tanypodinae	4.6	4.5	12.6
	Zygoptera	0	0.3	0.1
	Total % carnivores in each life cycle division	8.7	11.8	15.5
3. Browsers or Carnivores	Caratopogonidae	0	0.7	0.3
	Copepoda	15.0	1.4	0.9
	Total % from this category	15.0	2.1	1.2
4. Miscellaneous; probably Browsers	Unidentified arthropod prey and unclassified minor prey species	1.7	7.3	3.0

Table 30 Percentage by dry weight of each main prey type in diet of *Pyrrhosoma* for three life cycle divisions: main food of prey also included. See text for details.

Table 28 shows the mean length measurements made on faecel pellet remains and table 29 the estimated dry weights of each prey type. Table 30 shows the percentage contribution on a dry weight basis of each prey type in the diet of Pyrrhosoma, not on a monthly basis but for the three life cycle divisions.

The data in table 30 were calculated from the numbers of each prey species consumed each month multiplied by their mean dry weights given in table 29 and then the totals for each life cycle division calculated on a percentage basis. The actual procedure was slightly more complex because the total number of prey items identified differed from month to month: some samples were particularly large (e.g. April in the final instar, when 342 prey items were identified). In order to avoid undue bias from such large samples, the data were adjusted so that the total number of prey items identified each month was constant for each life cycle division. For example, for growing larvae (June - October) the maximum number of prey items identified in one month was in October (231 prey items identified.) The numbers of all prey captured in the other months within this life cycle division were, therefore, multiplied by the following factors:-

June	231/25	=	9.24
July	231/123	=	1.88
Aug.	231/135	=	1.71
Sept.	231/171	=	1.35

The corrected numbers were then converted to dry weights and the percen-

tages for each life cycle division calculated.

The numerical analysis (tables 26 and 27) suggests that Simocephalus, Copepoda and Chironomidae were taken regularly throughout development, though small larvae took more Copepoda than larger larvae. Both Chydorus and Oligochaeta were taken almost exclusively by small larvae. Ostracoda were surprising; they were not taken by small larvae, probably because the carapace was too smooth or hard to be pierced, but despite their small size (see tables 28 and 29) they were the most frequent prey item captured by final instars during the winter and many faecal pellets contained nothing else. It was interesting to observe that final instars picked them up by small movements of the labial palps and hardly extended the mask (labium) at all. Emphasis on Ostracoda may have been due to lack of other suitable or available prey.

None of the other prey species were taken regularly in any numbers and in combination rarely formed more than 10 - 15 percent of the prey items captured. Of these less important species, the unidentified arthropods formed the largest group, whilst of the types identified the most important were probably Tanypodinae and Cloeon dipterum.

The data indicate that Pyrrhosoma is an opportunistic carnivore taking whatever it was able to capture. However, within this wide range of species and types, most were taken only infrequently and the majority of prey items were Entomostraca and Chironomidae, with Entomostraca predominating.

Table 30 clearly shows how the conclusions based on numerical estimates must be modified when the effects of prey size are considered

Chironomids contribute about 70 percent of the dry weight (and therefore, of the calories) eaten by Pyrrhosoma, whilst the Entomostraca are a great deal less important than the numerical analysis suggests and, on a dry weight basis, form less than 10 percent of the diet. The Ostracoda particularly illustrate this point. In final instars in winter they often formed over 50 percent of the prey items captured (see table 27) but because of their small size contributed only 1.5 percent to the diet in terms of dry weights. Virtually all other prey species become similarly significantly reduced in proportion to the chironomids, which were obviously the most important single energy source and more important than all the remaining prey species combined.

The similarity between the three life cycle divisions in the percentage contribution of each prey type is also interesting. Despite the fact that smaller larvae captured smaller chironomids and that the number and percentage of chironomids captured varied from month to month, their final percentage contribution by weight to the diet was almost identical (70.4, 70.1 and 74.7 percent) for small, growing and final instar larvae respectively. The proportion by dry weight of the other prey types was also similar: Copepoda showed the greatest difference, ranging from 15.0 percent by weight in small larvae to 0.9 percent in final instars, though part of this difference may be due to no allowance being made for the Copepoda captured by small larvae being smaller than those taken by large larvae. It may be concluded that the proportion of energy derived by Pyrrhosoma from each prey type appears to be similar throughout development.

A few species that were common in the pond were rarely or never present in the faeces and their absence requires explanation.

Asellus was recorded in September, November and December (see tables 26 and 27) but even then was only infrequently captured by Pyrrhosoma. Its absence in spring and summer can probably be attributed to a lack of suitable sized animals available for capture by small larvae. In the study area Asellus had two reproductive periods (Fitzpatrick 1968), one in May and June giving rise to a generation which grew rapidly and reproduced again in September and October. Only after this second autumn reproduction were large Pyrrhosoma and small Asellus present in the pond together and a few Asellus were captured. No explanation can be offered for their absence from final instar faeces in winter. In the laboratory, they were readily taken and assimilation, though rather low, appeared to be normal with Asellus prey (chapter 9). The faeces were also distinctive and remains could not have been overlooked.

Fitzpatrick (1968) pointed out that Asellus was one of the most important component species of the small pond eco-systems at Brasside, both in terms of biomass and total energy flow, and further argues that it probably formed a primary food source for most of the carnivore species. Why Pyrrhosoma as a key carnivore species should now be shown to ignore this primary food source requires further investigation.

Cloeon was also captured infrequently in proportion to its abundance: in the laboratory it was taken readily but usually managed to avoid capture for long periods by rapid movement when attacked and by

periods of inactivity during which Pyrrhosoma was unable to detect it: both these factors probably reduced the number captured in the field.

The number of other Zygoptera caught by Pyrrhosoma was extremely low, suggesting that cannibalism was virtually non-existent. This may be partially attributed to the spatial separation of the large and small Pyrrhosoma larvae in the pond (chapter 5, section 5.2a) and to the fact that most Zygoptera remain immobile for long periods.

Haliplus spp (and other adult Coleoptera), Sigara spp Notonecta spp and Trichoptera were never recorded in the faeces: nor were they taken in the laboratory. Adult Coleoptera could not be held if attacked probably because of their heavy, chitinised exoskeletons. The protective cases and slow movements of Trichoptera were obviously adequate protection from attack. Sigara spp. and Notonecta spp. are rarely taken by a number of aquatic predators, though earlier suggestions that Sigara were distasteful (Pritchard 1964) have been questioned by Staddon and Griffiths (1967). Large size and rapid movements may be responsible for their apparent immunity from attack by Pyrrhosoma.

Finally, it should be mentioned that many of the pellets analysed contained surprising amounts of detritus, usually present as fine brown material, but plant fragments were also recognised. Part was probably derived from the guts of prey organisms but in the laboratory, Pyrrhosoma was frequently observed to pick up accidently part of the substrate when striking at small prey species moving near to or on the bottom of aquaria, and detritus was obviously consumed in this way in the field. It probably passed through the gut unaltered, but may have been partly

responsible for the low calorific value of field faeces noted in chapter 3 (table 5, sample 30).

8.3 THE CONTRIBUTION OF BROWSERS AND CARNIVORE PREY SPECIES TO THE FOOD ENERGY CONSUMED BY PYRRHOSOMA

The main prey species probably all had calorific values slightly greater than 5,00 calories per gram dry weight (see chapter 3, table 5 and Cummins 1967). The percentage contribution of each prey type in terms of dry weight was therefore approximately equal to its contribution in terms of energy in the food eaten by Pyrrhosoma.

The prey species were grouped into categories according to their own feeding habits. Table 30 shows these categories, which are based on information taken from the literature (see appendix 3), supplemented by personal observation.

Prey in category 1. were termed Browsers following Lindeman (1941) who stated "the distinction between plant browsers and ooze browsers does not appear justified: many species which are primarily herbivorous during the summer are saprophagous during winter. Others appear to feed indiscriminately upon both living and dead plant material." Therefore, in the present study following Lindeman, browsers were defined as herbivorous or saprophagous animals feeding primarily on or near to the substrate. (Tilly 1968 suggested that the aquatic detritus feeders in cone spring resembled carnivores rather than herbivores in their patterns of abundance. His proposals are largely speculative, however, and without more evidence it seems more logical to include aquatic

herbivores and detritus feeders together as "browsers".)

Category 2. included the carnivores.

Category 3. included those groups of prey types which were either carnivores of browsers depending on the species or, alternatively, omnivores. In apportioning the energy consumed by Pyrrhosoma to either that derived from browsers or that derived from carnivores, the prey placed in category 3. were divided equally between these two groups.

Category 4. included the small percentage of unidentified arthropods and infrequently captured prey species in which the main food source was not determined with certainty. The majority of category 4. were probably also browsers.

Table 31 shows the total percentage contribution by dry weight (and therefore energy) of browsers and carnivores in the diet of Pyrrhosoma according to the assumptions made above and the data presented in table 30.

In general terms, it is probable that 85 percent of the food energy in Pyrrhosoma was derived from browsers and 15 percent from carnivores and that this proportion remained constant throughout most of development.

8.4 DISCUSSION

Faecal pellet analysis is subject to a number of errors. Of these, failure to recognise and identify important prey items was not thought to be significant in the present study (see section 8.2b).

Fisheries biologists have pointed out (e.g. Hess and Rainwater 1939, Gerking 1962) that different prey species may pass through the gut at different rates. Consequently, slowly moving prey tend to be overestimated

Classification in table 30	Life cycle division		
	Small larvae Nov-May	Growing larvae June-Oct	Final instars Nov-May
Category 1.	74.7	78.8	80.3
Category 4.	1.7	7.3	3.0
50% of category 3.	7.5	1.1	0.6
Total percentage from browsers	83.9	87.2	83.9
Category 2.	8.7	11.8	15.5
50% of category 3.	7.5	1.1	0.6
Total percentage from carnivores	16.2	12.9	16.1

Table 31 Proportion of food energy obtained from browsers and carnivores in three life cycle division of Pyrrhosoma.

compared with more rapidly moving (usually more easily digested) prey types. In Pyrrhosoma, chironomids took twice as long to pass through the gut as Daphnia when both were fed as single prey species in the laboratory. However, it was shown that under field conditions with a mixed diet, differences between rates of movement of prey through the gut were reduced and all prey moved at the rate of the slower dominant chironomids (gut clearance experiments, chapter 12). The fact that after collection larvae usually produced one large pellet containing most if not all of the prey remains and not several pellets containing more rapidly digested prey types first, supports this conclusion. It

was therefore concluded that errors due to different rates of passage through the gut with different prey types were insignificant.

Laboratory food preference experiments with instars 2 and 3 proved valuable because they provided information on the type and size of prey taken by small Pyrrhosoma. This data was unobtainable by faecal pellet analysis. In larger larvae, food preference experiments were known to have little value except to show that Odonata larvae will generally take any available moving prey of suitable size (Wright 1946, Pritchard 1964). It was therefore desirable to determine which of the range of potential prey species were actually taken in the field. Radiotracer techniques using labelled prey (Baldwin et. al. 1955, James 1965, Odum and Kuenzler 1963) in the field of seriological methods (Hall et. al. 1953, Dempster 1960) could have been employed, but gut or faecal pellet analysis based on larvae collected in the field appeared to offer the least complicated solution. The latter was eventually chosen because it did not involve killing the larvae.

In Odonata, Corbet (1957a) reported analysis of Anax faeces, whilst more detailed studies were those of Pritchard (1964) for some Canadian Anisoptera and Staddon and Griffiths (1967) for Aeshna juncea (L.). The present study was the first attempt to analyse zygopteran faeces though Chutter (1961) made a detailed study of prey captured by the South African zygopteran Pseudagrion salisburyense (Ris.) from analysis of foregut contents and Macan (1964) analysed gut contents in Pyrrhosoma and Enallagma cyathigerum (Charp.). Fischer (1966; 1967b) studied food

selection in Lestes sponsa (Hans.) by gut content analysis and calculated the percentage contribution of each prey type in terms of biomass (calories) as well as numerically. Fischer (1966 and 1967b) is the only one, apart from the present study, to have attempted this.

Macan (1964) found that the range of animals eaten by Pyrrhosoma was wide, but that chironomids and entomostraca were the main food of larvae of all sizes, a result confirmed by the present study. Further, Pyrrhosoma appears to be typical of most of the other dragonfly larvae studied where chironomids and entomostraca also formed the main prey. The importance of converting data to biomass is emphasised by Fischer's (1966, 1967b) work on Lestes where plankton formed the main food component by number but where chironomid larvae were the most important prey type in terms of calories throughout larval life, a situation identical to that found for Pyrrhosoma in pond B.

Chutter (1961) and Pritchard (1964) showed that seasonal changes in the numbers of each prey type captured were frequently closely correlated with changes in prey abundance. This was not examined in detail in the present study, but was probably the explanation for many of the observed monthly changes in the numerical proportions of each prey type in the faeces. Chutter and Pritchard also reported that a number of apparently suitable organisms were rarely or never captured. These included adult Coleoptera, Corixids, Notonecta and Ephemeroptera, much as in the present study. However, whereas Pyrrhosoma did not take Trichoptera Pritchard found that they were regularly taken by Aeshna. This may possibly be explained by the size difference between Pyrrhosoma

and Aeshna.

The virtual absence of cannibalism in most Odonata larvae is confirmed by nearly all studies e.g. Macan (1964), Pritchard (1964), Chutter (1961) and Corbet (1957a). Only Lestes (Fischer 1961) is reported as cannibalistic, particularly young larvae at high density.

Paine (1965) employed detailed faecal pellet analysis in his energetics study of the carnivorous marine opisthobranch Navanax inermis (Cooper). Relationships between length of prey remains in the faeces and total calorific content of each prey type were determined and used to estimate the calories of each prey type consumed. Pearson (1966) utilised faeces remains in a similar way in an ingenious study of predation by unspecified mammalian carnivores on two mice Microtus californicus and Reithodontomys megalotis. The works of Paine (1965), Pearson (1966), Fischer (1966) and that for Pyrrhosoma reported here are the only studies where faecal pellet analysis has been used to provide information on the energy utilisation by carnivore populations. Where clearly recognisable remains are present, as in these four studies, the method appears to be highly satisfactory.

Stability in ecological units above the population level increases as the number of alternative energy flow pathways within the system increases (MacArthur 1955). Although Pyrrhosoma obtained most of its energy from chironomid populations, several species were involved within this general category, whilst the wide range of other prey species taken further increased the number of potential pathways through which energy could be obtained. Such a system is potentially very stable. This

potential stability is probably reflected in the constant proportion of food derived by Pyrrhosoma throughout development from browsers and carnivores, since each of these groups contained numerous species. This could have been due to a constant selection of certain prey types, for which there is some evidence in Odonata (Fischer 1964, 1966). Most Odonata however, appear to attack all available prey within certain size limits equally and show no evidence of selection (Pritchard 1964, 1965, Wright 1946 and the present study). Prey captured by Pyrrhosoma was therefore a random sample of "available" species and the constant proportion of browsers and carnivores in the food probably reflected a stable ratio between these two main trophic divisions in the pond.

There appeared to be no distinction between a decomposer and herbivore food chain in pond B of the type suggested by Odum (1962). The lack of distinction between the two food chains was probably due to the almost complete intermixing of living and dead plant material in the small ponds.

However, at least two energy utilising pathways were present in pond B. Asellus aquaticus was taken only exceptionally by Pyrrhosoma but was a key browser in the small ponds (Fitzpatrick 1968). One known predator of Asellus was Triturus palustris which appeared never to take Pyrrhosoma (see Chapter 5). Tilly (1968) argued that the aquatic fresh water community in Cone Spring was divided into a number of discrete energy utilising pathways (groups of primary consumers and their particular predators) with relatively little connection between groups. Similarly Wiegert et al. (1967) demonstrated that a one year old field

was divided into two discrete, apparently unconnected food chains. Division of communities into relatively unconnected energy utilising pathways could, therefore, be commoner than has hitherto been suspected and may have important consequences for the study of total community energy flow and community stability.