ADAPTIVE ASPECTS OF BIOENERGETICS IN SEXUAL AND ASEXUAL SPECIES OF FRESHWATER TRICLADS

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SUMMARY

The effects of temperature and starvation on 3 species of freshwater triclad with contrasting life cycles, distribution and methods of reproduction were investigated, to examine the relative merits of each species' strategy in relation to its environment. The 3 species - Polycelis tenuis (Iijima), Polycelis felina (Dalyell) and Dugesia tigrina (Girard) - were cultured at 5°, 10°, 15° and 20°C, under fed and starved conditions. Food intake, respiration, growth and reproductive rates, were measured for each species under each set of conditions. A study of a population of P. felina was also undertaken to seek information on field conditions.

The results from fed triclads show that in general food intake, and growth and respiration rates, increase with temperature. At low temperatures (below 15°C), D. tigrina ingests little food, and growth and respiration rates are much lower than in the 2 other species. Similarly D. tigrina does not reproduce below 15°C, confirming its thermophilic nature.

P. tenuis is more temperature sensitive than P. felina. In P. tenuis, food intake, and growth and respiration rates, reach a maximum at 20°C, whereas they are greatest in P. felina at 15°C. These differences between the 2 species are reflected in the higher Q_{10} values observed in P. tenuis.

During starvation, the exponential rates of degrowth increase with temperature. Except for D. tigrina at 5°, 10° and 15°C, respiration rates are significantly lower in starved than in fed individuals. The respiration rates also increase with temperature, although this is less pronounced in P. felina than in P. tenuis or D. tigrina. In all 3 species, reproduction ceased soon after the onset of starvation.

The field study of P. felina identified seasonal changes in temperature and food supply. An examination of potential mortality factors demonstrated that predation was likely to be negligible, and that death was most likely to occur through being washed away.

In conclusion, it was suggested that the indigenous asexual reproducer (P. felina) was successful because it was eurytolerant, and because it occurred in streams where biotic stress (i.e. competition and predation) was low. Under such circumstances, the adoption of a low cost method of reproduction such as fission, was argued to be prudent. In contrast, D. tigrina, the immigrant asexual reproducer, could compete successfully with indigenous populations of lake-dwelling triclads such as P. tenuis by growing and reproducing at a very high rate.

The following species of freshwater triclad are referred to by these abbreviations in the text:

Planaria torva	*P. torva*
Planaria fissipara	*P. fissipara*
Polycelis tenuis	*P. tenuis*
Polycelis felina	*P. felina*
Polycelis nigra	*P. nigra*
Phagocata vitta	*Ph. vitta*
Phagocata gracilis	*Ph. gracilis*
Crenobia alpina	*C. alpina*
Dugesia tigrina	*D. tigrina*
Dugesia lugubris	*D. lugubris*
Dugesia polychroa	*D. polychroa*
Dugesia gonocephala	*D. gonocephala*
Dugesia dorotocephala	*D. dorotocephala*
Dugesia izuensis	*D. izuensis*
Dendrocoelum lacteum	*D. lacteum*
Bdellocephala punctata	*Bd. punctata*

"I ask charity for ..... imperfections in view of the labour involved."

(L.H. Hyman, 1940, p.9)

Chapter 1

INTRODUCTION

There has been much debate concerning the evolution and ubiquitous occurrence of sex in plants and animals (Maynard Smith 1971a, 1971b, 1978; Williams 1975). Sex must have evolved at a very early stage in the history of life, since some of the most primitive eukaryotes reproduce sexually. A variety of methods of sexual reproduction, for example, are employed by such simple animals as Protozoa (Sleigh 1973).

Models have been constructed which confer both short-term and long-term advantages to organisms that reproduce sexually (e.g. Williams and Mitton 1973). Sex involves meiosis and fertilisation, resulting in gene reshuffling. As a result, sexual species are more variable and better able to survive environmental changes. In this way "sexual reproduction facilitates evolution by making extinction less likely" (Williams 1975, p.154).

Long-term advantages are seen by some biologists to arise from recombination. Recombination means that (in theory) different beneficial mutations arising in separate individuals can come together in future generations (Fisher 1930; Muller 1932; Maynard Smith 1971a; Kimura and Ohta 1971).

Despite this, other methods of reproduction exist. Balancing arguments in favour of asexual reproduction point out that sex may be more efficient at breaking up gene combinations than it is at constructing them (Eshel and Feldman 1970), and that in such cases this will result in the loss of "adaptively proven combinations" (Williams 1975). Also, sex is expensive in energy terms, involving such costs as the search for a mate.

If a mutation arose in an individual that resulted in it becoming parthenogenetic, then it would increase in numbers twice as

quickly as its sexual counterparts (Maynard Smith 1971b). It is argued that under certain circumstances, parthenogenesis would be the fitter strategy (Richards 1973; Congdon, Vitt and Hadley 1978; Glesener and Tilman 1978). Generalisations about the exact conditions under which parthenogenesis is favoured are not clear. For example, species colonising a new area could be either sexual or asexual. Sexual species would do better moving into K-type environments with high biotic diversity, and parthenogenetic species could do well colonising r-type environments where abiotic mortality factors were more important.

Most models which have been constructed to explain the relative merits of different reproductive strategies have concerned themselves with sexual and parthogenetic reproduction (Williams and Mitton 1973; Williams 1975; Maynard Smith 1978). Little attention, however, has been paid to fission. This is largely due to a sparseness of data which this thesis has set out to help rectify.

The aims of this thesis are to examine the relative adaptive merits of asexual (fission) and sexual strategies in several species of free-living freshwater flatworms (Turbellaria: Tricladida). They are an excellent group of animals to study, since they show considerable inter- and intra- specific variation in life cycles (Reynoldson 1978) and mode of reproduction (Reynoldson 1961). Prerequisites, such as their taxonomy (e.g. Ball 1971, 1974), physiology (e.g. Hyman 1951), and general ecology (e.g. Reynoldson 1966a) have been well studied. Triclads are readily cultured in the laboratory (McConnell 1967), and are also easily observed in the field (Reynoldson 1966a).

Eleven species of freshwater triclad are found in Britain (Reynoldson 1978). In this study I chose to look at 3 species which

differed in their taxonomy, distribution and mode of reproduction : Polycelis tenuis (Iijima), Polycelis felina (Dalyell) and Dugesia tigrina (Girard). P. felina and D. tigrina reproduce mainly by fission, but have different distributions in the British Isles. P. felina is a stream-dwelling triclad with an extremely widespread distribution (L. Bellamy, pers. comm.), whereas D. tigrina is restricted to the warmer, more eutrophic lakes and rivers of England (Reynoldson 1978). P. tenuis is an iteroparous lake-dwelling species which is widely distributed in Great Britain (Reynoldson ibid).

Several studies have suggested a link between environment and mode of reproduction in freshwater triclads (Kenk 1937; Hyman 1941; Dahm 1958; Reynoldson 1961; Kawakatsu, Yamada and Iwaki 1967; Calow 1977b). Reynoldson (1961) suggested that species which reproduced asexually were restricted to unproductive habitats and that sexual reproduction was more important in eutrophic conditions.

Reynoldson (ibid) further suggested that under oligotrophic conditions, a poor food supply exacerbated by the maintenance of high activity levels (even at low temperatures) could result in insufficient energy being available to reproduce sexually. By reproducing asexually (fissioning), stream-dwelling triclads could solve this problem. Calow (1977b) corroborated the theories of Reynoldson by demonstrating that P. felina was very sensitive to temperature fluctuations and starvation conditions, resulting in high rates of degrowth. Calow further suggested that this explained why P. felina was restricted to habitats with low and stable temperatures.

However, several inconsistencies are apparent in the above arguments. D. tigrina reproduces asexually, and is restricted in the U.K. to warm, eutrophic waters (Reynoldson 1978). P. felina has a

more widespread distribution than previously reported (Calow 1977b), being ubiquitous in streams throughout Britain (L. Bellamy, pers. comm.).

Other conclusions concerning the high metabolic rates (activity levels) and the relative costs of different methods of reproduction have been drawn from scanty data. Little work has been done on energy balance in the asexual species of triclad. It was felt that a rigorous investigation into the energy partitioning of both sexual and asexual species at different temperatures and feeding regimes would result in a clearer picture of why asexual reproduction is common and apparently successful amongst triclads.

The aims of this study, then, are to examine the energy partitioning of fed and starved groups of worms at different temperatures. Constant temperature regimes were chosen. In order to avoid some of the pitfalls of extrapolating measurements made at constant temperatures in the laboratory to field situations (Bullock 1955; Odum 1975), observations concerning acclimation rates were also made. A population of P. felina was studied in the field, and observations concerning temperature, water flow, food availability, reproductive rate, and mortality collected.

A part of this thesis has already been published (Calow, Beveridge and Sibly 1979) and is included in the Appendices. The paper is largely theoretical and is based on results from preliminary studies on P. felina, and includes little information about fission in D. tigrina. This thesis contains much more data on energy partitioning in all 3 species.

Chapter 2

LITERATURE REVIEW

2.1 Introduction

The considerable interest that has been shown by scientists in freshwater triclads over the years, is reflected in the existence of a vast literature concerned with almost every aspect of their taxonomy, physiology, ecology and behaviour. A review of all these studies is clearly beyond the cope of this thesis, and instead I will concentrate on those areas most pertinent to the present work.

In this chapter, I propose to review the more important studies that have been done on the taxonomy, distribution and ecology of the 3 species I have been concerned with: *P. tenuis*, *P. felina* and *D. tigrina*. In each of the following chapters, a relevant literature review will preceed the results and discussion sections.

2.2 Taxonomy and Zoogeography

The earliest descriptions of freshwater triclads were by Dana (1766) and Müller (1774, 1776). Müller (1774) first described all triclads under the generic name of *Fasciola*, but later changed this to *Planaria* (1776). In Zoologicae Danicae (ibid), a description is given of *Planaria nigra*. Ehrenberg (1831) formed a new genus *Polycelis* for the species, and further studies by Iijima (1884) suggested, on the basis of the structure of the penis and the colour of the whole animal, that there were two distinct species, *P. tenuis* and *P. nigra*. Reynoldson (1948) confirmed Iijima's observations, and strengthened the argument by submitting data on ecological and colour differences.

P. felina was first described as *Planaria felina* by Dalyell in 1814. Johnson (1822) renamed the species *Planaria cornuta*, and it was later described under this name by Johnson (1832). However, in

keeping with Ehrenberg's (1831) description of the genus Polycelis, Schmidt (1860; in Dahm 1958) renamed the species Polycelis cornuta. The species was finally given the name of Polycelis felina by Johnson in 1865 (Kenk 1974).

The earliest description of D. tigrina was by Leidy (1847), who gave it the name Planaria maculata. (Several years prior to this Darwin (1844) used the same name to wrongly describe a species of terrestrial planarian). In 1850 Girard, in his survey of the fresh-water triclads of the United States, replaced the specific name by tigrina. The name Dugesia tigrina was finally adopted by Hyman (1939).

A full list of the taxonomic names, by which these 3 species have been described, appears in Kenk (1974). From this it is apparent that there has been considerable confusion. Early taxonomic descriptions were based solely on morphological characteristics, which have since proved to be unreliable. Large variations in colour and form are now known to exist in most species (Hyman 1951).

Dahm (1958) believed that cytological studies are essential to a proper understanding of triclad taxonomy. However, he also stated that such studies should only be used in conjunction with the more traditional taxonomic methodology (Dahm 1967). Nevertheless cytological investigations by Dahm and others have done much to clarify triclad taxonomy.

Not only can P. tenuis and P. nigra be differentiated on morphological grounds (see above), but evidence produced by Benazzi (1963) demonstrates that cytological differences also exist. Cytological evidence, used in conjunction with morphological and ecological observations, has led most workers to conclude that P. felina and D. tigrina are superspecies. Dahm (1958) defined this as "categories of

a higher systematic rank than species". Both P. felina and D. tigrina can be thought of as existing in several different races, which differ in chromosome number, colour, shape, mode of reproduction and distribution.

Vandel (1921a, 1921b) believed that 3 sub-species of P. felina exist, which differ in the number and position of adenodactyls. Thienemann (1926, 1949) separated P. felina into 2 different sub-species on the basis of reproductive method (an asexual and a sexual sub-species). Dahm (1958) looked at samples of P. felina from more than 60 sites in Western Europe, and found no variation in the number and position of the adenodactyls. He did, however, find considerable variation in colour (grey to black, pale brown to dark brown), shape of the tentacles, and chromosome number. The haploid chromosome number is 9, but up to 27 chromosomes may be present. (Most animals had between 18 and 27 chromosomes). No relationship has yet been found in P. felina, between chromosome number and mode of reproduction, or any other character.

A series of papers summarised by Hyman (1951) and Kenk (1972) discuss the variations in colour, form and method of reproduction of D. tigrina in the United States. Dahm (1955, 1958) found variations in the chromosome number of 7 European populations he examined, and concluded that the haploid chromosome number is 8, and that individuals with 16 or 24 chromosomes were most common.

The distribution of freshwater triclads has been of considerable interest to biologists, and this is reflected in the great number of publications that exist. The 3 species being studied here are no exception, and thus there is a reasonably accurate picture of their past and present-day distribution.

D. tigrina is an American species. Within the United States it is ubiquitous, and is found in streams, rivers and lakes from the northern to the southern states (Kenk 1972). From here it has spread to South America (Marcus 1946; Ball 1971), Japan (Kawakatsu 1970), Israel (Kenk 1974) and Europe (Dahm 1955; Gourbalt 1969; Reynoldson 1978). The rapid spread of D. tigrina in recent years has undoubtedly been due to the activities of aquarists, and Reynoldson (ibid) states that it can be seen in the aquaria of London dealers.

Early records of D. gonocephala in Britain by Whitehead (1915) and others have subsequently been thought to be erroneous. Reynoldson (1955) believes that the species was probably D. tigrina. However, the earliest positive record of D. tigrina in Europe was by Meinken (1925, 1927) who first recorded seeing it in Bremmen. From there it rapidly spread throughout Germany (Tu 1938; Menthe 1939) and Western Europe. It is now found in France (Beauchamp 1946; Lender 1951; Tuzet and Perrugia 1957; Alause 1968, in Gourbalt 1969), Switzerland (Dahm 1955, 1958), the Netherlands (Hartog 1959, 1962), Roumania (An der Lan 1962, in Gourbalt 1969) and in Italy and Spain (Gourbalt ibid). Reynoldson (1955) recorded that the species was found in only 4 places in England: Tenley Lake and Conniston Hall Lake in Yorkshire, the River Wye and the River Thames. Since then Pickavance (1968) has also recorded it in Colemere (Shropshire) and in Virginia Waters in Surrey. The most recent records are summarised by Reynoldson (1978) who states that it has now spread in Southern England and is also found in the Norfolk Broads. It is likely to spread further in the British Isles, although whether it will invade Scotland is debatable (see below).

Due to the difficulties that early researchers had of

distinguishing P. tenuis from P. nigra, the distribution of this species is somewhat uncertain. Kenk (1974) has attempted to distinguish between those publications which clearly identify P. nigra and those which have erroneously ascribed the name to P. tenuis. We can, however, be certain that P. tenuis is an indigenous European species which has been definitely recorded in France (e.g. Lender 1936; Lascombe 1974), Germany (e.g. Iijima 1884), the Netherlands (e.g. Hartog 1962), several Eastern European countries and Western Siberia (Kenk 1974). P. tenuis is found in most parts of the British Isles, although it is uncommon north of the Great Glen Fault as are all other lake-dwelling species (Reynoldson 1966, 1978).

P. felina is also an indigenous European species, and it is found in almost every European country from Denmark in the north, to Spain and Italy in the south (Dahm 1958). Eastwards, its range covers most of the Eastern European countries (Kenk 1974). It has also been recorded in North Africa (Kenk ibid). In Britain, its distribution has been described as widespread and locally abundant (Wright 1968). P. felina occurs commonly in North Wales and Anglesey (Carpenter 1928; Wright 1968; Reynoldson and Jaques 1976), mainland Scotland and England (Reynoldson 1978), and on some of the Western Isles (Bertram 1939; Reynoldson 1953; Baird and Beveridge, unpublished data).

The interpretation of the present day distribution of freshwater triclads is open to debate. Ullyot (1936) states that the dispersal of triclad species must have been active since passive dispersal cannot possibly account for their present-day distribution. He supports this statement by presenting evidence concerning the large differences between triclad faunas on opposite sides of narrow sea straits e.g. the English Channel, Menai Straits (North Wales) and

Skaggerrak and Kattegat (Reynoldson 1966). Ball and Fernando (1969) concur with this view.

However, there are exceptions. There is evidence of passive dispersal of freshwater triclads by birds (Dahm 1958; Reynoldson 1966a), and egg capsules by floodwaters (Leloup 1944). Other exceptions are the dispersal of D. tigrina (see above) and P. tenuis (Reynoldson 1978) through fish stocking, and of P. tenuis by students engaged in freshwater biology field courses (Reynoldson and Jaques 1976). Many of these cases are discussed by Ball (1974).

2.3 Ecology and Distribution

The ecology of freshwater triclads in the British Isles is well documented largely as a result of the research undertaken by Reynoldson and his students. A review of the ecology of lake-dwelling triclads is given in Reynoldson (1966a), and summaries of the ecologies of all British species appear in Reynoldson (1978).

P. tenuis is one of the 9 lake-dwelling species of triclad recorded in the British Isles (P. felina and C. alpina may be occasionally found in lakes [Reynoldson 1953]). It commonly occurs in the slower stretches of rivers, and most types of lakes and ponds (Reynoldson 1978). The general ecology of this species has been studied by Reynoldson (1960, 1964, 1966a) and others in Britain, and by Bengtsson (1971), Pattee (1972, 1975) and Lascombe (1973, 1974) in Europe.

P. tenuis is a perennial, iteroparous species, which generally begins to breed and deposit cocoons in February, when the temperature rises above 5°C (Reynoldson 1960, 1964, 1966a, 1978; Calow and Woollhead 1977a). The emergence of the young results in increased intraspecific

competition, and a gradual disappearance of the adults through shrinkage (Reynoldson 1966b). Reynoldson (1964) and Reynoldson and Bellamy (1971) have proposed intraspecific competition for food as a major regulator of the numbers of P. tenuis. Aggregation of triclads on a prey item during feeding may result in intraspecific competition, even when food is abundant (Reynoldson and Bellamy ibid). The diet and feeding behaviour of freshwater triclads is reviewed in Chapter 4).

Interspecific competition for food also occurs, particularly between P. tenuis and the closely related species P. nigra (Reynoldson 1975). Intense interspecific competition may force P. tenuis to modify its diet, and under exceptional conditions, to depend on a single prey species (food refuge). The outcome of such competitive interactions depends on the species competing, and the nature of the prey, but it undoubtedly has a major influence on the distribution of P. tenuis (Reynoldson 1966a).

The role of predators in regulating the population size of P. tenuis is likely to be unimportant. Indeed, Young and Reynoldson (1965) have shown that under laboratory conditions only some dragonfly nymphs, sticklebacks and the water beetle D. marginalis predated on P. tenuis. Serological studies by Davies (1969a, 1969b) and Davies and Reynoldson (1969, 1971) extended the list of predators of P. tenuis, but confirmed that the incidence and intensity of predation in the field is insufficient to reduce their numbers below the level at which intraspecific competition for food operated.

P. felina is one of the 4 species of stream-dwelling triclad which occurs in the British Isles. Bellamy (pers. comm.) has described its distribution as widespread, and it has been recorded almost everywhere in the U.K., from the Inner Hebrides (Bertram 1939) to the South

of England. Indeed, Reynoldson (1978) states that it may generally be found "wherever suitable habitats are present". It commonly occurs on the undersides of stones in small streams where the gradient does not rise above 27% (Wright 1968). In such streams it may be found as far upstream as the source. Its lower limits would appear to be governed by temperature, although the picture is confused, e.g. Thienemann (1912) found that P. felina occurred at temperatures up to 17.5°C, whereas Stankovic (1934) claimed it was not found in streams where the temperature rose above 16.5°C. Such discrepancies may be partially accounted for by taking into consideration the polytypic nature of the species (Dahm 1958). Recent work by Pattee (1966) has demonstrated the ability of P. felina to withstand much higher temperatures for short periods of time.

In streams where C. alpina is also found, P. felina is restricted to those areas where the gradient is less than 16% (Wright 1968, 1974). Wherever low gradients are found, and where temperature is not a limiting factor, P. felina is dominant over C. alpina and Wright (ibid) has suggested that intense interspecific competition for food is the cause. Both species feed primarily on oligochaetes and arthropods (Wright ibid).

Reynoldson (1953) has also recorded P. felina during the summer months in Loch Lossit on Islay. Baird and Beveridge (unpublished data) observed that it was still present in Loch Lossit in the winter of 1979, and we can conclude that it is a permanent member of that lake community. However, records of P. felina in Windermere (Reynoldson 1978) suggest that it may only be a temporary member of the community, being washed in from streams during floods. The general ecology of P. felina has been studies by Beauchamp (1932),

Beauchamp and Ullyott (1932), Wright (1968, 1972, 1974, 1975) and Lock (1972) in the U.K., and by Thienemann (1922), Dahm (1958), Pattee (1966) and Russier-Delolme (1974) in Europe.

Although populations which reproduce sexually have been found in Europe (Dahm 1958), all the British populations of P. felina which have been examined so far, reproduce asexually by fission (Reynoldson 1978). Studies on populations in North Wales by Wright (1968), suggest that fissioning occurs all year round, with no distinctive breeding season. (The literature concerning the reproduction of P. felina is reviewed more thoroughly in Chapter 7).

Wright (ibid) found no seasonal maxima in numbers, and no evidence that intraspecific competion for food had an important regulatory effect on the population size of P. felina. Several species of Plectoptera and Trichoptera larvae do however prey on P. felina, and under certain circumstances may depress the population numbers, and limit the microdistribution (Davies 1967; Wright 1975). The parasite T. pyriformis may also affect the population density, although Wright (1968) believes that it does not influence the microdistribution of the species.

In the U.S.A., the ecology and distribution of D. tigrina has been studied by workers, including Curtis (1902), Kenk (1937, 1940, 1941), Hyman (1939, 1941), Armstrong (1964), Chandler (1966) and Folsom and Clifford (1978). In Europe, where it is a recently introduced species, its ecology and distribution has been studied principally by Dahm (1955, 1958), Reynoldson (1956), Pickavance (1968, 1971, 1971b) and Russier and Lascombe (1970).

As stated in Section 2.2, the distribution of D. tigrina in the U.K. is peculiar, being principally found in those areas into which

it was first introduced. There is, however, some evidence that it is becoming more widespread (Reynoldson 1978). It has been recorded mainly from artificial habitats, and a few lakes and stretches of slow moving rivers in England (Reynoldson ibid).

Most of our knowledge about the ecology of D. tigrina in the U.K. is based on the studies of Pickavance (1968, 1971a, 1971b) on a population in Colemere, Shropshire. He found that its ecology and life-cycle were largely governed by temperature, and concluded that it is a warm-water species. It does not start feeding until the temperature rises above 6°C. Its diet is catholic, and it will readily feed on naids, tubificids, chironomid larvae, gastropods and crustaceans (Pickavance 1971b). Its diet closely resembles that of P. tenuis, and Pickavance (ibid) concludes that the 2 species compete severely.

Although a sexually reproducing population of D. tigrina has been recorded in Virginia Waters, all other populations in the U.K. reproduce asexually by fission (Pickavance 1968; Reynoldson 1978). Pickavance (1968) found no viable eggs produced by the Colemere population and only 2 capsules out of 300 laid by laboratory stock proved viable. Reproduction also appears to be controlled by temperature, and fission will not start in the field until the water temperature exceeds 16°C (Pickavance ibid).

Some evidence also exists that intraspecific competition for food during the autumn when peak populations have been recorded, results in the stabilisation of numbers (Pickavance ibid). Predators of D. tigrina have not yet been identified, and so their impact on population size cannot be assessed. Cannibalism has been observed under laboratory conditions by some authors (Hull 1947; Best 1960; Armstrong 1964) but its relevance to field situations remains unclear.

Spontaneous tumours have been observed in D. tigrina by Stéphan (1960), although Pickavance (1968) claimed that only 0.1% of the animals in Colemere were affected. It is unlikely that these tumours play any significant role in regulating numbers.

Chapter 3

MATERIALS, METHODS AND PRELIMINARY OBSERVATIONS

3.1 Materials and Field Methods

As stated in the introduction, it was decided to investigate 3 species of freshwater triclad with contrasting distributions and modes of reproduction. P. tenuis and D. tigrina are primarily lake-dwelling species, whereas P. felina is restricted to streams (Reynoldson 1978). P. tenuis reproduces exclusively by sexual means (Reynoldson ibid), whereas (in Great Britain) P. felina reproduces asexually by fission (Wright 1968). All but one of the British populations of D. tigrina reproduce asexually (Pickavance 1968).

Both P. felina and P. tenuis were collected locally (in and around Loch Lomond), but D. tigrina had to be collected from a site in England due to its restricted distribution. The exact locations and characteristics of the collection sites are given in Table 1.

Loch Lomond is a very large, oligotrophic loch (Slack 1957), and 3 species of freshwater triclad are found there: P. tenuis, P. torva and D. lacteum (Slack ibid; Ball, Reynoldson and Warwick 1969; Woollhead pers. comm.). The population of P. tenuis is thought to be long-established (Woollhead 1979). During the late summer and early autumn large adult P. tenuis were collected from under stones near Glasgow University Field Station, which lies on the East shore of the loch, approximately 2 miles from Rowardennan.

P. felina was collected throughout the spring, summer and autumn months from the outflow of Balmaha Pond. The pond was drained and left empty for some time during 1974, by the Forestry Commission, so the history of the triclad population is unknown. However, it is probably of recent origin (since 1974), having been colonised by individuals from the pond inflow. A full description of the stream is given in Chapter 9.

	Lake/ Stream	Grid Reference	Ca^{2+} (mg/l)	Mg^{2+} (mg/l)	pH	Conductivity (mhos cm^{-1})	Total No. of Triclad Species	Presence of *Asellus*
Polycelis tenuis	Loch Lomond	NS 377959	3.6	1.05	6.4	121.2	3	+
Polycelis felina	Balmaha Pond Outflow	NS 425908	-	-	6.6	8.7	1	+
Dugesia tigrina	Colemere, Salop	SJ 4333	40.7	-	7.6	7.6	5	+

Table 1 : The location and some characteristics of the collection sites for *P. tenuis*, *P. felina* and *D. tigrina* used in the study

D. tigrina was collected from Colemere, Shropshire, during the warmer months of the year. The population was first reported by Pickavance (1968). The lake is large and eutrophic, and contains several other species of triclad: P. tenuis, P. nigra, D. lacteum, P. torva (Pickavance ibid).

Animals were collected from the undersides of stones, using a paintbrush or micro-spatula. The animals are easily injured, and great care was taken to avoid damaging them. Nevertheless, on return to the laboratory the specimens were closely examined and damaged or unhealthy (obviously infected with parasites) animals picked out and discarded.

Chapter 9 of this thesis is concerned with a field study of P. felina and the field methods used are described there.

3.2 Laboratory Methods

In this section I will describe the general conditions under which the triclads were cultured, and the technique used to estimate their size. These methods have been used throughout this study. More specific methods will be found in the relevant chapters.

All P. tenuis used in this study came from laboratory stock. Animals collected from the field (see above) were incubated in groups of 10 at 10°C and 15°C in large evaporating dishes containing 300ml of filtered lake water, and fed every other day on Asellus aquaticus (L.) (see below). The egg capsules produced at 10°C were incubated at 5°C and 10°C, whilst those produced at 15°C were incubated at 15°C and 20°C. This procedure was carried out in order to minimise any effects which a dramatic change in temperature might have had on the embryos, and the subsequent growth and development of the hatchlings.

(An evaluation of temperature acclimation in triclads is described in Chapter 5). When the young emerged from the egg capsules, they were randomly sorted into individual perspex pots containing 30ml of filtered lake water.

D. tigrina and P. felina were cultured in exactly the same manner. On return from the field, groups of 10 were placed in glass evaporating dishes, containing 300ml of filtered lake or stream water. Samples of water were taken from Colemere, and filtered and frozen on return to Glasgow, so that D. tigrina could be cultured in conditions approximating to those found in the field. Dishes of animals were incubated at 5°C, 10°C, 15°C and 20°C, and these animals were fed twice weekly on Asellus. After a period of at least 60 days, adult animals (i.e. animals which had not recent fissioned) were transferred to individual perspex pots containing 30 ml of filtered lake or stream water. In all cases, the water was changed weekly, and the dishes wiped clean of mucus and food debris.

Attempts were made to culture P. felina in aerated water. It is a stream-dwelling species, and so is exposed to water with high levels of dissolved oxygen. I confirmed this by measuring the oxygen concentration in the field under different conditions, and found no appreciable difference between measurements made in mid-summer and measurements made in mid-winter (see Chapter 9).

Ten pots, identical to those described above, were filled with 30ml of filtered stream water, and placed in an incubator at 10°C. Holes were drilled in the lids of the pots and a short length of 4mm glass tubing drawn to a fine point, carefully pushed through. The tubes were connected, via plastic tubing, to an air pump, and the flow of air through each of the pots set at ca. one bubble per second.

One animal was placed in each pot, and fed normally (2 Asellus per week). However, the mortality rate was high. After 4 weeks, 6 of the 10 animals were dead. Three weeks later, the remaining animals had also died.

Although the causes of death are uncertain, it seems unlikely that high oxygen concentrations could be to blame. However, the air bubbles themselves may have been in part responsible. During the period of observation the animals fed irregularly, and all shrank in size. Only 1 triclad fissioned. Although small, the bubbles may have agitated the water and disrupted normal feeding and behaviour sufficiently to contribute to their deaths. In the field, P. felina lives on the undersides of stones. This is an area of dead or reduced current (Ambuhl 1959). Laboratory observations by Lock (1972) have shown that it prefers areas of low current to areas of high current velocity.

The animals were cultured in Griffin incubators. A thermometer (Range -10°C to +50°C), which had been inserted into a sealed water-filled conical flask was placed in each of the incubators. In this way the actual temperatures in the triclad dishes could be checked. The ranges of temperature recorded by thermometer are given in Table 2. The incubators were defrosted regularly, but despite this, temperature fluctuations persisted.

Previous studies have shown that triclads were very sensitive to light (Marriot 1958) and were negatively phototrophic (Walter 1907; May and Burukow 1966). In the field they live under stones, or in amongst vegetation, and are probably subject to very low light levels. Nevertheless, the effect of culturing P. felina when subjected to strong light was tested.

Reading on Incubator Temperature Gauge	Range of Temperatures measured in the incubators by thermometer
5°C	4° - 6°C
10°C	8.5° - 11°C
15°C	14° - 16°C
20°C	19° - 21°C

Table 2 : Results of thermometer and temperature gauge readings from Griffin incubators used in this study

Two dishes of 10 P. felina were placed in incubators set at 10°C, in a dimly-lit room. One incubator was illuminated by 6 x 50 watt fluorescent lights, and the other remained unlit. Every hour, the doors of the incubators were carefully opened, and the numbers of moving triclads recorded. The results are given in Table 3.

Light increased the activity level of those animals kept in constant light, although this effect decreased with time. After ca. 8 hours, no difference between the two dishes was observed.

Recent studies have demonstrated that strong light can reduce the rate of fission (Legner, Tsai and Medved 1976) and affect feeding (Yu and Legner 1976) and movement (Bellamy and Reynoldson 1974). The triclads were therefore cultured in the dark, and exposure to strong light kept to a minimum, in order to reduce the effects of adverse laboratory conditions on their energy expenditure and life cycles.

Triclads appear to feed readily on a number of different foods under laboratory conditions (e.g. Reynoldson and Sefton 1976). However, because it was hoped to extrapolate from laboratory to field conditions, a food normally encountered in the field was chosen.

Reynoldson and Young (1963) and Reynoldson and Davies (1976) have shown that Asellus is a major food item in the diet of P. tenuis. It is commonly found in Loch Lomond (Slack 1957), the source of P. tenuis used here. Woollhead (1979) successfully used Asellus in a laboratory-based investigation into the rate of food consumption in this species.

Pickavance (1968, 1971b) found Asellus in Colemere, and recorded it as part of the natural diet of D. tigrina. Further studies on feeding under laboratory conditions confirmed this (Pickavance 1971a).

Time (Hrs)	Number of animals moving	
	Dark	Light
0	10	10
1	3	8
2	2	8
3	1	6
4	2	4
5	1	3
6	0	3
7	1	1
8	1	0
9	2	1
10	2	2

Table 3 : The effect of light on the activity of P. felina

Asellus is not a common member of stream communities (Morgan and Egglishaw 1964). It is, however, found in Balmaha Pond, and is occasionally washed into the outflowing stream, where there is a large population of P. felina.

In preliminary laboratory observations, all 3 species of triclad fed readily and grew well on Asellus. Tubifex was also used in initial feeding experiments involving P. felina, but poor results were obtained and the mortality rate of P. felina was high. This may have been due to the rapid death of Tubifex in still water, which resulted in fouling. Kouyoumjian (1975) reports that P. felina is extremely sensitive to organic pollution. Woollhead (1979) states that P. tenuis did not grow when fed on Tubifex.

The Asellus used in the feeding studies were collected at regular intervals from the field. Two sites were used: Woodend Loch (G.R. NS 703665) and Bardowie Loch (G.R. NS 579734) near Glasgow.

The measurement of triclad size was an important part of this study, as I wished to express growth rates, respiration and feeding as a function of animal size. Several of the techniques for estimating triclad size that had been used previously were unsuitable or too inaccurate to be of use here. Many of the methods described below were precluded, since they involved killing the animals. Løvtrup (1953) and Pedersen (1956) used nitrogen content, but this proved impractical in the present study due to the time and quantities of material required. Reynoldson (1960) and Adams (1979, 1980a, 1980b) among others, used length. However, this is not an accurate measurement of size during starvation, as differential shrinkage is known to occur (Child 1915). Volume was used by Greenburg and Schmidt (1936), and triclad area by Calow (1977b). In addition to assessing the

repeatability of results using the above methods, Woollhead (1979) tested wet weight and dry weight as indices. He found that area was the most reliable and easily measured parameter.

The plan area of the triclads was measured photographically, using the same equipment as Calow (1977b) and Woollhead (1979). The animals were photographed (X1 - X2) with a 35mm Zeiss Icon camera adapted for close up photography. Kodak Panatomic X (ASA 32, 16 DIN) black and white film was used.

The triclads were transferred from the incubator to a petri dish of water and photographed against a background of black 1cm graph paper. The animals were measured while moving in a straight line, with head and tail gliding at the same speed (i.e. no photographs were taken while animals were at rest, or during periods of looping behaviour). The actual area of the triclads was then measured planimetrically from enlargements (ca. X 10).

The reliability of the results was tested by taking several photographs of an animal over a short time period. Table 4 shows that there is very little variation between measurements (P. felina : mean = 17.34, S.E. = 0.26; P. tenuis : mean = 8.17, S.E. = 0.14).

At all times, fed animals were photographed on the second day after feeding.

However, as triclads grow, they increase in thickness (Woollhead 1979). Moreover, there may be interspecific differences in thickness, and this could have resulted in erroneous conclusions being drawn from the data. The relationship between area and dry weight was investigated. Animals were photographed, then dried in a vacuum oven at 45°C for 7 days, before being weighed on a Mettler electro-microbalance (± 0.001 mg). The plan areas of the worms (mm^2)

Species	Size (Area) mm^2	Number of Photographs	Mean Size	S.E.
P. felina	16.57	12	17.34	0.26
	17.08			
	18.04			
	17.96			
	16.52			
	18.04			
	16.21			
	18.17			
	16.30			
	18.34			
	18.14			
	16.75			
P. tenuis	8.50	10	8.17	0.14
	8.71			
	8.65			
	8.67			
	8.26			
	7.97			
	7.46			
	7.91			
	7.83			
	7.81			

Table 4 : The repeatability of results of measuring triclad size (area in mm^2) photographically

were then plotted against dry weight (mg), and the results are shown in Figure 1.

Significant linear relationships (correlation coefficient, $r, \geq 0.94$) were found between area and dry weight:

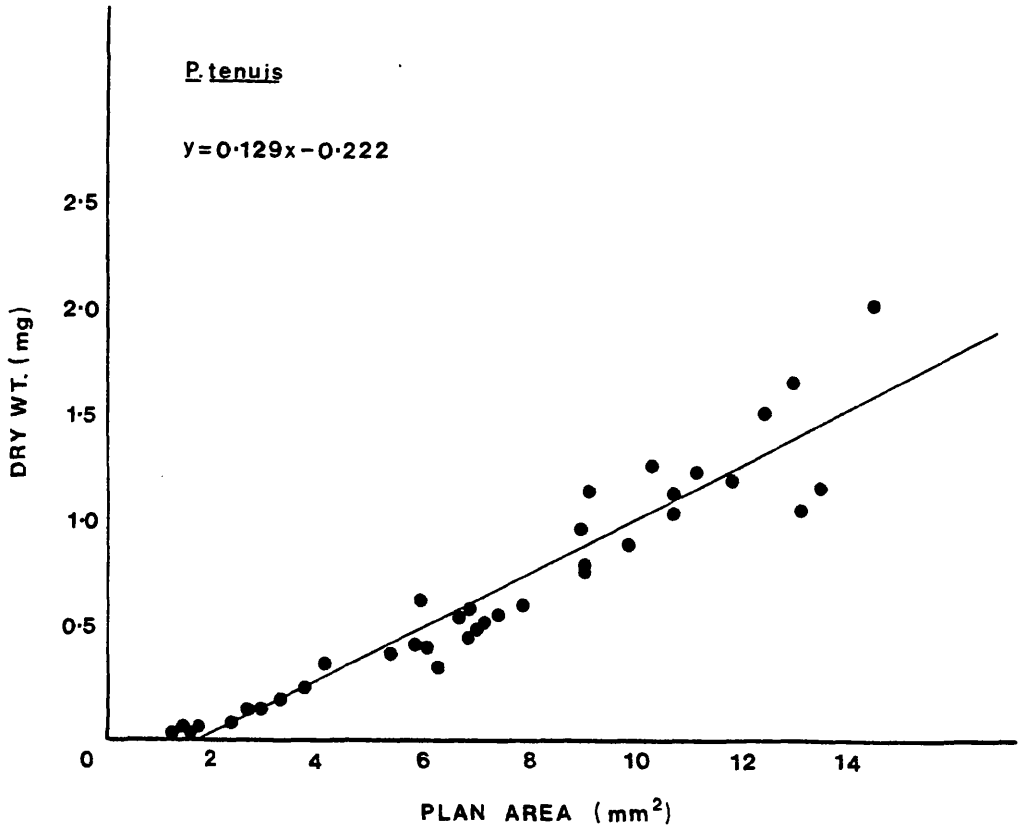
P. tenuis	$y = 0.129x - 0.222$	$r = 0.95$
P. felina	$y = 0.068x - 0.041$	$r = 0.94$
D. tigrina	$y = 0.059x - 0.046$	$r = 0.97$

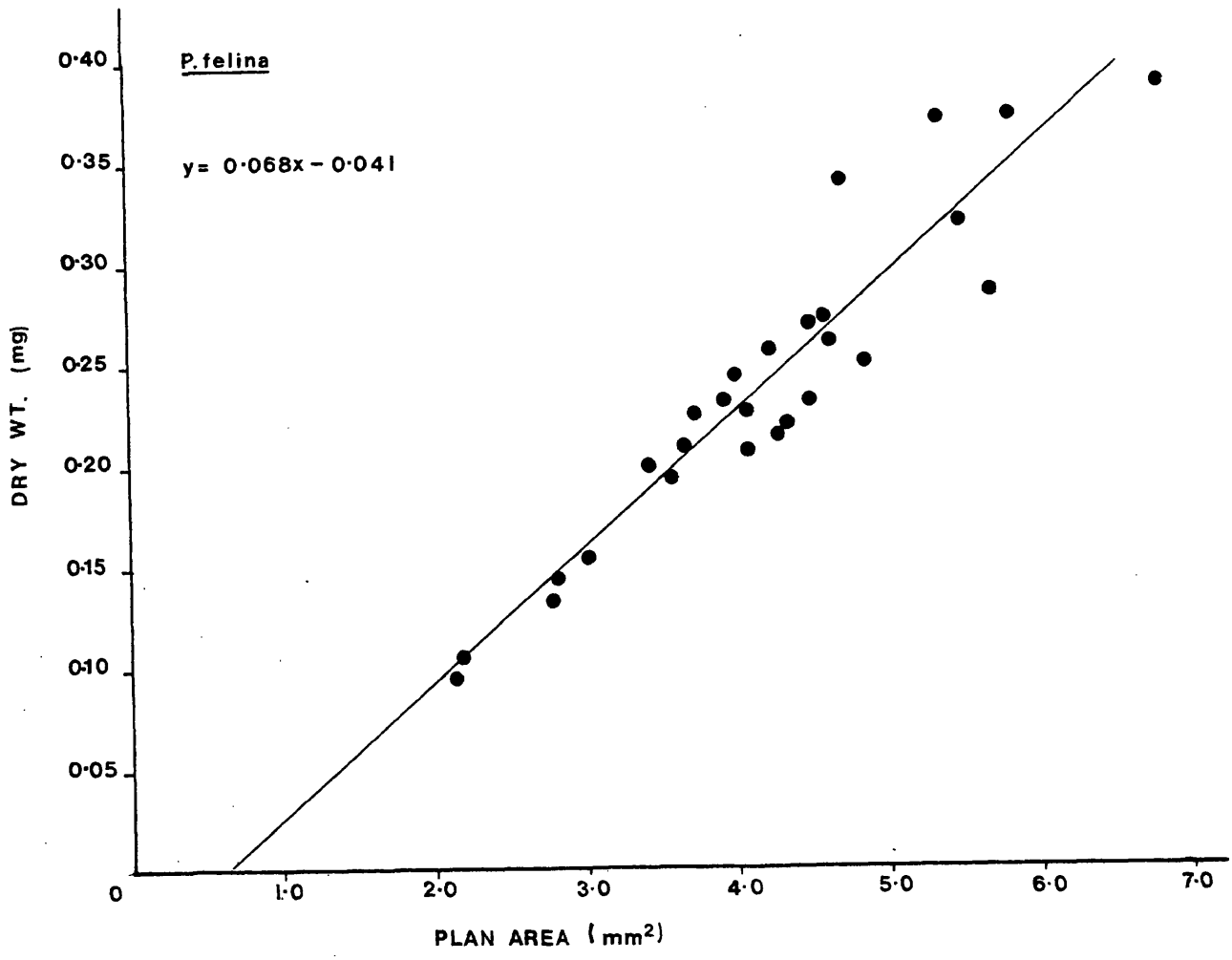
Woollhead (*ibid*) observed a curvilinear relationship between area and dry weight, but this was probably because he was investigating larger species in which changes in thickness with size were correspondingly greater.

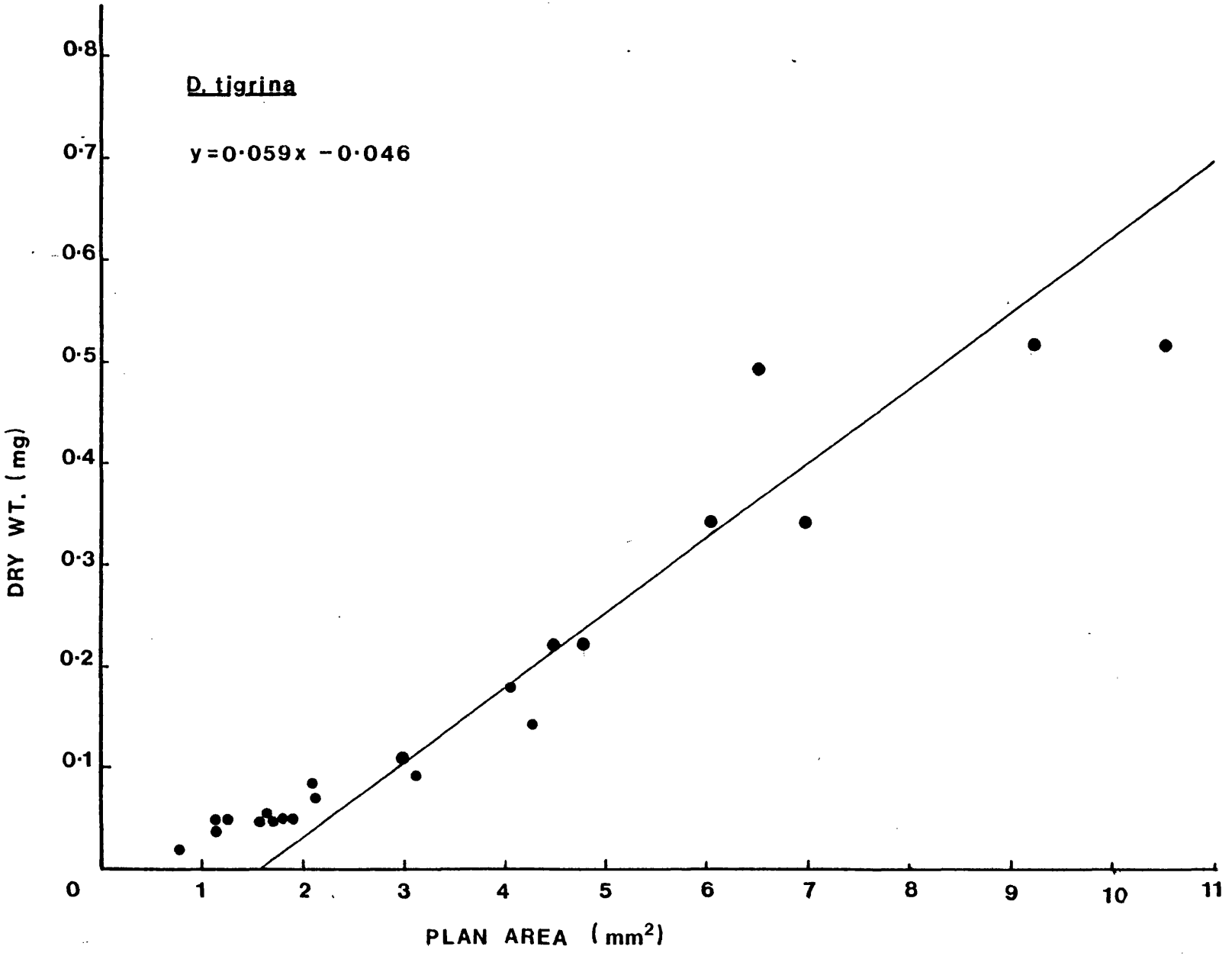
The above equations were used to convert triclad plan area to dry weight, and used to analyse interspecific differences in feeding, growth and respiration rates.

Figure 1

The relationship between plan area (mm^2) and dry weight (mg) in *P. tenuis*, *P. felina* and *D. tigrina*


P. tenuis
y=0·129x-0·222
DRY WT. (mg)
2·5
2·0
1·5
1·0
0·5
0
2
4
6
8
10
12
14
PLAN AREA (mm²)


P. felina
y= 0·068x - 0·041
DRY WT. (mg)
0·40
0·35
0·30
0·25
0·20
0·15
0·10
0·05
0
1·0
2·0
3·0
4·0
5·0
6·0
7·0
PLAN AREA (mm²)


D. tigrina
y = 0·059x − 0·046
DRY WT. (mg)
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0
1
2
3
4
5
6
7
8
9
10
11
PLAN AREA (mm²)

Chapter 4

FEEDING AND INGESTION

4.1 Introduction

This chapter is concerned with an examination of the feeding and ingestion rates of P. tenuis, P. felina and D. tigrina. The influence of size and temperature on the food intake of these species is looked at in detail, as part of a general investigation into energy partitioning in freshwater triclads.

4.2 Literature Review

Feeding in animals is not indiscriminate, although there are some exceptions. Filter feeders and deposit feeders appear to feed haphazardly on what is available, although Darwin (1898) demonstrates that earthworms, which are detritus feeders, show preferences for certain types of deciduous leaves.

A great deal of literature has been published concerning the nutritional requirements of animals and the diets of domestic animals (Crampton 1965; Blaxter 1965, 1967) and insects (Matthews 1976) are particularly well known.

Hyman (1951) states that triclads will not feed on plant or decaying material. Gut content analysis by Reynoldson and Young (1963) and subsequent laboratory and field studies (Pickavance 1970, 1971; Reynoldson 1975; Reynoldson and Bellamy 1975; Reynoldson and Sefton 1976; Reynoldson and Piearce 1979; etc.), confirmed the opinion of Hyman (1951) that freshwater triclads are predatory carnivores.

The nutritional requirements of animals are not constant, but vary with time. There are 3 principal factors affecting food consumption:

a. Seasonal abundance or scarcity,

b. Morphological and physiological limitations,

c. Physiological demands.

The seasonal abundance of food is primarily influenced by temperature and competition, and may force consumers to reduce consumption, or switch food supply (Krebs 1978). The morphological and physiological limitations of the consumer may affect the ability to capture, handle or digest food. These restrictions may change with time, and affect the choice and range of food available to the consumer (see Klekowski and Duncan 1975, p.231).

Physiological demands, too, may change with time. Seasonal changes in temperature can affect metabolic costs, and so necessitate a change in food consumption (e.g. Gophen 1976). Increased food intake during reproduction is a widespread phenomenon (e.g. Anthony and Kunz 1977; Hislop, Robb and Gauld 1978).

The diets of most British species of freshwater triclad have been studied in detail and their dietary preferences have been shown to be partly innate, and partly opportunistic (Reynoldson 1975). They feed on a wide variety of annelids, molluscs and arthropods (Reynoldson and Young 1963; Reynoldson and Davies 1970), although they may sometimes feed on newly spawned fish eggs (Newburg 1974). Cannibalism has also been observed in some species under certain conditions (Hull 1947; Best 1960; Armstrong 1964; Fox 1975).

Variations in the diet of a species are found with habitat (Reynoldson and Davies 1970), and season (Reynoldson and Sefton 1976), and both interspecific and intraspecific competition, play a crucial role (Reynoldson and Bellamy 1971, 1973; Bellamy and Reynoldson 1974).

Pickavance (1971a, 1971b) studied the diet of D. tigrina under laboratory and field conditions. The food of P. felina was studied by Lock and Reynoldson (1976) and that of P. tenuis by Reynoldson and Davies (1970), using serological techniques.

As triclads grow, their nutritional requirements change and this has been demonstrated in D. lacteum by De Silva (1976a), and Calow and Woollhead (1977b). Woollhead (1979) also showed that food consumption increased with size in P. tenuis, P. torva, D. lugubris and B. punctata. Changes in diet are partly due to the triclads' ability to capture prey. For example small D. lacteum are unable to capture large Asellus (De Silva 1976a). As triclads grow, increased physiological demands also necessitate an increase in food consumption.

Prey capture in triclads has been described by several authors (Hyman 1951; Jennings 1957, 1962; Pickavance 1971a; De Silva 1976a; Adams 1979) and usually involves the trapping and subsequent entanglement of small or wounded invertebrates, in a film of mucus. During feeding, the triclad inserts its pharynx into the victim, and withdraws the body contents into its gut. This happens even when the prey is small enough to be swallowed whole (Jennings 1974). Calow, Beveridge and Sibly (1979) reported that post-fission P. felina tails were unable to feed until the pharynx was regrown. The food in the gut is mechanically broken down, before being phagocytosed for intracellular digestion (Arnold 1910; Willier, Hyman and Rifenburgh 1925; Jennings 1957, 1962, 1974; Rosenbaum and Rolon 1960a; Osborne and Miller 1962; Horne and Darlington 1967; Sakharova and Sheimann 1978). Some evidence also exists that flatworms can absorb fatty acids, nucleic acid precursors, sucrose and amino acids through the body wall (Bernett, Herbert and Hughes 1967).

Measurements of the amount of food eaten have been made by Woollhead (1979), who studied the influence of various feeding regimes on the food intake of 5 species of freshwater triclad.

Carbohydrates, proteins and fats can all be digested and stored by triclads (Kelley 1931; Brand 1936; Jennings 1957; Osborne and Miller 1962). These reserves are called upon during starvation (Boddington and Mettrick 1971; Calow and Woollhead 1977a; Woollhead 1979). Studies on P. torva (Brand 1936), P. felina (Jennings 1957), D. tigrina (Boddington and Mettrick 1971), and D. lacteum and P. tenuis (Calow and Woollhead 1977a) have shown that glycogen stores are the first food reserves to be utilised. As starvation proceeds, other reserves are then used (Boddington and Mettrick ibid; Calow and Woollhead ibid). The importance of these food reserves in triclads is discussed in Chapter 5.

4.3 Materials and Methods

From 5 to 10 individual triclads of each species were cultured at each temperature, and fed on Asellus. However, Reynoldson and Young (1963) reported that live Asellus remained inaccessible to most triclads unless prevented from escape. Further studies by Bellamy and Reynoldson (1974) confirmed this. Thus the triclads were fed on Asellus which had been immobilised in a standard manner by lightly crushing the head with a pair of fine forceps. Woollhead (1979) successfully cultured a number of triclad species in this way.

The triclads were fed twice per week on a single immobilised Asellus. The food was left for 12 hours before being removed, and the remains dried as described below. The feeding periods alternated between nighttime (2000-0800 hrs) and daytime (0800-2000 hrs), to avoid possible diurnal feeding cycles (Best 1960).

The amount of food eaten was calculated from the difference in dry weight of Asellus, before and after feeding. Dry weight was

determined by drying the Asellus in a vacuum oven at 45°C for 72 hours, before weighing on a Mettler electro-microbalance (accuracy ± 0.001 mg). Since it was impossible to directly measure the dry weight prior to feeding, a parameter directly related to dry weight was used. Volume, length, uropod width, head width and wet weight were all tested as suitable indices by Woollhead (1979). Wetweight proved to be the most satisfactory index and therefore was used in this study.

The wet weight was determined by anaesthetising the Asellus in 30 mls of water to which 1 ml of 1:10 chloroform:methanol mixture had been added. Once immobilised, they were removed from the anaesthetic and blotted dry with filter paper, in a standard fashion. The Asellus were then immediately weighed on a Mettler electro-microbalance (accuracy ± 0.001 mg) before being returned to a dish of lake water to recover.

Slight seasonal variations in the wet weight/dry weight relationships were recorded (Table 5), and for accuracy's sake, the appropriate regression equations were used at each time of year.

In calculating the amount of food eaten, complications arose due to the loss of Asellus body contents to the medium through seepage. The method of Asellus immobilisation may have caused significant losses (Woollhead 1979). These were estimated as follows:

The wet weights of a number of Asellus were determined as described above. They were then immobilised and left in perspex pots, containing 30 mls of filtered lake water for 12 hours, before being removed, dried in a vacuum oven at 45°C for 72 hours and weighed. Estimates of the loss of body contents were then made by comparing the results with the standard regression lines given in Table 5. Measurable

January	D.W. = 0.228 W.W. + 0.081	r = 0.96
April	D.W. = 0.283 W.W. - 0.409	r = 0.98
July	D.W. = 0.214 W.W. + 0.1463	r = 0.97
October	D.W. = 0.219 W.W. + 0.0651	r = 0.95

Table 5 : Regression equations with correlation coefficients (r), relating dry weight to wet weight in Asellus, from Woodend Loch, at different times of the year.

Where D.W. = Dry weight
W.W. = Wet weight

decreases in dry weight were observed, and mean values, expressed as percentage loss of initial dry weight, are given in Table 6.

The amount of seepage recorded varied greatly at any one temperature. An increase in temperature caused an increase in seepage - possibly due to the greater viscosity of body fluids at higher temperatures.

Dagg (1974) reported that copepods may lose up to 25% of their body contents to the medium, whilst being fed on by predatory amphipods. However the copepod body is broken up during feeding. Jennings (1957) observed the feeding behaviour of P. felina on various arthropod species, and stated that the triclad's pharynx was inserted into its prey through weak points in the exoskeleton (e.g. between sclerites or through the areas of limb articulation). Thus seepage through feeding is likely to be insignificant.

Woollhead (1979) used radiotracers to measure seepage, and found the percentage loss of body contents to the medium in wounded Asellus was less when being fed on by triclads.

The triclads were fed with Asellus ≤ 17 mg wet weight. Above this size, the wet weight/dry weight correlation became unacceptable. In general, small triclads (< 10 mm^2) were fed small Asellus (< 10 mg), and large triclads (> 10 mm^2), large Asellus (10-15 mg).

4.4 Results

The food intake of P. tenuis, P. felina and D. tigrina, which were fed twice per week on immobilised Asellus was measured as described above. Between 5 and 10 animals were observed at each temperature (5^o, 10^o, 15^o and 20^oC), and their growth rates measured simultaneously (see Chapter 6). The influence of temperature and size on the amount

Temperature	No. of Observations	Mean Loss through seepage	Range
5°C	19	18.1%	4 - 35%
10°C	24	20.4%	0 - 44%
15°C	22	22.3%	3 - 39%
20°C	20	24.0%	2 - 41%

Table 6 : Estimates of weight changes in Asellus over 12 hours, due to loss of body contents through seepage, expressed as percentage loss of initial dry weight

of food ingested are examined. Although data were collected throughout the growth cycle, (and during regrowth in the sexual species), only data collected on non-reproducing triclads will be described here, since after this, complications due to reproduction arise (see Chapter 7).

In order to investigate the effect of size, food intake per week was plotted against triclad size (Fig. 2), and straight lines fitted by regression analysis. The data is summarised in Table 7.

In general, there is a great deal of scatter, and the correlation between triclad size and food intake is poor. In several cases (P. felina at 5°C, and D. tigrina at 5°, 10° and 20°C), the correlation coefficient is not significant ($r \leq 0.16$; p 0.05).

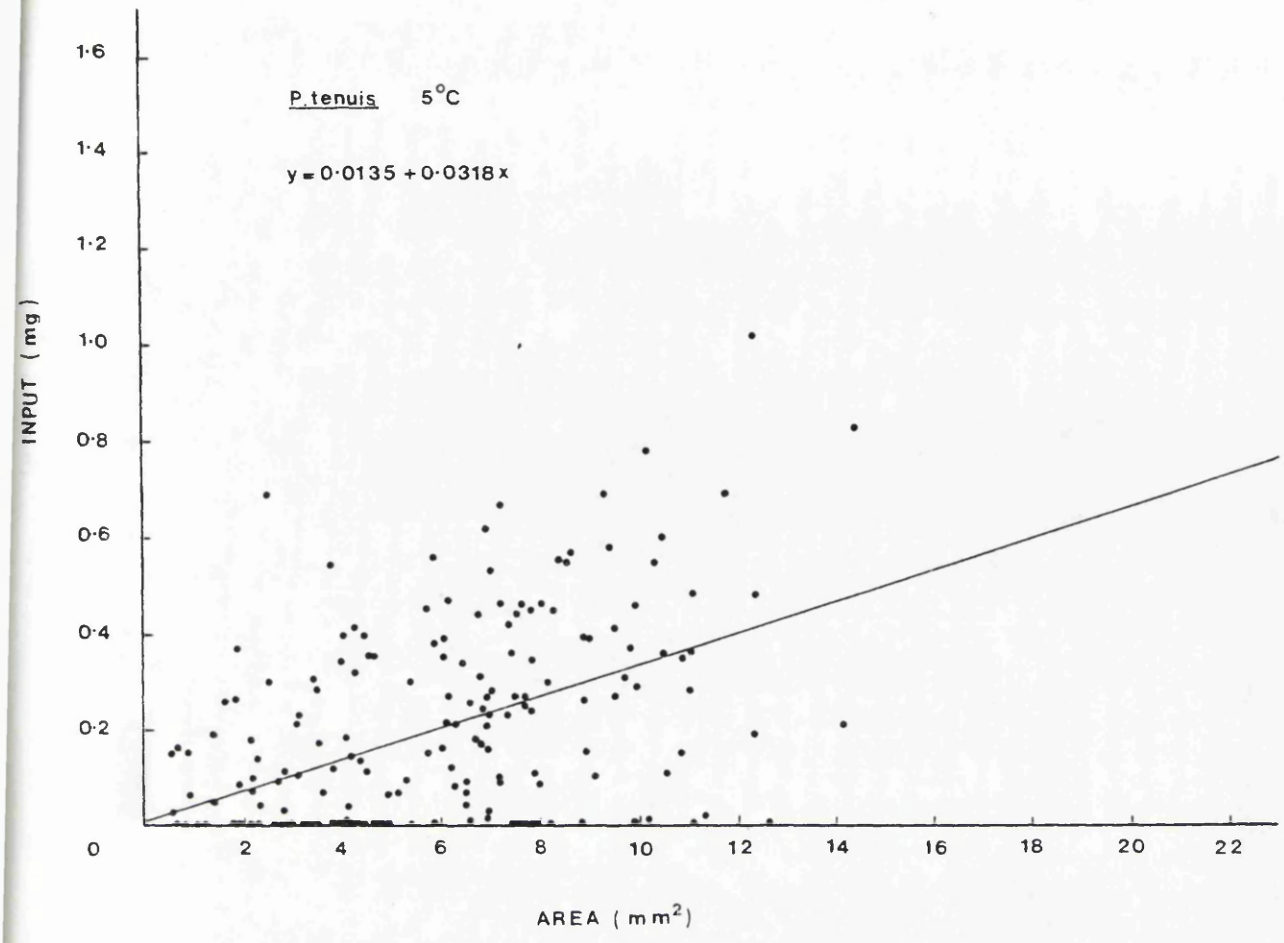
For P. tenuis at each temperature, the slope, b, is significantly different from 0, which shows that there is an increase in food intake with size. The influence of food intake on size is most clearly seen at 20°C, where b is greatest.

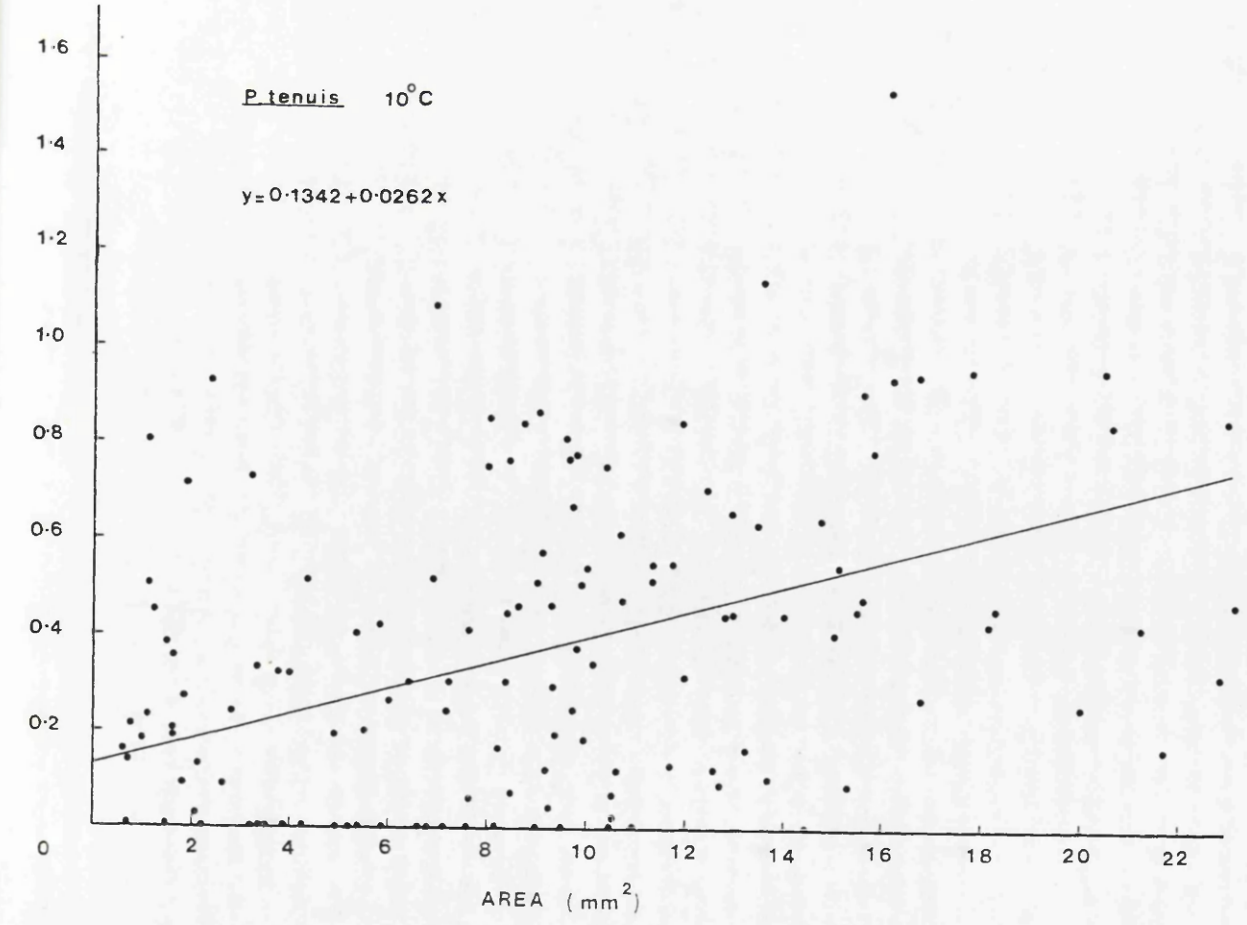
In P. felina, the influence of size on food intake is only apparent at certain temperatures. The slope, b, is significant ($p < 0.05$) at 10°, 15° and 20°C, although at 20°C, food intake and triclad size are inversely related. In D. tigrina, the relationship between triclad size and food intake is only significant at 15°C.

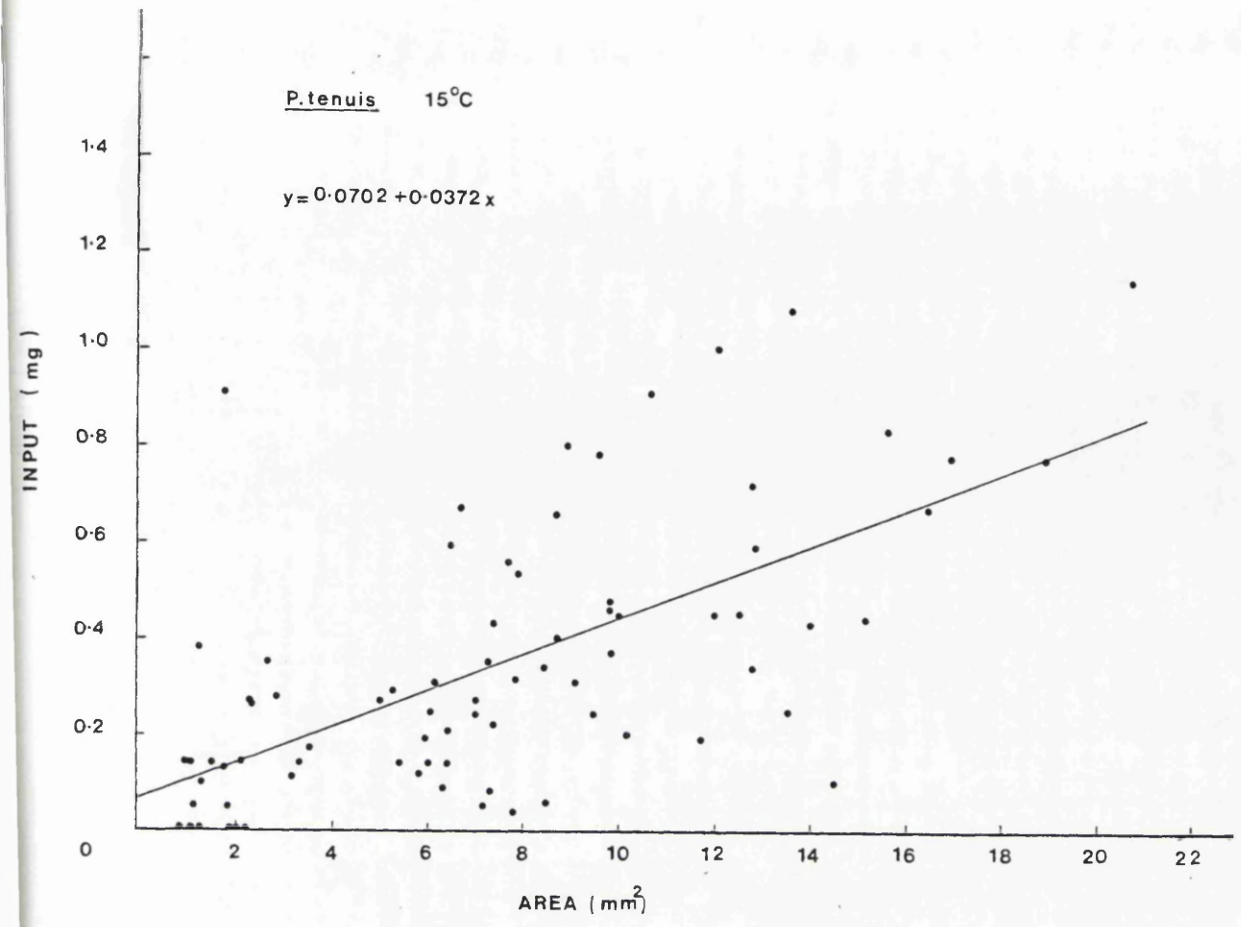
The b values from the regression equations in Table 7 were plotted in order to see how food intake was affected by temperature (Fig. 3). In P. tenuis, there is no significant difference in b values between 5° and 10°C ($d = 1.2$; $p > 0.05$). However, there are significant differences between 10° and 15°C ($d = 2.20$; $p < 0.05$) and 15° and 20°C ($d = 2.83$; $p < 0.05$) i.e. food intake per unit triclad size increased significantly with increasing temperature.

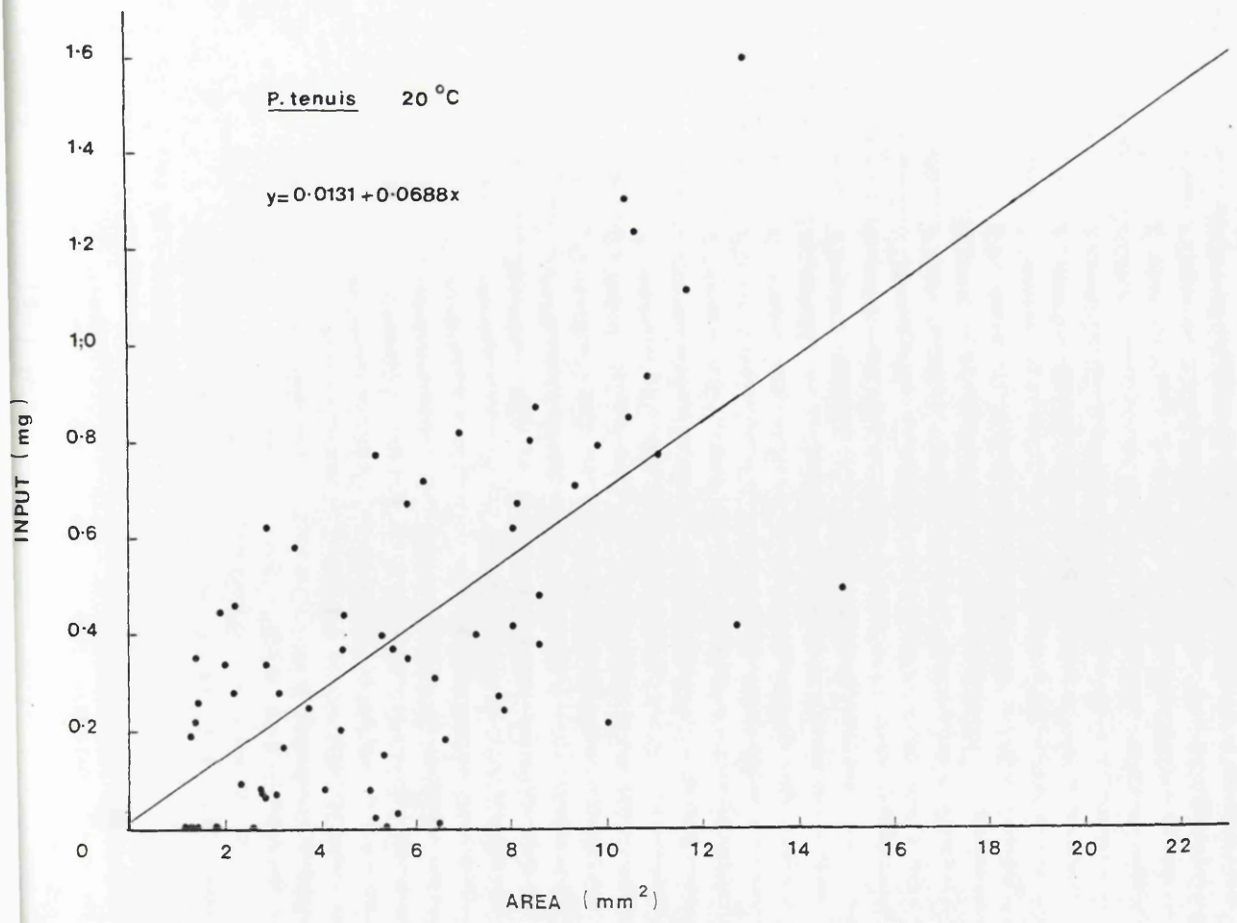
Figure 2

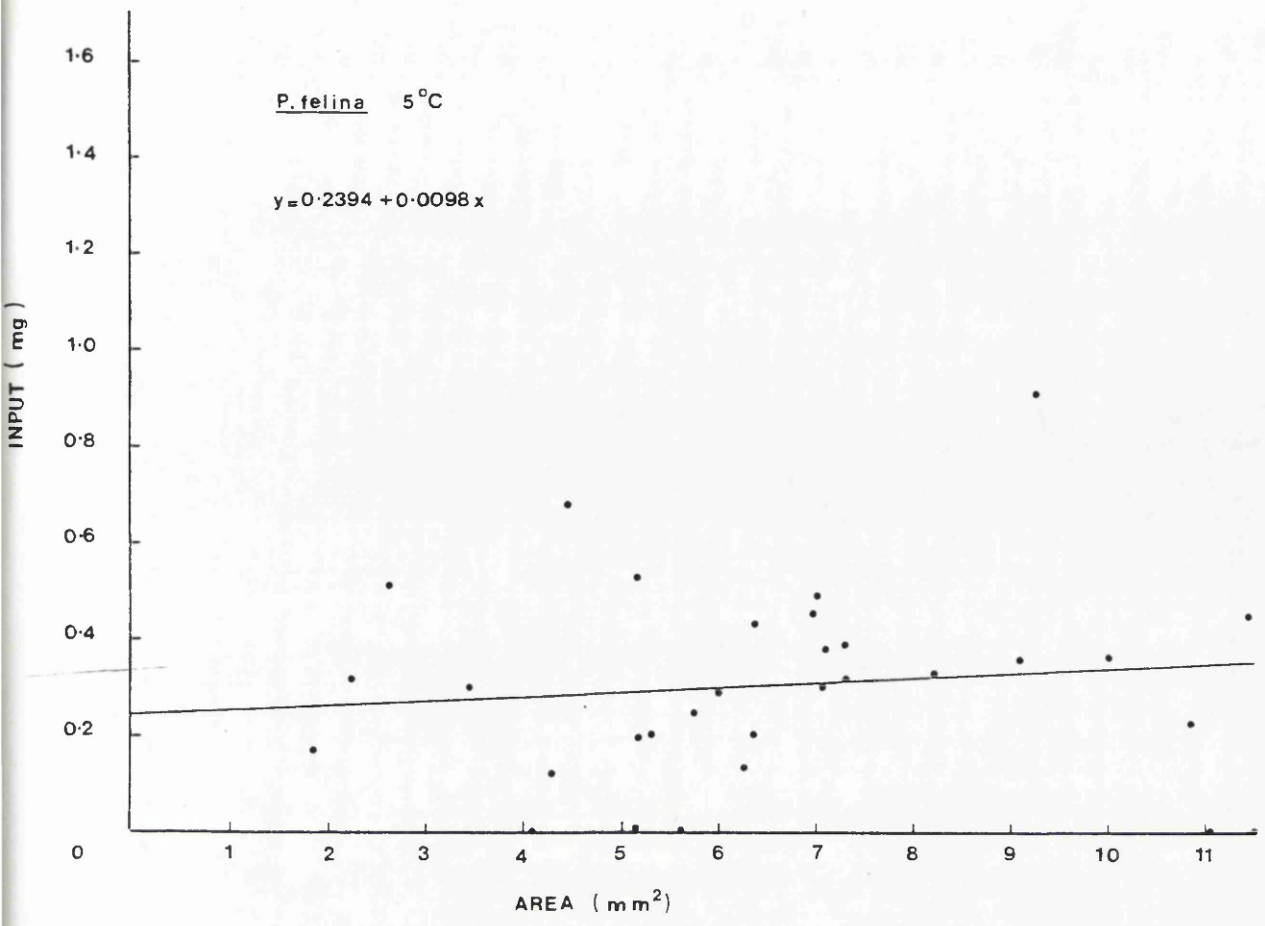
The relationship between weekly food intake, expressed as mg dry *Asellus* per week, and triclad size (area in mm^2) for *P. tenuis*, *P. felina* and *D. tigrina* at 5^{o}, 10^{o}, 15^{o} and $20^{o}C$.

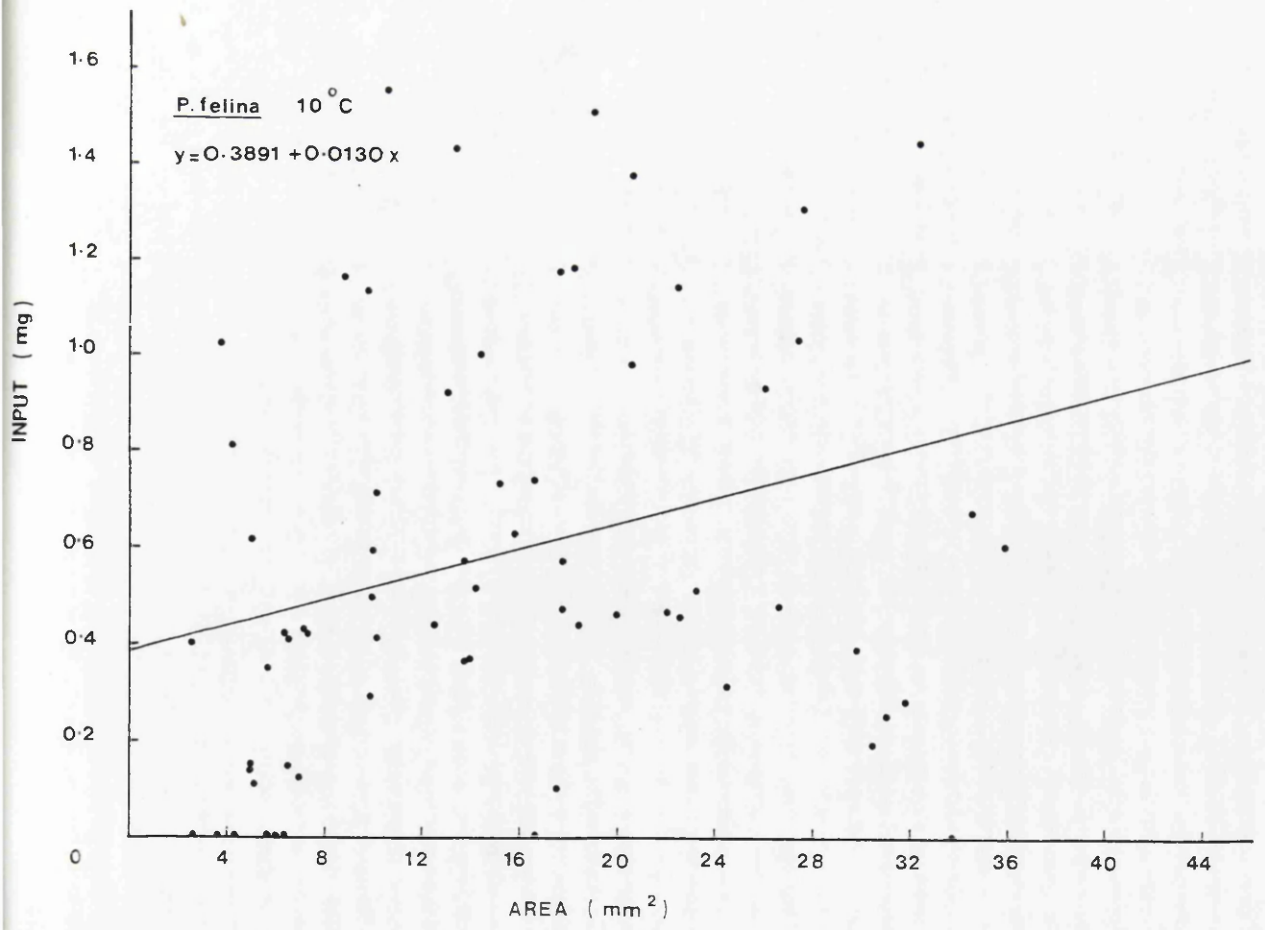

P. tenuis 5°C
y = 0·0135 + 0·0318x
INPUT (mg)
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
0
2
4
6
8
10
12
14
16
18
20
22
AREA (mm²)

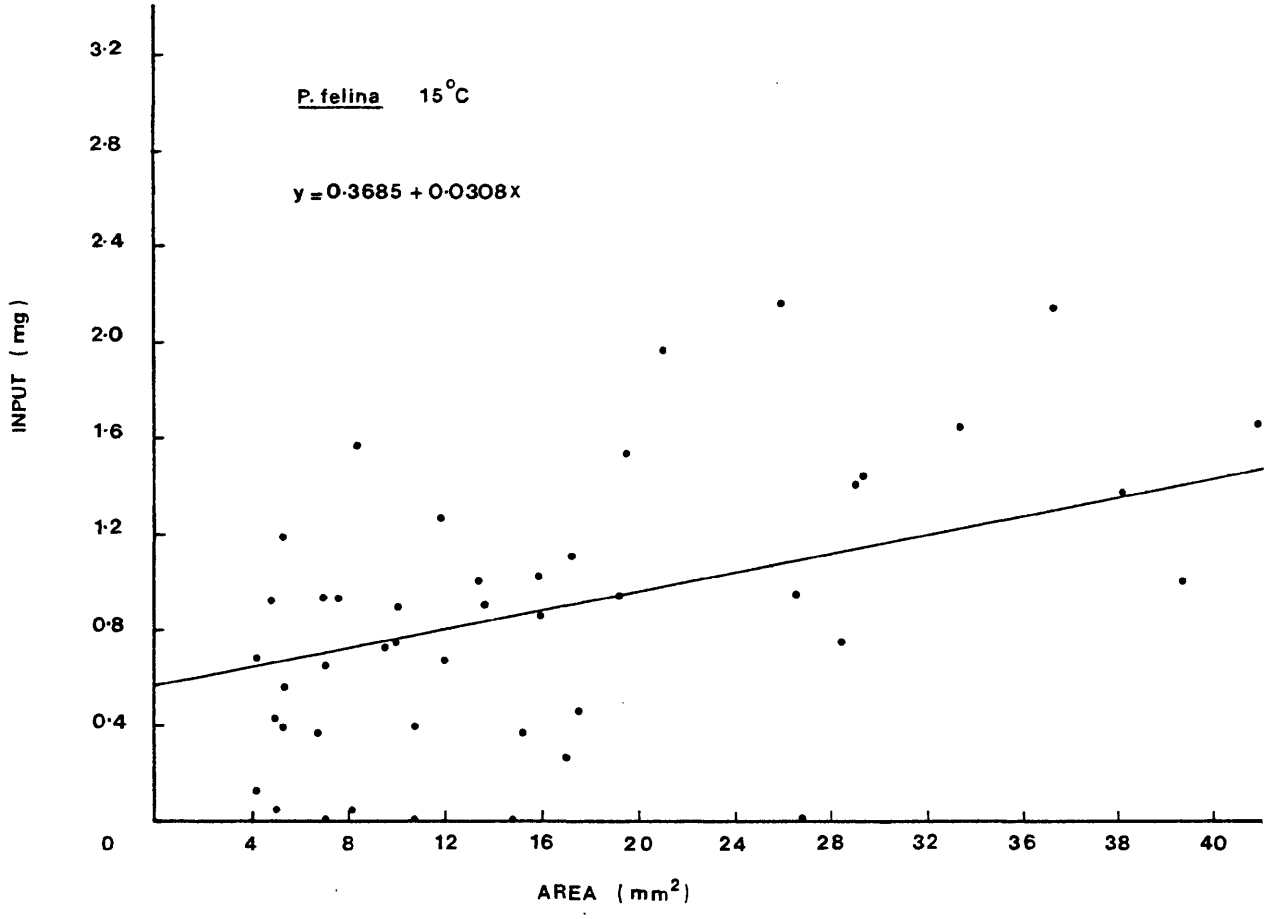

P. tenuis 10°C
y = 0·1342 + 0·0262x
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
0
2
4
6
8
10
12
14
16
18
20
22
AREA (mm²)

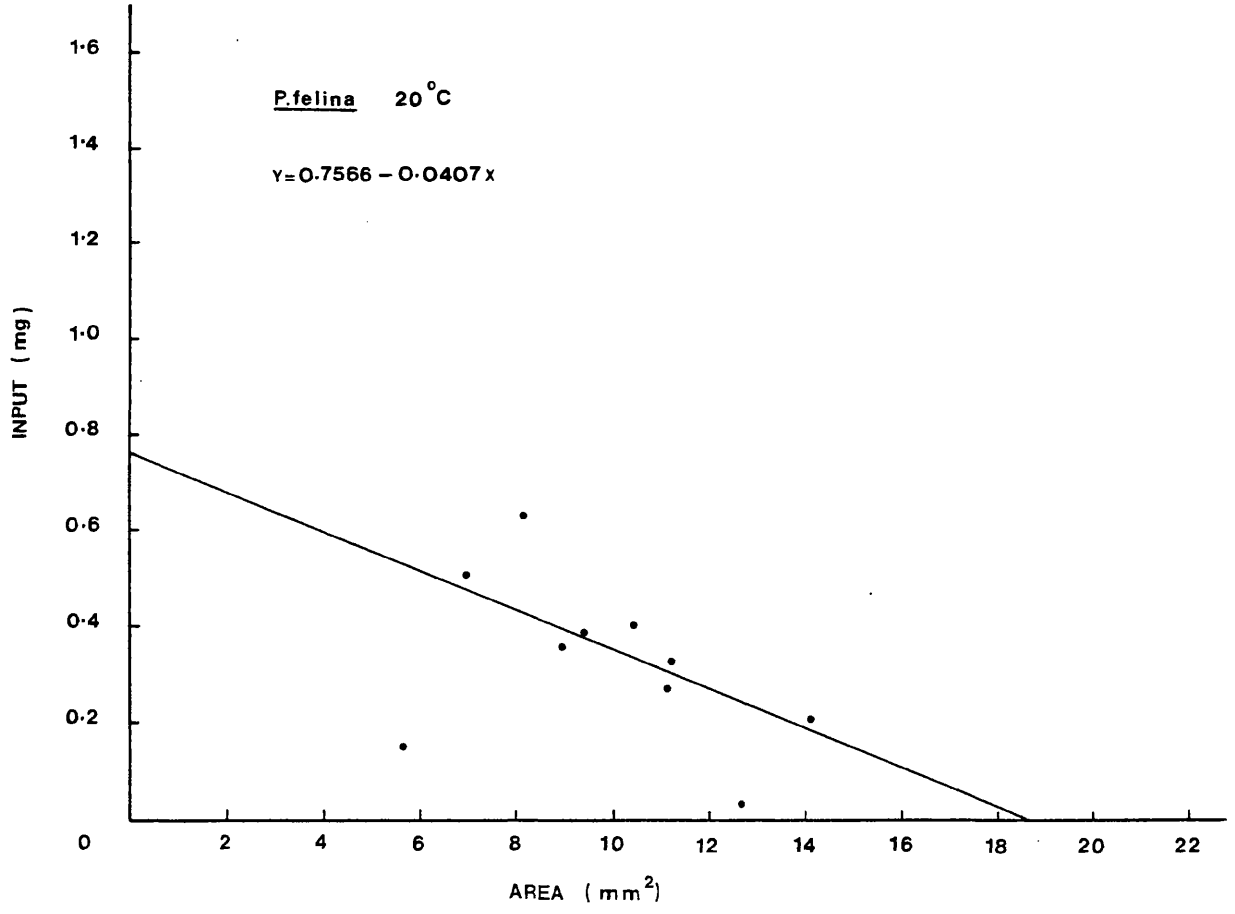

P. tenuis 15°C
y = 0·0702 + 0·0372 x
INPUT (mg)
AREA (mm2)
0
0·2
0·4
0·6
0·8
1·0
1·2
1·4
2
4
6
8
10
12
14
16
18
20
22

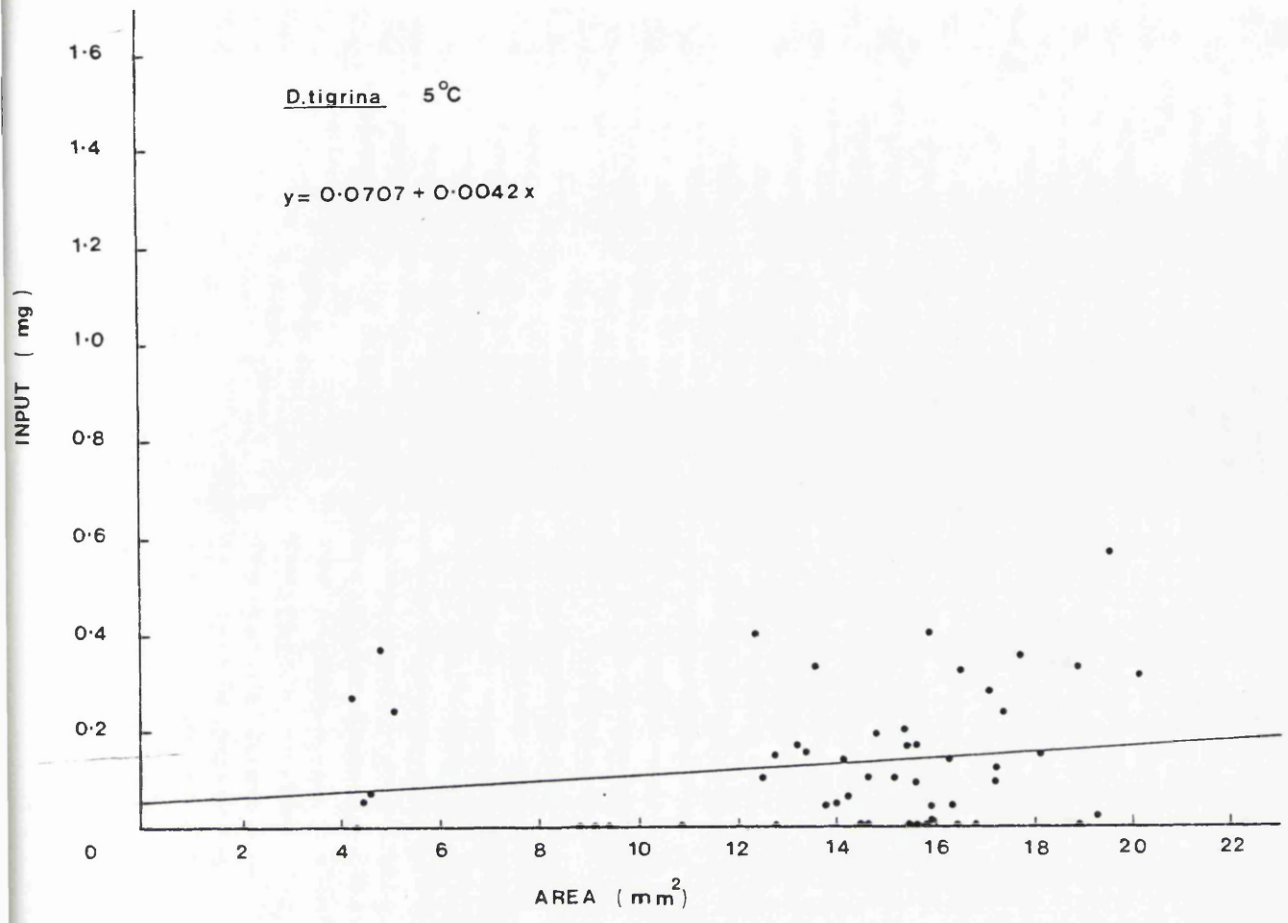

P. tenuis 20 °C
y = 0·0131 + 0·0688x
INPUT (mg)
AREA (mm2)
0
0·2
0·4
0·6
0·8
1;0
1·2
1·4
1·6
2
4
6
8
10
12
14
16
18
20
22

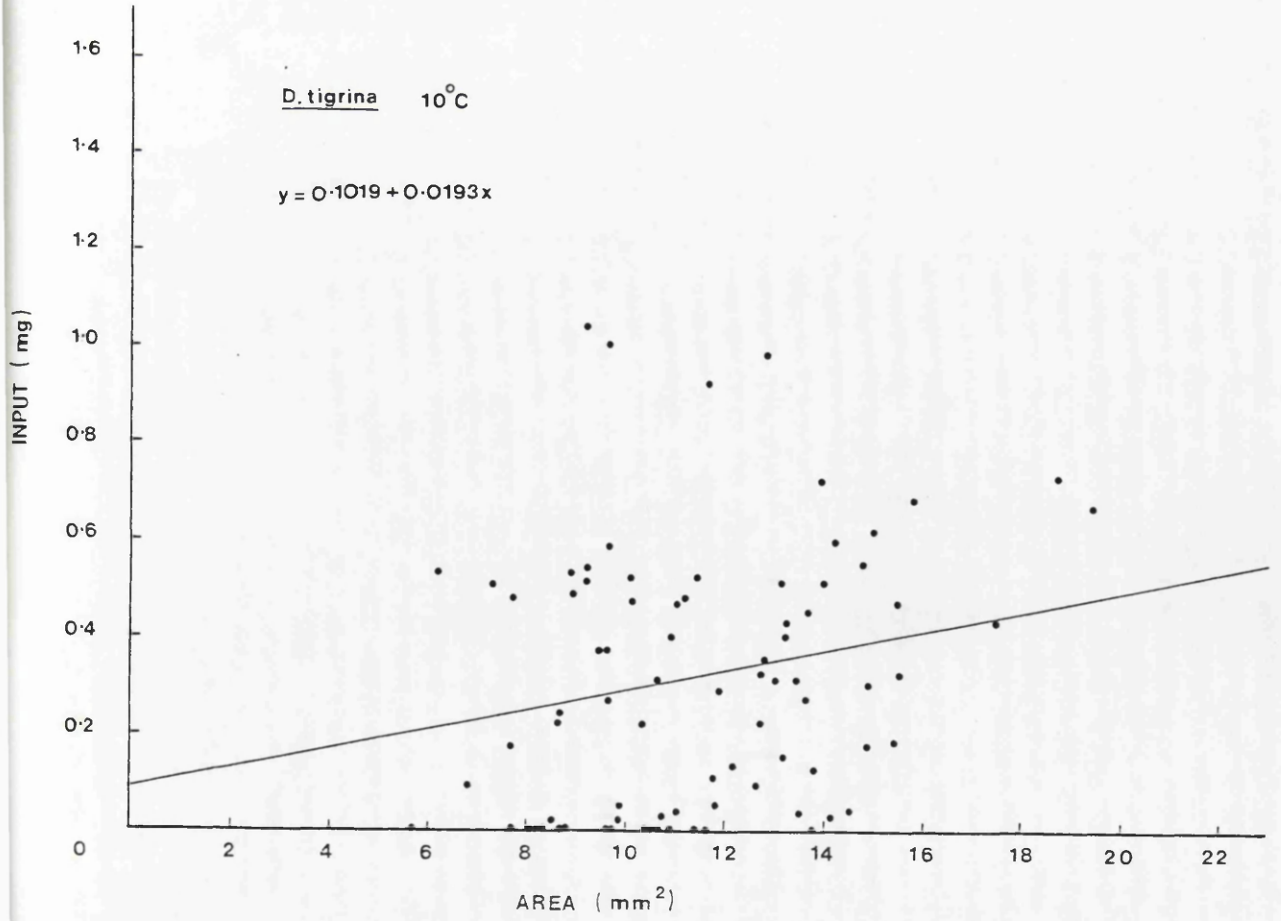

P. felina 5°C
y=0·2394 +0·0098 x
INPUT (mg)
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
0
1
2
3
4
5
6
7
8
9
10
11
AREA (mm²)

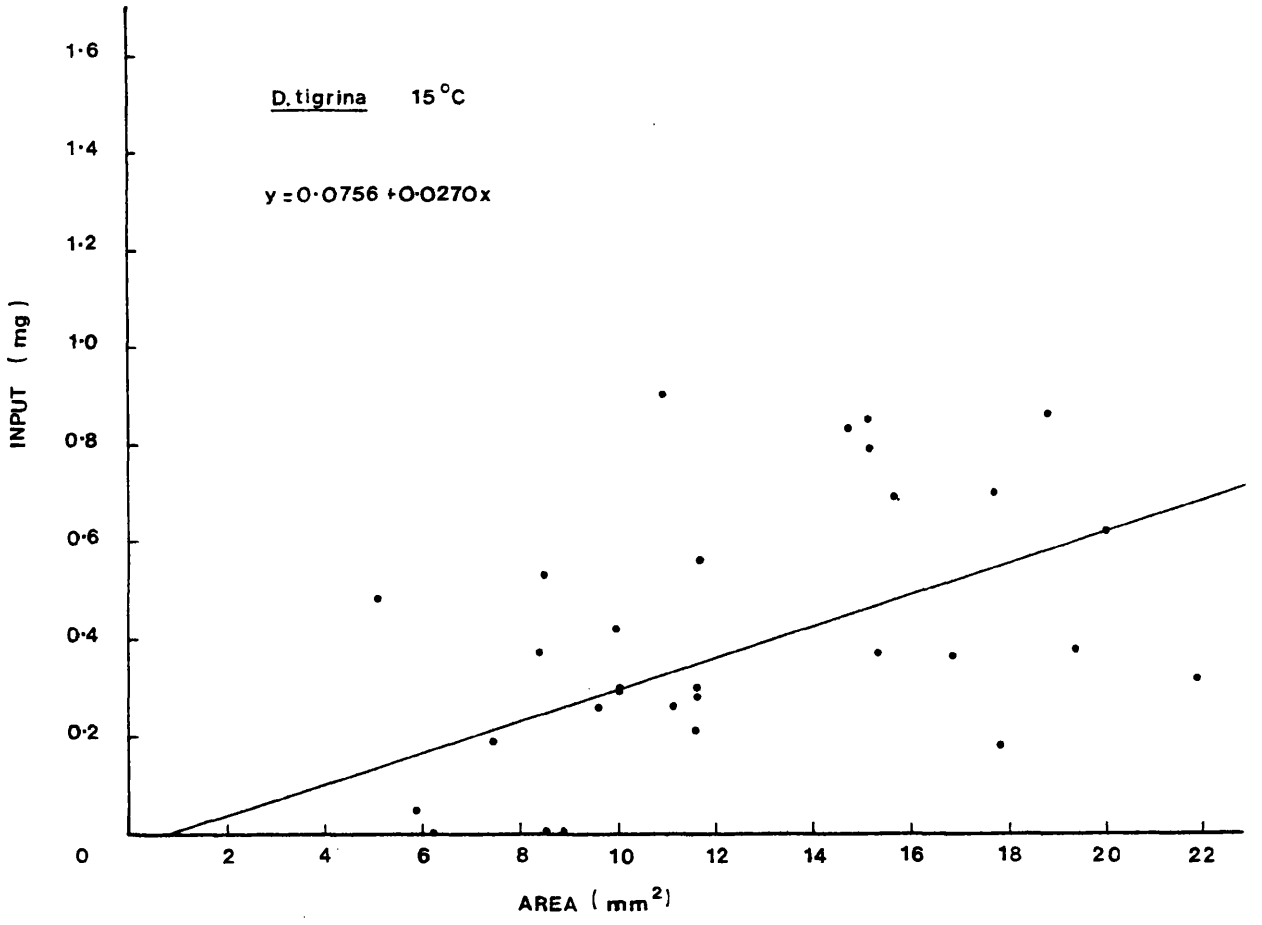

P. felina 10°C
y=0·3891 +0·0130 x
INPUT (mg)
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
0
4
8
12
16
20
24
28
32
36
40
44
AREA (mm²)

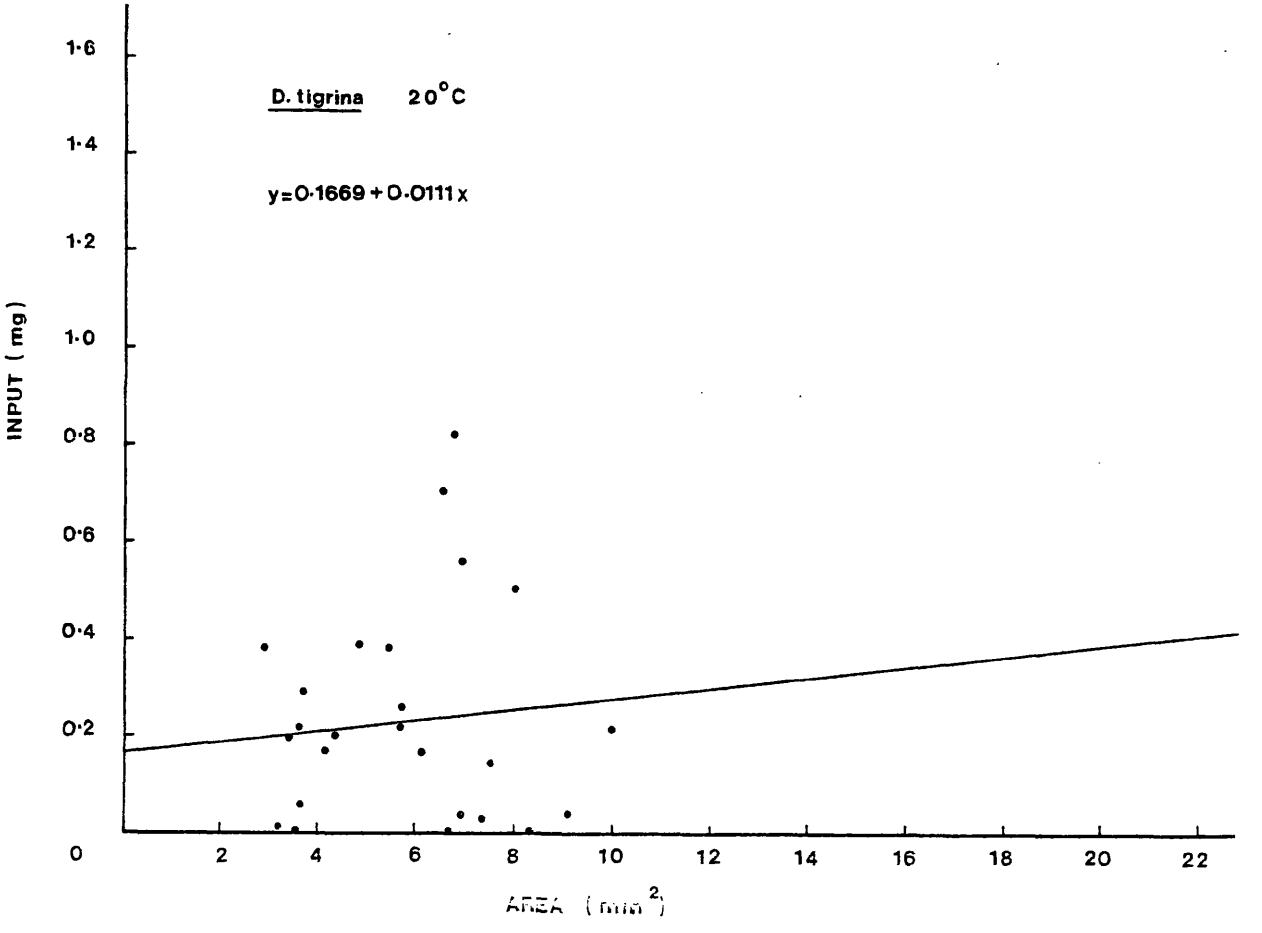

P. felina 15°C
y = 0·3685 + 0·0308x
INPUT (mg)
3·2
2·8
2·4
2·0
1·6
1·2
0·8
0·4
0
4
8
12
16
20
24
28
32
36
38
40
AREA (mm²)


P.felina 20°C
Y=0·7566 – 0·0407x
INPUT (mg)
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
0
2
4
6
8
10
12
14
16
18
20
22
AREA (mm²)


D.tigrina 5°C
y= 0·0707 + 0·0042 x
INPUT (mg)
AREA (mm²)
0
0·2
0·4
0·6
0·8
1·0
1·2
1·4
1·6
2
4
6
8
10
12
14
16
18
20
22


D. tigrina 10°C
y = 0·1019 + 0·0193x
INPUT (mg)
AREA (mm²)
0
0·2
0·4
0·6
0·8
1·0
1·2
1·4
1·6
2
4
6
8
10
12
14
16
18
20
22


D. tigrina 15°C
y = 0·0756 + 0·0270x
INPUT (mg)
AREA (mm²)
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
0
2
4
6
8
10
12
14
16
18
20
22


D. tigrina 20°C
y=0·1669 + 0·0111x
INPUT (mg)
AREA (mm²)
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
0
2
4
6
8
10
12
14
16
18
20
22

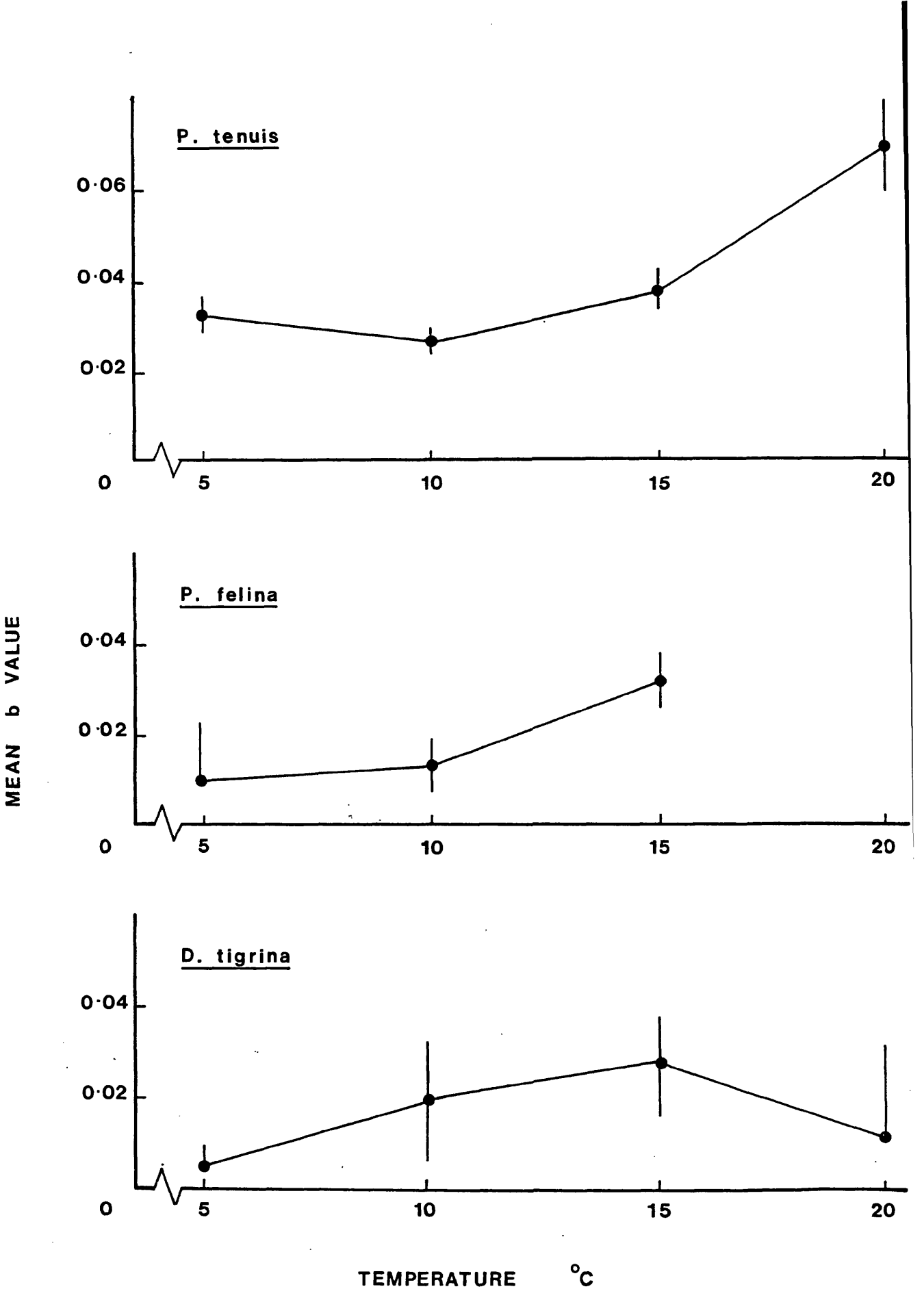
Species	Temperature	Regression Equation	No. of Observations	S.E.	Correlation Coefficient	T(b=0)	p(b)
P. tenuis	5°C	y = 0.0135 + 0.0318x	190	0.004	0.49	7.26	
	10°C	y = 0.1342 + 0.0262x	141	0.003	0.56	7.85	
	15°C	y = 0.0702 + 0.0372x	78	0.005	0.62	6.80	
	20°C	y = 0.0131 + 0.0688x	66	0.010	0.67	7.20	
P. felina	5°C	y = 0.2394 + 0.0098x	35	0.013	0.13*	0.82	n.s.
	10°C	y = 0.3891 + 0.0130x	68	0.006	0.27	2.30	
	15°C	y = 0.3685 + 0.0308x	45	0.006	0.60	4.51	
	20°C	y = 0.7566 - 0.0401x	10	0.029	-0.44	-1.38	
D. tigrina	5°C	y = 0.0707 + 0.0042x	55	0.005	0.12*	0.90	n.s.
	10°C	y = 0.1019 + 0.0198x	84	0.013	0.16*	1.48	n.s.
	15°C	y = 0.0756 + 0.0270x	28	0.011	0.44	2.49	
	20°C	y = 0.1669 + 0.0111x	26	0.021	0.11*	0.53	n.s.

Table 7 : The equations of the lines plotted by regression analysis for the data in Figure 2. The significance of the correlations (r) and the slopes (b) are also tested.

* Not significant

Figure 3

The effect of temperature on the regression coefficients (b values) given in Table 7.


P. tenuis
0·06
0·04
0·02
0
5
10
15
20
P. felina
0·04
0·02
0
5
10
15
20
D. tigrina
0·04
0·02
0
5
10
15
20
MEAN b VALUE
TEMPERATURE °C

P. felina shows a significant increase in food consumption between 10^{o} and $15^{o}C$ ($d = 3.72$; $p < 0.05$). The relationship between size and food intake at most temperatures in D. tigrina is not significant (see above) and therefore nothing may be concluded about the effects of temperature.

4.5 Discussion

In general, the relationships between food intake and size, and food intake and temperature in the 3 species of triclad are weak. This is in part due to the limitations of the techniques employed to measure food intake, and in part due to a number of factors which can affect feeding and feeding behaviour (see below). The correlations between food intake, triclad size and temperature are poorest where small amounts of food are being measured (see Table 7), which suggests that the methods used here have limitations, and are best used when measuring food intake in large triclads, or at high temperatures.

There are numerous other sources of variability. The degree of satiation may have a considerable influence on food intake and dietary preferences in triclads. In this way, a large intake of food on one occasion may inhibit a triclad from feeding when next presented with food. Another source of variability is temperature. The proportion of occasions on which triclads did not feed is not constant, but varies with temperature, and this is summarised in Table 8. In all 3 species, there is an inverse relationship between the percentage of triclad weeks (number of triclads x number of weeks of feeding observations) in which no feeding occurred, and temperature, although this pattern is much less obvious in P. felina. This suggests that less food is eaten, and that feeding is more irregular at low temperatures

Species	Temp.	Total No. of Observations	No. of Observations in which no feeding occurred	% of Observations in which no feeding occurred
P. tenuis	5°C	190	60	32
	10°C	141	22	16
	15°C	78	4	5
	20°C	66	4	6
P. felina	5°C	35	4	11
	10°C	68	8	12
	15°C	45	4	9
	20°C	10	0	0
D. tigrina	5°C	55	15	27
	10°C	84	16	19
	15°C	28	3	11
	20°C	26	3	12

Table 8 : The effect of temperature on feeding in *P. tenuis*, *P. felina* and *D. tigrina*

in P. tenuis and D. tigrina. In P. felina at low temperatures, the proportion of weeks in which no feeding occurred was less than in the other 2 species.

In P. tenuis, a clear relationship was established between triclad size and food intake, and triclad size and temperature. Large P. tenuis eat more than small P. tenuis, and in general food intake per unit size increases with increasing temperature. However, this pattern is different in P. felina and D. tigrina. Pickavance (1968) described D. tigrina as a warm-water species, and states that it does not feed below 5°C. This study agrees with Pickavance's conclusions. Food intake and size are not significantly related at low temperatures. At 15°C, however, food intake increased with triclad size, as anticipated. At 20°C, however, the high rate of reproduction in D. tigrina resulted in a reduction in the exponential growth phase (see Chapter 6), and hence few observations on food intake. Frequently, triclads reproduced within a week or 10 days, and feeding patterns may well have been disrupted. Thus, triclad size and food intake are not significantly related in D. tigrina at 20°C.

There was a significant relationship between size and food intake in P. felina at 10° and 15°C, and also a significant increase in food intake per unit triclad size between these temperatures. At 5°C, P. felina ate little, and there was a low correlation between triclad size and the amount of food eaten. At 20°C, there was an inverse relationship between triclad size and food intake. However, at this temperature P. felina does not reproduce (Dahm 1958) and those triclads which fed only did so intermittently (see Chapter 7).

These results will be discussed more fully in the light of data on growth, reproduction, and respiration rate in Chapter 10.

Chapter 5

RESPIRATION

5.1 Introduction

The purpose of this series of experiments was to determine the effects of temperature and starvation on the respiration rates of P. tenuis, P. felina and D. tigrina. The rate of acclimation was also evaluated by recording the effects of temperature change on respiration in the 3 species. This is part of the more general inquiry into the effects of these environmental influences on the bioenergetics (see Chapter 8).

5.2 Literature Review

There are 2 distinct aspects of respiration: internal and external. Internal respiration refers to "the sum of enzymatic reactions, both oxidative and non-oxidative, by which energy is made available for biological work" (Prosser and Brown 1961). Some studies have been done on triclads concerned with respiration at the cellular and sub-cellular levels (Allen 1919, 1920; Lund 1921a, 1921b; Fraps 1930; Huble and Van Grembergen 1948-49; Løvtrup 1953).

External respiration may be defined as "the exchange of oxygen and carbon dioxide between the organism and the external environment" (Barrington 1967). In this section, we are primarily concerned with external respiration.

The Platyhelminthes rely exclusively on respiratory exchange by diffusion through the body surface. This holds true for terrestrial as well as aquatic species (Hyman 1951).

A variety of methods have been used to investigate the respiratory metabolsim of freshwater triclads. Anaerobic respiration is of little or no importance (Brand 1945), and so the methods used have been concerned with the measurement of aerobic respiratory

exchange. Only Behre (1918), Child (1920), Robbins and Child (1920) and Allen (1920) measure carbon dioxide production. All other researchers have measured oxygen uptake.

Much research has been carried out on the factors affecting the rate of oxygen consumption in animals (for review see: Prosser and Brown 1961; Newell 1970). These may be divided into endogenous and environmental factors. The influence of endogenous factors such as size, (Kleiber 1932; Brody 1945; Hemmingson 1950, 1960; Bertalanffy 1957; and Newell 1970), activity (Fry and Hart 1948; Halcrow and Boyd 1967; Newell and Roy 1973) and nutritional state (Barnes Barnes and Finlayson 1963; Marsden 1973; Calow 1974) have all been studied in detail.

Previous studies have shown that all these factors can affect the respiration rate of freshwater triclads. The work of Allen (1918) on D. dorotocephala and D. tigrina, Hyman (1919) on D. dorotocephala and D. tigrina and Whitney (1942) on C. alpina, P. felina and D. polychroa demonstrated an increase in oxygen consumption with weight. The work of Hyman and Whitney also showed that the respiration rate per unit weight of smaller individuals was higher than in larger animals. However, Pederson (1956) found no change in weight-specific respiration for Ph. vitta. Later work by Calow and Woollhead (1977a) and Woollhead (1979) on D. lacteum, P. tenuis, D. lugubris, Ph. torva and Bd. punctata, showed that there was a linear relationship between oxygen consumption and size measured in terms of plan area (Calow 1977).

Calow and Woollhead (1977a) demonstrated that during the early stages of starvation the activity level of P. tenuis rose, whereas that of D. lacteum fell. This resulted in a relatively greater

reduction in the respiration rate of D. lacteum.

Hyman (1919) found that there was an immediate increase in the respiration rate of D. dorotocephala after feeding. This was later confirmed by Hyman, Willier and Riffenburgh (1924). Bolen (1937) found that feeding caused an approximate 2-fold increase in the respiration rate of D. dorotocephala, which he ascribed to specific dynamic action (S.D.A.).

Many studies on different species of freshwater triclad have shown a reduction in the respiration rate during starvation (Child 1919; Hyman 1919, 1920; Bolen 1937; Calow and Woollhead 1977a). Bolen attributed this to a reduction in S.D.A. Calow and Woollhead agreed with this conclusion, but added that differences in locomotory strategies adopted during food shortages exerted a modifying effect.

Woollhead (1979) investigated the respiration rates of freshwater triclads during reproduction. Of the 5 sexually reproducing species he studied, he found that there was no significant difference between the respiration rate per unit area of reproducing and non-reproducing adults.

Pedersen (1956) measured the rate of oxygen consumption in Ph. vitta during the early stages of asexual reproduction and found the respiration rates of reproducing and non-reproducing adults to be almost identical. Calow, Beveridge and Sibly (1979) found no difference between the respiration rates of head and tail fission products in P. felina, and concluded that the process of forming a new head/tail involved considerable metabolic costs.

The literature concerning the effects of regeneration on the rate of oxygen uptake in flatworms is more confusing. Child (1914), Hyman (1919) and Robbins and Child (1920) found that the respiration

rate of triclads increased during regeneration, whilst Pedersen (1956) found no such increase.

The influence of environmental factors on oxygen consumption in animals is well known (see Newell (1970) for review). Following the nomenclature adopted by Fry (1947) these factors may be divided into two types: limiting and non-limiting factors. The former of these are actually involved in the chain of metabolic processes. Since the overall metabolic rate is determined by the slowest step in the chain (Blackman 1905; Barton 1936; Fry 1947), factors such as oxygen availability can affect the respiration rate.

In contrast, controlling factors act in such a way as to "govern the maximal and minimal metabolic rates" (Newell 1970). Such factors include temperature, salinity and pH, several of which may act simultaneously.

There have been fewer studies on the influence of environmental factors on the respiration rates of freshwater triclads. This is because much of the work done on the respiratory physiology was a result of investigations into metabolic gradients (Child 1911; Hyman 1932; Allen 1920; Shearer 1930). In conjunction with these, experiments were carried out on the effects of certain poisons on the metabolism of the triclads (Allen 1919; Child 1923; Buchanan 1926).

Another limiting factor investigated was the effect of oxygen availability on the respiration rate. Lund (1921) found no change in rate of consumption of oxygen in D. dorotocephala down to $^1/_3$rd air saturation. Hyman (1929) working on D. dorotocephala confirmed these results. Abbott (1960) observed that Ph. gracilis and D. tigrina tolerated oxygen tensions as low as 0.6 µg/l (approx. $^1/_{15}$th air saturation). Russier-Delolme (1974) showed that at that level, the

oxygen consumption of D. tigrina and P. felina was reduced to about ¼ of its value at air saturation. Russier-Delolme (ibid) also found the lethal limit for these species to be about 0.30 mg/l (approximately $^{1}/_{30}$ th air saturation).

Among many other workers, Newell (1970) is of the opinion that temperature is the most important controlling factor which operates on the respiration rates of poikilotherm animals. Despite this, little work has been done on freshwater triclads. Behre (1918) looked at carbon dioxide production as influenced by temperature and found that transferring D. dorotocephala from low to high temperatures caused an immediate increase in respiration rate, although this was later modified by acclimation.

Buchanan (1931) investigated the influence of solutions of different osmotic pressures on oxygen consumption in D. dorotocephala and showed that it was modified by hypotonic and hypertonic solutions. Hess (1929) also found the respiration rates of the same species to be affected by the addition of metal chlorides to the culture medium.

I now intend to look at the influences of temperatures and starvation on the respiration rates of P. tenuis, P. felina and D. tigrina.

5.3 Materials and Methods

P. tenuis eggs were collected and incubated at 5°, 10°, 15° and 20°C, as described previous (see Chapter 3). Newly-emerged young were randomly sorted, before being transferred to individual plastic pots containing 30 mls of filtered lake water.

D. tigrina and P. felina were collected from the field and acclimated to laboratory conditions, as previously described (see

Chapter 3). Individuals were then transferred to plastic pots containing 30 mls of filtered lake or stream water.

The respiration rates of both heads and tails and the subsequent developmental stages of P. felina and D. tigrina were measured. No attempt was made to differentiate between them, since Calow, Beveridge and Sibly (1979) demonstrated that there was no significant difference between the respiration rates of whole animals, or regenerating fission products.

Two separate feeding regimes (fed and starved) for each species at each temperature were chosen, and between 5 and 10 individual animals were used.

The effect of acclimation was investigated by culturing groups of P. felina, P. tenuis and D. tigrina at 5° and 15°C for at least 100 days, before transferring them to 10°C. The respiration rates were measured 1 day, 2 days, 4 days, 8 days and 16 days after being transferred, in order to determine the rate of change in the respiration rates. The final rates of oxygen uptake were compared, in order to see if there was any difference between cold and warm acclimated groups.

Bolen (1937) has demonstrated that an approximate 2-fold increase in the rate of oxygen uptake occurred in triclads immediately after feeding. However, this period of high respiration rate lasted only a few hours, and returned to normal within 24 hours. Hence the respiration rate of fed triclads was measured between 24 and 48 hours after feeding. The rate of O_2 consumption was measured regularly during starvation.

Many methods for measuring the respiratory metabolism of animals exist, and these are reviewed by Kleiber (1961) and Klekowski

(1975). It was decided to measure oxygen uptake by the Winkler method, employing the modifications of Fox and Wingfield (1938), for measuring dissolved oxygen in a small volume of water. This method has been recently reappraised by Carpenter (1965a, 1965b) and has been used successfully on triclads by Calow and Woollhead (1977a).

The use of oxygen electrodes was precluded because they involve agitation. Lock (1972) showed that water currents affected the behaviour of C. alpina and P. felina and Woollhead (1979) stated that D. lacteum was adversely affected by disturbance. Russier-Delolme (1974) demonstrated that the rate of oxygen uptake in D. tigrina and P. felina varied significantly according to whether the water was stirred or not.

Glass syringes were used as respiration chambers (Calow and Woollhead 1977), 2 ml syringes for small and 10 ml syringes for large worms. These were filled with filtered, air-saturated water that had previously been conditioned at the required temperature. An animal was placed in each syringe, the nozzle sealed with a water filled plastic cap, and the chamber checked to see that no air bubbles were present. The syringes were then weighed on a Mettler top-pan balance (accuracy ± 0.05 mg) to determine the amount of water they contained, before being incubated in a water bath, pre-set to a specific temperature (accuracy ± 0.5^{o}C) for ca. 24 hours. In addition, a control syringe containing only filtered water was always included.

At the end of the incubation period, the syringes were removed and a sample of water transferred from the respiration chamber to a calibrated syringe pipette via a short piece of plastic tubing. In this way the risk of reaeration was minimized. The Winkler reactions

were then carried out in the syringe pipettes, using exactly the same concentrations of reagents as described in Fox and Wingfield (1938).

Manganous chloride was drawn into the syringe pipette at the same time as the water sample was transferred from the reaction chamber. Alkaline, iodide solution was introduced to the pipette which was shaken gently and left for 3 minutes in order that all the oxygen present could be absorbed by the precipitate. Concentrated ortho-phosphoric acid was then drawn into the pipette which is agitated gently until the precipitate has disappeared and the iodine is liberated. The solution is then transferred to a 25 ml conical flask and the liberated iodine titrated against sodium thiosulphate from a micrometer burrette (accuracy ± 0.00005 ml; Calow and Woollhead 1977a). Starch solution was used as indicator.

All the reagents used in the experiments were prepared from Analar chemicals and distilled/deionised water. Fresh, $^{N}/_{40}$ sodium thiosulphate was prepared for each experimental run from $^{N}/_{10}$ stock solution, and the concentration accurately determined as described in Fox and Wingfield (1938). Using the above procedure, Calow and Woollhead (1977a) obtained 98% repeatability in estimating the oxygen concentration of air saturated water.

The amount of oxygen consumed by the triclads was calculated by subtracting the amount of oxygen contained in the experimental sample from that found in the control. The experiments were always accurately timed, so that oxygen consumption could be expressed as $\mu l O_2^{-1}$ individual^{-1}hour.

All experiments in which the oxygen had been severely depleted by the worms were discarded.

In order to evaluate the effects of size on oxygen consumption, the triclads were measured immediately after the 24 hour incubation period. Triclad size was measured photographically (see Chapter 3) and expressed in terms of plan area (mm^2).

5.4 Results

5.4.1 Fed animals

The following results were obtained from worms that had been fed twice weekly on immobilised Asellus.

Initially, the relationship between size and respiration rate was investigated. A linear relationship ($P < 0.05$) between oxygen consumption and plan area for each species at each temperature was found (Fig. 4).

Regression lines of the form $R = a + bA$ were calculated for each species at each temperature, where R = oxygen consumption (μl^{-1} $individual^{-1}hour^{-1}$); A = plan area (mm^2) (Table 9). The regression coefficients (b) were significantly different from zero ($P < 0.05$), except for D. tigrina at $5^{o}C$.

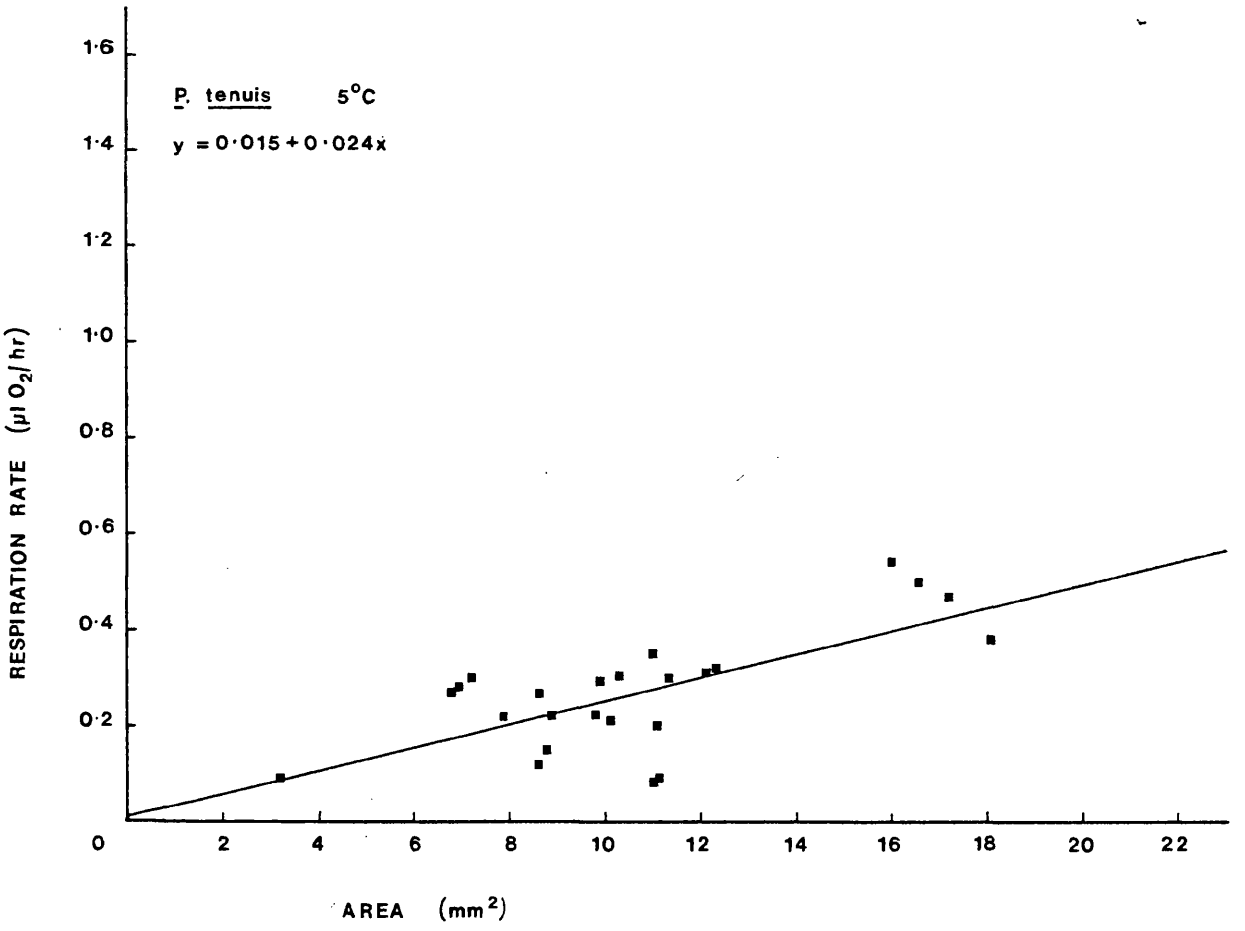
Using the equation from Davies and Goldsmith (1972) there was no significant difference at the 5% level between a and 0.

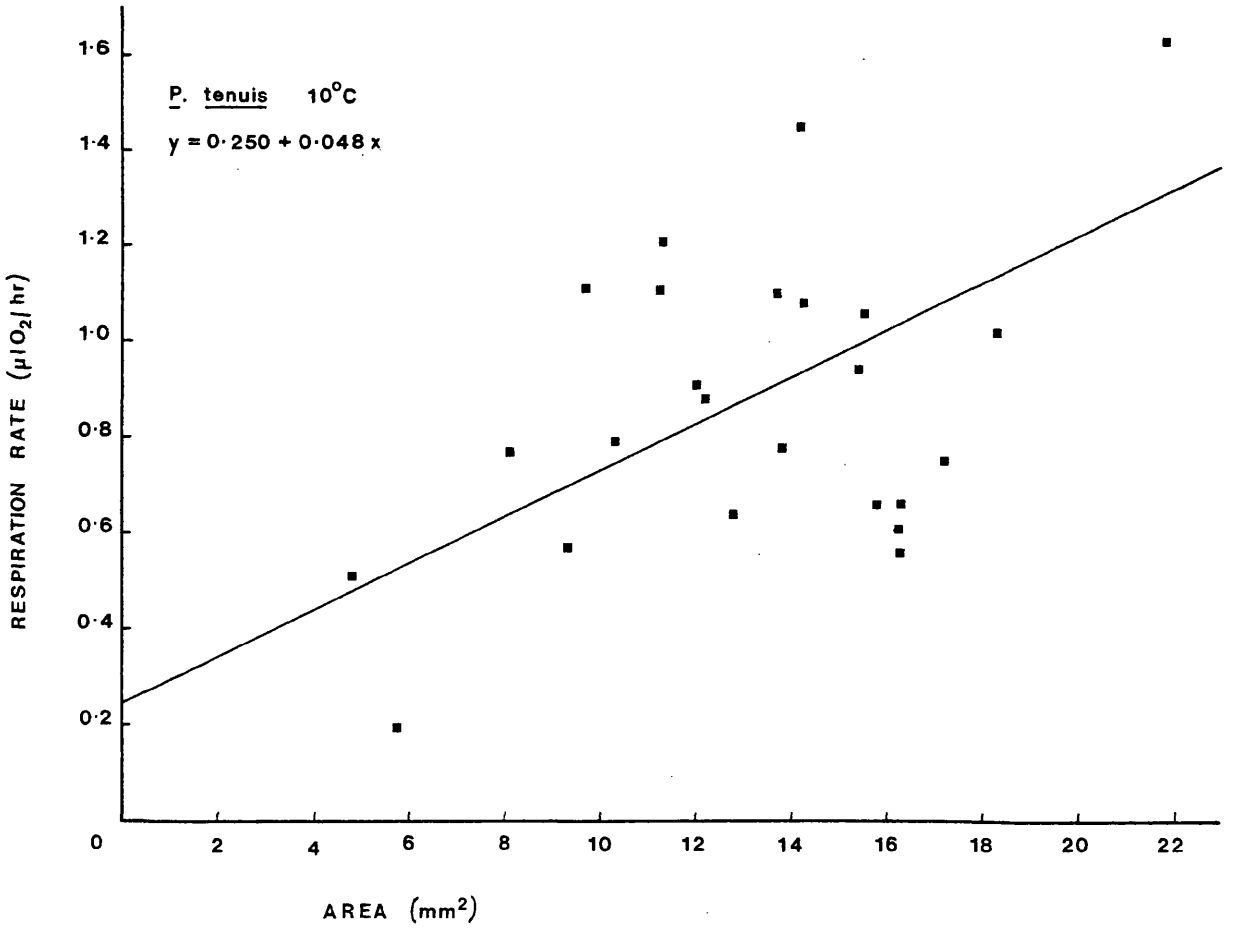
Hence, the relationship between respiration rate and plan area is isometric, and may be described by a single parameter, the respiration rate per unit area (R/A), and this can be used as a size independent index of oxygen consumption.

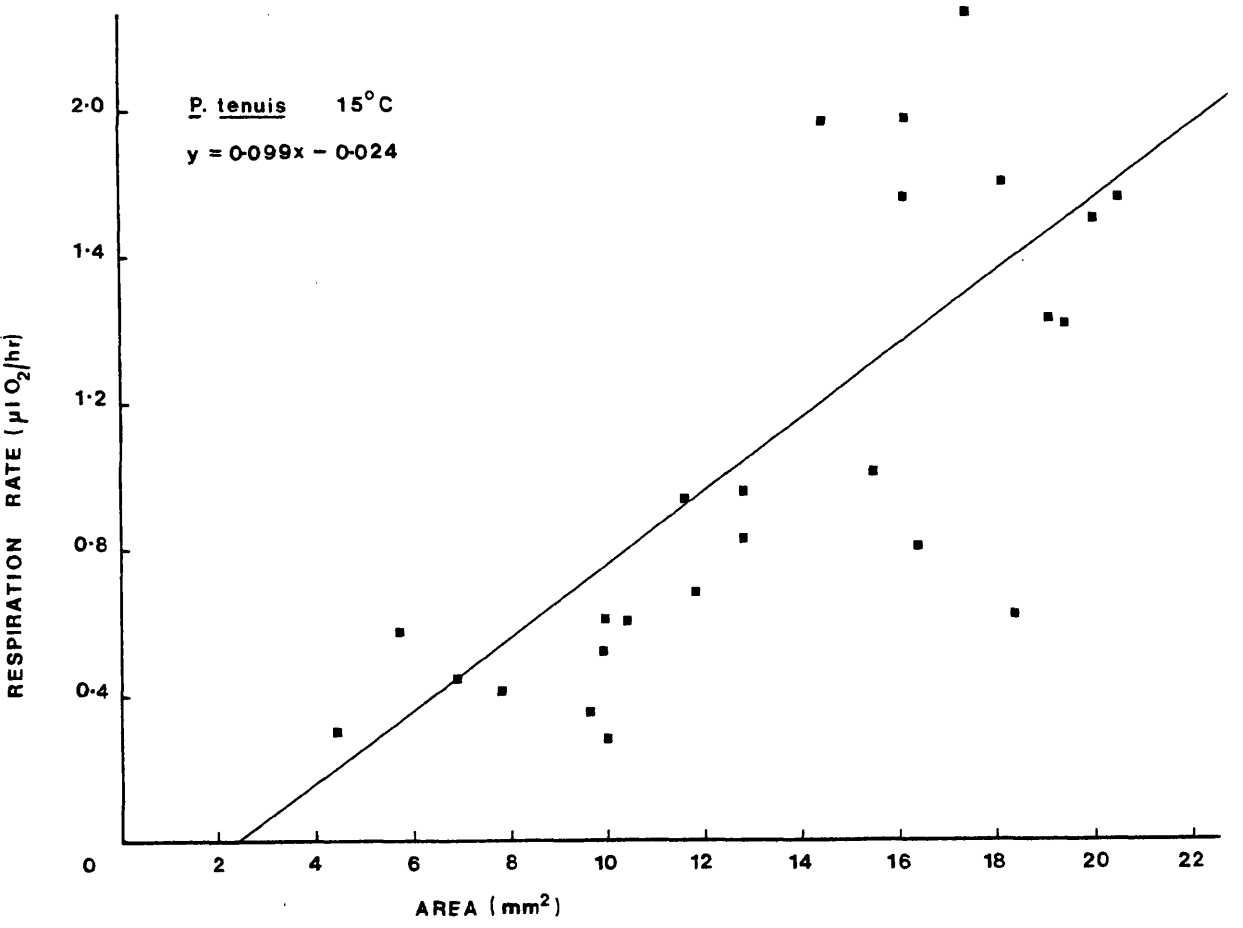
Mean R/A values were computed for each species at each temperature (Table 10a). An analysis of variance (Snedecor 1956) was performed on the individual values for each species, to discover what effects temperature had on the rate of oxygen consumption (Table 10b).

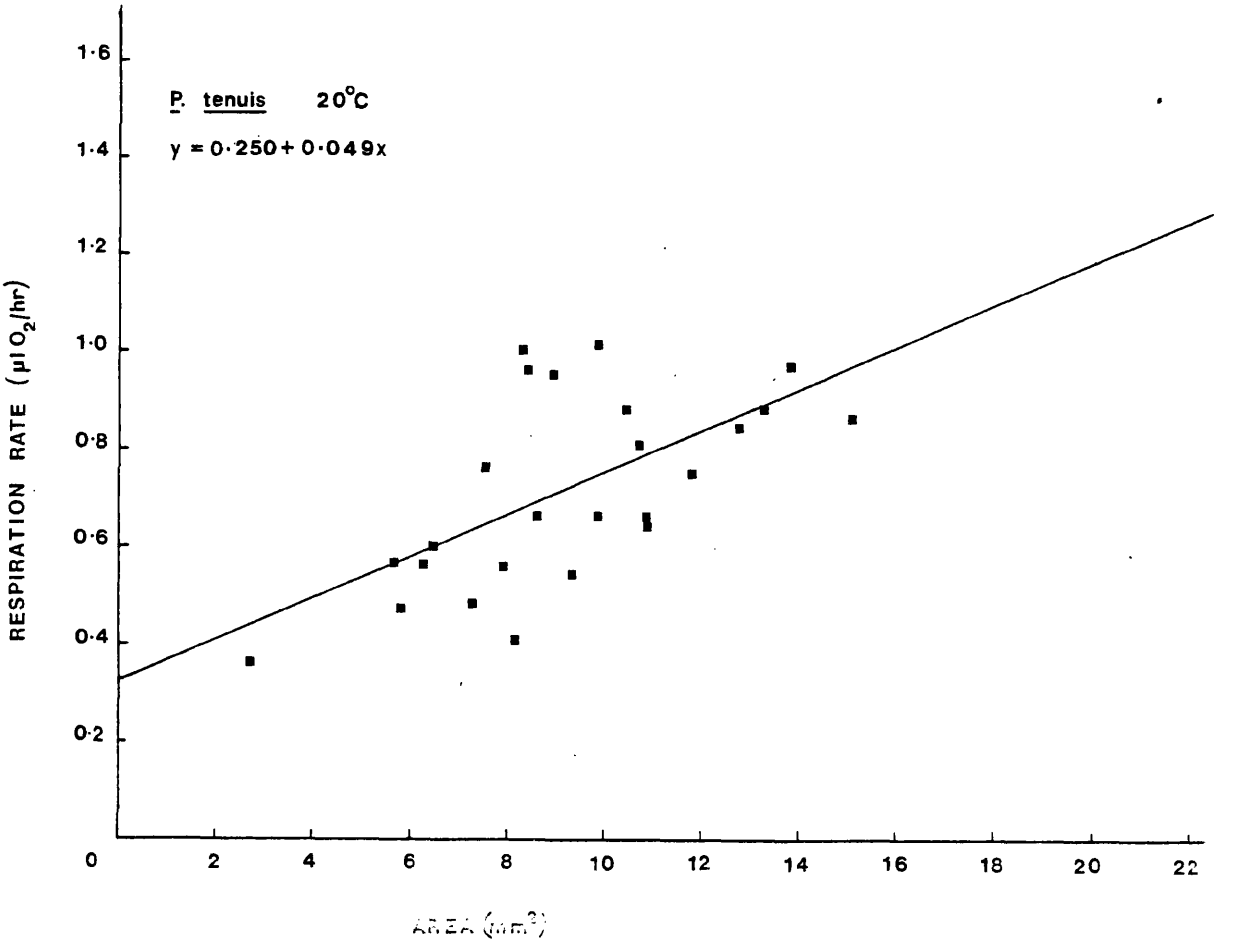
Figure 4

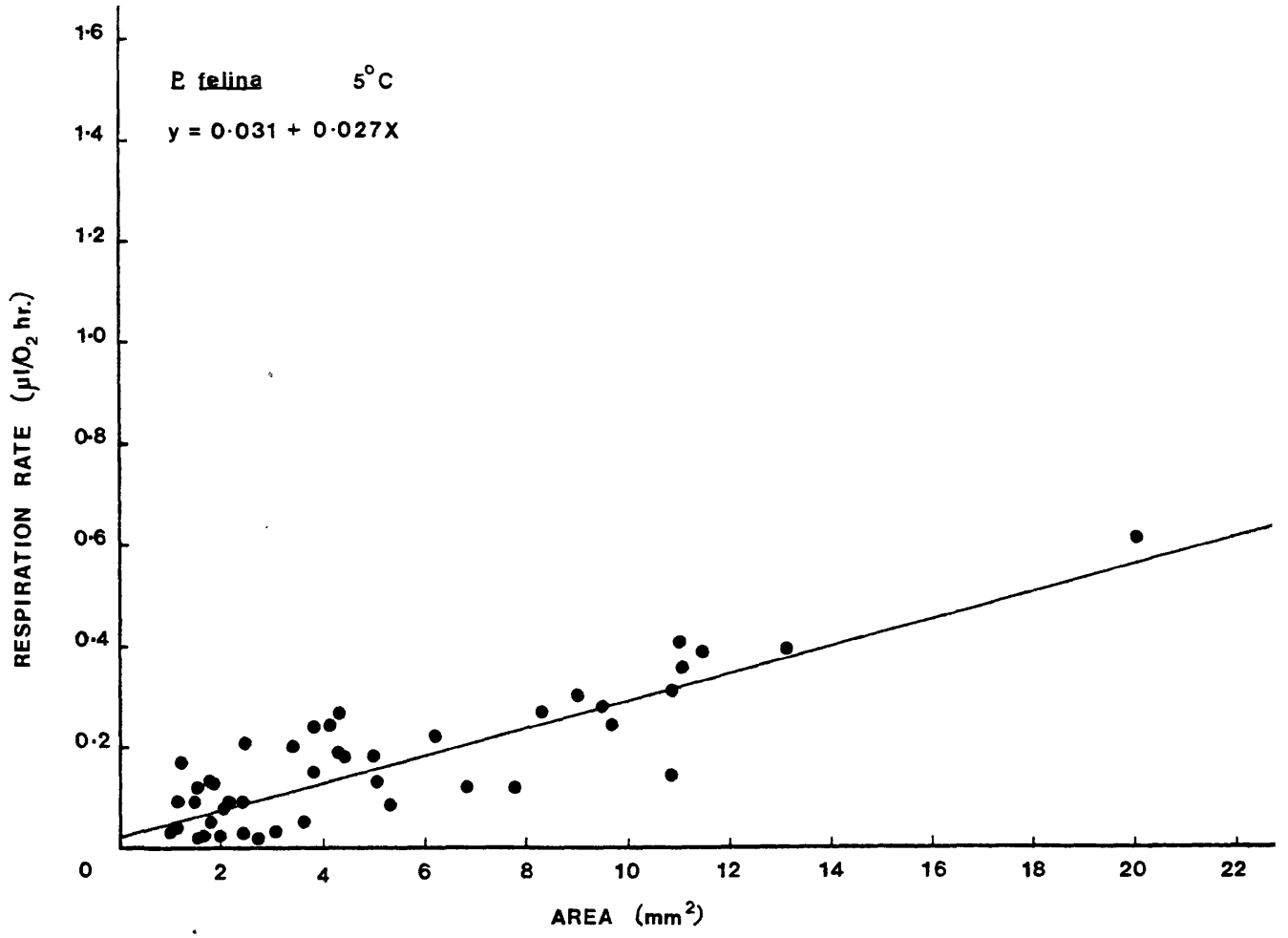
The relationship between oxygen consumption and plan area (mm^2) of fed *P. felina*, *P. tenuis* and *D. tigrina*

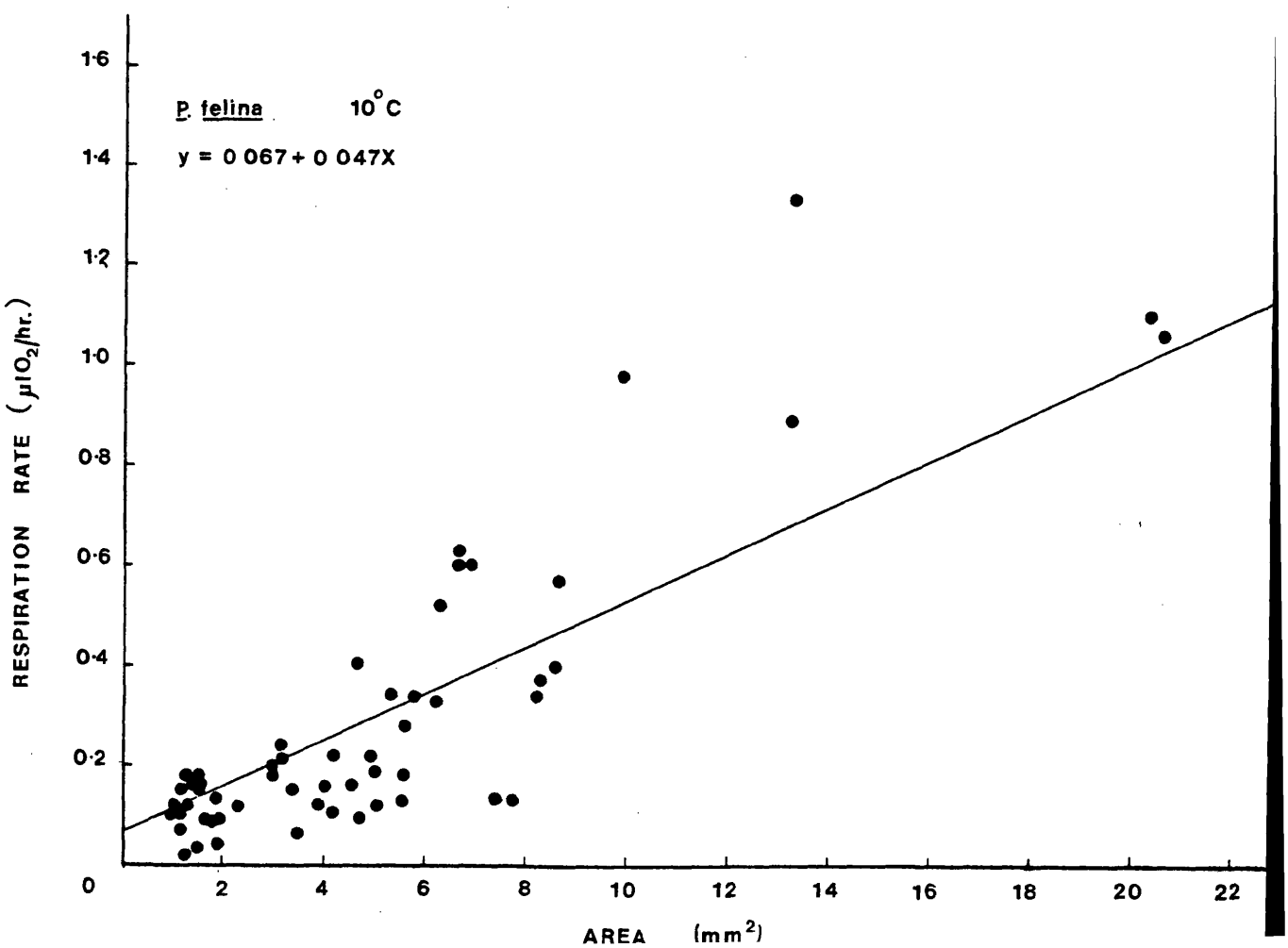

P. tenuis 5°C
y = 0·015 + 0·024x
RESPIRATION RATE (µl O2/ hr)
AREA (mm2)
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
0
2
4
6
8
10
12
14
16
18
20
22

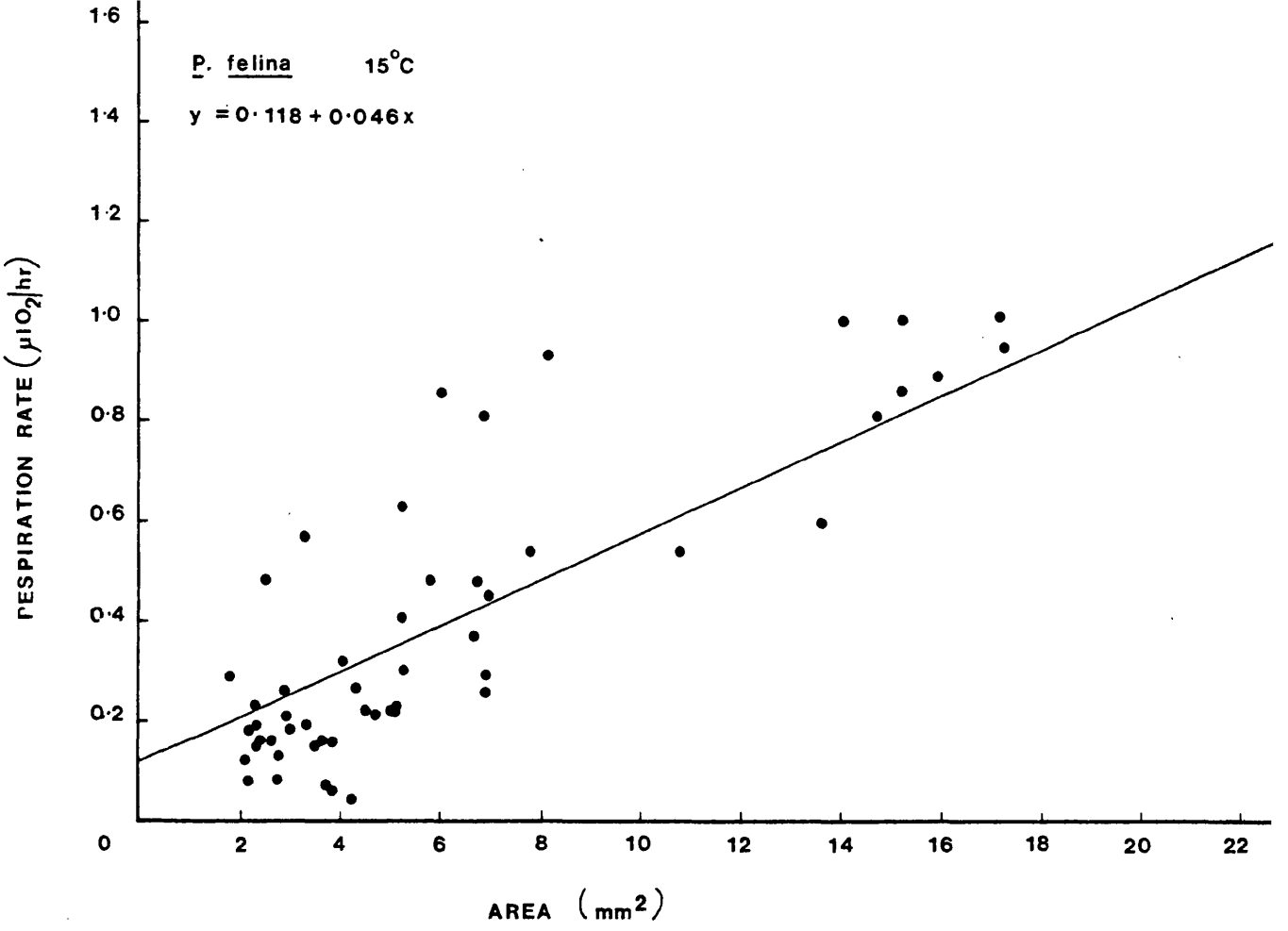

P. tenuis 10°C
y = 0·250 + 0·048 x
RESPIRATION RATE (µlO2/ hr)
AREA (mm2)
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
0
2
4
6
8
10
12
14
16
18
20
22

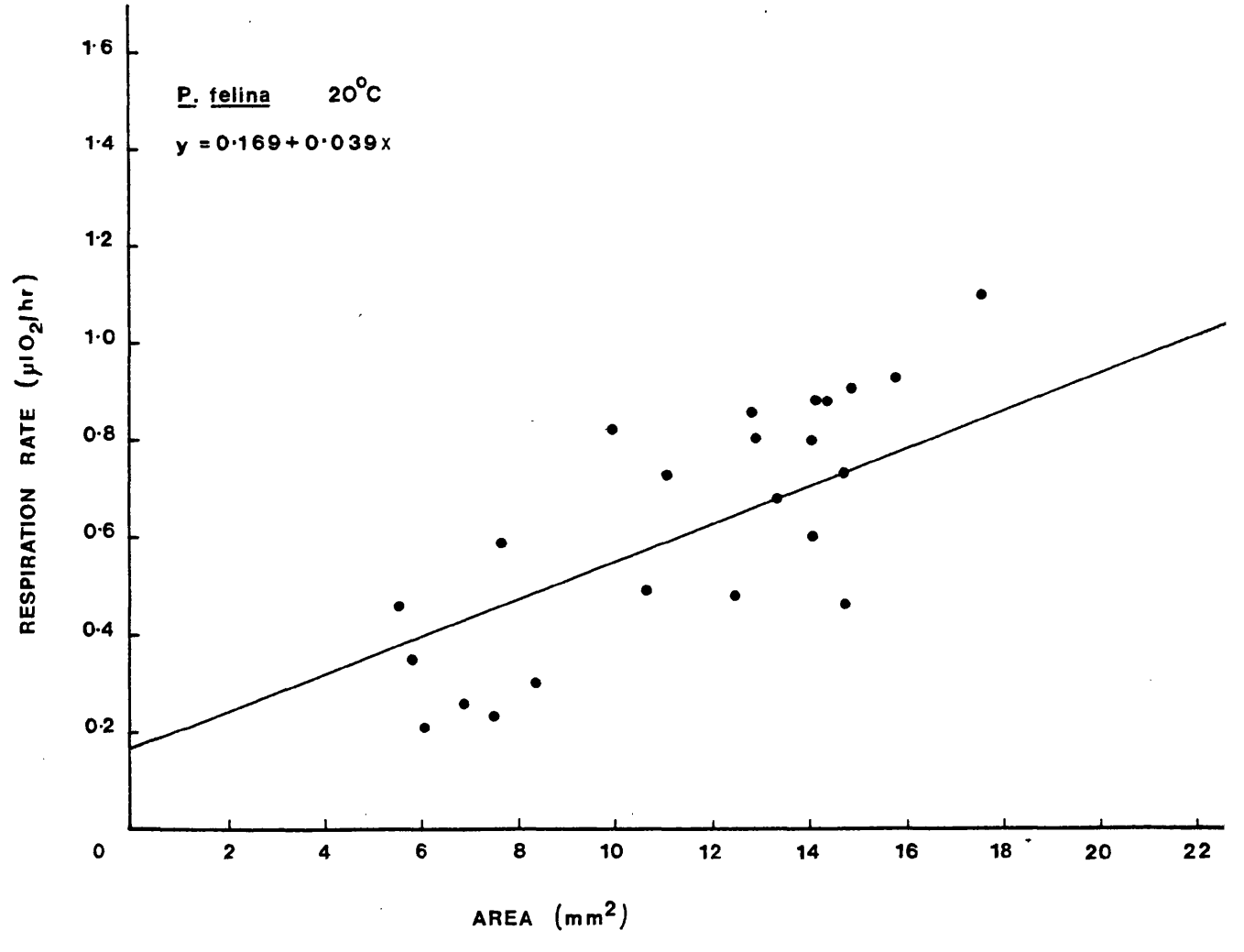

P. tenuis 15°C
y = 0·099x − 0·024
RESPIRATION RATE (µl O2/hr)
AREA (mm²)
0
0·4
0·8
1·2
1·4
2·0
2
4
6
8
10
12
14
16
18
20
22

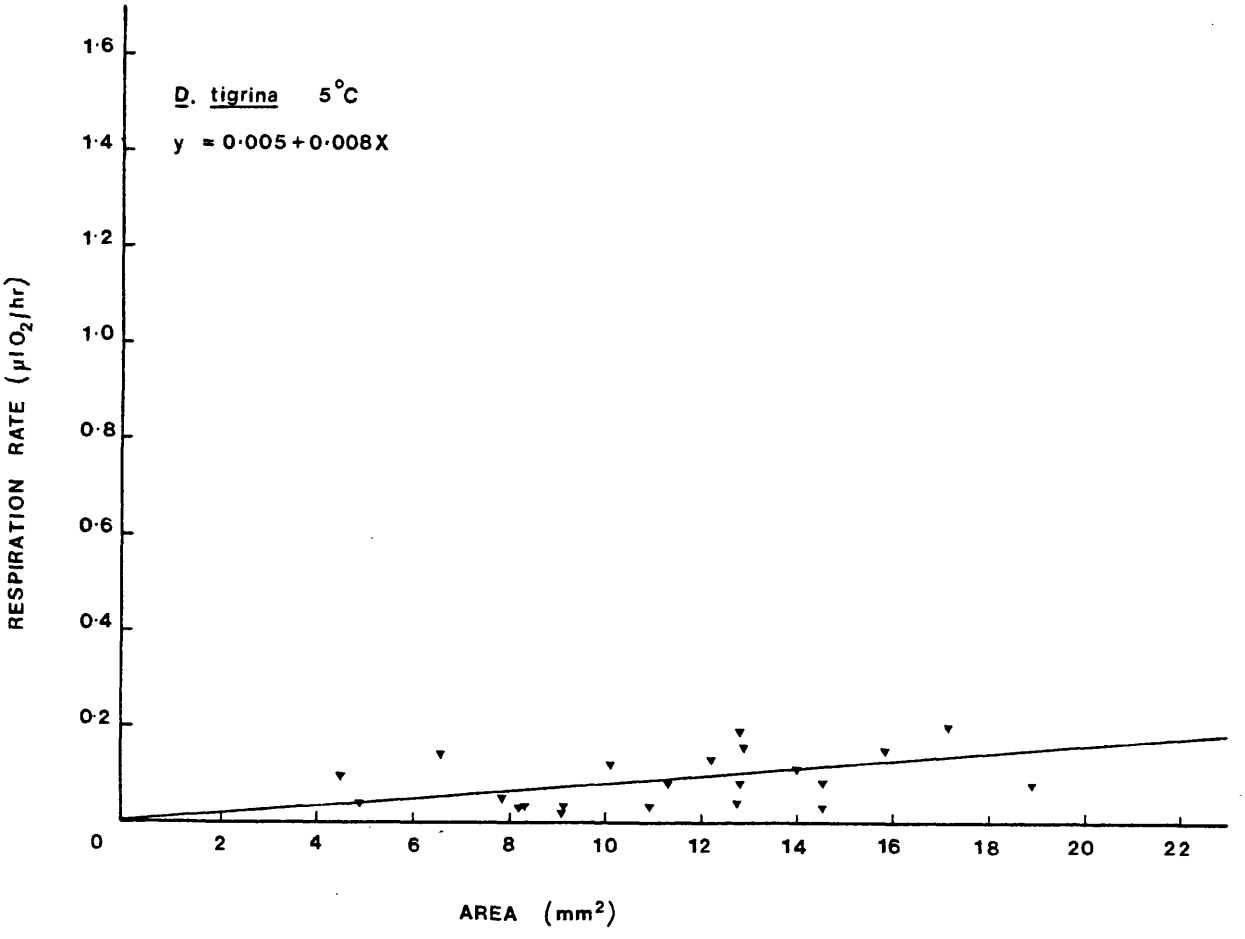

P. tenuis 20°C
y = 0·250 + 0·049x
RESPIRATION RATE (µl O2/hr)
AREA (mm²)
0
0·2
0·4
0·6
0·8
1·0
1·2
1·4
1·6
2
4
6
8
10
12
14
16
18
20
22

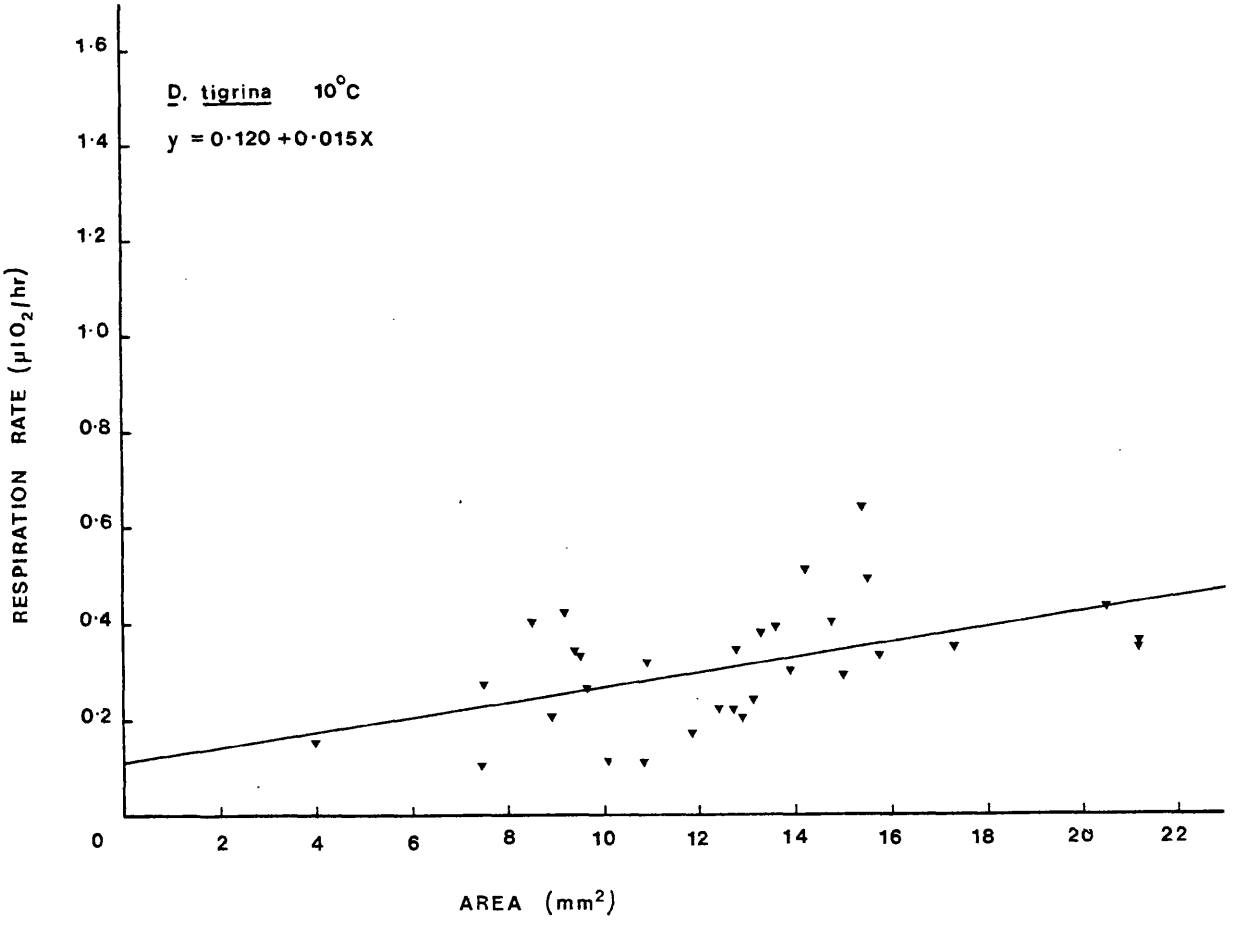

P. felina 5°C
y = 0·031 + 0·027X
RESPIRATION RATE (µl/O2 hr.)
AREA (mm2)
0
0·2
0·4
0·6
0·8
1·0
1·2
1·4
1·6
2
4
6
8
10
12
14
16
18
20
22

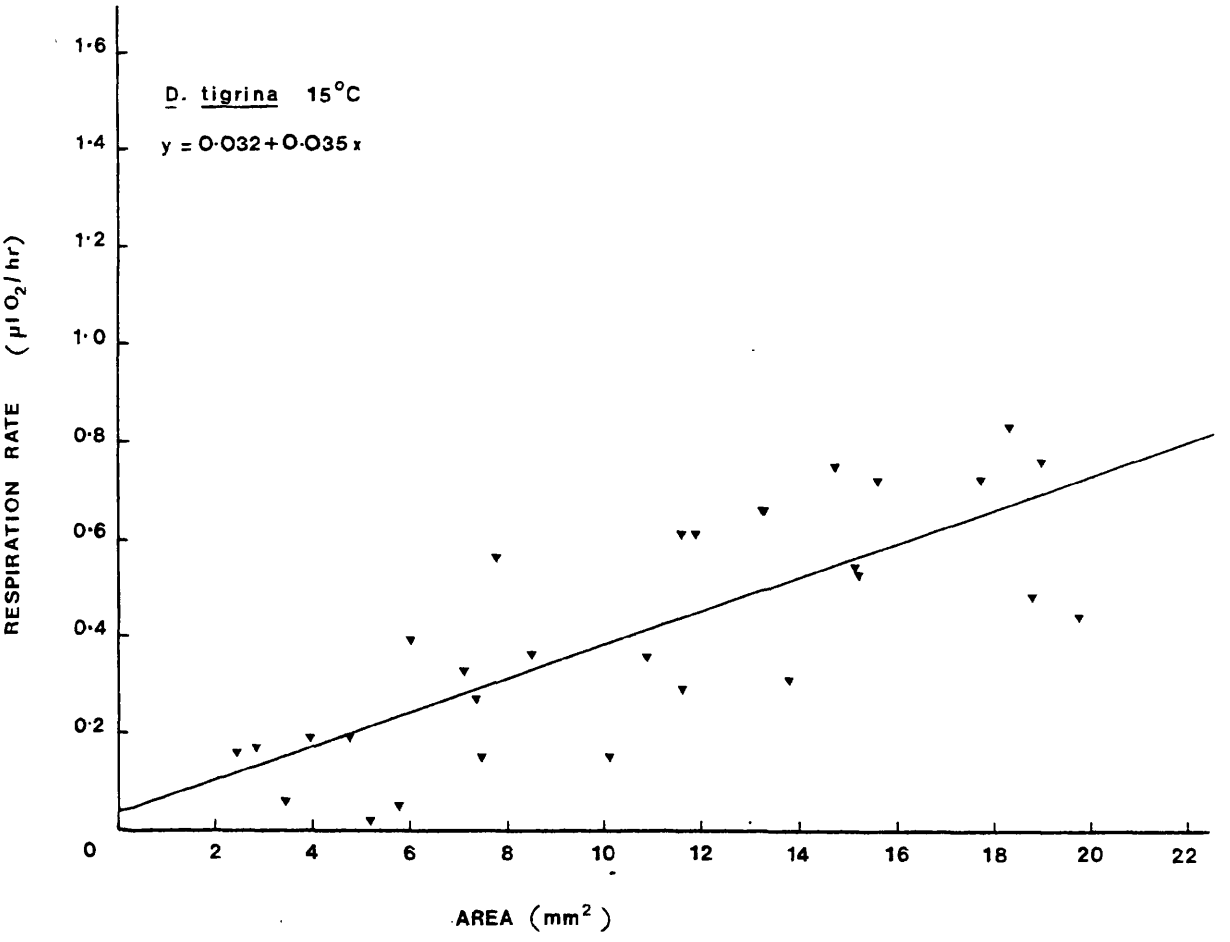

P. felina 10°C
y = 0 067 + 0 047X
RESPIRATION RATE (µlO2/hr.)
AREA (mm2)
0
0·2
0·4
0·6
0·8
1·0
1·2
1·4
1·6
2
4
6
8
10
12
14
16
18
20
22

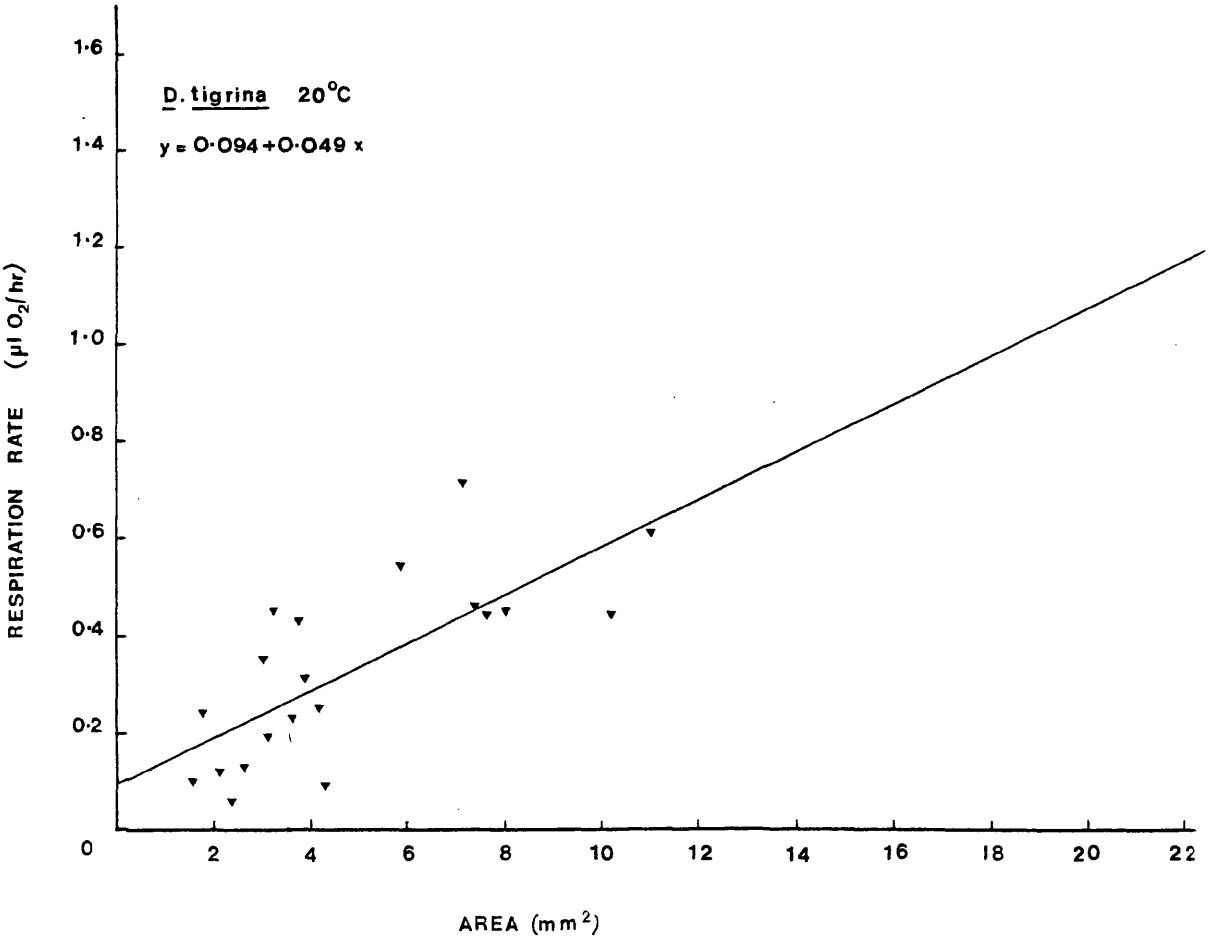

P. felina 15°C
y = 0·118 + 0·046x
RESPIRATION RATE (µlO2/hr)
AREA (mm2)
0
0·2
0·4
0·6
0·8
1·0
1·2
1·4
1·6
2
4
6
8
10
12
14
16
18
20
22


P. felina 20°C
y = 0·169 + 0·039x
RESPIRATION RATE (µlO2/hr)
AREA (mm2)
0
0·2
0·4
0·6
0·8
1·0
1·2
1·4
1·6
2
4
6
8
10
12
14
16
18
20
22


D. tigrina 5°C
y = 0·005 + 0·008X
RESPIRATION RATE (µlO2/hr)
AREA (mm2)
0
0·2
0·4
0·6
0·8
1·0
1·2
1·4
1·6
2
4
6
8
10
12
14
16
18
20
22


D. tigrina 10°C
y = 0·120 + 0·015X
RESPIRATION RATE (µlO2/hr)
AREA (mm2)
0
0·2
0·4
0·6
0·8
1·0
1·2
1·4
1·6
2
4
6
8
10
12
14
16
18
20
22


D. tigrina 15°C
y = 0·032 + 0·035 x
RESPIRATION RATE (µl O2/hr)
AREA (mm2)
0
0·2
0·4
0·6
0·8
1·0
1·2
1·4
1·6
2
4
6
8
10
12
14
16
18
20
22


D. tigrina 20°C
y = 0·094 + 0·049 x
RESPIRATION RATE (µl O2/hr)
AREA (mm2)
0
0·2
0·4
0·6
0·8
1·0
1·2
1·4
1·6
2
4
6
8
10
12
14
16
18
20
22

Species	Temperature	n	Regression Equation	t. (a=0)	t. (b=0)	r	(P)r
P. tenuis	5°C	24	y = 0.0149 + 0.0241x	0.007 (n.s.)	4.52	0.69	0.001
	10°C	25	y = 0.2496 + 0.0487x	0.085 (n.s.)	3.57	0.60	0.002
	15°C	28	y = 0.0998x - 0.02354	0.276 (n.s.)	5.49	0.73	0.001
	20°C	25	y = 0.2496 + 0.0486x	0.127 (n.s.)	3.85	0.63	0.001
P. felina	5°C	44	y = 0.0312 + 0.0266x	0.039 (n.s.)	10.80	0.86	0.001
	10°C	61	y = 0.0671 + 0.0466x	0.012 (n.s.)	6.46	0.64	0.001
	15°C	60	y = 0.1175 + 0.0461x	0.059 (n.s.)	16.90	0.92	0.001
	20°C	24	y = 0.1693 + 0.0385x	0.052 (n.s.)	4.76	0.71	0.001
D. tigrina	5°C	24	y = 0.0049 + 0.0079x	0.002 (n.s.)	1.85	0.37	n.s. (p=0.01)
	10°C	31	y = 0.1202 + 0.0150x	0.050 (n.s.)	3.00	0.49	0.01
	15°C	30	y = 0.0322 + 0.0349x	0.029 (n.s.)	6.66	0.73	0.001
	20°C	20	y = 0.0938 + 0.0490x	0.091 (n.s.)	4.64	0.74	0.001

Table 9 : The relationship between triclad size and respiration rate in P. tenuis, P. felina and D. tigrina, at 5°, 10°, 15° and 20°C. The regression equations were derived from the data in Fig. 4.

Species	Temperature	n	Mean R/A	S.E.
P. tenuis	5°C	24	0.027	0.006
	10°C	25	0.070	0.006
	15°C	28	0.081	0.006
	20°C	25	0.083	0.006
P. felina	5°C	44	0.039	0.005
	10°C	61	0.065	0.004
	15°C	60	0.067	0.004
	20°C	24	0.054	0.006
D. tigrina	5°C	24	0.009	0.004
	10°C	31	0.026	0.004
	15°C	30	0.039	0.004
	20°C	20	0.074	0.004

Table 10a : Mean R/A values ($\mu l O_2 mm^{-2} hr^{-1}$) for P. tenuis, P. felina and D. tigrina at different temperatures

	P. tenuis	P. felina	D. tigrina
F	35.98	9.00	41.75
d.f.	3/98	3/185	3/101
P	<0.01	<0.01	<0.01

Table 10b : Results of one-way analysis of variance on the above data

The variance ratios were all highly significant at the P = 0.01 level. Hence there were significant differences between R/A values at each temperature within a species.

A plot of the mean R/A values (see Table 10a), with confidence limits (± 2 Standard Errors) illustrates those differences (Fig. 5).

In general terms, the mean R/A value increased with temperature in P. tenuis to a plateau of about $0.082\mu l^{-1}mm^{-2}hr$. There was no significant difference in the rate of oxygen uptake per unit area between 10° and 15°C (d = 1.29; p > 0.05), or 15° and 20°C (d = 0.34; p > 0.05). A large increase in the R/A value was observed between 5° and 10°C (d = 5.10; p < 0.05).

Similarly, the R/A value increased with temperature in P. felina. A plateau of about $0.066\mu l^{-1}mm^{-2}hr$ was reached between 10° and 15°C. However, the rate of oxygen consumption fell significantly between 15° and 20°C (d = 1.96; p < 0.05).

In contrast to P. felina and P. tenuis, the R/A values observed in D. tigrina continued to rise from a low figure of 0.009 $\mu l^{-1}mm^{-2}hr$ at 5°C, to 0.074 $\mu l^{-1}mm^{-2}hr$ at 20°C without an obvious plateau being reached.

The impact of temperature on the respiration rate of the 3 species can be clearly seen by looking at the Q_{10} values. The Q_{10} may be defined as "the factor by which a reaction velocity is increased for a rise in temperature of 10°C" (Prosser and Brown 1961), and is widely used in assessing the effects of temperature on the metabolism of animals, viz:

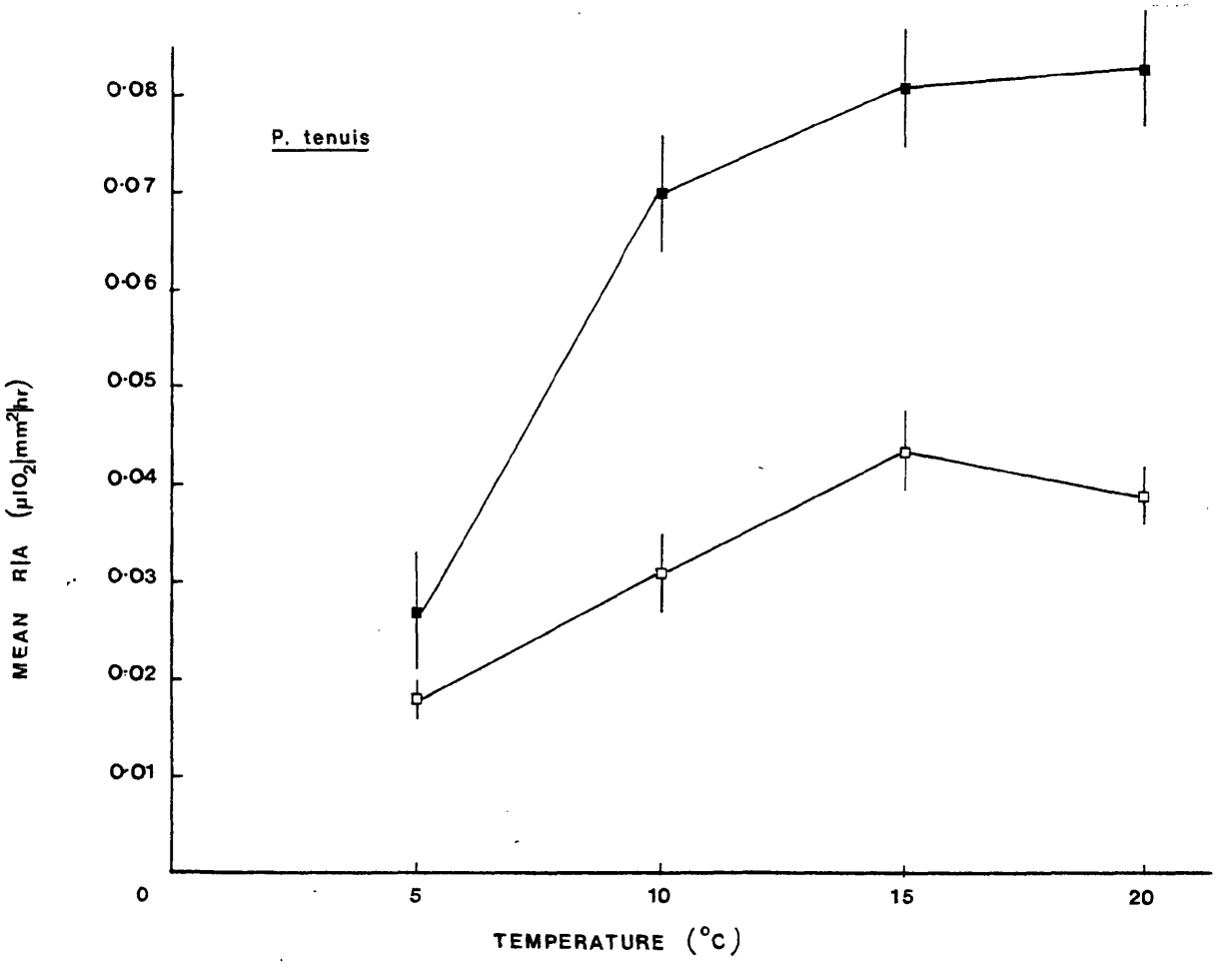
$$Q_{10} = \left(\frac{R_1}{R_2}\right)^{\frac{10}{t_1-t_2}}$$

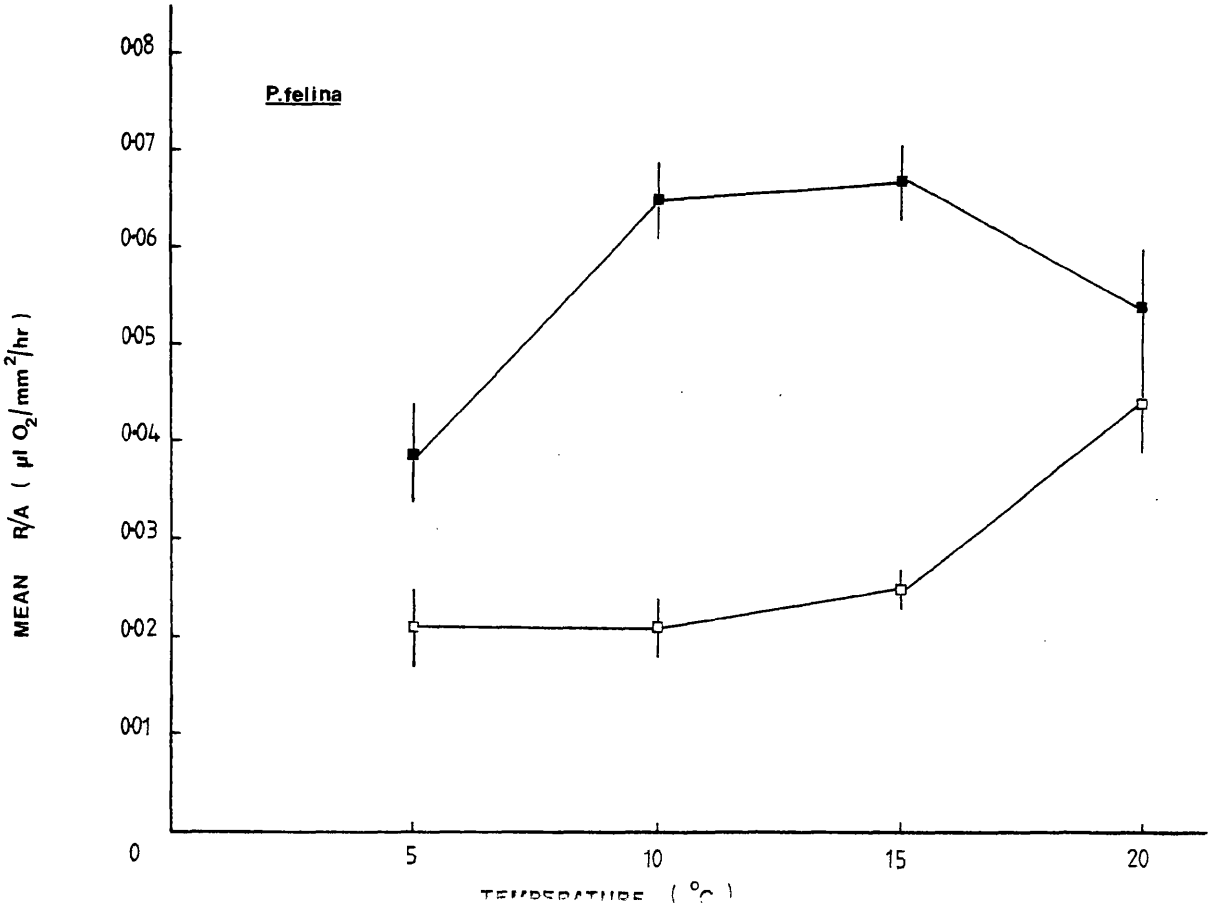
Figure 5

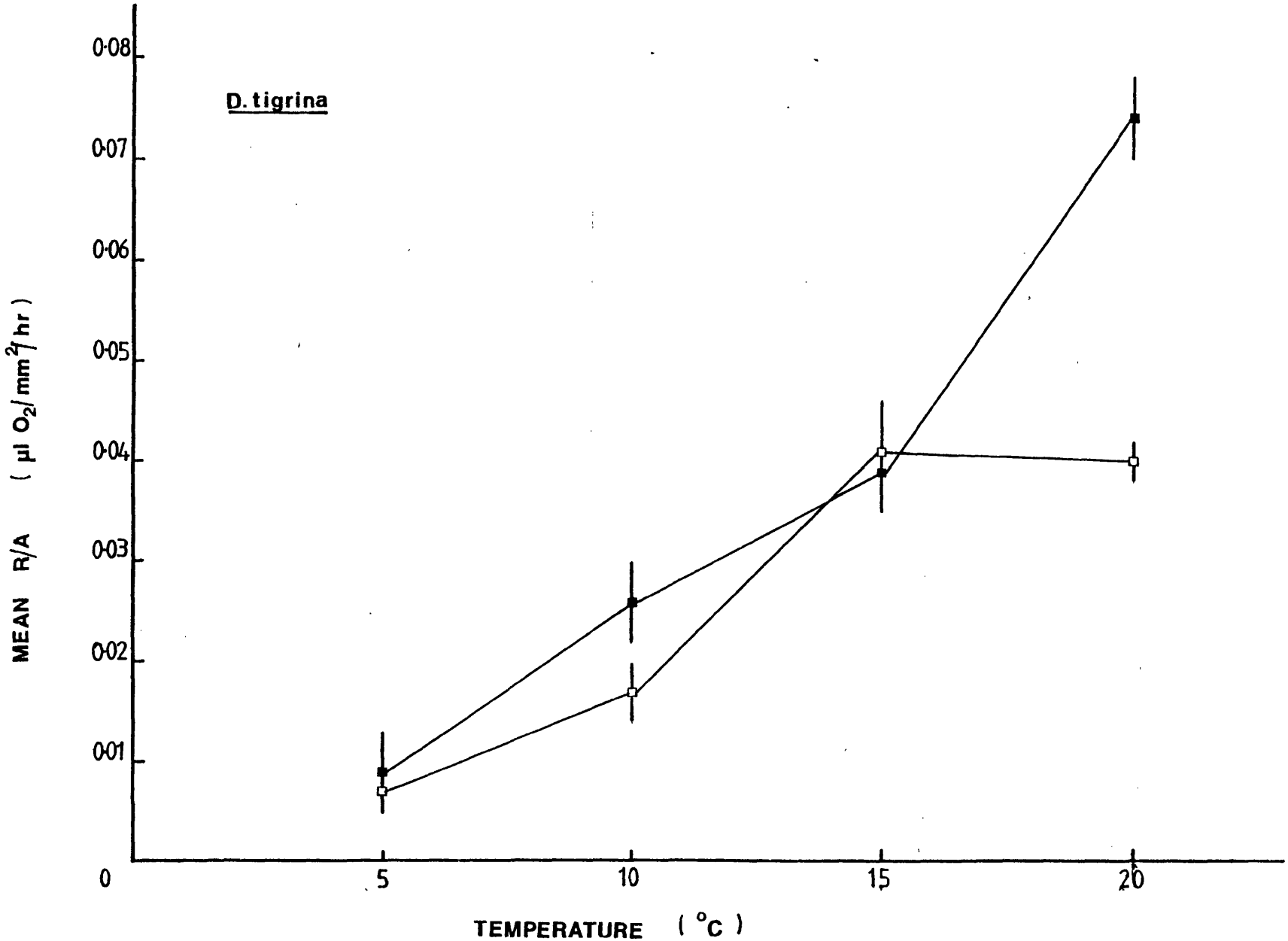
Mean R/A values (± 2 Standard Errors) for fed and starved *P. tenuis*, *P. felina* and *D. tigrina* at 5°, 10°, 15° and 20°C.

□ = starved

■ = fed


P. tenuis
MEAN R/A (µlO2/mm2/hr)
0·08
0·07
0·06
0·05
0·04
0·03
0·02
0·01
0
5
10
15
20
TEMPERATURE (°C)


P.felina
MEAN R/A (µl O2/mm2/hr)
0·08
0·07
0·06
0·05
0·04
0·03
0·02
0·01
0
5
10
15
20


D. tigrina
0·08
0·07
0·06
0·05
0·04
0·03
0·02
0·01
0
MEAN R/A (µl O_2/mm²/hr)
5
10
15
20
TEMPERATURE (°C)

where: R_1 and R_2 are the rates of reaction corresponding to temperatures t_1 and t_2 (Newell 1970).

Table 11 below shows the Q_{10} values over different temperature ranges for the 3 species. The Q_{10} values are greatest for P. tenuis and D. tigrina at low temperatures, and least in P. felina over intermediate temperatures.

5.4.2 Starved animals

The relationship between R/A and temperature was investigated for starved animals. Values were determined over a period of weeks and these data are summarised in Table 12. Also listed are mean R/A values for unstarved triclads (data from Table 10a).

Inspection indicates that there are obvious and consistent differences between starved and unstarved R/A values. However, there are no consistent differences between starved animals at different starvation times. Hence mean R/A values for starved animals were computed by averaging all data at all starvation times for each species at each temperature. These are summarised in Table 13a.

The results indicate that temperature also affects the respiration rate of starving animals, and that in general, R/A increases with temperature. Results from a one-way analysis of variance on the data in Table 13a, are presented in Table 13b. For each species, significant differences at the $p = 0.01$ level were found between R/A values over the temperature range studied. Mean R/A (± 2 Standard Errors) are plotted in Fig. 5.

No significant difference (Bailey 1964) was found between the mean R/A values at 5° and $10^{\circ}C$ ($d = 0$; $p > 0.01$) or 10° and $15^{\circ}C$ ($d = 1.09$; $p > 0.01$) in P. felina. However, at $20^{\circ}C$, the R/A value was

Species	5-10°C	10-15°C	15-20°C	5-15°C	10-20°C
P. tenuis	6.72	1.34	1.05	3.00	1.19
P. felina	2.77	1.06	0.65	1.72	0.83
D. tigrina	8.35	2.25	3.60	4.33	2.85

Table 11 : Q_{10} values (respiration) for 3 species of triclad fed 2 X per week, and fully acclimated to respective temperatures. Values dereived from data in Table 10a.

Species	Temperature	Mean R/A Unstarved*	Weeks of Starvation	No. of Observations	Mean R/A	S.E.
P. tenuis	5°C	0.027	3	4	0.015	0.004
			19	3	0.013	0.003
			21	4	0.025	0.004
			23	5	0.012	0.002
			29	5	0.022	0.002
	10°C	0.070	4	5	0.035	0.006
			7	9	0.045	0.006
			11	9	0.020	0.004
			19	6	0.023	0.003
	15°C	0.081	4	7	0.046	0.003
			5	8	0.054	0.005
			10	4	0.027	0.001
			14	5	0.036	0.006
	20°C	0.083	2	10	0.038	0.003
			4	5	0.036	0.007
			6	10	0.044	0.008
			7	5	0.044	0.009
P. felina	5°C	0.039	1	1	0.078	-
			11	8	0.071	0.002
			23	4	0.025	0.008
			24	5	0.023	0.005
			41	1	0.017	-
	10°C	0.065	1	7	0.045	0.007
			2	12	0.022	0.003
			3	4	0.022	0.007
			4	1	0.023	-
	15°C	0.067	1	2	0.024	0.003
			2	13	0.024	0.003
			7	5	0.028	0.010

Species	Temperature	Mean R/A Unstarved*	Weeks of Starvation	No. of Observations	Mean R/A	S.E.
P. felina (cont.)	20°C	0.054	1	2	0.032	0.010
			2	7	0.052	0.007
			3	6	0.026	0.005
			6	4	0.039	0.025
			8	5	0.075	0.013
			9	2	0.028	0.002
D. tigrina	5°C	0.009	1	4	0.009	0.002
			2	4	0.011	0.003
			7	4	0.009	0.006
			18	2	0.003	0.001
			19	3	0.003	0.002
			20	4	0.007	0.001
			22	4	0.004	0.001
			23	4	0.007	0.002
	10°C	0.026	1	6	0.025	0.003
			12	5	0.008	0.002
			14	4	0.024	0.009
			17	7	0.012	0.003
	15°C	0.039	1	4	0.042	0.014
			3	4	0.022	0.005
			4	4	0.029	0.003
			12	4	0.049	0.018
			14	6	0.049	0.010
			16	2	0.047	0.015
	20°C	0.074	1	8	0.050	0.012
			2	9	0.039	0.010
			5	10	0.055	0.016
			6	3	0.049	0.014

Table 12 : The effect of starvation on the respiration rate of *P. tenuis*, *P. felina* and *D. tigrina* at different temperatures

* Data from Table 10a

Species	Temperature	Mean R/A	S.E.
P. felina	5°C	0.021	0.004
	10°C	0.021	0.003
	15°C	0.025	0.002
	20°C	0.044	0.005
P. tenuis	5°C	0.018	0.002
	10°C	0.031	0.004
	15°C	0.044	0.004
	20°C	0.039	0.003
D. tigrina	5°C	0.007	0.001
	10°C	0.017	0.003
	15°C	0.041	0.005
	20°C	0.040	0.002

Table 13a : Mean R/A values ($\mu l O_2^{-1} mm^{-2} hr$) of starved P. tenuis, P. felina and D. tigrina, at different temperatures

	P. tenuis	P. felina	D. tigrina
F	14.68	7·47	37.49
d.f.	3/103	3/87	3/103
P	<0.01	<0·01	<0.01

Table 13b : Results of one-way analysis of variance on the above data

significantly higher ($d = 3.59$; $p < 0.01$) than at 15°C. A significant increase in mean R/A values was observed in P. tenuis between 5° and 10°C ($d = 2.87$; $p < 0.01$) and 10° and 15°C ($d = 2.25$; $p < 0.01$). Between 15° and 20°C, however, the increase in R/A values levelled off, and no significant difference could be found ($d = 1.00$; $p > 0.01$). In D. tigrina, there is also an increase in the mean R/A value with temperature, reaching a plateau of about 0.040 $\mu l O_2^{-1} mm^{-2} hr$ between 15° and 20°C. Significant increases were observed between 5° and 10°C ($d = 3.20$; $p < 0.01$) and 10° and 15°C ($d = 4.18$; $p < 0.01$).

In Table 14, the Q_{10} values for the 3 species over a range of temperatures have been calculated. The Q_{10} is smallest for P. tenuis at high temperatures, and P. felina at low temperatures. The lowest Q_{10} values were observed in D. tigrina between 5° and 15°C.

A two-way analysis of variance (Snedecor 1956; Sokal and Rohlf 1973) was performed on the data in Tables 10a and 13a, in order to test if temperature affected the respiration rate of starved and fed triclads differently. Since equal numbers of observations per cell are required for the analysis, data was deleted at random, using random number tables (Fisher and Yates 1963). The values used in the analysis are given in Appendix I, and the results are summarised in Table 15. In all 3 species, temperature affects starved and fed groups in significantly different ways ($p < 0.01$).

5.4.3 Respiration in groups with different thermal histories

The respiratory responses of P. tenuis, P. felina and D. tigrina to changes in temperature are shown in Fig. 6. In each species the change in temperature resulted in an immediate change in the respiration rate. The rate of oxygen uptake fell dramatically during

Species	5-10°C	10-15°C	15-20°C	5-15°C	10-20°C
P. tenuis	2.97	2.01	0.79	2.44	1.26
P. felina	1.00	1.42	3.10	1.19	2.10
D. tigrina	5.90	5.82	0.95	5.86	2.35

Table 14 : Q_{10} values (respiration) for fully acclimated, starved, P. tenuis, P. felina and D. tigrina

a. *P. tenuis*

Source	D.F.	S.S.	M.S.	F
Total	159	0.1418		
A	1	0.0377	0.0377	100.3902
B	3	0.0398	0.0133	35.2971
Int.	3	0.0071	0.0024	6.3017
Error	152	0.0571	0.0004	

b. *P. felina*

Source	D.F.	S.S.	M.S.	F
Total	159	0.1248		
A	1	0.0245	0.0245	44.7074
B	3	0.0080	0.0027	4.8687
Int.	3	0.0091	0.0030	5.5226
Error	152	0.0832	0.0005	

c. *D. tigrina*

Source	D.F.	S.S.	M.S.	F
Total	159	0.1241		
A	1	0.0034	0.0034	9.2950
B	3	0.0598	0.0199	54.4783
Int.	3	0.0054	0.0018	4.8832
Error	152	0.0556	0.0004	

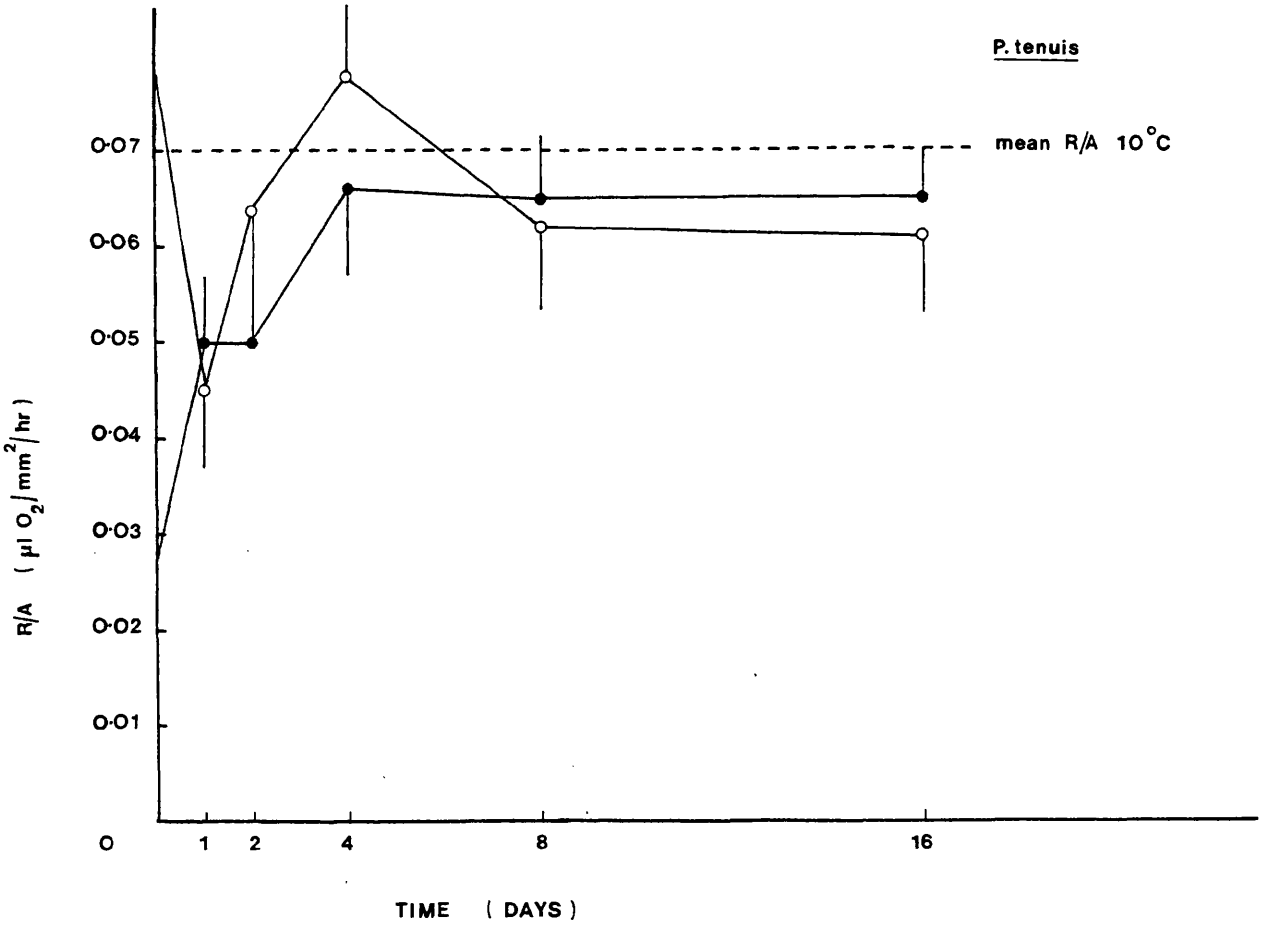
Table 15 : Results from 2-way analyses of variance of R/A values in starved and unstarved triclads. Data from Appendix I (see text)

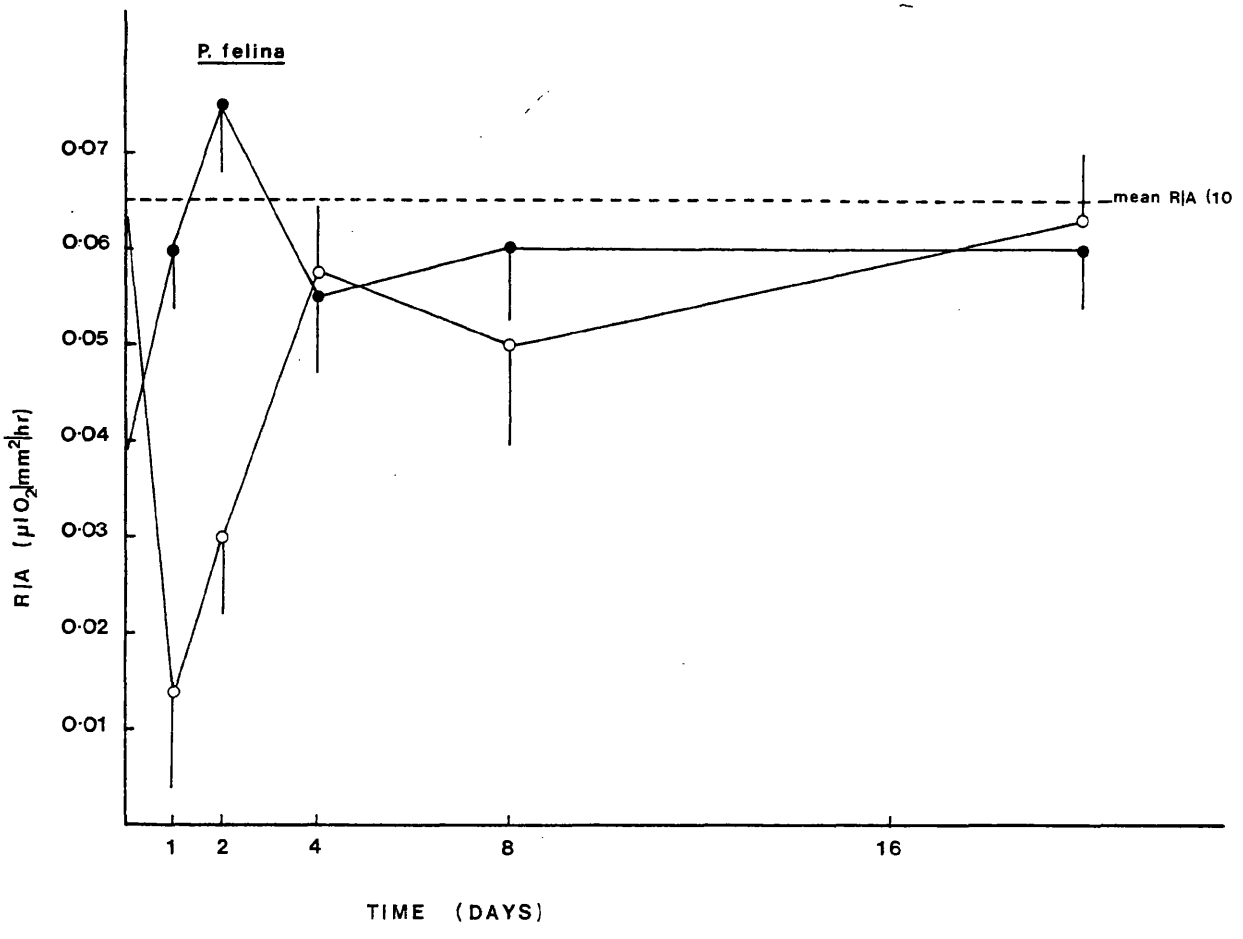
Figure 6

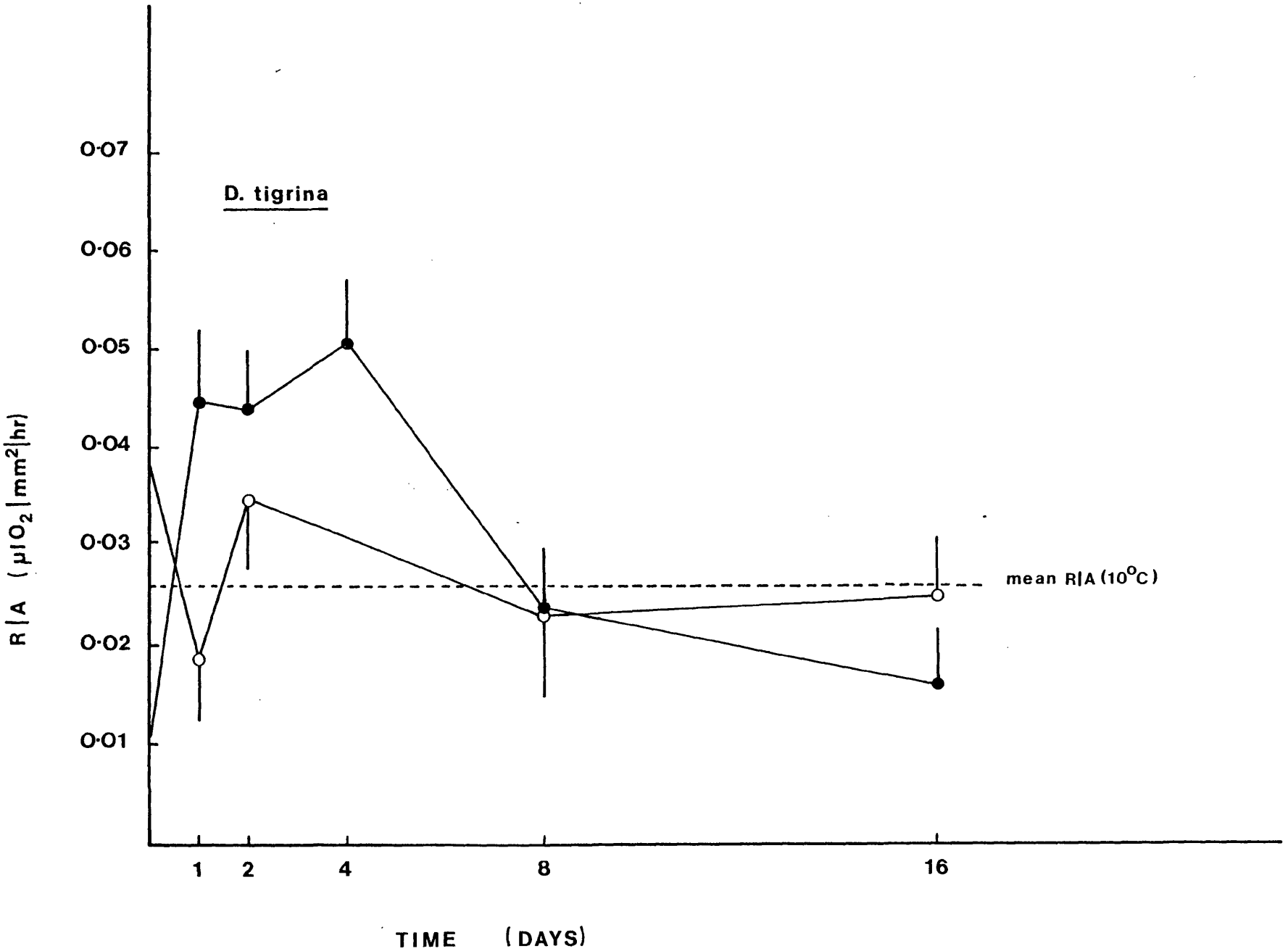
The acclimation responses of groups of P. tenuis, P. felina and D. tigrina when transferred from 5°C and 15°C to 10°C.

○ from 15°C

● from 5°C


P. tenuis
mean R/A 10°C
0·07
0·06
0·05
0·04
0·03
0·02
0·01
0
R/A (µl O2/mm2/hr)
1
2
4
8
16
TIME (DAYS)


P. felina
mean R/A (10
0·07
0·06
0·05
0·04
0·03
0·02
0·01
R/A (µlO2/mm2/hr)
1
2
4
8
16
TIME (DAYS)


D. tigrina
0·07
0·06
0·05
0·04
0·03
0·02
0·01
R|A (µlO2|mm2|hr)
mean R|A (10°C)
1
2
4
8
16
TIME (DAYS)

the first 24 hours after the triclads had been transferred from 15° to 10°C. However, after 16 days, there was no significant difference ($p > 0.05$) between the respiration rates of these groups and the mean R/A values derived from laboratory stock acclimated to 10°C in the standard manner (data from Table 10a) (see Table 16).

A corresponding initial rapid rise in the rate of oxygen uptake was measured in all species, when transferred from 5° to 10°C. However, there was no significant difference ($p > 0.05$) between the R/A values measured on day 16, and the R/A values measured in the standard manner described above (see Table 16).

5.5 Discussion

To be able to use a single, size independent parameter to investigate the effects of different variables on the respiration rate of an animal is very useful, since it allows certain comparisons to be made. The correlations between the rate of oxygen consumption and plan area were acceptable in most cases. The lowest correlation coefficients (0.37 and 0.49) for R/A were found in *D. tigrina* at low temperatures (5° and 10°C). This may be because *D. tigrina* is a warm water species (Dahm 1958), and at 5° and 10°C, the respiration rate was much lower and more difficult to measure than in the other 2 species. This isometric relationship between respiration rate and plan area has been found in all other species of freshwater triclad that have been studied (Calow and Woolhead[1] 1977a; Woollhead 1979).

Using R/A values, only intraspecific comparisons can be made. Difficulties arise in making direct interspecific comparisons, since area/dry weight relationships are different in the 3 species. However, by using Q_{10} values, this problem is overcome, since the relationship

Species	Mean R/A at 10°C	Final R/A of Animals transferred from 15° to 10°C	t-test		Final R/A of Animals transferred from 5° to 10°C	t-test	
P. tenuis	0.070	0.061	t = 2.03	p > 0.05	0.065	t = 2.00	p > 0.05
P. felina	0.065	0.063	t = 0.57	p > 0.05	0.060	t = 1.67	p > 0.05
D. tigrina	0.026	0.025	t = 1.33	p > 0.05	0.021	t = 1.67	p > 0.05

Table 16 : The effect of acclimation temperature on the R/A values ($\mu l O_2^{-1} mm^{-2} hr$) of P. tenuis, P. felina and D. tigrina at 10°C

between area and dry weight is the same at all temperatures, i.e. the respiration rates of 2 species at the same temperature cannot be compared, whereas differences between temperatures (Q_{10} values) between 2 species can.

Since the fed animals were given food twice per week, this approximates to the food supply index (F.S.I.) of 0.25 used by Woollhead and Calow (1979) (see Chapter 4). Woollhead (1979) found the R/A of P. tenuis under an F.S.I. of 0.25 to be 0.087 ± 0.010 $\mu l O_2/mm^2/hr$. This is not significantly different from the R/A value of 0.070 ± 0.012 $\mu l O_2/hr/mm^2$ measured in this study ($t = 1.214$, d.f. = 50, $p > 0.05$).

In warm-acclimated (15°C) groups of P. tenuis, P. felina and D. tigrina, an initial undershoot in the respiration rate was observed, when transferred to 10°C. In contrast to this, an initial overshoot was measured when cold-acclimated (5°C) groups of P. felina and D. tigrina were transferred to the new temperature regime. These responses were characteristic of most poikilotherms (Prosser and Brown 1961). Although no overshoot was observed in P. tenuis when transferred from 5°C to 10°C, there was a rapid increase in the respiration rate.

In all 3 species, there was complete acclimation to 10°C within the period of observation. Measurements made on day 16 showed that there was no significant difference between the R/A values of the warm and cold acclimated groups, and the R/A values of animals acclimated to 10°C. Acclimation in the 3 species of triclad would therefore appear to be fairly rapid, although some care should be taken when extrapolating these results to discuss acclimation over other temperature ranges.

Because all measurements were made over a 24 hour period, no account of diurnal variations in oxygen consumption need be taken.

Therefore, all respiration rates measured may be described as the average hourly rate of oxygen uptake under different feeding regimes. Following the definitions of Fry (1947, 1957), Fry and Hart (1948) and Kausch (1969), the respiration rates measured may be defined as the routine and starvation metabolic rates.

Both the routine metabolic rate versus temperature curves for P. felina and P. tenuis exhibited plateau effects, although if the respiration rates had been measured at higher temperatures, then the respiration rate/temperature plateau for P. tenuis might have been extended. Both P. felina and P. tenuis exhibit a certain degree of temperature independence, particularly over the higher temperature range (10^{o}-20^{o}C), and have Q_{10} values < 2 (0.83 and 1.19 respectively).

By contrast, the routine metabolic rate for D. tigrina shows no evidence of temperature independence over the range studied. However, it is well known that D. tigrina is a thermophilic species (Dahm 1958; Pattee, Lascombe and Delolme 1973) and a plateau would not be expected below 20^{o}C.

The switch from routine metabolic rate to starvation rate appears to occur during the first week in all species. This is in agreement with the findings of Calow and Woollhead (1977a) who reported a sharp decrease in the rate of oxygen uptake within 5 days of starvation. No change in the locomotory or metabolic strategies can be detected during the period of starvation.

The starvation metabolism of P. felina exhibits a wide tolerance over the lower temperature range, whereas that of P. tenuis between 5^{o} and 15^{o}C, appears to be more dependent on temperature. In general, however, the starvation metabolism of both species is less sensitive to temperature than the routine metabolism.

The difference between the metabolic rate of starved and fed animals is a widespread phenomenon (Saunders 1963; Barnes, Barnes and Finlayson 1963; Kausch 1969). Calow and Woollhead (1977a) reported similar differences in the metabolic rates of triclads. These differences are apparent at most temperatures in P. felina and P. tenuis, but only found in D. tigrina at high temperatures (Fig. 5).

The difference in metabolic rates between fed and starved triclads reveals the ability of the species to adapt their metabolic rates to food shortages. Bolen (1937) states that during starvation there is a reduction in specific dynamic action (S.D.A.) resulting in lower metabolic expenditure. There is evidence, however, that the energy savings through a reduction in S.D.A. are likely to be negligible (e.g. Needham 1964). Calow (1977b) and Calow and Woollhead (1977a) have suggested that the differing abilities of triclads to conserve energy during starvation are due to the adoption of contrasting locomotory strategies, governed by their methods of feeding. Both Reynoldson (1961) and Calow (1977a) suggest that not only does P. felina have a higher level of activity than P. tenuis, even under famine conditions, but that it is unable to control its activity with temperature. Considering the evidence from this chapter alone, I must disagree. The Q_{10} values for starved P. felina between 5^{o} and $15^{o}C$ are lower than in P. tenuis, and only under unusual conditions (high temperatures combined with starvation) does the respiratory metabolism of P. felina show the effects of thermal stress.

The above results also contradict the conclusions arrived at by other workers. Pattee (1972) described P. tenuis as a eurythermic species on the basis of the high intrinsic rate of natural increase of populations over a wide temperature range, and using the same criterion,

P. felina and other rheophilic species were described as being stenothermic (Pattee 1969). However, considering the low Q_{10} values for both fed and starved triclads, P. felina is eurythermic.

The results concerning D. tigrina confirm the data of Russier - Delolme (1970) and Pattee, Lascombe and Delolme (1973) that it is a thermophilic species.

Further discussion of the differences between species must await the results obtained from the construction of energy budgets for fed and starved animals (see Chapter 8).

Chapter 6

GROWTH AND DEGROWTH

6.1 Introduction

In the previous chapter, I have presented data which show that temperature affects the routine and starvation metabolism of the 3 species of triclad under study.

In this chapter, as part of the investigation into the bio-energetics and ecology of *P. felina*, *P. tenuis* and *D. tigrina*, I am going to examine the effects of temperature on the rates of growth and degrowth.

6.2 Literature Review

Organic growth was first defined as an "increase in volume (Davenport 1899), or an "increase in size" (T.H. Huxley, quoted in Davenport *ibid*). Morgan (1907), Thompson (1942), and others initiated much research on the metabolic and cellular basis of growth, which is now well understood (for review, see Brody 1945; Needham 1964).

However, as Needham pointed out, growth was very amenable to mathematical analysis from an early date, and hence much of the literature has been concerned with measuring rates of growth in plants and animals (Huxley 1932; Le Gros Clark and Medawar 1945; Weiss and Kavanau 1957; Ursin 1967; Parks 1973). The study of growth rates has been further stimulated by recent work on energy balance in animals (e.g. Palaheimo and Dickie 1965; Winberg 1971; Lawlor 1976).

A wide variety of factors are known to influence growth in poikilotherms (Needham 1964; Iles 1974). However the most important are temperature and food supply. The former has been studied by Clarke and Sardesai (1959), and Edwards *et al.* (1979), among other. Many studies, including those of Calow (1973) and Hislop *et al.* (1978) have looked at the influence of food supply on growth rate.

Comparatively few studies on triclads have concerned themselves with growth. It is important here to clearly define the terms growth and regeneration, since some confusion exists in the literature (Argyris 1968). Regeneration is a repair process which occurs in triclads after wounding or decapitation. It is a developmental process, involving the formation of a blastema of undifferentiated cells which subsequently differentiate to replace the missing tissue or head (Abeloos 1930; Brøndsted 1969). Growth, on the other hand, involves an increase in size which does not involve morphollaxis (remodelling of existing tissue).

Over the years, a great deal of interest has been shown in regeneration, and this has stimulated some research into the influence of temperature and nutrition on the regenerative rates in triclads (Brøndsted 1961, 1969; Nentwig and Schauble 1974).

C.M. Child carried out a series of investigations into growth in triclads, which he summarised in "Senescence and Rejuvenescence" (1915). He found that nutrition profoundly influenced the rate of growth. Further studies by Bahrs (1929, 1931), Pettibone and Wulzer (1934), Greenburg and Schmidt (1936), and Calow and Woollhead (1977b) corroborated these findings in a number of different triclad species.

Few studies have investigated the influence of temperature on growth in triclads. Balazs and Burg (1962) produced a growth curve for D. lugubris at 20°C. Reynoldson, Young and Taylor (1965) examined the change in growth rate (increase in length, in mm per 4 weeks) with temperature in P. tenuis, P. nigra, D. lacteum and D. lugubris.

The phenomenon of negative growth or degrowth is common in animals, and is a frequent response to starvation in the lower metazoa (Needham 1964). Degrowth has been observed in Paramecium by Wichterman

(1953), in the ascidian Clavellina by Huxley (1921), in Coelenterate medusae by De Beer (1924), and in the nemertine Lineus by Dawydoff (1924). Degrowth in triclads has been reported by many workers (Child 1915), and numerous studies were performed on their metabolism during degrowth. Bowen, Ryder and Dark (1976) investigated the effects of starvation on P. tenuis using a histochemical and biochemical approach.

Abeloos (1930), who worked on D. gonocephala, and Calow and Woollhead (1977a) who studied D. lacteum and P. tenuis, demonstrated that degrowth was exponential and the reverse of growth. However, the relevance of degrowth in triclads was not fully appreciated until the work of Reynoldson (1966b, 1968) established the importance of being able to withstand long periods of starvation.

In many animals, there are limits to the degree of shrinkage possible and the reasons may be connected with dedifferentiation which is known to occur during degrowth (Needham 1964). However, there is evidence that triclads have considerable capacity for degrowth. P. tenuis and D. lacteum have been found to shrink by 50% in 7 weeks during starvation, and Calow (1977b) has further demonstrated that some species can degrow to beyond their initial size at hatching.

Several factors appear able to influence degrowth. Reynoldson (1968) has shown in P. tenuis and D. lugubris that there is a direct relationship between size at the onset of starvation, and the survival period, and Calow (1977b) has further demonstrated the metabolic sensitivity of degrowth to temperature in 8 British species of triclad.

I now intend to examine the rates of growth and degrowth in P. tenuis, P. felina and D. tigrina at different temperatures in relation to their ecology.

6.3 Materials and Methods

Due to differences in life-cycles, any comparison between species is complicated. Hence I intend to analyse the growth and degrowth rates of each species separately, before looking for inter-specific differences.

P. tenuis eggs were collected and incubated at 5°, 10°, 15° and 20°C, as described previously (see Chapter 3). When the young emerged, they were transferred into plastic pots (one animal per pot) containing 30 mls of filtered lake water, and their size measured photographically (see Chapter 3). About 10 animals were used at each temperature, and their subsequent growth followed closely by measuring them each week (see Chapter 3). They were fed twice weekly on punctured Asellus and their water changed every week.

In order to measure degrowth in P. tenuis, large non-reproducing adults that had been grown from hatchling in the laboratory were starved. From 5 to 10 animals were used at each temperature (the same temperature that they had been grown at). They were cultured individually, and water was changed every week. The rate of degrowth was monitored photographically as described in Chapter 3.

D. tigrina and P. felina were collected from the field and acclimated to laboratory conditions, as previously described (Chapter 3). In order to measure the rates of growth at 5°, 10°, 15° and 20°C between 5 and 10 animals were transferred to plastic pots (one animal per pot) containing 30 mls of filtered lake or stream water. They were fed twice per week on punctured Asellus, and water was changed every week. The growth rates of both heads and tails that were produced during the experimental period were regularly monitored.

Simultaneous measurements of food intake were made in all 3 species and the results presented in Chapter 4.

Animals that were being used in the degrowth experiments were randomly selected to avoid any size or genetic bias. From 5 to 10 animals were degrown at each temperature.

6.4 Results

6.4.1 Growth

(a) P. tenuis

The plan area for individual P. tenuis at each temperature was plotted against time. The resultant plots were sigmoid (e.g. Fig. 7) and could be analysed in several ways:

(a) Overall growth rate

(b) Time taken to reach maximum size

(c) Growth rate during the exponential phase

(d) Growth rate during the decelerating phase

However, occasionally animals died before reaching maximum size, so that less data existed from which (a), (b) or (d) could be calculated. In order to make maximum use of the data collected, it was decided to analyse the rates measured during the exponential phase.

Regression lines, of the form $y = a + bx$, of $\log_{10}$ area against time (days) were calculated for individual animals at each temperature, over the period of exponential growth (Table 17). Good correlations ($r > 0.90$) were obtained in almost all cases, which confirmed the logarithmic nature of the data. The slope, b, corresponds to K, the coefficient of exponential growth, given in the equation:

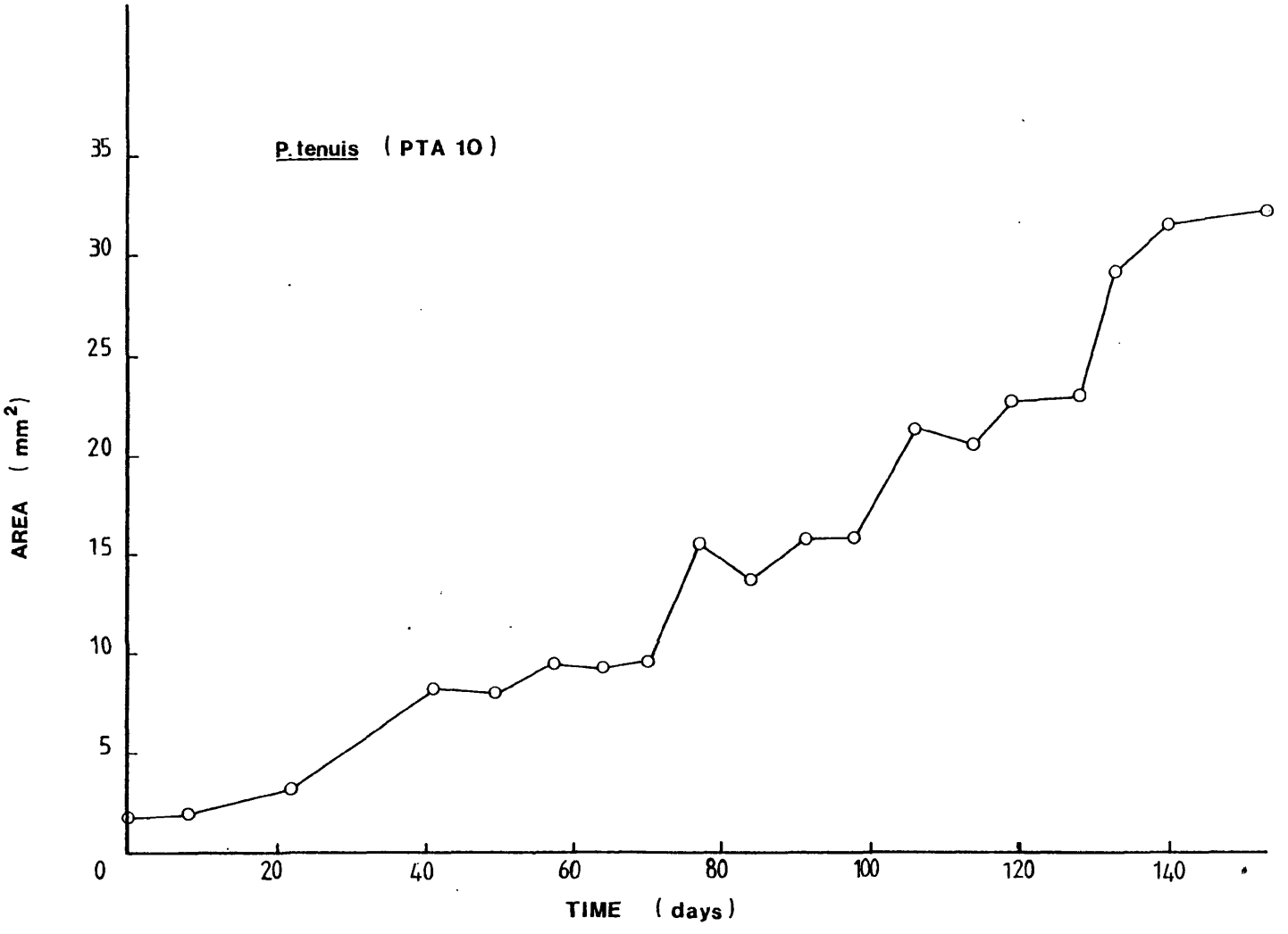
$$A_t = A_o e^{Kt}$$

where A_t = Area at time t; A_o = Area at time 0;

e = base of natural logarithms

Figure 7

The plan area (mm^2) of an individual P. tenuis plotted against time, at 10^oC.


P. tenuis (PTA 10)
AREA (mm2)
0
5
10
15
20
25
30
35
20
40
60
80
100
120
140
TIME (days)

Species	Temperature	Animal	Regression Equation	Correlation Coefficient	K	Initial Size
P. tenuis	5°C	PTB 1	y = 0.0632 + 0.0063x	0.95	0.0145	1.73 mm^2
		2	y = 0.1742 + 0.0041x	0.93	0.0094	1.60 mm^2
		3	y = 0.0153 + 0.0050x	0.95	0.0115	0.80 mm^2
		4	y = 0.2823 + 0.0046x	0.88	0.0106	0.66 mm^2
		5	y = 0.4386 + 0.0029x	0.90	0.0067	2.53 mm^2
		6	y = 0.3033 + 0.0047x	0.95	0.0108	1.80 mm^2
		7	y = 0.3525 + 0.0036x	0.96	0.0083	2.26 mm^2
		8	y = 0.1906 + 0.0054x	0.92	0.0124	0.93 mm^2
		9	y = 0.4150 + 0.0031x	0.94	0.0070	1.86 mm^2
		10	y = 0.1231 + 0.0036x	0.89	0.0083	0.54 mm^2
	10°C	PTA 1	y = 0.2475 + 0.0108x	0.96	0.0249	1.20 mm^2
		2	y = 0.2349 + 0.0101x	0.96	0.0233	0.96 mm^2
		3	y = 0.1259 + 0.0111x	0.96	0.0256	1.11 mm^2
		4	y = 0.2215 + 0.0090x	0.92	0.0207	1.11 mm^2
		5	y = 0.0984 + 0.0118x	0.97	0.0272	0.60 mm^2
		6	y = 0.1158 + 0.0092x	0.91	0.0212	0.70 mm^2
		7	y = 0.3142 + 0.0105x	0.98	0.0242	1.61 mm^2
		8	y = 0.4929 + 0.0075x	0.96	0.0173	1.81 mm^2
		9	y = 0.0464 + 0.0105x	0.96	0.0242	1.61 mm^2
		10	y = 0.3944 + 0.0084x	0.97	0.0193	1.81 mm^2

Species	Temperature	Animal	Regression Equation	Correlation Coefficient	K	Initial Size
P. tenuis	15°C	PTC 1	y = 0.2177 + 0.0134x	0.99	0.0309	1.33 mm^2
		2	y = 0.1033 + 0.0188x	0.99	0.0433	1.00 mm^2
		4	y = 0.4582 + 0.0084x	0.94	0.0193	2.08 mm^2
		5	y = 0.2132 + 0.0105x	0.93	0.0242	1.30 mm^2
		6	y = 0.2519 + 0.0103x	0.85	0.0237	0.87 mm^2
		7	y = 0.0231 + 0.0182x	0.98	0.0419	1.16 mm^2
		8	y = 0.2819 + 0.0092x	0.91	0.0212	1.13 mm^2
		10	y = 0.2210 + 0.0188x	0.99	0.0433	0.94 mm^2
	20°C	PTD 1	y = 0.2405 + 0.0173x	0.96	0.0398	1.27 mm^2
		2	y = 0.3565 + 0.0121x	0.89	0.0279	1.46 mm^2
		3	y = 0.2972 + 0.0177x	0.94	0.0408	1.37 mm^2
		4	y = 0.2744 + 0.0192x	0.93	0.0422	1.27 mm^2
		5	y = 0.3077 + 0.0152x	0.91	0.0350	1.28 mm^2
		6	y = 0.2034 + 0.0243x	0.92	0.0560	1.41 mm^2
		7	y = 0.0922 + 0.0186x	0.97	0.0428	1.19 mm^2
		8	y = 0.2150 + 0.0144x	0.98	0.0332	1.32 mm^2
		9	y = 0.3357 + 0.0179x	0.97	0.0412	1.81 mm^2
		10	y = 0.1331 + 0.0161x	0.95	0.0371	0.91 mm^2

Table 17 : The regression equations and coefficients of exponential growth (K) for individual P. tenuis at different temperatures (I = Initial size at hatching)

However, the b values must be multiplied by a correction factor (2.303) to convert them from $\log_{10}$ to $\log_e$ (Table 17).

There is no significant relationship ($r < 0.50$) between K, the coefficient of exponential growth, and the initial size of P. tenuis (Fig. 8). However, a one-way analysis of variance performed on all data shows that there are significant differences between K values at different temperatures ($F = 40.2$; d.f. = $^3/_{34}$; $P < 0.001$).

The mean values for K ($\overline{K}$) at each temperature was calculated (Table 18a), and these are illustrated in Fig. 9. $\overline{K}$ increases with temperature from 0.0100 at 5°C, to 0.0396 at 20°C. The Q_{10} values were then computed (see Chapter 5), and used to measure the sensitivity of growth to temperature. The overall Q_{10} between 5°C and 20°C was 0.60 (Table 18b). The highest value was found between 5° and 10°C, whereas Q_{10} was lowest between 15° and 20°C.

(b) P. felina

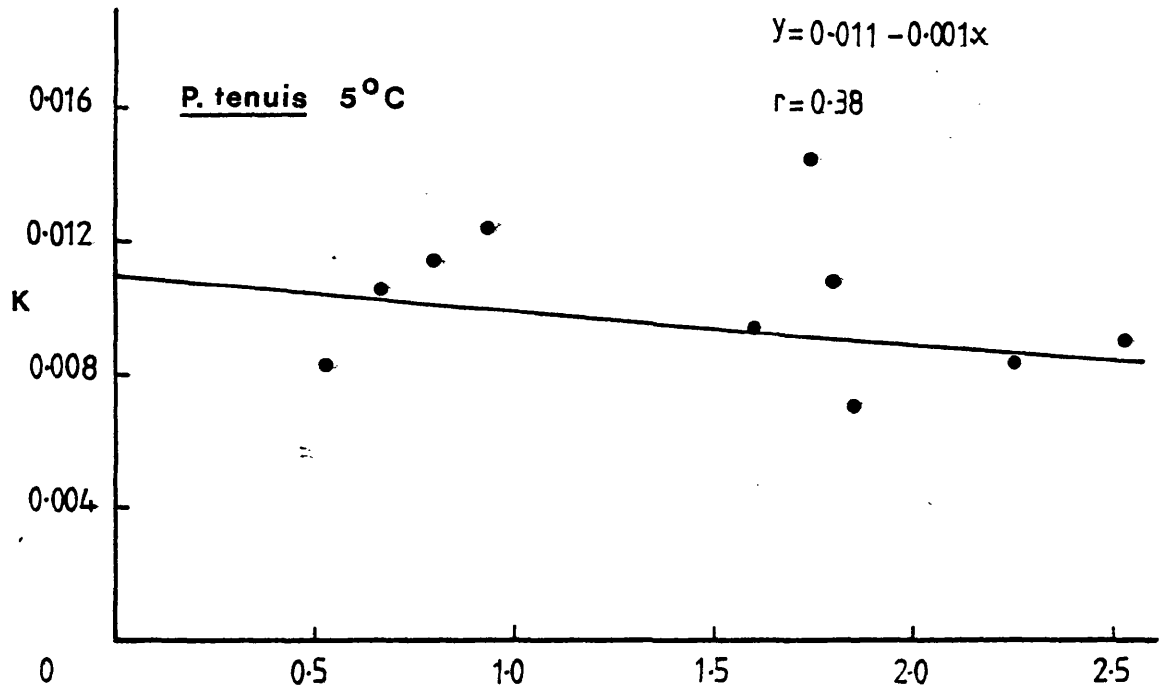
The typical growth pattern of P. felina is different from that of P. tenuis. Except at 20°C, it would appear that at any point during the growth cycle, fission may occur. The resultant pattern of growth and fission is illustrated in Fig. 11.

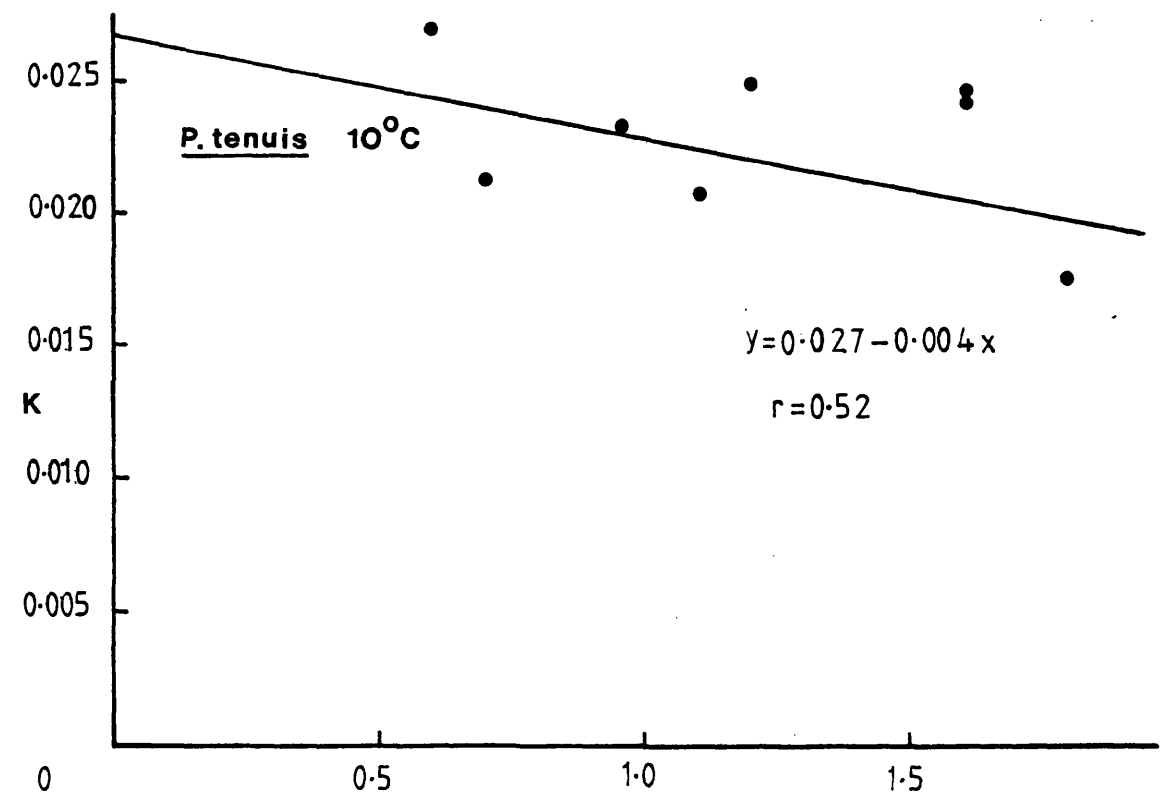
Before the effects of temperature on growth can be assessed, it is necessary to look at differences that may exist between head and tail growth.

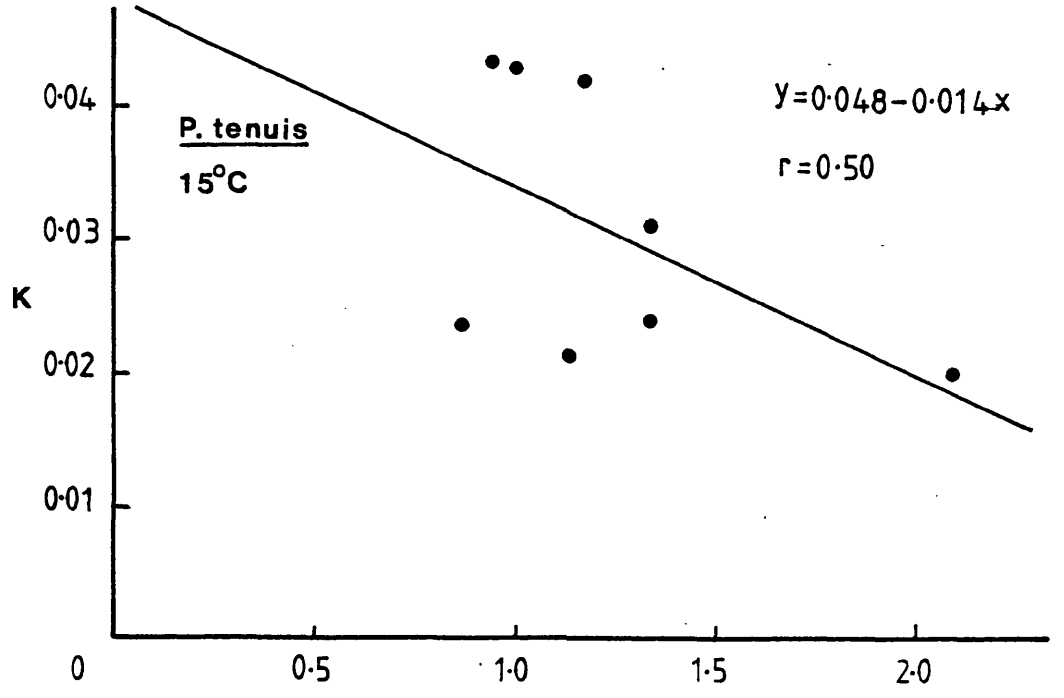
Five individual triclads were grown at each temperature, and their resulting fates followed. "Dendrograms", which illustrate the increase in numbers and successive growth of individual fission products, were plotted for each clone (e.g. Fig. 10). From these "dendrograms" plots of size (area in mm^2) against time (days) were drawn. Examples are given in Fig. 11.

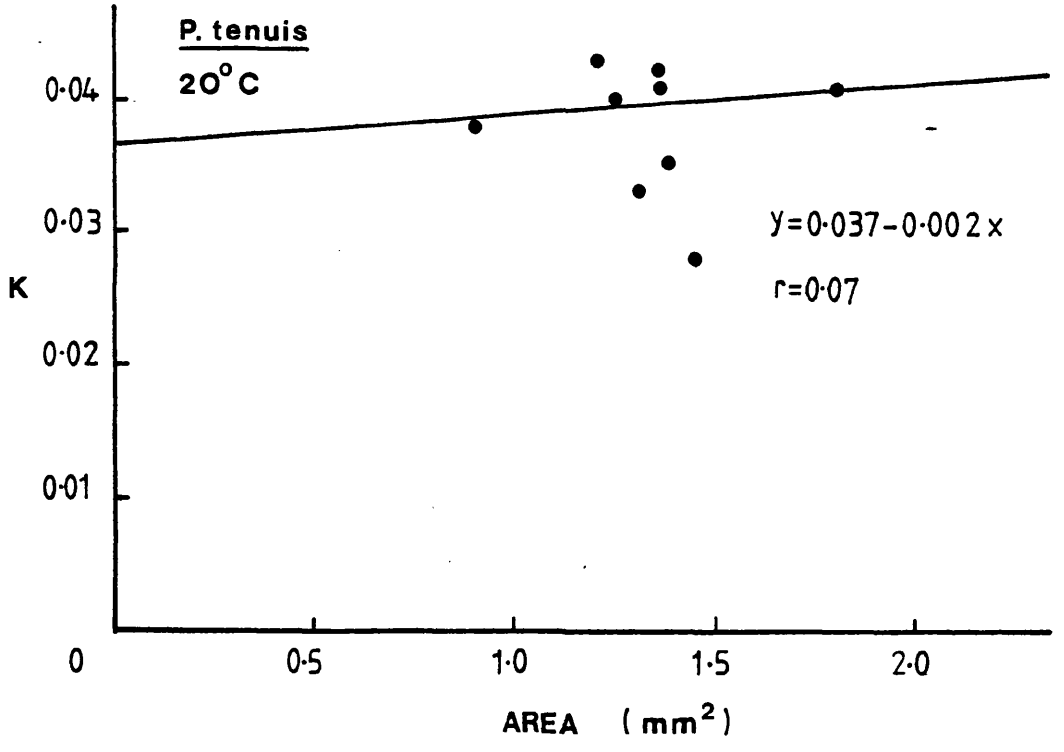
Figure 8

The relationship between the coefficient of exponential growth (K) and initial size at hatching in P. tenuis at different temperatures.


y = 0·011 − 0·001x
r = 0·38
P. tenuis 5°C
0·016
0·012
K
0·008
0·004
0
0·5
1·0
1·5
2·0
2·5


P. tenuis 10°C
y = 0·027 − 0·004x
r = 0·52
0·025
0·020
0·015
K
0·010
0·005
0
0·5
1·0
1·5
AREA mm²


P. tenuis
15°C
y=0·048-0·014x
r=0·50
K
0·04
0·03
0·02
0·01
0
0·5
1·0
1·5
2·0


P. tenuis
20°C
y=0·037-0·002x
r=0·07
K
0·04
0·03
0·02
0·01
0
0·5
1·0
1·5
2·0
AREA (mm²)

Species	Temperature	Mean K ($\overline{K}$)	S.E.
P. tenuis	5°C	0.0100	0.0008
	10°C	0.0228	0.0010
	15°C	0.0310	0.0037
	20°C	0.0396	0.0023
P. felina	5°C	0.0222	0.0030
	10°C	0.0420	0.0056
	15°C	0.0426	0.0060
	20°C	0.0303	0.0021
D. tigrina	5°C	-	-
	10°C	-	-
	15°C	0.0362	0.0012
	20°C	0.0852	0.0182

Table 18a : Mean values of K (rate of exponential growth) for P. tenuis, P. felina and D. tigrina at 5°, 10°, 15° and 20°C (calculated from data in Tables

Species	5-10°C	10-15°C	15-20°C	5-15°C	10-20°C	5-20°C
P. tenuis	5.20	1.85	1.63	3.10	1.74	0.60
P. felina	3.58	1.03	0.51	1.92	0.72	0.14
D. tigrina	-	-	5.54	-	-	-

Table 18b : Q_{10} values (growth) calculated over different temperature intervals for P. tenuis, P. felina and D. tigrina

Figure 9

A plot of mean K values ($\overline{K}$) against temperature in *P. tenuis*, *P. felina* and *D. tigrina*.

□ = P tenuis

○ = P felina

△ = D tigrina

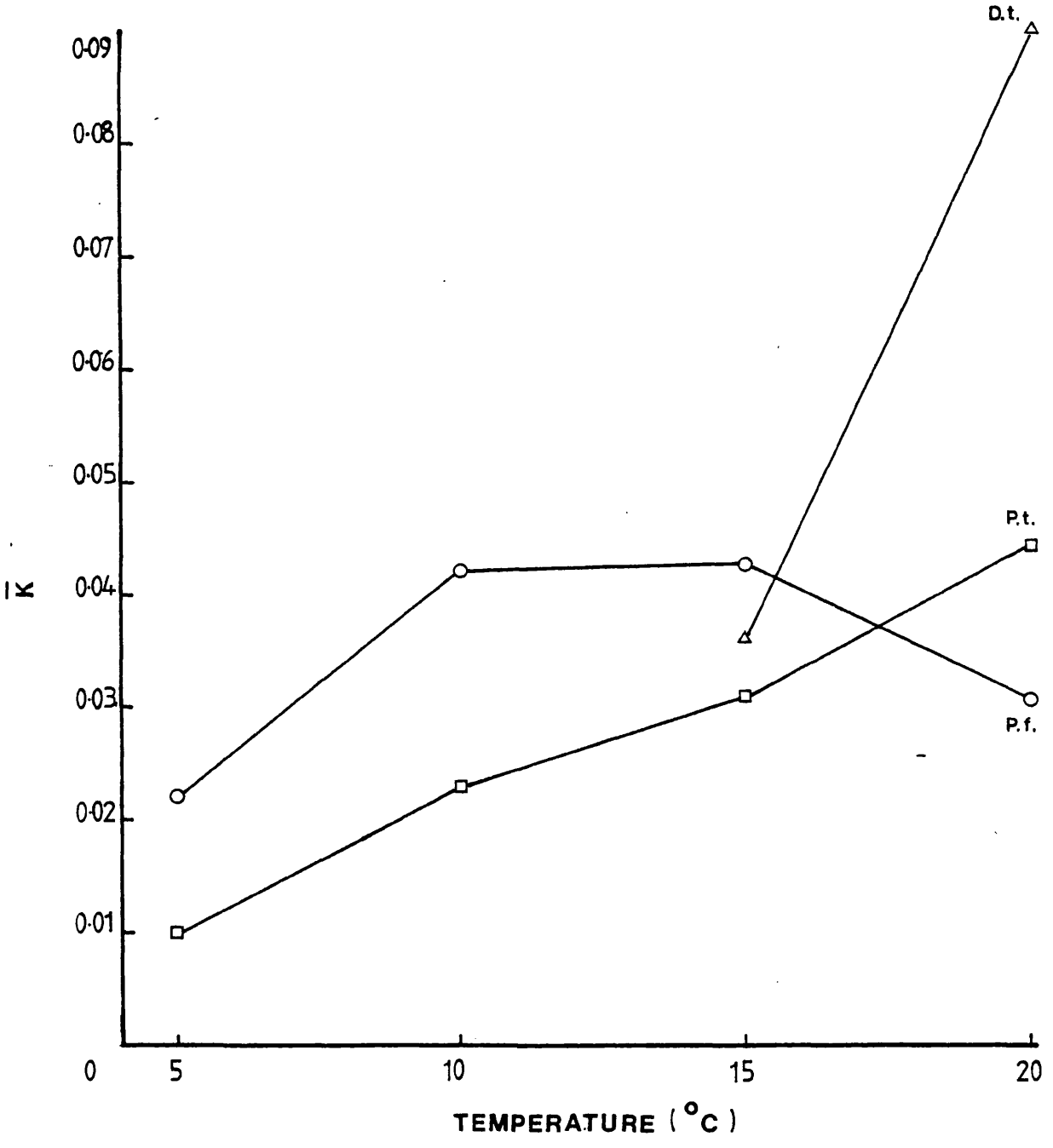

D.t.
P.t.
P.f.
0·09
0·08
0·07
0·06
0·05
0·04
0·03
0·02
0·01
0
5
10
15
20
I K
TEMPERATURE (°C)

Figure 10

An example of a typical dendrogram produced for P. felina. The nomenclature used is described in more detail in Section 7.3.

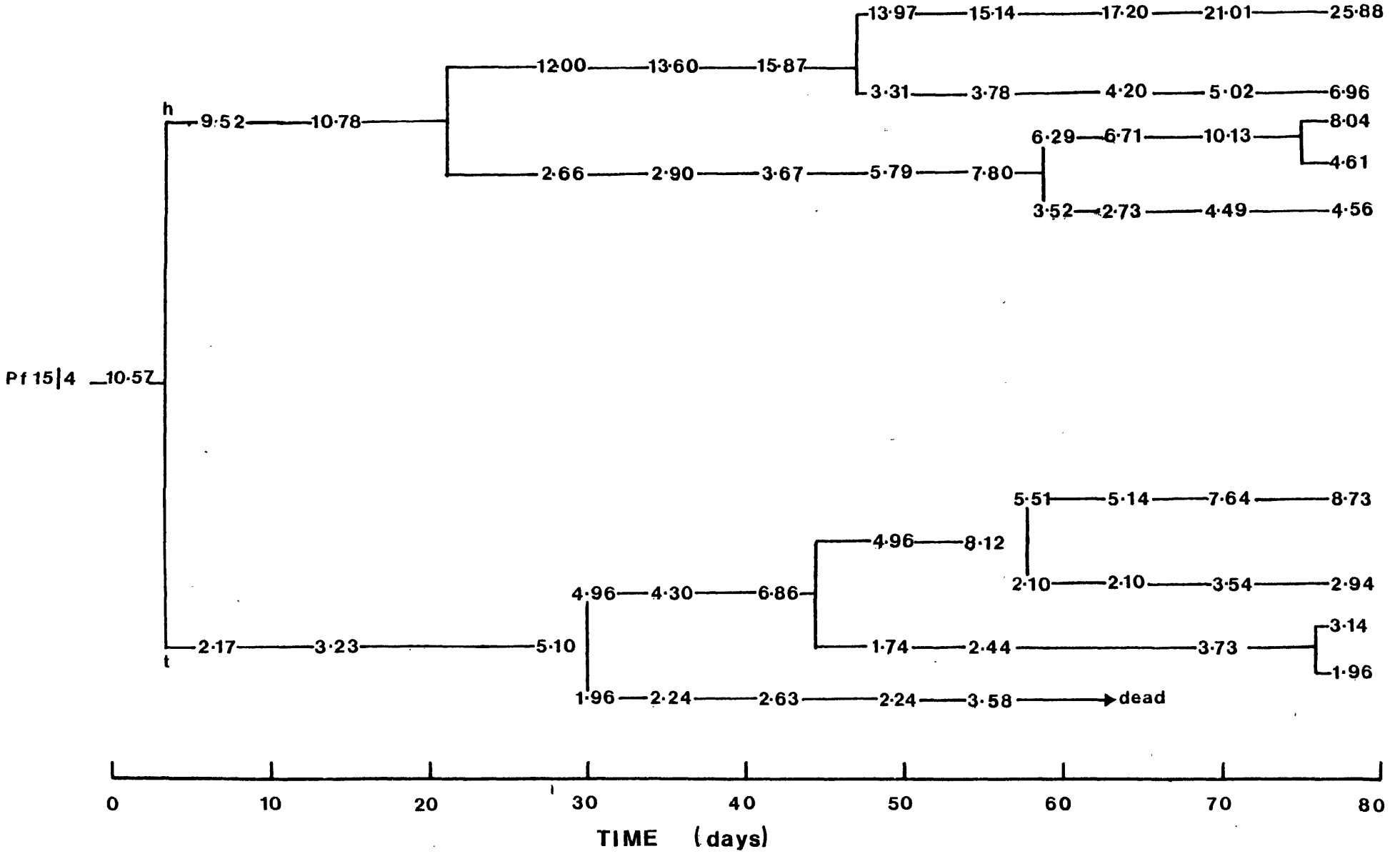

Pf 15|4
10·57
h
9·52
10·78
12·00
13·60
15·87
13·97
15·14
17·20
21·01
25·88
3·31
3·78
4·20
5·02
6·96
2·66
2·90
3·67
5·79
7·80
6·29
6·71
10·13
8·04
4·61
3·52
2·73
4·49
4·56
t
2·17
3·23
5·10
4·96
4·30
6·86
4·96
8·12
5·51
5·14
7·64
8·73
2·10
2·10
3·54
2·94
1·74
2·44
3·73
3·14
1·96
1·96
2·24
2·63
2·24
3·58
dead
0
10
20
30
40
50
60
70
80
TIME (days)

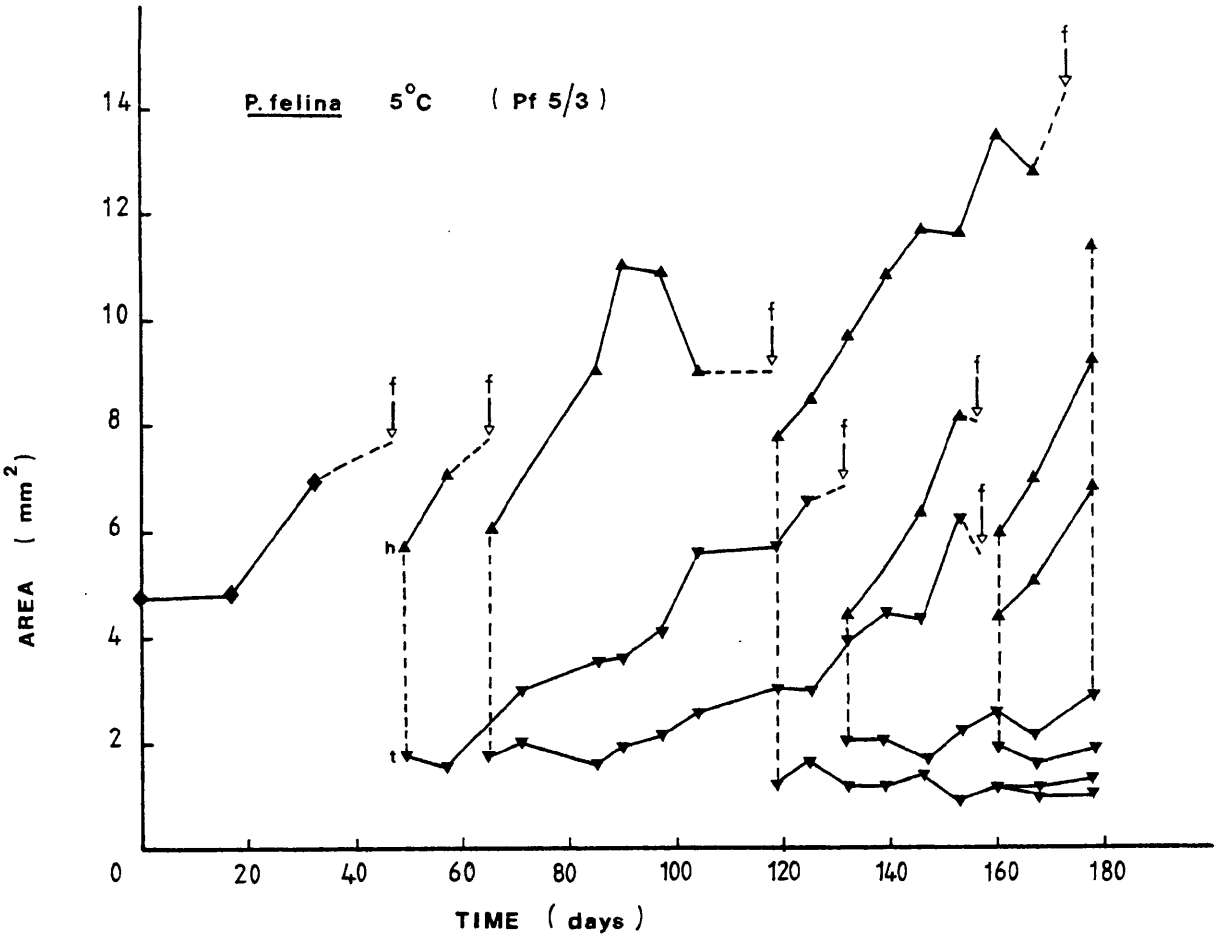
Figure 11

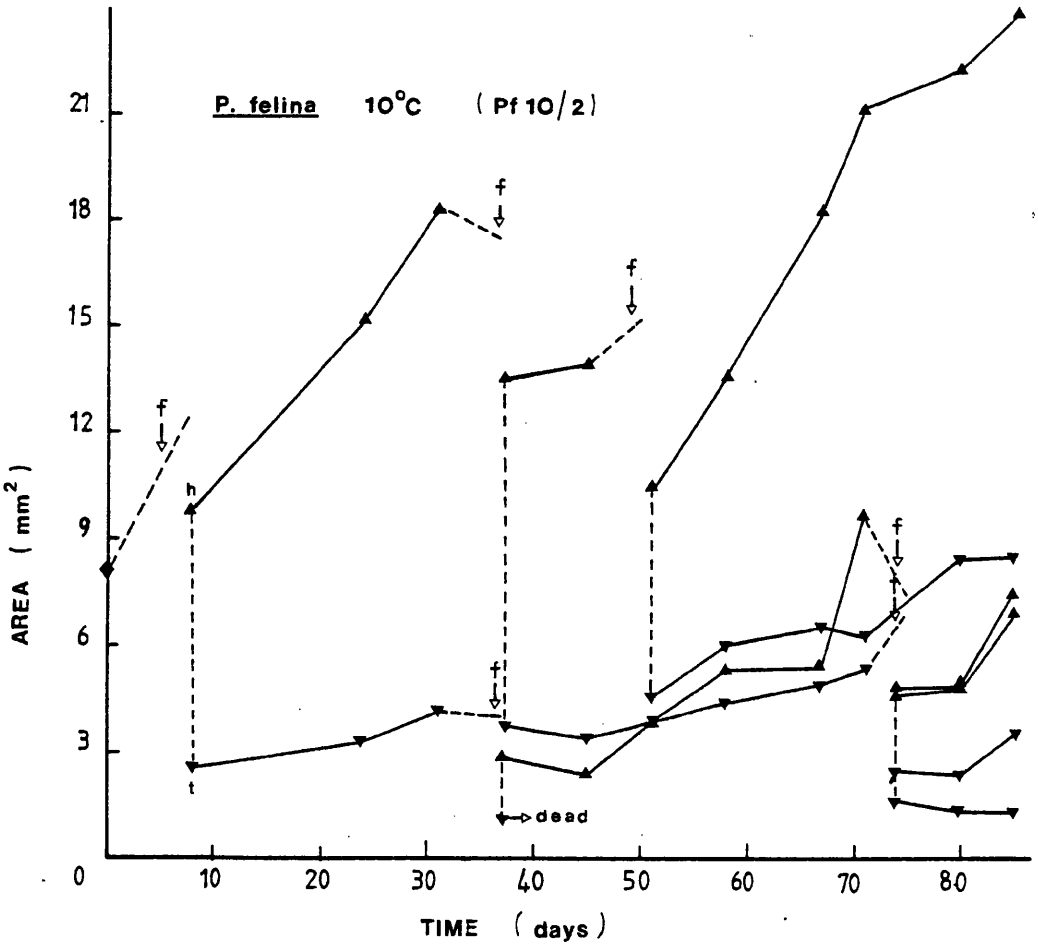
Examples illustrating the growth patterns of P. felina at 5^{o}, 10^{o}, 15^{o} and 20^{o}C.

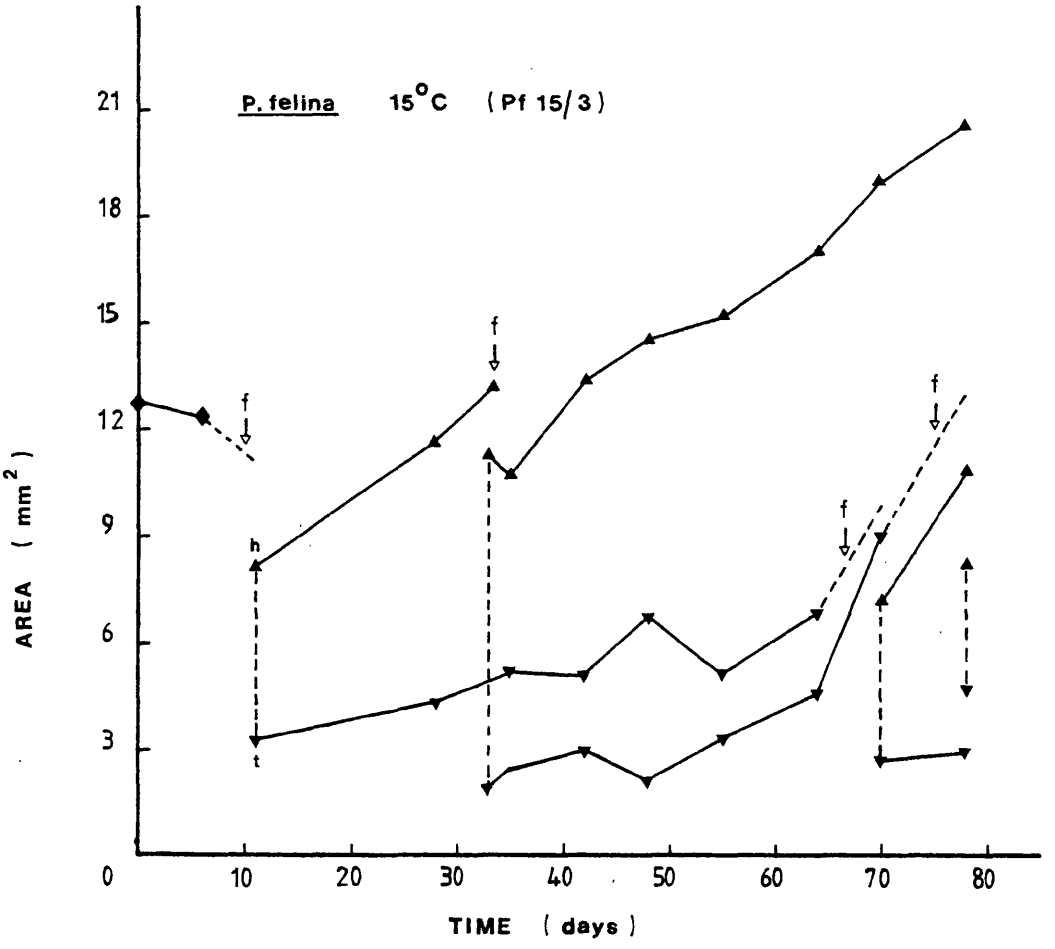
h = head

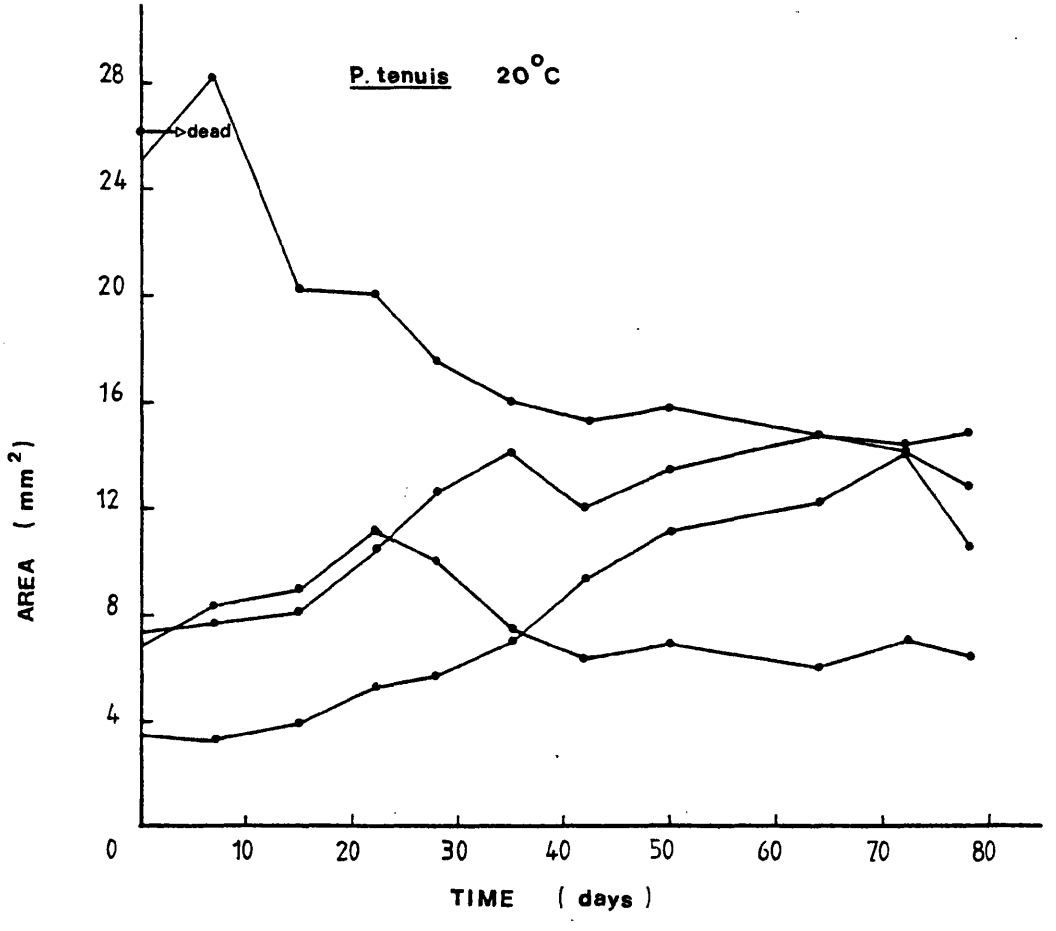
t = tail

f = fission


P. felina 5°C (Pf 5/3)
AREA (mm²)
TIME (days)
0
2
4
6
8
10
12
14
20
40
60
80
100
120
140
160
180
f
h
t


P. felina 10°C (Pf 10/2)
AREA (mm²)
TIME (days)
0
3
6
9
12
15
18
21
10
20
30
40
50
60
70
80
f
h
t
dead


P. felina 15°C (Pf 15/3)
AREA (mm²)
TIME (days)
f
h
t
0
3
6
9
12
15
18
21
10
20
30
40
50
60
70
80


P. tenuis 20°C
dead
AREA (mm²)
TIME (days)
0
4
8
12
16
20
24
28
10
20
30
40
50
60
70
80

The overall growth rates of heads and tails appear to be different. However, differences between the growth patterns of the fission products are partly responsible. In general, tails produced from fission are smaller than heads, and take considerably longer to regrow their missing halves (Calow, Beveridge and Sibly 1979). This period of head formation is to a large extent responsible for the long lag period observed in tails after fission (see Chapter 7). In Pf 5/4, an unusual fission resulted in a tail, mid-piece and head being formed. The tail and head were the same size, and the subsequent growth patterns appear to be identical (Fig. 12).

The rates of exponential growth (K) were used to describe the growth rates of P. felina (including heads and tails), and were calculated from the "dendrogram" data in exactly the same manner as described above for P. tenuis. Due to long lag phases, the fates of few tails could be followed through from fission to fission, and therefore more data was collected about K in heads (K_h) than in tails (K_t). The K_h data is summarised in Table 19. Also included are data from 20°C (non-dividing animals).

At any one temperature, a range of K values is found. The influence of initial head size after fission on K_h was examined by plotting head size against K_h (Fig. 13). A curvilinear relationship is found, showing that the value of K_h is inversely related to initial head size. A constant value for K_h can be observed where the initial head size is greater than 10 mm^2.

The influence of initial head size on K_h explains much of the variation observed at a particular temperature, without involving possible interclonal (i.e. genetic) differences.

The growth rates of P. felina at different temperatures were

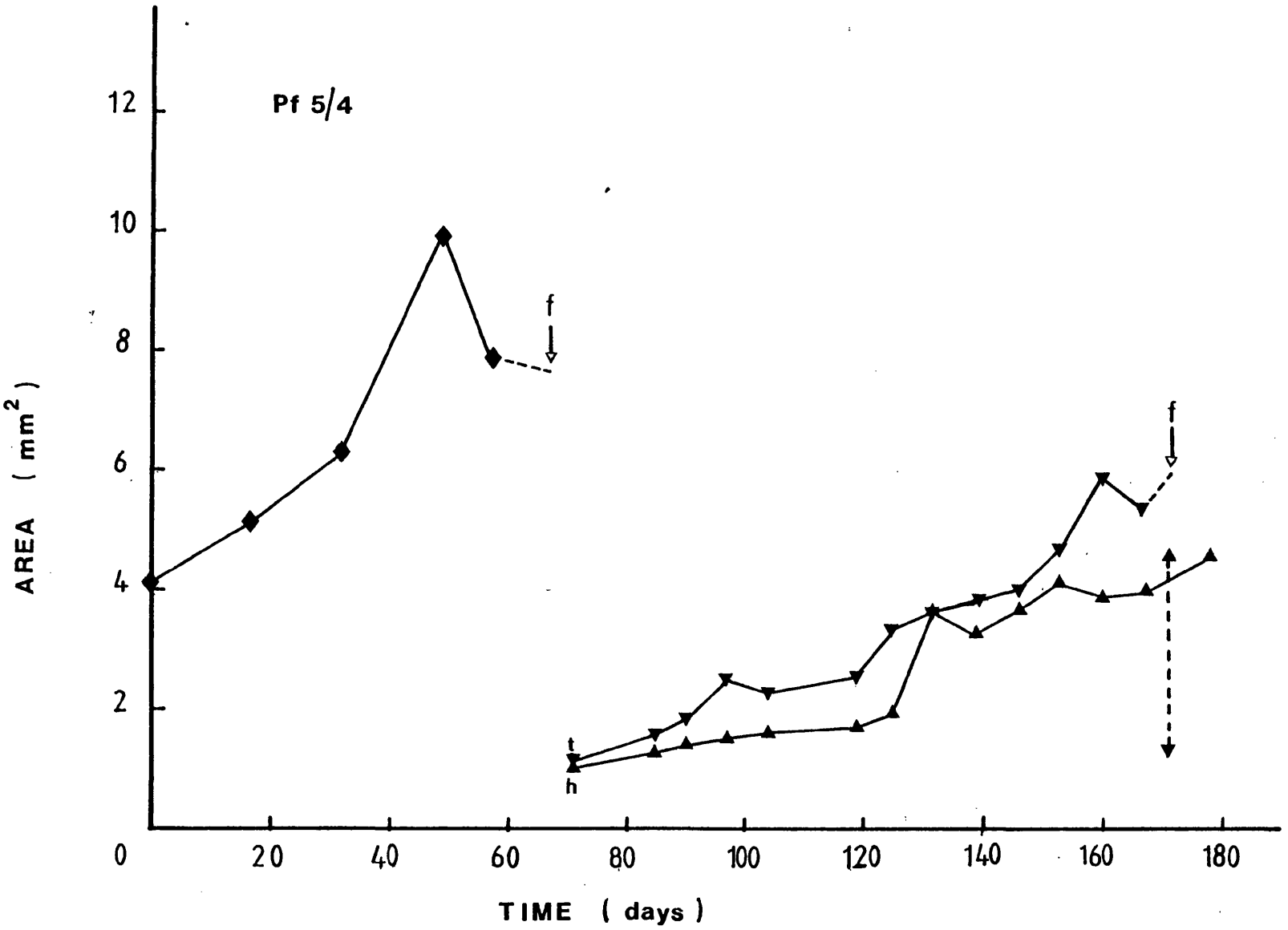
Figure 12

The growth of Pf 5/4 head and tail fragments, after an unusual fission (see text).

h = head

t = tail

f = fission


Pf 5/4
AREA (mm^2)
TIME (days)
0
2
4
6
8
10
12
20
40
60
80
100
120
140
160
180
f
f
t
h

Species	Temp.	Animal	Days between which growth measured	Slope(b)	K_h	Initial Size
P. felina	5°C	Pf 5/2h	57-71	0.0226	0.052	3.44 mm^2
		2hth	153-178	0.0187	0.043	1.86 mm^2
		3h	49-57	0.0144	0.026	5.73 mm^2
		3hh	65-90	0.0100	0.023	6.01 mm^2
		3hhh	119-160	0.0056	0.013	7.81 mm^2
		3th	132-153	0.0126	0.029	4.42 mm^2
		3thh	160-178	0.0104	0.024	6.01 mm^2
		3hth	160-178	0.0109	0.025	4.43 mm^2
		2	17-32	0.0034	0.008	4.48 mm^2
		3	17-32	0.0106	0.024	4.75 mm^2
		4	17-49	0.0091	0.021	4.13 mm^2
	10°C	Pf 10/1h	10-85	0.0081	0.019	9.77 mm^2
		1th	51-58	0.0258	0.059	4.21 mm^2
		1thh	67-85	0.0177	0.041	4.88 mm^2
		2h	10-31	0.0127	0.029	9.97 mm^2
		2hhh	51-93	0.0115	0.026	10.51 mm^2
		2hth	80-93	0.0229	0.053	4.93 mm^2
		2th	45-71	0.0189	0.044	2.91 mm^2
		2thh	74-93	0.0175	0.040	4.88 mm^2
		3	0-10	0.0098	0.023	9.97 mm^2
		3h	24-80	0.0079	0.018	13.83 mm^2
		4h	8-45	0.0083	0.019	10.12 mm^2
		4hh	51-93	0.0083	0.019	17.77 mm^2
		4thh	67-93	0.0081	0.019	4.34 mm^2
		5	0-10	0.0249	0.057	7.33 mm^2
		5h	24-31	0.0096	0.022	14.23 mm^2
		5hh	45-93	0.0070	0.016	13.45 mm^2
		5hth	71-85	0.0175	0.040	5.07 mm^2
		4th	51-58	0.0193	0.044	2.28 mm^2
	15°C	Pf 15/1	6-78	0.0051	0.012	19.84 mm^2
		2	0-13	0.0086	0.020	4.28 mm^2
		2th	55-64	0.0105	0.024	4.22 mm^2

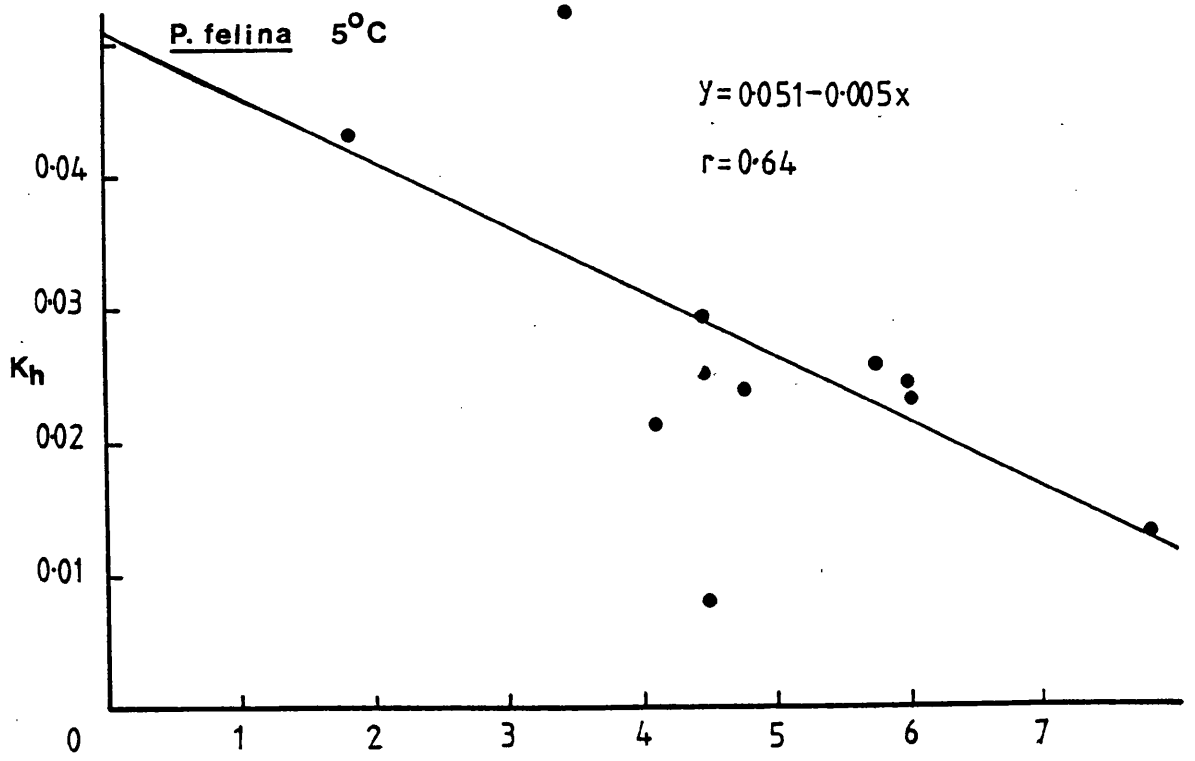
Species	Temp.	Animal	Days between which growth measured	Slope(b)	K_h	Initial Size
P. felina	15°C	Pf 15/2thh	70-78	0.0234	0.054	4.78 mm^2
		2hh	28-35	0.0166	0.038	5.32 mm^2
		3h	13-28	0.0104	0.024	8.26 mm^2
		3hh	35-78	0.0057	0.013	10.75 mm^2
		3th	55-64	0.0135	0.031	5.28 mm^2
		3thh	70-78	0.0239	0.057	7.17 mm^2
		4h	6-13	0.0077	0.018	9.52 mm^2
		4hh	28-42	0.0087	0.020	12.00 mm^2
		4hhh	64-78	0.0170	0.043	13.97 mm^2
		4hth	64-70	0.0298	0.069	6.29 mm^2
		4th	35-42	0.0290	0.067	4.19 mm^2
		4thh	48-55	0.0306	0.070	4.96 mm^2
		4thhh	64-78	0.0159	0.037	5.51 mm^2
		5	0-78	0.0049	0.011	16.87 mm^2
	20°C	Pf 20/1	15-35	0.0120	0.028	7.36 mm^2
		4	28-50	0.0140	0.032	3.53 mm^2
		5	15-22	0.0135	0.031	6.77 mm^2

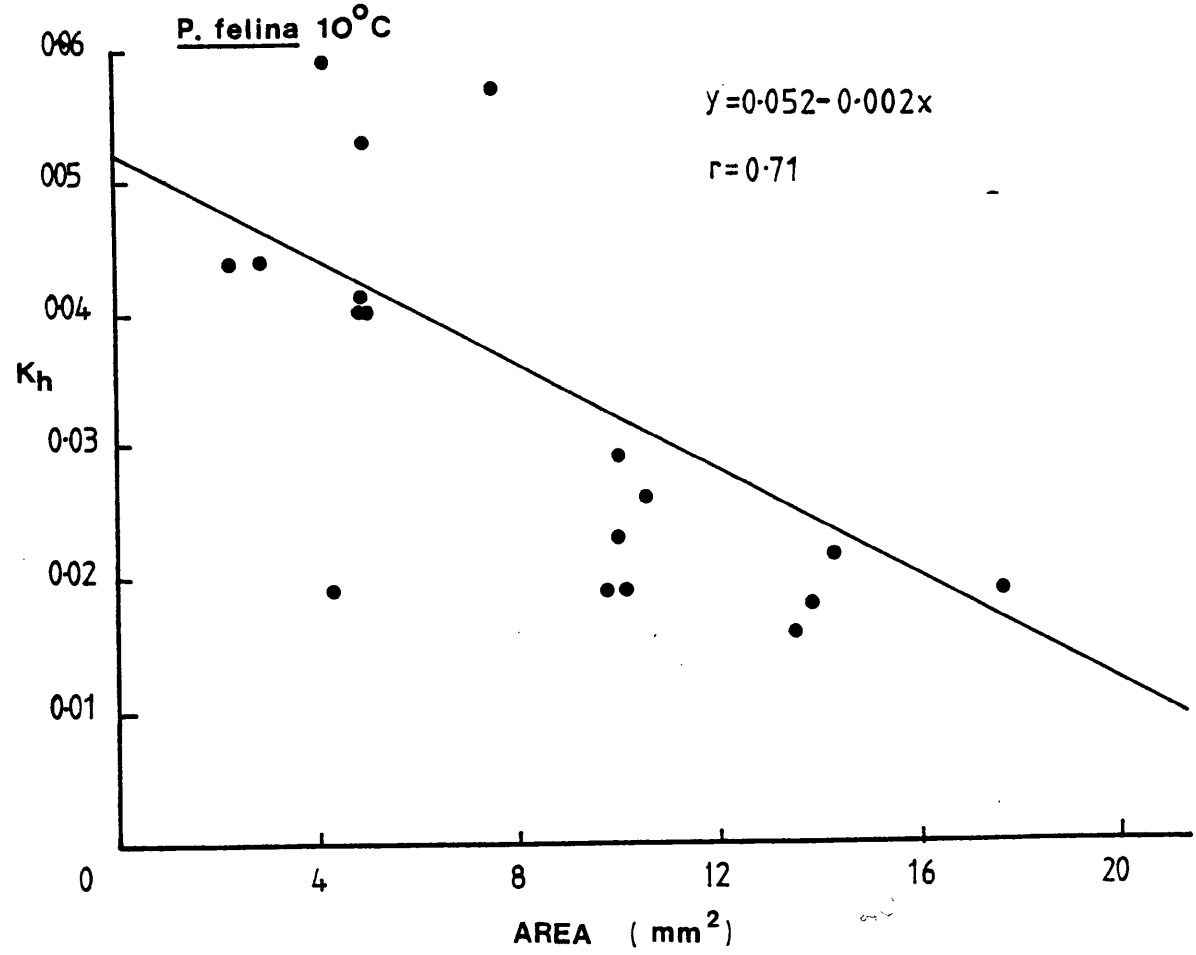
Table 19 : The K_h values for P. felina heads* at different temperatures. The period during which K_h was measured and the size at fission are also given.

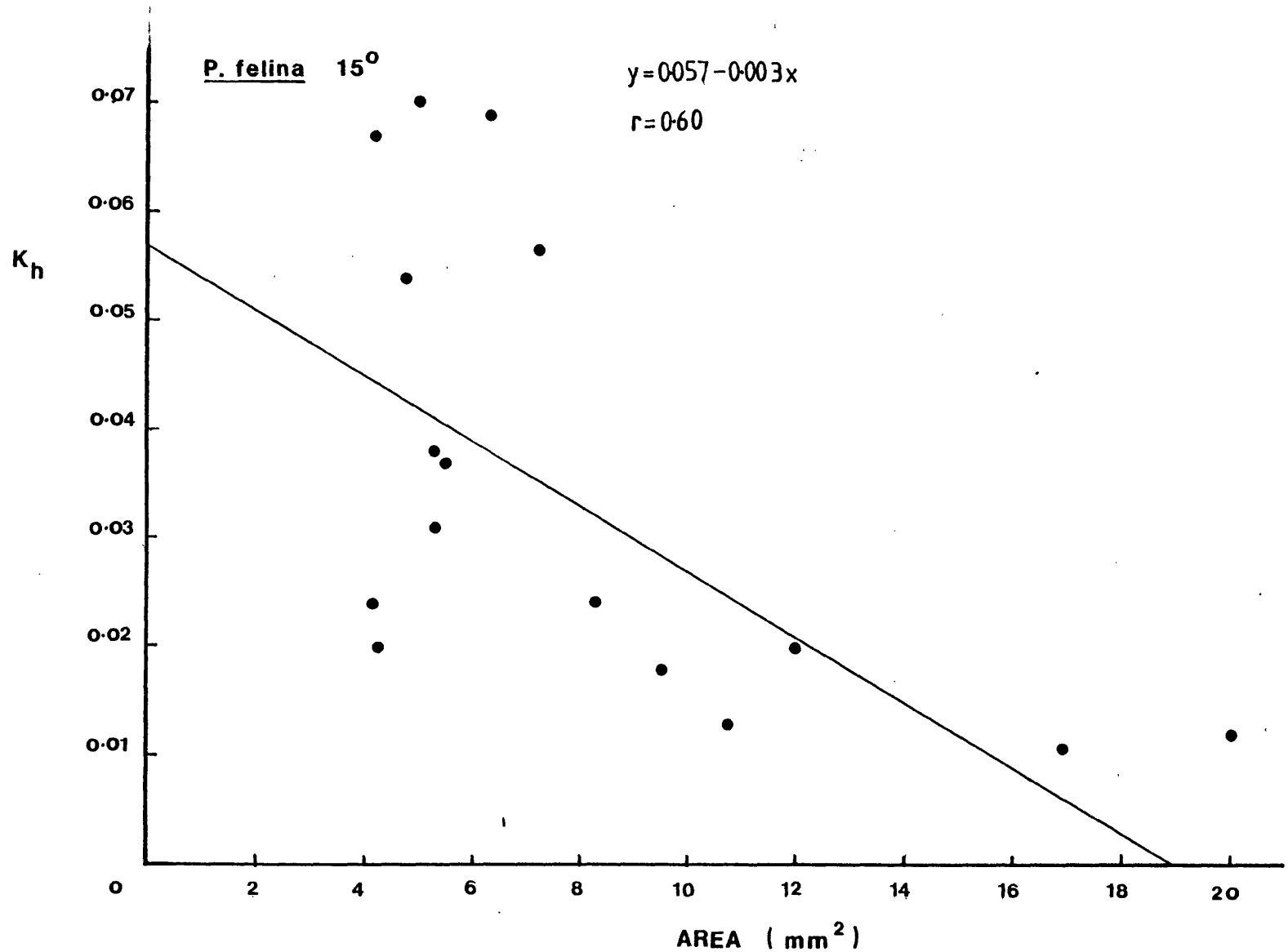
* Also included are K values for undivided animals (see text).

Figure 13

The influence of initial head size after fission on K_h in P. felina at 5°, 10° and 15°C.


P. felina 5°C
y = 0·051 − 0·005x
r = 0·64
0·04
0·03
Kh
0·02
0·01
0
1
2
3
4
5
6
7


P. felina 10°C
y = 0·052 − 0·002x
r = 0·71
0·06
0·05
0·04
Kh
0·03
0·02
0·01
0
4
8
12
16
20
AREA (mm²)


P. felina 15°
y=0·057-0·003x
r=0·60
K_h
0·07
0·06
0·05
0·04
0·03
0·02
0·01
0
2
4
6
8
10
12
14
16
18
20
AREA (mm²)

compared using K_h values obtained over a small range of initial head sizes (area = 4 to 6 mm^2). This size range corresponds to the highest K_h values observed at all temperatures. The data is given in Table 20. A one-way analysis of variance demonstrates that there are significant differences in K_h at different temperatures ($F = 3.50$; d.f. = $^3/_{19}$; $P < 0.10$).

Mean K_h values ($\overline{K}_h$) were then calculated from the data in Table 20 and these are illustrated in Fig. 9. $\overline{K}_h$ increases from 0.0222 at 5°C to 0.0426 at 15°C. $\overline{K}_h$ then falls to 0.0303 at 20°C. The Q_{10} values are given in Table 18b. The overall Q_{10} between 5° and 20°C was 0.14. The Q_{10} was highest between 5° and 10°C (3.58) and lowest between 15° and 20°C (0.51).

(c) D. tigrina

Between 6 and 8 triclads were grown at each temperature, and plan area (mm^2) plotted against time (days). Two distinct growth patterns were observed. At 5°C and 10°C, no fission occurred. At 15°C and 20°C, growth was interrupted by fissioning.

Fig. 14 shows the change in size with time of D. tigrina at 5°C. At this temperature, a net decrease in size occurred over the period of observation (165 days). Only occasionally were any increases in plan area observed between one measurement and another.

At 10°C (Fig. 14), 6 of the 8 animals showed a net increase in size during the period of observation (110 days). However the plots appear to be linear, with superimposed oscillations, and no period of exponential growth exists.

At 15°C, infrequent fissioning occurs, but at 20°C, the rate of fissioning dramatically increases (see Chapter 7). Distinct lag

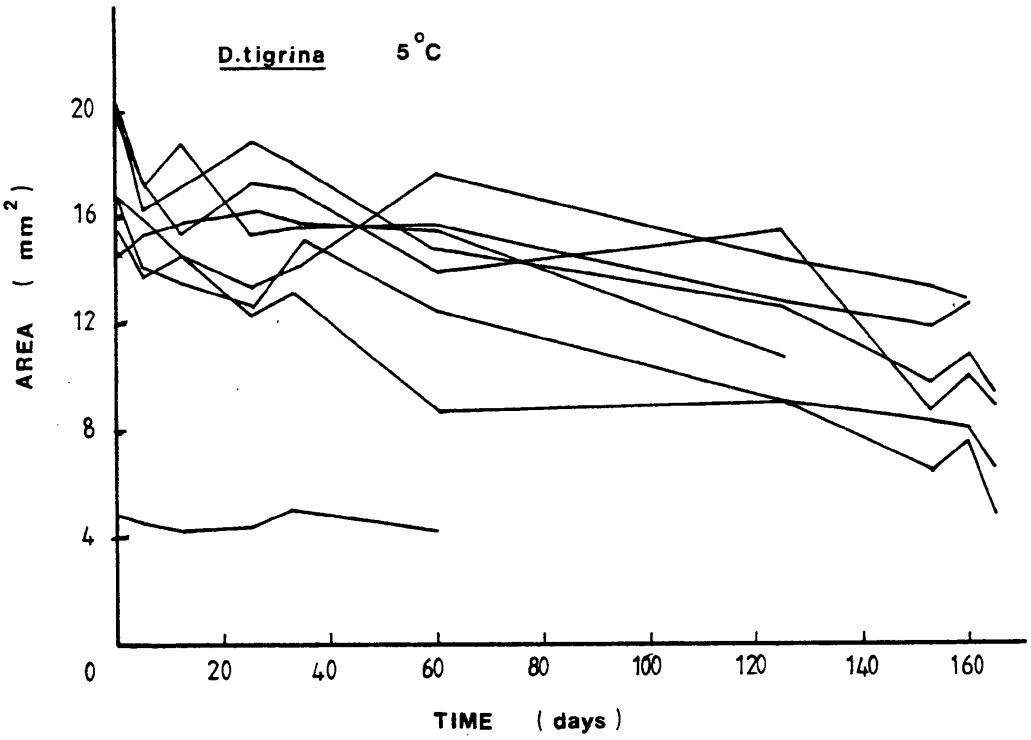
Temperature	Animal	Days between which k_h measured	Size range between which k_h measured	Min. Size	Slope(b)	k_h
5°C	Pf 5/3h	49-57	5.73 - 7.07 mm^2	5.73 mm^2	0.0114	0.026
	3th	132-153	4.42 - 8.19 mm^2	4.42 mm^2	0.0126	0.029
	3hth	160-178	4.43 - 6.92 mm^2	4.43 mm^2	0.0109	0.025 M = 0.022
	2	17-32	4.48 - 5.77 mm^2	4.48 mm^2	0.0034	0.008
	3	17-32	4.75 - 6.92 mm^2	4.75 mm^2	0.0106	0.024
	4	17-49	4.13 - 8.98 mm^2	4.13 mm^2	0.0091	0.021
10°C	Pf 10/1th	51-58	4.21 - 6.38 mm^2	4.21 mm^2	0.0258	0.059
	1thh	67-85	4.88 - 10.54 mm^2	4.88 mm^2	0.0177	0.041
	2hth	80-93	4.93 - 10.17 mm^2	4.93 mm^2	0.0299	0.053 M = 0.042
	2thh	74-93	4.88 - 10.57 mm^2	4.88 mm^2	0.0175	0.040
	4thh	67-93	4.34 - 7.86 mm^2	4.34 mm^2	0.0081	0.019
	4hth	71-85	5.07 - 9.02 mm^2	5.07 mm^2	0.0175	0.040
15°C	Pf 15/2	0-13	4.28 - 5.54 mm^2	4.28 mm^2	0.0086	0.020
	2th	55-64	4.22 - 5.25 mm^2	4.22 mm^2	0.0105	0.024
	2thh	70-78	4.78 - 7.36 mm^2	4.78 mm^2	0.0234	0.054
	2hh	28-35	5.32 - 6.95 mm^2	5.32 mm^2	0.0166	0.038 M = 0.043

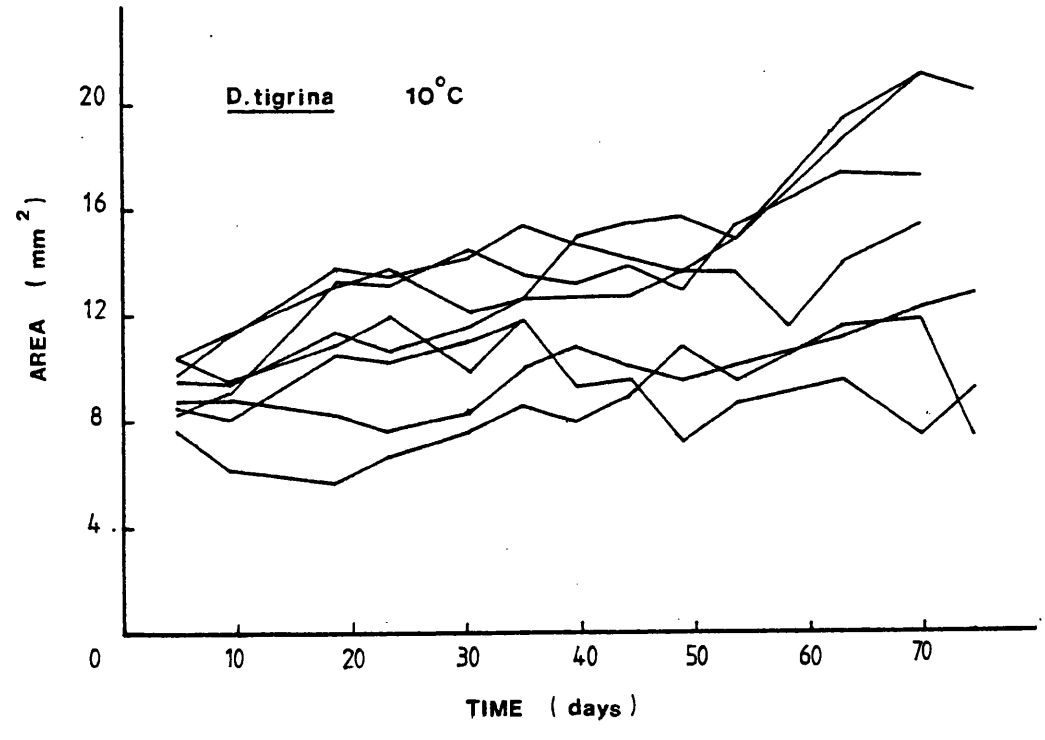
Temperature	Animal	Days between which k_h measured	Size range between which k_h measured	Min. Size	Slope(b)	k_h
15°C	Pf 15/3th	55-64	5.26 - 6.98 mm^2	5.28 mm^2	0.0135	0.031
	4th	35-42	4.30 - 6.86 mm^2	4.19 mm^2	0.0290	0.067
	4thh	48-55	4.96 - 8.12 mm^2	4.96 mm^2	0.0306	0.070
	4thhh	64-78	5.51 - 8.73 mm^2	5.51 mm^2	0.0159	0.037
20°C	Pf 20/1	15-35	8.10 - 14.10 mm^2	7.36 mm^2	0.0122	0.028
	4	28-50	5.16 - 11.15 mm^2	3.53 mm^2	0.0139	0.032 M = 0.030
	5	15-22	8.99 - 11.18 mm^2	6.77 mm^2	0.0135	0.031

Table 20 : K values obtained over a small initial size range (4 to 6 mm^2 area) at 5°, 10° and 15°C for *P. felina*

Figure 14

Growth of D. tigrina at 5° and 10°C


D.tigrina
5°C
AREA (mm²)
20
16
12
8
4
0
20
40
60
80
100
120
140
160
TIME (days)


D.tigrina
10°C
AREA (mm²)
20
16
12
8
4
0
10
20
30
40
50
60
70
TIME (days)

and log phases in growth per unit time can be observed, and there is some evidence that tails have a different growth pattern from heads (Fig. 15). The long initial lag phase, which is characteristically found in tails, is absent in most cases in heads, except where fission has resulted in a small head fragment (< 3 mm^2) being produced (e.g. Fig. 15, Dt 20/1).

The rate of exponential growth (K) was used as a measure of the overall growth rates of heads and tails. Unfortunately, insufficient data is available from these experiments to test whether heads and tails have different growth rates. However, tails were usually smaller than heads, and the evidence suggests that the initial size of the fission fragment is important in determining K. The K_h values for D. tigrina at 15° and 20°C are given in Table 21, and a plot of K_h against initial head size (Fig. 16) shows that there is an inverse relationship between the 2 factors.

Since the smallest heads have the largest K_h values, it was necessary to compare the K_h values of the smallest heads at 15°C, with those at 20°C. This meant comparing the K_h values of 2-4 mm^2 heads at 20°C with 8-10 mm^2 heads at 15°C (Table 22). A one-way analysis of variance between these data showed that there was a significant difference between the rates of exponential growth ($F = 3.9387$; d.f. 1/10; $p < 0.10$).

The means of the K_h values given in Table 21 were calculated and plotted (Fig. 9). The Q_{10} for K_h between the 2 temperatures is 5.54 (Table 18b).

6.4.2 Degrowth

The rates of degrowth or shrinkage were measured by plotting

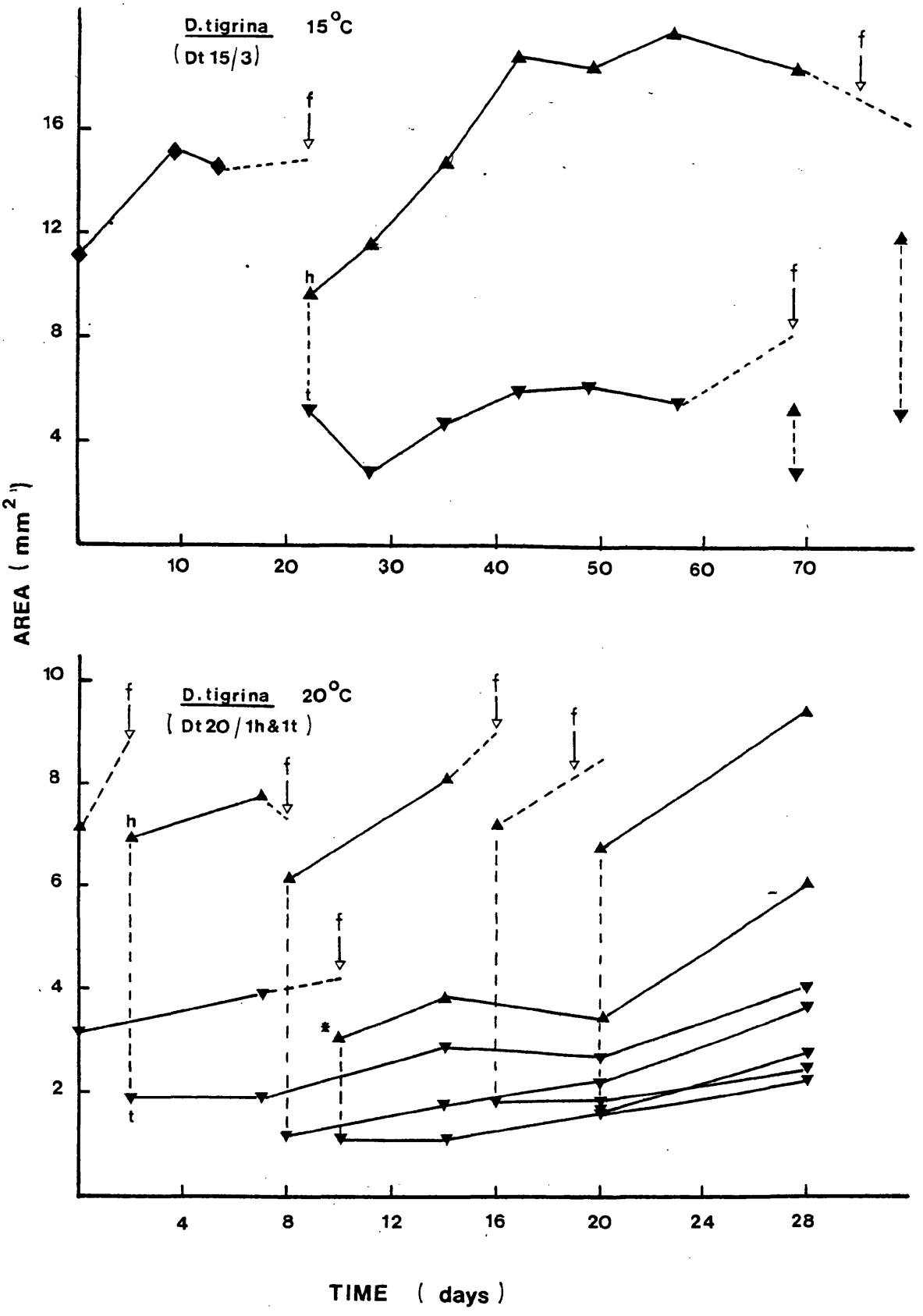
Figure 15

Examples illustrating growth in D. tigrina at 15° and 20°C. Note that the pattern of * in Dt 20/1 is different from the rest (see text).

n = head

t = tail

f = fission


D.tigrina 15°C
(Dt 15/3)
f
h
t
16
12
8
4
10
20
30
40
50
60
70
D.tigrina 20°C
(Dt 20/1h&1t)
10
8
6
4
2
4
8
12
16
20
24
28
AREA (mm²)
TIME (days)

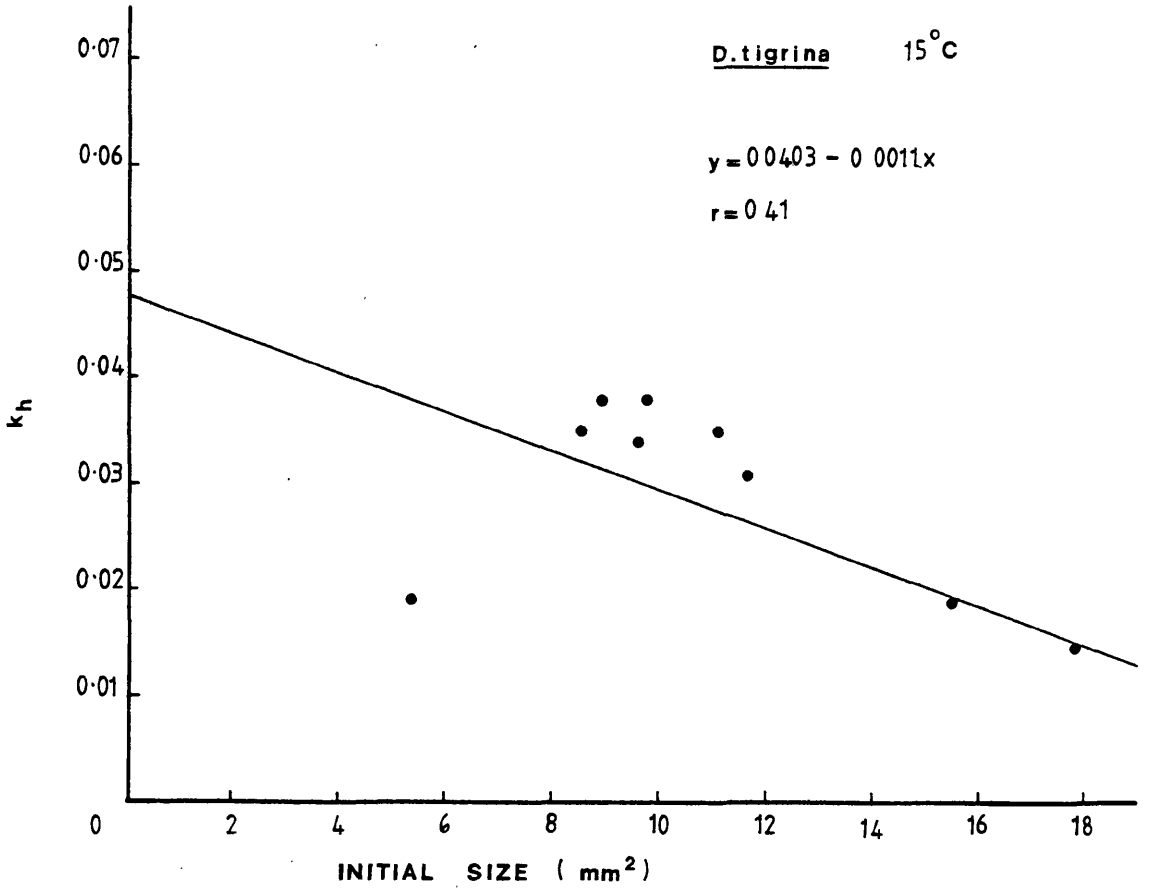
Species	Temp.	Animal	Days between which growth measured	Slope (b)	K_h	Initial Size
D. tigrina	15°C	Dt 15/2	0-13	0.0064	0.0147	17.81 mm^2
		3	0-9	0.0153	0.0352	11.12 mm^2
		4	9-49	0.0069	0.0158	5.33 mm^2
		5	0-9	0.0150	0.0345	8.52 mm^2
		6	9-13	0.0167	0.0385	9.68 mm^2
		2h	28-35	0.0081	0.0187	15.54 mm^2
		3h	22-42	0.0147	0.0339	9.56 mm^2
		5h	35-49	0.0133	0.0306	11.58 mm^2
		6h	28-42	0.0165	0.0380	8.92 mm^2
	20°C	Dt 20/1hh	2-7	0.0095	0.0219	6.95 mm^2
		1hhh	8-14	0.0195	0.0449	6.10 mm^2
		1hhhhh	20-28	0.0177	0.0408	6.76 mm^2
		1th	20-28	0.0315	0.0725	3.05 mm^2
		2hh	4-7	0.0162	0.0373	5.80 mm^2
		2th	2-7	0.0087	0.0200	2.86 mm^2
		2hhhh	12-14	0.0297	0.0684	6.63 mm^2
		3hhhh	10-14	0.0194	0.0447	8.50 mm^2
		3hhhhh	16-20	0.0279	0.0643	8.30 mm^2
		3hth	18-20	0.0411	0.0947	3.60 mm^2
		3hhth	9-14	0.0736	0.1695	1.86 mm^2
		3hhthh	16-18	0.0513	0.1181	3.79 mm^2
		3hhthhh	27-28	0.0707	0.1628	5.54 mm^2
		5hh	5-7	0.0194	0.0447	9.30 mm^2
		5hhhh	14-20	0.0218	0.0502	7.55 mm^2
		5hhhhh	22-28	0.0186	0.0428	8.16 mm^2
		5hth	11-14	0.0248	0.0571	3.80 mm^2
		5hthh	17-20	0.0250	0.0576	4.50 mm^2
		5hhth	24-28	0.0279	0.0643	3.68 mm^2
		6h	0-7	0.0074	0.0170	7.35 mm^2
		6hh	14-20	0.0110	0.0253	6.87 mm^2
		6hhh	22-28	0.0235	0.0541	6.65 mm^2

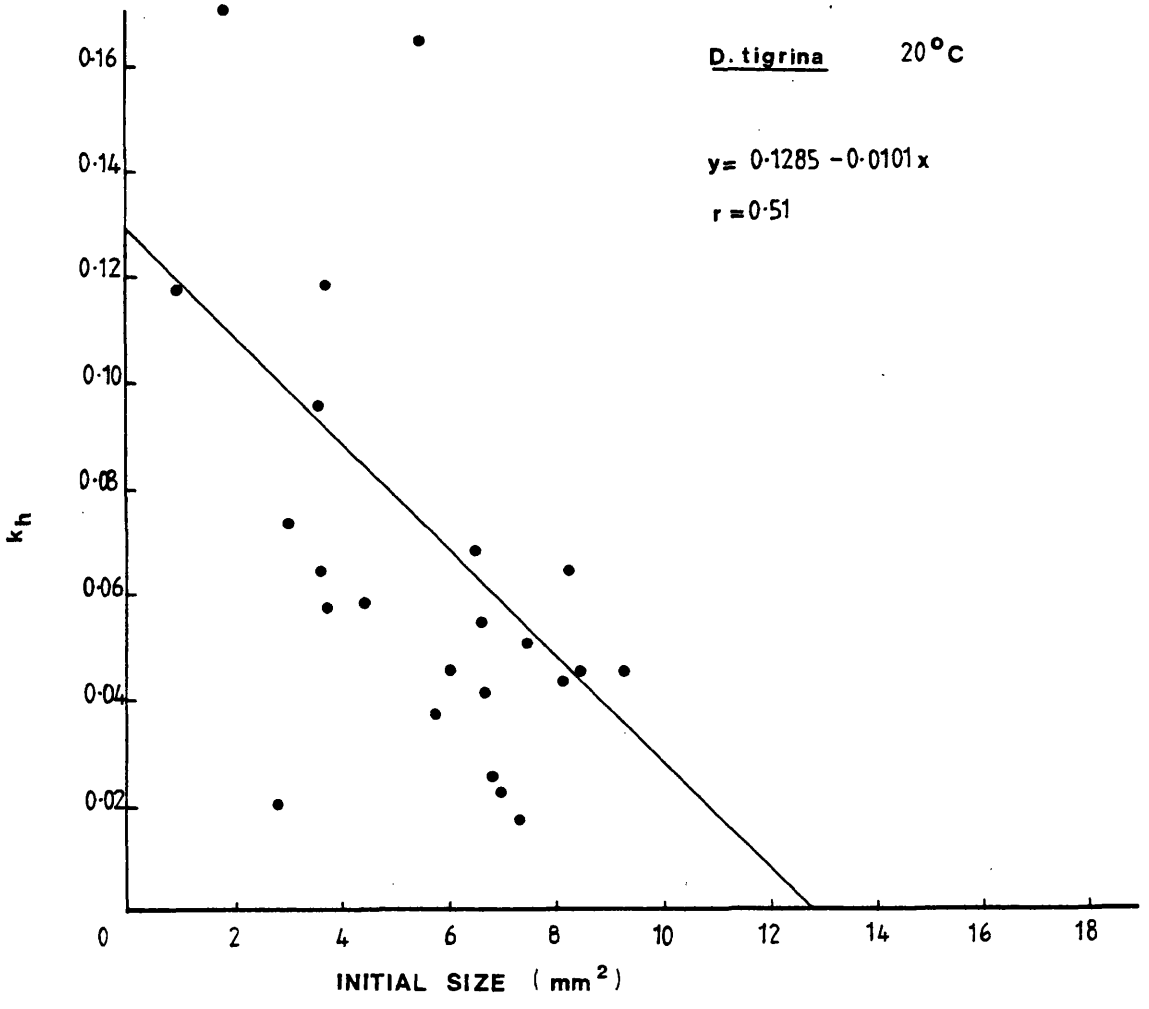
Table 21 : The K_h values for D. tigrina heads* at different temperatures. The period during which K_h was measured and the size at fission are also given.

* Also included ar K values for undivided animals.

Figure 16

The relationship between initial head size after fission and K_h in D. tigrina at 15° and 20°C.


D. tigrina 15°C
y = 0 0403 - 0 0011x
r = 0 41
0·07
0·06
0·05
0·04
0·03
0·02
0·01
0
2
4
6
8
10
12
14
16
18
k_h
INITIAL SIZE (mm^2)


D. tigrina 20°C
y = 0·1285 - 0·0101 x
r = 0·51
0·16
0·14
0·12
0·10
0·08
0·06
0·04
0·02
0
2
4
6
8
10
12
14
16
18
k_h
INITIAL SIZE (mm^2)

Animal	Temperature	Size	K_h
Dt 15/5	15°C	8.52 mm^2	0.0345
6		9.68 mm^2	0.0385
3h		9.56 mm^2	0.0339
6h		8.92 mm^2	0.0380
Dt 20/1th	20°C	3.05 mm^2	0.0725
2th		2.86 mm^2	0.0200
3hth		3.60 mm^2	0.0947
3hhth		1.86 mm^2	0.1695
3hhthh		3.79 mm^2	0.1181
5hth		3.80 mm^2	0.0571
5hhth		3.68 mm^2	0.0643

Table 22 : D. tigrina K values on which a comparison of of growth rates is based (see text for full explanation).

$\log_{10}$ size against starvation time, and straight lines fitted by regression analysis (Bailey 1969). The regression equations are given in Table 23. In most cases, the correlation coefficients are high, and this augments the argument that the rate of degrowth reduces exponentially with starvation time.

Since degrowth is exponential, the coefficient of exponential degrowth (k) may be used to compare species at different temperatures. k is calculated from the exponential equation:

$$A_t = A_o e^{-kt}$$

where A_t = Area at time t; A_o = Area at time 0

e = base of natural logarithms

k can be calculated from b, the slope, given in the regression equations (Table 23) by:

$$k = \log_e 10 \; b$$

$$= 2.303 \; b$$

The influence of size on the rate of degrowth was examined by plotting k against initial size. The graphs are shown in Fig. 17.

There appears to be no correlation between I and k in P. tenuis or D. tigrina. In P. felina, however, correlations may exist at certain temperatures. Initial size and k are inversely related at 10°C, and positively related at 20°C, although these correlations are weak (P. felina at 10°C, r = 0.63; P. felina at 20°C, r = 0.87). Neither of the above correlations can be attributed to differences in k values between heads and tails.

A one-way analysis of variance demonstrates that there are significant differences in degrowth rates between temperatures within a species (Table 24; $p < 0.01$). Mean k values ($\bar{k}$) for the 3 species were derived from the data in Table 23, and Q_{10} values were calculated

Species	Temperature	Animal	Regression Equation	Correlation Coefficient	k	Initial Size
P. tenuis	5°C	TR 5/1	y = 1.2870 - 0.0016x	-0.95	-0.0037	20.26 mm^2
		2	y = 1.2489 - 0.0015x	-0.98	-0.0035	18.01 mm^2
		3	y = 1.3081 - 0.0017x	-0.98	-0.0039	20.84 mm^2
		4	y = 1.3365 - 0.0017x	-0.98	-0.0039	20.55 mm^2
		5	y = 1.3263 - 0.0023x	-0.93	-0.0053	21.06 mm^2
	10°C	TR 10/1	y = 1.1875 - 0.0044x	-0.98	-0.0101	19.70 mm^2
		2	y = 1.3353 - 0.0037x	-0.99	-0.0085	20.78 mm^2
		3	y = 1.3571 - 0.0035x	-0.99	-0.0081	21.23 mm^2
		4	y = 1.3199 - 0.0036x	-0.99	-0.0083	21.30 mm^2
		5	y = 1.3546 - 0.0034x	-0.98	-0.0078	20.78 mm^2
		6	y = 1.2505 - 0.0035x	-0.99	-0.0081	17.64 mm^2
		7	y = 1.3549 - 0.0033x	-0.99	-0.0076	19.44 mm^2
		8	y = 1.3971 - 0.0031x	-0.99	-0.0071	23.73 mm^2
		9	y = 1.3276 - 0.0031x	-0.99	-0.0071	18.18 mm^2
		10	y = 1.2978 - 0.0037x	-0.99	-0.0085	18.63 mm^2
	15°C	TR 15/1	y = 1.1875 - 0.0039x	-0.98	-0.0090	23.07 mm^2
		2	y = 1.2356 - 0.0049x	-0.97	-0.0113	17.72 mm^2
		4	y = 1.2056 - 0.0041x	-0.98	-0.0094	19.10 mm^2
		6	y = 1.1841 - 0.0037x	-0.98	-0.0085	19.83 mm^2
		7	y = 0.9711 - 0.0044x	-0.94	-0.0101	19.01 mm^2
		8	y = 1.2255 - 0.0041x	-0.99	-0.0094	19.20 mm^2
		9	y = 1.2169 - 0.0042x	-0.98	-0.0097	20.77 mm^2
		10	y = 1.2263 - 0.0059x	-0.98	-0.0136	19.94 mm^2
	20°C	TR 20/1	y = 1.1655 - 0.0035x	-0.97	-0.0081	16.24 mm^2
		2	y = 0.9035 - 0.0042x	-0.86	-0.0097	12.61 mm^2
		3	y = 1.0846 - 0.0043x	-0.90	-0.0099	12.99 mm^2
		4	y = 1.2644 - 0.0055x	-0.93	-0.0127	26.65 mm^2

Species	Temperature	Animal	Regression Equation	Correlation Coefficient	k	Initial Size
P. tenuis	20°C	TR 20/5	y = 1.0788 - 0.0050x	-0.95	-0.0115	19.87 mm^2
		6	y = 1.2525 - 0.0034x	-0.97	-0.0078	22.54 mm^2
		7	y = 1.2263 - 0.0028x	-0.86	-0.0064	19.63 mm^2
		8	y = 1.1020 - 0.0047x	-0.95	-0.0108	13.75 mm^2
		9	y = 0.8379 - 0.0031x	-0.81	-0.0071	9.55 mm^2
		10	y = 1.0953 - 0.0029x	-0.96	-0.0067	15.00 mm^2
P. felina	5°C	F 5/1	y = 1.0322 - 0.0030x	-0.89	-0.0069	14.59 mm^2
		3	y = 0.7686 - 0.0019x	-0.82	-0.0044	4.75 mm^2
		4	y = 0.6470 - 0.0032x	-0.97	-0.0074	4.39 mm^2
		5	y = 0.2731 - 0.0028x	-0.90	-0.0064	1.58 mm^2
		6	y = 0.4562 - 0.0032x	-0.92	-0.0074	3.16 mm^2
	10°C	F 10/1	y = 0.6872 - 0.0045x	-0.93	-0.0104	4.87 mm^2
		3	y = 0.0949 - 0.0102x	-0.91	-0.0235	0.97 mm^2
		4	y = 0.0819 - 0.0079x	-0.97	-0.0182	1.17 mm^2
		5h	y = 0.6227 - 0.0042x	-0.94	-0.0097	6.23 mm^2
		6	y = 0.5802 - 0.0053x	-0.97	-0.0122	3.70 mm^2
		8	y = 0.6500 - 0.0044x	-0.94	-0.0101	5.35 mm^2
		5t	y = 0.2854 - 0.0063x	-0.87	-0.0145	0.85 mm^2
		7t	y = 0.4903 - 0.0042x	-0.99	-0.0097	2.14 mm^2
	15°C	F 15/1	y = 0.2826 - 0.0088x	-0.99	-0.0203	1.67 mm^2
		3	y = 0.7286 - 0.0058x	-0.99	-0.0134	5.01 mm^2
		4	y = 0.3356 - 0.0056x	-0.92	-0.0129	1.50 mm^2
		5	y = 0.7610 - 0.0066x	-0.98	-0.0152	5.54 mm^2
	20°C	F 20/1	y = 0.3598 - 0.0043x	-0.80	-0.0099	1.94 mm^2
		2	y = 0.3525 - 0.0070x	-0.95	-0.0161	2.00 mm^2

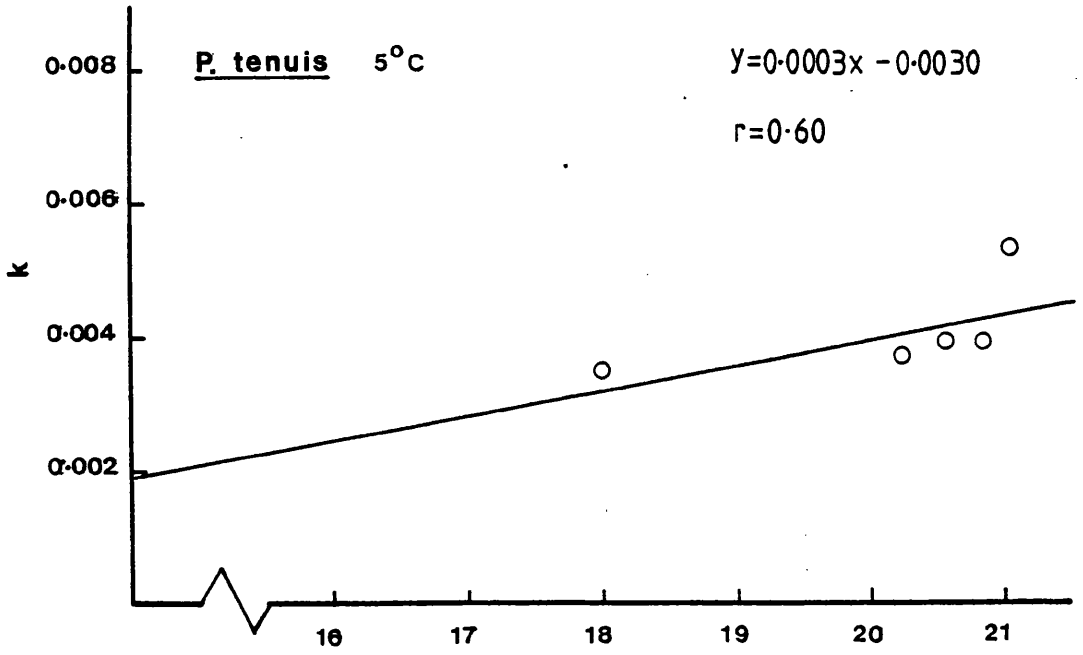
Species	Temperature	Animal	Regression Equation	Correlation Coefficient	k	Initial Size
P. felina	20°C	F 20/3	y = 0.4313 - 0.0051x	-0.96	-0.0117	2.21 mm^2
		4	y = 0.4632 - 0.0046x	-0.84	-0.0106	2.10 mm^2
		5	y = 0.4182 - 0.0051x	-0.95	-0.0117	2.36 mm^2
		6	y = 0.8014 - 0.0114x	-0.96	-0.0263	5.87 mm^2
		10	y = 0.7568 - 0.0082x	-0.97	-0.0189	5.72 mm^2
D. tigrina	5°C	TS 5/1	y = 1.3107 - 0.0022x	-0.98	0.0051	17.38 mm^2
		2	y = 1.2948 - 0.0021x	-0.90	0.0048	20.61 mm^2
		3	y = 1.1658 - 0.0026x	-0.83	0.0060	13.31 mm^2
		4	y = 1.1230 - 0.0016x	-0.89	0.0037	14.02 mm^2
		5	y = 1.2362 - 0.0026x	-0.95	0.0060	15.68 mm^2
		6	y = 1.0747 - 0.0026x	-0.92	0.0060	10.45 mm^2
		7	y = 1.2003 - 0.0022x	-0.92	0.0051	16.26 mm^2
		8	y = 1.0779 - 0.0029x	-0.94	0.0067	10.60 mm^2
		9	y = 1.0182 - 0.0016x	-0.89	0.0037	9.55 mm^2
		10	y = 0.8950 - 0.0022x	-0.93	0.0051	7.68 mm^2
	10°C	TS 10/2	y = 1.3731 - 0.0052x	-0.95	0.0120	19.70 mm^2
		3	y = 1.2802 - 0.0043x	-0.95	0.0099	18.77 mm^2
		4	y = 1.2774 - 0.0055x	-0.98	0.0127	17.45 mm^2
		5	y = 1.2714 - 0.0052x	-0.97	0.0120	17.45 mm^2
		6	y = 1.2365 - 0.0027x	-0.94	0.0062	19.52 mm^2
		7	y = 1.3330 - 0.0058x	-0.95	0.0134	16.70 mm^2
		8	y = 1.3320 - 0.0045x	-0.95	0.0104	17.26 mm^2
		9	y = 1.2136 - 0.0048x	-0.98	0.0111	15.01 mm^2
		10	y = 1.4227 - 0.0044x	-0.98	0.0101	19.89 mm^2

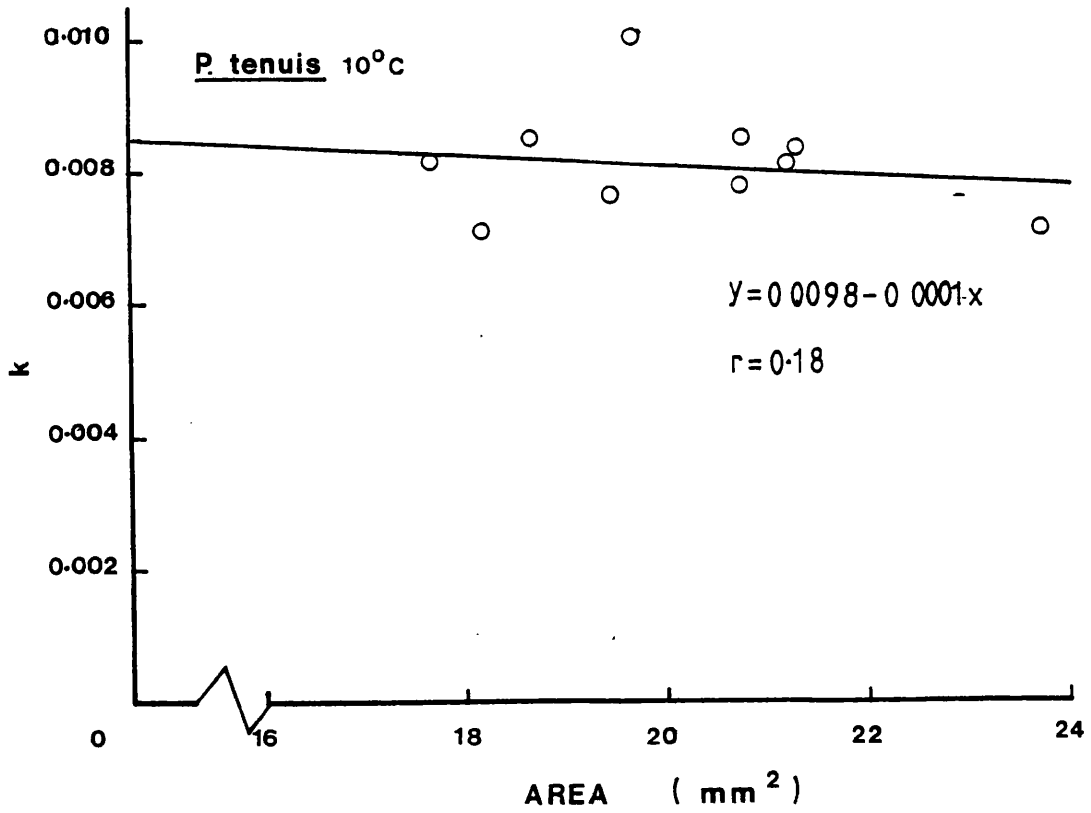
Species	Temperature	Animal	Regression Equation	Correlation Coefficient	k	Initial Size
D. tigrina	15°C	TS 15/1	y = 1.2840 - 0.0079x	-0.99	0.0182	22.89 mm^2
		2	y = 1.2465 - 0.0088x	-0.99	0.0203	17.63 mm^2
		3	y = 1.2463 - 0.0087x	-0.99	0.0200	16.29 mm^2
		4	y = 1.2394 - 0.0097x	-0.98	0.0223	16.29 mm^2
		5	y = 1.2913 - 0.0087x	-0.99	0.0200	19.54 mm^2
		6	y = 1.2709 - 0.0081x	-0.99	0.0187	20.64 mm^2
		8	y = 1.2854 - 0.0081x	-0.99	0.0187	20.27 mm^2
		9	y = 1.2469 - 0.0092x	-0.98	0.0212	18.01 mm^2
		10	y = 1.1882 - 0.0075x	-0.98	0.0173	17.45 mm^2
	20°C	TS 20/1	y = 1.2586 - 0.0162x	-0.98	0.0373	19.12 mm^2
		2	y = 1.2794 - 0.0161x	-0.99	0.0371	16.37 mm^2
		3	y = 0.9527 - 0.0173x	-0.99	0.0398	8.31 mm^2
		4	y = 0.9250 - 0.0145x	-0.98	0.0334	9.34 mm^2
		5	y = 1.0787 - 0.0161x	-0.99	0.0371	10.00 mm^2
		6	y = 1.2391 - 0.0224x	-0.92	0.0516	13.34 mm^2
		8	y = 0.8987 - 0.0158x	-0.99	0.0364	7.31 mm^2
		9	y = 0.9861 - 0.0164x	-0.99	0.0378	9.12 mm^2

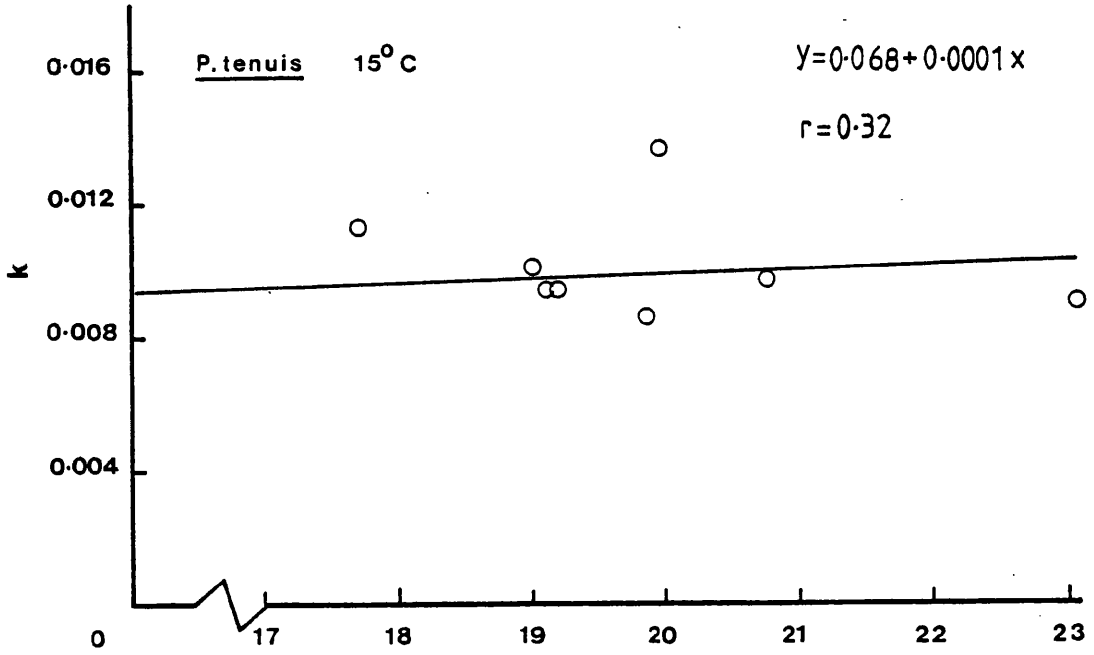
Table 23 : The regression equations and coefficients of exponential degrowth (k) for individual P. tenuis, P. felina and D. tigrina at different temperatures

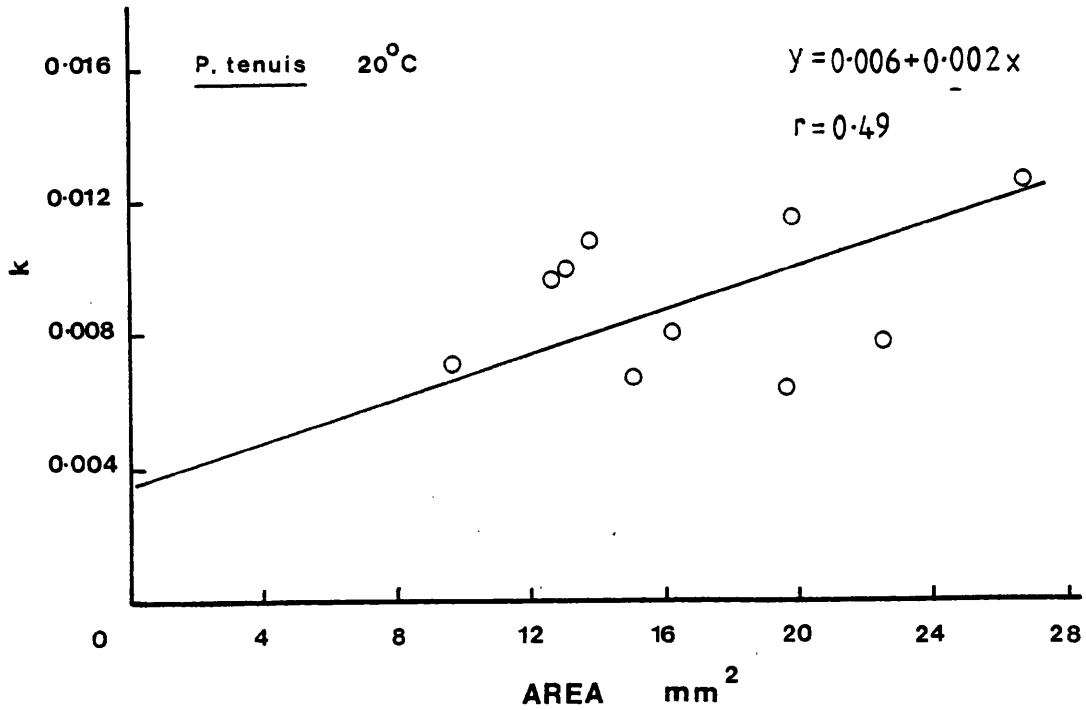
Figure 17

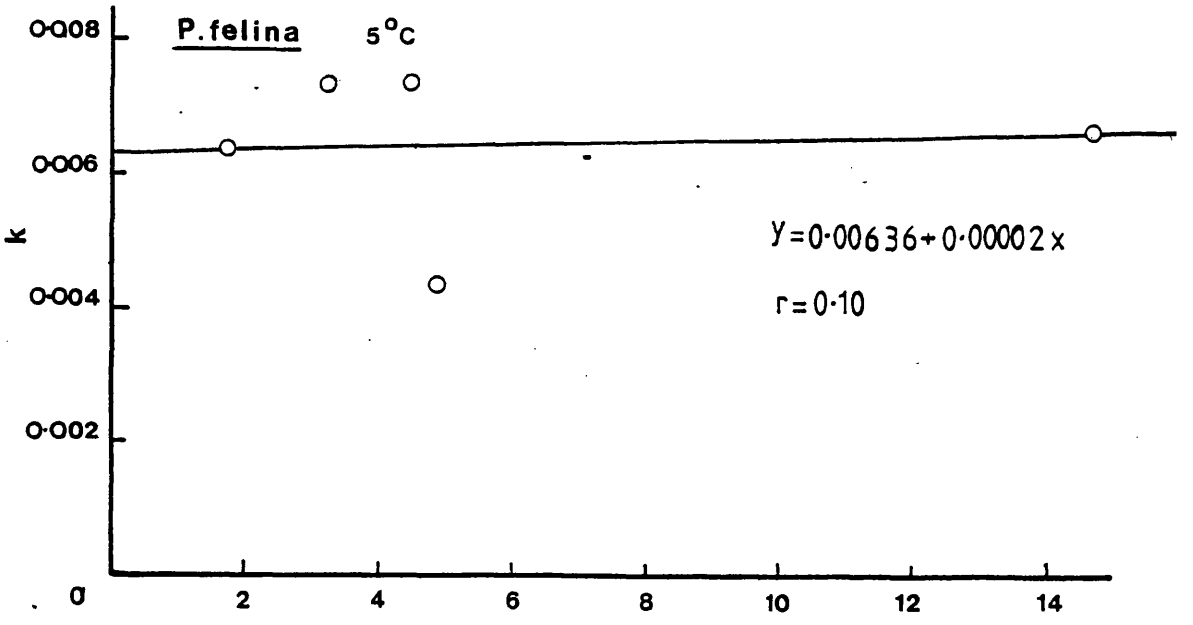
The relationship between initial size prior to starvation, and k, the coefficient of exponential $\frac{de}{dt}$ growth.

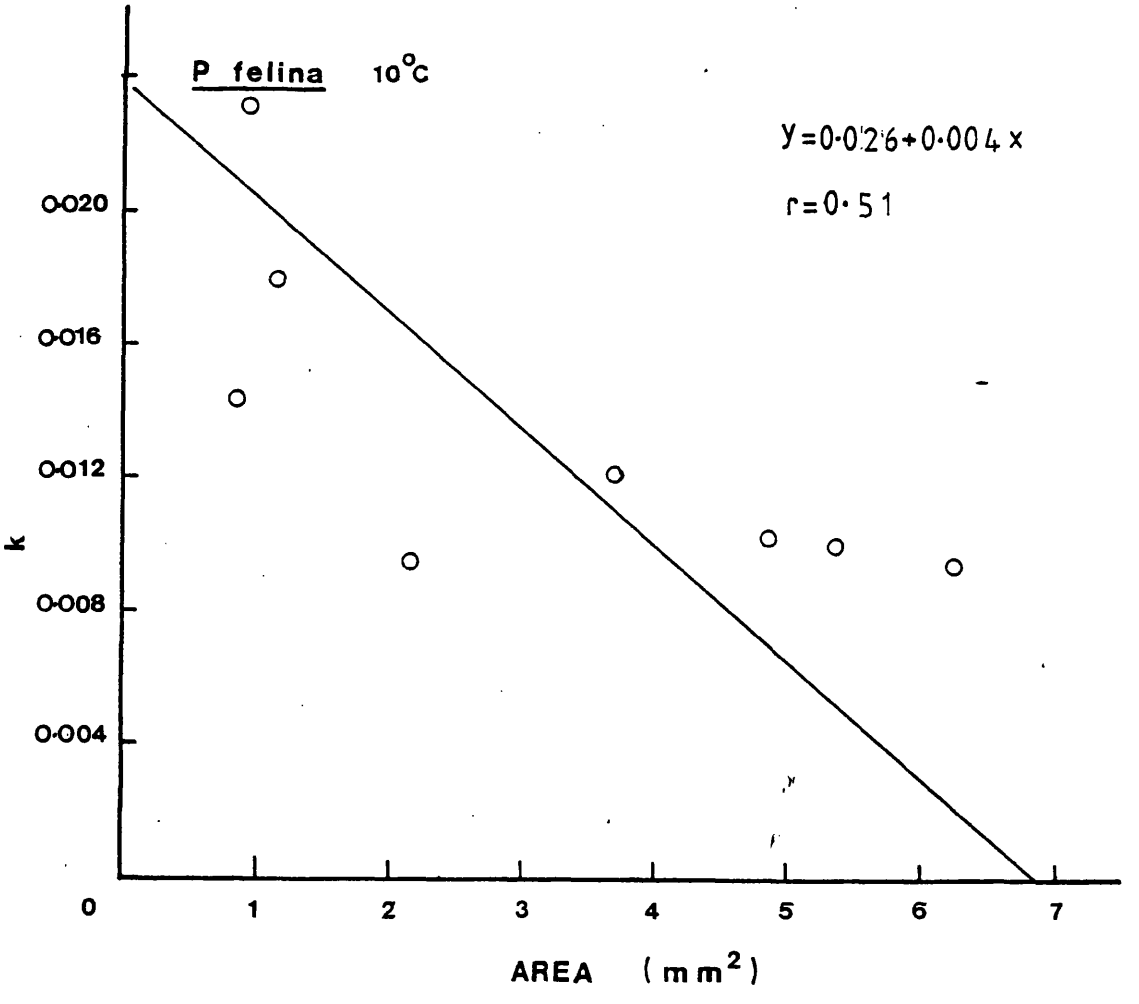

P. tenuis 5°C
y=0·0003x - 0·0030
r=0·60
k
0·008
0·006
0·004
0·002
16
17
18
19
20
21

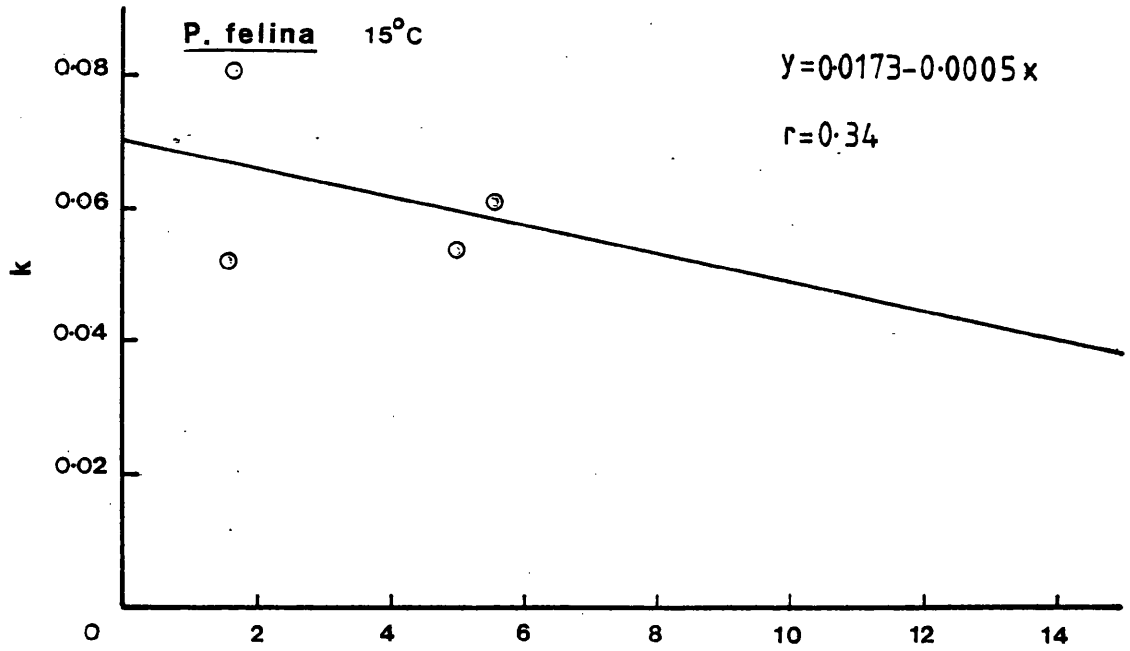

P. tenuis 10°C
y=0 0098 - 0 0001x
r=0·18
k
0·010
0·008
0·006
0·004
0·002
0
16
18
20
22
24
AREA (mm 2)

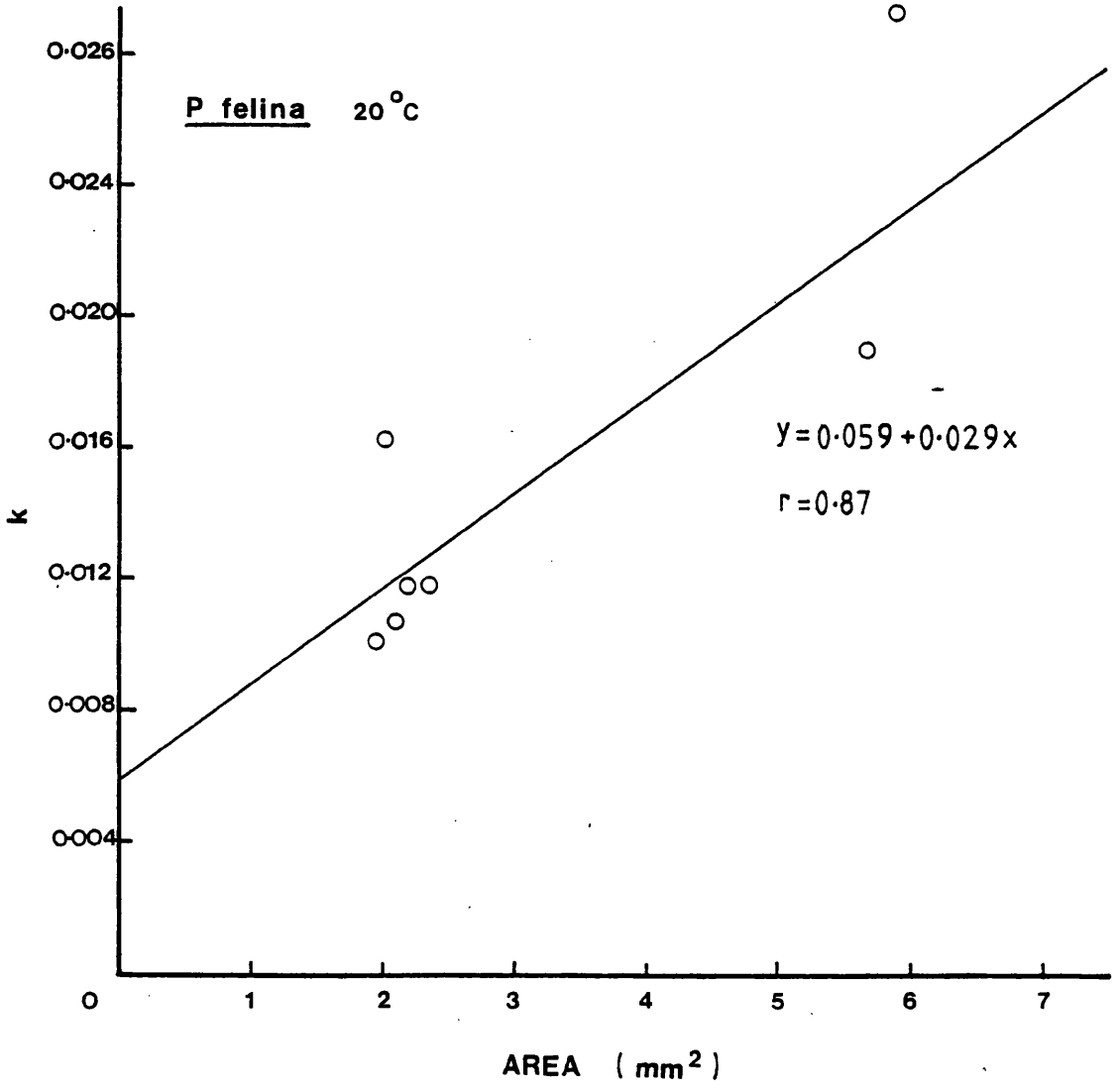

P. tenuis 15°C
y=0·068+0·0001x
r=0·32
0·016
0·012
0·008
0·004
k
0
17
18
19
20
21
22
23

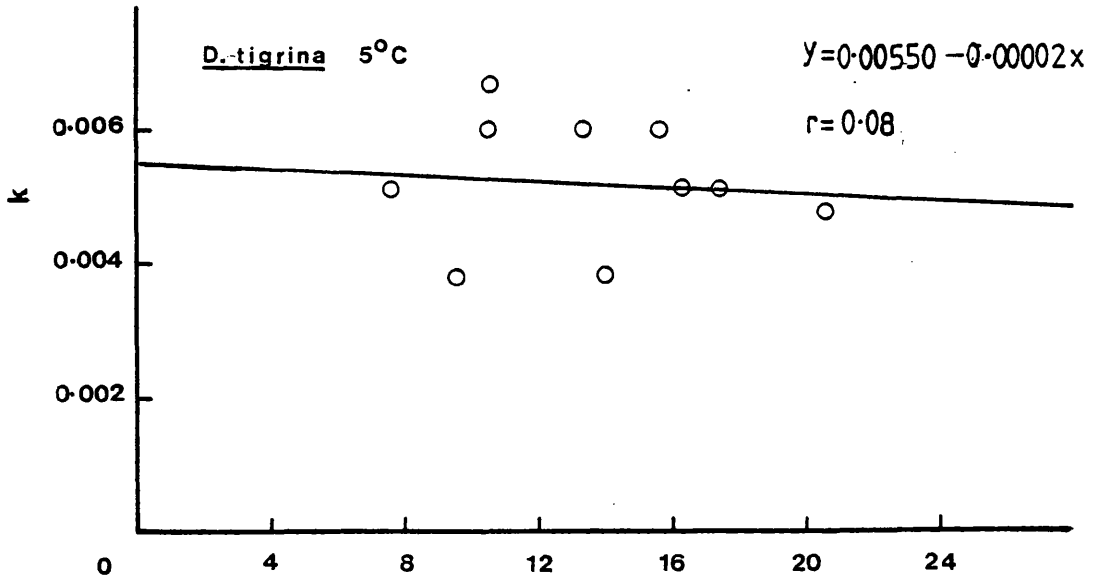

P. tenuis 20°C
y=0·006+0·002x
r=0·49
0·016
0·012
0·008
0·004
k
0
4
8
12
16
20
24
28
AREA mm²

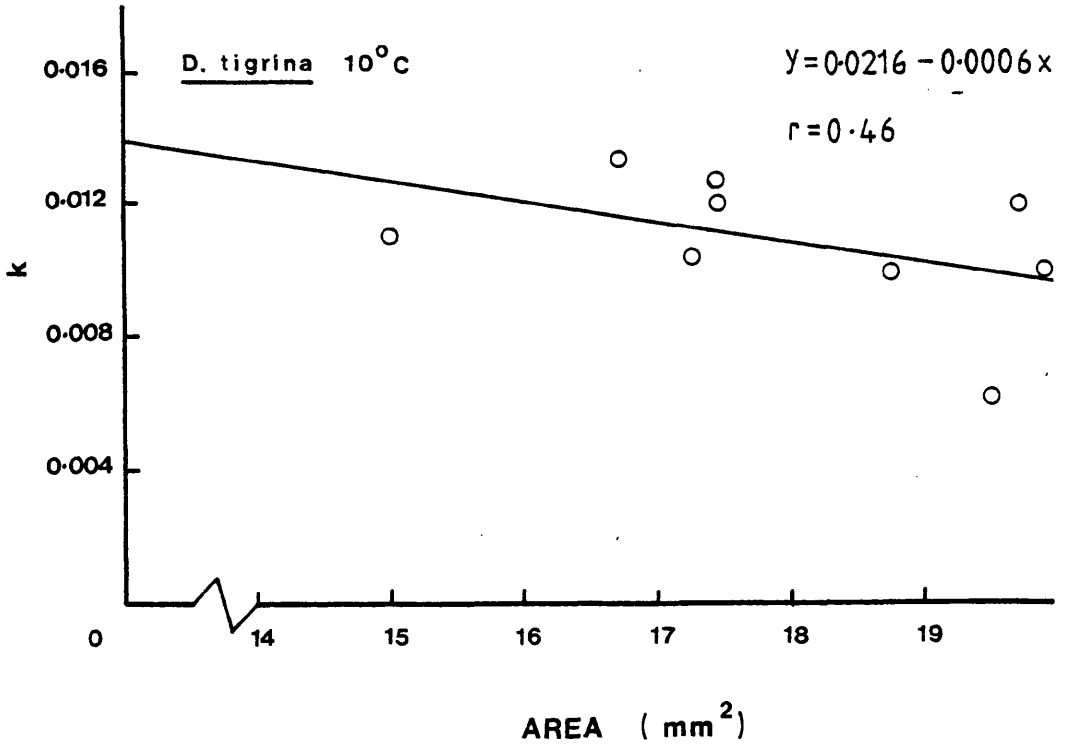

P.felina 5°C
0·008
0·006
0·004
0·002
0
k
y=0·00636+0·00002x
r=0·10
0
2
4
6
8
10
12
14

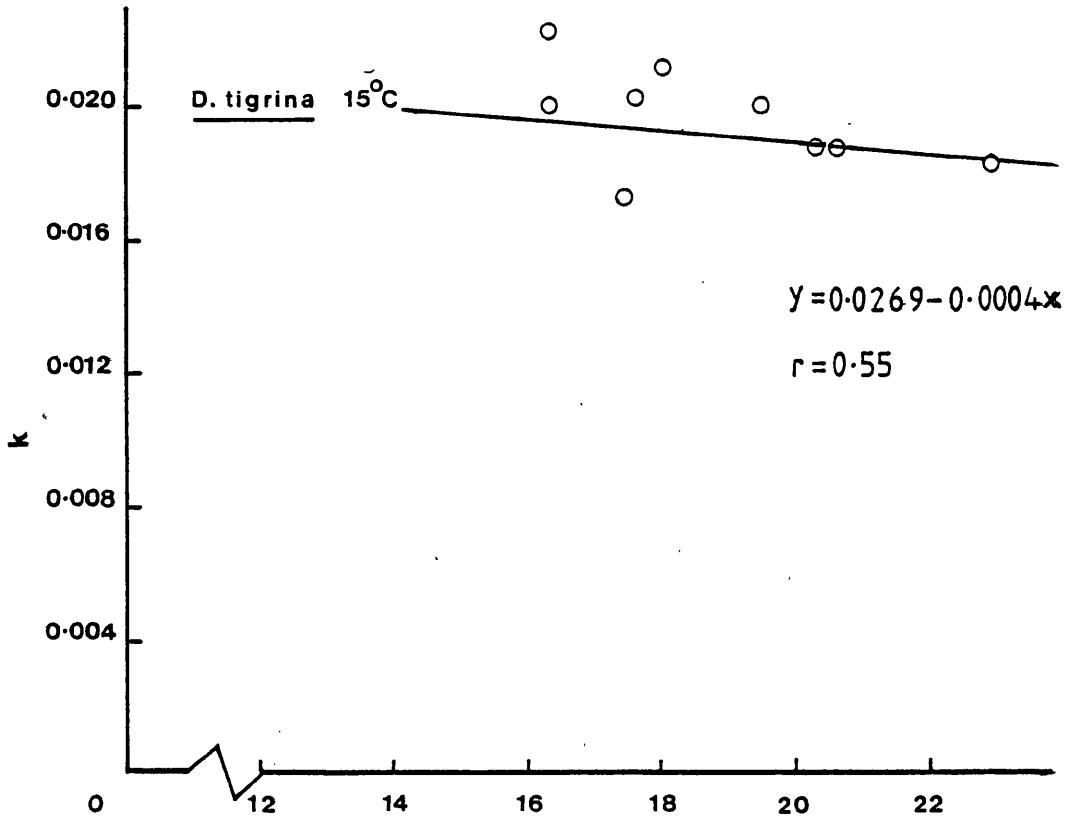

P felina 10°C
y=0·0126+0·004x
r=0·51
0·020
0·016
0·012
0·008
0·004
0
k
0
1
2
3
4
5
6
7
AREA (mm²)

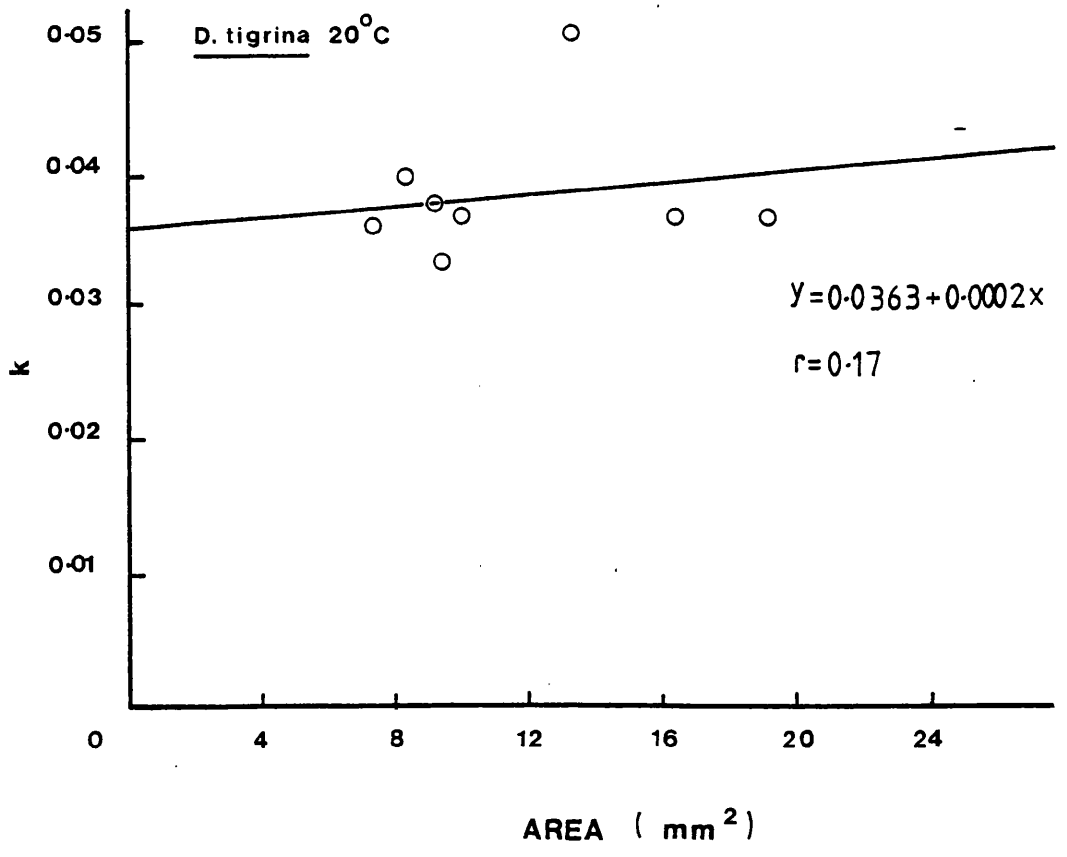

P. felina 15°C
y=0·0173-0·0005x
r=0·34
k
0·08
0·06
0·04
0·02
0
2
4
6
8
10
12
14


P felina 20°C
y=0·059+0·029x
r=0·87
k
0·026
0·024
0·020
0·016
0·012
0·008
0·004
0
1
2
3
4
5
6
7
AREA (mm²)


D.-tigrina 5°C
y=0·00550 −0·00002x
r=0·08
k
0·006
0·004
0·002
0
4
8
12
16
20
24


D. tigrina 10°C
y=0·0216 − 0·0006x
r=0·46
k
0·016
0·012
0·008
0·004
0
14
15
16
17
18
19
AREA (mm²)


D. tigrina 15°C
0·020
0·016
0·012
0·008
0·004
0
k
12
14
16
18
20
22
y=0·0269-0·0004x
r=0·55


D. tigrina 20°C
0·05
0·04
0·03
0·02
0·01
0
k
4
8
12
16
20
24
y=0·0363+0·0002x
r=0·17
AREA (mm²)

	P. tenuis	*P. felina*	*D. tigrina*
F	17.04	4.15	217.85
d.f.	3/32	3/23	3/35
p	< 0.01	< 0.01	< 0.01

Table 24 : Results of a one-way analysis of variance on the effect of temperature on the rates of exponential degrowth in *P. tenuis*, *P. felina* and *D. tigrina* (k values from Table 23).

over different temperature intervals, in order to analyse these differences in greater detail (Tables 25a and 25b).

A plot of the $\bar{k}$ values given in Table 25a summarises the differences between species (Fig. 18). The rate of exponential degrowth increases with temperature to a plateau at around 15°C in P. tenuis and P. felina. However, in D. tigrina the rate of exponential degrowth continues to increase with temperature, up to 20°C.

These results are borne out by the Q_{10} values (Table 25b). Both P. tenuis and P. felina have similar Q_{10} values over the same temperature ranges. The Q_{10}'s decrease with temperature. In contrast, D. tigrina shows consistently similar and higher Q_{10}'s over the whole temperature range studied.

5.5 Discussion

The growth patterns of the 3 species studied appeared to be sigmoid in nature, which is consistent with results from previous studies (Abeloos 1930; Woollhead 1979; Calow, Beveridge and Sibly 1979). Both P. felina and D. tigrina showed characteristic sigmoid growth curves at each temperature. The long lag phase normally observed in tails, and the short or non-existent lag phase which normally occurs in heads, results in differences in growth pattern between heads and tails. Tail buds take considerably longer to regrow their missing halves, except when an unusual fission results in the fission plane shifting towards the head (see Chapter 7). Then a similar lag period is observed in both heads and tails (Fig. 12).

Exceptions to the sigmoid patterns described above are found in D. tigrina at 5° and 10°C. Here degrowth or zero growth was

Species	Temperature	Mean k ($\bar{k}$)	S.E.
P. tenuis	5°C	0.0041	0.0003
	10°C	0.0081	0.0003
	15°C	0.0101	0.0006
	20°C	0.0091	0.0007
P. felina	5°C	0.0065	0.0006
	10°C	0.0135	0.0018
	15°C	0.0155	0.0017
	20°C	0.0150	0.0022
D. tigrina	5°C	0.0052	0.0004
	10°C	0.0109	0.0007
	15°C	0.0196	0.0005
	20°C	0.0388	0.0019

Table 25a : Mean values of k (rate of exponential de-growth) for P. tenuis, P. felina and D. tigrina at 5°, 10°, 15° and 20°C (calculated from data in Table 23)

Species	5-10°C	10-15°C	15-20°C	5-15°C	10-20°C	5-20°C
P. tenuis	3.90	1.55	0.81	2.46	1.12	0.23
P. felina	4.31	1.32	0.94	2.38	1.11	0.24
D. tigrina	4.39	3.23	3.92	3.77	1.98	0.58

Table 25b : Q_{10} values calculated over different temperature intervals for P. tenuis, P. felina and D. tigrina

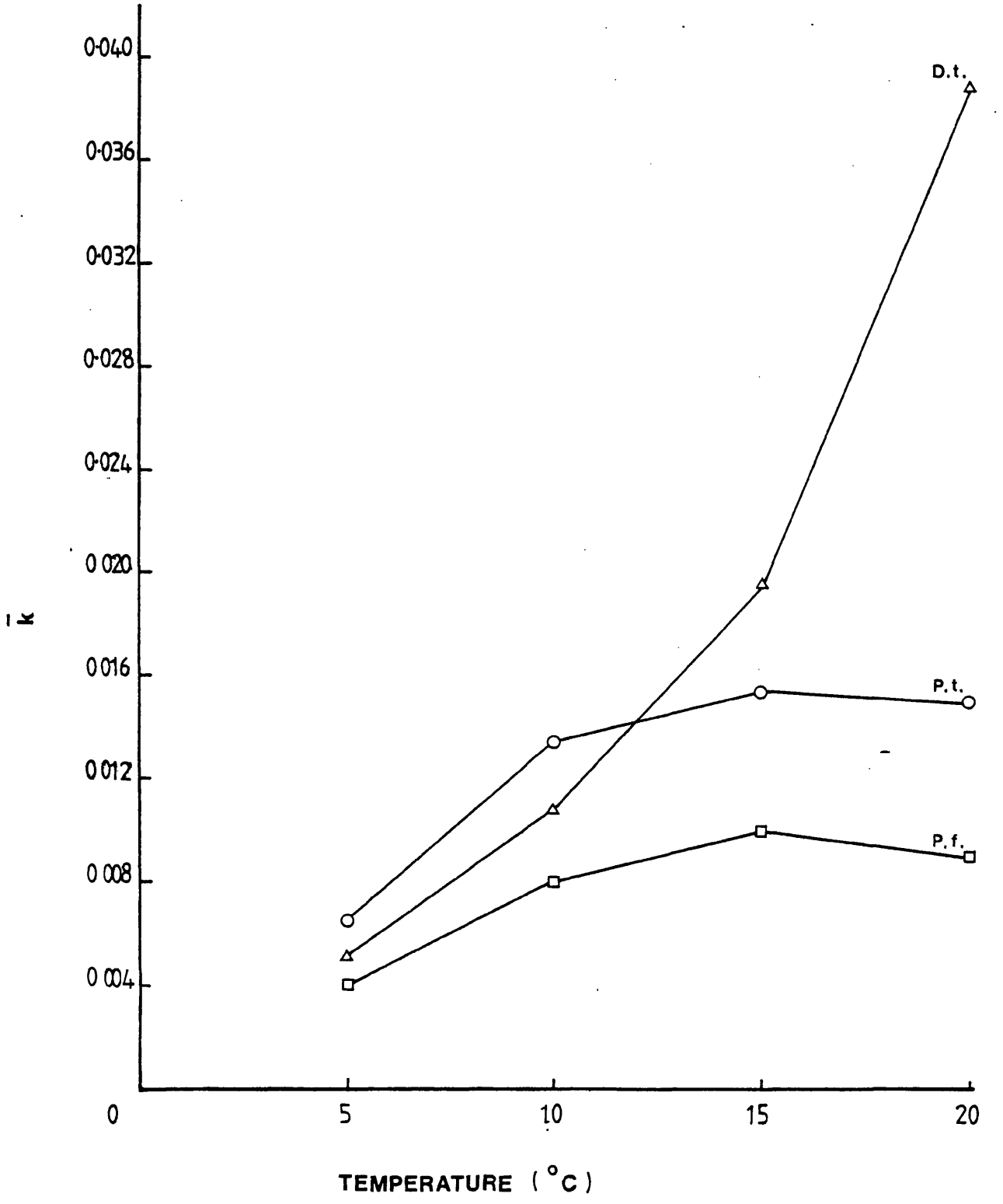
Figure 18

Mean k values ($\bar{k}$) plotted against temperature for P. tenuis, P. felina and D. tigrina.

○ = P tenuis

□ = P felina

△ = D tigrina


0·040
0·036
0·032
0·028
0·024
0 020
0 016
0 012
0 008
0 004
0
5
10
15
20
k
D.t.
P.t.
P.f.
TEMPERATURE (°C)

observed, which was probably due to cold inhibition of feeding (Chapter 4), since D. tigrina is known to be a warm water species (Dahm 1958).

Since differences exist between species in the relationship between plan area and dry weight (see Chapter 3), caution must be used when comparing the growth and degrowth rates of the 3 species. For the reasons stated in Section 5.5 above (i.e. that area/dry weight relationships are unaffected by temperature) only intraspecific comparisons between temperature, and interspecific comparisons of Q_{10} values may be made. Comparisons between species will be made in Chapter 8, where for the purposes of constructing energy budgets, growth/degrowth is measured in terms of changes in weight per unit time.

Growth rates, in terms of area, have been measured by Woollhead (1979). He found that P. tenuis, when fed on 1 Asellus per day, at 10°C, had a K value of 0.0189 ± 0.0058. In this study, under a feeding regime of 1 Asellus per 3.5 days, the rate of exponential growth of P. tenuis at 10°C was very similar (0.0228 ± 0.0040).

In general, the growth rates increased with temperature in all 3 species, although differences in temperature sensitivity were observed. The rate of exponential growth increases in P. tenuis from 0.0100 at 5°C to 0.0396 at 20°C. The decreasing Q_{10} values suggest that a plateau of maximum growth rate is reached at 20°C. Lascombe, Pattee and Bornard (1975) present corroborating evidence as regards the intrinsic rate of natural increase, which shows that P. tenuis does not survive or reproduce for long above 20°C. The rate of exponential growth in P. felina reaches a maximum at 15°C, but appears to be maintained at a high level between 9° and 16°C. This corresponds

to a range of temperatures - both diurnal and seasonal - commonly encountered in the field (see Chapter 9). In D. tigrina, the rate of exponential growth increased dramatically between 15° and 20°C. The maximum growth rate was not observed, but according to other studies (Dahm 1958; Russier-Delolme 1972) it probably corresponds to a temperature of approximately 23°C.

The highest Q_{10} value was measured in D. tigrina between 15° and 20°C. However, zero growth or degrowth is observed below 10°C, and therefore growth is restricted to only a few months in the year. (The adaptive significance of growth is discussed below). The species in which degrowth is least sensitive to temperature change is P. felina. The Q_{10} values observed in the other 2 species are much higher. This contradicts the views of Reynoldson (1961) and Calow (1977a) who state that the metabolism of P. felina is very sensitive to temperature changes. This point will be discussed at greater length in Chapter 10.

An increase in growth rates results in 2 things: a reduction in the length of the life cycle, and a larger increase in size with time. Since growth rates are subjected to selection pressure, like any other physiological process, we must assume that they are at an optimum for the conditions encountered by the species in the field. Thus a reduction in the length of the life cycle may not always result in an increase in fitness, since the timing of maturity is often crucial to successful reproduction (Russell-Hunter 1961; Calow 1978a).

An increase in size through increased growth may however confer several advantages on the triclads. Although predation on triclads is not a major source of mortality, Young and Reynoldson (1965) showed that juvenile (i.e. small) triclads were more likely

to be eaten than large triclads. However, data given in Chapter 9 suggests that sticklebacks find both small and large triclads equally distasteful.

Also, larger sized animals are better able to withstand the effects of starvation (Reynoldson 1968). Results from this study show that initial size does not affect the rate of degrowth in a species (except in P. felina at 10° and 20°C), but merely the length of time taken to reach minimum size. Beyond the minimum size recovery is impossible, and mortality certain. Minimum sizes for P. felina and P. tenuis were measured by Calow (1977b). It is likely that temperature affects this, but no measurements were made in my study.

Temperature does affect the rate of degrowth, and thus the survival time of a triclad subjected to starvation. In general, the rate of degrowth, k, increases with temperature. D. tigrina appears to be very sensitive to temperature, and degrowth is highest at 20°C. In P. felina and P. tenuis, the k values are less temperature dependent and a plateau is observed between 10° and 20°C. The Q_{10} values confirm this. The Q_{10} values for P. felina and P. tenuis are less than Q_{10}'s for D. tigrina.

In general, k increased in a similar manner to K, although k is less temperature sensitive than K (except for P. felina). In other words, the advantages of rapid growth at high temperatures are partly lost by the increased susceptibility to death by starvation. This will be discussed at greater length in the concluding chapter (Chapter 10).

Chapter 7

REPRODUCTION

7.1 Introduction

In this chapter I intend to look at reproduction in P. felina, P. tenuis and D. tigrina in the U.K. P. tenuis reproduces sexually, whereas D. tigrina and P. felina usually reproduce asexually. Part of this study is concerned with the rates and costs of reproduction as part of the overall investigation into the bioenergetics of the 3 species. However, I also intend to look at reproduction in triclads in the light of the general debate on sexual and asexual reproduction (Williams 1975; Maynard Smith 1978). Part of these data have already been published (Calow, Beveridge and Sibly 1979) and are included in Appendix 3.

7.2 Literature Review

There are 3 principal methods of reproduction found in plants and animals: sexual reproduction, parthenogenesis and fission. Sexual reproduction is the most widespread method, and occurs in almost every major group of eukaryotes. Organisms that reproduce sexually have haploid and diploid stages in the life cycle, and a meiotic division at the transition from diploid to haploid. Usually during sexual reproduction, haploid sex cells from different "parents" fuse to form a new individual and restore ploidy.

In parthenogenesis, a haploid ovum develops without fertilisation into a new individual. Parthenogenetic development may be induced artificially under laboratory conditions (for review see Balinsky 1970), but it has also been adopted by a relatively small number of organisms throughout the plant and animal kingdoms (Ghiselin 1974) e.g. dandelions (Harper 1976), soft corals (Hartnell 1977), rotifers (Birkby and Gilbert 1971), molluscs (Morton 1967), aphids

(Narzikulov 1970), fish (Schultz 1973), and lizards (Congdon, Vitt and Hadley 1978).

According to Abercrombie, Hickman and Johnson (1973) asexual reproduction may be defined as "reproduction without gametes". In animals the most common method of asexual reproduction is fission. Fission occurs by the budding off of new individuals from the parent organism. Many plants (e.g. strawberries, Harper 1976) and lower invertebrates (e.g. sponges, Bowerbank 1864; Hydra, Lenhoff and Loomis 1961; sea-anemones, Hoffman 1976; polychaetes, Gibson 1977; Turbellaria, Hyman 1951) reproduce asexually. A few of the simpler chordates also reproduce in this manner (Ivanova-Kazazs and Konopisteva 1972; Fujimoto and Hiroshi 1977).

Cyclic changes from one mode of reproduction to another are fairly common. For example, several species of Daphnia are known to reproduce parthenogenetically for large parts of the year, before switching to sexual reproduction to produce overwintering eggs (Bacci 1965, in Williams 1975). The importance and timing of each mode of reproduction in cyclic sexual/asexual, and sexual/parthenogenetic organisms in general is discussed by Bowler and Rundel (1975), Davison (1976) and Muenchow (1978).

A variety of methods of reproduction have been observed in the Turbellaria. Most species are hermaphrodite, with simultaneous development of eggs and sperm (Hyman 1951; Russell-Hunter 1979). This "simultaneous hermaphroditism" (Russell-Hunter ibid) contrasts with the sequential hermaphroditism often seen in snails (Morton 1967) and fish (Warner 1975, 1978).

There are numerous descriptions of the reproductive apparatus of the Turbellaria (Hyman 1951, Barnes 1974, Russel-Hunter 1979). A typical sexually mature triclad will possess a pair of ovaries from

which 2 oviducts lead to a common vagina. Associated with the oviducts are many vitelline glands. The testes are widely dispersed organs found in the lateral margins of the body. Numerous ducts lead from the testes to a pair of vasa deferentia, which fuse to form the muscular penis. Both the penis and the vagina discharge into a common genital atrium, situated in the posterior ventral surface of the animal.

This system is typical of the Turbellaria, although variations are found in the more "primitive" acoels, rhabdocoels and alloeocoels. The "higher" turbellarians (polyclads and some triclads) may even possess uteri and musculoglandular organs (Russel-Hunter ibid). There are several recent publications concerned with the anatomy and ultrastrucutre of the reproductive organs in triclads (Farnesi, Marinelli, Tei and Vagnetti 1977; Marinelli and Vagnetti 1977).

Cross-fertilisation is usual in most turbellarians (Hyman 1951), and effective mechanisms exist in many species to prevent self-fertilisation (Gelei 1924; Goetsch 1925; Ullyott and Beauchamp 1931). The eggs produced by freshwater triclads are laid in capsules (or cocoons) and often cemented onto the undersides of stones and plants (Kenk 1972; Reynoldson 1978). The egg capsules are transparent when laid, but darken rapidly within a few hours (Nurse 1950). The capsules may contain up to 20 embryos (Kawakatsu, Yamada and Iwaki 1967; Kenk 1972; Reynoldson 1978) together with nurse cells and yolk reserves (Hyman 1951).

Both semelparous (breed once then die) and iteroparous (breed repeatedly) species of freshwater triclad exist in Great Britain (Reynoldson 1966a). Egg production in iteroporous species is affected by age (Balazs and Burg 1962).

Capsule production shows a distinct seasonal pattern. In Japan, some species lay cocoons in late autumn and early winter, although most species restrict breeding to spring and early summer. In Britain, most species also deposit cocoons during the spring and early summer (Reynoldson 1966a). Temperature has a major influence on the timing of cocoon production (Reynoldson, Young and Taylor 1965) e.g. D. polychroa cannot produce egg capsules below 10°C, or above 23°C. At low temperatures, retardation of the testes occurred and at high temperatures both ovaries and testes failed to develop. Copulation was also adversely affected (Reynoldson, Young and Taylor ibid).

Reynoldson, Young and Taylor (ibid) further demonstrated the influence of temperature on the rate of cocoon production and the viability of cocoons. The results published for P. tenuis showed that cocoon production was highest at 10°C, although the proportion of viable cocoons was greatest at 20°C. Temperature affected the cocoon incubation period, and in the case of P. tenuis, decreased from 151 days at 3.5°C to 18 days at 20°C. Studies on Canadian populations of D. tigrina by Folsom and Clifford (1978) have also shown that cocoon production increases with temperature.

Food has been identified in several studies as an important influence on capsule production (e.g. Dubois 1946). Calow and Woollhead (1977b), Woollhead and Calow (1979) and Woollhead (1979) showed that perennial and annual species differed in their response to reduced food supply. In perennial species, capsule production decreased, whereas in annual species it was maintained, even under conditions of total starvation.

Some turbellarians reproduce asexually (Hyman 1951) and a review of the mechanisms employed is given in Ax and Schultz (1959).

Two methods of asexual reproduction are used by the turbellaria : fragmentation and fission.

A few triclads, such as members of the freshwater genus Phagocata (Kawakatsu, Yamada and Iwaki 1967; Kenk 1972), and the terrestrial genus Bipalium (Chandler 1976) fragment. In Phagocata species, each piece forms a cyst from which a small worm eventually emerges.

There are 2 types of fission, termed paratomy and architomy after Wagner (1890). Paratomy is by transverse fission, and the fission products remain attached until a fairly complete degree of development has occurred. This may result in the formation of chains of zooids. Paratomy is common in rhabdocoels, e.g. in the genera Catenula, Stenostomum and Microstomum (Child 1915; Hyman 1951; Barnes 1974), but appears to be rare among freshwater triclads. Kennel (1888, cited in Curtis 1902) described the paratomous fission of P. fissipara, in which complete formation of the eyes, brain, etc. occurred before the separation of the tail. Partial paratomy has also been described by Zacharias (1886) in P. subtentaculata (D. gonocephala). Partial formation of the gut and pharynx occurred in the tail before separation. However, these descriptions remain unsubstantiated.

Among freshwater triclads, architomy is the most common method of asexual reproduction. Transverse fission, usually below the level of the pharynx (Curtis 1902; Child 1915; Nentwig 1978), results in a head and tail, which then regrows the missing half. In P. albissima (Ph. vitta) fissioning may occur to the anterior of the pharynx (Sekera 1888, cited in Curtis 1902).

Several authors had reported the occurrence of asexual reproduction in freshwater triclads, before Curtis (1902) described

the process in detail in D. tigrina. Early accounts of asexual reproduction may be found in Dalyell (1814), Johnson (1822), Dugès (1828) and Faraday (1832) amongst others.

The actual process of fission is surrounded by controversy. Fission occurs without the prior appearance of a division furrow, and without the preformation of any organs, or parts of organ systems (Curtis 1902; Child 1915). However, Child (ibid) claims to have found a series of metabolic gradients in D. dorotocephala, which predetermines where the division will occur. Recent ultrastructural studies by Nentwig (1978) failed to find preformation of organs prior to fission. However, Boguta (1947) disagrees and states that in D. tigrina the nervous system undergoes morphological reconstruction accompanied by the formation of supernumerary additional nerve stems prior to fission.

After fission, both head and tail regrow their missing half. The similarity of regrowth after fission to regeneration is remarked on by Curtis (1902) among others. However, Kenk (1937) and Nentwig (1978) disagree, and claim that head formation after fission involves an internal remodelling process (morphollaxis) whereas decapitation results in regeneration through epimorphosis. Both processes involve neoblast cells (Brønsted 1969; Spiegleman and Dudley 1973; Krichinskaya and Martynova 1974; Gremigni and Puccinelli 1977; Nentwig 1978), although Nentwig (ibid) states that the number of neoblasts which are involved in each case are different. She also shows that the changes in the shape of the animal are different during regeneration and regrowth. Blastema formation is also absent after fission.

Although exclusively asexual populations are common, few freshwater triclad species reproduce by asexual means alone. Extensive

surveys of the freshwater triclad fauna of the United States, Europe, Japan and Australia (Dahm 1958; Reynoldson 1961, 1978; Kawakatsu 1968; Kawakatsu, Yamada and Iwari 1967; Kenk 1972; Hay and Ball 1979) have unearthed only 1 solely fissiparous species, D. izuensis, which has a very narrow range of distribution. This species is described by Kato (1950), and occurs in Japan. In all other fissiparous species, sexual populations, or populations in which both sexual and asexual methods occur, are known to exist.

In Great Britain, 4 species of freshwater triclad reproduce asexually: C. alpina, Ph. vitta, P. felina and D. tigrina. Sexual reproduction is common in C. alpina. Some populations reproduce exclusively by asexual means, however it is more common to find populations which reproduce by both methods (Dahm 1958; Reynoldson 1961, 1978; Wright 1968).

Dahm (1958) looked at the methods of reproduction employed by 47 different populations of Ph. vitta from Europe, and found 32 populations reproduced by asexual means alone. Only 13 populations reproduced sexually, but all of those were in Great Britain. The remaining 2 populations reproduced by both means. Thus sexual populations may be more common than asexual populations in Great Britain. Carpenter (1928) found all 3 types (sexual, asexual and sexual/asexual populations) in Wales, but Baird (pers. comm.) has so far (June 1980) only found asexual populations in Scotland.

Dahm (1958) records a population of P. felina from near Pitlochry, Scotland, which reproduces by both means. However, no other records of P. felina reproducing sexually in Great Britain occur in the literature. Wright (1968) states that there are no sexual populations of P. felina occurring in Britain, and personal observations confirm this.

Although sexual, asexual and facultative sexual/asexual populations of D. tigrina occur in the United States (Kenk 1940, 1972) the European populations reproduce almost exclusively asexually. Dahm (1955, 1958) and Reynoldson (1956) report that no populations which reproduce sexually have been found in Britain or in the rest of Europe. Okugawa (1957) was apparently in error when he referred to a sexual population in Europe, and Pickavance (1968) suggests that this might have been due to a misreading of Lender's (1951) report on a population of D. tigrina in France. However, Pickavance (ibid) describes a population of D. tigrina which is found in Virginia Waters, England, and is known to reproduce sexually. This is the only reference to a European sexual population of D. tigrina in the literature.

The 4 British species described above all reproduce by architomy, although some evidence exists that P. felina and Ph. vitta may occasionally reproduce by fragmentation (Faraday 1832; Baird pers. comm.).

Dugès (1828) first observed that fission and asexuality in freshwater triclads, were related. Benazzi (1973) went as far as to say that "fissiparous individuals are always devoid of the sexual apparatus", despite earlier observations that in some individuals, traces of gonads and the copulatory organ may be present (Benazzi 1938). In populations which reproduce both sexually and asexually, the onset of fission is preceeded by the disappearance of the copulatory apparatus (Curtis 1902).

There have been many different opinions as to the relative importance of environmental and internal factors on controlling sexuality (Kenk 1937, 1940; Hyman 1939, 1941; Goldsmith 1942; Benazzi

1973). However, it is now clear from the work done by Benazzi and others (e.g. Benazzi 1973; Benazzi and Grasso 1977; Grasso and Benazzi 1973) that the sexual/asexual state found in different populations of the same species is under genetic control. Laboratory cultures have shown the sexual or asexual character of a population to be stable over several generations, despite environmental manipulations: Kenk (1937) and Goldsmith (1942) for D. tigrina; Benazzi (1936, 1938, 1942) for D. gonocephala; Okugawa and Kawakatsu (1954, 1956, 1957) for D. japonica.

The existence of separate sexual, asexual, and facultative sexual/asexual races of a particular species is now widely accepted, and is supported by convincing cytological evidence. Dahm (1958) first indicated that there is a strong relationship between chromosome number and asexuality. Further studies by Benazzi Lentati (1964), Iwashiro and Sachiko (1975), and Bromley (1977) have produced strong evidence that polyploidy is primarily responsible for the lack of sexual reproduction in field populations. However, some contradictory work on D. lugubris has been produced by Benazzi, Baguna and Ballester (1970) and Benazzi, Ballester, Baguna and Puccinelli (1972).

The fissiparous gene or genes may stop sexualisation by preventing the transformation of neoblasts into germ cells (Benazzi 1973).

Numerous studies have shown that environmental factors are primarily responsible for governing the timing and rate of fission. In populations which reproduce by both methods, temperature and light play a crucial part in switching reproduction from asexual to sexual (Castle 1928; Okugawa 1957; Vowinckel 1970; Vowinckel and Marsden 1971). Light is also one of the factors known to affect the rate of fission,

and Legner, Tsai and Medved (1976) have shown that high light levels reduce the rate of fission in D. dorotocephala. Other factors such as oxygen tension and the nature of the substrate may also be important (Legner, Tsai and Medved ibid).

However, the most important factors governing fission in the field are likely to be population density, food availability and temperature. As the population density increases, the rate of fission falls (Kantani 1957a, b; Wowinckel, Wolfson and Marsden 1970; Legner, Tsai and Medved 1976), and this is thought to be due to the influence of neurosecretory ectocrines (Lender and Zghal 1968; Best, Goodman and Pigon 1969). However whether the action of ectocrines is important in the field (e.g. in running water) is difficult to assess.

The nutritional state of a triclad is known to greatly influence the rate of fission. Fissioning increases with increased food availability (Dahm 1958; Legner, Tsai and Medved 1976), and ceases during starvation (Nentwig and Shauble 1974).

Below certain temperatures, fissioning does not occur. Okugawa (1957) found that asexual reproduction in D. gonocephala was inhibited below 11°C. As temperature increases, the rate of fissioning increases (Dahm 1958; Legner, Tsai and Medved 1976).

In the above review, it is apparent that there is a considerable body of literature on reproduction in triclads. However, what has not yet emerged from this is the adaptive significance of different modes of reproduction. To this end, I intend to investigate the effects of temperature and starvation on reproduction in P. tenuis, P. felina and D. tigrina. In particular, I will look at how the rate of fission and the partitioning of resources during fission is affected by these 2 factors. I will also look at differences between heads and tails.

Part of these results has already been published (Calow, Beveridge and Sibly 1979) and where this is the case, the reader will be referred to Appendix

7.3 Materials and Methods

P. tenuis hatchlings, obtained from laboratory cultures, were randomly sorted into individual perspex pots at 5°, 10°, 15° and 20°C, and cultured as described in Chapter 3. The time taken for each animal to reach sexual maturity was noted. After they became sexually mature, the rate of capsule production at each temperature, and the dry weight of each egg capsule was determined. Egg capsules were removed from the perspex pots and dried in a vacuum oven at 40°C for a week, before being weighed.

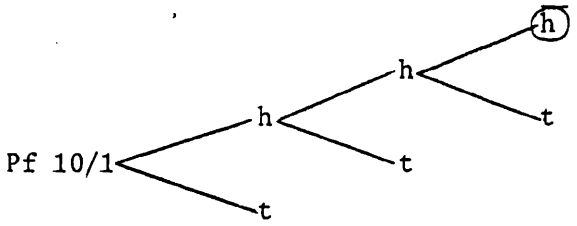
Groups of 10 adult P. tenuis were also kept at each temperature and cultured in the normal manner (see Chapter 3). The egg capsules produced were collected and incubated individually in small glass tubes filled with filtered loch water. They were checked daily, and the time of hatching and number of hatchlings recorded.

The joint effects of starvation and temperature on cocoon deposition was also measured in 10 P. tenuis cultured separately at different temperatures.

P. felina and D. tigrina collected from the field were acclimated to laboratory conditions as described previously, before being transferred to perspex pots and culture separately in the usual manner (see Chapter 3). After each division, either the resultant head or tail was transferred to a new pot, so that the culture of 1 triclad per dish was maintained. Many of the observations on reproduction in P. felina and D. tigrina were made in conjunction with observations on growth (Chapter 6).

In order to describe what happens during and after fission, the following terminology has been developed. Triclads which are not fissioning and which bear no signs of recent fission, are described as adults. After fission, the resulting heads and tails regrow their missing halves. The term regrowth is used here in preference to the term regeneration, since recent research suggests that the process of regeneration is different from post-fission processes (see above).

A system of following the fates of fission products has been developed, and is described below using an example:-



This is a schematic representation of an animal fissioning (P. felina at 10°C), and the resulting fission products. The letters h and t denote heads and tails. The animal circled is referred to as Pf 10/1hht. This notation summarises the known history of the animal. Thus Pf 10/1hht is a P. felina tail, which is the result of 3 previous fissions. At each temperature, the rate of fission and the size of the resulting heads and tails were recorded. The process of regrowth was observed in P. felina by photographing a head and tail daily after fission, and by regular observations using a low-power microscope. As Pickavance (1968) has described regrowth in D. tigrina, and since I recorded no significant difference from Pickavance, no detailed observations will be recorded here.

The effect of starvation on fission is also noted.

7.4 Results

7.4.1 Sexual reproduction in P. tenuis

The time taken for P. tenuis from hatching to reach sexual maturity and begin laying capsules was found to vary with temperature. The results are given in Table 26. The time taken decreased from 502 days at 5°C to 141 days at 15°C. No data were obtained for animals cultured at 20°C, as all either died or started to degrow after the 8th week.

Since few of the above triclads cultured through from hatchling reached sexual maturity, capsule production was estimated in a different group of animals. P. tenuis hatchlings produced in the laboratory at 10°C were reared at that temperature to adult size (prior to egg production), before being transferred to other temperatures. The rate of production was estimated from the time all animals started to produce capsules. Capsules produced during the period of observation were collected, dried and weighed (No data were obtained for P. tenuis at 15°C due to problems with this particular culture). The data are summarised in Table 27.

There was no significant difference between 5° and 10°C in the rate of capsule production ($t = 0.75$; d.f. = 18; $p > 0.05$), or in the dry weights of capsules produced ($t = 0.95$; d.f. = 18; $p > 0.05$).

The viability of those capsules produced by groups of P. tenuis at different temperatures (see Section 7.3 above) was measured, and the results are presented in Table 28a. Viability was estimated from the percentage of cocoons laid, from which hatchlings emerged. The number of non-viable eggs in cocoons was not investigated. The viability of P. tenuis cocoon was highest at 10°C (81%), and lowest at 5°C (69%).

Temperature	No. of Animals which produced Eggs	Mean time taken to reach egg production from hatching	Range
5°C	1	502	-
10°C	5	170	141 - 187
15°C	4	141	129 - 173
20°C	0	-	-

Table 26 : Results of time taken from hatching to egg production of 10 P. tenuis kept at different constant temperatures

Temperature	Period of Observation	Triclad	No. of Capsules Produced	Mean Dry Weight
5°C	68 days	PTB 1	8	0.38 mg
		2	3	0.42 mg
		3	2	0.31 mg
		4	8	0.31 mg
		5	4	0.33 mg
		6	3	0.29 mg
		7	5	0.35 mg
		8	4	0.33 mg
		9	10	0.39 mg
		10	5	0.35 mg
10°C	49 days	PT 1	4	0.47 mg
		2	4	0.38 mg
		3	3	0.40 mg
		4	4	0.45 mg
		5	4	0.31 mg
		6	3	0.32 mg
		7	2	0.35 mg
		8	5	0.34 mg
		9	1	0.24 mg
		10	3	0.45 mg

Mean rate of capsule production at 5°C = 0.75 capsules/10 triclads/day
Mean weight of capsule produced at 5°C = 0.35 mgs

Mean rate of capsule production at 10°C = 0.68 capsules/10 triclads/day
Mean weight of capsule produced at 10°C = 0.37 mgs

Table 27 : The rates of capsule production and weights of capsules produced by P. tenuis at 5° and 10°C

Temperature	Number of Cocoons	Viable No.	Viable %
5°C	16	11	69
10°C	26	21	81
15°C	23	17	74

Table 28a : The viability of P. tenuis cocoons at 5°, 10° and 15°C

Temperature	Mean Number of hatchlings/capsule	Mean Development Time (days)	S.D.
5°C	4.1	102.18	9.84
10°C	4.2	34.91	3.16
15°C	4.5	25.35	1.62

Table 28b : The development time of P. tenuis cocoons at 5°, 10° and 15°C

All capsules produced by the groups of P. tenuis were incubated, and the time taken for the hatchlings to emerge (development time) carefully recorded. In order to test if development time was related to the number of embryos in a capsule, the number of emerging hatchlings was plotted against development time at 10°C (Fig. 19). No significant correlation was found ($r = 0.25$; $t = 1.07$; $p > 0.05$). Hence the incubation period is independent of the number of eggs in a capsule.

The development time of P. tenuis capsules at different temperatures is given in Table 28b. The development time was longest at 5°C (mean incubation 102.18 days), but decreased with increasing temperature to a mean value of 23.35 days at 15°C. There was little difference in the number of hatchlings that emerged from viable capsules at different temperatures (Table 28b).

During starvation capsule production ceased. Although no specific data were collected on the joint effects of temperature and starvation on the rate of capsule production, P. tenuis was observed laying capsules for up to 4 weeks after the onset of starvation at 5°C, 3 weeks at 10°C and approximately 10 days at 15°C. All capsules produced by the starving P. tenuis were collected, dried and weighed in the standard manner. The results are given in Table 29.

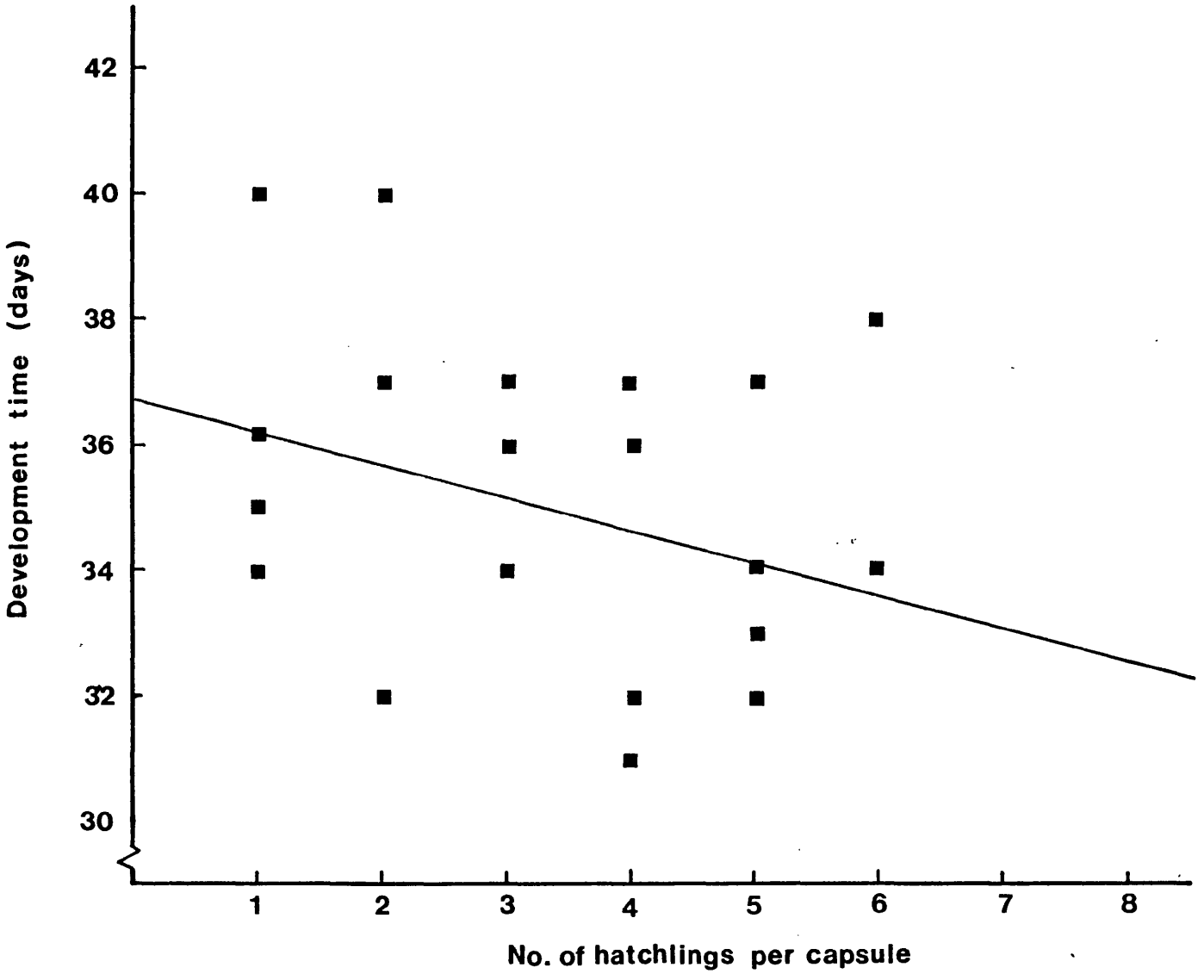
There is a significant reduction in the overall mean weight of capsules laid by P. tenuis at 5° and 10°C during starvation ($t = 17.04$ and 21.17 respectively; $p < 0.01$). Capsules produced at 15°C were also much lighter than those produced by fed P. tenuis at 5° and 10°C.

7.4.2 Asexual reproduction in P. felina

The process of asexual reproduction by fission was observed

Figure 19

The relationship between number of P. tenuis hatchlings and incubation period at 10°C


Development time (days)
42
40
38
36
34
32
30
1
2
3
4
5
6
7
8
No. of hatchlings per capsule

Temperature	Weeks from the Onset of Starvation	No. of Capsules Produced/ 10 Triclads	Mean Capsule Weight
5°C	1	8	0.32 mg
	2	4	0.26 mg
	3	4	0.21 mg
	4	1	0.24 mg
10°C	1	10	0.23 mg
	2	6	0.20 mg
	3	1	0.23 mg
15°C	1	8	0.20 mg
	2	1	0.14 mg

Table 29 : Capsule production by P. tenuis during starvation

only once in P. felina. The head end of the animal was seen to tear itself free from the attached tail end. This took several attempts, during which the anterior end of the animal extended itself greatly, while the tail end remained firmly attached to the substrate. The force of the movements produced by the head end caused the tail to move slightly across the bottom of the dish. A small transverse tear appeared approximately $^3/_4$ of the way down the animal, near the tail end. This widened, with each successive extension of the anterior end. After 6 or 7 attempts, the head end tore itself free from the tail, and moved off rapidly, leaving the tail adhering to the bottom of the dish. The whole event lasted just over 1 minute.

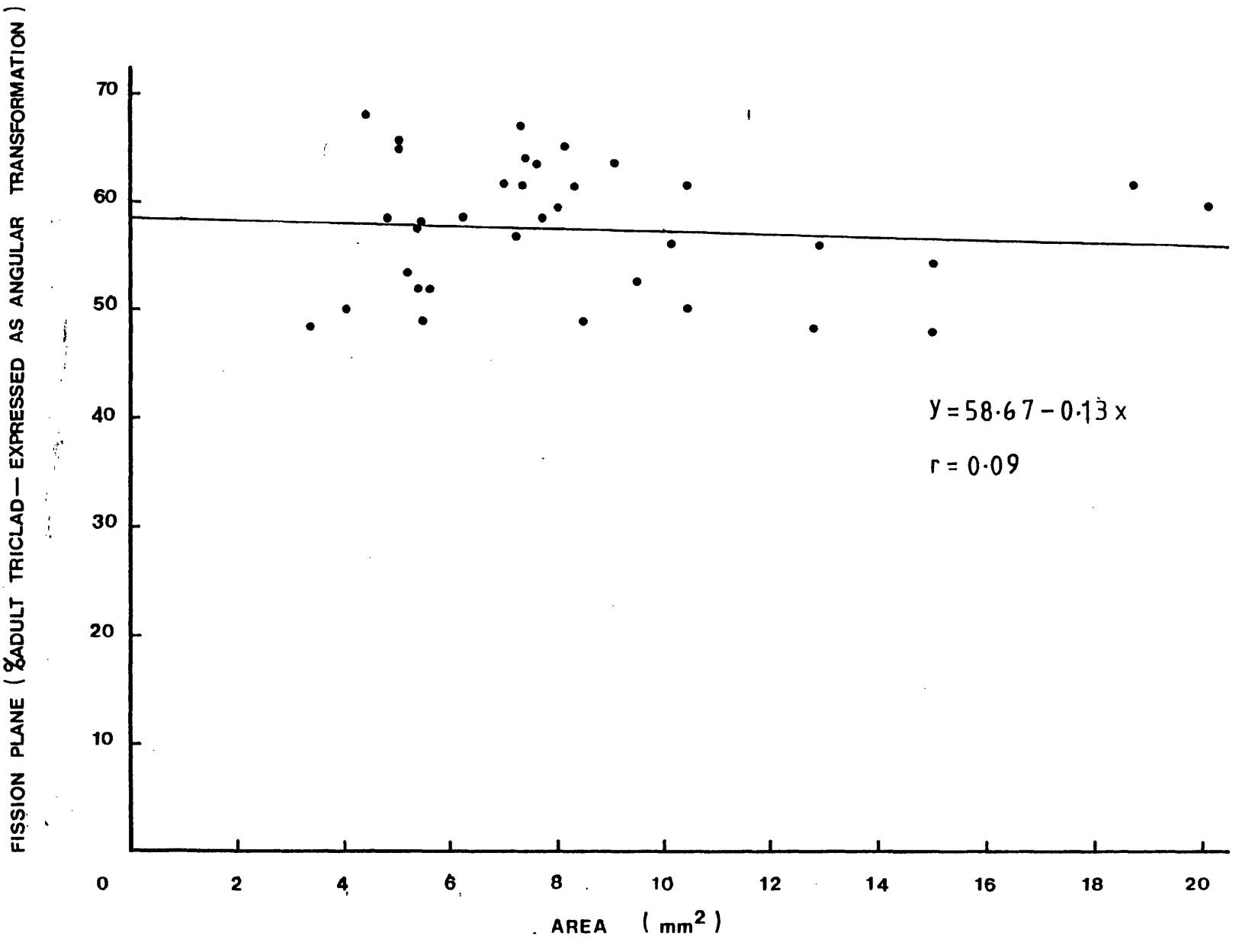
Fission was recorded in P. felina at 5°, 10° and 15°C, but never at 20°C. Although there was some variation, the fission plane was usually post-pharyngeal, and was not significantly influenced by temperature (see Appendix 3), or size. In Fig. 20, the influence of size on the position of the fission plane is illustrated, and there is no signficant relationship between the 2 factors ($r = 0.09$; $t = 0.50$; $df = 33$; $p > 0.01$).

The position of the fission plane is also independent of genetic control. The division planes were observed in 5 different clones from Balmaha Pond, and 1 clone from Lossit Loch, Islay. The results are given in Table 30. An analysis of variance was performed on the results (after Angular Transformation) and shows that there is no signficant difference between clones ($F = 0.49$; d.f. = 21; $p > 0.01$).

After fission the head and tail regrow the missing halves. Although it is continuous, regrowth may be broken down into several stages, which are summarised in Fig. 21. The head: during Stage I

Figure 20

The influence of triclad size (area in mm^2) on the position of the fission plane in P. felina (normally expressed as a percentage of the pre-fission animal. Here the percentage values have been changed to degrees by Angular Transformation [Fisher and Yates 1963]).


FISSION PLANE (%ADULT TRICLAD— EXPRESSED AS ANGULAR TRANSFORMATION)
70
60
50
40
30
20
10
0
2
4
6
8
10
12
14
16
18
20
AREA (mm2)
y = 58·67 – 0·13 x
r = 0·09

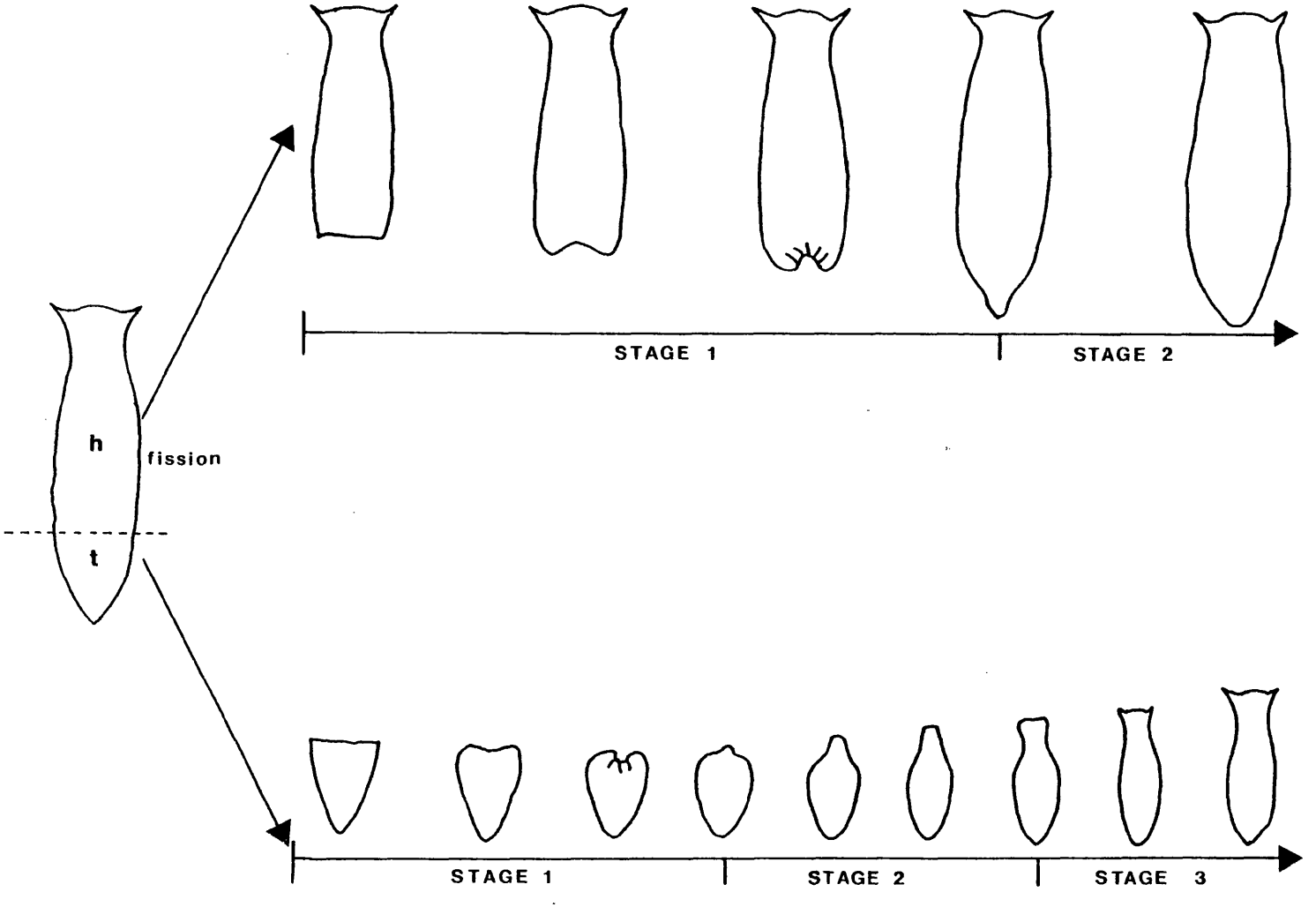
Clone	Size of Head after Fission (mm^2)	% Size of Adult	Degrees
1h	3.83	70.66	57.21
1hh	3.50	72.31	58.24
1hhh	1.93	56.10	48.50
2h	8.13	54.20	47.41
2hh	4.54	72.94	58.66
2hhh	5.94	80.05	63.47
2hhhh	5.98	85.40	67.54
3h	5.92	73.93	59.29
3hh	5.38	70.98	57.42
3hhh	5.04	70.29	56.98
3hhhh	9.98	66.71	54.76
4h	3.33	62.01	51.94
4hh	5.78	73.63	59.09
4hhh	6.99	69.28	56.35
4hhhh	4.86	56.98	49.02
5h	2.41	59.36	50.40
5hh	3.92	72.32	58.25
5hhh	5.69	73.23	58.83
5hhhh	3.39	64.94	53.70
*6h	4.21	69.02	56.18
*6hh	4.55	75.71	60.47
*6hhh	7.16	69.79	50.66

Table 30 : Fission in 6 clones of P. felina at 10°C. The sizes of head fragments are expressed in Column 3 as a percentage of the "adult" triclad. In Column 4, the percentage values have been changed to degrees, by Angular Transformation (Fisher and Yates 1963).

* Clone from Lossit Loch, Islay (see Chapter 3).

Figure 21

Illustration of the subsequent development of P. felina heads and tails after fission.


h
fission
t
STAGE 1
STAGE 2
STAGE 1
STAGE 2
STAGE 3

the end where fission occurred takes on a crescent-like appearance. The crescent gradually increases in size until it appears slightly puckered (this varies in degree from animal to animal). This marks the end of Stage 1.

During Stage 2, the puckered end of the head quickly becomes square-cut, then triangular in shape. The onset of triangulation marks the earliest stage at which fission may commence. The new tissue is still unpigmented, but pigment gradually appears as the post-pharyngeal end of the head becomes normal in outline.

The tail: during Stage 1, the end where fission occurred changes from square-cut to puckered in appearance. Tissue then appears to fill the hollow in the pucker till it appears slightly convex. This marks the end of Stage 1.

During Stage 2, the new head becomes bud-like and more pronounced. Towards the end of this stage, the rudimentary new pharynx becomes visible.

The onset of Stage 3 is marked by the flattening out of the new head, which becomes spatula-like in appearance. The new head has eye spots around the margin, but no auricles and no pigmentation. From this point in development, fission may occur. Gradually, the auricles appear, and the new head becomes pigmented.

As the tail regrows a new head, it decreases in width. No such consistent change was observed in heads undergoing tail regrowth. In contrast to the heads which are mobile throughout regrowth, the immobile tails only become mobile during Stage 2 (see Calow, Beveridge and Sibly 1979).

The process of regrowth is greatly influenced by temperature. In Table 31, the ranges of regrowth times at 5^{o}, 10^{o} and $15^{o}C$ are shown.

Temperature	Stage 1	Stage 2	Stage 3
5°C	12 - 18 days	30 - 40 days	50 days+
10°C	6 - 8 days	14 - 16 days	25 days+
15°C	5 - 6 days	8 - 9 days	14 days+

Table 31 : The development times of P. felina 'tails' at different temperatures.

Temperature	Stage 1	Stage 2
5°C	8 days	35 days+
10°C	6 days	26 days+
15°C	4 days	20 days+

The development times of P. felina 'heads' at different temperatures.

The whole process of regrowth by a P. felina tail takes only 14 days at 15°C, whereas it takes more than 50 days at 5°C.

Fissioning appears to occur over a wide size range in P. felina, as is illustrated in Table 32a. The influence of temperature, however, has little effect on the size range at which fissioning occurs. An analysis of variance performed on the data shows that there is no significant difference between the ranges observed at 5° and 15°C, although animals at 10°C tend to be bigger when they fission (Table 32b).

The rate of fissioning in P. felina is also greatly affected by temperature. The fates of 5 clones at 5°, 10°, 15° and 20°C were followed for at least 75 days (see Chapter 5). The changes in numbers over this period are summarised in Table 33. At 5°C, 3 of the clones reproduced. Pf 5/5 died shortly after observations began, and Pf 5/1 did not feed, but shrank and died after 104 days. At 10°C, all 5 clones fissioned. At 15°C, 2 of the clones, Pf 15/1 and Pf 15/5, did not fission. Both these animals fed and grew beyond the maximum size at which fissioning had been observed in the laboratory (see above). Fissioning was not observed at 20°C.

The rate of fissioning increased from 5°C to a maximum at 15°C. Differences between clones at each temperature were partly due to animals dying.

When degrowth in P. felina was observed, fissioning did not occur after the first week of starvation at 10° and 15°C. At 5°C, no fissioning occurred at all.

7.4.3 Asexual reproduction in D. tigrina

Under the conditions described above, D. tigrina reproduced only at 15°C and 20°C. The actual process of fission, however, was not observed. Data on fission in D. tigrina is summarised in Table 34.

Temperature (°C)	Animal	Size prior to Fission (mm^2)
5	Pol 5/9	8.39
	3	7.38
	1	11.63
	5	6.84
	3h	7.55
	2	4.55
	4	7.76
	2h	2.80
	10	7.18
	1h	6.45
	8h	9.62
	5h	6.39
	2t	6.12
	3t	10.82
	6h	5.33
	8h	19.41
	7h	8.52
	2h	10.20
	3h	10.50
	4h	9.82
	2ht	13.62
	7t	4.72
	5hh	4.47
	10h	8.69
	3hht	7.63
	3th	5.40
	9t	7.96
	Pf 5/2	5.82
	2h	7.12
	2ht	3.81
	3	6.92
	3h	7.07
	3hh	9.00
	3hhh	12.84
	3ht	6.33
	3t	6.58
	3th	8.19
	4	7.90
	4h	5.35
10	Pol 10/7	12.41
	7h	9.53
	8	31.05
	8t	18.71
	9th	7.33
	8hh	25.66
	1	5.42
	1h	4.84
	1hh	3.44
	2	15.00
	2h	6.22
	2hh	7.42

Temperature (°C)	Animal	Size prior to Fission (mm^2)
10	Pol 10/2hhh	7.00
	3	8.01
	3h	7.58
	3hh	7.17
	3hhh	14.96
	4	5.37
	4h	7.85
	4hh	10.09
	4hhh	8.53
	5	4.06
	5h	5.42
	5hh	7.77
	5hhh	5.22
	6h	12.93
	6htt	4.39
	6hh	10.35
	6ht	5.51
	10h	7.02
	10hh	8.10
	6t	9.08
	5th	7.27
	3th	4.96
	9t	8.30
	1t	12.81
	Pf 10/1	9.80
	1t	4.60
	1th	6.38
	1thh	10.54
	2	8.14
	2h	18.35
	2hh	14.13
	2ht	5.66
	2hht	8.83
	2t	4.31
	2th	9.86
	3	12.50
	4h	10.06
	4h	20.48
	4t	2.60
	4th	3.66
	5	13.00
	5h	15.68
	5ht	8.76
	5hth	9.02
15	Pol 15/1	4.31
	4	6.33
	5	6.35
	2	8.31
	7	10.05
	3	11.00
	1h	4.10

Temperature (°C)	Animal	Size prior to Fission (mm^2)
15	Pol 15/1th	4.31
	4h	6.33
	Pf 15/2	5.54
	2h	4.06
	2ht	5.93
	2t	6.70
	2th	5.25
	3	12.41
	3h	11.85
	3ht	9.08
	3t	6.86
	3th	6.98
	4	10.57
	4h	10.78
	4hh	15.87
	4ht	7.80
	4t	5.10
	4th	6.86
	4thh	8.12
	4th	3.73

Table 32a : The sizes prior to fission of P. felina at 5°, 10° and 15°C

Temperatures	F	d.f.	p
5 - 10°C	2.98	1/94	> 0.01
10 - 15°C	3.00	1/82	> 0.01

Table 32b : One-way analysis of variance performed on data in Table 32a (above) to illustrate differences between size ranges at different temperatures

Temperature	Clone	No. of Animals Produced during 78 days	Mean no. of Animals per Clone at each temperature
5°C	Pf 5/1	No fission	
	2	3	
	3	3	2.67 ± 0.34
	4	2	
	5	dead	
10°C	Pf 10/1	4	
	2	7	
	3	2	4.40 ± 0.81
	4	5	
	5	4	
15°C	Pf 15/1	No fission	
	2	6	
	3	6	7.33 ± 1.74
	4	10	
	5	No fission	
20°C	Pf 20/1	No fission	
	2	dead	
	3	No fission	-
	4	No fission	
	5	No fission	

Table 33 : The influence of temperature on the number of triclads produced by fission during 78 days in different clones of P. felina

Temperature	Animal	Size	% Adult	Degrees
15°C	Dt 15/2h	15.54	73.72	54.15
	2hh	13.12	76.73	61.14
	2th	6.02	63.23	52.65
	2thh	6.03	80.94	64.09
	3h	9.56	64.68	53.55
	3hh	11.90	72.56	58.44
	3th	5.25	64.33	53.31
	4h	9.22	72.14	58.12
	5h	11.58	75.39	60.27
	5hh	14.43	76.96	61.34
	5hhh	12.69	75.04	60.00
	6h	8.92	75.21	60.13
	6hh	13.21	70.15	56.89
20°C	Dt 20/1hh	6.95	78.18	62.17
	1hhh	6.10	84.02	66.42
	1hhhh	7.18	79.96	63.43
	1hhhhh	6.76	80.48	63.79
	1th	3.05	73.14	58.76
	2hh	5.81	77.06	61.41
	2hhh	8.73	87.13	68.95
	2hhhh	6.63	78.65	62.51
	2hhhhh	6.80	80.00	63.43
	2hhhhhh	8.55	86.89	63.94
	2th	2.86	70.09	56.85
	2thh	3.17	76.39	60.44
	2thhh	4.57	83.55	66.08
	2thhhh	5.19	85.08	67.29
	3hh	9.60	73.85	59.26
	3hhh	10.11	84.04	66.42
	3hhhh	8.56	81.99	64.90
	3hhhhh	8.30	78.38	62.31
	3hhhhhh	9.17	83.82	66.27
	3hhhhhhh	9.67	84.53	66.82
	3hhtth	4.97	82.01	64.90

Temperature	Animal	Size	% Adult	Degrees
20°C	Dt 20/3hhth	1.86	60.00	50.77
	3hhthh	3.79	72.19	58.18
	3hhthhh	5.54	84.45	66.82
	3hth	3.60	78.26	62.24
	3th	4.03	79.17	62.87
	5h	9.40	73.55	59.08
	5hh	6.95	78.00	62.03
	5hhh	7.55	85.02	67.21
	5hhhh	8.16	80.16	63.54
	5hhth	3.68	73.75	59.21
	5hth	3.80	73.08	58.76
	5hthh	4.50	78.95	63.41
	5hthhh	6.14	79.64	63.18
	5th	5.00	74.63	59.76
	5thh	4.97	82.28	65.12
	6h	6.87	85.86	67.92
	6hh	6.65	84.07	66.40

Table 34 : The size of D. tigrina heads after fission at 15° and 20°C. Size is expressed in terms of area (mm^2) and as a percentage of total area of fission products (head and tail)

The fission plane is usually post-pharyngeal, and an analysis of variance performed on the data shows that temperature has no effect on the position of the fission plane ($F = 0.56$; d.f. = 1/49; $p > 0.01$).

D. tigrina appears to fission over a considerable size range. In Table 35, the sizes of the animals prior to fission are shown. The range of sizes at which fission occurred is between 6.02 and 22.64 mm^2 at 15°C, and between 1.92 and 10.98 mm^2 at 20°C. An analysis of variance shows that there is a significant difference between the sizes of animals prior to fission at 2 temperatures, and this suggests that at 20°C, D. tigrina fissions at a smaller size ($F = 28.99$; d.f. = 1/49; $p < 0.01$).

Temperature also affects the rate of fission. At 15°C, fission only occurred in 3 out of the 5 clones during the first 28 days of observation (see Table 35). During the same time period at 20°C, fissioning had occurred in all 9 clones, and the rate of fission was considerably higher. The data collected at 20°C show that clones produced from heads, fission at a significantly faster rate than clones from tails ($F = 10.10$; d.f. = 1/6; $p < 0.01$).

Fissioning ceased soon after the onset of starvation (see Chapter 6). Only 1 animal out of 10 fissioned at 15°C (during the first 6 days) and 2 out of 20 at 20°C (during the first 15 days).

7.5 Discussion

Sexual reproduction in P. tenuis is greatly influenced by temperature. Under the imposed laboratory conditions, P. tenuis only produced capsules at 5°, 10° and 15°C. No capsules were produced at 20°C. Viability is highest at 10°C, where only 19% of the capsules are non-viable. At 5° and 15°C, the proportion of viable capsules is considerably lower (31 and 26% non-viable, respectively).

Temperature (°C)	Animal	Size prior to Fission (mm^2)
15	Dt 15/2	22.64
	2h	19.76
	2t	6.02
	3	14.61
	3h	18.36
	3t	5.75
	4	10.11
	5	13.24
	5h	16.68
	5hh	14.43
	6	12.12
	6h	18.93
20	Dt 20/1h	7.08
	1hh	7.75
	1hhh	7.99
	1hhhh	7.18
	1t	3.92
	2h	7.54
	2hh	6.50
	2hhh	8.73
	2hhhh	7.60
	2hhhhh	6.80
	2hhhhhh	8.55
	2t	3.72
	2th	2.86
	2thh	3.17
	2thhh	4.57
	3h	10.98
	3hh	9.60
	3hhh	10.11
	3hhhh	10.23
	3hhhhh	10.73
	3hhhhhh	9.17
	3ht	3.61
	3hht	1.92
	3hhth	4.24
	3hhthh	4.80
	3hhht	2.30
	3t	5.65
	5h	10.89
	5hh	10.28
	5hhh	6.95
	5hhhh	10.20
	5hht	3.25
	5ht	3.43
	5hth	4.51
	5hthh	5.35
	5t	4.27
	5th	5.00
	6	8.28
	6h	8.00

Table 35 : The sizes of D. tigrina prior to fissioning at 15° and 20°C

Temperature	Clone	No. of Animals Produced during 28 days	Mean no. of Animals per Clone at each temperature
15°C	Dt 15/1	dead	
	2	2	
	3	2	2.00
	4	No fission	
	5	No fission	
	6	2	
20°C	Dt 20/1h	5	
	1t	2	
	2h	7	
	2t	5	
	3h	12	
	3t	2	5.33 ± 1.14
	4	dead	
	5h	9	
	5t	3	
	6	3	

Table 36 : Reproduction in D. tigrina at 15° and 20°C

Temperature also exerts an influence on the development of hatchlings. The development time and temperature are inversely related, and capsules produced at high temperatures (10° and 15°C) result in larger numbers of hatchlings. Temperature exacerbates the effects of starvation on reproduction. As temperature is increased, there is a reduction in the period of reproduction after the onset of starvation, and a fall in the numbers of capsules produced. Capsule weight also decreases during this period, and is adversely affected by temperature.

Reynoldson, Young and Taylor (1965) and Pattee (1972) found that P. tenuis does produce capsules at 20°C, but comparatively few. Pattee (ibid) claims that P. tenuis can produce capsules at temperatures up to 22.5°C, but at 20°C and above, the viability of the capsules is very low (< 24%). Both Reynoldson, Young and Taylor (1965) and Pattee (1972) reported a decrease in the incubation period with increasing temperature. However, Pattee (ibid) recorded a much higher value for the mean number of young per capsule at 5°C, than is observed in this study (c.f. 8.2/capsule and 4.1/capsule). Pattee also found that the numbers per capsule fell with increasing temperature.

Differences between studies are probably due to differences between populations, and feeding regimes.

Fissioning in P. felina and D. tigrina is fairly similar, and usually post-pharyngeal. Although temperature affects the position of the fission plane in D. tigrina, nevertheless no pre-pharyngeal fissions were observed in this species. There appears to be a larger variation in the position of the fission plane in P. felina (Calow, Beveridge and Sibly 1979), and recent observations by Baird (unpublished data) on P. felina collected from streams around Loch Baile à Ghobhainn,

Lismore, corroborate this. Baird noted that in 12% of those animals that had recently fissioned, the fission plane was pre-pharyngeal.

The few observations made on the Balmaha Pond and Lossit Loch populations suggest that there may be differences between populations in the position of the fission plane, but this is complicated by the fact that there is a considerable amount of variation within any 1 population.

The process of regrowth in P. felina is similar to that in D. tigrina (Curtis 1902; Pickavance 1968) and D. dorotocephala (Nentwig 1978), which suggests that the same mechanisms may be involved, although ultrastructural studies would be necessary to prove this. P. felina and D. tigrina differ in the range of temperatures over which they are capable of fissioning.

Within the range at which fissioning may occur, food and temperature govern the rates of fission in both species. When food is removed, and animals starved, fissioning ceases almost immediately. In this respect the reproductive strategy of the fissioning species studied here resemble that of perennial sexual reproducers such as P. tenuis. Annual species (e.g. D. lacteum) can continue reproducing for a considerable period of time after the onset of starvation (Calow and Woollhead 1977b).

Within the temperature ranges at which they reproduce, an increase in temperature results in an increase in the rate of fission in both species. Other factors can also affect fission : heads fission faster than tails, which may in part be due to differences in the rates of growth.

In order to compare fissioning in the 2 species, and with previously published figures, R, the intrinsic rate of natural increase

has been calculated for both species at each temperature, where

$$R = \frac{d\log_e N}{dt} = \text{rate of change in numbers per unit time}$$

The results are summarised in Table 37. Although D. tigrina is incapable of fissioning below 15°C, it nevertheless is able to reproduce at a much higher rate than is found in P. felina.

Some care is required when comparing these figures with published data, since conditions under which reproductive performance has been measured, vary. Pickavance (1968) recorded a higher value for R in D. tigrina at 15-16°C (0.022), and a lower value at 18-20°C (0.023). Although working on the same population, differences in temperature and culture conditions are probably responsible.

Pattee (1969) recorded much lower R values in P. felina at 5° and 10°C. However, at 15°C the R values are very similar (c.f. 0.019 and 0.020). Even under different culture conditions, it is likely that inter-population differences are responsible i.e. the northern (Balmaha Pond Outflow) population reproduces faster at lower temperatures. Dahm (1958) observed large intraspecific variations in the rates of reproduction in both P. felina and D. tigrina, which he attributed to basic genetic differences between populations.

Comparisons between the sexual and asexual reproducers, and the adaptive significance of asexual reproduction will be considered in Chapter 10, when the results from the sections on physiology, ecology and energy partitioning will be examined together.

Species	5°C	10°C	15°C	20°C
P. felina	0.010	0.019	0.020	
D. tigrina	-	-	0.017	0.060

Table 37: The R values (change in numbers with time) for P. felina and D. tigrina at different temperatures. Data is derived from Tables 33 and 35. Mortalities are included.

Chapter 8

BIOENERGETICS

8.1 Introduction

Reynoldson (1961) has suggested that there is a relationship between reproduction and environment in freshwater triclads, and that asexual reproduction is the result of poor food supply exacerbated by high metabolic costs.

In previous chapters I have looked at the influence of temperature on feeding, respiration, growth and reproduction in P. tenuis, P. felina and D. tigrina. In this section, I wish to draw together the results from all those chapters, and to consider the effects of temperature and food on the energy partitioning in the 3 species. Thus, I hope to illuminate any differences that exist between species, in the energy intake and the subsequent partitioning of energy among growth, metabolic costs, and reproduction.

8.2 Literature Review

Klekowski and Duncan (1975) define bioenergetics as "the study of energy transformations in living organisms". The study of bioenergetics may be according to any one of 3 approaches: the molecular-biochemical, the physiological and the ecological. The molecular-biochemical approach is concerned with energy transformations at the molecular and sub-molecular level. There is, however, some disagreement as to the definitions of physiological and ecological energetics. Wiegert (1968) chooses to rigorously differentiate between the 2 terms, and defines physiological energetics as dealing "with the utilisation of energy by the resting, post-absorbtive individual", whereas ecological energetics "encompasses the energy costs of the individual in a growing, reproducing population of organisms". Others (e.g. Phillipson 1975) doubt whether the 2 approaches can be entirely

separated, since both are concerned with whole animals.

The study of bioenergetics is comparatively recent, and initially developed through interest in the production of domestic animals (see Brody 1945 for review). However its potential contribution to the understanding of how nature functions, was quickly grasped by ecologists, and stimulated the classic studies of Ivlev (1939, 1945), Lindeman (1942), Teal (1957) and Odum (1957). The recent International Biological Programme has furthered this line of research, through the publication of a series of handbooks, principally concerned with ecological bioenergetics.

The bioenergetics of many freshwater invertebrate groups have now been studied in detail, e.g. Crustacea (Conover 1966; Prus 1972; Adcock 1979), Odonata (Lawton 1970, 1971; Fischer 1972), Trichoptera (Otto 1975; McCulloch, Minshall and Cushing 1979) and Diptera (Kimmerle and Anderson 1971; McCulloch, Minshall and Cushing 1979).

Few studies have examined the bioenergetics of freshwater triclads. Teal (1957) computed an approximate energy budget for Ph. vitta. In a series of papers, Calow (1977a), Calow and Woollhead (1977a, b), Woollhead and Calow (1979) and Woollhead (1979) have examined the bioenergetics of several lake-dwelling species of triclad and contrasted the energy partitioning strategies of semelparous, annual species (e.g. D. lacteum) with iteroparous, perennial species (e.g. P. tenuis), under various food peturbations. Among reproducing adult triclads, they found that D. lacteum partitioned more energy (in absolute terms, and relative to ingested energy) into reproduction than P. tenuis, and that during conditions of food deprivation, D. lacteum increased its reproductive output, whereas P. tenuis reduced

its investment under the same conditions (Woollhead 1979; Woollhead and Calow 1979).

Differences were also found in the partitioning of resources between starving, non-reproducing individuals of both species. P. tenuis continued searching for prey, whereas D. lacteum became quiescent. This difference in locomotory strategy when faced with starvation resulted in the consistently higher metabolic rate of P. tenuis (Calow and Woollhead 1977a).

No work, however, has been carried out on the bioenergetics of D. tigrina or P. felina. The influence of temperature on the bioenergetics of triclads has also not been established. In this part of the study I intend to look at the influence of temperature on energy partitioning in P. tenuis, P. felina and D. tigrina in non-reproducing adults.

8.3 Materials, Methods and Preliminary Obsevations

8.3.1 Input (I)

The methods used in estimating input (food ingested) are detailed in Chapter 4, Section 3. Estimates of input were expressed as mg dry Asellus, and then converted to joules, employing a mean joule-equivalent obtained by bomb-calorimetry using a Phillipson microbomb and the standard procedure described by Phillipson (1964). Several samples were taken in order to measure any seasonal variation. Since the triclads fed on the body contents of Asellus (see Chapter 4), it was assumed that the input would be free of exoskeleton, and therefore energy ingested was calculated in terms of joules per ash-free dry gram of tissue. The ash content was estimated from 2 separate samples of

Asellus (a winter and a summer sample) taken from the field, and heated to 600°C for 3 hours in a muffle furnace. The results are summarised in Tables 38a and 38b.

Seasonal variations in energy content have been measured in many freshwater invertebrates (e.g. Comita, Marshall and Orr 1966; Schindler, Clark and Fray 1968; Wissing and Hassler 1971), including Asellus (Rodgers and Quadri 1977). However, differences between the maximum and minimum joule-equivalents recorded in summer and winter populations were not significantly different ($F = 8.76$; $df = 31$; $p > 0.01$), and therefore a final mean value of 23.60 Jmg^{-1} ash-free dry Asellus was used. This value is similar to that of 22.65 Jmg^{-1} used by Woollhead (1979) and Woollhead and Calow (1979).

8.3.2 Growth (G) and Degrowth (Dg)

The photographic methods used in measuring triclad size in terms of area, and the conversion equations which were subsequently used in converting plan area into dry weight, are detailed in Chapter 3. Joule-equivalents were then computed by combusting groups of triclads in a Phillipson microbomb. The results are presented in Table 39. The ash-free values presented in the Table were calculated by assuming a 5% ash content. Calow and Woollhead (1977a) found that the ash content of D. lacteum varied between 4.88 and 4.98%, and that ash accounted for 4.74 - 5.09% dry weight of P. tenuis. Caspers (1975) reported similar values for C. alpina (5.77%) and D. gonocephala (6.35%).

The calorific value of P. tenuis is slightly higher than that of the other 2 species, and at 10°C differs little from the value obtained by Calow and Woollhead (1977a) using biochemical methods (c.f. 28.43 and 28.32 Jmg^{-1} ash-free).

Time of Sample	No. of *Asellus*	Mean Energy Equivalent (Joules/mg dry *Asellus*)	Mean Energy Equivalent (Joules/ash-free mg dry *Asellus*)
July	10	17.85 ± 1.43	24.59
December	12	15.97 ± 1.47	22.37
April	10	17.30 ± 1.61	23.83

Table 38a : The joule-equivalents of *Asellus* at different times of the year, expressed in terms of dry weight and ash-free dry weight

Time of Sample	No. of Animals	Mean Ash Content of Sample	Range
Winter	20	28.6%	27.5 - 29.8
Summer	20	27.4%	26.2 - 28.9

Table 38b : The ash content of groups of *Asellus*, expressed in terms of percentage of dry body weight

Species	Temperature	Approx. Size Range Combusted	No. Combusted	Energy Equivalent (J mg^{-1})	Energy Equivalent (J mg^{-1} ash-free*)
P. tenuis	5°C	10 - 30 mm^2	10	26.94	28.36
	10°C	10 - 30 mm^2	10	26.97	28.39
	15°C	10 - 30 mm^2	10	26.82	28.23
	20°C	10 - 30 mm^2	7	26.79	28.39
P. felina	5°C	5 - 15 mm^2	10	25.58	26.86
	10°C	5 - 15 mm^2	9	25.41	26.68
	15°C	5 - 15 mm^2	10	25.36	26.63
	20°C	10 - 20 mm^2	7	25.39	26.66
D. tigrina	5°C	5 - 15 mm^2	5	25.47	26.74
	10°C	5 - 15 mm^2	8	25.81	27.10
	15°C	5 - 15 mm^2	10	25.76	27.05
	20°C	5 - 15 mm^2	10	25.32	26.59

Table 39 : The energy equivalents (J mg^{-1}) of groups of P. tenuis, P. felina and D. tigrina cultured at 5°, 10°, 15° and 20°C combusted in a Phillipson microbomb

* Ash content assumed to be 5% (see test)

8.3.3 Respiration (R)

Energy losses through respiration were estimated by the methods detailed in Chapter 5. The linear relationships between respiration rate (R), and triclad size (plan area; A) were described by R/A values ($\mu lO_2^{-1}mm^{-2}hr^{-1}$), which were influenced by temperature, and by starvation. Therefore, respiratory heat losses may be derived from:

$$\text{Resp.} = \sum_{n}^{o} (R/A.Ox.A)$$

where: $\sum_{n}^{o}$ = the period over which the observations were made (between day 0, and day n); R/A = daily oxygen consumption of a particular species under defined feeding and temperature regimes; Ox. = oxyjoule equivalent = 19.5 x $10^{-3}J\mu L^{-1}$oxygen inspired (Calow and Woollhead 1977a); A = area of worm on a particular day (from growth/degrowth curves).

8.3.4 Excretion (Exc) and Secretion (Sec)

These 2 components of the energy budget are notoriously difficult to measure in triclads, and have in the past been estimated from the difference between energy input and energy loss (Teal 1957; Calow and Woollhead 1977a; Woollhead 1979; Wollhead and Calow 1979) i.e. (I + Dg) - (Resp. + Rep. + G) = Exc. + Sec. Woollhead and Calow (1979) argue that energy losses through excretion are likely to be negligible, since there is little evidence of faecal production by triclads, and since excretion is in the form of ammonia (Hyman 1951), a low-energy compound. Thus absorbed energy is equivalent to energy ingested. The imbalance between energy derived from food and tissue catabolism, and that used in reproduction, growth and respiration, has

been assumed to be due to losses through the secretion of mucus (Calow and Woollhead 1977a; Woollhead 1979; Woollhead and Calow 1979). Triclads also secrete rhabdites (Hyman 1951; Jennings 1957), ~~but~~ and these are believed to be produced in significant numbers ~~only~~ during anti-predator defense (see Chapter 9).

In this study, for the purpose of constructing energy budgets, excretory and secretory losses will be estimated indirectly as described below, and the results compared with those computed indirectly.

Triclads which had been acclimated to 10°C, were transferred to sterile perspex pots (1 per pot) containing 30 mls of boiled, filtered loch water. Each triclad had been fed and allowed to digest its meal for 12 hours before being transferred to the clean pots. The dishes of triclads were then incubated at 10°C. After a period of time, each triclad was removed from its dish, and the contents poured into a measuring cylinder. The sides and bottom of the dish were carefully cleaned with distilled water, and washed into the cylinder where the volume was made up to 75 mls. (Previous trials had shown that this method removed all trace of mucus. When carmine red was added to a dish after cleaning, none adhered to the sides). The mucus and water was filtered through a Millipore filter paper (Type HA; 0.45µ pore size), which was then dried in a vacuum oven at 40°C for at least 72 hours. An analysis of the filtrate, using the standard materials and methods described by Golterman (1970), in which any polysaccharides are first reduced to simple sugars, showed that no sugars were present. It was therefore assumed that triclad mucus is largely insoluble, and that filtration by the method described above, removed all the suspended mucus.

Millipore filter papers lose weight during use (A.D. Pickering,

pers. comm.). In this case, the papers were 0.2 mg lighter after 75 mls of distilled water had been filtered through. The mean weight of a used filter paper was 24.70 ± 0.26 mg.

The energy content of mucus was estimated by combusting filter papers and mucus in a Phillipson microbomb, using standard methods (Phillipson 1963). Pre-washed papers were combusted in order to determine their energy content. The mean energy equivalent of the Millipore filter papers (4 samples) was 12.64 ± 0.88 Jmg^{-1} dry weight. The energy content of an average Millipore paper (after 75 mls of distilled water has been filtered through), is therefore 312.21 ± 25.24 J.

Table 40 summarises the energy equivalent of P. tenuis and P. felina mucus. Both values are lower than the joule-equivalents of carbohydrate, fat or protein (Cummins and Wuychek 1971), which suggests that triclad mucus is a low energy compound. The joule-equivalent value of P. tenuis mucus is slightly higher than that of P. felina, although this is not significant ($t = 1.42$; d.f. 3.77; $p > 0.05$). In a biochemical analysis of mucus, Rahemtulla and Løvtrup (1974) found little difference between cestodes and turbellaria.

Mucus production in P. felina was estimated by culturing 3 similar-sized triclads (8 - 10 mm^2) at 10°C for 24, 48 and 96 hours. The mucus produced was collected and weighed as described above. The results are summarised in Table 41a. P. felina produced approximately 0.6 mg dry wt. of mucus during the first 24 hours. However, production appears to decrease with time. This could be due to the growth of bacteria in the dishes, and the subsequent breakdown of the triclad mucus, although boiled, filtered loch water, and sterile dishes were used in order to minimise the risk of infection, and Calow (1978b)

Species	Weight of Paper and Mucus	Joule Equivalent of Paper and Mucus	Joule Equivalent of Mucus	Mean Joule Equivalent of Mucus
P. tenuis	27.45 mg	364.64 J	19.07 J mg^{-1}	
	28.15 mg	367.45 J	16.01 J mg^{-1}	
	27.10 mg	369.38 J	23.82 J mg^{-1}	
				19.63 ± 2.27 J mg^{-1}
P. felina	27.05 mg	346.56 J	14.62 J mg^{-1}	
	27.15 mg	362.88 J	20.68 J mg^{-1}	
	27.60 mg	360.25 J	16.57 J mg^{-1}	
				17.29 ± 1.76 J mg^{-1}

Table 40 : The energy equivalents (J mg^{-1} dry wt) of mucus produced by *P. tenuis* and *P. felina*

Time Period over which Mucus Production Measured	Weight of Paper and Mucus	Weight of Mucus Produced
24 hours	25.32 mg	0.62 ± 0.26 mg dry wt.
48 hours	26.05 mg	1.35 ± 0.26 mg dry wt.
96 hours	25.55 mg	0.85 ± 0.26 mg dry wt.

Table 41a : Weight of mucus produced by 3 similar-sized *P. felina* (8-10 mm^2) at 10°C, after 24, 48 and 96 hours incubation

Triclad Size	Weight of Mucus Produced (mg) in 24 hours
3.11 mm^2	0.29 ± 0.26 mg
3.15 mm^2	0.29 ± 0.26 mg
4.35 mm^2	0.54 ± 0.26 mg
4.48 mm^2	1.10 ± 0.26 mg
4.92 mm^2	0.88 ± 0.26 mg
8.78 mm^2	0.07 ± 0.26 mg
8.87 mm^2	0.68 ± 0.26 mg
9.52 mm^2	0.35 ± 0.26 mg
10.05 mm^2	0.52 ± 0.26 mg

Table 41b : Weight of mucus produced by starved *P. felina* at 10°C, over a period of 24 hours

reported that triclad mucus is highly resistant to bacterial breakdown. A more plausible explanation is that the introduction of a triclad into a clean dish stimulates the animal to explore its new environment, and thus cover the dish in mucus trails. After an initial period of high activity, the animal would become relatively quiescent. This is borne out by observations on triclad activity under different light conditions (see Chapter 3 above). Thus the effects of frequently changing or cleaning dishes would be to stimulate activity, and dramatically increase mucus production, which could have a considerable effect on the energy budget.

The influences of triclad size and starvation on mucus production was studied in a group of P. felina. Nine triclads, which had been starved at 10°C, for 6 weeks were photographed, before being transferred to clean perspex pots. After 24 hours, the worms were removed, and the mucus collected in the manner described above. The results are shown in Table 41b.

No significant relationship was found between triclad size, and amount of mucus produced ($r = 0.19$; $p(r) > 0.01$). However, the amount of mucus produced by the starved P. felina is much less than that produced by similar-sized animals which were fed twice per week (Compare the results from Tables 40a and 40b).

8.4 Results

The energy budgets of fed and starved triclads will be considered separately. Differences between species and between temperatures have been statistically evaluated in the chapters dealing with feeding, respiration and growth. Therefore, no statistical analysis of the results is necessary here.

8.4.1 Fed triclads

The energy budgets of P. tenuis, P. felina and D. tigrina at 5°, 10°, 15° and 20°C are presented in Tables 42a, 42b and 42c. These were constructed as described above, using regressing lines for I, R/A and k values, converted into energy terms (J). The budgets are concerned with the partitioning of energy over a 10 day period. The rapid growth and subsequent fission of D. tigrina at 20°C, and P. felina at 15°C, restricted the time interval over which a relevant energy budget could be constructed. The computations are based on triclads whose initial size was 5 mm^2.

In P. tenuis, food intake, metabolic expenditure and the amount of energy partitioned into growth, increase with temperature. However, temperature also exerts a considerable effect on the partitioning of absorbed energy. The proportion of ingested food partitioned into growth (G/I) is used here as a measure of growth efficiency. Growth efficiency increases with temperature to a maximum of 64.42% at 15°C. The relative proportion of input energy expended in respiration (R/I) is also at a maximum at 15°C.

Input increases with temperature to a maximum at 15°C in P. felina (Table 42b). Energy partitioned into growth and metabolic costs are also highest at 15°C. However, temperature has little effect on growth or metabolic efficiencies. R/I values, for example, remain between 8.54 and 12.15% throughout the temperature range. At 5°C, D. tigrina degrows over the 10 day budget period, thus making more energy available for partitioning into respiration, mucus production, and excretion. The R/I value in this case has been derived from R/(I + Dg). Food intake, growth and respiration all increase with temperature. Growth efficiency also increases to a maximum at 15°C.

	5°C	10°C	15°C	20°C
Initial Size	5 mm^2	5 mm^2	5 mm^2	5 mm^2
$\overline{K}$	0.0100 ± 0.0016	0.0228 ± 0.0020	0.0310 ± 0.0074	0.0396 ± 0.0046
Final Size	5.52 ± 0.10 mm^2	6.28 ± 0.12 mm^2	6.81 ± 0.50 mm^2	7.43 ± 0.34 mm^2
Increase in Weight	0.067 ± 0.013 mg	0.165 ± 0.016 mg	0.235 ± 0.064 mg	0.313 ± 0.044 mg
Joule Equivalent (triclad)	26.94 J mg^{-1}	26.97 J mg^{-1}	26.82 J mg^{-1}	26.79 J mg^{-1}
J	1.80 ± 0.35	4.45 ± 0.043	6.30 ± 1.72	8.39 ± 1.18
b value (Input)	0.0318 ± 0.0080	0.0262 ± 0.0080	0.0372 ± 0.0100	0.0688 ± 0.0200
I (7 days)	0.181 ± 0.042 mg	0.282 ± 0.034 mg	0.290 ± 0.059 mg	0.441 ± 0.123 mg
I (10 days)	0.258 ± 0.034 mg	0.403 ± 0.048 mg	0.414 ± 0.084 mg	0.630 ± 0.176 mg
Joule Equivalent (food)	23.60 J mg^{-1}	23.60 J mg^{-1}	23.60 J mg^{-1}	23.60 J mg^{-1}
J	6.09 ± 1.42	9.51 ± 1.14	9.78 ± 1.99	14.86 ± 4.15
Mean R/A	0.027 ± 0.006	0.070 ± 0.006	0.081 ± 0.006	0.083 ± 0.006
Respiration (10 days)	34.22 ± 7.60 µl	95.42 ± 8.18 µl	115.73 ± 8.57 µl	124.62 ± 9.01 µl
Oxyjoule Equivalent	19.5 x 10^{-3} J µl^{-1}	19.5 x 10^{-3} J µl^{-1}	19.5 x 10^{-3} J µl^{-1}	19.5 x 10^{-3} J µl^{-1}
J	0.67 ± 0.15	1.86 ± 0.16	2.26 ± 0.17	2.43 ± 0.18
Mucus + Excretion (J)	3.62 ± 1.89	3.20 ± 1.73	1.22 ± 3.88	4.04 ± 5.51
Muc + Exc/I	59.44%	33.65%	12.47%	27.18%
G/I	29.56%	46.79%	64.42%	56.46%
R/I	11.00%	19.56%	23.11%	16.35%

Table 42a : Ten-day energy budgets constructed for P. tenuis at 5°, 10°, 15° and 20°C, fed 2 x per week on Asellus. Figures include confidence limits (± 2 Standard Errors)

	5°C	10°C	15°C	20°C
Initial Size	5 mm^2	5 mm^2	5 mm^2	5 mm^2
$\overline{K}$	0.0222 ± 0.0060	0.0420 ± 0.0112	0.0426 ± 0.0120	0.0303 ± 0.0042
Final Size	6.24 ± 0.39 mm^2	7.61 ± 0.90 mm^2	7.66 ± 0.97 mm^2	6.77 ± 0.29 mm^2
Increase in Weight	0.084 ± 0.027 mg	0.177 ± 0.062 mg	0.181 ± 0.066 mg	0.120 ± 0.020 mg
Joule Equivalent (triclad)	25.58 J mg^{-1}	25.41 J mg^{-1}	25.36 J mg^{-1}	25.39 J mg^{-1}
J	2.15 ± 0.69	4.50 ± 1.58	4.59 ± 1.67	3.05 ± 0.51
b value (Input)	0.0098 ± 0.0260	0.0130 ± 0.0120	0.0308 ± 0.0120	0.401 ± 0.0580
I (7 days)	0.295 ± 0.145 mg	0.471 ± 0.076 mg	0.564 ± 0.076 mg	0.521 ± 0.342 mg
I (10 days)	0.421 ± 0.207 mg	0.673 ± 0.109 mg	0.806 ± 0.109 mg	0.744 ± 0.488 mg
Joule Equivalent (food)	23.60 J mg^{-1}	23.60 J mg^{-1}	23.60 J mg^{-1}	23.60 J mg^{-1}
J	9.94 ± 4.89	15.88 ± 2.57	19.02 ± 2.57	17.56 ± 11.44
Mean R/A	0.039 ± 0.005	0.065 ± 0.004	0.067 ± 0.004	0.054 ± 0.006
Respiration (10 days)	52.98 ± 6.72 µl	99.00 ± 6.05 µl	102.41 ± 6.09 µl	76.83 ± 8.47 µl
Oxyjoule Equivalent	19.5 x 10^{-3}Jµl^{-1}	19.5 x 10^{-3}Jµl^{-1}	19.5 x 10^{-3}Jµl^{-1}	19.5 x 10^{-3}Jµl^{-1}
J	1.03 ± 0.13	1.93 ± 0.12	2.00 ± 0.12	1.50 ± 0.17
Mucus + Excretion (J)	6.76 ± 5.71	9.45 ± 4.27	12.43 ± 4.36	13.01 ± 12.12
Muc + Exc/I	68.01%	59.51%	65.35%	74.09%
G/I	21.63%	28.34%	24.13%	17.37%
R/I	10.36%	12.15%	10.52%	8.54%

Table 42b : Ten-day energy budgets constructed for P. felina at 5°, 10°, 15° and 20°C, fed 2 x per week on Asellus. Figures include confidence limits (± 2 Standard Errors)

	5°C	10°C	15°C	20°C
Initial Size	5 mm^2	5 mm^2	5 mm^2	5 mm^2
$\overline{K}$	-0.0015 ± 0.0018	0.0029 ± 0.0004	0.0362 ± 0.0024	0.0852 ± 0.0364
Final Size	4.93 ± 0.09 mm^2	5.15 ± 0.02 mm^2	7.18 ± 0.18 mm^2	11.72 ± 5.11 mm^2
Increase in Weight	-0.004 ± 0.009 mg	0.009 ± 0.001 mg	0.129 ± 0.011 mg	0.396 ± 0.301 mg
Joule Equivalent (triclad)	25.47 J mg^{-1}	25.81 J mg^{-1}	25.76 J mg^{-1}	25.32 J mg^{-1}
J	0.10 ± 0.23	0.23 ± 0.03	3.32 ± 0.28	10.03 ± 7.62
b value (Input)	0.0042 ± 0.0100	0.0193 ± 0.0260	0.0270 ± 0.0220	0.0110 ± 0.0420
I (7 days)	0.092 ± 0.049 mg	0.200 ± 0.132 mg	0.240 ± 0.134 mg	0.260 ± 0.350 mg
I (10 days)	0.131 ± 0.070 mg	0.286 ± 0.189 mg	0.343 ± 0.191 mg	0.371 ± 0.500 mg
Joule Equivalent (food)	23.60 J mg^{-1}	23.60 J mg^{-1}	23.60 J mg^{-1}	23.60 J mg^{-1}
J	3.10 ± 1.65	6.75 ± 4.45	8.09 ± 4.52	8.76 ± 11.80
Mean R/A	0.009 ± 0.004	0.026 ± 0.004	0.039 ± 0.004	0.074 ± 0.004
Respiration (10 days)	10.71 ± 4.76 µl	31.69 ± 4.86 µl	60.27 ± 6.18 µl	146.22 ± 7.90 µl
Oxyjoule Equivalent	19.5 x 10^{-3} J µl^{-1}	19.5 x 10^{-3} J µl^{-1}	19.5 x 10^{-3} J µl^{-1}	19.5 x 10^{-3} J µl^{-1}
J	0.21 ± 0.09	0.62 ± 0.10	1.18 ± 0.12	2.85 ± 0.15
Mucus + Excretion (J)	2.99 ± 1.97	5.90 ± 10.48	3.59 ± 4.92	
Muc + Exc/I	93.45%	87.40%	44.38%	
G/I	-	3.41%	41.04%	
R/I	6.55%	9.19%	14.59%	

Table 42c : Ten-day energy budgets constructed for D. tigrina at 5°, 10°, 15° and 20°C, fed 2 x per week on Asellus. Figures include confidence limits (± 2 Standard Errors)

(N.B. No efficiences could be calculated for D. tigrina at 20°C, due to an imbalance between energy income, and energy expenditure. See Section 8.5 below, for discussion).

8.4.2 Starved triclads

Energy budgets have been calculated for triclads shrinking from 20 mm^2 to 10 mm^2 (i.e. 50% initial size). The initial triclad size of 20 mm^2 was chosen for several reasons. D. tigrina cultured in the laboratory or collected from the field rarely exceeded 20 mm^2, and P. felina also usually fissioned before reaching this size. Previous budgets constructed for P. tenuis by Calow and Woollhead (1977a, 1977b) were based on animals of initial size = 20 mm^2.

The results are summarised in Tables 43a, 43b, 43c. The time taken to degrow from 20 mm^2 to 10 mm^2 (i.e. from A_i to $0.5A_i$) is given by $\log_e 0.5/\overline{k}$. Respiratory losses were derived from size-time curves using R/A values.

The time taken to reach $0.5A_i$ in P. tenuis fell from 169 days at 5°C, to 69 days at 15°C. Temperature had little influence on the partitioning of energy derived from tissue catabolism. At all temperatures, over half of the energy made available during degrowth was used up in respiration.

In P. felina the time taken to shrink to $0.5A_i$ fell from 101 days at 5°C to 45 days at 15°C. Temperature affected energy partitioning dramatically. At 5°C, over 90% of the energy derived from catabolism was respired. At 10° and 15°C, respiratory demands were much lower - 43.52 and 45.82% respectively. However, respiratory demands rose again at 20°C, where over 82% of catabolised energy was used in respiration.

	5°C	10°C	15°C	20°C
Initial Size	20 mm^2	20 mm^2	20 mm^2	20 mm^2
Final Size	10 mm^2	10 mm^2	10 mm^2	10 mm^2
$\bar{k}$	0.0041 ± 0.0006	0.0081 ± 0.0006	0.0101 ± 0.0012	0.0091 ± 0.0014
Decrease in Weight	1.29 mg	1.29 mg	1.29 mg	1.29 mg
Joule Equivalent	26.94 J mg^{-1}	26.97 J mg^{-1}	26.82 J mg^{-1}	26.79 J mg^{-1}
J	34.75	34.79	34.60	34.56
Time Interval	169 ± 12 days	86 ± 6 days	69 ± 8 days	76 ± 10 days
R/A	0.018 ± 0.004	0.031 ± 0.008	0.044 ± 0.008	0.039 ± 0.006
O_2 inspired over this time	1177.55 ± 346.61 µl	959.76 ± 314.64 µl	1092.96 ± 325.44 µl	1067.04 ± 304.58 µl
Oxyjoule Equivalent	19.5 x 10^{-3}J μl^{-1}	19.5 x 10^{-3}J μl^{-1}	19.5 x 10^{-3}J μl^{-1}	19.5 x 10^{-3}J μl^{-1}
J	22.96 ± 6.76	18.72 ± 6.14	21.31 ± 6.35	20.81 ± 5.94
Mucus + Excretion (J)	11.79 ± 6.76	16.07 ± 6.14	13.29 ± 6.35	13.75 ± 5.94
Muc + Exc/C	33.93%	46.19%	38.41%	39.79%
R/C	66.07%	53.81%	61.59%	60.21%

Table 43a : Energy budgets constructed for starving P. tenuis during degrowth from 20 mm^2 to 10 mm^2, at 5°, 10°, 15° and 20°C. Figures include confidence limits (± 2 Standard Errors)

	5°C	10°C	15°C	20°C
Initial Size	20 mm^2	20 mm^2	20 mm^2	20 mm^2
Final Size	10 mm^2	10 mm^2	10 mm^2	10 mm^2
$\overline{k}$	0.0065 ± 0.0012	0.0135 ± 0.0036	0.0155 ± 0.0034	0.0150 ± 0.0044
Decrease in Weight	0.68 mg	0.68 mg	0.68 mg	0.68 mg
Joule Equivalent	25.58 J mg^{-1}	25.41 J mg^{-1}	25.36 J mg^{-1}	25.39 J mg^{-1}
J	17.39	17.28	17.24	17.27
Time Interval	101 ± 17 days	51 ± 10 days	45 ± 8 days	46 ± 10 days
R/A	0.021 ± 0.008	0.021 ± 0.006	0.025 ± 0.004	0.044 ± 0.006
O_2 inspired over this time	808.92 ± 436.68 µl	385.56 ± 185.76 µl	405.00 ± 136.80 µl	728.64 ± 257.76 µl
Oxyjoule Equivalent	19.5 x 10^{-3}Jµl^{-1}	19.5 x 10^{-3}Jµl^{-1}	19.5 x 10^{-3}Jµl^{-1}	19.5 x 10^{-3}Jµl^{-1}
J	15.77 ± 8.52	7.52 ± 3.62	7.90 ± 2.67	14.21 ± 5.03
Mucus + Excretion (J)	1.69 ± 8.52	9.76 ± 3.62	9.34 ± 2.67	3.06 ± 5.03
Muc + Exc/C	9.32%	56.48%	54.18%	17.72%
R/C	90.68%	43.52%	45.82%	82.28%

Table 43b : Energy budgets constructed for starving P. felina during degrowth from 20 mm^2 to 10 mm^2, at 5°, 10°, 15° and 20°C. Figures include confidence limits (± 2 Standard Errors)

	5°C	10°C	15°C	20°C
Initial Size	20 mm^2	20 mm^2	20 mm^2	20 mm^2
Final Size	10 mm^2	10 mm^2	10 mm^2	10 mm^2
$\bar{k}$	0.0052 ± 0.0008	0.0109 ± 0.0014	0.0196 ± 0.0010	0.0388 ± 0.0038
Decrease in Weight	0.59 mg	0.59 mg	0.59 mg	0.59 mg
Joule Equivalent	25.47 J mg^{-1}	25.81 J mg^{-1}	25.76 J mg^{-1}	25.32 J mg^{-1}
J	15.03	15.23	15.20	14.94
Time Interval	133 ± 17 days	64 ± 8 days	35 ± 1 day	18 ± 2 days
R/A	0.007 ± 0.002	0.017 ± 0.006	0.041 ± 0.010	0.040 ± 0.004
O_2 inspired over this time	335.16 ± 138.60 µl	391.68 ± 187.20 µl	516.68 ± 140.76 µl	259.20 ± 29.12 µl
Oxyjoule Equivalent	19.5 x 10^{-3}Jµl^{-1}	19.5 x 10^{-3}Jµl^{-1}	19.5 x 10^{-3}Jµl^{-1}	19.5 x 10^{-3}Jµl^{-1}
J	6.54 ± 2.70	7.64 ± 3.65	10.07 ± 2.74	5.05 ± 0.57
Mucus + Excretion (J)	8.49 ± 2.70	7.59 ± 3.65	5.13 ± 2.74	9.89 ± 0.57
Muc + Exc/C	56.49%	49.84%	33.75%	66.20%
R/C	43.51%	50.16%	66.25%	33.80%

Table 43c : Energy budgets constructed for starving D. tigrina during degrowth from 20 mm^2 to 10 mm^2, at 5°, 10°, 15° and 20°C. Figures include confidence limits (± 2 Standard Errors)

The time taken to reach $0.5A_i$ by D. tigrina was greatly influenced by temperature, and fell from a maximum of 133 days at 5°C to 18 days at 20°C. As temperature increased, the partitioning of energy derived from catabolism into R and Muc + Exc, changed. Respiratory demand rose from 43.51% at 5°C to 66.25% at 15°C. At 20°C, however, respiration only accounted for 33.80% of the available energy.

8.5 Discussion

The validity of using mean values to calculate energy budgets in triclads has been tested by Woollhead and Calow (1979), who showed that there was no significant difference between values derived from individual budgets and budgets constructed from mean values.

Before discussing the above results, it is important to draw attention to several assumptions implicit in the budgets. First, the energy equivalent of O_2 consumption used, is based on the figures obtained by Calow and Woollhead (1977a) for catabolised triclad tissue. The energy equivalents for each µl of O_2 inspired differ depending on the substrate utilised (Elliott and Davidson 1974) and are therefore likely to be different when Asellus is used as the food source. Second, it was assumed that all tissues were catabolised equally during degrowth. However, there are differences between species of triclad in their chemical composition, and in the nature of their food reserves (Jennings 1957; Boddington and Mettrick 1971; Mettrick and Boddington 1972; Calow and Woollhead 1977a). During starvation, the utilisation of these reserves differs from species to species, thus affecting the amount of energy released during various stages of degrowth. The catabolism of different substrates during degrowth will also affect the oxy-joule equivalent.

These sources of error, together with the large Standard Errors associated with some of the figures in Tables 42 and 43, mean that differences between species in their energy partitioning must be interpreted with care. The following discussion, therefore, will concern itself with major differences between species.

Since all measurements of growth and respirations have been converted into energy terms (J), interspecific differences in growth and respiration may now be discussed more fully, i.e. differences between species at the same temperature may now be analysed (see Sections 5.5 and 6.5 above).

During growth, food intake at all temperatures in P. felina is higher than in the other 2 species. However, although the amount of energy respired by P. felina over the 10 day period at each temperature is similar to that respired by P. tenuis, R/I values are much lower. Growth efficiency, G/I, is also much lower in P. felina than in P. tenuis, over the whole temperature range, although differences between species are greatest at 15° and 20°C. Energy expended by P. felina on mucus and lost through excretion (Muc + Exc) is much higher than in P. tenuis.

Temperature has little effect on growth efficiency or metabolic efficiency in P. felina. As temperature increases, so does food intake, and the amount of energy subsequently partitioned into the various metabolic outputs. In contrast to this, temperature has a much greater influence on P. tenuis and D. tigrina. At 5°C growth efficiency in P. tenuis is low (< 30%). Above this temperature, most of the energy is partitioned into growth. In D. tigrina at 5°C, degrowth occurred, and at 10°C growth was negligible over the 10 day period. Input was much lower at 5° and 10°C than in the other 2

species. Thus, although 90% of the input energy at 5° and 10°C was partitioned into Muc + Exc, the net amounts were much less than in P. felina or P. tenuis at the same temperatures. At 15°C, growth efficiency increases dramatically to over 40%. G/I was likely to be even higher at 20°C, since there was a considerable increase in energy partitioned into growth (c.f. 3.32 J at 15°C and 10.03 J at 20°C).

However, the energy budget did not work in the case of D. tigrina at 20°C. Metabolic outputs exceeded input. The high errors associated with I (8.76 ± 11.80 J) are due to the low numbers of observations on feeding (see Chapter 4). I is probably much higher than estimated here, since D. tigrina is very active at this temperature (Pickavance 1968) and likely to incur considerable losses through mucus.

The figures for energy losses through mucus production derived from the difference between I + Dg and R + G, are impossible to compare with those obtained by direct measurement, since there are many variables, and potential sources of error, e.g. triclad size, the frequency of cleaning dishes. However, if we assume that P. felina produces 0.85 mg dry wt. of mucus in 4 days (from Table 41a), then it will produce 2.13 mg dry wt of mucus in 10 days. The energy loss through mucus over this period, is 36.74 J. This is much more than the 9.5 J calculated in the budgets (Table 42b).

Few conclusions may be drawn from this, except that if anything, the budgets tend to give conservative estimates of mucus production. Calow (1977a) has stated that the energy transactions of organisms will maximize fitness and that this "should lead to a maximisation of food conversion into somatic and gametic tissue, unless other metabolic demands are important for the survival of the animal,

and its reproductive success". It follows from this that the high levels of mucus production in P. felina are important for survival and reproductive success. Mucus production in triclads is necessary in facilitating locomotion, and may also play an important role in prey capture (Hyman 1951; Jennings 1957; Pickavance 1971a; De Silva 1976b, 1978; Adamas 1980a). Calow, Beveridge and Sibly (1979) have shown that the rate of movement in P. felina is significantly higher than in P. tenuis, and that this is a necessary adaptation for life in a fast flowing stream. Similar high values (70%) have been reported by Teal (1957) for stream dwelling triclads in the USA. Mucus may also be important in prey capture by P. felina, although it is unlikely to be very effective in lotic conditions.

Calow (1977a) has shown that mucus producers (snails and triclads) have lower metabolic rates than predicted by Hemmingsen's standard regression equation for poikilotherms (Hemmingsen 1960). Thus, the commitment to expending large amounts of energy on mucus by P. felina might result in economies elsewhere, such as in growth or metabolism. There is some evidence for this at higher temperatures (15° and 20°C) where the net energy partitioned into growth and respiration is considerably less in P. felina than in P. tenuis (see Chapters 5 and 6). However at 5° and 10°C, differences between species are less pronounced. An alternative way of providing energy for mucus production is to increase food intake, and I in P. felina is much higher than in either P. tenuis or D. tigrina.

In contrast to P. tenuis and D. tigrina, the energy partitioning strategy of P. felina remains relatively unaffected by temperature, i.e. the proportion of I partitioned into G, R, and Muc + Exc in P. felina changes little with temperature. In P. tenuis the increase

in G/I with temperature affects the growth rate, and therefore the time taken to reach sexual maturity. In the asexual P. felina and D. tigrina, growth and reproduction are undoubtedly connected (see Chapter 7), yet there are differences between species in energy partitioning. However, D. tigrina is a warm water species, and growth and reproduction are largely restricted to a short period in summer (Pickavance 1968, 1971a, 1971b), when it must capitalise on favourable conditions. Thus I and G/I are likely to be high at temperatures of 15°C and above, otherwise it would be unable to compete with other lake-dwelling species such as P. tenuis.

As in the budgets derived for feeding triclads, temperature affected D. tigrina most, and the time taken to reach $0.5A_i$ fell dramatically between 5° and 20°C. There are marked differences between species in energy partitioning during degrowth. In P. tenuis, between 34 and 46% of energy derived from catabolism is expended on mucus. This range of values corresponds closely to that of 44% found by Calow and Woollhead (1977a) for P. tenuis starving at 10°C. Temperature has little influence on the energy partitioning strategy of P. tenuis during degrowth. In comparison, P. felina loses relatively little energy through mucus secretion at 5° or at 20°C (9% and 18% respectively). However, at 10° and 15°, 55% of the energy available is expended on mucus. D. tigrina partitions proportionally less energy into mucus as temperature increases between 5° and 15°C. At 20°C, though, mucus production rises dramatically to 66% of the energy derived from tissue catabolism.

Calow and Woollhead (1977) believe that energy losses through mucus secretion during starvation may be explained by differences in relative activity levels. This may not be entirely true, since the

secretion of mucus (or rhabdite material) by a sedentary triclad during starvation may offer some protection against attack by parasites or predators. Indeed, in several cases after prolonged periods of starvation individual P. tenuis were observed encased in capsule-like material.

However, assuming that mucus losses are largely incurred through activity it seems that P. felina in particular employs different strategies at different temperatures. The inactivity at 5^{o} and $20^{o}C$ seems unwise in view of the effect of current on mortality (Calow, Beveridge and Sibly 1979), i.e. mobility is important in stream-dwelling triclads in avoiding being washed away. However, conflicting pressure to conserve energy during starvation may compromise the animal into being less active at certain temperatures. The results from a field study of a P. felina population described in Chapter 9, will be discussed together with the above results, in the Discussion chapter (Chapter 10) in order to try and understand the adaptive significance of bioenergetics in asexual species.

Chapter 9

A FIELD STUDY OF *P. FELINA*

9.1 Introduction

In previous chapters, I have examined the physiology and energy partitioning of sexual and asexual triclads, and have discussed differences in approach to the problems of acquiring energy and distributing it among various metabolic outputs. By manipulating temperature and food availability under laboratory conditions, insights have been gained into the costs and benefits of different strategies. However, before attempting to explain the adaptive significance of these strategies, information is required concerning field conditions. For this reason, I have studied a single field population of *P. felina*.

The purpose of this part of the study is to investigate temperature and food fluctuations in the field, and their effects on the rate of fission; to identify mortality factors, and to assess their influence on the relative survivorship of fission products.

9.2 Literature Review

There have been few specific studies of stream-dwelling triclads. In the United States, they have been looked at by Jenkins and Miller (1962), and Chandler (1966), and in Europe by Hubault (1927), Gislen (1946), Dudziak (1956) and Oomen and Geelen (1966) and others. In Britain they have been studied by Carpenter (1928), Beauchamp (1932), Beauchamp and Ullyott (1932), Wright (1968, 1972, 1974, 1975), Lock (1972, 1975) and Lock and Reynoldson (1976), and many of these authors have discussed *P. felina*.

The literature concerning the general ecology of *P. felina* was reviewed in Chapter 2, and therefore little will be added here. Most of the previous studies have concerned themselves with the debate over the distribution of *P. felina* (e.g. Thienemann 1912, 1922; Vandel

1921a, 1921b; Beauchamp and Ullyott 1932; Stankovic 1934; Wright 1968, 1972, 1974; Lock 1972a, 1972b, 1975; Pattee, Lascombe and Delolme 1973). These studies have demonstrated the inability of P. felina to withstand strong currents, and steep gradients, and its restriction to habitats where the temperature does not rise above 20°C for long periods.

The diet of P. felina has been cursorily examined by Wright (1968, 1975) using squash techniques, and he concludes that oligochaetes and arthropods are the principle food items. Further, more detailed studies by Lock (1972), involving serological techniques, suggest that the diet of P. felina may be more general, and that Daphnia and occasionally Ephemeroptera are eaten. No quantitative studies have as yet been made.

The only mortality factor investigated so far is predation. The predators of P. felina, and the impact of predation have been assessed by Wright (1968, 1975), who concluded that under certain circumstances predation may have a regulatory effect on distribution and population density. In general, however, stream-dwelling triclads have few enemies which may be because potential predators find them distasteful (Jennings 1957; Young and Reynoldson 1965).

9.3 Site Description

I will describe the sampling site in some detail, as several of the features are unusual, and may have an important influence on the population of P. felina.

The population studied was from the outflow of Balmaha Pond (for details, see Table 1, Chapter 3). The pond itself is man-made, and was used in the 18th and 19th centuries as a reservoir supplying

local industry. However, before the Forestry Commission bought the surrounding land, prior to the last war, the pond had fallen into disuse, and had become choked with vegetation. About 1970 the Forestry Commission drained the pond, banked up the sides, and deepened it considerably. Two main drainage channels also run into the pond. The Commission have constructed several forest walks as part of the Balmaha Forest Park, and these pass close to the pond and outflow.

The pond is now triangular in shape, measuring approximately 25 x 40 x 40 m. It is steep-sided, and almost 2m deep in the middle. In addition to drainage ditches, two streams run into the pond, and in one of these there is a population of P. felina. There is one outflow, through a 30cm diameter pipe. The water falls approximately 1 m. from the pipe into a stream which flows into Loch Lomond, some 300 m. away.

The stream is 1.5 m. wide at the broadest part, just below the outflow pipe, but for most of its length, it is much narrower. The overall gradient is shallow, although the upper part is slightly steeper. The lower stretch of the stream has been diverted into a ditch, bypassing a car park. This section is muddy, with few stones. In contrast to this, the upper part of the stream is very stony, although the stones are generally small and do not exceed 200 mm in diameter.

The population of P. felina appears to be restricted to the upper stony section of the stream. Regular sampling of the lower reaches proved fruitless and few triclads were ever found there. The population extends as far upstream as the pond outflow. The area chosen for investigation was a 10 m. stretch, immediately below the outflow.

9.4 Materials, Methods and Preliminary Observations

9.4.1 Physical parameters

Temperature and water flow were recorded regularly for 15 months of the study, though additional observations were made prior to this period. Dissolved oxygen levels were measured on several occasions during the study, although not on a regular basis. Observations were always made between 1030 and 1330 hours.

The water flow was estimated from the time taken for the water from the outflow pipe to fill a 10 l. plastic bucket. This method was found to be convenient, and accurate (i.e. it measured almost all the water entering the stream). The temperature was measured with a thermometer. This was not entirely satisfactory; several attempts were made to use maximum/minimum thermometers but these were always lost within a few days of installation. Nevertheless, some data concerning diurnal temperature changes were recorded. The Winkler technique was used to measure dissolved oxygen (see Chapter 5).

9.4.2 The population of *P. felina*

Observations were made on the numbers of triclads found in the study area and the relative proportions of fissioning and non-fissioning worms.

The following index of density was used to describe the size of the population of *P. felina*. Ten stones from within the 10 m. study area were marked with red paint and each week the numbers of triclads found on the undersides of the stones were recorded. Initially, stones were chosen at random, but floods following heavy rain resulted

in many or all of the stones being washed away. Eventually 10 stones of average to larger than average size (between 100 mm and 180 mm diameter) were randomly selected. The approximate position of these stones is shown in Figure 22. Observations were made for 20 weeks, before several of the stones were washed away during a spate.

In order to estimate the proportion of heads and tails in the population, and thus assess any seasonal changes in the rate of reproduction, the study area was sampled every month for 20 months. All animals encountered during a 5 minute search were removed with a paint brush, put into a jar of stream water, and sorted on return to the laboratory. Sampling was restricted due to the small size of the population.

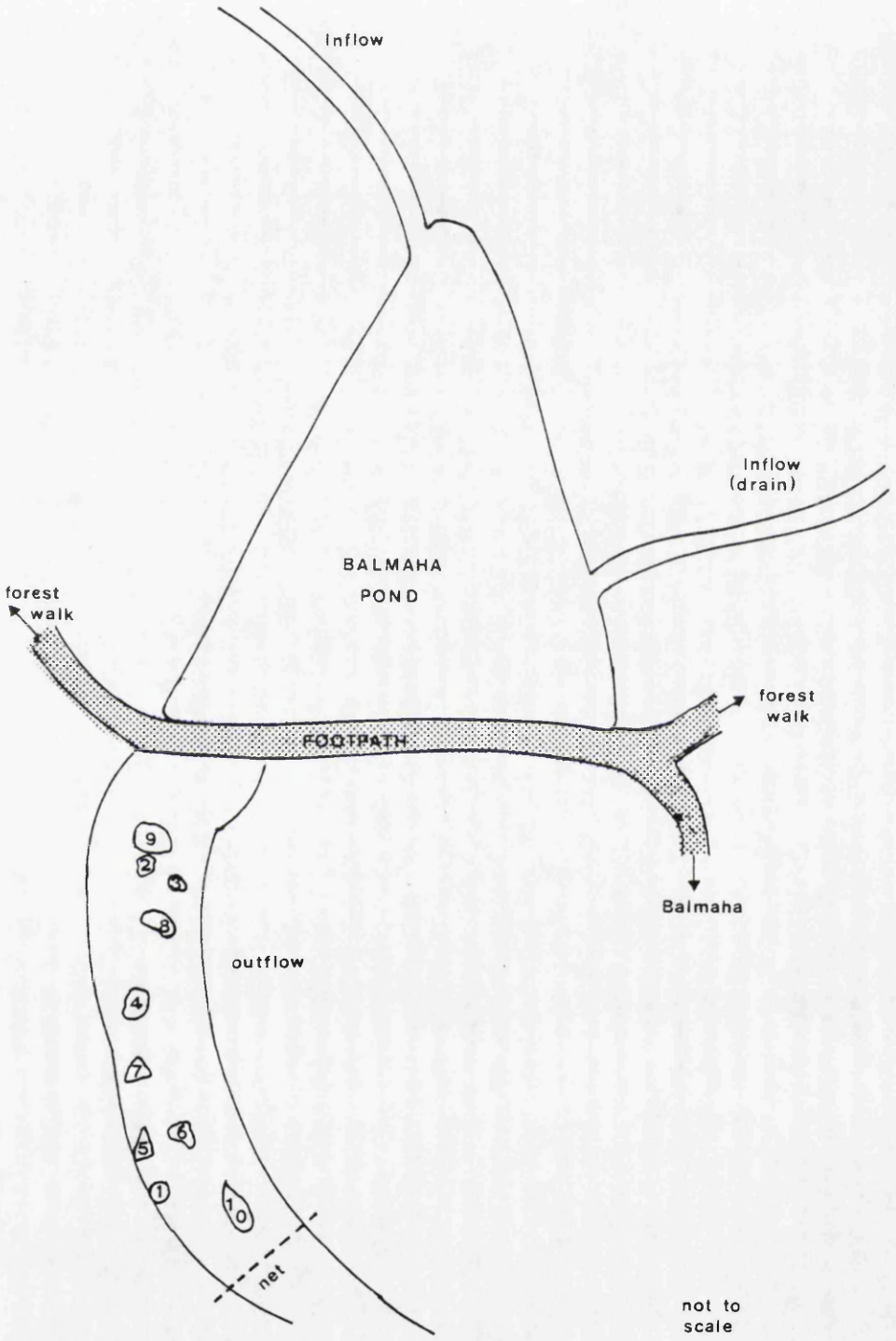
Initially, 4 different types of P. felina were distinguished: heads, tails, regrowing heads, and adults. Regrowing heads are defined as adults, which have distinctive unpigmented tail regions. From laboratory observations this situation is found in heads which have recently regrown a missing tail. However, after some time, it became apparent that tails which have recently regrown a new head could be distinguished on the same basis, and these were classed as regrowing tails. More precise methods would have necessitated involvement in an ambitious field study of the population dynamics, and this study required only an indication of general trends in the population.

9.4.3 Food

Successful serological techniques were devised by Davies (1967) for investigating the diet of triclads in the field. Unfortunately, these methods are both difficult and time consuming. An alternative method is to look at feeding in the laboratory and extra-

Figure 22

Balmaha Pond outflow, and the approximate position of the stones used to observe changes in the P. felina population (see text).


Inflow
Inflow
(drain)
BALMAHA
POND
forest
walk
forest
walk
FOOTPATH
Balmaha
outflow
net
not to
scale

polate to field conditions. Although this is a somewhat less satisfactory approach, it nevertheless gives an insight into the range of dietary preferences in the field.

Dishes of water at 10°C, containing single, starved (no food for 10 days) triclads, were placed in a dimmed room, and left for 15 minutes prior to the introduction of an immobilised prey item. Ten potential foods from Balmaha Stream were tested on 30 different triclads (10 different foods; 3 triclads offered each food). During 15 minutes of observation, 2 factors were carefully noted: the success of the triclad in locating the prey, and whether it subsequently fed on the prey or not. The results are shown in Table 44. It is clear that P. felina has a catholic diet, and feeds readily on oligochaetes, Gammarus and Asellus, although it sometimes feeds on Simulidae, Ancylus and Sphaerium. It may also prey on caddis larvae when removed from their cases. On no occasion did they feed on water mites.

In general, these results agree with the findings of Lock (1972) and Wright (1968, 1975), except that Wright states that molluscs are generally ignored. This study suggests that molluscs are eaten, although the importance in the diet of P. felina remains unclear.

If we assume from these and other observations (see Chapter 4, concerning feeding behaviour) that the Balmaha stream population of P. felina are opportunistic generalists, and not restricted to 1 particular prey type, then the problem arises as to the source of their food in the field. Prey could either be derived from the benthos, or from animals washed down from the pond in drift and deposited in the stream. No direct evidence exists to support either of these hypotheses.

It was decided to measure the macroinvertebrates in the drift

Potential Prey Item	Located			Fed On		
	1	2	3	1	2	3
Oligochaeta	+	+	+	+	+	+
Sphaerium	+	+	+		+	+
Ancylus	+	+	+	+	+	+
Gammarus	+	+	+	+	+	+
Asellus	+	+	+	+	+	+
Trichoptera*	+		+		?	
Simuliidae		+	+			
Stickleback	+	+	+			
Tadpole	+	+	+			
Acarina	+	+	+			

Table 44 : Results of presenting starved P. felina with immobilised potential food items. Observations recorded over 15 minutes.

* Caddis removed from case

as an index of food availability, and thus avoid the destruction of the habitat normally associated with traditional benthic sampling methods. An advantage of using this approach is that there is a correlation between the numbers of macroinvertebrates found in the drift, and the total numbers of benthic macroinvertebrates. This correlation is high in some cases (e.g. Stoneburner and Smock 1979), but much lower in others. The situation appears to be complicated by a number of variables, such as precipitation (for review see Waters 1972; Muller 1974).

A net was constructed with a mesh size of 500 μ. The bag was 75cm deep and the mouth was held open by a wire hoop. The hoop could be bent to closely match the substrate, and was designed to fit into a narrow section of the stream at the lower end of the study area (see Fig. 22). It could be secured by 2 stones, and was large enough to allow all the water to flow through, even in spate conditions.

Samples were collected every fortnight for a year, and the net was left in place for 24 hours when possible, in order to account for the diurnal nature of the drift (Anderson 1966; Elliott 1967). However, during flood conditions, the net filled quickly and had to be removed after only a few hours. The contents of the net were carefully sorted on return to the laboratory.

9.4.4 Mortality factors

Two potential mortality factors were investigated: predation and water flow.

Observations on the predators of stream-dwelling triclads by Wright (1968, 1975) identified only 4 species (3 plecopterans and 1 trichopteran) which prey on P. felina. None of these occurred in

Balmaha Pond stream. Of the predators of lake-dwelling triclads identified by Young and Reynoldson (1965) and Davies and Reynoldson (1971), only the stickleback Gasterosteus aculeatus (Linn.) was found in the stream. Sticklebacks were often washed down into the stream from the pond above. Their importance as predators of P. felina was assessed in the laboratory. Under these controlled conditions, the role of mucus/rhabdite secretions in anti-predator defense was also examined.

Twenty sticklebacks were removed from Balmaha Pond with a sweepnet and transferred to the Field Station, where they were kept outside in a large tank without food for 24 hours. Each stickleback was then measured before being transferred to a small experimental tank (20 x 40 x 30 cms) filled with filtered loch water. The tank contained a small clump of artificial weed. The sticklebacks were left to settle for 5 minutes.

Each stickleback was then presented sequentially with a Tubifex worm (approximately the same size as the triclads being used), a Tubifex worm coated with mucus material, and a P. felina. The mucus was removed from a dish in which 30 P. felina had been kept for a week. After the prey item was dropped into the tank, the subsequent reactions of the stickleback were observed for a minute, before the next prey item was introduced.

Laboratory investigations of the effects of water current on heads and tails were summarily described in Calow, Beveridge and Sibly (1979) (see Appendix 3).

In the field, P. felina recovered from the fortnightly drift samples (see above) were sorted, counted and examined for damage.

9.5 Results

9.5.1 Physical parameters

The temperature of Balmaha Pond stream was measured at least once a week between November 1977 and July 1979, except for a period between 23 December 1977 and 13 April 1978. In order to remedy this, extrapolations were made from the Clyde River Purification Board's air temperature records for Arrochymore station, near Balmaha. The relationship between Balmaha Pond stream temperature and the air temperature at Arrochymore are shown in Figure 23. A straight line was plotted by regression analysis:

$$y = 0.207 + 1.083\,x. \qquad r = 0.92$$

where y = water temperature, x = air temperature, r = correlation coefficient.

In Figure 24, the temperature of the stream is plotted. The temperature rarely rose above 20°C, and only once dropped below 1°C. During the summer months the temperature could fluctuate by as much as ± 4°C (e.g. in June 1978) from day to day, although in winter these fluctuations were much less severe. A diurnal variation in temperature of 5°C (14-19°C) was recorded during the summer months. In winter however the diurnal variation was usually less than 1°C.

The water flow was measured regularly at least once per week for 14 months between May 1978 and July 1979, and the results are shown in Figure 25. Although a stopwatch was used, it was difficult to accurately measure the time taken to fill the bucket, if less than a second. Hence, in Figure 25, the water flow does not appear to exceed 10 l. $second^{-1}$.

As expected, the water flow in the stream was highest during

Figure 23

The relationship between the water temperature of Balmaha Pond outflow, and local air temperature.

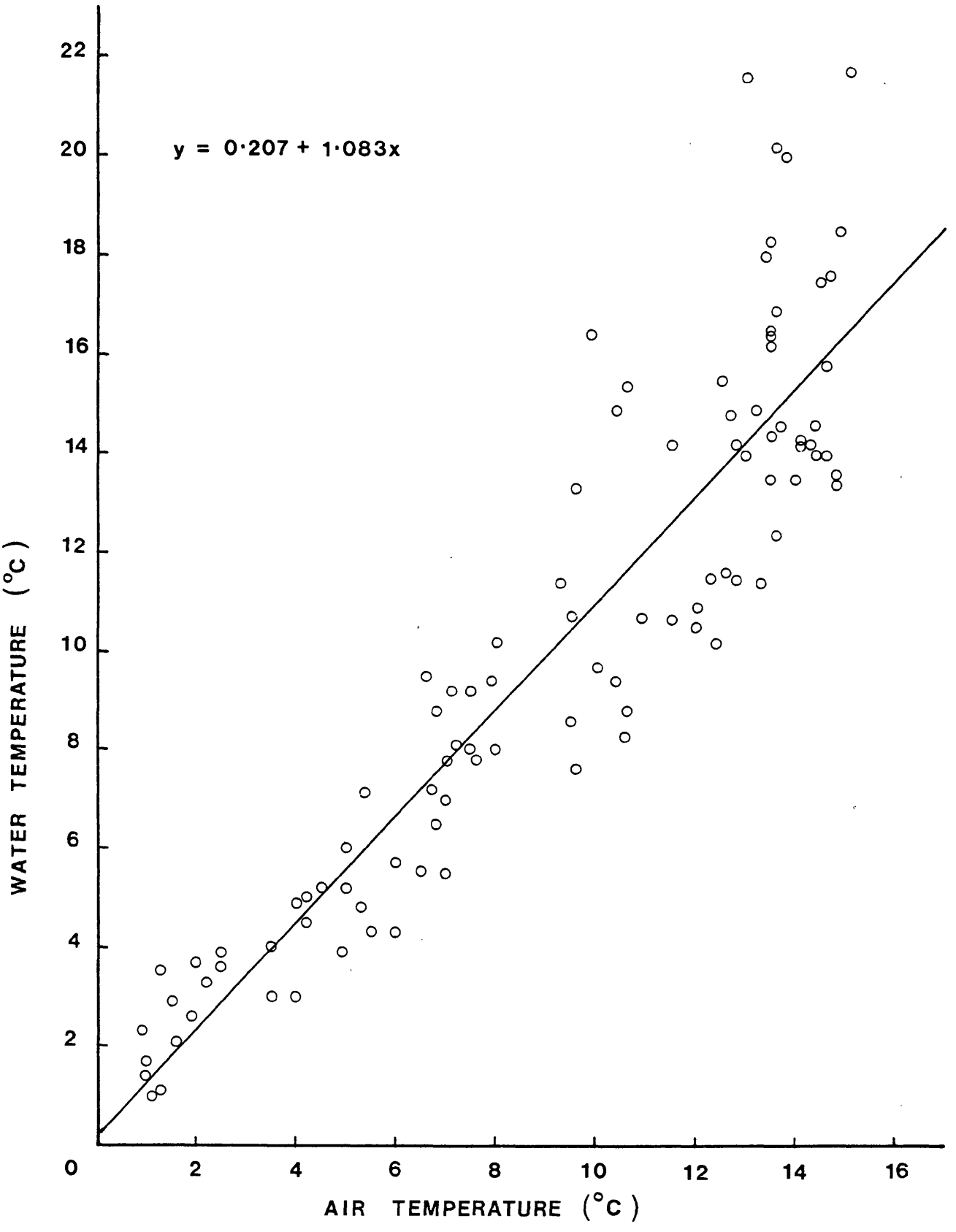

y = 0·207 + 1·083x
WATER TEMPERATURE (°C)
AIR TEMPERATURE (°C)
0
2
4
6
8
10
12
14
16
18
20
22

Figure 24

The water temperature of Balmaha Pond outflow, between October 1977 and July 1979.

..... extrapolated from air temp.

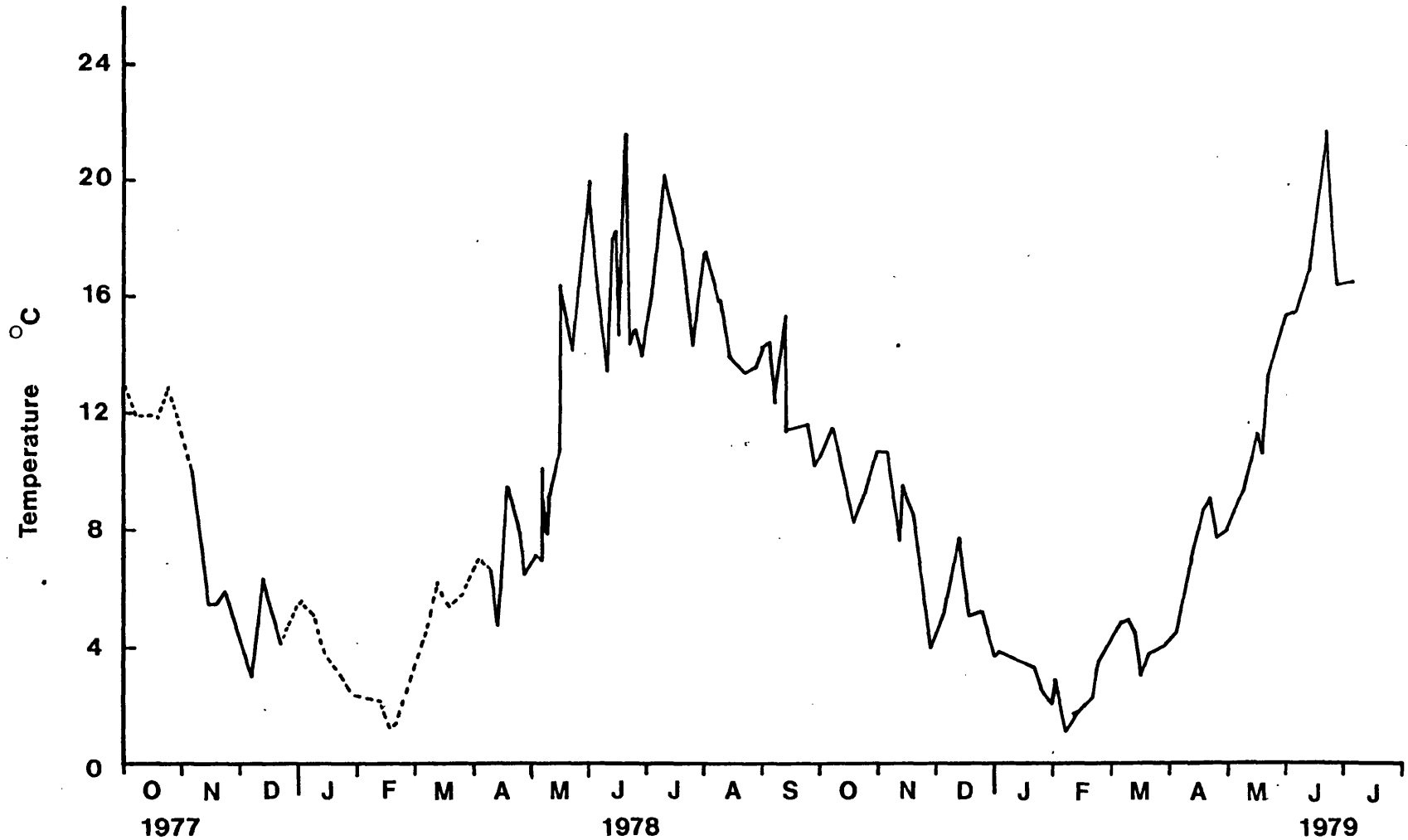
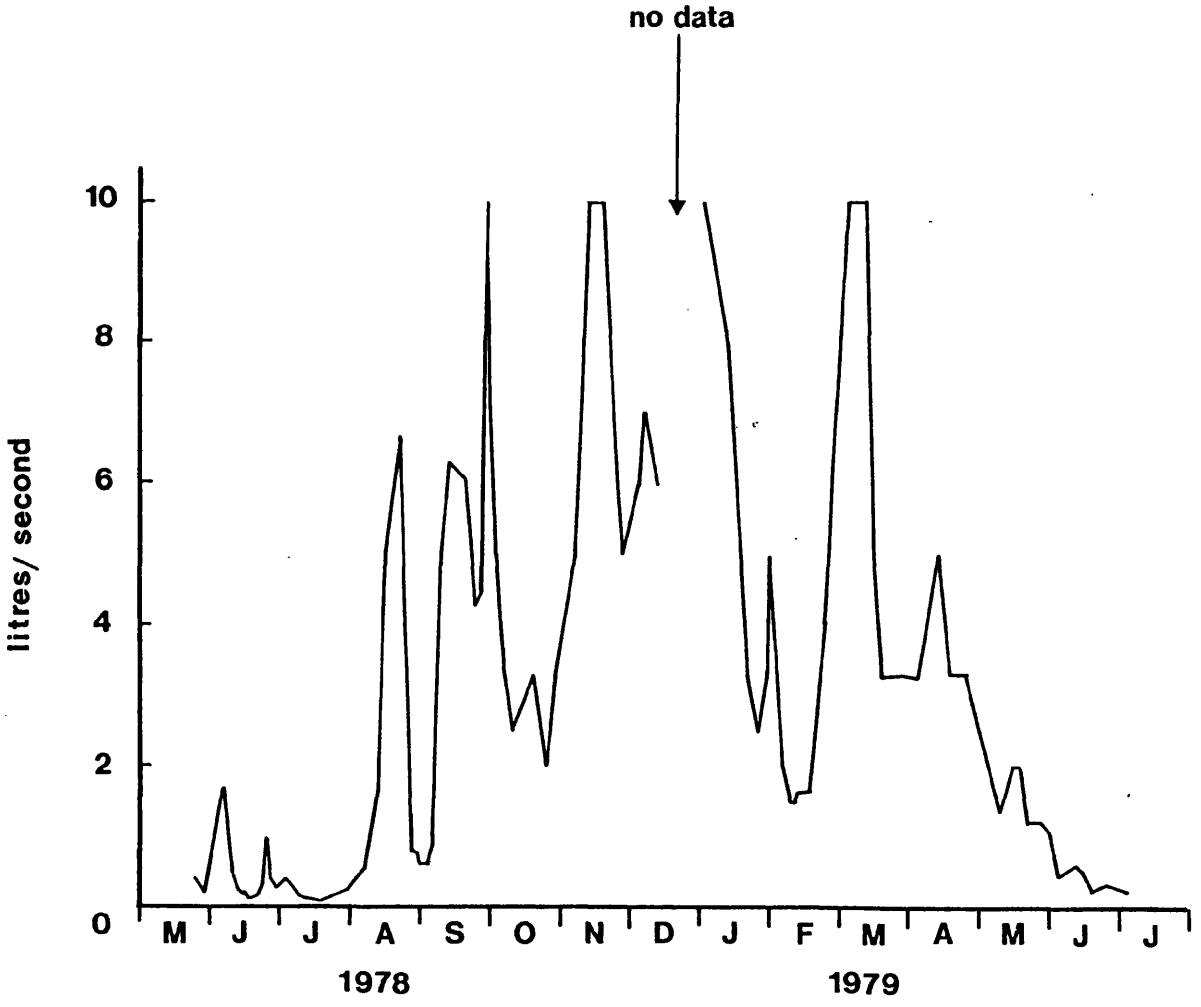

Temperature °C
24
20
16
12
8
4
0
O N D J F M A M J J A S O N D J F M A M J J
1977
1978
1979

Figure 25

The water flow in Balmaha Pond outflow, between May 1978 and July 1979.


no data
litres/ second
10
8
6
4
2
0
M
J
J
A
S
O
N
D
J
F
M
A
M
J
J
1978
1979

the winter months, although peaks were recorded in April 1979. Water flow during the summer months was generally less than 2 l. second^{-1}.

The concentration of dissolved oxygen in the stream was found to vary little from summer to winter (Table 45), and was always close to saturation level. This is in part due to aeration of the water entering the stream (see 9.3 above).

9.5.2 The population of P. felina

An index of P. felina population size was constructed from observations on the changes in numbers of triclads recorded on 9 marked stones between February and July 1979. (Two weeks after the project began, 1 of the stones disappeared and was not replaced). The results are summarised in Figure 26.

From mid-February until the beginning of May, the total number of triclads found on the stones fluctuated, but never exceeded 20 (or approximately 2 animals per stone). In the second week of May, the numbers rose dramatically, and there was a 5-fold increase in 1 week. The numbers fluctuated during May, but increased steadily throughout June, and into July. Over this 5 week period, there was a 20-fold increase in the population size. The highest mean density of triclads per stone was recorded at the beginning of July (> 20 P. felina per stone).

On 2 occasions, the stones were examined on consecutive days. The results are shown in Table 46. Not only did the population density change dramatically, but the distribution pattern also altered from day to day.

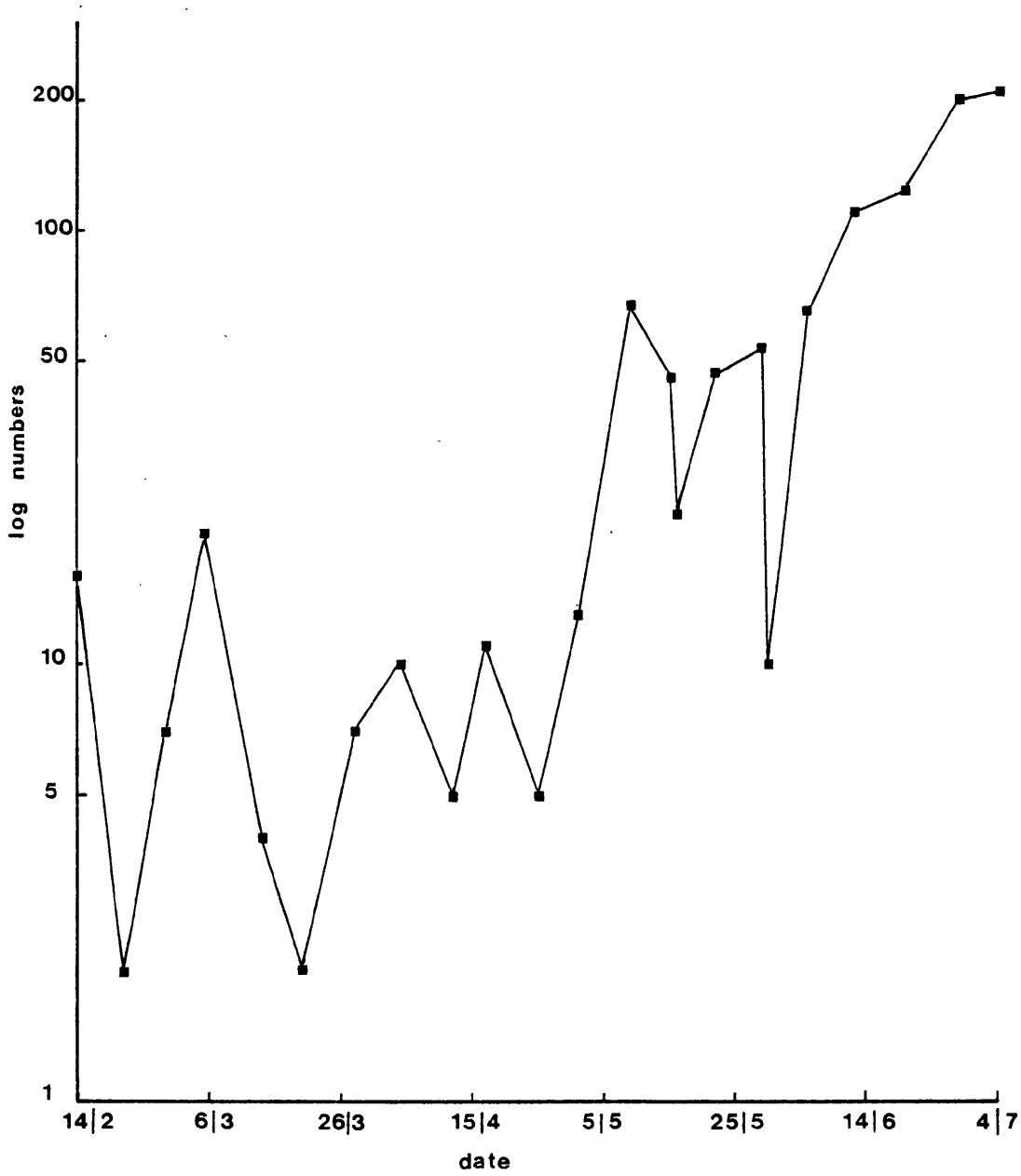
The numbers of heads, tails, regrowing heads, regrowing tails and adults occurring in the monthly population samples are given in Appendix 2. From these data, the percentage of heads and tails

Date	Temp. of Stream (^{o}C)	100% O_2 Saturation at Stream Temperature	No. of Samples	Mean Amount of Dissolve O_2
6 December 1976	3	13 mg $O_2 l^{-1}$	4	13.10 mg l^{-1}
5 June 1978	17	9.3 mg $O_2 l^{-1}$	5	9.10 mg l^{-1}

Table 45 : The oxygen concentrations of water samples taken from Balmaha Pond outflow.

Figure 26

Changes in the size of the Balmaha Pond outflow population of *P. felina* between February and July 1979.


200
100
50
10
5
1
log numbers
14|2
6|3
26|3
15|4
5|5
25|5
14|6
4|7
date

Stone No.	Date			
	15 May 1979	16 May 1979	29 May 1979	30 May 1979
1	11	5	0*	0*
2	4	3	24	3
3	4	3	8	2
4	1	1	2	1
5	7	1	0	0
6	4	0	0	1
7	3	0	7	3
8	5	0	13	0
9	M	M	M	M
10	7	9	0	0
Total	46	22	54	10

Table 46 : The number of P. felina found on stones on different days. Two sets of consecutive observations shown. Stone numbers refer to Figure 22.

* Stone is no longer submerged as width of stream has contracted during a dry spell

(i.e. the products of recent asexual reproduction) in the population were calculated, and the results are summarised in Figure 27.

The percentage of heads and tails (fission products) varied from month to month, indicating changes in the rate of reproduction. Consistent seasonal maxima were observed in the autumn months, when the percentage of fission products exceeded 35%. However, there are inconsistencies in the rates of reproduction during the spring and summer months of 1978 and 1979.

9.5.3 Food

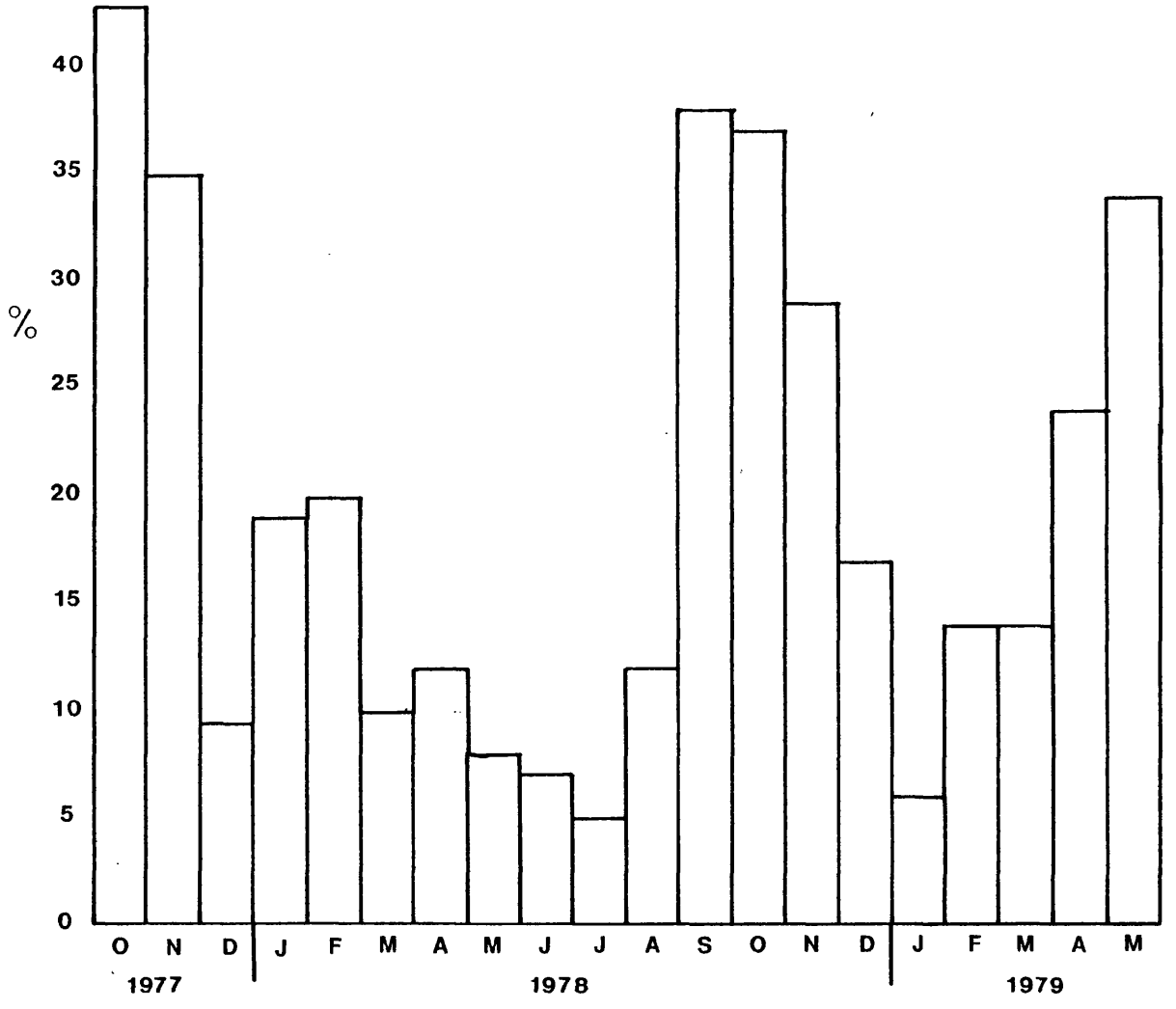
Animals recovered from the drift nets were sorted into taxonomic groups and counted. Any aquatic mites that were found were ignored, since laboratory studies had shown that P. felina did not feed on them (see above). All samples after 4 December 1978 were dried and weighed after identification, although Trichoptera larvae, G. aculeatus and B. bufo tadpoles were excluded in order to reduce bias.

There were marked differences between consecutive samples in the numbers and species recovered (Table 47). Certain organisms were absent from the drift for large parts of the year (e.g. Zygoptera larvae and G. aculeatus) whilst others were present in large numbers throughout the sampling period (e.g. Simulium larvae, which accounted for almost 25% of the total numbers of organisms recovered from the drift). Numbers were lowest during the summer months, although drift appears to be present throughout the year.

There is a good relationship between numbers of organisms, and dry weight ($y = 3.58x - 91.53$; $r = 0.98$).

Figure 27

Numbers of P. felina heads and tails (expressed as a percentage of total) recorded from the monthly samples collected from Balmaha Pond outflow


%
40
35
30
25
20
15
10
5
0
O N D J F M A M J J A S O N D J F M A M
1977
1978
1979

Species \ Date	23/6	17/7	1/8	15/8	30/8	12/9	27/9*	17/10	31/10	19/11*	4/12*	22/1
Oligochaeta	2			1	1	4	3	1		1	2	1
Gammarus	12		3	17	24	9	12	3	4	11	6	16
Asellus	1					2		1			2	
Arachnida						1	3	1	1			
Diplopoda						1						
Anisoptera				1				1			2	
Zygoptera	1			1		3	2					
Ephemeroptera	1						4				1	3
Plecoptera				4	4			4	11	10	4	6
Trichoptera			1	4		2	7	3	2	3	5	3
Coleoptera	3		1	2	2	4				1	2	
Tipulidae				2				1	1	1	1	
Simulidae		1		36	28	50	20	4	14	12		7
Chironomidae	2		1			1	4	4	1		1	
Formicidae									1			
Misc. Diptera		2	1	1	1	6	1			1		
Gastropoda	9	2			4	16						
Bivalvia	1				1	8						
Bufo tadpoles					1	1						
Triturus sp.											1	
G. aculeatus						1						
Total (Nos./ 24 hours)	32	3	8	69	66	109	84	23	34	164	108	36
Dry wt/24 hours (mg)											220.64	94.86

Table 47 : Changes in the numbers of animals found in the drift of Balmaha Pond outflow.
Total numbers are = numbers/24 hours

* Sampling done for shorter period of time, and numbers corrected for 24 hours

Species \ Date	8/2	22/2	6/3*	20/3	5/4	18/4	2/5	16/5	30/5	13/6
Oligochaeta	1	1		1			1			1
Gammarus	10	4	37	2		2	2	11		4
Asellus										
Arachnida				1			1			
Diplopoda										
Anisoptera										
Zygoptera				1						
Ephemeroptera	20	5								
Plecoptera	8	11	14	9	7	5	1	6	1	
Trichoptera	2	2	4		4	1		11	1	
Coleoptera	1		7	5	5	2		2	1	
Tipulidae		2		1	1				1	1
Simulidae	11	16	4	1	6	1	8	81	3	1
Chironomidae		4	2	2	6	1	3	1	2	14
Formicidae										1
Misc. Diptera	2		2	1	3		4			
Gastropoda				1	1	1				
Bivalvia										
Bufo tadpoles									46	9
Triturus sp.										
G. aculeatus				1		1		13	6	3
Total (Nos./ 24 hours)	55	45	280	26	35	14	20	125	61	34
Dry wt/24 hours (mg)	61.45	44.59	959.56	32.46	22.49	8.11	9.30	53.06	11.59	-

Table 47 (cont) : Change in the numbers of animals found in the drift of Balmaha Pond outflow. Total numbers are = numbers/24 hours

* Sampling done for shorter period of time, and numbers corrected for 24 hours.

9.5.4 Mortality

Predation on P. felina was assessed in the laboratory as described above. On the introduction of a worm into the fish tank, the subsequent behaviour of the stickleback was recorded. The behaviour of the fish has been broken down into discrete units for analysis (see Fig. 28). The terminology used is defined below:

See : a change in the orientation of the fish, in the direction of the worm;

Approach : the fish moved towards the worm;

Nip : in which the stickleback took part of the worm into its mouth;

Spit out : worm violently ejected;

Spit fit : fish rapidly flushed water through gills and out mouth.

The results are summarised in Table 48.

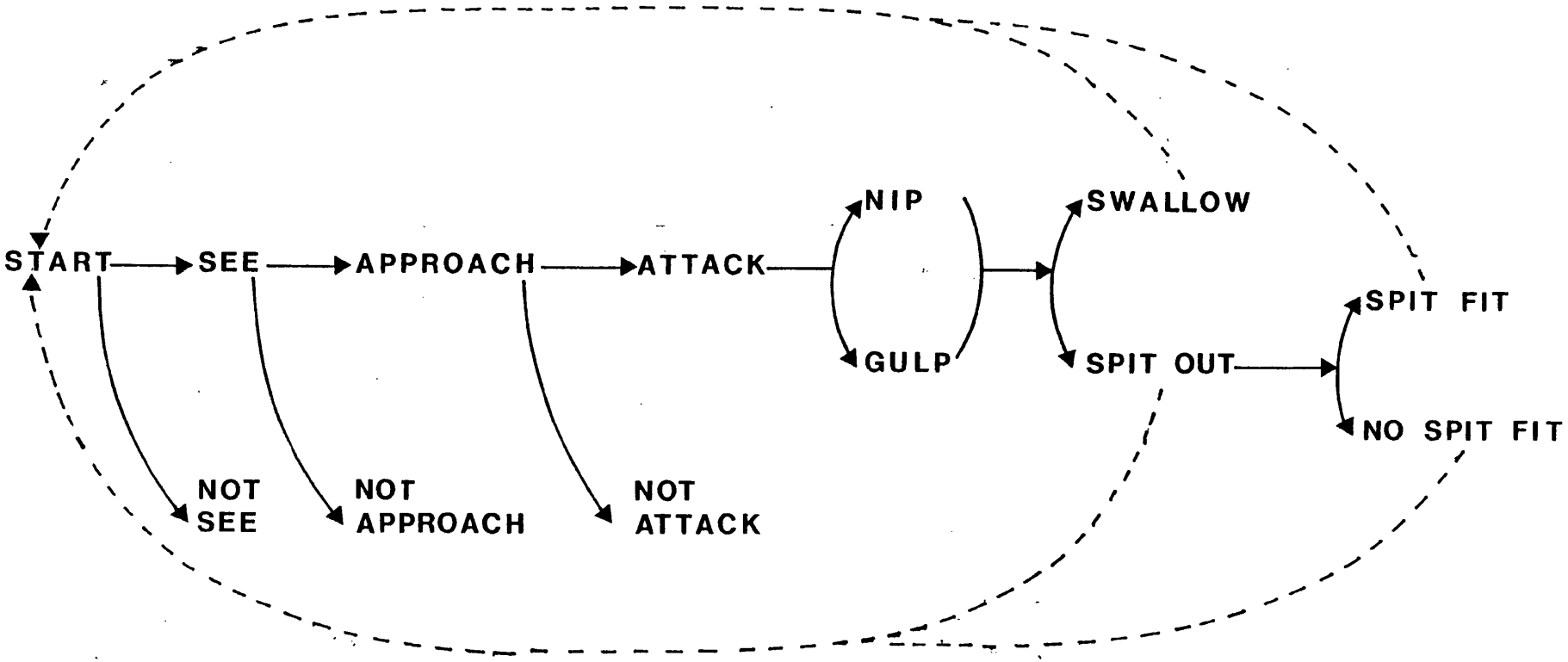
In each case, the sticklebacks saw the introduced prey item. In 10 out of 20 trials, the sticklebacks then approached and swallowed the Tubifex whole. P. felina was approached 8 out of 20 times, although none were subsequently eaten. In some cases the whole triclad was taken into the fish's mouth and at other times the stickleback nipped at it. Invariably the sticklebacks spat the P. felina out, and thereupon had a spit-fit (see above). On many occasions a second or third attack was attempted before the end of the trial.

On only 5 occasions did the stickleback approach a Tubifex coated in triclad mucus. On 4 of these trials, the Tubifex was eaten.

The results from the laboratory investigations on the effects of water current on heads and tails are published in Calow, Beveridge and Sibly (1979) (see Appendix 3).

Figure 28

The behavioural sequence of a stickleback when a potential food item is introduced into the experimental tank (see text).


START
SEE
APPROACH
ATTACK
NIP
GULP
SWALLOW
SPIT OUT
SPIT FIT
NO SPIT FIT
NOT SEE
NOT APPROACH
NOT ATTACK

Run No.	Size (length of fish)	See	Approach	(A)Nip In (B)In Whole	(A)Spit Out (B) Swallow	Repeat	Spit -fit
1.W	3.0 cm	+	+	+ (B)	+ (B)		
1.W+M	"	+	+	+ (B)	+ (B)		
1.FW	"	+	+	+ (B)	+ (B)	+ (1)	+
2.W	2.0 cm	+				dive to	
2.W+M	"	+				weed	
2.FW	"	+				'Ignore'	
3.W	2.0 cm	+				dive to	
3.W+M	"	+				weed	
3.FW	"	+				'Ignore'	
4.W	4.0 cm	+	+	+ (B)	+ (B)		
4.W+M	"	+	+	+ (B)	+ (B)		
4.FW	"	+	+	+ (B)	+(2xA)(1xB)	+ (2)	+
5.W	2.5 cm	+	+	+ (A)	+ (A)		
5.W+M	"	+	+	+ (B)	+ (B)		
5.FW	"	+	+	+ (A)	+ (A)	+ (1)	+
6.W	2.5 cm	+					
6.W+M	"	+					
6.FW	"	+					
7.W	4.0 cm	+	+	+ (B)	+		
7.W+M	"	+					
7.FW	"	+					
8.W	2.0 cm	+					
8.W+M	"	+					
8.FW	"	+					
9.W	2.0 cm	+	+	+ (B)	+ (B)		
9.W+M	"	+					
9.FW	"	+					
10.W	1.5 cm	+	+				
10.W+M	"	+					
10.FW	"	+					
11.W	2.5 cm	+	+	+ (B)	+ (B)		
11.W+M	"	+	+	+ (B)	+ (A)		
11.FW	"	+	+	+ (A)	+ (A)	+ (2)	+
12.W	3.5 cm	+	+	+ (B)	+ (B)		
12.W+M	"	+	+	+ (B)	+ (B)		
12.FW	"	+	+	+ (B)	+ (A)	+ (1)	+

Run No.	Size (length of fish)	See	App-roach	(A) Nip In (B) IN Whole	(A) Spit Out (B) Swallow	Repeat	Spit -fit
13.W	1.5 cm	+					
13.W+M	"	+					
13.FW	"	+	+				
14.W	2.0 cm	+					
14.W+M	"	+					
14.FW	"	+					
15.W	2.0 cm	+	+	+ (B)	+ (B)		
15.W+M	"	+					
15.FW	"	+	+	+ (A)	+ (A)		+
16.W	3.5 cm	+	+	+ (A)	+ (B)		
16.W+M	"	+					
16.FW	"	+	+	+ (A)	+ (B)		+
17.W	2.0 cm	+					
17.W+M	"	+					
17.FW	"	+					
18.W	2.0 cm	+					
18.W+M	"	+					
18.FW	"	+					
19.W	2.5 cm	+	+	+ (B)	+ (B)		
19.W+M	"	+					
19.FW	"	+					
20.W	1.5 cm	+					
20.W+M	"	+					
20.FW	"	+	+	+ (A)	+ (A)	+ (1)	

Table 48 : The results of presenting tubifid worms (W), worms coated in triclad mucus (W+M), and triclads (FW) to starved sticklebacks

The numbers of triclads recovered from the fortnightly drift net collections are shown in Table 49. Up to 74 P. felina were recovered from the drift net after a period of 24 hours. Tails seemed to be least susceptible to current, and rarely comprised more than 10% of the triclads collected. The largest numbers of worms recovered from the net were during the summer months (23 June 1978 and 16 May 1979), when the water flow was correspondingly high (between 1 and 2 ls^{-1}). However, spate conditions did not always result in large numbers of triclads being washed away. When the numbers of triclads recovered from the drift are plotted against water flow (Fig. 29) the correlation is poor ($r = 0.33$).

The proportion of damaged P. felina recovered varied from 0 to 100%. In general, high flow values resulted in proportionately large numbers of damaged triclads.

9.6 Discussion

It is in the nature of this study that the programme of field observations on the population of P. felina evolved gradually and this has resulted in a few unfortunate gaps in the data. In other parts, the observations have been, of necessity, rather few. Where this is the case, conclusions will be tentatively drawn.

From the data, it appears that the Balmaha stream population of P. felina is subject to dramatic fluctuations in the environment. There are differences of over 20°C between summer and winter daytime temperatures. The few observations made on diurnal variations suggest that temperatures can change by as much as 4°C in 24 hours. However, the stream temperature appears to be predictably unstable. From January until mid-March, the temperature is generally below 5°C, and

Date	Current Flow	No. of Adults	No. of Heads	No. of Tails	No. of Regrowing Heads	No. of Regrowing Tails	Total	% Damaged
9. 6.78	0.38 l/s	9	0	0	0	0	9	0
23. 6.78	1.00 l/s	38	14	7	7	5	69	28
17. 7.78	0.07 l/s						NONE	
1. 8.78	0.27 l/s	0	0	0	0	1	1	0
15. 8.78	5.00 l/s	6	2	0	1	1	10	10
30. 8.78	0.63 l/s	8	3	1	2	1	15	40
12. 9.78	3.33 l/s	11	2	0	3	3	26	27
26. 9.78	3.33 l/s	9	6	0	5	1	32*	38
30.10.78	3.33 l/s	1	0	0	5	4	10	20
19.11.78	>10.00 l/s	7	5	0	2	0	56*	64
4.12.78	5.00 l/s	3	1	0	0	0	16*	25
19. 1.79	3.33 l/s	14	3	1	3	4	25	32
8. 2.79	1.82 l/s	3	1	0	2	0	7	14
14. 2.79	1.67 l/s	4	0	0	0	0	4	0
. 3.79	>10.00 l/s	6	2	1	0	0	54*	100

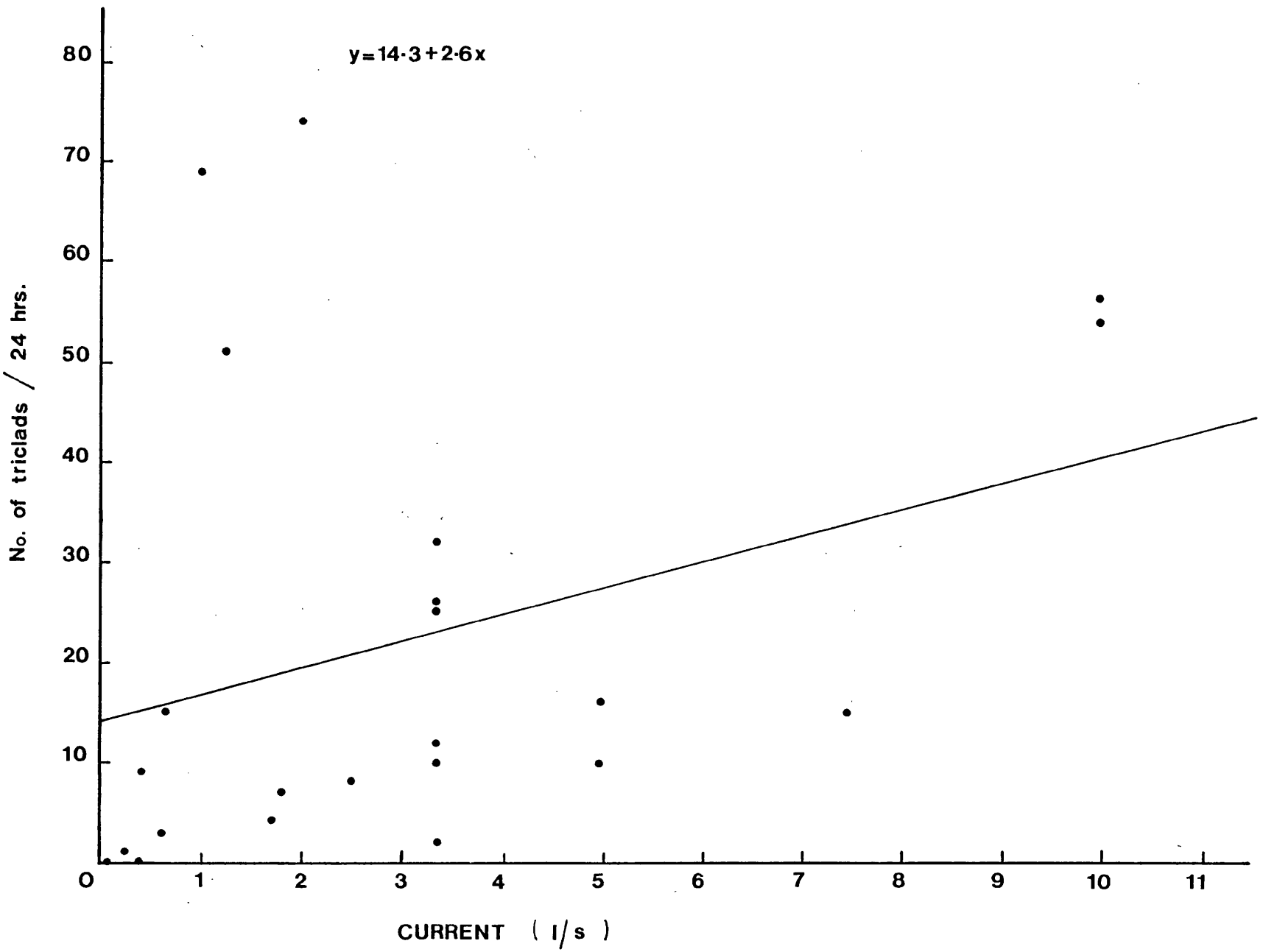
Date	Current Flow	No. of Adults	No. of Heads	No. of Tails	No. of Regrowing Heads	No. of Regrowing Tails	Total	% Damaged
14. 3.79	7.50 l/s	4	2	1	2	1	15*	30
5. 4.79	3.33 l/s	10	1	1	0	0	12	25
18. 4.79	3.33 l/s	1	0	1	0	0	2	50
2. 5.79	2.50 l/s	6	1	0	0	1	8	13
16. 5.79	2.00 l/s	37	6	1	16	11	74	13
29. 5.79	1.25 l/s	25	5	0	5	16	51	6
13. 6.79	0.59 l/s	3	0	0	0	0	3	0

Table 49 : Numbers of P. felina recovered from the drift (No/24 hours) in Balmaha Pond Outflow

* Sampling done for shorter periods of time, and numbers corrected for 24 hours

Figure 29

The influence of water flow on the numbers of triclads recovered from the drift.


y=14·3+2·6x
No. of triclads / 24 hrs.
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
CURRENT (l/s)

diurnal variations are small. From the end of May until mid-August, the temperature is likely to be above 15°C, and diurnal variation large.

The volume of water flowing down the stream is also subject to violent fluctuations, although these are less predictable. In general, though, spates (defined here as flow rates > 10 ls^{-1}) were more common in winter and spring than during the summer. In the summer, rainfall was lower, and consequently, the volume of water flow was less. Water flow during May, June and July was more or less stable, and did not exceed 2 ls^{-1}.

Drift has been used here as a measure of food availability. However, caution is required in drawing any conclusions from the data. Unfortunately the feeding behaviour of P. felina has not been studied, hence we do not know how it finds or captures prey. From laboratory observations, it appears to feed readily on many of the animals found in the drift. The drift data suggests that food may be available throughout the year.

The size of the P. felina population does not remain constant. Observations made over the spring weeks of 1979 (15 February to 1 May) show that the population fluctuates around a mean level of ∿ 1 triclad per stone. However, from May through until observations ceased in early July, the population increased dramatically to ∿ 23 animals per stone.

Only one possible predator of triclads is found in the stream: G. aculeatus. However laboratory observations demonstrate that it does not prey on P. felina, even when hungry. Because none of the P. felina were damaged during these trials, it is concluded that a secretion was responsible for their unpalatability to the sticklebacks. Triclads

secrete both mucus and rhabdites, although their structures and functions are still imperfectly known (Hyman 1951; Pedersen 1963; Rahemtulla and Løvtrup 1974). Mucus is known to be important in locomotion (Hyman 1951; Kaestner 1964) and prey capture (De Silva 1976b). Since Tubifex coated in mucus proved palatable to the sticklebacks, we may conclude that it serves no function in anti-predator defense (It may play some role in defense against bacterial or fungal infections). It seems likely, therefore, that the sticklebacks' reactions to P. felina were due to the secretion of spicule-like rhabdites. Dr J. Jennings (pers. comm.) states that rhabdites are produced in P. felina when it is irritated. Similar results were found in D. dorotocephala by Coward and Piedilato (1972).

Parasitism is also likely to play a minor role in controlling P. felina numbers, since all triclads removed from the stream during the study were found to be free from parasites. These results agree with other studies (Wright 1968, 1975), which demonstrate that parasitism and predation are unlikely to have much influence on the numbers of stream-dwelling triclads.

Any short-term fluctuations in numbers are therefore the result of animals being washed away. During the 20 weeks of observation on the marked stones in the study area, several stones moved considerable distances downstream. This was particularly apparent after heavy rainfall, when the stream was swollen. Indeed the scouring effect of the stream often resulted in the drift net containing large numbers of small stones.

During 2 periods of rainfall, observations were made on the population on consecutive days (see Table 46). Results show that P. felina numbers decreased dramatically during these periods when fairly

small volumes of water were flowing down the stream (see Fig. 29). This is undoubtedly in part due to triclads moving from one stone to another. However, it is also in part due to animals being washed away. This hypothesis is supported by the numbers of triclads recovered from the drift net during the 24 hours between counts e.g. between May 15 and May 16, 1979, the numbers of P. felina on marked stones decreased from 46 to 22. During the same period, 74 triclads - the highest number recorded during the study - were recovered from the drift net. The stream flow was 2 ls^{-1}, which is much lower than the flow recorded during winter spates ($> 10\ ls^{-1}$).

When the stream flow is plotted against numbers of P. felina recovered from the drift, then the relationship is weak (r = see Fig. 29). It seems likely that stream flow acts in a density dependent manner, washing away those triclads in sub-optimal sites. i.e. the larger the triclad population, the more likely individuals are to take up sub-optimal sites. These sites are, by definition, more prone to being washed away during spates. Hence water flow is likely to have most effect when the population is large. This is borne out by the data in Table 46, in which the triclads on certain of the marked stones were more affected by the stream flow than others. However, the optimality of a site is likely to change with time, e.g. as the stream contracts, the stones at the edge are no longer in contact with water. In the study area, marked stone number 1 was dry for over two weeks in May/June 1979.

Water flow also acts selectively on heads and tails. Laboratory observations (see Appendix 3) suggest that large triclads are more prone to being washed away then small triclads, and that tails are more susceptible than heads. However, evidence from the field

does not support this. If the triclads recovered from the drift net are compared with those present in the population at the time, then the drift contains proportionately more heads and fewer tails. This is illustrated in Figure 30, where the ratio of heads to tails in the drift has been plotted against the ratio of heads to tails in the population. If the ratios are the same (i.e. H/T drift = H/T population), then the points plotted would like along a line of slope b = 1. However, all but two of the points lie above this line, which demonstrates that H/T drift > H/T population. Thus in the field, heads are more susceptible to being washed away than tails.

The relationship between H/T drift and H/T population is likely to be complicated by a number of factors : population size, volume of flow, triclad mobility. This is borne out by the amount of scatter in Figure 30. (No regression line can be plotted since several of the y coordinates have value = ∞).

Triclads washed away in the drift are either killed or carried into unsuitable areas. When flow rate is plotted against % injured P. felina recovered from the drift, there is a positive relationship (Fig. 31). Tails appear to be most susceptible to injury : 64% of all those recovered (9/14) were injured, which may explain the discrepancy between field and laboratory observations on the effects of flow on heads and tails.

The lower reaches of the stream are unsuitable for triclads (see Section 9.3), and few of those washed downstream ever return. Elliott (1971) claims that only 8% of those P. felina washed away ever return.

Although in this case water flow is the major mortality factor, it may not be so in other situations, e.g. Minshall and

Figure 30

The relationship between H/T drift and H/T population from Balmaha Pond outflow (refer to points whose coordinate = ∞).

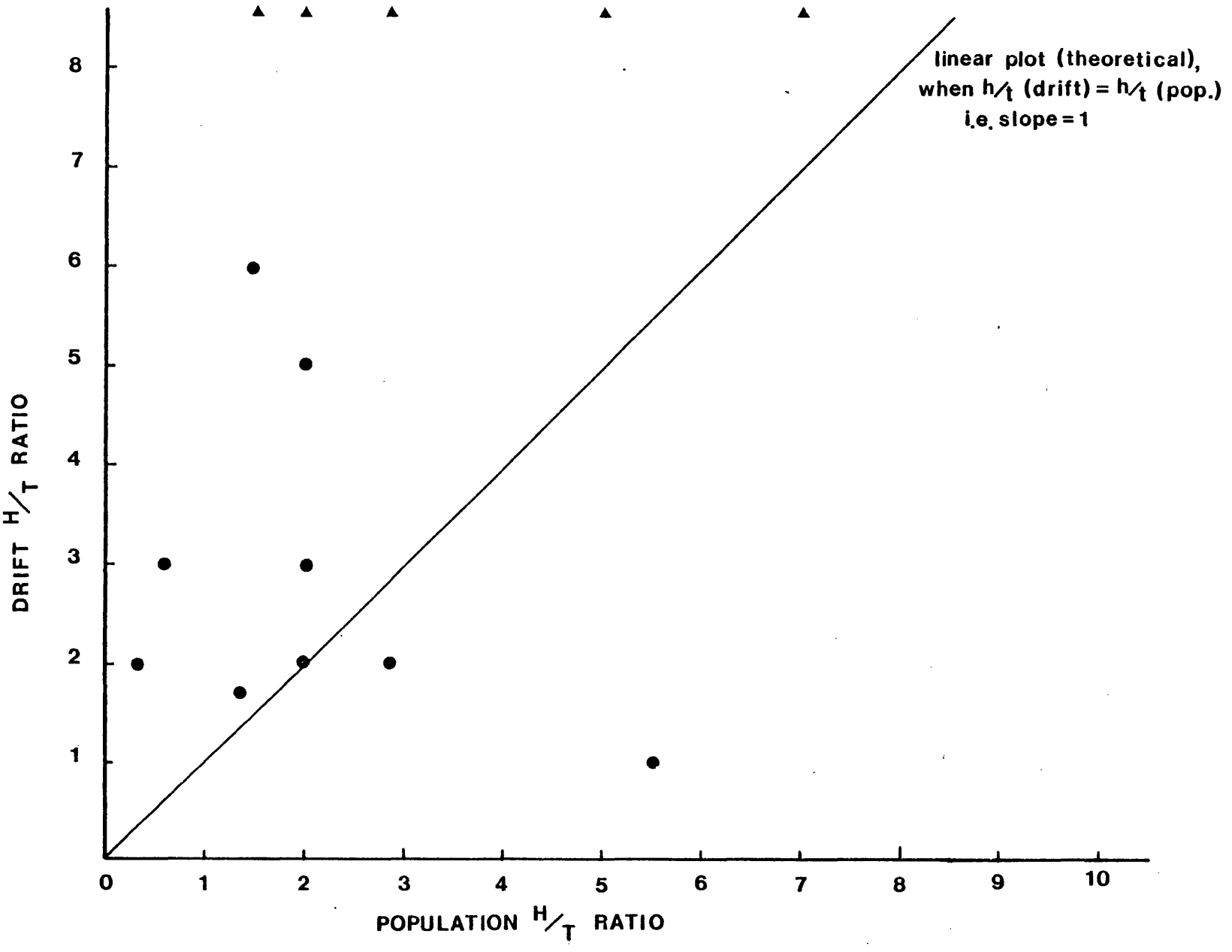
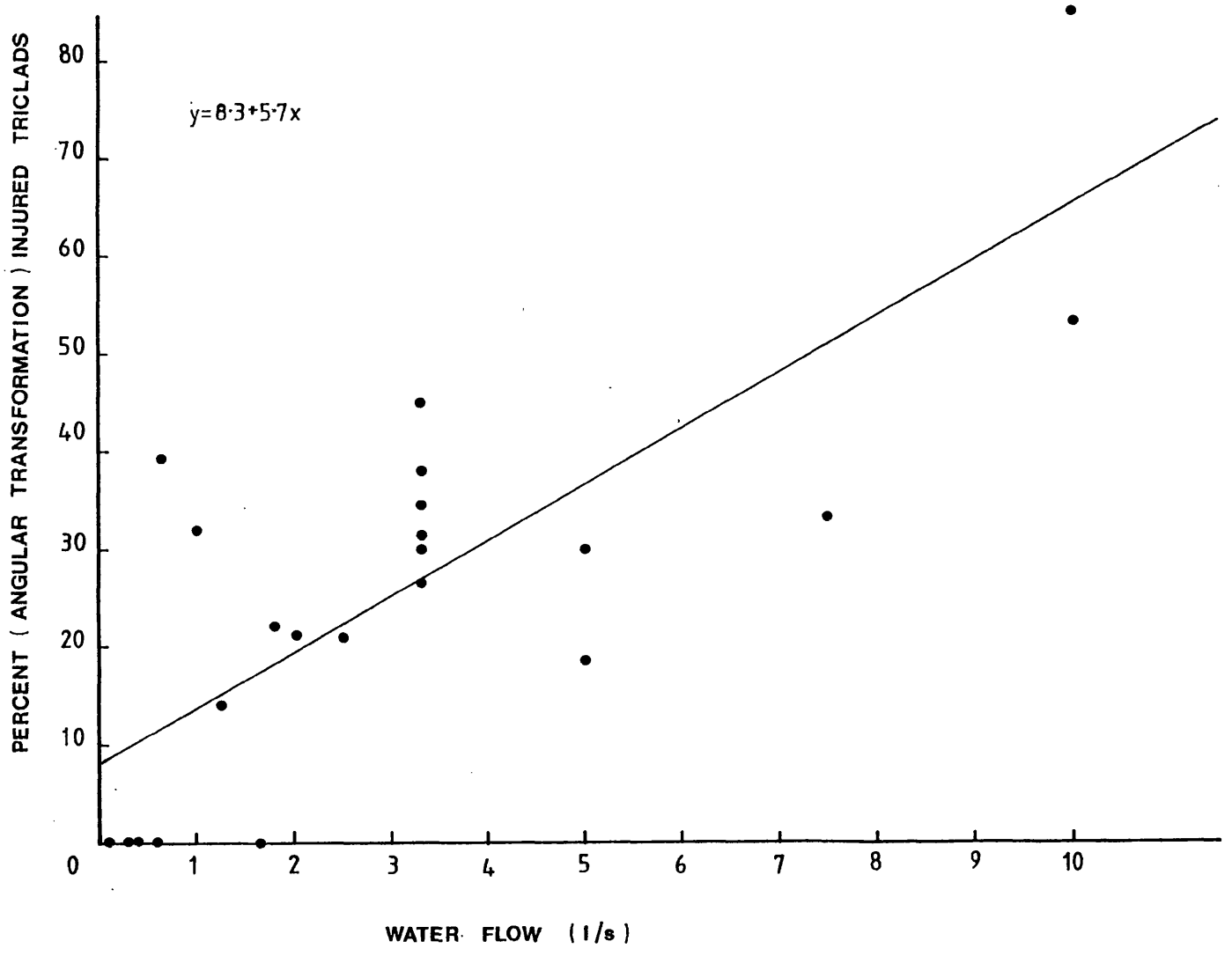

linear plot (theoretical),
when h/t (drift) = h/t (pop.)
i.e. slope = 1
DRIFT H/T RATIO
POPULATION H/T RATIO
0
1
2
3
4
5
6
7
8
9
10

Figure 31

The relationship between stream velocity ($1s^{-1}$), and the percentage of injured triclads recovered from the drift.


y=8·3+5·7x
PERCENT (ANGULAR TRANSFORMATION) INJURED TRICLADS
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
WATER FLOW (l/s)

Winger (1968), working in the USA, state that "the occurrence of freshwater triclads in the invertebrate drift of streams is a rare event."

Long term (i.e. seasonal) fluctuations in the P. felina population are probably due to changes in the rate of fission. From February 1979 through to early May 1979, both the proportion of heads and tails in the population and the size of the population remained fairly constant. However, during May 1979 the population increased dramatically and there is a concomitant increase in the proportions of heads and tails. Although there is no further direct evidence, it seems likely that the continuing population growth observed through June and July 1979 are due to increased fission.

The observed seasonal variations in the rate of fission show a degree of consistency from one year to another, with one exception: the rate of fission during the spring and early summer of 1978 is much lower than in the corresponding period of 1979. The observed seasonal and annual differences in fission rates are I believe the result of temperature and food fluctuations. The field data will therefore be discussed in conjunction with laboratory observations in the next chapter (Chapter 10).

Chapter 10

DISCUSSION

This thesis has been concerned with comparing and contrasting the energy partitioning strategies of several triclad species, with a view to sorting out the relative merits in different ecological circumstances. The results show marked differences between species in their ability to regulate their metabolic rates over a wide range of temperatures, and during starvation conditions. The evidence from measurements of growth, degrowth and respiration rates, suggest that of the 3 species, P. felina is the most eurythermic (temperature tolerant). The energy partitioning of fed P. felina is also relatively unaffected by temperature, and the proportions of the energy intake partitioned into growth, respiration, and mucus + excretion, changes little between 5° and 20°C. A similar pattern of temperature independence is apparent over the mid-range of temperatures (10°-15°C) in starving P. felina.

This view of P. felina as a eurythermic species appears contrary to opinions expressed in earlier papers (e.g. Dahm 1958; Reynoldson 1961; Pattee, Lascombe and Delolme 1973). These differences in opinion stem largely from 3 sources. Populations from different geographic areas have been examined using different methods, and judged by different standards. There is evidence that populations from different areas show differences in temperature tolerance (Dahm 1958). The upper temperature limit tolerated by P. felina is in dispute, and is claimed to be 17.5°C by Thienemann (1912), 16.5°C by Stankovic (1934) and 17°C by Carpenter (1928). However, the upper temperature limit of tolerance is greatly modified by exposure time e.g. P. felina can tolerate temperatures of 23°C and above for several hours (Pattee, Lascombe and Delolme 1973). It must also be remembered that the term eurythermic is a subjective one and not well defined,

and I use it here to describe the physiological responses of P. felina in comparison with P. tenuis and D. tigrina.

The respiration, growth and degrowth rates of P. tenuis are more temperature dependent than P. felina, and this is illustrated by the relatively high Q_{10} values. The proportion of the energy ingested, that is partitioned into the various metabolic outputs, is also temperature sensitive.

This study supports the views expressed by Dahm (1958) and Pickavance (1968), that D. tigrina is a thermophilic (i.e. warm-water) species. It feeds sporadically below 10°C, and respiration and growth rates increase dramatically above 15°C.

What is the adaptive significance of these differences in response to temperature and food availability? The Balmaha Pond population of P. felina commands a high degree of metabolic stability, and this is important, since it is exposed to wide diurnal and annual fluctuations in temperature. Problems arise, though, in extrapolating from the conditions occurring in Balmaha Pond outflow, to other lotic environments. In general, however, P. felina inhabits the mid-region of streams (Wright 1968, 1972, 1974; Reynoldson 1978), where the annual and daily temperature fluctuations tend to be greater than at the source (Ruttner 1963). Nevertheless, considering the widespread distribution of P. felina, there must be populations which inhabit streams with greater and lesser degrees of thermal stability, and it would be interesting to compare the metabolic sensitivities of populations from both extremes.

In contrast to P. felina, P. tenuis is a lentic species and as such will be subject to thermal fluctuations of a different nature. Unfortunately, it is impossible to make direct comparisons

between the lentic and lotic environment, since there is no data on the temperature fluctuations for the littoral region of Loch Lomond, or any other local loch.

Balmaha Pond outflow is subject to rapid changes in temperature and water flow, which seem at first glance unpredictable. This pattern is common in many streams, particularly in the mid-sections (Ruttner ibid). In environments such as this, animals might be expected to reproduce sexually, since sex increases variability, and thus improves the chances of survival of any progeny (Williams 1975). This paradox between the persistence of asexual and parthenogenetic reproduction in fluctuating environments is apparent in many plants and animals (Levin 1975; Glesener and Tilman 1978), and in order to resolve the problem, Hamilton (1975) has suggested that the concept of environmental uncertainty be examined more closely.

Balmaha Pond outflow was studied over an 18 month period, and measurements of temperature, water flow and food availability, established that the environment was predictably unpredictable, i.e. fluctuations were unpredictable in the short term, but when measured over a longer period of time, a pattern could be discerned.

Hamilton (ibid) further suggested that the biotic component of an environment was particularly important in determining environmental uncertainty. Following on from this, Glesener and Tilman (1978), in a review of the geographic distribution of parthenogenesis in terrestrial animals, showed that sexuality is favoured in conditions of high biotic diversity, where the interspecific interactions (predation, competition, parasitism) are unpredictable. The uncertainty they propose, stems from the type of competition, predator or parasite, an organism may encounter at various times, and the uncertainty of the

genotype of these organisms. This latter component of unpredictability is likely to be sufficient to select for sexuality (Maynard Smith 1971b).

From this viewpoint, then, Balmaha Pond outflow is not a highly unpredictable environment. There are very few predators present, and parasitism and interspecific competition are also likely to be negligible. The major sources of mortality are through being swept downstream into an unsuitable environment, or being badly injured by the current. Current may also be important in reducing intraspecific competition, since it acts in a density-dependent manner i.e. when the P. felina population is large, high numbers of triclads are recovered from the drift after a comparatively small increase in the volume of water flowing down the stream (see Chapter 9). The adoption of an opportunistic, catholic diet by P. felina seems prudent, for despite the fact that a source of food (drift) may be available throughout the year, it is unlikely to be plentiful, since streams are often less productive than lakes (Ruttner 1963). In such circumstances, a bonus of reproducing asexually is that it costs less in energy terms, since there is no need to build sexual apparatus, or to produce gametes (see Calow, Beveridge and Sibly 1979, for discussion). The adoption of this less expensive means of replication is found in all 3 species of rheophilic triclad (P. felina, Ph. vitta and C. alpina) which occur in the U.K. (Reynoldson 1978).

In contrast to streams, lakes tend to be more biotically saturated, and interspecific competition for food between different species of triclads is often intense (Reynoldson and Bellamy 1971). All British species of lake-dwelling triclad - with the exception of D. tigrina - reproduce sexually (Reynoldson 1978). D. tigrina is, however, a recent immigrant to the U.K., and hence has a rather

peculiar distribution. It is likely that the present day distribution is the result of the rapid proliferation and subsequent dispersal of a few colonists of unknown genotype and origin. Despite its thermophilic nature, asexual D. tigrina is able to compete successfully with the indigenous lake-dwelling species such as P. tenuis, because it can capitalise on the short summer by growing and fissioning at a very fast rate. This strategy may be less successful in exceptionally eutrophic lakes, where biotic stress is high. In Virginia Waters, for example, which has a very high biotic diversity, and where competition is particularly intense, a sexual population of D. tigrina has become established (Pickavance 1968).

All asexual triclads are polyploid, and this is believed to be primarily responsible for the lack of sex, or asexual condition (Iwashiro and Sachiko 1975; Bromley 1976). Polyploidy has also been found to enhance genetic variability in many species of plant and animal (e.g. Salisbury 1942; Baker 1965; Levin 1975). Thus the lack of variability often associated with populations which reproduce asexually may be partially compensated for by being polyploid. Among triclads, the polyploid asexual races may be more eurytolerant (i.e. tolerant of a wide range of environmental conditions) than the sexual populations. Pattee and Persat (1978) demonstrated that the asexual races of P. felina and D. gonocephala are more tolerant of high temperatures than their sexual counterparts. Preliminary results from an electrophoretic study of a number of enzyme systems in the Balmaha Pond outflow population of P. felina suggests that there is a high degree of enzyme variation present, both within and between individuals, which may explain the eurythermic nature of this population (unpublished data).

Both D. tigrina and P. felina are excellent colonisers. P. felina is found throughout the U.K., and is certainly the most common lotic species of triclad in Britain. The spread of D. tigrina to large parts of southern England and western Europe has been rapid. Asexual reproduction is a common feature of colonising species (Baker 1965), since it provides a mechanism for the rapid establishment of a population in a new environment, through the production of copies of a genotype which has proved successful under local conditions (Shick and Lamb 1977). Colonisers are also often eurytolerant (Lewontin 1965), possessing a good "general-purpose genotype" (Baker 1965).

A survey of American, Australian, European and Japanese species of freshwater triclads (literature cited in Chapter 7) shows that asexuality has only arisen in certain families, most notably the Planariidae and the Dugesiidae. Many of these species are common, particularly in lotic conditions, where for the reasons stated above, they have become particularly successful.

APPENDIX 1

The respiration rates of starved and fed P. tenuis, P. felina and D. tigrina at 5°, 10°, 15° and 20°C, on which 2-way ANOVARS were performed (see Section 5.4.2.)

R/A values were obtained from Tables 10a and 13a. Values were deleted using random number tables (Fisher and Yates 1963), so that there were equal numbers of observations per cell. Two-way analyses of variance were performed on the data, and the results are given in Table 15.

FED TRICLADS

Species	5°C		10°C		15°C		20°C	
P. felina	0.016	0.077	0.059	0.041	0.043	0.045	0.077	0.051
	0.084	0.008	0.053	0.096	0.043	0.109	0.053	0.050
	0.036	0.037	0.036	0.120	0.045	0.172	0.036	0.031
	0.029	0.035	0.031	0.052	0.085	0.043	0.067	0.039
	0.034	0.037	0.091	0.098	0.048	0.066	0.063	0.061
	0.025	0.011	0.025	0.084	0.122	0.051	0.083	0.046
	0.030	0.061	0.024	0.093	0.043	0.016	0.043	0.063
	0.013	0.059	0.045	0.052	0.090	0.052	0.038	0.061
	0.012	0.034	0.042	0.016	0.056	0.010	0.031	0.057
	0.070	0.010	0.120	0.067	0.076	0.045	0.051	
							0.066	
P. tenuis	0.042	0.021	0.076	0.069	0.104	0.076	0.091	0.068
	0.030	0.032	0.034	0.056	0.110	0.053	0.100	0.094
	0.030	0.021	0.077	0.044	0.117	0.064	0.067	0.064
	0.026	0.010	0.095	0.115	0.139	0.065	0.082	0.076
	0.023	0.028	0.061	0.076	0.129	0.052	0.133	0.058
	0.040	0.008	0.075	0.057	0.087	0.060	0.062	0.071
	0.028	0.026	0.041	0.081	0.085	0.049	0.051	0.067
	0.031	0.027	0.062	0.103	0.084	0.028	0.067	0.103
	0.034	0.014	0.075	0.031	0.062	0.036	0.102	0.121
	0.017	0.029	0.107	0.073	0.034	0.103	0.058	0.085
D. tigrina	0.012	0.015	0.046	0.027	0.047	0.046	0.101	0.058
	0.025	0.013	0.038	0.030	0.047	0.073	0.142	0.065
	0.004	0.007	0.020	0.021	0.060	0.066	0.093	0.026
	0.006	0.004	0.028	0.014	0.021	0.046	0.116	0.022
	0.010	0.020	0.018	0.018	0.052	0.004	0.056	0.051
	0.011	0.022	0.019	0.018	0.041	0.035	0.117	0.064
	0.008	0.003	0.023	0.031	0.051	0.023	0.063	0.059
	0.004	0.002	0.011	0.042	0.040	0.023	0.055	0.061
	0.007	0.002	0.036	0.036	0.025	0.010	0.137	0.059
	0.003	0.003	0.022	0.016	0.033	0.049	0.081	0.043

STARVED TRICLADS

Species	5°C		10°C		15°C		20°C	
P. felina	0.021	0.027	0.019	0.060	0.029	0.021	0.040	0.005
	0.011	0.006	0.047	0.029	0.032	0.078	0.020	0.018
	0.005	0.070	0.006	0.004	0.039	0.014	0.050	0.063
	0.008	0.018	0.022	0.016	0.012	0.035	0.079	0.058
	0.013	0.034	0.042	0.028	0.028	0.023	0.043	0.031
	0.015	0.020	0.013	0.029	0.020	0.024	0.059	0.066
	0.007	0.024	0.012	0.017	0.026	0.005	0.055	0.082
	0.006	0.034	0.020	0.026	0.033	0.037	0.040	0.049
	0.046	0.018	0.015	0.002	0.024	0.025	0.009	0.026
	0.017	0.017	0.012	0.006	0.037	0.019	0.010	0.030
P. tenuis	0.018	0.031	0.031	0.018	0.032	0.047	0.032	0.058
	0.014	0.010	0.023	0.015	0.055	0.060	0.024	0.037
	0.004	0.007	0.026	0.020	0.047	0.062	0.031	0.028
	0.024	0.011	0.044	0.007	0.051	0.026	0.041	0.071
	0.013	0.018	0.061	0.038	0.044	0.024	0.054	0.058
	0.015	0.013	0.043	0.004	0.044	0.027	0.028	0.040
	0.011	0.020	0.031	0.017	0.081	0.038	0.049	0.028
	0.034	0.019	0.065	0.020	0.030	0.056	0.035	0.046
	0.021	0.022	0.040	0.029	0.057	0.024	0.024	0.045
	0.016	0.030	0.081	0.015	0.046	0.020	0.027	0.024
D. tigrina	0.007	0.004	0.020	0.008	0.056	0.080	0.047	0.035
	0.009	0.010	0.013	0.011	0.034	0.041	0.044	0.067
	0.005	0.004	0.030	0.011	0.095	0.031	0.032	0.032
	0.019	0.004	0.052	0.005	0.010	0.034	0.034	0.053
	0.012	0.002	0.007	0.007	0.013	0.024	0.038	0.027
	0.007	0.006	0.005	0.009	0.070	0.014	0.046	0.032
	0.009	0.001	0.016	0.025	0.026	0.024	0.027	0.055
	0.008	0.009	0.003	0.022	0.069	0.032	0.033	0.039
	0.001	0.005	0.049	0.023	0.050	0.025	0.050	0.029
	0.005	0.010	0.012	0.029	0.032	0.061	0.046	0.032

APPENDIX 2

Numbers of *P. felina* heads, tails, regrowing heads, regrowing tails, and adults, found in samples from Balmaha Pond outflow between October 1977 and May 1979.

Note that prior to March 1978 samples collected from Balmaha Pond outflow were divided into only 4 categories.

	18.10.77		14.11.77		14.12.77		17. 1.78		22. 2.78		22. 3.78		25. 4.78		23. 5.78		17. 6.78	
	No.	% Total	No.	% Total	No.	% Total	No.	% Total	No.	% Total	No.	% Total	No.	% Total	No.	% Total	No.	% Total
Heads	22	32	21	29	7	6	6	9	4	10	3	7	5	10	10	6	4	4
Tails	8	11	4	6	4	4	7	10	4	10	1	3	1	2	2	2	3	3
Regrowing heads	24	35	33	46	61	53	25	37	14	38	20	47	18	34	51	29	17	15
Regrowing tails	-	-	-	-	-	-	-	-	-	-	4	10	7	14	29	17	18	16
Adults	15	22	14	19	43	37	30	44	16	41	15	35	21	42	85	48	74	64
Total sampled	69		72		115		68		39		43		53		177		116	

	17. 7.78		15. 8.78		19. 9.78		14.11.78		12.12.78		21. 1.79		14. 2.79		15. 3.79		17. 4.79		16. 5.79	
	No.	% Total	No.	% Total	No.	% Total	No.	% Total	No.	% Total	No.	% Total	No.	% Total	No.	% Total	No.	% Total	No.	% Total
Heads	3	3	4	8	17	28	10	19	5	14	3	2	7	12	1	4	11	20	12	20
Tails	2	2	2	4	6	10	5	10	1	3	5	4	1	2	3	10	2	4	8	14
Regrowing hds.	17	17	10	19	12	20	9	17	7	19	17	12	4	7	3	10	6	11	7	12
Regrowing tls.	12	12	6	12	4	7	7	13	4	11	12	8	9	16	3	10	8	15	8	14
Adults	67	66	32	60	23	37	23	43	21	56	115	76	38	65	20	67	29	52	25	42
Total sampled	101		54		62		54		38		152		59		30		56		60	

APPENDIX 3

Published Paper

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Heads and Tails: Adaptational Aspects of Asexual Reproduction in Freshwater Triclads

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SYNOPSIS. This paper uses energy relations to compare asexual and sexual reproduction, and fission and egg production in freshwater triclads. Egg production is more efficient at converting food to "reproductive energy" but fission is more efficient at converting this to offspring. Fission is therefore favored in ecological circumstances where food is always limiting and egg production in ecological circumstances where food is not always limiting. In principle, sex is the least efficient method of all the reproductive strategies, yet parthenogenesis and self-fertilization are rare. We cannot be sure, however, to what extent the metabolic losses incurred by sperm production in hermaphrodites are made good by resorption of the unused sperm derived from the mate. Furthermore, possible short-term and long-term balancing advantages of the sexual strategy can be identified in triclads.

We also consider how, in those triclads which take the fission option, the body biomass should be divided between the head and tail in order to maximize the fitness of the system. If mortality is independent of size, a midway fission plane should be favored. However, strong size-dependent mortality occurs during spates in the streams in which fissiparous triclads are found, "pushing" the optimum fission plane tailwards.

INTRODUCTION

Freshwater triclads are a good group for investigating theories associated with the adaptational biology of life-cycles and reproductive patterns since much is known about their general ecology (*e.g.*, Reynoldson, 1966*a*) and physiology (*e.g.*, Hyman, 1951). They can also be studied with comparative ease in both laboratory culture (*e.g.*, Calow and Woollhead, 1977*a*) and field populations (*e.g.*, Reynoldson, 1966*a*) —and they show considerable inter- and intraspecific variation in their life-cycle and reproductive patterns, adopting sexual and/or asexual strategies.

The sexual triclads are hermaphrodite, usually practice cross-fertilization, produce egg capsules (containing several to many developing embryos, nurse cells and yolk reserves) (Hyman, 1951) and may either be semelparous (breed once and then die) or iteroparous (breed repeatedly) (Reynoldson, 1966*a*). The asexual forms usually practice binary fission, dividing into head and tail fragments each of which then regenerates the other. Following Wagner (1890) this method of fission is called architomy and is to be distinguished from paratomy in which organs, in particular the head apparatus of the tail, are regenerated prior to fission. Ax and Schultz (1959) made a short survey of the fission mechanism in the Turbellaria and found architomy to be the most important method in the triclads while paratomy was the predominant form of fission in catenulid and macrostomid rhabdocoels. A particular population of triclads may practice either asexual or sexual reproduction exclusively or a combination of both. There has been much controversy on whether such within-species variations in reproductive strategies between populations is a direct consequence of environmental factors or genetic control (*cf.*, Kenk, 1941; Hyman, 1951), but a large genetic element is now suspected (Grasso, 1974). Despite widespread intraspecific variation, certain species seem predisposed to either the sexual or asexual strategies; for example, semelparous triclads rarely practice fission and have poor regenerative powers (Brøndsted, 1969).

In this paper our main concern will be with the adaptational aspects of the asexual strategies in freshwater triclads and these we will consider against the background of the general debate on sexual and asexual reproduction (Williams, 1975; Maynard Smith, 1978). The main focus of attention

in this debate has been on the so-called cost of sex: that is, because there are no males the rate of increase of asexually produced offspring should be more than twice that of sexually produced offspring. Given this disadvantage, sex should be rare and, since it is not, there is an anomaly which has stimulated the search for balancing advantages. Models constructed to explain the anomaly usually depend on the fact that meiosis and fertilization allow genetic mixing which, in principle (though some would not say in practice; Maynard Smith, 1978), may bring both long-term and short-term benefits to organisms living in variable habitats. The foregoing arguments are based, of course, on the assumption that asexual and sexual systems are similar in every way except that sex involves meiosis and fertilization: *i.e.*, the asexual forms are presumed to be parthenogenetic. In fissiparous organisms, however, the comparison between sexual and asexual reproduction becomes less straightforward, for the reproductive potential of the sexual and asexual systems then differs not only in terms of the amount of energy made available for the production of the new offspring but in terms of the way that this energy is packaged and delivered. Our first concern will, therefore, be a comparison between fission and egg production and only later will we consider the complication of sex. We will concentrate on the metabolic aspects of each strategy, since we believe that in resource-limited organisms, particularly top carnivores, the metabolic constraints inherent in each method of reproduction will be important as general constraints in deciding which strategy is most appropriate. More specific explanations of the ecological distribution of fission and egg production, and asexual and sexual reproduction in the triclads will undoubtedly require more specific models and information on the relative survivorship, growth rates and reproductive potential of the offspring produced. This is the subject of on-going research and we can only indicate here the kinds of information required in such models.

Finally, the two specific questions with which we shall be concerned are: Why are some triclad species predominantly fissiparous, and why do the fissiparous forms divide in the way they do? The first question will lead to a consideration of the more general issues associated with the relative merits of sexual and asexual reproduction, whereas the second question considers how "having taken the fission option" a triclad should optimize its division strategy in order to maximize its fitness. Although for the most part we use data obtained from *Polycelis felina* (predominantly asexual in the U.K.), the closely related *Polycelis tenuis* and *Dendrocoelum lacteum* (exclusively sexual in the U.K.), we also discuss the other British triclads.

ENERGETICS OF FISSION AND EGG PRODUCTION

Organisms can be considered as energy transformers which partition an input (of food in animals) between respiratory metabolism, growth, storage materials and reproduction. It is to be expected that these energy transactions will maximize fitness (Calow, 1977*a*). This should lead to a maximization of the conversion of food to somatic and gametic tissues except when other metabolic demands (*e.g.*, arising out of movement) are important for the survival of the parent and its ultimate reproductive success. Reynoldson (1961*a*) was the first to suggest that metabolic constraints may be important in the evolution of fission and gamete production in triclads but was unable to be very explicit on this point. In this section, therefore, we review data now available on the energetics of reproduction in triclads and their progeny in order to investigate the potential relevance of metabolic constraints to the problem of when fission and gamete production are the most appropriate reproductive strategies.

Figures 1a to c show the partition of input energy between growth, reproduction and the rest of metabolism (= respiration + excretion + mucus secretion) in semelparous, iteroparous and fissiparous triclads. Figure 1a and b refer to *Polycelis tenuis* (= perennial and iteroparous) and *Dendrocoelum lacteum* (= annual and semelparous) respectively and are derived from data in

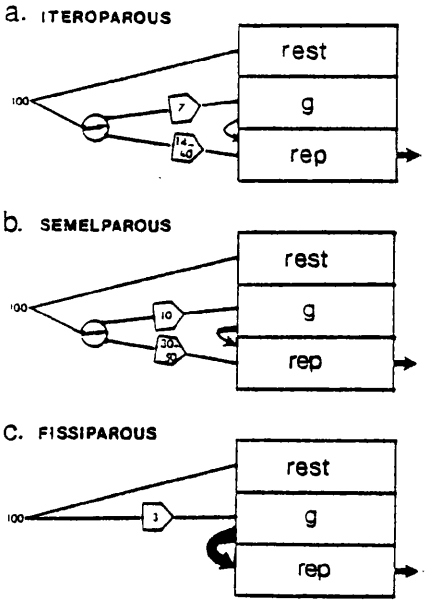

FIG. 1. Energy partitioning of input (= absorbed energy, = ingested energy) between reproduction (rep), growth (g) and the rest of metabolism in an iteroparous (a), semelparous (b) and fissiparous triclad (c). Figures in the arrowed boxes represent conversion efficiencies; those for growth are calculated over the last half of the growth cycle whereas those for reproduction are based on a fifty-day measurement period. All figures assume that food supply is non-limiting. See text for source of data.

Calow and Woollhead (1977*a*) and Woollhead and Calow (1979). Figure 1 c is for *P. felina*. The input in all cases is expressed in terms of 100 arbitrary energy units and is equivalent to absorbed energy. However, there is little defecation in triclads so the input can also be taken to approximate to ingested energy (Woollhead and Calow, 1979). Within each system there is the option of partitioning this food intake directly among the three metabolic compartments and of redistributing energy from one compartment to another. The efficiency of converting input to growth directly represents the average efficiency over the last half of the growth cycle. In this respect the semelparous *D. lacteum* is a more efficient converter than *P. tenuis* (possibly because of differences in feeding behavior and rates of searching, in preparation), and both egg producers are more efficient converters than *P. felina*—again possibly as a result of differences in activity levels. Under laboratory conditions, for example, we find that the rates of movement of similar-sized triclads at 10°C (for techniques see Calow, 1977*a*), are about twice as great in *P. felina* as in *P. tenuis* (rate of movement of *P. felina* = 352 ± 36.1 cm day^{-1} and for equivalent-sized *P. tenuis* = 163 ± 17.4 cm day^{-1}; $t = 6.3$; $P < 0.001$). The high level of activity in *P. felina* puts constraints on conversion, but might, nevertheless, be a necessary adaptation for life in fast-flowing streams (see below).

Most of the energy available for reproduction (hereafter called "reproductive energy") in the gamete producers is derived directly from their food and as soon as reproduction is "switched on" in these triclads growth is "switched off." Some of the reproductive energy in the gamete producers might be derived from somatic tissues, particularly in *D. lacteum* under poor trophic conditions (Woollhead and Calow, 1979), and adjustments for this have been made in calculating the conversion efficiency of input to reproductive energy. These adjusted values are, nevertheless, still greater than the growth efficiencies which operate just prior to the time that reproduction is "switched on" and this difference seems to occur generally in animals (Calow, 1979). It is probably due to the fact that undifferentiated gametes can be produced much more easily and quickly than differentiated somatic tissue so that a larger amount of biomass production can be achieved in the gonads for a given amount of basal metabolism than in the organism as a whole. There is no significant change, for example, in the respiratory metabolism of triclads during the breeding season (Woollhead and Calow, 1979) but there is an increase in food intake (unpublished).

In fissiparous triclads, energy cannot be converted into reproductive propagules directly. Instead it is converted to somatic tissue through the normal processes of growth and then separated from the "parent" as a "tail fragment." This means that fission cannot make use of the same highly efficient conversion processes as gamete production. Because of this, a gamete producer can in principle render more energy

to reproduction per unit time than a fissiparous triclad.

The same point is made in the data presented in Table 1. This summarizes information on the size of adults, hatchlings and egg capsules produced by a variety of semelparous and iteroparous triclads. There is much variation in the data which will be discussed in detail elsewhere. From the present point of view, however, the most interesting information relates to the capsule and the energy it contains. Since the release of one capsule is a "quantum reproductive event" equivalent in a sense to the release of a tail fragment, it is relevant to compare the time required to produce a capsule via gamete production with the time that would be required to grow an equivalent amount of tissue to be released in fission. On average it would take a gamete producer about 9 times longer to produce an equivalent amount of tissue for fragmentation by fission than by capsule production. For comparison with the data in Table 1 it is of interest to note that a tail fragment produced by *P. felina* contains approximately 3.5J and that a head takes approximately 28 days to grow this amount of tissue.

Despite gamete production being more efficient in yielding energy to reproduction, however, it is less efficient in yielding that energy to the "finished" offspring. This is illustrated in Table 2 which compares the conversion process of an equivalent amount of reproductive energy to offspring in a fissiparous, *P. felina* (real data) and a gamete producer (hypothetical example). Both parthenogenetic and sexual egg production are considered. Egg production carries definite extra costs in building the apparatus necessary to form the gametes and in fertilization but it is not possible to be precise, at this stage, over the amounts involved. The cost for the sexual form might be as much as twice that of the parthogenetic form, however, because in triclads the male part of the hermaphrodite system is as extensive as the female (Hyman, 1951). Actual data on *P. felina* suggest that of all the reproductive energy involved in the tail fragment, only about 5% is used up in the initial non-feeding, developmental stage when the tail is reorganized into a complete worm. By contrast, as much as 40% of the reproductive energy may be used up in the more extensive embryonic development that goes on within the egg capsule (Calow, 1977*b*). In addition the sexual system carries extra costs in sperm production and the search for mates. We put the sperm cost as 50% of the reproductive energy because, despite hermaphroditism, the relative size of gonads in triclads seems to suggest an equal distribution of energy between ovaries and testes. However, we are unsure to what extent energy loss in sperm can be made good in hermaphrodites by absorption of unused sperm derived from a mate and we have no estimates on the cost of searching.

As a result of the definite extra costs incurred by gamete production, the final size of offspring produced is likely to be smaller for an equivalent amount of reproductive energy in the gamete-producers than in the fissiparous triclads. Hence the developmental time of offspring from fission or hatching to their own reproduction and the energy required to convert them to adults is likely to be greater for those produced from gametes than for those produced from fission. For *P. felina*, for example, which has a final size of 14J, the products of fission take about 25% less energy and 15% less time to develop than the products of gamete production.

ECOLOGICAL DISTRIBUTION OF FISSION AND EGG PRODUCTION

The previous section led to the conclusion that gametic tissue can be made more efficiently and more rapidly than somatic tissue (at the later stages of growth), but is converted less efficiently into "finished" offspring. Hence to compare fission with egg production we consider the rate of production of offspring and their subsequent rate of development. The fitness of individual offspring may also be at stake since large offspring are often less susceptible to mortality than small (Smith and Fretwell, 1974). On the other hand, genetic variability in sexually produced offspring

TABLE 1. *Comparison of the products of reproduction, their rate of production and the time required to grow an equivalent amount of somatic tissue in several freshwater triclads at 10°C.*

	Adult size mm²[a]	Hatchling size		Capsule size J	Days to produce one capsule	Days to grow equivalent biomass of somatic tissue[b]
		mm²	J			
SEMELPAROUS						
Bdellocephala punctata	60	2.4	2.8	48	7	100
Planaria torva	30	1.5	1.6	11	6	40
Dendrocoelum lacteum	50	2.0	2.4	46	7	92
ITEROPAROUS						
Dugesia lugubris	50	1.0	0.8	12	7	40
Polycelis tenuis	35	0.9	0.3	2.5	3	16

[a] Approximate.
[b] Calculated roughly from growth efficiencies and feeding rates, assuming a superabundant food supply. All figures are rounded up to nearest whole number.

TABLE 2. *Comparison of efficiencies of converting reproductive energy to offspring and then to reproductive adults for different modes of reproduction of* P. felina *at 10°C.*[a]

	Fission	*Parthenogenesis*[b]	*Sex*[b]
Energy available	3.5	3.5	3.5
Cost of reproductive apparatus[c]	0	x	2x
Cost of sperm	0	0	0.5 × 3.5
Energy available for development	3.5	3.5	1.75
Cost of development	3.5 × 0.95	3.5 × 0.6	1.75 × 0.6
Energy/offspring	3.33	2.1	1.05
Final size to be reached by adult	14	14	14
Approximate J that must be ingested to reach final size[d]	300	396	430
Min. gen. time (days)	75	85	90

[a] All energy data in Joules.
[b] Hypothetical.
[c] See text for further explanation.
[d] x = unknown quantity—see text. Calculated on basis of conversion efficiencies assuming a non-limiting food source.

might raise the overall fitness of the genes they carry despite the reduced size (Williams, 1975). The ecological distribution of fission and egg production should depend on the relative advantages of each of these properties in different habitats and possibly on the metabolic properties of the different species of triclad (*e.g.*, determined by activity levels).

Before we can evaluate the ecological distribution of egg production and fission we will need to know the survivorship of the different developmental stages of triclads in the habitats in which they live. Such information is not yet available, but some qualitative predictions are, nevertheless, possible on the basis of information that is on hand.

The trade-off between the two modes of reproduction is between their ability to exploit good conditions of food supply to make energy available for reproduction and their ability to convert this reproductive energy into a reproductively competent adult. The success of egg production therefore depends on the free availability of food. In conditions of poor food supply, the effect of the inefficient conversion of reproductive energy to offspring and then the slower more expensive conversion of this to adults is likely to become more serious. Consequently, as trophic conditions deteriorate, egg production is likely to be less favored and fission more favored. Reynoldson (1961*a*) suggested that trophic conditions were worse for triclads in streams (lotic habitats) than lakes (lentic habitats) and noted that fission is most

common in stream-dwelling as opposed to lake-dwelling triclads. Primary production is certainly low in lotic conditions because of the scouring action of water movements (Douglas, 1958) and this is likely to have adverse effects on secondary production and therefore on prey availability for predatory triclads. Furthermore, these trophic conditions may be exacerbated since stream-dwelling triclads, with higher activity levels, are less efficient converters than lake-dwelling forms (Reynoldson, 1961*a*). However, such metabolic constraints may not apply to all stream-dwelling species (Calow, 1977*c*).

It would be a mistake to suggest, however, that the littoral regions of lakes in which sexual triclads are more common than asexual triclads are consistently richer than the head-waters of streams. Typical temperature profiles from each habitat suggest, in fact, that the littoral habitats are more strongly seasonal than the lotic habitats (Calow and Beveridge, unpublished). Furthermore, life-cycle patterns obtained from seasonal sampling (in the iteroparous *Polycelis tenuis*, Reynoldson, 1960; *Dugesia lugubris/polychroa*, Reynoldson, 1961*b* and *Polycelis nigra*, Taylor and Reynoldson, 1962; and the semelparous *Dendrocoelum lacteum*, Young and Reynoldson, 1965; and *Planaria torva*, Reynoldson and Sefton, 1972) also suggest strong seasonality (see summary chart; Fig. 2) with conditions for at least half the year being unsuitable for growth and perhaps even causing degrowth (shrinkage). Conditions over the rest of the year are sufficient to allow rapid growth of both the hatchlings and post-wintering adults and to allow continuous egg-capsule production over three to four month intervals. The progeny of most egg producers on hatching are not very different in size from those of *P. felina* (see Tables 1 and 2). Hence, egg production wins because it can result in rapid numerical enrichment of populations and can therefore more fully exploit a relatively short pulse of trophic enrichment. The relative merits of semelparity and iteroparity depend on the ability of hatchlings to cope with deteriorating trophic conditions as a result of the recruitment of more "mouths" into the population at the end of the breeding season, and this has been discussed elsewhere (Calow and Woollhead, 1977*a*; Woollhead and Calow, 1979).

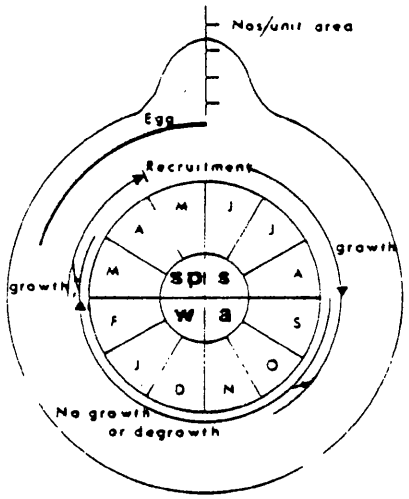

FIG. 2. Schematic representation of annual cycle in lake-dwelling triclads. Recruitment most often occurs in May/June when the egg-capsules hatch. Triclad density increases dramatically at this time, competition is intensified and there is much mortality (particularly in the semelparous species). Those hatchlings that survive through this period grow until September/October. Thereafter trophic conditions deteriorate and there is a period of no growth or degrowth. Growth resumes in spring and egg production occurs shortly after. Most of the density adjustments in the population occur in May-June, thereafter biomass adjustments predominate. N.B. precise timing may vary from species to species and from year to year.

The only other case that has not been considered so far is the one where fission and egg production are used at different times in the life-cycle. This would seem most likely when conditions of food supply switch from moderately poor (sufficient for some growth) to good. Switching of this sort perhaps occurs in the U.K. in *Crenobia alpina* which, interestingly, is not as metabolically inefficient as *P. felina* (Calow, 1977*c*) and which may also undertake considerable seasonal migrations within streams to exploit optimal trophic and temperature conditions at different seasons (Beauchamp and Ullyott, 1932; Beauchamp, 1937; but see also Wright, 1968).

WHY SEX?

We have suggested why, in certain eco-

logical circumstances, egg production is favored but the question now arises as to why this should occur by sexual and not parthenogenetic means. Furthermore why is self-fertilization rare in triclads even though there are no apparent morphological barriers to it (Hyman, 1951) and though genetically-identical triclads (from the same clones) can cross-fertilize to produce viable offspring (Dahm, 1958)? At present we are only able to give a glimpse at the possible solutions to these problems.

In the first place it is necessary to re-emphasize that because of sperm absorption the cost of sex for triclads may not be as great as the two-fold cost usually discussed (Maynard Smith, 1978). Consequently the balancing advantages of sex need not be as great as is usually required.

Of these balancing advantages it is difficult to reconcile the life histories of the British freshwater triclads with the sib-competition models of Williams (1975). The most likely candidate is *Crenobia alpina*, which at least in one stream (Macan, 1974) is found in discrete patches (*viz.*, the headwaters of minor streams that adjoin a bigger stream). Some workers hold that *Crenobia alpina* reproduces sexually in winter and asexually during the rest of the year (Reynoldson, 1961*a*; but see also Wright, 1968), which would fit Williams' aphid-rotifer model. Whether competition occurs within a patch to the extent that just one genotype prevails by the end of the year, and whether that genotype is randomly selected from those initially supplied, is at present unknown.

On the other hand the ecological data discussed above do suggest that sex is found in triclads occupying ecological circumstances of greatest biotic stress. This supports the suggestion of Glesener and Tilman (1978) that biological components of environmental uncertainty have been important for the origin and evolution of sex. Thus the faunal diversity of lentic habitats is much greater than that of lotic habitats so that the biotic interactions and environmental uncertainty should be greater for triclads in lentic ecosystems. However, lake-dwelling triclads are not significantly parasitized or predated (Reynoldson, 1966*a*) so that the other organisms encountered are prey (usually dead or dying) and other triclads. The implication of these kinds of biological interaction for the hypothesis proposed by Glesener and Tilman have not so far been considered (see also paper in present volume) and it will therefore be necessary to produce a population genetics model to incorporate the new features. The most obvious characteristic that might affect the fitness of conspecifics competing for food is their size. This is because size at reproduction is very variable, even between genetically identical individuals, with obvious drastic consequences for generation time and therefore reproductive rate. In addition, we can imagine many ways that size might affect an individual's success in interactions with other triclads. Whether these are plausible in the field (or even in principle) remains to be demonstrated.

STRATEGIES OF FISSION

In this section we consider what criteria are important when a triclad divides up its body during fission. We are particularly interested in how tissue is divided between the head and the tail in binary fission, but we will also consider architomy *versus* paratomy.

Fission occurs by a tearing process in which the tail part holds firmly onto the substratum and the head moves away from it (Child, 1915). However, there is physiological evidence that the plane of fission is determined prior to separation so that the process is probably not haphazard (Child, 1915). After fission the head regrows a tail and the tail develops a new head apparatus. Since the mouth and pharynx are useless without the ability to find food, and since the latter depends on an intact head with sensory eyes and possibly tentacles, the split invariably occurs below the mouth and pharynx. This means that the head with the mouth can continue feeding without interruption whereas the tail must regenerate a new pharynx. In the latter case the mouth and head apparatus develop almost simultaneously and only then does the worm become active (Fig. 3). However, the energy costs of active development must be as great as those for active feeding and movement, for we can find no significant difference

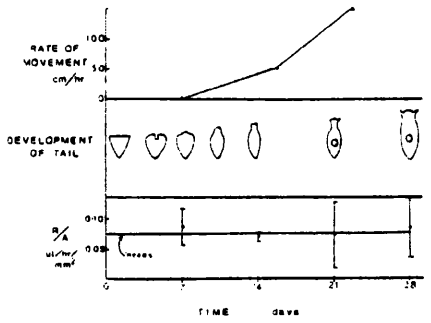

FIG. 3. Development of a tail fragment of *P. felina* at 10°C and associated rates of movement (determined as in Calow, 1977*a*) and respiratory rates (R/A = μL oxygen intake per hour per mm² of triclad; for rationale see Calow and Woollhead, 1977*b*). Solid horizontal line = R/A for heads. Points are for tails. Vertical bars = 95% confidence intervals.

between the respiratory rates of the inactive and active tail fragments (Fig. 3), and indeed there is no significant difference between these data for tails and the respiratory rate per unit area of heads ($P < 0.05$) at any temperature.

The overall growth process of *P. felina* at 10°C for both heads and tails is as summarized in Figures 4a and b, Figure 4b being a plot of developmental time, from fission to fission, for head and tail fragments. Both growth curves suggest no major differences in either the growth pattern (over equivalent parts of the curve) or size at splitting for heads and tails—again implying no major metabolic differences between the two halves.

A reasonable initial assumption is that the fitness of a particular clone depends upon its intrinsic rate of natural increase (r) and that the division strategy is optimally adapted to maximize r. Now for a particular clone, r depends on the developmental times (defined in Fig. 4b) of heads (t_h) and tails (t_t) and (as shown in the Appendix) can be defined by:

$$1 = e^{-rt_h} s_h + e^{-rt_t} s_t \qquad (1)$$

where s_h and s_t are the probabilities of head and tail fragments surviving through to undergo fission themselves; e = base of natural logs. If it is assumed that the mortality of heads and tails is independent of size, then this mortality can be modeled as a

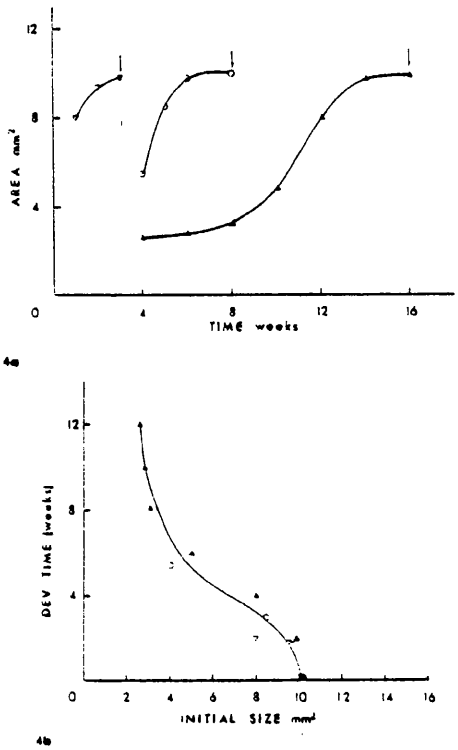

FIG. 4. a. Growth curves (triclad area in mm² *versus* time in weeks) for *P. felina* at 10°C. ▽ = "parent," ○ = head, ▲ = tail. b. Relationship between developmental time (fission to fission) and size; derived from Figure 4a.

Poisson function (see Appendix) and eq. 1 becomes:

$$1 = e^{-(r+\lambda)t_h} + e^{-(r+\lambda)t_t} \qquad (2)$$

where λ = probability of dying per unit time. By inserting t_h and t_t (derived from data as given in Fig. 4b) for different head and tail fission ratios it is possible to solve for r with different values of λ. For *P. tenuis* we find that all cases of λ result in r being maximized at a head to tail fission ratio of 50% (Fig. 5 gives results for $\lambda = 0$ and $\lambda = 0.5$). This result seems to be fairly general since it can be arrived at in other ways, using different assumptions on population growth and mortality (see Appendix).

The actual data diverge from this prediction, however. Fig. 6 shows the frequency-distribution of head to tail fission ratio from 50 individuals (mixed clones) kept at 10°C on super-abundant food. Here, the mean fission ratio is 0.76 head to

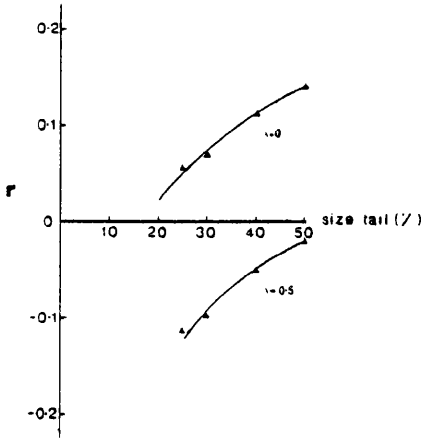

FIG. 5. Relationships between coefficient of exponential growth (r) and size of tail as % initial "parent" at different levels of mortality λ. See text for further explanation.

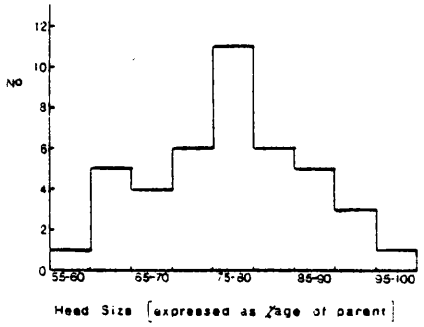

FIG. 6. Frequency distribution of head sizes (% initial "parent") after fission in a cohort of fifty at 10°C.

0.24 tail. Mean ratios at 5 and 15°C were 77.8% head and 75.3% head respectively and did not differ from the result at 10°C ($P < 0.05$). Some of our data do suggest a variation in the fission ratio with food supply (this ratio increasing to a higher value on smaller rations), but we have never found a 50% fission ratio to be normal under any conditions. Furthermore, the mouth is usually situated nearer the tail than the head, which suggests a "developmental presupposition" that the 50% fission would be disadvantageous. It is clear, therefore, that there is a significant discrepancy between the theoretical prediction and the actual findings. Since it is unlikely that the fission patterns of *P. felina* are suboptimal (with respect to fitness) some of the assumptions incorporated into eq. 1 and 2 must be incorrect. In this context there would seem to be two major possibilities: (1) that what is lost to r through the increased t_t associated with the smaller tail is more than counterbalanced by an increased tail survivorship; *i.e.*, mortality is not random and small tails are less susceptible than large; (2) that what is lost to r through an increased t_t is more than counter-balanced by an increased s_h; *i.e.*, mortality is not random, and any increase in head size enhances s_h more than s_t is reduced with reductions in tail size. Another possibility is that an off-center position of the mouth and pharynx could be more effective in dealing with prey and that physiological advantages so accruing could outweigh the disadvantages to fission. Hence, mouth position would determine all else. However, we cannot think of any reason why this should be so and the more plausible explanation would seem to be that other factors affecting survivorship favor a head to tail fission ratio of greater than 50% and that this determines the position of the mouth.

There are two major potential sources of mortality in stream-dwelling triclads which must be considered in terms of criteria 1 and 2 listed above. These are being washed away, crushed and battered to death by watercurrents (particularly in spate), and being starved to death in the poor trophic conditions which are likely to prevail in lotic habitats (see above). Predators also play a part in the population regulation of stream-dwelling triclads and may perhaps feed selectively on larger individuals, but, at this stage, their impact is difficult to assess (Wright, 1975).

On the effect of water currents, we have found from investigation on a relatively small population (= 10^3 to 10^4 individuals) of *P. felina* in a circumscribed portion of a small, moderately rapid stream that, dependent on the rate of water flow, between 1 and 100 worms can be collected daily in drift nets. This indicates how important water current might be in effecting mortality and regulating population size and growth. To investigate the nature of this effect more precisely we have measured the critical flow rates at which heads and tails of different sizes became dislodged in the lab-

oratory. This experiment will be described in more detail elsewhere but some of the results are summarized in Figure 7. There was a linear negative relationship between critical flow rate (CFR) and triclad size (measured as plan area—Calow, 1977*c*). The equations for the head and tail lines are:

$$\text{for the head, CFR} = 57.4 - 3.1\ \text{Area}$$
$$(t\,(b = 0) = 7.8, P < 0.001)$$
$$\text{for the tail, CFR} = 69.1 - 12.8\ \text{Area}$$
$$(t\,(b = 0) = 4.9, P < 0.001)$$

The lines differed in slope ($P < 0.01$) but not in intercepts ($P > 0.05$). Clearly, large triclads were more susceptible to flow rate than small (possibly because the small ones can fit more easily into the static water layer which runs near to the water-substratum interface; Ambühl, 1959), but larger heads were less susceptible to being washed away than larger tails. Our observations suggest that this difference between heads and tails may be due to differences in mobility because the heads, unlike the tails, can rapidly orient their bodies with regard to current and can swim against it. This could account for the increased activity rates found generally in stream-dwelling triclads (see above and Pattee and Bournaud, 1970). The main conclusion, however, is that mortality through water-movement is likely to conform to criterion 1 as listed above (*i.e.*, that small tails will be less susceptible to mortality than larger) and this would therefore tend to favor an increased head to tail fission ratio in moving water.

Triclad size is also important in the effect of starvation on mortality because: (a) starving triclads subsist on their own tissues and as a result shrink or degrow, and (b) shrunken triclads may recover upon re-feeding but there is a minimum size beyond which recovery is impossible (Reynoldson, 1966*b*; Reynoldson, 1968). Degrowth of the head and tail fragments of *P. felina* is of the usual exponential kind (Fig. 8) and the coefficients of exponential degrowth for each fragment (ignoring the first few days when developmental changes result in tissue reorganization and alterations in plan area without any significant change in volume) do not differ significantly (coeff. head = 0.025 (± 0.008); coeff. tail = 0.036 (± 0.011); $F = 2.35, P > 0.05$). The minimum size for recovery of *P. felina* at 10°C is approximately 0.4 mm² for both heads and tails (Calow, 1977*c*).

The actual head-to-tail fission ratio is therefore likely to be a compromise between four major influences: reproductive potential, susceptibility of fission products to water-currents, susceptibility of fission products to starvation and possibly predation (Fig. 9). Details of the compromise must await information on the frequency of starvation and critical flow rates for natural populations, but from the data we have on head-to-tail fission ratios we suspect that the water-current is the major selective force on *P. felina*.

Finally, we note that one way of reducing the risks of starvation for both head and tail

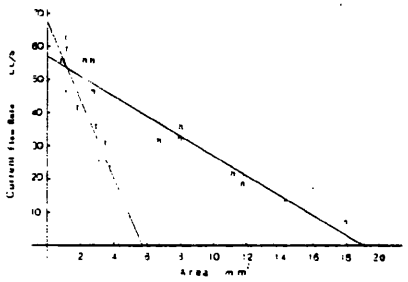

FIG. 7. Relationship between critical flow rate at which a fragment is dislodged and size (mm²) of fragment for heads (h) and tails (t) of *P. felina*.

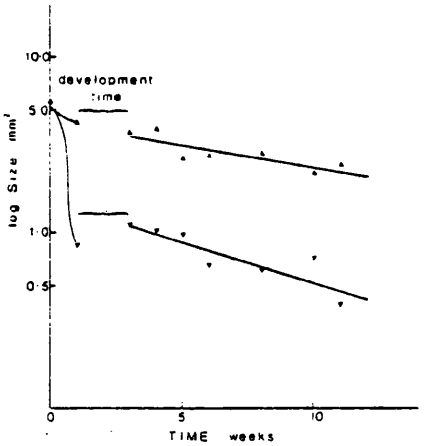

FIG. 8. Degrowth during starvation in heads ▲ and tails ▼ for *P. felina* at 10°C.

is to maintain a connection between both until the tail has developed sufficiently to be less vulnerable. That is, to practice paratomy (a kind of parental care) rather than architomy. However, the developing tail might impede and hinder the head and thereby render it more prone to current action and make it less efficient at finding food. Interestingly, paratomy is almost entirely restricted to rhabdocoel turbellarians (Hyman, 1951) which live generally in the interstices of sediments. These habitats are protected from water movements and carry a rich food supply of bacteria and protozoa (Meadows and Anderson, 1967). Annelids which live in the same habitats also show a similar method of asexual reproduction (McElhone, 1978). There are a few records of paratomy or intermediate forms of fission in freshwater triclads (*e.g.*, Kennel, 1889; Keller, 1894; Fuhrmann, 1914) but little is known of the ecology of these aberrant species.

CONCLUSIONS AND PROSPECTS

In this paper we have built a general explanatory framework for the occurrence of fission and egg production in triclads based on an understanding of the physiology, and more specifically the bioenergetics, of each process. More precise explanatory models of the ecological distribution of each reproductive type and of the optimum head-to-tail fission ratio will depend on more precise information about environmental variables like food supply and current-flow, about survivorships of the different life-stages of the triclads themselves, and about the relationship between enviornmental variables and survivorships. Ideally this will require an intensive study either on those species which show inter-

FIG. 9. Factors influencing positioning of fission plane (F) between head (H) and tail (T) in *P. felina*. Numbers represent head-to-tail fission ratios (%). See text for further explanation.

population variation in reproductive pattern or on a single population which cycles between fission and egg production. Good subjects for this kind of research would be *Crenobia alpina* in the U.K. (see Reynoldson, 1961*a*) and *Dugesia tigrina* in the U.S.A. (see Kenk, 1941). The latter species is also a recent immigrant to the U.K. (Dahm, 1955).

The relative merits or demerits of sex in the triclads remain unexplained. At present we are unsure of the cost of sex over parthenogenesis in terms of extra reproductive apparatus, sperm production and mate location. In particular, we need a more careful appraisal of the bioenergetics of the sexual parents. On the other hand it is possible to suggest potential balancing advantages of sex both in terms of long-term group effects and short-term individual effects. Being more precise about these will require more extensive information about the geographical distribution of sex in triclads, the construction of population genetics models incorporating the pecularities of the triclad system and laboratory data on such things as the size-dependence of competition for food.

APPENDIX

In a fissiparous clone individuals reproducing at time t are either head-derived (that is, derived from the head end of an individual that underwent binary fission time t_h previously) or tail-derived (at time t_t previously). Let $N(t)\Delta t$ be the number reproducing between t and $t + \Delta t$. If the survivorship from fission to fission of head-derived individuals is s_t we have

$$N(t)\,\Delta t = N(t - t_h)\,\Delta t\, s_h + N(t - t_t)\,\Delta t\, s_t$$

If the population is growing exponentially at intrinsic rate of increase r then

$$N(t - t_h) = N(t)\, e^{-r t_h},$$
$$N(t - t_t) = N(t)\, e^{-r t_t}, \text{ so}$$
$$1 = e^{-r t_h} s_h + e^{-r t_t} s_t \qquad (1)$$

If mortality of heads and tails occurs independent of size according to a Poisson process with parameter λ then the chance of a head-derived individual surviving for time t_h is $e^{-\lambda t_h}$, $= s_h$. Similarly $s_t = e^{-\lambda t_t}$.

Thus equation (1) becomes

$$1 = e^{-r t_h} e^{-\lambda t_h} + e^{-r t_t} e^{-\lambda t_t}$$

or

$$1 = e^{-(r+\lambda) t_h} + e^{-(r+\lambda) t_t} \qquad (2)$$

Other models of population growth may sometimes be useful. For example $N(t - t_h)$ can in general be expanded as a Taylor series as

$$N(t - t_h) = N(t) - t_h \frac{\partial N}{\partial t} - \frac{t_h^2}{2!} \frac{\partial^2 N}{\partial t^2} + \cdots$$

This yields

$$1 = s_h + s_t - r(t_h s_h + t_t s_t) + \cdots \qquad (1')$$

since

$$\left[\frac{1}{N}\frac{\partial N}{\partial t}\right] = r$$

by definition. If terms in t_h^2 and t_t^2 can reasonably be ignored (*i.e.*, population growth is linear)

$$r = \frac{s_h + s_t - 1}{t_h s_h + t_t s_t}$$

Let the pattern of growth, and size just prior to splitting, be considered as "evolutionary constraints." Then developmental time is a given function $t(x)$ of size x just after splitting, and $x_h + x_t = X$, where X is the size just before splitting, and subscripts h and t denote heads and tails as before. Hence $t_t = t(X - x_h)$, so

$$r = \frac{s_h + s_t - 1}{t(x_h) s_h + t(x_t) s_t}$$

The optimal life history maximizes r (by definition) and can be characterized by x_h such that

$$\frac{\partial r}{\partial x_h} = 0$$

For illustrative purposes suppose that $s_h = s_t = 1$. Then

$$r = \frac{1}{t(x_h) + t(X - x_h)}$$

and

$$\frac{\partial r}{\partial x_h} = -\frac{1}{r^2}\left\{\frac{\partial t}{\partial x_h} + \frac{\partial t(X - x_h)}{\partial x_h}\right\}$$

$= 0$ if and only if

$$\left.\frac{\partial t}{\partial x_h}\right|_{x_h} = \left.\frac{\partial t}{\partial x_t}\right|_{x_t}$$

By inspection (Fig. 4b) this only holds if $x_h = x_t$.

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