THE PHYSIOLOGICAL ECOLOGY AND LIFE HISTORY STRATEGIES OF THE NUDIBRANCH MOLLUSCS *ADALARIA PROXIMA* (ALDER & HANCOCK) AND *ONCHIDORIS MURICATA* (MULLER)

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The physiological ecology and life history strategies of the nudibranch molluscs *Adalaria proxima* (Alder & Hancock) and *Onchidoris muricata* (Müller) (Gastropoda: Opisthobranchia)

by

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being a thesis submitted to the University of St Andrews in candidature for the degree of Doctor of Philosophy.

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Declaration

I declare that the work reported in this thesis is my own and has not been submitted for any other degree. Due acknowledgement has been given for any assistance received.

Supervisors Certificate

I certify that Jonathan Neil Havenhand has fulfilled the conditions laid down under Ordinance General Number 12 and Resolution of the University Court 1967 Number 1, of the University of St Andrews and is accordingly qualified to submit this thesis for the degree of Doctor of Philosophy.

Curriculum Vitae

I graduated from the University of Stirling in 1981 with a B.Sc. in Biology. The work described in this thesis was carried out between October 1981 and September 1986.

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ABSTRACT

This study investigated the physiological ecology, larval biology and population genetics of the nudibranch molluscs *Adalaria proxima* (A & H) and *Onchidoris muricata* (Müller). These two species are annual, simultaneous hermaphrodites and are ecologically very similar with the exception that *A.proxima* reproduces by means of pelagic lecithotrophic larvae whereas *O.muricata* has long-term planktotrophic larvae. The aim of the study was therefore to determine the selective pressures which resulted in the evolution of different larval types in these two species, and to ascertain the ecological and population genetic consequences thereof.

Simple energy budgets comprising the major components (consumption, growth, respiration and reproduction) were constructed for laboratory populations of each species. In both *A.proxima* and *O.muricata*, feeding rate displayed an asymptotic increase with body size. Mean feeding rates of *A.proxima* were greater than those of comparable *O.muricata* individuals, and overall assimilation efficiency was higher in *A.proxima* than in *O.muricata*. This difference was reflected in the somatic growth rates which were correspondingly greater in *A.proxima* than in *O.muricata*. Net growth efficiencies were broadly comparable between the two species, however, growth of *A.proxima* was approximately linear over time whilst that of *O.muricata* displayed a curvilinear, almost exponential, pattern. This is interpreted as demonstrating that some form of constraint (possibly feeding rate) operated on the growth rates of *A.proxima* but not on those of *O.muricata*.

Respiration rates were found to be relatively constant within given animals, but significant differences were found between individuals. The allometry of respiration rate was not constant; *O.muricata* demonstrated a more rapid increase in respiration rate with increasing body size than did *A.proxima*. Individual variations in respiration rate did not reflect variations in the energy partitioned to either growth or reproduction.

Reproductive patterns in the two species were dissimilar. *A.proxima* laid fewer spawn masses containing fewer, larger ova than those laid by *O.muricata* individuals. In addition, the spawning period of *A.proxima* was shorter than that of *O.muricata* (60 days and 105 days respectively). Both species exhibited a similar (proportional) degree of somatic catabolism over these periods. The consequently more rapid "degrowth" of *A.proxima* is interpreted as the necessary utilization of an energy resource (*i.e.* the soma) caused by an inability to meet the energy demands of reproduction through feeding alone. This was not the case in *O.muricata* individuals which exhibited a much smaller maximum body size and were able to feed at a sufficiently rapid rate to maintain reproduction. In the latter case, the longer reproductive period served to maximise the total reproductive output.

Several different measures of "Reproductive Effort" (RE) were calculated. These generally indicated that the RE of *O.muricata* was considerably greater than that of *A.proxima*. Although such differences have been used in the literature to classify the respective costs of different larval types or "reproductive strategies", the variability of the RE's obtained from the different measures used here has led to the suggestion that the general lack of association between RE and reproductive strategy which has been reported elsewhere may (partially) be attributable to the different measures of RE employed in different studies.

Studies of the embryonic and larval period showed that the egg-to-juvenile period of *O.muricata* was approximately 50% longer than that of *A.proxima*. This difference was primarily attributable to the extended pelagic development of *O.muricata* larvae. Estimates of the degree of dispersal, and hence gene-flow, between populations of these species were tested by investigating the biochemical genetics of such populations. No data were available for *O.muricata*, but *A.proxima* populations proved to be more genetically heterogeneous than had been expected. It is therefore concluded that actual pelagic dispersal may be considerably abbreviated over that expected on the basis of larval culture data alone.

A model is developed to explain the possible consequences of different egg-to-juvenile periods (which accrue from different larval types) on both the ecology of the benthic adult, and on overall energy partitioning to reproduction. However, although (probable) proximate causes and effects of the different reproductive traits exhibited by *A.proxima* and *O.muricata* are shown, it has not been possible to determine the exact selective pressures which caused *A.proxima* to diverge from the ancestral *"O.muricata"* stock through the evolution of a pelagic lecithotrophic larva.

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CHAPTER 1

GENERAL INTRODUCTION

The nudibranch molluscs *Adalaria proxima* (Alder & Hancock) and *Onchidoris muricata* (Müller) are exclusively predators of marine Bryozoa. Although both species are known to take a variety of species, the preferred prey in each case is the cheilostome bryozoan *Electra pilosa* (L.) (Todd, 1981; and refs. therein). The adult nudibranchs of either species are comparatively small, *A.proxima* reaching a maximum size of approximately 18 - 20 mm in length (≈ 40 mg dry weight) but *O.muricata* rarely exceeding ~ 10 mm in length (≈ 14 mg dry weight).

The geographic distributions of both species have been described as "Boreo-arctic" (see Thompson & Brown, 1984). *A.proxima* has only been recorded from the Atlantic extending as far south as Plymouth (Garstang, 1894) and Massachussetts (Franz, 1970). In contrast, *O.muricata* has been recorded as far south as Wimereux, France (Bouchet & Tardy, 1976) and Connecticut (Clark, 1975) in the Atlantic. *O.muricata* has also been reported from the Pacific coasts of Alaska (Bergh 1880, cited in Thompson & Brown, 1984), British Columbia (Millen, 1983) and the San Juan Islands, Washington State, U.S.A. (Hurst, 1967). Smith & Sebens (1984) found *Onchidoris aspera* to be a relatively abundant predator of *Electra pilosa* at Nahant, Massachussetts, U.S.A.. This dorid is morphologically and ecologically very similar to *O.muricata*, and the precise taxonomic distinctions between these two species are unclear. Care must therefore be exercised when interpreting distributional data for "*O.muricata*" from such locations.

Throughout the northern and western parts of Britain, both *A.proxima* and *O.muricata* can be found associated with their bryozoan prey in the lower intertidal zone. Subtidally they can be found to depths of 60 m (*A.proxima*) and 70 m (*O.muricata*), (Thompson & Brown, 1976). The local distribution of both of these species can vary considerably, and either or both may be absent from some localities. Nonetheless, these are generally common animals which may, occasionally, be found in abundance. Although

PLATES 1.1 & 1.2 *Adalaria proxima* (top) and *Onchidoris muricata* (bottom)

(Photographs by B.E. Picton)

A.proxima and *O.muricata* are largely sympatric, their fine-scale distributions tend toward neighbouring rather than biotic sympatry, (*sensu* Lincoln *et al.*, 1982). For example, in the Menai Straits, *A.proxima* individuals are found grazing bryozoan communities epiphytic on *Fucus serratus* whereas *O.muricata* are more commonly found preying upon understone bryozoan communities (C.D. Todd, pers. comm.). Additional micro-habitat differences have been observed on the west coast of Scotland (pers. obs.). However, these are usually quantitative rather than qualitative differences and may be a result of temporal and/or spatial variation in recruitment. Notwithstanding the above, some subtle form of niche separation may operate between these two species.

The first thorough, systematic treatment of the anatomy of *Doris* (= *Adalaria*) *proxima* and *Doris diaphana* (= *D.aspera* = *Onchidoris muricata*) was undertaken by Alder & Hancock (1845-55). More recently, Thompson & Brown (1984) have revised the systematics of the Order Nudibranchia and reviewed the biology and (especially radular) morphology of the nudibranchs.

Particular aspects of nudibranch ecology have been investigated by a number of researchers from various parts of the world, and the relevant references are made in the introductions to each Chapter of this work. More general reviews of nudibranch ecology have been provided by Harris (1973), Clark (1975), Hadfield & Switzer-Dunlap (1984), and Todd (1981, 1983). Reports relating specifically to the species under study here include those of Thompson, (1958a,b) on the larval / post-larval biology and the radular morphology of *A.proxima*, and Behrentz (1931), Swennen (1960), Miller (1960, 1961), Thompson (1961) and Todd (1978a,b) on various aspects of the life-cycle, distribution and reproduction of *O.muricata*.

The reproductive ecology of *A.proxima* and *O.muricata* has been investigated by several workers (refs. above and Todd, 1979a). Both of these two species are strictly annual simultaneous hermaphrodites, and have semelparous life-histories. Fertilization is internal and exchange of spermatozoa is usually reciprocal (see Hadfield & Switzer-Dunlap, 1984). In both species, the adult populations undergo total post-spawning mortality prior to the

establishment of the succeeding generation of newly settled juveniles in the spring/early summer.

Both *A.proxima* and *O.muricata* lay benthic spawn masses. These consist of a spiral of gel matrix (or stroma) which is applied to the substratum (usually the *Fucus* or rock upon which the bryozoan prey are located). Within this gel are embedded large numbers of egg capsules each containing a single ovum. (For a more thorough description see Thompson, 1976; Hadfield & Switzer-Dunlap, 1984). The major difference between the two species is that *A.proxima* spawns ova of ~ 165 µm in diameter whilst *O.muricata* ova are only ~ 80 µm diameter. This difference is reflected in the larval development: *A.proxima* larvae are pelagic lecithotrophic (type 2 of Thompson, 1976) while in contrast, *O.muricata* larvae are planktotrophic (type 1, Thompson, 1976). Therefore, the central purpose of this study becomes apparent: "Why, given such notable morphological and ecological similarities between these two species, have they evolved different larval types?"

Much of ecological theory concerns the evolution of particular life-history strategies (*e.g.* Stearns, 1976, 1977; Schaffer, 1974; Gadgil and Bossert, 1970; Pianka, 1970). *Adalaria proxima* and *Onchidoris muricata* present a ready opportunity to test some aspects of this body of theory since, due to their obligate semelparity, these species "cannot afford reproductive failure .... in any one year" (Todd, 1979a). *i.e.* by virtue of their very existence the life-historical and reproductive characteristics which they exhibit must be consistently successful. As in the majority of marine invertebrates, nudibranchs display a variety of larval types (*i.e.* planktotrophy, and pelagic or non-pelagic lecithotrophy). Consequently, any study of life-history evolution in marine invertebrates must also account for the selective pressures which led to the evolution of the observed larval type, and for the interactions between these two processes. Theoretical treatments include the seminal work of Vance (1973a,b), and studies by Christiansen & Fenchel (1979), Strathmann (1978a,b) and Obrebski (1979) on the evolution of different larval types. In addition, Pechenik (1979), Caswell (1981), and Grant (1983) have considered the evolution of "mixed" life-histories. This work has been reviewed recently by Day & McEdward (1984), Grahame & Branch (1985), Strathmann, (1985) and Todd (1985). It is not the intention of the present study to

evaluate this body of theory with regard to nudibranch molluscs, but rather to investigate the proximate causes and consequences of these different larval types and place these within the context of the current theory.

Todd (1979a) undertook preliminary investigations of the reproductive energetics of *A.proxima* and *O.muricata* and concluded that the different larval types may be an evolutionary response to the "predictability/unpredictability" of energy allocation to reproduction. He concluded that reproductive resources were "predictable" in *O.muricata*, but "unpredictable" in *A.proxima*. One of the aims of the present study is to further clarify this specific question. The approach has involved analysis of the major energy budget parameters for both juveniles and adults of each species, the dynamics of growth and reproduction in laboratory-held populations, and the derivation of a variety of measures of reproductive "effort". Throughout this work, energy content has been used as a 'common currency' for inter-specific comparisons. The use of energy as a measure has been criticised (*e.g.* Russell-Hunter and Buckley, 1983), however, some of these criticisms can also be levelled against more complex measures such as Carbon or Nitrogen content. Given the relatively great body of data with which to compare energy content, and the comparative ease with which such data can be obtained, this methodology is considered justifiable.

In addition to studies of energetics, investigations of the embryonic and larval biology, and the population genetics of both species have been attempted in order to quantify the wider implications of the evolution of different larval types between these two species.

CHAPTER 2

INTRODUCTION TO ENERGETICS

2.1 INTRODUCTION

The partitioning of energy utilization within an organism is conventionally represented by the energy balance equation:

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

where: C = consumption (*i.e.* ingestion); P = production as somatic growth (P_g) and/or reproduction (P_r); R = metabolic (*i.e.* respiratory) losses; F = faecal losses and U = excretory losses. Traditionally, this equation is considered in terms of energy units, although balances of Nitrogen or Carbon may be (and frequently are) used as alternative measures (Crisp, 1971).

Any, or all, of the individual components of the energy balance equation may vary in response to changes in the environment. The nature of this variation and its consequent effects on the remaining components is the essence of physiological adaptation. Understanding these responses is, therefore, a prerequisite to understanding how a given organism is (physiologically) adapted to its environment, (Bayne & Newell, 1983). In this respect, the present study falls under the definition of "physiological ecology" given by Bayne & Newell (1983), or alternatively, "ecophysiology" (Lincoln *et al.*, 1983).

With particular regard to reproductive ecology, an analysis of the energy balance equation is, perhaps, fundamental. Many attempts have been made to study what association exists between energy allocation to reproduction and a variety of life-history and larval "strategies". Most of these studies have employed simple indices of reproductive effort (e.g.

Hughes, 1972; Stickle, 1973; Menge, 1974, 1975; Grahame, 1977; Clarke, 1979; Todd, 1979a,b; Hughes & Roberts, 1980; Perron, 1982; De Freese & Clark, 1983). However, no consistent pattern has emerged, and the utility of these indices is therefore questionable (Calow, 1983; Todd & Havenhand, 1983). Tinkle & Hadley (1975) have suggested that the only valid measure of reproductive effort is that fraction of the total assimilated energy budget which is expended in (all) reproductive processes. Clearly, to obtain such measures requires information regarding the magnitude of the individual energy budget components. Therefore, in addition to understanding physiological adaptations, energy budget information is of value in determining the processes involved in the evolution of life-histories in general.

Energy budgets have been determined for many mollusc species (for a review see Bayne & Newell, 1983). Studies of gastropods include those of Hughes (1971) on *Nerita* spp., Huebner & Edwards (1981) and Ansell (1982) on two *Polinices* species, Wright & Hartnoll (1981) on *Patella vulgata* and Grahame (1982) who studied two species of *Lacuna* . Energy budgets of opisthobranchs have been less comprehensively studied. Paine (1965) provides an energy budget for the bullomorph *Navanax inermis* , while Carefoot (1967) gives energy budgets for non-reproducing *Archidoris pseudoargus, Dendronotus frondosus* and *Aplysia punctata* individuals. Smith & Sebens (1983) studied all the components of the energy budget of the dorid nudibranch *Onchidoris aspera* , but failed to present their data in a manner which would permit the construction of an energy balance equation. Other workers have concentrated on individual components of the energy budget. These studies are cited in the appropriate section.

The present work has centred on the most (ecophysiologically) important energy budget components, namely consumption, growth, respiration and reproduction. The two remaining components (defaecation and excretion) have not been quantified.

Defaecation has been studied in other nudibranch species (Carefoot, 1967; Hall, 1983), but these studies concerned large nudibranch species which produce relatively large and solid faecal pellets. Although similar investigations may have been technically possible here (using the method of Conover (1966), for example), the small size and large number of

individuals studied, in addition to the largely liquid composition of the faeces ruled out such an investigation from a practical standpoint.

Nitrogenous excretion and mucus production have also been measured in molluscs (reviewed by Russell-Hunter & Buckley, 1983; Bayne & Newell, 1983). However, for the reasons outlined above relating to the number of individuals studied, these factors have not been quantified here.

In order to observe the major effects on energy partitioning caused by reproduction, energy budgets have been constructed for both pre-gametogenic nudibranchs (in September/October), (Todd, 1978), and reproductively active nudibranchs (in the following spring).

The data presented in the following chapters are derived from seven *Adalaria proxima* individuals and seven *Onchidoris muricata* individuals in which growth, reproduction and respiration were measured. These individuals were sampled *ad hoc* from laboratory populations of 28 *A.proxima* and 23 *O.muricata* which were monitored continuously. Measures of growth and reproduction were therefore obtained for the entire laboratory population of each species from collection in late summer until post-reproductive death in the following spring. These data are presented in Chapters 4 and 6. Consumption was not measured solely in the above individuals, but was quantified for a large number of animals. The precise methodology and its underlying rationale are presented in Chapter 3. Chapter 5 presents the results of the respirometric determinations and the overall energy budgets are compiled and discussed in Chapter 7.

2.2 GENERAL METHODOLOGY

For the present investigations of energy budgets of *Adalaria proxima* and *Onchidoris muricata* the same general methodology has been employed throughout. For convenience these methods are outlined here.

Mass Determinations

All determinations of mass (either in air or underwater) have been made on a Mettler ME22 analytical microbalance. In the case of live nudibranchs, all determinations were made under water by means of a modification to the floor of the balance chamber which permitted weighings to be obtained from beneath the chamber. In this way, any stress or damage which may otherwise be caused by damp weighing was minimised. Moreover, this technique eliminated any variation in damp weight which may have been attributable to varying degrees of surface water removal from the animals concerned, or of internal water content.

Any materials which were to be dried were first frozen and then dried in a Vacuum Freeze-Drier (Chemlab Instruments) for 24 hours. All marine organisms were rinsed in 0.9% (w/v) Ammonium Formate (HCO_2NH_4) before being frozen. (0.9% Ammonium Formate is isotonic with sea-water and actively displaces marine salts. Upon freeze-drying it sublimes with the water ice leaving no residue).

Inorganic Ash Determinations

All ash determinations were obtained by igniting pre-weighed dried samples of material for four hours at 520^{o}C in a muffle furnace (Crisp, 1971).

Calorimetry

A Phillipson Oxygen microbomb calorimeter (Gentry Instruments Inc.) was used throughout. A standard procedure was adopted (Crisp, 1971), although ash content was derived for an independent sample of pills rather than from the residue in the microbomb.

This practice avoided errors which may have otherwise been caused by sputtering of biological material and fusion of ash to the ignition wire which was occasionally observed. All calibrations were performed with freeze-dried pills of Benzoic Acid (energy content = 26.4345 $kJ.g^{-1}$).

Use of Regression Equations

The very nature of this study requires that measurements of one parameter are converted to estimates of other parameters through the use of regression equations. The accepted methodology is to use a model I or ("Y on X") regression analysis. A number of assumptions are inherently accepted when performing such an analysis. These include the assumption that the Y-variate is dependent on the X-variate but that the converse does not apply; that the independent variable (X) is measured without error; and that the dependent variable (Y) is normally distributed (no such assumption is made with regard to the independent variable X).

Clearly there are many biological situations where these assumptions are violated. In the present context it is worthy of note that the assumption of independence of the X-variate is never met. In all cases involved in this study, both variables are measured with error and both are usually inter-dependent (weight under water to dry weight, for example). The general case where both variables exhibit random variation is called model II regression (Sokal & Rohlf, 1981). The utility of model II, or "functional", regressions in biology has been outlined by Ricker (1973). The latter paper highlights the different methods of estimating a functional regression and the criteria for their adoption. According to Ricker's rationale, when both X and Y are subject to natural variability and to measurement error, a geometric mean (GM) estimation procedure is the most consistently appropriate. In this procedure, the sums of squares of both X and Y are minimised, rather than just those of Y (as in a model I, Y on X regression). Furthermore, whilst acknowledging that extrapolation from a data set is error-prone, the imprecision resulting from extrapolation of a GM regression is considerably reduced in comparison to that from a model I regression. This arises because as the sample size increases, the model I regressions X on Y and Y on X approach the GM regression (Ricker, 1973). Thus, whilst a model I regression is generally

used for predictive purposes, a model II regression will yield more reliable estimates when the sample size is small and when both variables are subject to error. Although the technique of model II regression is still a matter of statistical research, for the above reasons it is felt to be more appropriate within the context of the present work. Therefore, all regressions used in this study are GM estimates of model II regressions.

CHAPTER 3

FEEDING

3.1 INTRODUCTION

By definition, the greatest energy budget component is ingestion. Clearly, the sum of the energy expended on all the individual components of the budget cannot exceed the energy which is acquired by ingestion. That the ingestion, or feeding, rate will increase in some manner with body size is perhaps, therefore, intuitive. The precise form of this relationship is not so readily apparent however, and several different models have become established in ecological theory. The object of the present study was not to test observations of feeding rate in nudibranchs against the theory, (although theoretical considerations will undoubtedly apply), but to obtain realistic estimates of average caloric intake for nudibranchs of known size. These estimates were then utilised in an analysis of the gross energy budgets of individuals.

In common with most British dorid nudibranchs, *Adalaria proxima* and *Onchidoris muricata* are bryozoan grazers (Todd, 1979a, 1981). Many authors have noted these species preying upon a wide variety of Bryozoa (reviewed by Todd, 1981) but most sources have observed the most frequently taken prey species to be the anascan cheilostome *Electra pilosa* (L.), (Miller, 1961; Swennen, 1961; Thompson, 1958a,b, 1960, 1964; Todd, 1979a, 1981). Prey-selection experiments have further indicated that this is indeed the preferred prey (Chadwick & Thorpe, 1981). Both of the nudibranch species studied here remove the zooid of a given colony individually rather than grazing the entire colony and ingesting both zooids and the skeletal zooecia in the manner of, for example, *Polycera quadrilineata* (Todd, 1981). The methods by which *A.proxima* and *O.muricata* remove the bryozoan zooid from its calcareous exoskeleton do, however, differ. *A.proxima* uses the radula both to rasp through the frontal membrane and to remove the polypide with seven or eight strokes (Thompson, 1958b). By contrast, *O.muricata* apparently uses the powerful buccal pump to suck the polypide through the ruptured frontal membrane. In such cases the radula appears to be used

primarily in transportation of food to the oesophagus, (although it must be noted that these conclusions are deductive and some instances of feeding in a manner analogous to that of *A.proxima* have been recorded; Todd, 1981). Given these contrasts, the objective of the present analysis was to ascertain what, if any, differences in feeding rate pertain and more importantly, how such differences may affect total energy acquisition patterns throughout the animals' lifespan.

3.2 MATERIALS AND METHODS

Dry Weight and Ash Content of *Electra pilosa*

Colonies of *Electra pilosa* encrusting the brown alga *Fucus serratus* L. were collected from the lower intertidal zone of St Andrews Bay. Only healthy, intact specimens were used in the analysis. The number of zooids comprising each colony was determined with the aid of a *camera lucida* attachment to a Wild M8 stereo dissecting microscope. Once counted, each colony was carefully scraped off the *Fucus* substratum with a sharp spatula. Care was taken to ensure that only bryozoan tissue was removed. The removed zooecia were rinsed in 0.9% (w/v) Ammonium Formate. Dry weights and ash fractions were determined according to the methods outlined in the General Methodology (Section 2.2).

Calorimetry of *Electra pilosa*

Dried *E.pilosa* was finely ground and homogenised before being pressed into pills. All pills were re-frozen and re-dried for 24 hours before use.

All attempts to ignite pills of *E.pilosa* in the microbomb failed (presumably due to the high fraction of inorganic ash). To ensure ignition, Benzoic Acid was mixed with powdered *E.pilosa* in approximately 1 : 1 proportions. The precise proportion of *E.pilosa* in these 'spiked' pills was estimated from the ash fraction of both 'spiked' and 'pure' *E.pilosa* pill samples. The remainder of the 'spiked' pills were (successfully) ignited in the microbomb calorimeter.

Feeding Rates

All the nudibranchs used in feeding rate experiments were obtained from the same populations as those used in the remainder of the energy budget experiments. *Adalaria proxima* were collected from Loch Creran, Argyll, and *Onchidoris muricata* were obtained from Robin Hood's Bay, North Yorkshire. All animals were maintained in small nylon mesh cages (Toby 'Teaboys') in the Gatty Marine Laboratory aquarium. The aquarium system pumped fresh seawater twice daily into large holding tanks from which water was fed by gravity into the aquarium system. In all experiments through-flow (non-recirculating) tanks were used. No attempt was made to regulate inflowing (or tank) seawater temperature which was in general within 1 - 2 ^{o}C of the local ambient seawater temperature.

In order to provide mass determinations, all animals used in the feeding rate experiments were weighed under water. These weights were converted to dry weight (dwt) and thence to Joule equivalents using the equations presented in Chapter 4 (Section 4.3). Individual *Electra pilosa* colonies encrusting *Fucus serratus* were inspected and counted in the manner already described, and were each placed in a 'Teaboy' with a particular nudibranch. In all cases, the nudibranch was placed on the *E.pilosa* colony itself or on the *F.serratus* substratum at the colony edge.

The nudibranchs were left to graze the bryozoan colonies for periods ranging from one to 48 hours. At the end of the experimental period the container was inspected to ensure that the nudibranch had not become detached from the *F.serratus* and thus isolated from the prey organism. The *E.pilosa* colonies were subsequently counted to ascertain the number of zooids consumed by the nudibranch. (In cases where the nudibranch had become detached from the *Fucus* and no zooids had been consumed it was deemed that the animal may have become inadvertently detached from the outset. These results were therefore excluded from the analysis. Nonetheless, some cases where no *E.pilosa* zooids were consumed but the nudibranch remained in contact with the *F.serratus* were recorded. These were included in the analysis). At all times other than during feeding experiments, nudibranchs were supplied with an excess of *E.pilosa* .

3.3 RESULTS

Gravimetry and Calorimetry of *Electra pilosa*

The total number of zooids, dry weights, and ash fractions of *E.pilosa* colonies used in this analysis are presented in Table 3.1. A geometric mean estimate of a Model II regression of zooid number (y) and dry weight (x) was calculated:

$$y = 111.759\,x + 27.385 \qquad r^2 = 0.986,\ n = 19,\ p << 0.001$$

The ash-fraction of the *E.pilosa* / Benzoic Acid 'spiked' pills was found to be 26.099% ± 0.154 (s.e) (n = 10). Since the ash-fraction of whole *E.pilosa* colonies is 67.481% (Table 3.1), the *E.pilosa* content of the 'spiked' pills was estimated to be (26.099 / 67.481)% = 38.676%. *E.pilosa* ash almost wholly consists of the calcareous exoskeleton. For the purposes of this study, the ash was assumed to be 100% Calcium Carbonate. This compound undergoes endothermic decomposition to Calcium Oxide and Carbon Dioxide at temperatures in excess of 675°C (Duval, 1963). The energy lost in this decomposition is equivalent to 1.779 J.mg $CaCO_3^{-1}$ ($\Delta H = +179$kJ), and this was consequently accounted for in estimating total caloric content of the 'spiked' pills. Thus the "corrected" pill Joules comprises the measured energy output (by calorimetry) plus an endothermy correction of 1.799 J.mg ash^{-1}. The energy content of the 'spiked' pills attributable to the Benzoic Acid was calculated as the pill weight multiplied by the Benzoic Acid content (1 - 38.688% = 61.312%) expressed in caloric terms (1mg Benzoic Acid = 26.4345 J). This figure was subtracted from the "corrected" pill Joules to obtain the caloric content attributable to *E.pilosa*. The number of zooids present in the 'spiked' pills was estimated from the weight fraction of *E.pilosa* using the GM regression of zooid number on dry weight (above).

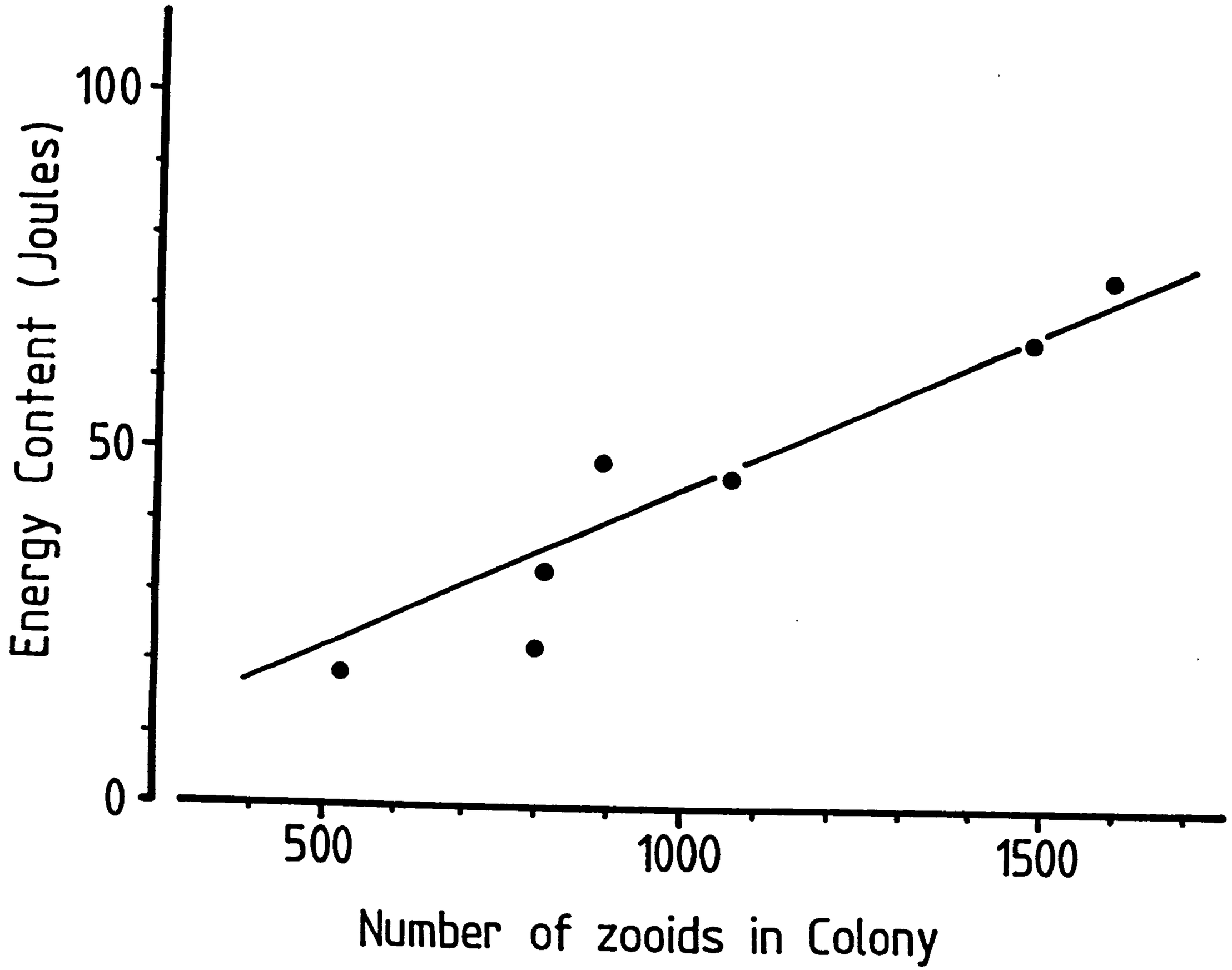
The energy content of the 'spiked' pills which was due to *E.pilosa* is shown against the equivalent zooid numbers in Fig. 3.1. A GM regression of *E.pilosa* Joules and zooid numbers indicated that the intercept was not significantly different from zero (t = 1.05, n = 7, n.s.). Moreover, an analysis of variance indicated that the inclusion of a constant (*i.e.*

TABLE 3.1 Zooid numbers, dry weights and inorganic ash content of *Electra pilosa* colonies

Zooid number	Dry weight (mg)	Ash weight (mg)	% Ash
70	0.511	0.357	69.86
229	1.467	1.004	68.44
83	0.494	0.332	67.21
115	0.899	0.617	68.63
125	1.050	0.695	66.19
155	1.036	0.697	67.28
142	0.907	0.630	69.46
64	0.534	0.363	67.98
68	0.428	0.295	68.93
835	7.673	4.797	62.52
704	7.268	4.967	68.34
798	5.844	3.983	68.16
578	5.165	3.522	68.19
859	6.280	4.126	65.70
446	4.086	2.657	65.03
1268	10.343	-	-
1361	12.955	-	-
1946	17.624	-	-
1543	12.687	-	-
Mean	599.4	5.119	1.936	67.481
s.e.	133	1.19	0.47	0.50

FIGURE 3.1 Energy content of *Electra pilosa* colonies

(*E.pilosa* $J = 4.4616.10^{-2}$.zooid number $r^2 = 0.91$, $p << 0.01$)


Energy Content (Joules)
100
50
0
500
1000
1500
Number of zooids in Colony

intercept) in the regression equation did not explain a significantly higher proportion of the overall variance (F = 1.099, n.s.). Therefore, a GM regression constrained to pass through the origin was fitted to the data and used in all subsequent estimations of energy content of *E.pilosa* colonies. This equation took the form:

$$E.pilosa \text{ Joules} = 4.4616 \times 10^{-2}. \text{Zooid no.} \quad r^2 = 0.910, p << 0.001$$

This line is depicted in Figure 3.1.

Feeding rates

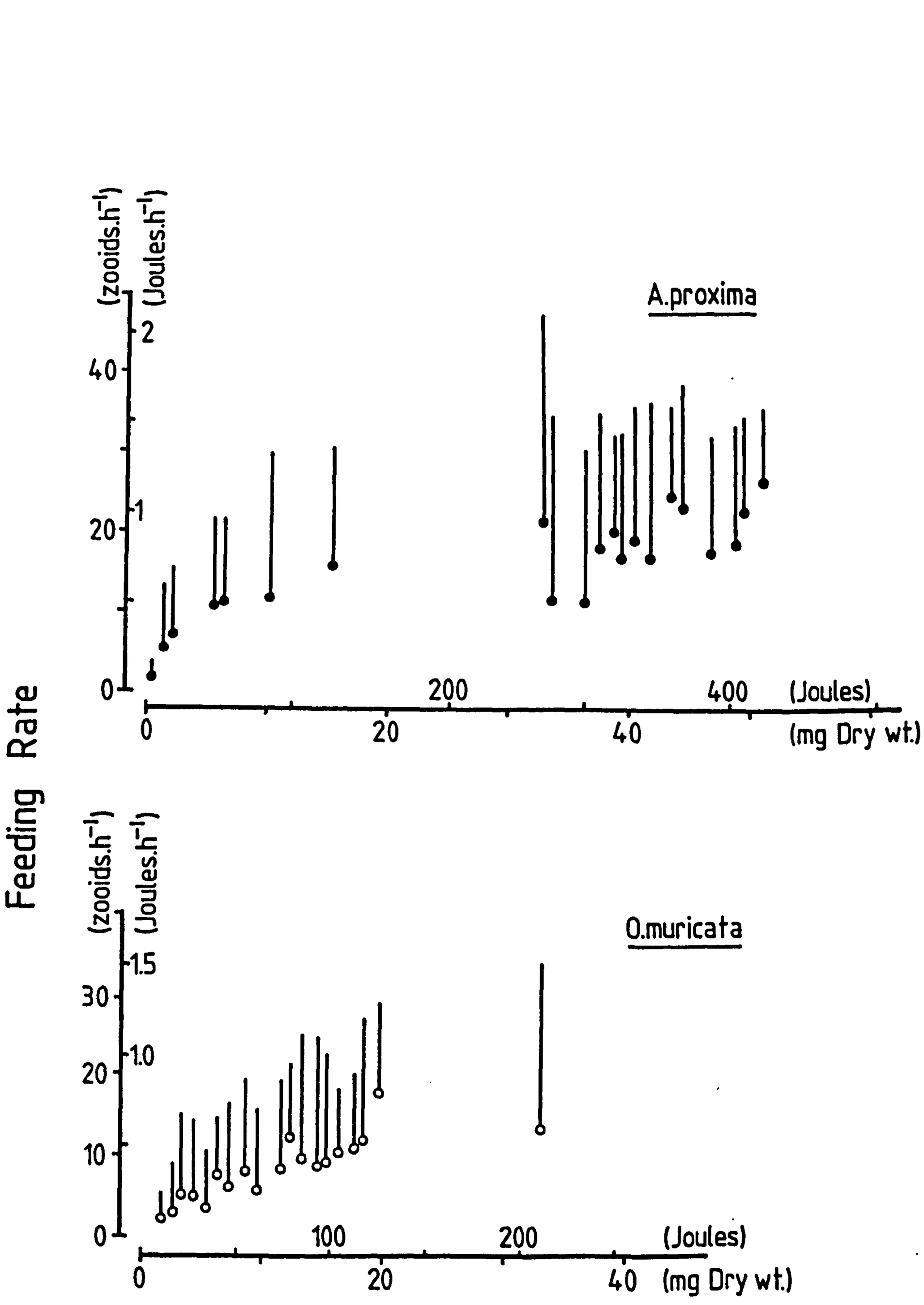
Feeding rate determinations were made for 87 *A.proxima* over a total experimental period of 4403 hours, comprising 363 observations. Similar studies were made of 90 *O.muricata* over 4971 hours (398 observations).

For the purpose of graphical presentation, the data were sorted according to nudibranch weight. The mean and maximum feeding rates are plotted against mean body size for each mg (dry weight equivalent) size class in Figure 3.2. Note that only size classes containing more than seven observations have been presented, although all computations were performed on the complete data set. It is clear that the maximum observed feeding rates in *A.proxima* follow an approximately asymptotic pattern, rising to ~ 35 zooids.h^{-1} for an individual nudibranch. Such a relationship is less apparent in the case of *O.muricata* . However, the trend is still markedly non-linear and not dissimilar from the comparable region of the *A.proxima* data. For the present purposes therefore, it has been assumed that the basic properties of the relationship between feeding rate and body size are the same for both species.

Given that maximum feeding rate is an asymptotic function of body size, an hypothesis was generated in order to explain the observed relationship. This supposes that maximum feeding rate is limited by both the buccal and radular structure of the nudibranch and by the prey defences. As the nudibranch grows in size, the former components become less of a limitation in overcoming the latter. That is to say the nudibranch can feed more

FIGURE 3.2 Feeding rates of *Adalaria proxima* and *Onchidoris muricata*

(mean and maximum for all 1 mg size-classes where n ≥ 7)


Feeding Rate
(zooids.h⁻¹)
(Joules.h⁻¹)
A.proxima
0
20
40
2
1
200
400
(Joules)
(mg Dry wt.)
O.muricata
30
20
10
1.5
1.0
100
200
(Joules)
Body Size

rapidly. However, there will be a finite limit to the rate at which an individual can feed. This is set by the minimum time necessary to overcome prey defences, consume the polypide and move on to the next prey item, *i.e.* the 'handling time'. Thus, as body size increases, maximum feeding rate will rise to an asymptote which reflects the minimum handling time. The mean feeding rate will not necessarily show the same general pattern however, and several possibilities exist. These possibilities were tested against the data by examining the proportion of the overall variance explained by their equivalent mathematical models.

The first possibility is that the mean feeding rate follows the same pattern as the maximum feeding rate. This was modelled as a rectangular hyperbola of the general form

$1/y = \alpha + \beta/x$, where y is the feeding rate; x is body dry weight (equivalent) and $1/\alpha$ is the asymptotic feeding rate.

The second possibility is that the mean feeding rate is proportional to radular size up to the point where this function meets the maximum feeding rate curve.

This point of intersection is likely to occur at a body size considerably in excess of those naturally encountered, and therefore the qualification with regard to maximum feeding rate can be ignored for modelling purposes. The radula is a ribbon of teeth which grows as a unit structure by elongation. Radula size was therefore assumed to be a linear function of length and thus a function of the cube root of weight. The model therefore took the form

$y = \alpha + \beta x^{1/3}$.

The last possibility considered here is that the mean feeding rate is proportional to both radular size and the rate of digestion. Digestion may be an important factor in that observed feeding rate may be constrained by loading of the gut. Digestion was assumed to be proportional to the gut surface area and therefore proportional to weight to the power of 2/3. This possibility was consequently modelled as $y = \alpha + \beta x^{1/3} + \gamma x^{2/3}$.

These models were fitted to the raw data using GLIM (Generalised Linear Models, Royal Statistical Society, London) on the University's DEC VAX 11/780 computer. Fits were obtained using an iterative least-squares regression procedure. For this procedure each feeding observation was weighted according to the period (hours) over which it was obtained, since the probability of a given observation accurately reflecting the overall mean feeding rate will be proportional to the duration of that observation.

The mean feeding rate for each size class was found to be roughly proportional to its variance. Consequently, the Y-variates were assumed to conform to the Poisson distribution. Therefore, when fitting the models, the variance of Y was assumed to be proportional to the estimated value of Y. (The assumption that the feeding rates conformed to a Gamma distribution was also investigated, but all such fits accounted for significantly smaller proportions of the overall variance than those obtained with a Poisson distribution).

A summary of the fits and parameters of the various models is given in Table 3.2. The '% "Variance" explained' in Table 3.2 is a measure of the reduction in deviations from the fitted value which is obtained when the model is fitted to the data. The 'Mean Square' is the residual deviation divided by the degrees of freedom, and is analogous to the Mean Square in an analysis of variance. A low mean square, therefore, indicates a good fit. The increase in '% "Variance" Explained' brought about by weighting the observations can be seen in Table 3.2. Clearly, the model gives a poorer fit to the unweighted data than to the weighted data. All the remaining fits in Table 3.2 were obtained with weighted data.

In order to test the statistical significance of including successive parameters in the general model $y = \alpha + \beta x^{1/3} + \gamma x^{2/3}$ an analysis of deviance was performed. The general form of the analysis was the same as that for an analysis of variance. This procedure indicated that significant reductions in the residual variation were obtained when each successive parameter was included in the model, up to the level $y = \beta x^{1/3} + \gamma x^{2/3}$ for *A.proxima* and $y = \alpha + \beta x^{1/3} + \gamma x^{2/3}$ for *O.muricata* . However, neither of these models

TABLE 3.2 Summary of Model parameters and fits for *A.proxima* and *O.muricata* feeding rates.

(y = zooids.h^{-1}, x = equivalent body dry weight)

Model	*A.proxima*					*O.muricata*				
	% "Variance" explained	"Mean Square"	α	β	γ	% "Variance" explained	"Mean Square"	α	β	γ
$1/y = \alpha + \beta/x$										
(unweighted)	19.6	-	-	-	-	18.9	-	-	-	-
(weighted)	43.9	46.6	.048	.329	-	30.7	46.8	.042	.847	-
$y = \beta x^{1/3}$	29.5	58.5	-	.849	-	28.4	48.3	-	.911	-
$y = \alpha + \beta x^{1/3}$	40.7	49.3	1.07	.529	-	28.4	48.4	.065	.885	-
$y = \gamma x^{2/3}$	0	-	-	-	-	3.3	65.2	-	-	.334
$y = \alpha + \gamma x^{2/3}$	37.1	52.3	1.69	-	.099	25.4	50.5	1.10	-	.181
$y = \beta x^{1/3} + \gamma x^{2/3}$	43.3	47.2	-	1.50	-.191	28.8	48.1	-	1.05	-.052
$y = \alpha + \beta x^{1/3} + \gamma x^{2/3}$	43.3	47.3	-.018	1.52	-.194	31.3	46.6	-2.23	2.96	-.452

gave a significantly better fit to the respective data sets than could be obtained from a rectangular hyperbola. Because the rectangular hyperbola is of a simpler design than the feeding and digestion based models, Occam's razor was applied and the former adopted as the estimator of mean feeding rates. These estimates are shown in Figure 3.3 superimposed on the data from Figure 3.2. The fitted value is shown for the size-range of nudibranch over which observations were made. Inspection of Figure 3.3 reveals that *A.proxima* individuals of all sizes generally feed at a faster rate than *O.muricata* . Perhaps the most striking feature however, is the relatively rapid rise in feeding rate during the early stages of growth (a feature common to all the models fitted here). Once the nudibranch has exceeded a body size equivalent to approximately 100 Joules (≈ 12.1 mg dwt.), any increase in feeding rate due to further increases in body size is relatively small. Although this feature is more pronounced in *A.proxima* than in *O.muricata* , it will clearly have consequences for the potential energy acquisition rates of larger nudibranchs of both species.

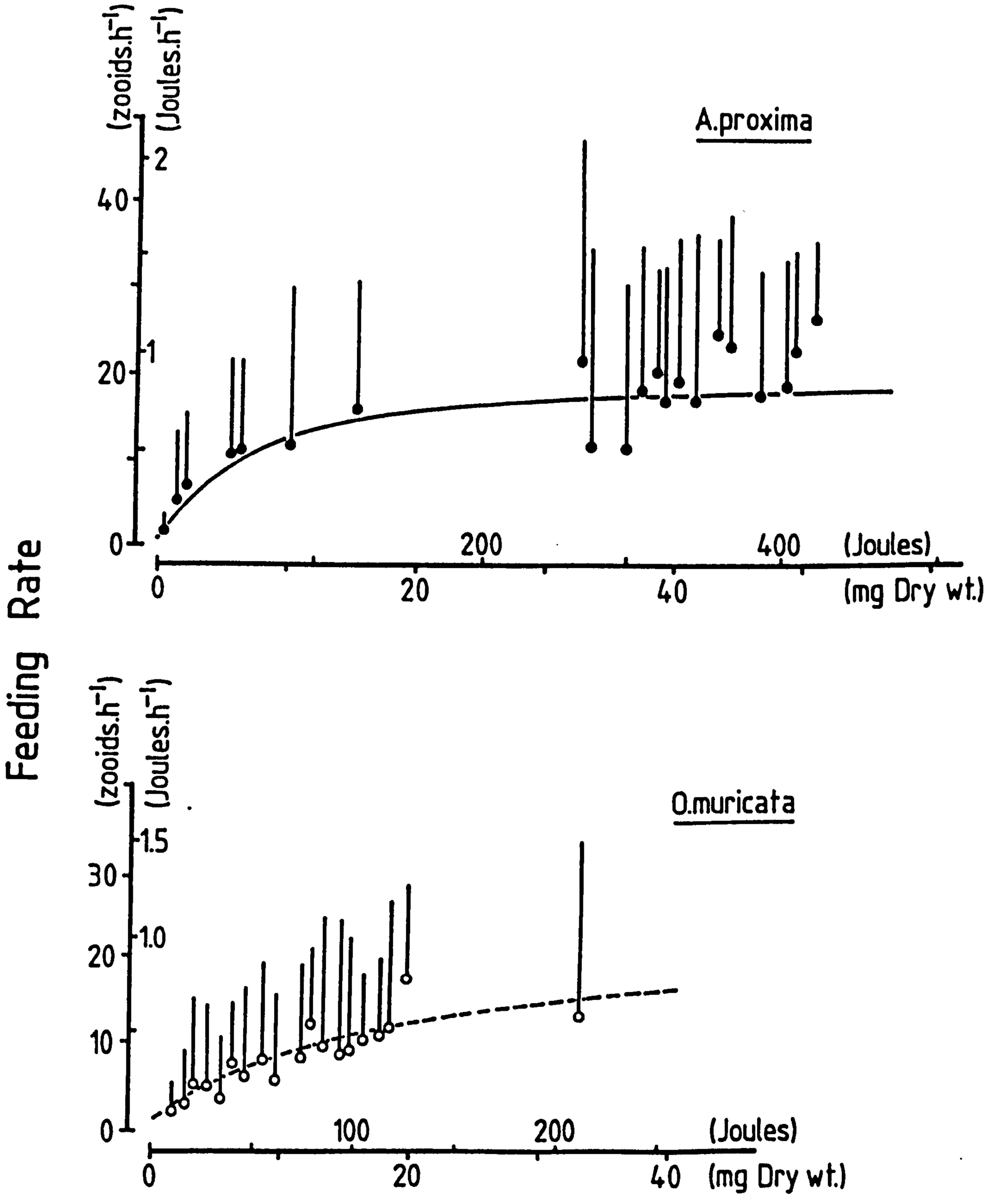
3.4 DISCUSSION

Analysis of sample *Electra* colonies has indicated that the energy content of a given colony can be predicted from the number of constituents zooids with a reasonable degree of accuracy. However, several potential sources of error exist when making such predictions. These errors have been minimised wherever possible (by avoiding extrapolation, for example), but some were necessarily incurred. Perhaps foremost of these are the assumptions concerning the endothermic properties of *Electra* ash. The methodology used here has relied on theoretical assumptions and is consistent with that of Todd (1979a). The assumption that *Electra* ash is wholly composed of Calcium Carbonate is clearly invalid. The true composition of the ash is unknown, but it is beyond doubt that the actual Calcareous fraction will be close to the 100% assumed here. A further theoretical assumption is that of 1.779 $J.mg^{-1}$ for the endothermic decomposition of Calcium Carbonate. Experimental observations of endothermy in artificial pills have yielded generally lower, (Paine, 1966) but also widely fluctuating values (R.Birse, unpubl. results; S.Kempf, pers. comm.). In the

FIGURE 3.3 Fitted estimates of feeding rates for *Adalaria proxima* and *Onchidoris muricata*

(notation as for Figure 3.2)

Feeding Rate



Body Size

absence of any consistent experimental results therefore, theoretical values have been applied and the possibility that endothermy was consequently over-estimated is acknowledged. Since the endothermy "correction" represents a minor proportion of the overall energy content of *E.pilosa* , what error does remain is likely to be of little significance.

A search of the literature revealed no comparable caloric data on either this or any other bryozoan species. Indeed, feeding rates of nudibranchs on Bryozoa have been reported only infrequently. Todd (1979a) states that *Adalaria proxima* consumes *E.pilosa* zooids at the rate of 2.3 - 17.8 polypides.h^{-1}, in contrast to *Onchidoris muricata* which fed at a rate of 0.4 - 5.2 polypides.h^{-1}, but he does not give body sizes for nudibranchs feeding at these rates. Chadwick & Thorpe (1981) have reported *O.muricata* feeding at rates of 0.59 and 0.49 *Electra* zooids.h^{-1} for nudibranchs of 20 mg and 10 mg wet weight (≈ 6.3 mg dwt and 3.1 mg dwt) respectively.

None of these values fall outside the range reported here, although the observations of Chadwick & Thorpe (1981) are remarkably low considering their animals had been starved for 24 hours prior to their observations.

Other nudibranch-bryozoan associations have been studied, and feeding rates estimated. For example, Miller (1958) observed a 7.5 mm *O.muricata* to consume 1.6 *Membranipora membranacea* zooids.h^{-1}. Seed (1976) observed the North Pacific nudibranch *Doridella steinbergae* preying upon *Membranipora villosa* at the rate of 3.3 zooids.h^{-1}, and cites other observations of 6.3 zooids.h^{-1}. Perhaps most surprising are the observations by Harvell (1984) for the North Pacific nudibranchs *Dirona albolineata* and *Triopha catalinae* grazing the margins of *Dendrobaenia lichenoides* colonies at the rate of 1000 zooids.h^{-1}. However, these species graze both zooid and zooecium and individual nudibranchs are of considerable size. (See also grazing rates of *Polycera quadrilineata* in Chadwick & Thorpe, 1981). Despite these observations, and extensive work by Hall (1983) on the predation rates of *Aeolidia papillosa* on sea anemones, no other studies of the allometric relationships of feeding rate in nudibranchs have been reported.

Theoretical treatments of ingestion rate have suggested that this should rise in direct proportion to body size to the power of 0.75 (Peters, 1983). Bayne & Newell (1983) have summarised published estimates of the allometric rate exponent in a variety of species of mollusc. They found this exponent varied between 0.3 and 0.85. Given this, and the variation observed in a variety of invertebrate taxa, with exponents ranging from 0.4 to 1.2 (summarised in Peters, 1983), the models adopted here seem acceptable. The assumptions which were inherent in the models tested against the data are supported by the literature. Thus, Thompson (1958b) found an increase in radula size with age in field samples of *A.proxima*. This increase is markedly curvilinear, although the scatter of the data is considerable. On this basis, the assumption that radula size increases in proportion to weight to the power of 1/3 seems warranted. The assumption that feeding rate may be (in part) proportional to digestion rate is substantiated by observations of juvenile nudibranchs spending significant proportions of their time in an inactive, non-feeding state. (Mean feeding rate ≠ max. feeding rate; Chadwick & Thorpe,1981; this study). Whilst it is possible that adult nudibranchs may use such periods for copulation or oviposition, no such demands are placed on the juvenile.

Despite the apparently sound biological basis of these feeding/digestion models, the variance in the data was such that they could not be statistically differentiated from the simpler rectangular hyperbola on the basis of the proportion of overall variance explained. Herein lies a potential source of error since the precise form of these models differs and therefore yields different estimates of feeding rate for a given nudibranch weight.

Several explanations can be proposed for the observed variations in feeding rate. Most important of these is that they are real. Given observations of varying duration, it is logical to expect variation in observed feeding rate - the greater the duration of that observation, the less that observation will deviate from the estimated value.

Secondly, Harvell (1984) has stressed that bryozoan colonies are rarely as homogeneous as they are often assumed to be. She found very different nudibranch feeding rates at the colony margins in comparison to central areas. Best & Winston (1984) have

emphasised this point in observing the central areas to be mechanically stronger than the new-growing margins in tropical bryozoans. Moreover, the energy content of a given zooid will vary according to its position in the colony, its age, and the time of year. None of these variables could be accounted for in the present study save that most colonies were moderately small and, presumably, comparatively young.

A final complication was incorporated by assuming that each *Electra pilosa* zooid which had been grazed had in fact been completely consumed. This may not be the case, although visual inspection of grazed colonies gave no indication to the contrary. Whilst such an error does not markedly affect estimates of feeding rate (in terms of zooids.h^{-1}), it is of relevance when calculating the energy equivalent of that feeding rate. Consequently the rate of energy acquisition may have been overestimated.

Despite these sources of variation, the observation that the fitted estimates of mean feeding rate accounted for 44% of the deviance observed for *A.proxima* and 31% of that for *O.muricata* indicates that reliance can be placed on these estimates.

Ideally, energy intake should be measured directly when estimating energy budgets. In the present study however, it was not possible to measure ingestion along with respiration, growth and (in adults) reproduction within a given time period. To have done so would have required counting approximately 4000 *E.pilosa* zooids per day. Consequently a degree of error will unavoidably be incorporated when the estimates presented here are used later to compare energy intake and allocation in the gross energy budgets of *A.proxima* and *O.muricata* . Nonetheless, the work detailed here has permitted the first estimates of the allometry of ingested energy and body size in nudibranch molluscs.

CHAPTER 4

GROWTH

4.1 INTRODUCTION

The ecological fitness of a given organism is best measured as the number of its offspring which survive to successfully reproduce in the next generation. For invertebrates in general, fecundity is a function of body size. Consequently the rate at which an organism can increase in body size is likely to be an important fitness component. The study of growth in invertebrates has, therefore, encompassed both theoretical and applied considerations and the resulting literature is extensive. Growth of molluscs in particular has not been neglected, although the majority of this work has concentrated on bivalves (*e.g.* Bayne & Worrall, 1980; Bayne, *et al.*, 1977; Griffiths, 1980, 1981; Vahl, 1980, 1981).

Growth in the opisthobranch molluscs has been less well studied and has generally been noted during field-based life-cycle investigations (for example, Thompson, 1958a, 1961; Miller, 1962; Potts, 1970; Todd 1979a,b). Some laboratory studies of growth in opisthobranchs have been reported (for example; Paine, 1965; Carefoot, 1967; Chia & Skeel, 1973; Smith & Sebens, 1983) and of these, Paine (1965) and Carefoot (1967) are especially relevant with regard to the energetics of growth. None of these studies however, have considered growth within the context of the current ecological theory. Indeed, relatively few reports of growth of molluscs in general consider theoretical models of growth, although several studies of *Mytilus* spp. are a notable exception (for example; Bayne & Worrall, 1980; Griffiths, 1981; Rodhouse *et al.*, 1984).

The present study attempts to relate the growth patterns of *A.proxima* and *O.muricata* to theoretical models of growth. To do this, all the individual nudibranchs maintained in the laboratory have been included in the analysis, rather than concentrating exclusively upon the seven individuals of each species chosen for the energy budget studies. Moreover, since energetic allocations to growth during the spawning period are inextricably linked to

reproduction, these allocations will be dealt with in Chapter 6. For the present, therefore, only pre-spawning growth will be considered.

In order to obtain comparative estimates of the caloric content of both *A.proxima* and *O.muricata* , extensive gravimetric analyses of both whole animals and their inorganic ash fractions have been performed. These are detailed here.

4.2 MATERIALS AND METHODS

Juvenile nudibranchs were collected and maintained according to the methods outlined in Chapter 3 (Section 3.2). Collections of *A.proxima* were made on 14th July 1983. *O.muricata* were collected on 16th August 1983. All the nudibranchs maintained in the laboratory were provided with an excess of the preferred prey *Electra pilosa* . This was collected fresh from St Andrews Bay.

Individuals were weighed under water at two week intervals up to the onset of spawning. The methods used have been outlined in the General Methodology (Section 2.2). A sample of individuals of each species was weighed under water, frozen, dried and re-weighed in order to calibrate the underwater weighing technique. Separate calibrations were obtained on five occasions for *A.proxima* and four times for *O.muricata* to ensure that estimates of dry weight from weight under water could be made without unnecessary extrapolation.

Ash fractions of field collected *A.proxima* were obtained throughout the pre-reproductive period. Insufficient animals could be collected to perform a similar analysis for *O.muricata* .

The elemental composition of the inorganic ash of both *A.proxima* and *O.muricata* was investigated by Energy Dispersive X-ray analysis (EDAX) and subsequently by X-ray ion probe analysis. These analyses were performed on pills of homogenised nudibranch ash

coated with either Gold or Carbon.

4.3 RESULTS

A total of 29 *A.proxima* and 23 *O.muricata* were monitored from collection up until the onset of spawning. Spawning commenced in February (*O.muricata*) and April (*A.proxima*). Mean sizes at collection (± one standard error) were 1.080 ± 0.070 mg dry weight (equivalent) for *A.proxima* and 0.512 ± 0.057 mg dry weight (equivalent) for *O.muricata*. Mean daily growth rates over the pre-spawning period were 0.156 ± 0.006 mg dwt.d^{-1} and 0.0769 ± 0.0047 mg dwt.d^{-1} for *A.proxima* and *O.muricata* respectively.

The calibration data sets of weight underwater and dry weight were investigated by an analysis of covariance (using Model I regression procedures). For both *A.proxima* and *O.muricata*, no significant differences between either slopes or intercepts of the lines for each calibration could be detected. The data sets were therefore combined and a GM regression equation of dry weight (y) against weight under-water (x) was calculated for each species:

$y = 2.98269.x - 0.059556$ $\quad r^2 = 0.994,\ n = 61,\ p << 0.001$ (*A.proxima*)

$y = 2.70353.x - 0.064604$ $\quad r^2 = 0.970,\ n = 38,\ p << 0.001$ (*O.muricata*)

Both of the correlation coefficients for these data are highly significant.

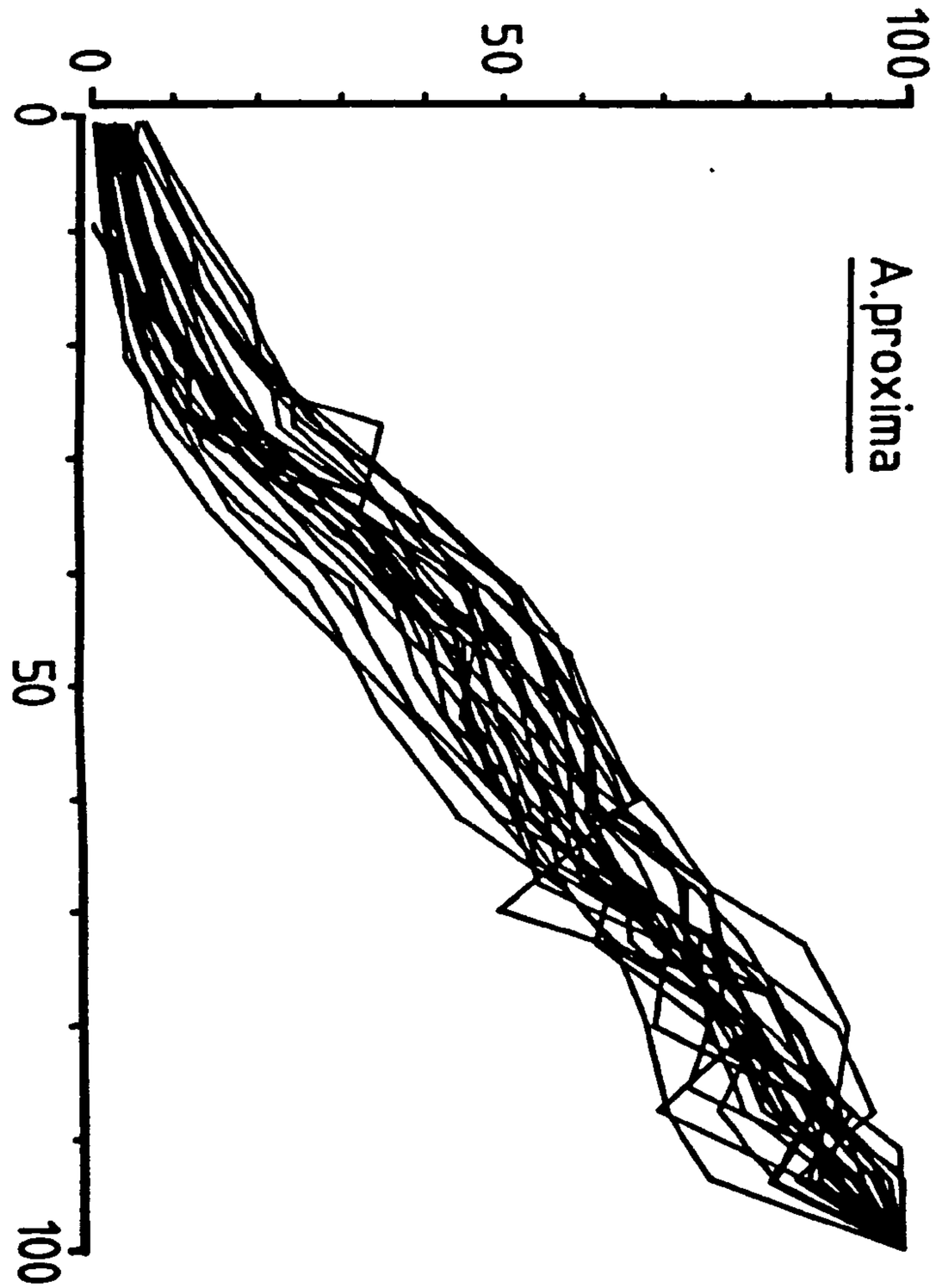
All measurements of weight under water were converted to dry weight (equivalent) using these equations.

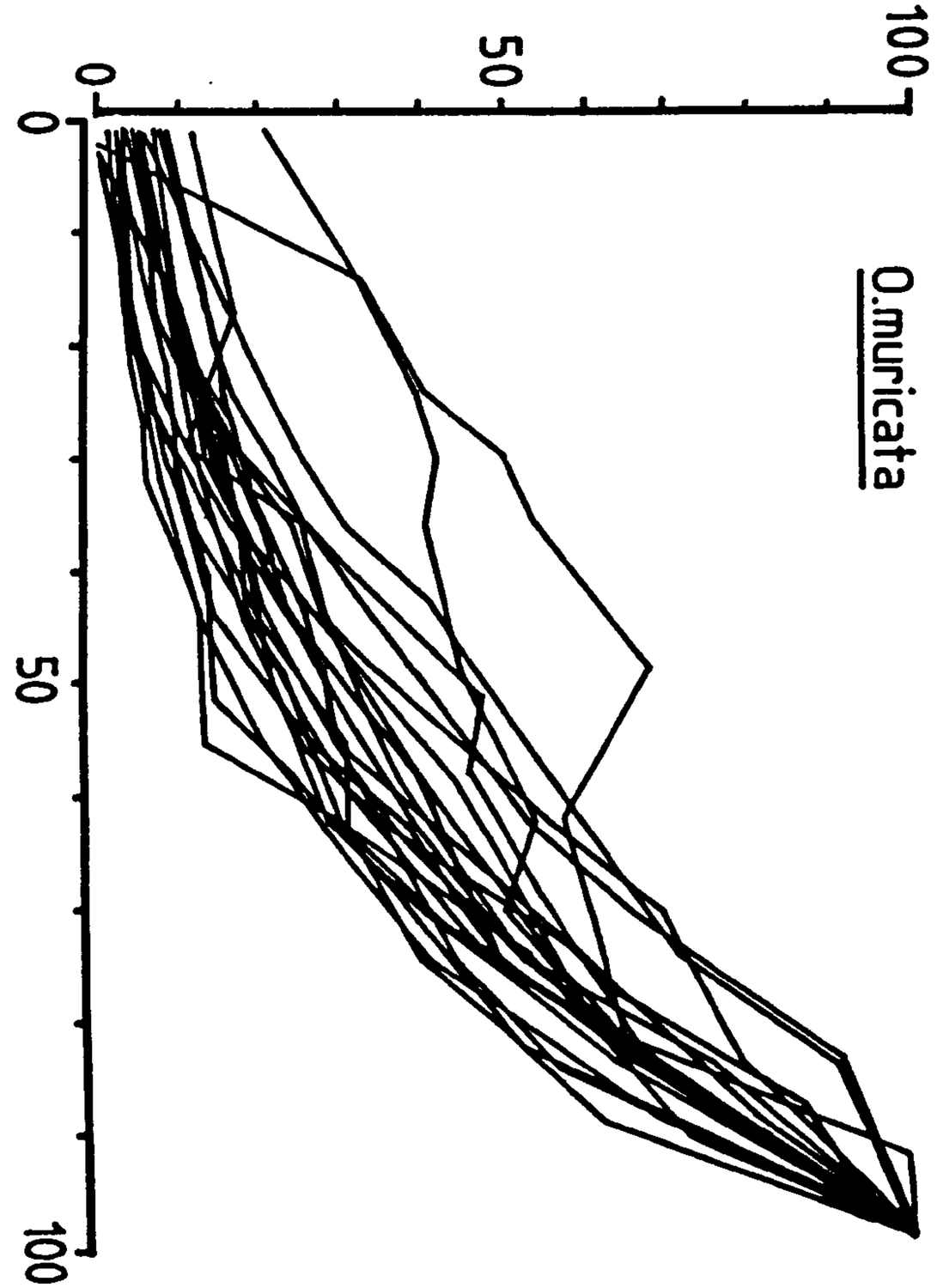
Figure 4.1 shows the increase in body size over time for both *A.proxima* and *O.muricata*. In order to best observe the pattern of growth, differences in both body weight and length of pre-spawning period have been accounted for by representing these as proportions of immediate pre-spawning weight and total pre-spawning time respectively. The consequent growth patterns presented in Figure 4.1 clearly show different relationships

FIGURE 4.1 Growth patterns for *Adalaria proxima* and *Onchidoris muricata*

(see text for explanation)

Proportion of Immediate Pre-spawning Weight (%)





Time (%)

for the two species. With few exceptions, *O.muricata* individuals generally displayed curvilinear, almost exponential growth over the entire period. Initially the growth pattern of *A.proxima* individuals was similar, but after a relatively short time growth became approximately linear.

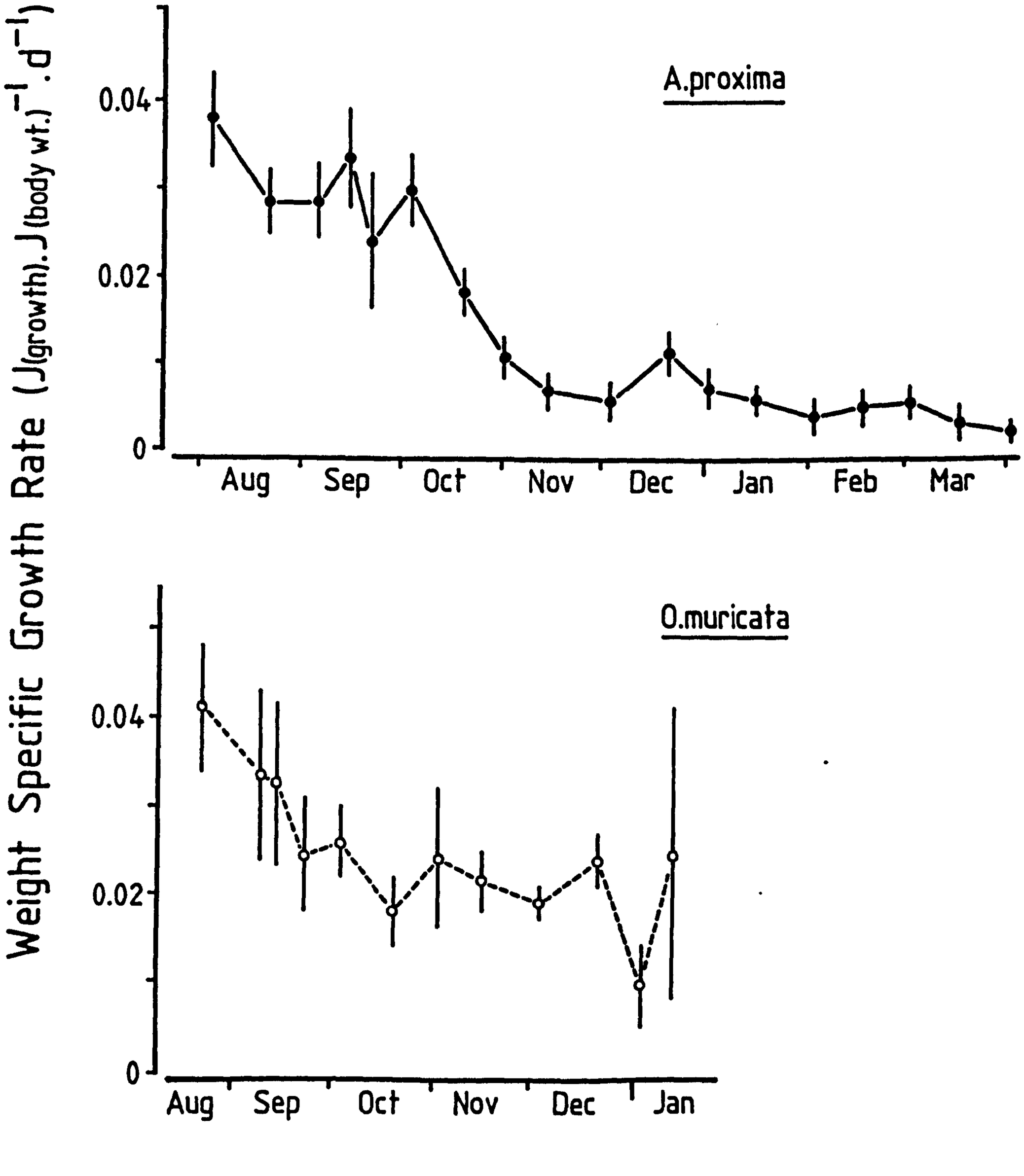
These differences in growth pattern are reflected in the mean weight-specific growth rates (mg growth.mg body weight^{-1}.day^{-1}) over the same period (Figure 4.2). Weight-specific growth rates were calculated using the mean body weight over each measurement interval. Since growth in *A.proxima* is approximately linear, the arithmetic mean body weight was used when calculating the weight-specific growth rate (WSGR). In *O.muricata* however, growth is curvilinear and the arithmetic mean body size represents an overestimate of the average body size over any given time interval. In this case therefore, the geometric mean body size was used as the denominator when calculating the WSGR.

Large confidence intervals around the mean WSGR for *O.muricata* (Figure 4.2) highlight the greater variation in growth pattern already seen in Figure 4.1. Despite this variation it is evident from Figure 4.2 that the mean WSGR declines over the pre-spawning period to an approximately constant rate of 0.02.d^{-1}. (The observed increase in confidence interval around the *O.muricata* mean WSGR after January is primarily due to the onset of spawning and consequent decrease in the number of pre-spawning individuals). By contrast, the mean WSGR of *A.proxima* falls steadily to a level approaching zero. Thus, the mean WSGR of *O.muricata* individuals is considerably in excess of that for *A.proxima* from November until the onset of spawning.

In order to evaluate the necessity for obtaining longitudinal growth data of the type collected here, an attempt was made to fit growth curves using the method of Kaufman (1981). This method uses cross-sectional data and therefore requires weight observations from the population on only two occasions. A variety of statistical transformations are then employed in order to linearise the relationship between WSGR and size. The growth curve most appropriate to the data is then determined by the transformations which were required to obtain linearity (Kaufman, 1981).

FIGURE 4.2 Weight-specific growth rates for *Adalaria proxima* and *Onchidoris muricata*

(means ± 95% C.I.)


Weight Specific Growth Rate (J(growth).J(body wt.)$^{-1}$.d^{-1})
0.04
0.02
0
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
A.proxima
0.04
0.02
0
Aug
Sep
Oct
Nov
Dec
Jan
O.muricata
Date

Four sets of transformations were used corresponding to the Logistic, Power, Gompertz and von Bertalanffy growth equations. These transformations were applied to sets of "cross-sectional" data abstracted from the longitudinal data set. Eleven *O.muricata* and 17 *A.proxima* analyses were performed. In addition, the method was applied to the entire longitudinal data set.

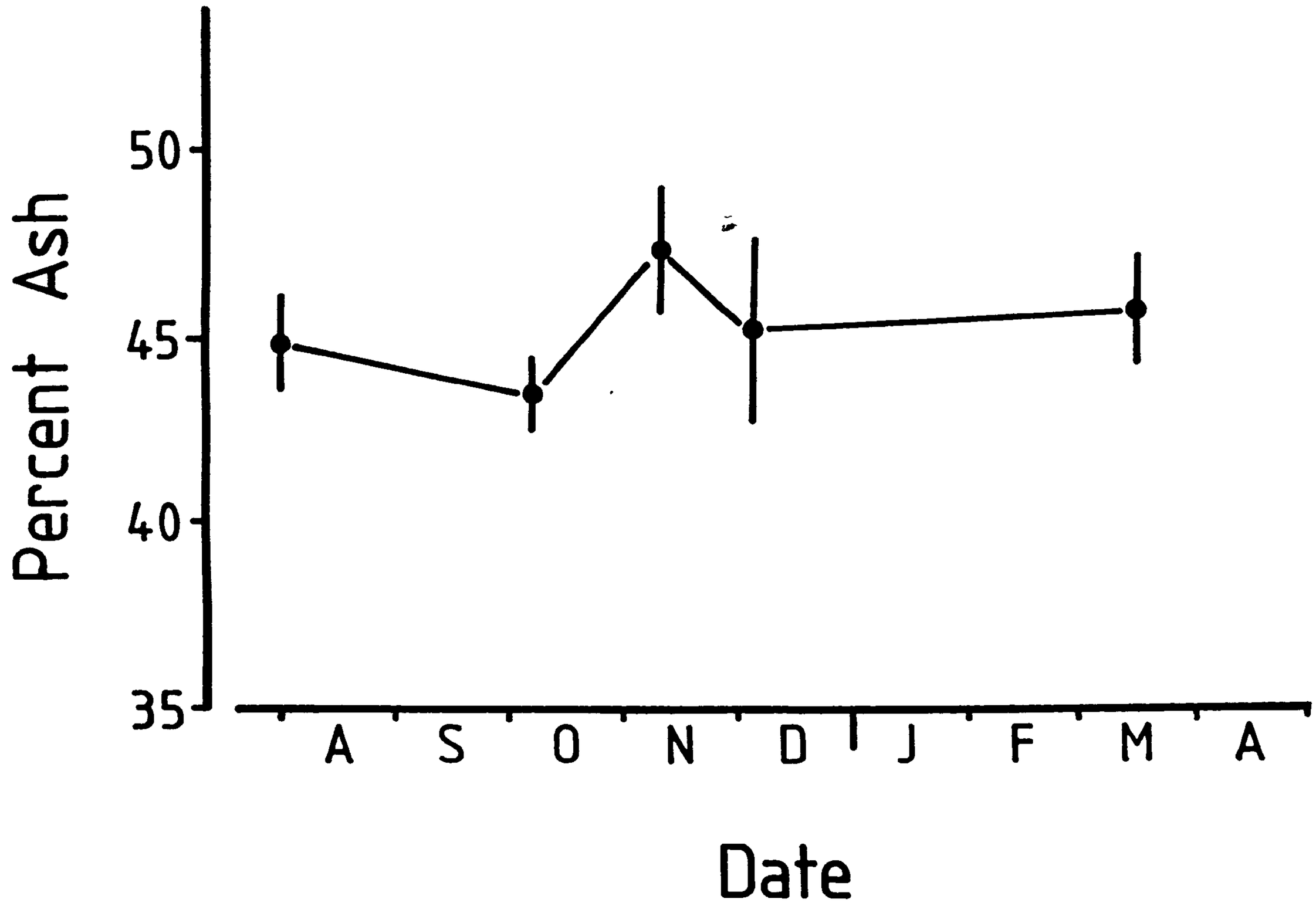
On no occasion was the scatter of data reduced to the level where reliable predictions of size at a given age could be made. The greatest degree of linearity was obtained from the entire data set using transformations for a Gompertz curve for *A.proxima* ($r^2 = -0.594$, $n = 462$, $p << 0.001$) and a von Bertalanffy curve for *O.muricata* ($r^2 = 0.207$, $n = 233$, $p << 0.001$). These curves provide estimates of size at 200 days of 51 mg and 0.55 mg dry weight for *A.proxima* and *O.muricata* respectively. The *A.proxima* estimate is significantly higher than the observed mean weight of 31.2 ± 1.12 (s.e) mg dry weight (equivalent) at 200 days. The estimated weight of *O.muricata* at 200 days is significantly lower than the observed mean after only 100 days (5.94 ± 0.37 (s.e.) mg dry weight (equivalent)). Therefore, in order to test the statistical precision of these estimates of size at age, 95% confidence intervals around the estimates were generated. These were 47.4 - 122.9 mg dry weight for *A.proxima* , and 0.45 - 0.78 mg dry weight for *O.muricata.* (Confidence intervals for other growth curves fitted to these data were considerably larger than those presented here).

In view of the relative imprecision of these estimates and the considerably greater scatter seen in all the abstracted "cross-sectional" data sets, no further analyses were performed.

The ash fractions of samples of *A.proxima* collected from the field throughout the pre-reproductive period are shown in Figure 4.3. A Kruskal-Wallis non-parametric test of variation failed to reject the null hypothesis of no significant trend in the data ($H = 8.809$, $\chi^2_{(0.05)\,[4]} = 9.488$). Therefore, the overall mean of 45.03% was taken as representative of

FIGURE 4.3 Seasonal variation in inorganic ash-fraction of *Adalaria proxima*

(means ± 95% C.I.)


Percent Ash
50
45
40
35
A
S
O
N
D
J
F
M
A
Date

the ash content of *A.proxima.*

An Energy Dispersive X-ray Analysis (EDAX) of *A.proxima* and *O.muricata* ash indicated that considerable quantities of Calcium, Magnesium and Chloride ions were present. Substantial amounts of Potassium and Sodium were also found. Emissions due to Phosphorus and Sulphur were very low and could only be distinguished from the background with difficulty. In view of the semi-quantitative nature of the EDAX technique, a quantitative X-ray ion probe analysis was performed. This analysis quantifies the X-ray emission due to specific elements or compounds and cannot readily study the emissions from all elements. Consequently, only the elements which yielded pronounced emissions in the EDAX were investigated. Moreover, since the primary aim of the investigation was to examine the potential endothermy of nudibranch ash caused by thermal decomposition of (primarily) Carbonate compounds, only the Oxygen containing compounds of the commonest elements were quantified. The proportions of these "Oxides" in the ash of *A.proxima* and *O.muricata* are shown in Table 4.1. Clearly, the most abundant "oxide" is that of Calcium representing 32% and 27% of the ash in *A.proxima* and *O.muricata* respectively. Magnesium "Oxides" are also relatively abundant, comprising 13% and 10% respectively. It is noteworthy however that the compounds studied here accounted for only 50% of the ash in both species. Presumably the high Chloride emissions seen in the EDAX account for a substantial proportion of the remainder.

Since relatively little Sulphur or Phosphorus was detected in the ash, the Oxygen containing compounds of Calcium and Magnesium are most probably either Nitrates or Carbonates. (Verification of this assumption is difficult since neither the EDAX nor the ion probe are readily capable of detecting elements with an atomic mass less than 15 Daltons). Odum (1951) found that the dermal spicules of nudibranchs were composed of Calcium Carbonate. Moreover, Calcium and Magnesium Nitrates are soluble, whereas the Carbonates are not. In all subsequent ash analyses therefore, the Oxygen containing compounds of Calcium and Magnesium have been assumed to be Carbonates. The energy changes involved in the thermal decomposition of Calcium and Magnesium Carbonates are outlined in Table 4.2. By combining the data in Tables 4.1 and 4.2 with the known ash

TABLE 4.1 "Oxide" composition of the inorganic Ash-Fraction of *Onchidoris muricata* and *Adalaria proxima.*

Compound	% Content (mean ± 1 s.e.)	
	A.proxima	*O.muricata*
CaO_x	32.45 ± 1.74	27.13 ± 2.16
MgO_x	12.87 ± 0.77	9.54 ± 1.39
NaO_x	5.38 ± 1.14	3.55 ± 1.31
SO_x	3.88 ± 0.46	2.59 ± 0.37
KO_x	1.69 ± 0.18	0.58 ± 0.15
Total	56.27%	43.39%

fraction of *A.proxima* (45.03% of dry weight) the endothermy involved in the thermal decomposition of *A.proxima* soma can be estimated (= 0.328 J.mg dwt^{-1}). By the same process using Todd's (1979a) figure of 48.90% for the ash fraction of *O.muricata*, the equivalent value for *O.muricata* soma is 0.292 J.mg dwt^{-1}.

Since the temperatures obtained in the microbomb are sufficient to cause the above thermal decompositions, the endothermy estimates presented here have been added to the caloric estimates for *A.proxima* and *O.muricata* soma given by Todd (1979a) to provide "corrected" estimates. Thus the "corrected" caloric content of *A.proxima* and *O.muricata* soma is estimated to be 8.273 J.mg dwt^{-1}, and 6.388 J.mg dwt^{-1} respectively. These values have been used throughout this study to obtain energy equivalents of nudibranch dry weights.

4.4 DISCUSSION

Perhaps the most striking result presented here is the observed difference in growth pattern between *A.proxima* and *O.muricata*. Although the data in Figure 4.1 show substantial variation, (especially in the case of *O.muricata*, the general trends are clear. Moreover, these trends are corroborated by the data presented in Figure 4.2. The association between these data sets is most easily illustrated with a theoretical example:

If an organism has a constant growth rate (*i.e.* growth is linear over time), the daily growth increment will represent a decreasing proportion of the body size as the organism grows larger. Thus, the weight-specific growth rate (WSGR) will decline steadily over time and approach, (but never reach) zero. In contrast, if an organism has a constant WSGR, it will gain a fixed proportion of its body weight per unit time. Thus growth will be curvilinear (in fact, exponential) over time.

Although the patterns observed here do not adhere to such clear cut models, the basic framework still applies. Growth in *A.proxima* is of the same general form as the first

TABLE 4.2 Thermal decomposition characteristics of Calcium and Magnesium Carbonates

Reaction	ΔH ($kJ.mol.^{-1}$)	"endothermy" ($kJ.g^{-1}$)	Temperature (C^0)
$CaCO_3 > CaO + CO_2$	+179	1.788	675
$MgCO_3 > MgO + CO_2$	+113	1.340	450

theoretical example; growth over time is essentially linear (Figure 4.1) and the mean WSGR declines to approach zero (Figure 4.2). The second theoretical example also bears some resemblance to the growth pattern of *O.muricata*. Growth is curvilinear over time (Figure 4.1) and although the mean WSGR is not constant throughout the period of study (Figure 4.2), it does fall to an approximately constant level from mid-October onwards, indicating that growth is approximately exponential at this time.

Both linear and exponential growth has been reported for opisthobranch species. Paine (1965) found linear growth, and declining WSGR, in the cephalaspidean *Navanax inermis*. Carefoot (1967) also found linear growth in the nudibranch species *Archidoris pseudoargus* and *Dendronotus frondosus*. In contrast, Thompson (1958) observed a linear increase in mantle length (= curvilinear increase in body weight) of field collected *A.proxima*, and both Miller (1962) and Todd (1981) report a similar relationship for *O.muricata*. Other workers have also noted curvilinear or exponential growth in opisthobranch species (for example Potts, 1970; Chia & Skeel, 1973; Todd 1979a) and Hall (1983) has reported both linear and curvilinear growth patterns for *Aeolidia papillosa*.

Smith & Sebens (1983) cite a WSGR for *Onchidoris aspera* equivalent to $0.016.d^{-1}$ for the period October to December. Given that their animals were of the same general size (mean dwt = 7.64 mg) as those studied here, this figure accords well with the WSGR of $\sim 0.02.d^{-1}$ taken from Figure 4.2.

In view of the observed variation in these growth patterns it is perhaps unsurprising that abstracted "cross-sectional" data sets failed to provide accurate estimates of size at age. Within the present context, such estimates would be of relatively little value given that a longitudinal data set was collected. However, the ability to accurately estimate size (*i.e.* total production) at age from such cross-sectional data would be of great significance to field-based life-history studies. The present data set therefore represented an opportunity to investigate the applicability of Kaufman's methods to nudibranch ecology.

Kaufman's (1981) method has been applied successfully to study growth in various marine phyla. For example, Jardine (1985) has recently applied this technique to study the prosobranch gastropod *Gibbula* with a considerable degree of success. However, no record was found of the method being used on species which do not possess overlapping generations, (although the example given by Kaufman (1981) is a possible exception). Undoubtedly, the greater the size range of animals used in the analysis, the more accurate any given prediction is likely to be. However, the method did not yield accurate results even when applied to the entire data set. It therefore seems likely that the utility of such methods in nudibranch ecology is limited.

It is evident from the results obtained here that growth processes in *A.proxima* operate under different constraints to those in *O.muricata*. The possible nature of these constraints is as yet unresolved and will therefore be dealt with in Chapter 7.

The observed lack of variation in the ash-fraction of *A.proxima* through the year (Figure 4.3) has also been reported for *O.muricata* (Todd, 1977). The assumption of a constant ash fraction for both of these species is therefore unlikely to have caused any systematic bias in the data presented here. It must be noted, however, that the ash fraction will not necessarily remain constant over the reproductive period (see Chapter 6). The supposition that all the Oxygen containing compounds of Calcium and Magnesium in the analysed nudibranch ash were in fact Carbonate compounds, has inevitably included some degree of error in the final endothermy estimates. However, under this supposition, the estimated Calcium Carbonate content of dried *A.proxima* and *O.muricata* is 14.6% and 13.3% by weight respectively. Carefoot (1967) estimated 15% of the dry weight of the dorid nudibranch *Archidoris pseudoargus* to be Calcium Carbonate, and cites values from other studies ranging from 20% - 34% of the dry weight. Thus the values obtained here are equivalent to those derived in other studies. Todd (1979a) assumed the entire ash fraction of both *A.proxima* and *O.muricata* to be Calcium Carbonate. The endothermy "corrections" given by him are therefore considerably in excess of those cited here, although the final caloric estimates for the whole body tissues of *A.proxima* and *O.muricata* differ only superficially between our two studies.

The adoption of Todd's (1979a) estimates of caloric content of *A.proxima* and *O.muricata* in the present study is perhaps questionable. Certainly, to use such conversions on a population different to that from which they were obtained, without verifying their applicability, is unsatisfactory. However, in the present study, *O.muricata* were collected from the same site as used by Todd (1979a), and although it was not possible to obtain a separate caloric conversion for the *A.proxima* collected from Loch Creran, an initial investigation indicated no significant difference between a Loch Creran sample and Todd's (1979a) figures. Whilst acknowledging therefore, that some bias may have been introduced by the use of these conversions, this will not markedly affect the overall conclusions of this study.

Before any attempt can be made to further explain the observed differences in growth pattern between *A.proxima* and *O.muricata*, more detailed information relating to energy partitioning is required. The consequent investigation into respiration which follows, relies heavily on the conversion factors derived here.

CHAPTER 5

RESPIRATION

5.1 INTRODUCTION

An important component of any energy budget is the amount of available energy which is necessarily dissipated as heat as a consequence of metabolic processes. The metabolic rate is the sum total of all these processes per unit time. Although Oxygen may not be required for all these processes, the rate of Oxygen consumption is generally taken to be an accurate measure of metabolic rate. Clearly, this methodology overlooks the potential contribution of anaerobic processes to metabolism, which in some cases may be considerable (Hammen, 1979; but see Clarke, 1983). Most authorities however, have assumed anaerobic metabolism to be zero and have therefore equated metabolic heat loss with respiration rate. The relative ease with which Oxygen consumption can be measured has led the present study also to adopt respiration rate as an estimate of metabolic rate, although the above considerations must be acknowledged.

It is perhaps self-evident that Oxygen consumption will be dependent on the metabolic processes active at the time of measurement. Many studies have discriminated between basal, resting and active metabolic rates on the basis of observed respiration rates at different activity levels. The majority of these studies however, have considered organisms with relatively high activity levels such as filter-feeding bivalves or swimming crustaceans (see Bayne & Newell, 1983, for a review). The primary concern of the present study was to obtain representative data on the overall respiration rates of individual *Adalaria proxima* and *Onchidoris muricata*. Therefore no attempt has been made either to quantify activity level or to correlate this with observed respiration rate. Given the relatively restricted locomotory capacity of these nudibranchs, it is unlikely that activity would influence respiration to any marked degree. Therefore observed respiration rate has been taken as a measure of daily metabolic expenditure.

Several methods exist for measuring declining Oxygen tension in a given water body.

These range from physico-chemical manometric methods, through chemical analyses such as Winkler titrations to electrochemical measurement. The latter comprise a variety of techniques but importantly include the commonly used "Oxygen electrodes", or more strictly, membrane-polarizing Oxygen detectors (MPOD's). Hitchman (1978) provides an extensive account of the characteristics and use of MPOD's, in addition to other methods for measuring dissolved Oxygen concentrations, and rightly emphasises the many practical considerations pertinent to their use. The application of MPOD's to respiration studies has further led to the development of a variety of techniques and equipment designed specifically for this purpose (*e.g.* de Wilde, 1973; Davenport, 1976; Crisp *et al.*, 1978; Propp *et al.*, 1982; Pearson *et al.*, 1984; reviewed by Gnaiger & Forstner, 1983).

The present study has utilized polarographic techniques for measuring Oxygen consumption of *A.proxima* and *O.muricata*. As such it represents the kernel of the energy budget study since all data relating to growth and reproduction presented in Chapter 7 have been obtained within the same framework as the respirometric observations. This study therefore comprises the quantification of respiration as an energy budget component rather than a physiological study of respiration *per se*. Nonetheless, some results may prove to be of more general interest.

5.2 MATERIALS AND METHODS

Apparatus

All respiratory observations were made using a Radiometer PHM 71 Mk II amplifier fitted with a PO_2 module and a Radiometer E5046 Oxygen electrode. Output from this apparatus was obtained using a Vitatron 2001 series flat-bed pen recorder.

The apparatus was calibrated at the beginning and end of each daily set of observations. Zero PO_2 was obtained using Sodium Sulphite / Sodium Tetraborate solution (Radiometer Product no. S4156) and air-saturated, 0.22 µm filtered, seawater (FSW)

provided a saturation calibration. Oxygen content of the water (allowing for Chlorinity, temperature and barometric pressure) was calculated according to the methods of Hitchman (1978).

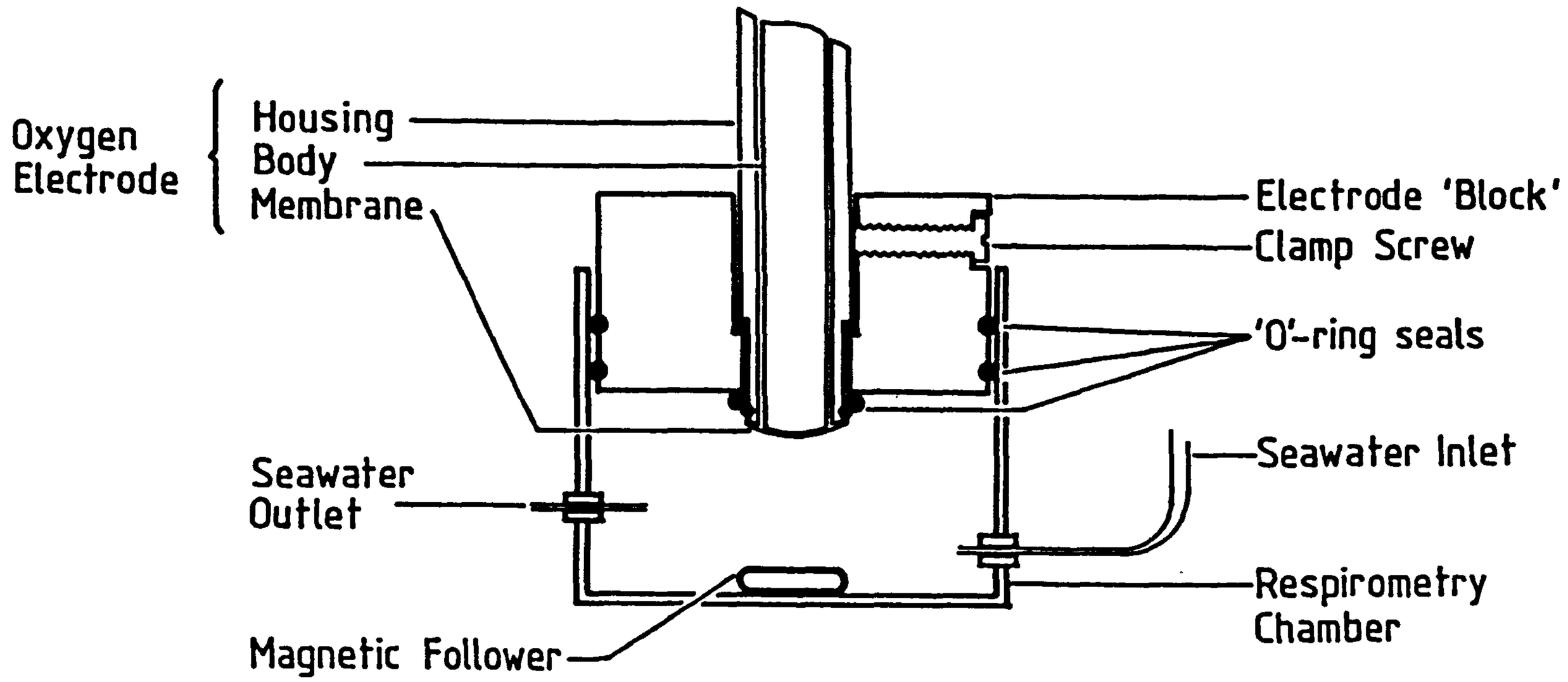
All observations were made in a constant temperature room which was maintained at the ambient laboratory seawater temperature. At these temperatures (5 - 12 °C), the observed air-temperature fluctuation within the room (± 0.5 °C) was found to be sufficient to markedly affect the electrode sensitivity. In order to counteract these fluctuations, all respiration rate determinations were obtained with the electrode and respiration chamber assembly submerged in a water bath (volume ~ 70 l). This had the effect of damping temperature fluctuations to a level which did not measurably affect the performance of the electrode.

Several experimental respirometry chambers were built before a successful design was obtained. The variable volume, semi-through-flow chamber which was finally used for all respiration rate determinations is shown in Figure 5.1. For this chamber, the Oxygen electrode was clamped into a solid "Perspex" electrode 'block' by means of three clamp screws (see Figure 5.1). The assembled electrode and block was then inserted into the water-filled respiration chamber (ensuring that no air bubbles were introduced) and lowered until the desired volume was obtained. Water-tight seals between electrode, block and chamber were obtained with semi-recessed rubber 'O'-rings and silicone grease sealant. Care was taken to ensure that the grease did not contaminate the chamber.

During initial investigations using a closed respirometry chamber it was often found that air was inadvertently introduced between the electrode block and chamber wall. The closed apparatus also required several minutes for stabilisation of the electrode response. This frequently resulted in the experimental organism depleting the available Oxygen in the chamber to less than 75% saturation before an accurate estimation of depletion rate could be obtained. Since many invertebrates demonstrate a modified respiration rate in low Oxygen tension environments, all estimates of respiration rate in this study were obtained at Oxygen tensions greater than 75% of saturation. In order to account for the above considerations, the

FIGURE 5.1 Diagrammatic representation of the respiration chamber

(not to scale)



Respirometry Chamber

chamber used here was fitted with a capillary inlet and outlet which permitted water to be pumped through the chamber at all times during assembly of the apparatus and during the initial stabilisation period. The capillaries were made from 2 mm internal diameter polyethylene tubing which was drawn over a flame until the internal diameter was approximately 100 µm. Experiments demonstrated that the decline in Oxygen tension due to Oxygen consumption by the electrode itself was constant both when the capillary inlet and outlet were open and when they were sealed with petroleum jelly. This indicated that Oxygen diffusion through the capillaries was not measurable. Thus, the chamber could be effectively closed by stopping the flow of water through the inlet, permitting determination of Oxygen depletion rates in a closed vessel. (At any time, water in the chamber could be replenished by pumping air-saturated FSW through the inlet. All excess water ran to waste through the outlet and thus into the water bath).

To obtain calibration values, air-saturated FSW was pumped continuously through the chamber by a Micro-metering Pump Mk II (Metering Pumps Limited) at an adjustable flow rate between 0.5 and 3.0 $ml.min^{-1}$. At all times a flow rate sufficient to ensure that dissolved Oxygen replacement rate exceeded depletion rate was used. For actual respirometric determinations, the chamber volume used during any given observation was largely determined by the size of the nudibranch under observation.

A magnetic follower placed in the bottom of the chamber and a magnetic stirrer (beneath the water bath) maintained a uniform Oxygen tension throughout the experimental chamber during measurement.

Methods

Respiration rate determinations were obtained for both pre-spawning (juvenile) and spawning (adult) *A.proxima* and *O.muricata*. The same seven individuals of each species were used throughout, and it is these nudibranchs for which overall energy budgets were constructed.

Pre-spawning respiration rates were obtained during September and October 1983. During this period, respiration rate determinations were made on an approximately weekly basis for a total duration of four weeks (five observations). Respiration rate observations of the seven individuals of either species were always obtained on the same day and the two species were always studied on consecutive days.

A similar methodology was adopted to study the respiration rates of spawning adults. Since *Adalaria proxima* spawns later in the spring than *Onchidoris muricata*, observations of the two species were not necessarily made at the same time (although the two periods do overlap). *O.muricata* individuals were studied from mid-February 1984 through to post-reproductive death in May 1984. All seven *O.muricata* had commenced spawning prior to the first observation. None of the seven *A.proxima* individuals had commenced spawning before the first respiration rate determinations were made in mid-April. These animals were also studied until post-reproductive death in June / July 1984.

All the nudibranchs studied were weighed under water immediately after each set of respiration rate determinations had been obtained (see General Methodology, Section 2.2).

'Blank' (chamber-only) respiration rates were obtained from the uncleaned (*i.e.* mucus-covered) chamber immediately following a nudibranch respiration rate determination. This was done for the first, and every alternate individual of each set of seven energy budget animals. These adjacent 'blank' values were subtracted from the observed nudibranch respiration rates to account for Oxygen depletion caused by the electrode itself and by bacteria in the nudibranch's mucus trail. Before every nudibranch respiration rate determination, the whole chamber and electrode block were sterilised in 10% "Milton" solution and then rinsed in FSW.

After each determination, the volume of water contained in the respirometry chamber was measured to the nearest 5 µl with an "Agla" micrometer syringe.

Q_{10} Determinations

The above apparatus and general methodology were used to investigate the effect of varying environmental temperature on respiration rates of *Adalaria proxima* and *Onchidoris muricata.*

Three pre-spawning individuals of each species were subjected to a range of temperatures from 2 °C to 13 °C. These individuals were maintained at the experimental temperature for 48 hours prior to each respiration rate determination.

5.3 RESULTS

A total of 14 (5 'pre-spawning' + 9 'spawning') respiration rate determinations were made for the seven *Onchidoris muricata* individuals, and 12 (5 'pre-spawning' + 7 'spawning') determinations were obtained for the seven *Adalaria proxima.*

The overall mean weight-specific respiration rate (± one standard error) were 0.2764 ± 0.0090 µl O_2.mg $dwt^{-1}.h^{-1}$ and 0.2194 ± 0.0075 µl O_2.mg $dwt^{-1}.h^{-1}$ for *A.proxima* and *O.muricata* respectively. However, respiration rate did not rise in direct proportion to body size in all cases, and therefore these figures are not strictly comparable. Both Model I (Y on X) and Model II (Geometric Mean) regressions of log respiration rate and log body weight were computed for each individual *A.proxima* and *O.muricata.* These regressions and the associated correlation coefficients are shown in Tables 5.1 and 5.2 respectively. Respiration rate was converted to Joule equivalent heat loss using the value of 13.661 J.mg O_2^{-1} (Elliott & Davidson, 1975). Body weight conversions were obtained using the equations given in Chapter 4 (Section 4.3).

For all *A.proxima* and *O.muricata* individuals, statistically significant correlations between log respiration rate and log body weight were found (Tables 5.1 and 5.2). However, the *A.proxima* data set is considerably more variable than that of *O.muricata,*

TABLE 5.1 Respiration Rate and Body Size in seven individual *Adalaria proxima*.

($y = \log_e$ (Respired $J.h^{-1}$); $x = \log_e$ (Equivalent Body J))

Animal no.	Model I Regression	Model II (GM) Regression	r	n
10	$y = 0.7414.x - 5.9882$	$y = 0.7891.x - 6.2362$	0.940^{***}	12
14	$y = 0.8865.x - 6.7733$	$y = 0.9744.x - 7.1958$	0.910^{***}	12
17	$y = 0.9452.x - 7.0034$	$y = 1.0876.x - 7.7628$	0.869^{***}	12
21	$y = 0.4389.x - 4.4157$	$y = 0.6873.x - 5.7301$	0.639^{*}	12
23	$y = 0.7084.x - 5.9554$	$y = 0.7235.x - 6.0284$	0.979^{***}	12
24	$y = 0.3488.x - 3.8800$	$y = 0.5925.x - 5.2491$	0.589^{*}	12
27	$y = 0.5700.x - 5.1524$	$y = 0.7033.x - 5.8549$	0.810^{**}	12

Note: Asterisks indicate statistical significance for $p \leq 0.05$ (*); $p \leq 0.01$ (**) and $p \leq 0.001$ (***).

TABLE 5.2 Respiration Rate and Body Size in seven individual *Onchidoris muricata*

(notation as for Table 5.1)

Animal no.	Model I Regression	Model II (GM) Regression	r	n
3	$y = 1.2733.x - 7.9148$	$y = 1.5901.x - 8.8209$	0.801^{***}	14
4	$y = 0.9922.x - 7.3049$	$y = 1.0269.x - 7.4259$	0.966^{***}	14
7	$y = 0.9501.x - 7.2797$	$y = 1.0238.x - 7.5477$	0.928^{***}	14
10	$y = 0.9938.x - 7.5723$	$y = 1.1835.x - 8.2487$	0.840^{***}	14
11	$y = 1.0029.x - 7.2647$	$y = 1.0591.x - 7.4711$	0.947^{***}	14
21	$y = 1.0262.x - 7.3063$	$y = 1.0484.x - 7.3869$	0.979^{***}	14
28	$y = 0.9945.x - 7.5859$	$y = 1.1095.x - 8.0128$	0.896^{***}	14

with respect to both slope and intercept of the individual regressions (although the latter are a considerable distance from the point mean-x, mean-y, and therefore may have amplified small deviations in the data). Greater within individual variation for *A.proxima* than for *O.muricata* was also found and is reflected in the correlation coefficients given in Tables 5.1 and 5.2.

To investigate these differences further, an analysis of covariance was performed on the respiration rate with individual animals as the factors and body size as the covariate. This tested the null hypothesis that the slopes and intercepts of the individual regressions did not differ significantly from the pooled trend for that species (*i.e.* that they were homogeneous). Analysis of variance can be subdivided into Model I and Model II procedures in a manner analogous to Regression analysis (see General Methodology, Section 2.2). (Indeed, analysis of variance and regression analysis are closely related techniques). However, no methods are yet available for Model II analysis of covariance, and therefore a Model I analysis was carried out.

The null hypothesis of homogeneity of slopes could not be rejected for the seven *O.muricata* data sets ($F = 0.417$, $p = 0.866$). However, after fitting a common slope to each data set, *O.muricata* individuals numbers 3 and 10 were found to have regression intercepts which deviated significantly from the overall relationship ($F = 4.198$, $p = 0.001$), number 3 being higher and number 10 lower.

A contrasting picture emerged for the seven *A.proxima* data sets. The null hypothesis of homogeneity of slopes was rejected ($F = 2.384$, $p = 0.037$) and therefore no test for the homogeneity of intercepts was undertaken. *A.proxima* individuals numbers 14 and 17 had regression slopes significantly steeper than the overall relationship, and *A.proxima* number 24 had a regression slope which was significantly shallower.

Inspection of Table 5.1 indicates that the variation of the Model I regression coefficients is considerably greater than that of the Model II regression coefficients. This is a reflection of the greater variability of respiration rate within individual *A.proxima*, (the Model

II coefficient is proportional to the Model I coefficient divided by the correlation coefficient, 'r'). Notwithstanding these differences, the allometry of respiration rate with body weight is clearly less consistent both within and between individual *A.proxima* than it is for any given *O.muricata* .

A more readily apparent dichotomy in the data is that of the Model II regression slopes for the two species. It can be seen from Tables 5.1 and 5.2 that the slopes of individual *A.proxima* data sets range from 0.59 to 1.09, while in contrast, the equivalent figures for *O.muricata* data sets are 1.02 to 1.59. This difference is still more evident from the GM regressions of Respired $J.h^{-1}$ (y) and Equivalent Body J (x) for the pooled data sets. These are:

$$\log_e y = 0.8332.\log_e x - 6.513$$

$$i.e.\ y = 0.001484.x^{0.833} \qquad r^2 = 0.648,\ n = 84,\ p << 0.001\ (A.proxima)$$

$$\log_e y = 1.077.\log_e x - 7.640$$

$$i.e.\ y = 0.0004808.x^{1.077} \qquad r^2 = 0.808,\ n = 98,\ p << 0.001\ (O.muricata)$$

The pooled raw data are shown in Figures 5.2 and 5.3 and clearly show the different slopes of the above regressions. Equally apparent in these Figures, is the relative lack of association between spawning respiration rate and body size for both *A.proxima* and *O.muricata.* Both of these data sets however lie in line with the respective pre-spawning data sets and thus indicate a relatively constant allometric relationship throughout the lifetime of both nudibranch species. Ambient temperatures over the experimental period ranged from a minimum of 4.5 °C to a maximum of approximately 12.5 °C.

Effects of Temperature on Respiration Rate

For the purposes of the present investigation, individual respiration rates are expressed on a weight-specific basis. Acknowledgement of the potential inaccuracies of such measures has been made in the preceding section. For the present, however, these

FIGURE 5.2 Respiration rate in relation to body size for *Adalaria proxima*

(Open circles = 'pre-spawning' data, closed circles = 'spawning' data)

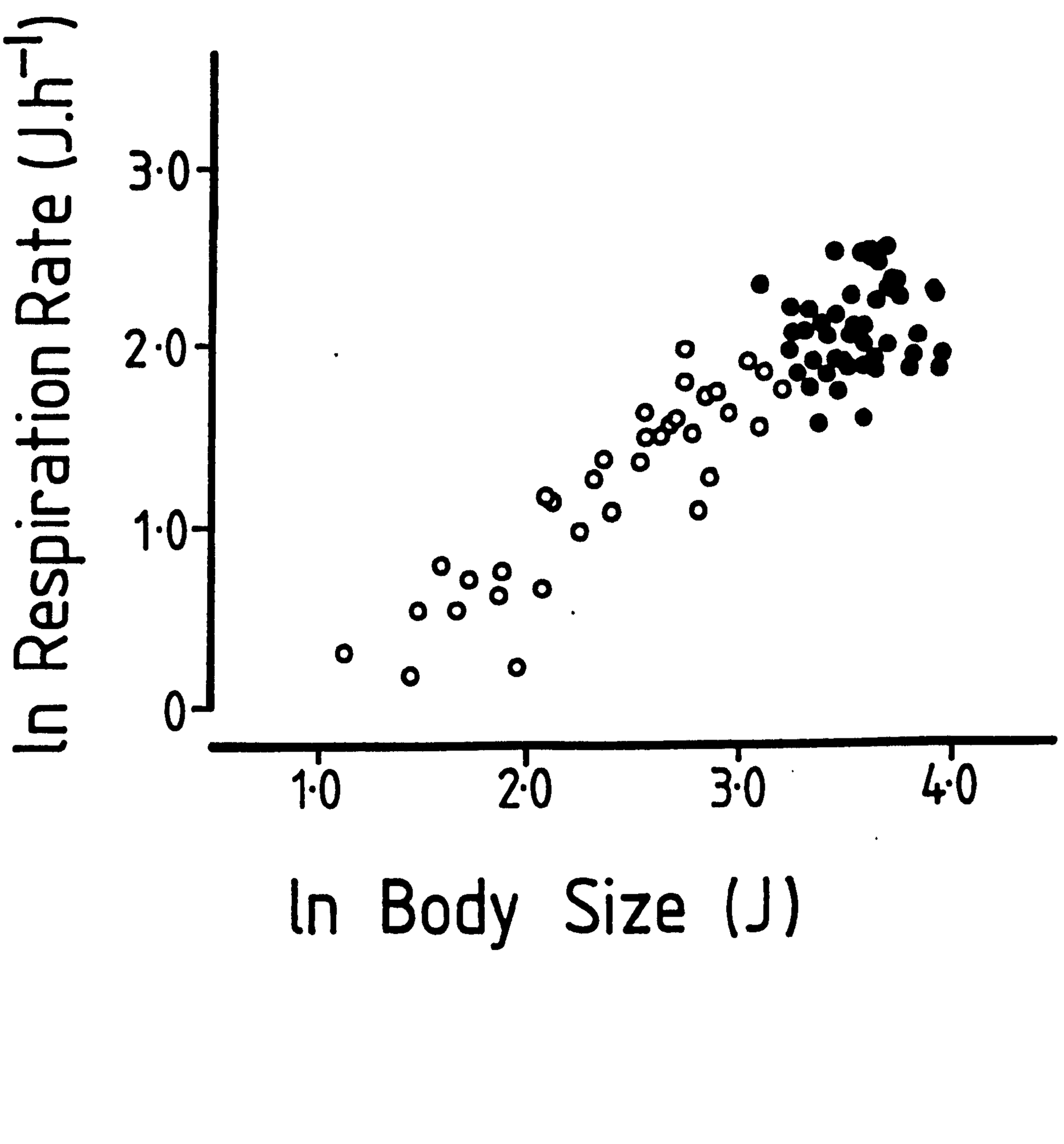
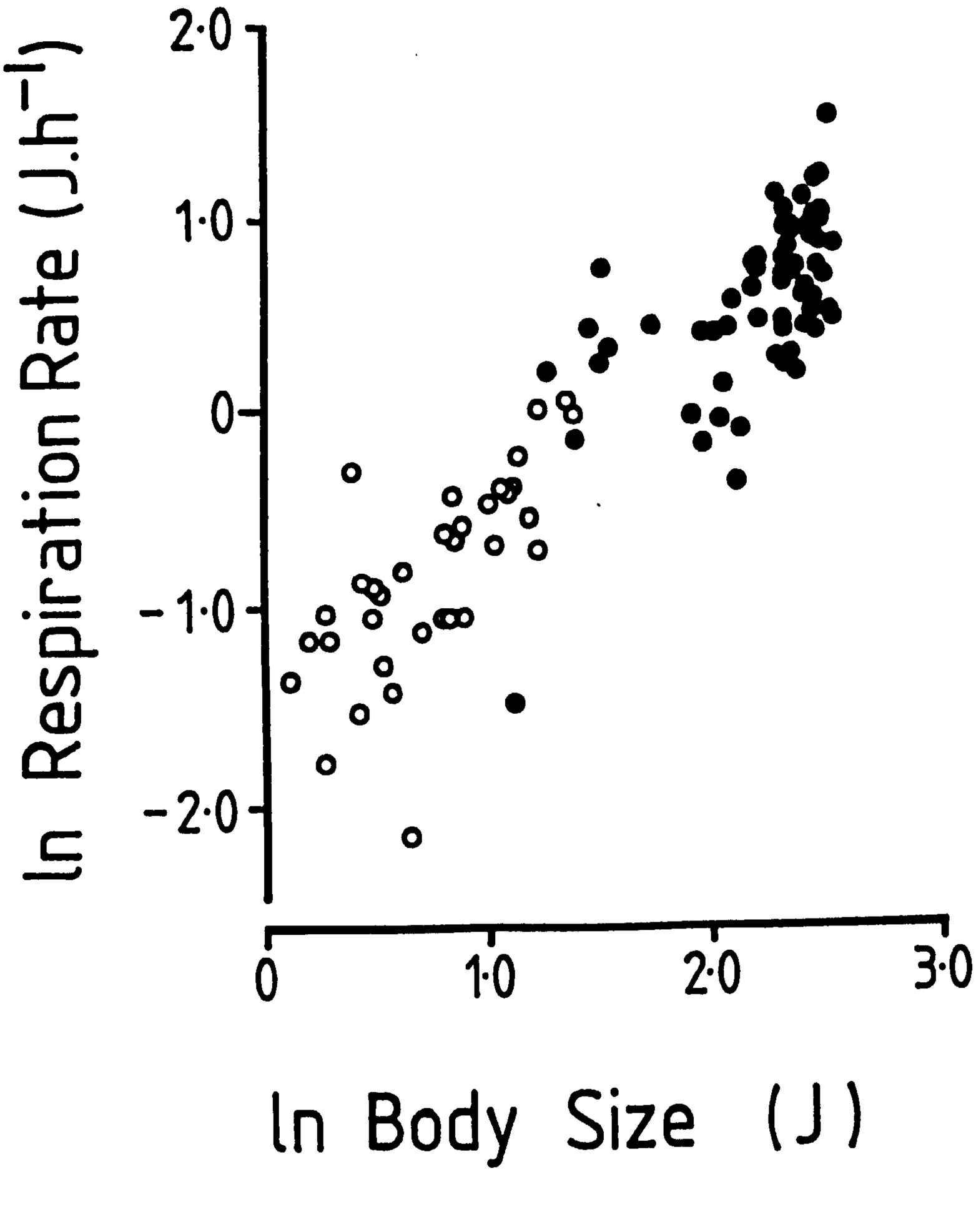

ln Respiration Rate (J.h⁻¹)
3·0
2·0
1·0
0
1·0
2·0
3·0
4·0
ln Body Size (J)

FIGURE 5.3 Respiration rate in relation to body size for *Onchidoris muricata*

(Open circles = 'pre-spawning' data, closed circles = 'spawning' data)


ln Respiration Rate (J.h⁻¹)
2·0
1·0
0
-1·0
-2·0
0
1·0
2·0
3·0
ln Body Size (J)

measures have been used primarily for the generation of Q_{10} estimates and therefore any errors arising from such inaccuracies cancel out and are consequently of little importance.

The mean weight-specific respiration rates of *A.proxima* and *O.muricata* at the different experimental temperatures are shown in Figure 5.4. Both nudibranch species display an increase in respiration rate with increasing temperature. However, the rate of this increase is less pronounced in the middle of the temperature range than it is at the extremities. Indeed, this 'plateau' effect is more marked in *A.proxima* than in *O.muricata*. These data and the associated Q_{10} estimates are also shown in Table 5.3. Again, the plateau in rate of respiration rate increase is reflected in the estimates. These Q_{10} estimates are also more variable in *A.proxima* indicating the greater temperature sensitivity of this species.

5.4 DISCUSSION

Although the primary concern of the present study was to obtain data for respiration within the context of the energy budget analysis, the results include several ecophysiological aspects which are interesting in their own right. Perhaps most notable of these is the variation in the allometry of respiration rate of individual *A.proxima*. The analysis of covariance has indicated that the slopes of the (log : log) regression lines of respiration rate on body size are essentially the same for all the *O.muricata* individuals in this study. The analysis has also found two of these individuals to respire at either significantly higher or lower rates than the pooled respiration rate for all individuals. Despite being highly significant ($p < 0.001$) these deviations from the pooled relationship are not great and the high significance level is undoubtedly a partial reflection of the strong correlations within the individual *O.muricata* data sets. Thus, although the absolute respiration rate of *O.muricata* may vary between animals of the same size, the rate of change of respiration rate as the animal grows is more or less constant for all individuals.

FIGURE 5.4 Effect of Temperature on respiration rate of *Adalaria proxima* and *Onchidoris muricata*

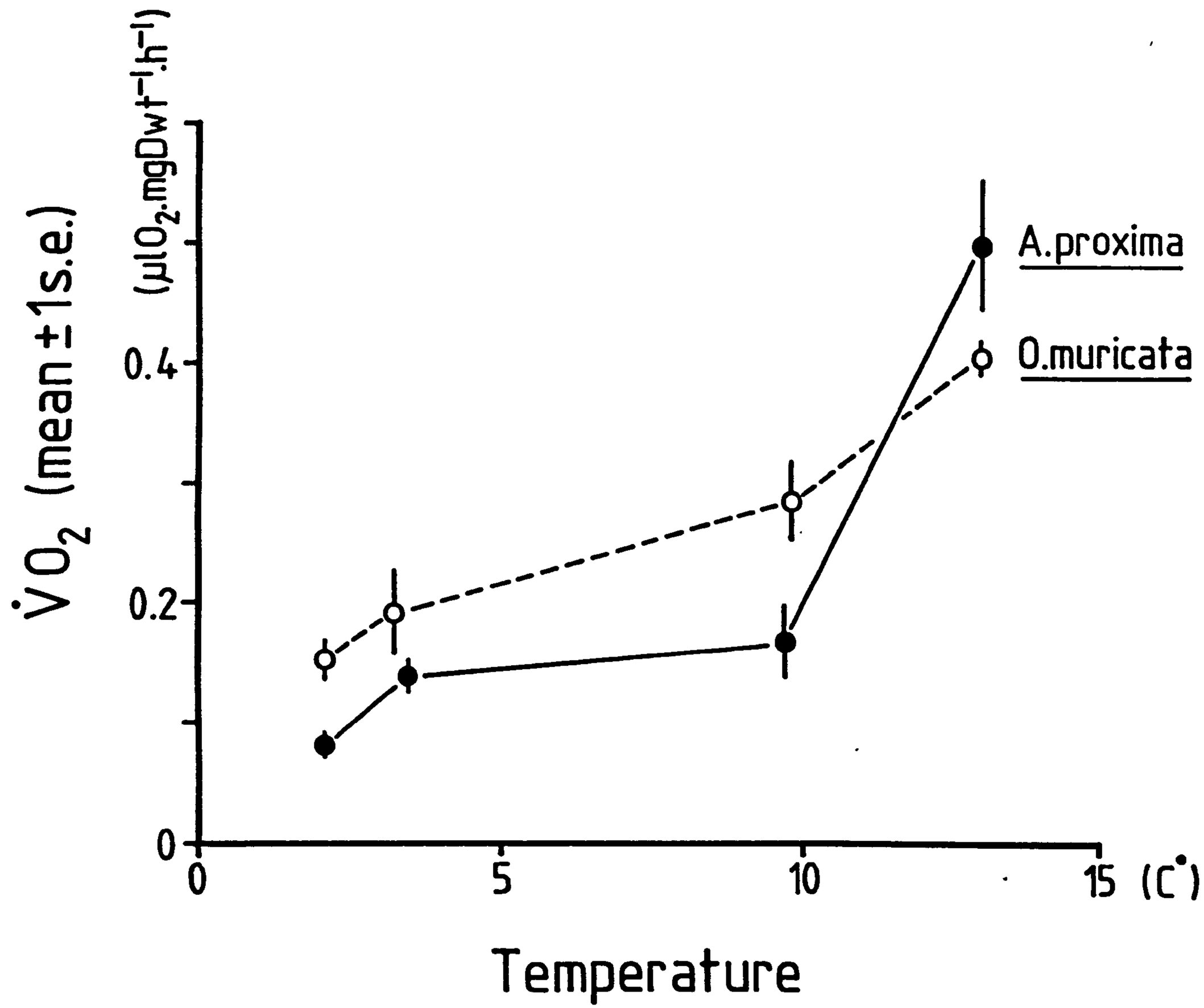

$\dot{V}O_2$ (mean ±1s.e.) ($\mu lO_2.mgDwt^{-1}.h^{-1}$)
0.4
0.2
0
0
5
10
15 (C°)
Temperature
A.proxima
O.muricata

TABLE 5.3 Q_{10} estimates for *A.proxima* and *Q.muricata*

(Figures given are means of 3 observations)

A.proxima			*Q.muricata*		
Temperature (°C)	Respiration (µl O_2.mgdwt.h^{-1})	Q_{10}	Temperature (°C)	Respiration (µl O_2.mgdwt.h^{-1})	Q_{10}
13.0	0.4991		13.0	0.4060	
		18.0			3.20
9.7	0.1694		9.8	0.2840	
		1.74			1.99
		5.20			2.45
3.5	0.1387		3.4	0.1904	
		35.6			3.11
2.1	0.0827		2.1	0.1528	

A very different pattern has emerged from the analysis of *A.proxima* data sets. The slopes of the allometric relationships for some individuals were shown to deviate significantly from the overall (pooled) relationship, and therefore suggest that different rate processes may operate in different individuals.

It is worth mentioning at this juncture that the previously noted difference between Model I and Model II regression slopes for the *A.proxima* data sets may be important. The rationale for adopting Model II regressions has been outlined earlier (see General Methodology, Section 2.2), and the relative lack of variation between the Model II regression coefficients for *A.proxima* individuals has already been noted.

Given the probability level of heterogeneity of slopes for the *A.proxima* data sets ($p = 0.037$), it is possible that a (hypothetical) Model II analysis of covariance would not have rejected the null hypothesis of homogeneous slopes. Thus, within the context of the adopted (Model II) rationale, the (Model I) analysis of covariance should be regarded as an indicator of trends rather than as a strict statistical test. Notwithstanding this qualification, the analysis has demonstrated a potentially fundamental difference in the constancy of respiratory allometry between these two nudibranch species.

Given the degree of individual variation already noted, the combination of these data to provide pooled allometric relationships for each species is perhaps questionable. Certainly, the primary interest of the overall study is to investigate individual variations in resource partitioning. For the present however, further points of interest are most easily considered in terms of the pooled data.

All the individual and pooled data sets have yielded allometric relationships with statistically significant correlation coefficients. What is perhaps remarkable, is that these allometric relationships have been obtained despite a temperature change of almost 8 °C over the observational period. Respiration rate observations were made at temperatures ranging from 12.2 - 9.8 °C and 4.3 - 9.9 °C for pre-spawning and spawning *O.muricata* respectively. Comparable values for *A.proxima* respirometry were 12.2 - 9.6 °C

(pre-spawning) and 4.8 - 11.9 °C (spawning).

The potential effects of such temperature fluctuations on respiration rate have been outlined in Figure 5.4 and Table 5.3. The data presented therein were derived from only six pre-spawning individuals, however, and consequently represent the results of a preliminary investigation rather than a definitive study.

Nonetheless, Figure 5.4 indicates that respiration rate in both *A.proxima* and *O.muricata* is considerably less sensitive to changes of environmental temperature in the range 3 - 10 °C than it is outside this range. Such a 'plateau' in respiratory response to temperature has been demonstrated for many marine invertebrates (for example; Newell & Northcroft, 1967; Brown & da Silva, 1978; reviewed by Bayne & Newell, 1983), though this effect appears to be more pronounced in *A.proxima* than in *O.muricata*.

Results similar to those given here for *O.muricata* have also been reported for the closely related *Onchidoris aspera* (Smith & Sebens, 1983). Clark (1975) also presents Q_{10} data for 18 nudibranch species from the western Atlantic. However, none of the species he studied (including *O.aspera*) demonstrated this same 'plateau'. This may be a real effect, but it may also be partially attributable to the different experimental technique employed by Clark (1975).

If the observed temperature sensitivity pattern is applicable throughout the lifetime of these nudibranchs and not just to the two-week period over which it was obtained, then it is logical to expect environmental temperature to explain some of the residual variation seen in Figures 5.2 and 5.3. To test this hypothesis, a stepwise multiple regression analysis was performed on the pooled data sets. For both the *A.proxima* and the *O.muricata* data, no significant increase in the explained variation was obtained when temperature was included with body size as a predictor of respiration rate. (Indeed, no increase whatsoever was obtained for the *O.muricata* data set). Analysis of the residuals however indicated that temperature might explain much of the variation in the respiration rates of spawning

A.proxima. To investigate this further, the pooled data sets were split into pre-spawning and spawning observations and the analysis was repeated on each subset. As before, temperature did not explain any of the residual variation in either pre-spawning or spawning *O.muricata* subsets, or for pre-spawning *A.proxima*. In spawning *A.proxima* however, temperature was far more important than body size in determining respiration rate (these variables explained 24% and 3% respectively of the total observed variation). Thus the initial hypothesis is largely refuted. Quite why the hypothesis is only supported by spawning *A.proxima* is unclear. Certainly, spawning *A.proxima* were exposed to environmental temperatures which were outside the 'plateau' region of Figure 5.4 and therefore may have been more sensitive to temperature than at other times. However, both *A.proxima* and *O.muricata* experienced environmental temperatures in excess of 10 °C for most of the pre-spawning observation period. Moreover, *O.muricata* does not appear to exhibit such a marked 'plateau' of respiratory response to temperature as does *A.proxima*. Consequently one might expect that temperature would exert a more consistent [?and therefore detectable?] effect on respiration rate than that which was found.

Since environmental temperature has clearly had little or no effect on respiration rate in these circumstances, it must be concluded that the observed temperature sensitivity of spawning *A.proxima* is a result of processes operating only at this time in this species. The consequences and possible identity of these processes will be dealt with in Chapter 7. For the present it remains clear that respiration in *O.muricata* has become largely independent of seasonal changes in temperature throughout the life-cycle, (although short-term temperature fluctuations may elicit some response). *A.proxima* demonstrates a similar adaptation up until the onset of spawning, after which respiration rate is largely dictated by environmental temperature.

In addition to the above, the pooled data also encapsulate the differences between the allometric relationships for respiration rate in the two species. Accepted physiological theory

states that respiration rate is proportional to the 3/4 power of body weight, or strictly;

$$R = a.W^{0.73}$$

where; R = respiration rate; W = body weight; and 'a' is a constant (Zeuthen, 1953). Much debate surrounds this relationship which is empirically, rather than theoretically based. Indeed, some authorities claim this relationship not to hold for invertebrates since the exponent in the above equation varies so widely between invertebrate species. Altman and Dittmer (1968) cite almost 200 allometric relationships for respiration in invertebrates. Of these, 48 are for gastropods amongst which the exponent varies from 0.45 to 1.10. The mean exponent for these data is, however, 0.76 indicating that despite interspecific variation, the general relationship may indeed hold true (Schmidt-Nielsen, 1984).

Bayne & Newell (1983) have reviewed the allometry of respiration rates in molluscs. Again, despite considerable variation, the mean exponent for carnivorous gastropods is 0.73. None of the data cited in the above studies are for nudibranchs however. Clark (1975) gives data on the allometry of respiration in 18 nudibranch species. The exponents of his data range from approximately 0.1 (*Onchidoris fusca* (= *bilamellata*)) to over 1.0 (*Acanthodoris pilosa*) but here, again, the overall mean exponent is 0.75. Potts (1983) gives allometric equations for respiration rate of the nudibranchs *Onchidoris bilamellata* and *Archidoris pseudoargus* at different acclimation temperatures. The exponents in these equations range from 0.38 (*A.pseudoargus*, 4 °C) to 0.97 (*O.bilamellata*, 18 °C). Carefoot (1967) also gives allometric equations for two species of nudibranch and an aplysiomorph respiring at 15 °C. The exponents of these equations were 0.25 (*Dendronotus frondosus*); 0.47 (*Aplysia punctata*) and 0.60 (*Archidoris pseudoargus*). Most of these values are below those obtained for the pooled *A.proxima* (0.83) and *O.muricata* (1.08) in this study. However, the data cited in the above studies were obtained from a number of individuals of varying size but approximately equivalent age. The present study has demonstrated that although the allometry of respiration rate in a known individual may remain relatively constant throughout the year, it may differ widely from that of another (conspecific) individual. Under such circumstances, the utility of allometric relationships for respiration of a given species is

limited, since such relationships may conceal ecologically important intraspecific variation. Nonetheless, the exponents of the individual allometric equations obtained in the present study reflect the values and variability reported for most investigations of molluscs if not for most opisthobranchs.

Perhaps the most important observation reported here is that the exponent of the allometric relationship for respiration in *O.muricata* is equal to, or greater than, unity. The consequence of this is that large *O.muricata* are no more (and possibly less) metabolically efficient than are small *O.muricata*. (In fact, some individuals may become less efficient as they grow larger). In contrast, almost all *A.proxima* individuals displayed allometric exponents smaller than unity and, therefore, do become more efficient as they grow larger. Given the body size differences between *A.proxima* and *O.muricata* (Figures 5.2 & 5.3) this result may have been expected on empirical grounds (Zeuthen 1953). However the observed variation of the allometric exponents is relatively high and indicates that the degree of change in efficiency which accompanies growth can vary widely between individuals. The consequences of such variation will be considered within the context of overall energy partitioning in Chapter 7.

CHAPTER 6

REPRODUCTION

6.1 INTRODUCTION

Ecological fitness can be defined as the contribution of a given genotype to the subsequent generation relative to that of other genotypes (Lincoln *et al.*, 1982). Clearly, many factors can affect the fitness of a given animal. However, the proportion of an animal's resources which is allocated to reproduction may be the most important fitness component. This is especially so in short-lived and semelparous species such as the nudibranch molluscs studied here. The allocation of energy to reproduction is therefore of interest not only as an energy budget component but also as a fitness component, and is consequently central to life-history theory in general (see, for example, Williams, 1966; Schaffer, 1974; Tinkle & Hadley, 1975; Hirshfield & Tinkle, 1975; Stearns, 1976).

Previous studies of reproduction amongst nudibranch molluscs have largely been of anatomical and taxonomic perspective (reviewed by Thompson, 1976; Thompson & Brown, 1985) or field-based life-cycle studies, of which Miller (1961) is a typical example. Recently, Todd (1981, 1983) has reviewed the reproductive ecology of the nudibranch molluscs, though again, these reviews concentrate on life-cycles and larval strategies. Reproductive energetics in nudibranchs has been studied by Todd (1979a,b), De Freese & Clark (1983), Smith & Sebens (1983) and Todd & Havenhand (1983). Of direct relevance to the present work are the studies of Todd (1979a) and Todd & Havenhand (1983) which include data on *Adalaria proxima* and *Onchidoris muricata.* All of the above studies cite a "Reproductive Effort" for various nudibranch species. However, only Todd (1979a) and Todd & Havenhand (1983) consider the allometry of reproductive allocation in nudibranchs. Hirshfield & Tinkle (1975) have stressed the importance of studying reproduction as a component within the energy budget, and in a brief review of the subject Todd & Havenhand (1983) concluded that little headway will be made unless this approach is adopted. However, none of the above studies of nudibranchs have concerned the quantification of

reproduction within the context of an energy budget investigation. The present chapter comprises such a study.

In order to obtain the maximum amount of information, all the *A.proxima* and *O.muricata* individuals which were maintained in the laboratory have been included. These are the same individuals as those studied in Chapter 4 and include the seven animals of each species which are dealt with in Chapters 5 and 7.

As a consequence of studying reproductive output (*i.e.* spawn production), the spawning characteristics and body weight changes of both *A.proxima* and *O.muricata* have also been recorded. These data are also presented here and interactions between the different characteristics are discussed. However, all consideration of energy fluxes to reproduction with respect to the other energy budget components is reserved for Chapter 7.

6.2 MATERIALS AND METHODS

In order to permit cross-fertilization between individually maintained experimental animals, nudibranchs were periodically paired in 'copulation trials'. Trials consisted of placing each nudibranch with a conspecific in a small, food-free chamber for between 6 and 8 hours. Nudibranchs were chosen on the basis of size and colour such that the individuals in any given pair could always be distinguished and separated. Every effort was made to provide a different mate for each animal in consecutive trials. *A.proxima* and *O.muricata* which had been maintained in pairs, were observed to copulate approximately every 5-6 days (Todd, 1986a). Therefore, in the present study, copulation trials were undertaken at this same frequency. Trials began when *A.proxima* and *O.muricata* were observed copulating in the field, and were continued throughout until post-reproductive death (*i.e.* March to July (*A.proxima*) and January to May (*O.muricata*)).

After the commencement of the copulation trials, each nudibranch and its container were examined daily. Any spawn-masses which had been deposited were carefully excised

using a scalpel and fine forceps. The excised spawn masses were rinsed in Ammonium Formate and placed in individual, pre-weighed Aluminium foil pans. These were then frozen prior to freeze-drying and subsequent weighing. On each day, all the nudibranchs which had spawned in the previous 24 hours were weighed under water (see General Methodology, Section 2.2) to determine the immediate post-spawning weight. This mass was taken as the best ('gonad-free') measure of somatic weight.

In addition to post-spawning weight-determinations, all individuals were weighed under water at approximately two week intervals.

6.3 RESULTS

For comparative purposes, all gravimetric data have been converted to energy (Joule) equivalents. This was achieved using the somatic regression and conversion coefficients given in Chapter 4 (Section 4.3) and the calorific values for spawn given by Todd (1979a). The latter are equivalent to 7.293 J.mg dry weight^{-1} for *Adalaria proxima* spawn and 9.138 J.mg dry weight^{-1} for *Onchidoris muricata* spawn.

Reproductive Characteristics

The major characteristics of both somatic and gonadal production during the spawning period are summarised for both *A.proxima* and *O.muricata* in Table 6.1. The major contrasts between the two species are readily apparent from this Table; *A.proxima* individuals generally attained a larger size, invested more energy in spawn, and spawned over a shorter period than *O.muricata* individuals. Table 6.1 also shows that the greater energy allocation to spawn by *A.proxima* individuals was represented in fewer spawn masses than in *O.muricata* individuals. The net result is that the mean spawn mass energy content differed widely between the two species (33.9 J for *A.proxima* and 10.8 J for *O.muricata*).

The relatively high energy content of *A.proxima* spawn masses is not reflected in the

TABLE 6.1 Summary Reproductive Output Data for *A.proxima* and *O.muricata*

(All figures are means ± one standard error)

	A.proxima	*O.muricata*
a) Maximum Body Size* (J)	324.1 ± 10.6	88.2 ± 4.2
Growth Rate ($J.d^{-1}$)	-1.658 ± 0.146	-0.248 ± 0.025
Length of Spawning Period (d)	60.6 ± 3.9	105.0 ± 4.0
First Spawn Mass (J)	95.5 ± 7.8	33.0 ± 2.6
b) Total Spawn Output (J)	301.0 ± 19.0	211.2 ± 13.0
Equivalent Egg No.†	6002 ± 393	64996 ± 3973
Spawning Rate ($J.d^{-1}$)	5.45 ± 0.43	2.02 ± 0.11
" " ($d.spawn^{-1}$)	7.40 ± 0.42	5.52 ± 0.23
No. of Spawn Masses	8.89 ± 0.71	19.61 ± 0.99
"Reproductive Effort" [(b/a).100]	94.6 ± 6.1	241.9 ± 12.3

* This is the equivalent energy content of the Maximum Body Size observed after the onset of spawning.

† Egg numbers were calculated from individual spawn mass weights using regression equations obtained by C.D.Todd (unpubl. obs.).

number of ova those spawn masses contain. From the data in Table 6.1 it is readily calculated that an 'average' *A.proxima* spawn mass contained an estimated 675 ova in comparison to the 3300 ova in an 'average' *O.muricata* spawn mass. These figures were calculated from derived totals and means for the data set and are therefore not a true measure of mean ova number per spawn mass. Nonetheless, they do illustrate that greater energetic investment is required for each lecithotrophic ovum of *A.proxima* in comparison to that for a planktotrophic (*O.muricata*) ovum, (see also Todd, 1979a).

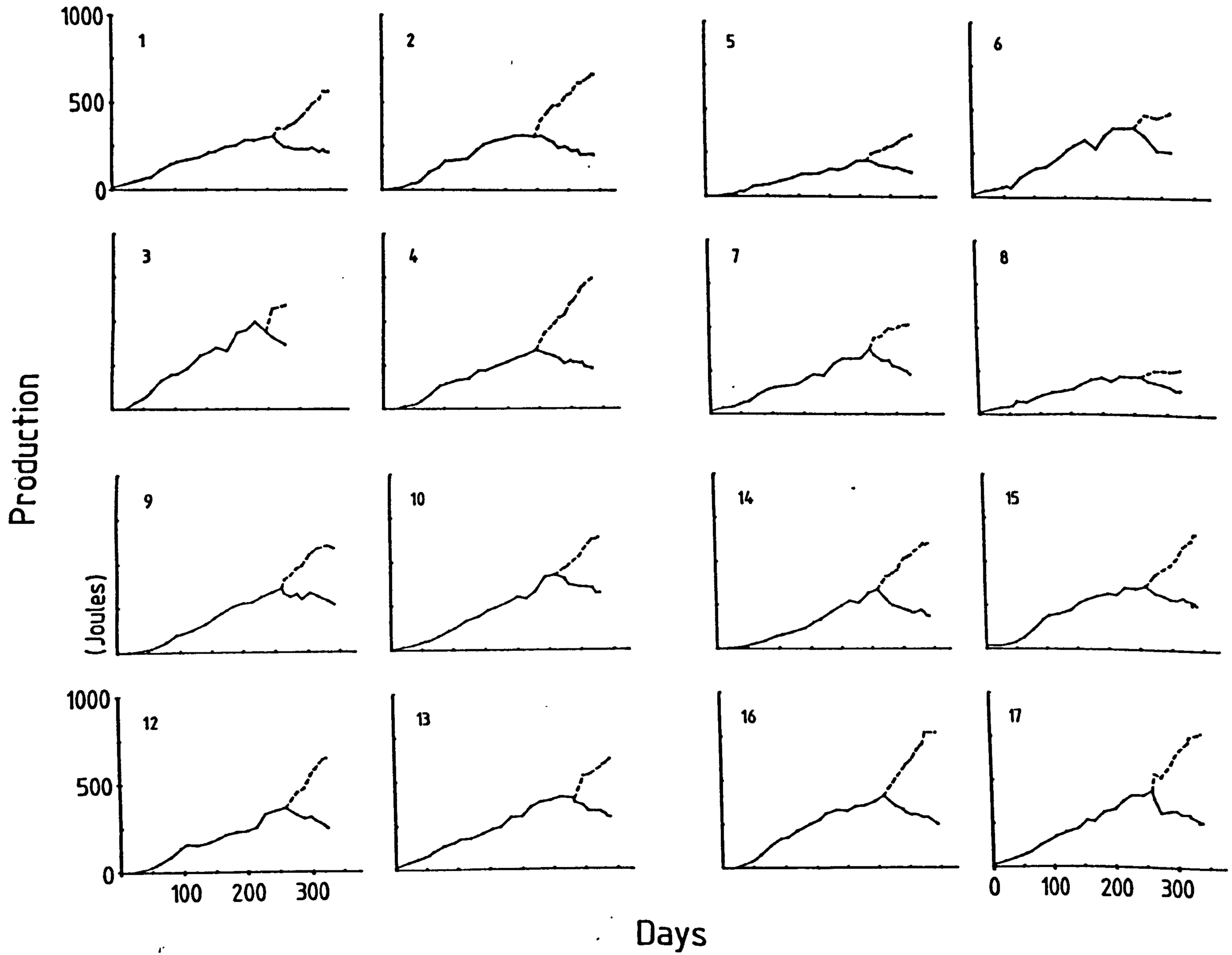
Despite the observation that *A.proxima* individuals invest more energy in (fewer) spawn masses than do *O.muricata* individuals, the proportionally greater difference between the mean maximum body sizes for these two species (Table 6.1) results in a lower mean "Reproductive Effort" for *A.proxima* than for *O.muricata* (Table 6.1). Indeed, the "Reproductive Effort" of *A.proxima* individuals ranged from 35.2% to 150.0% in comparison to the 111.1% to 374.8% range observed for *O.muricata* individuals. Given the lack of correlation between total spawn output and maximum body size however, (Table 6.2) the value of this ratio as a measure of reproductive investment is perhaps questionable. The utility of this, and alternative, measures of energetic allocation to reproduction are dealt with in detail in Chapter 8.

Production Patterns

Table 6.1 also summarises the observed weight loss by both *A.proxima* and *O.muricata* individuals throughout their spawning periods. The mean rate of weight loss by individual *A.proxima* is significantly in excess of that of *O.muricata* (Mann-Whitney test of rank order, $p < 0.001$). However, the consistency of this pattern both over time and between individuals is more readily seen in individual plots of growth. These plots are presented along with cumulative total production (soma and cumulative spawn) curves in Figures 6.1 and 6.2 for *A.proxima* and *O.muricata* individuals respectively. These Figures include pre-spawning growth data (already discussed in Chapter 4) in order to illustrate patterns of individual production throughout the year. The solid plots in Figures 6.1 and 6.2 represent body size, the broken lines represent cumulative total production (*i.e.* body size plus cumulative spawn production) and the bifurcation of these two lines represents the

FIGURE 6.1 Individual total Production (cumulative ΣP_g (solid line) plus cumulative ΣP_r (broken line)) for *Adalaria proxima*

(Numbers at top left of each Figure are individual identification nos.)


1000
500
0
1
2
5
6
3
4
7
8
Production
(Joules)
9
10
14
15
1000
500
0
12
13
16
17
100
200
300
0
100
200
300
Days

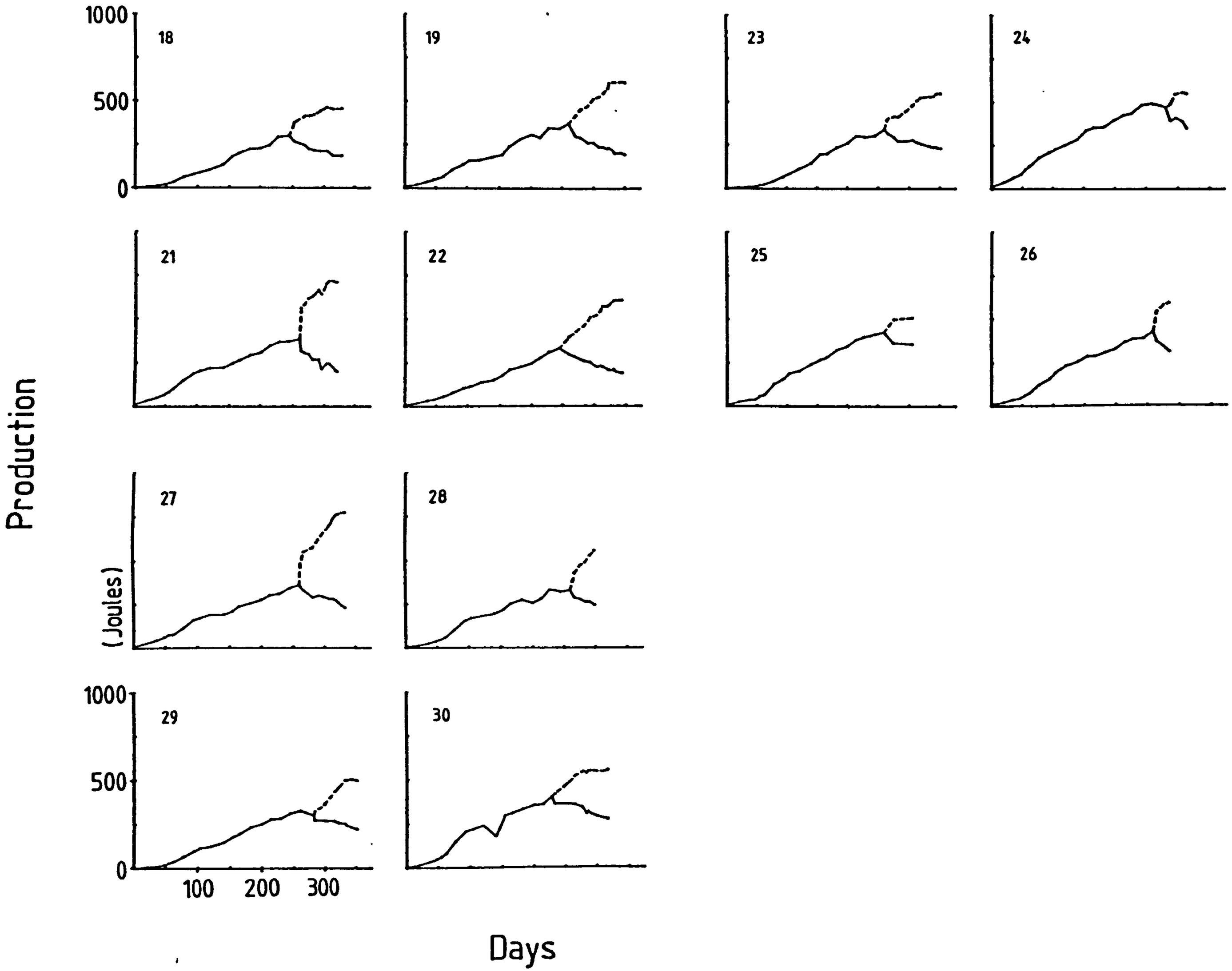
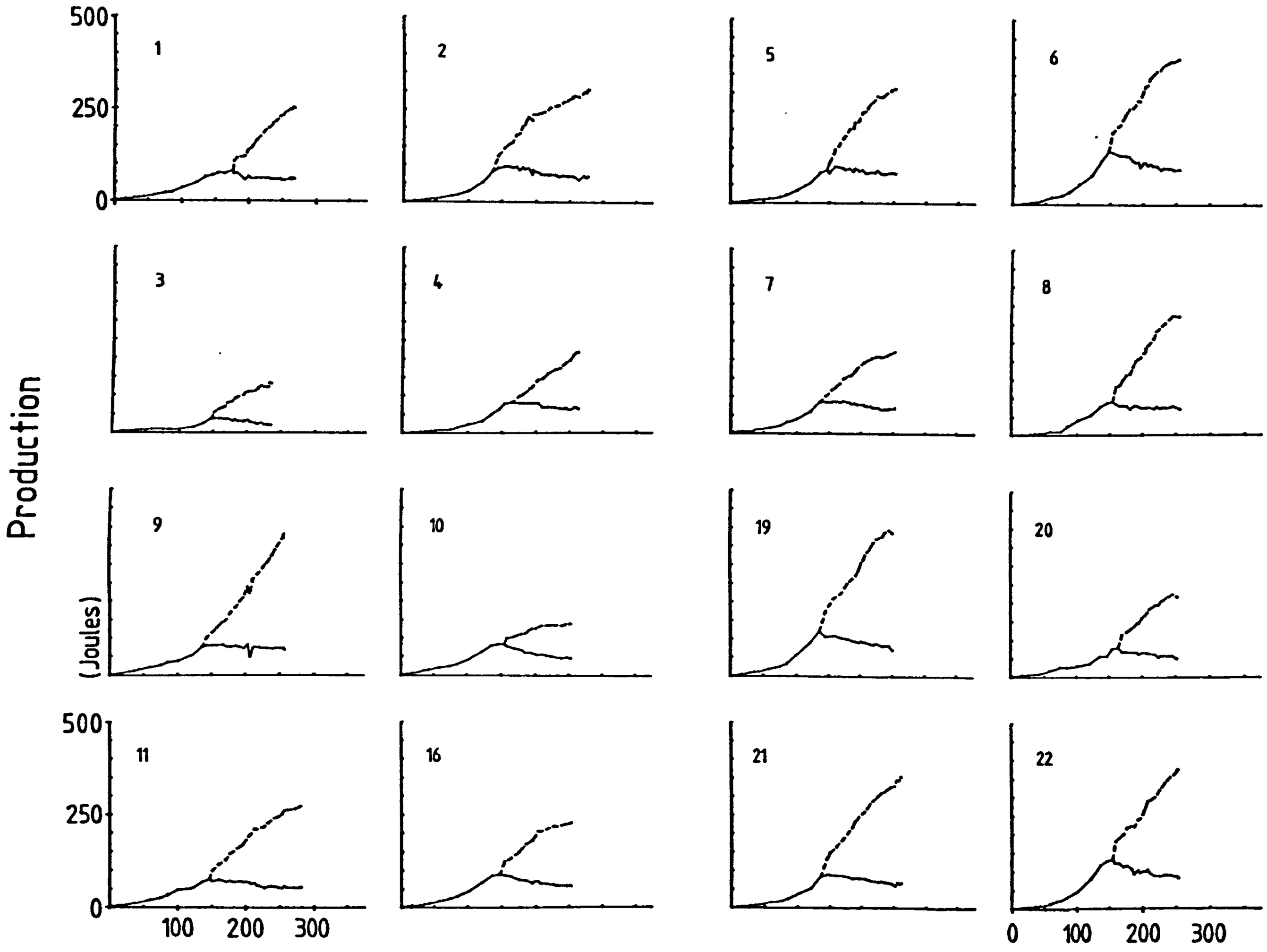
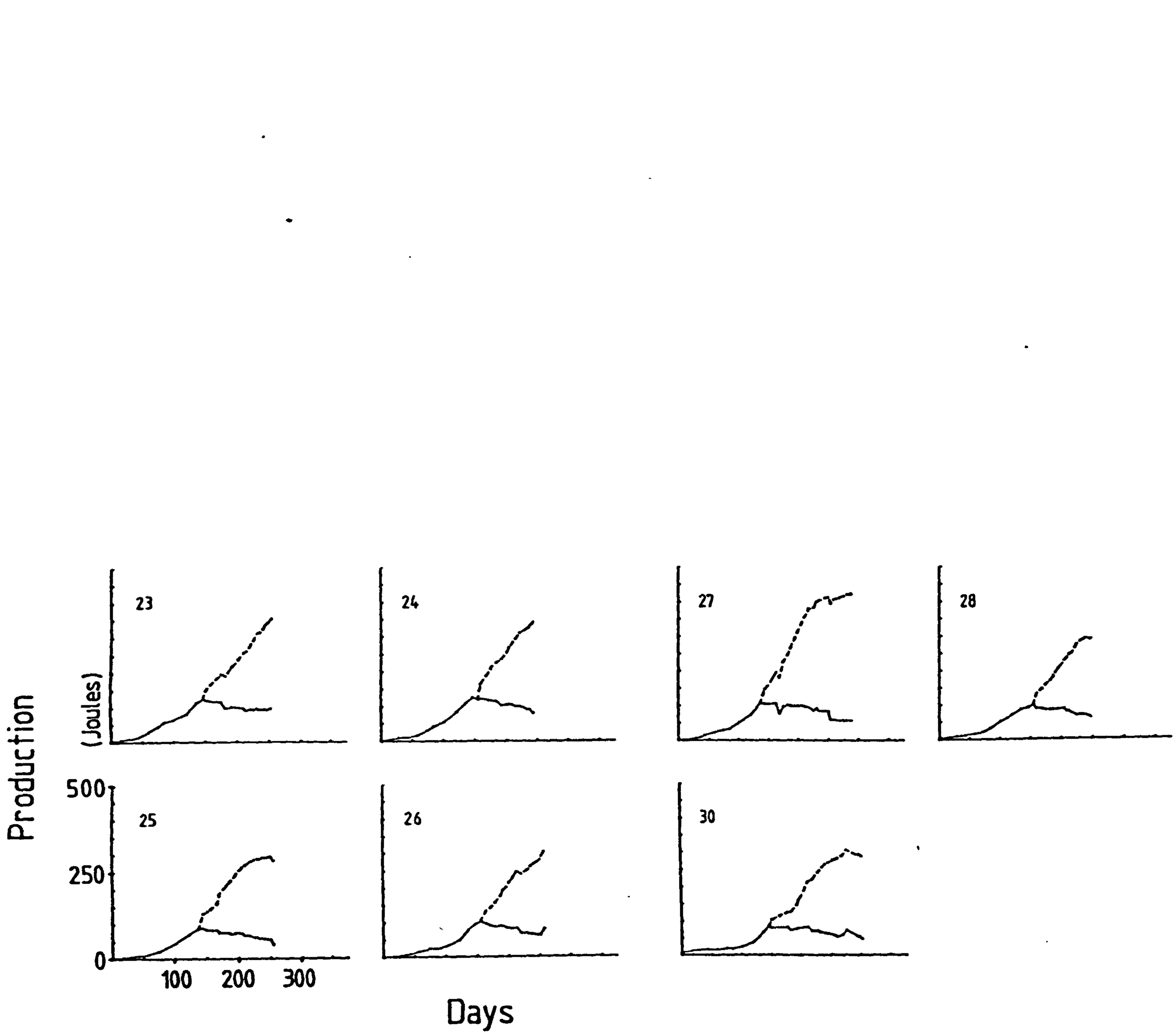

1000
500
0
18
19
23
24
21
22
25
26
Production
(Joules)
27
28
1000
500
0
29
30
100
200
300
Days

FIGURE 6.2 Individual total Production for *Onchidoris muricata*

(Format and notation as for Figure 6.1)


500
250
0
1
2
5
6
3
4
7
8
Production
(Joules)
9
10
19
20
500
250
0
11
16
21
22
100
200
300
0
100
200
300
Days


Production
(Joules)
500
250
0
23
24
27
28
25
26
30
100
200
300
Days

beginning of the spawning period.

Figure 6.1 shows that the pattern of weight loss for individual *A.proxima* during the relatively short reproductive lifespan is both consistent and considerable. Following the onset of spawning, total production continues to rise however, despite loss of body weight. Indeed, the total production rate is, in most cases, marginally greater than the pre-spawning growth rate. In many *A.proxima* individuals, this increased production rate is due mainly to the relatively large first spawn mass, this representing a substantial proportion of the average total spawn production (31.7%; Table 6.1). Comparable data for *O.muricata* show that the first spawn mass represents a much smaller proportion of the total spawn production (15.6%). This is reflected in the relative continuity between the pre-spawning somatic production curves and cumulative total production curves for a given individual (Figure 6.2). The relatively lower rate of weight loss by *O.muricata* during the spawning period can also be seen in Figure 6.2. The linearity of this pattern is quite striking in comparison to the curvilinear pattern of pre-spawning growth, and reflects the small error term for the mean growth rate given in Table 6.1.

The overall pattern of total production (pre-spawning growth and cumulative total spawning production) is remarkably consistent for all the individuals studied here. Although the absolute magnitude of the various components varies from one individual to another, the difference between the two species is clear:

A.proxima production follows an approximately linear pattern over the entire lifespan. Nonetheless, some individuals did show a marked discontinuity in this linearity at the onset of spawning, due to the production of a larger than average first spawn mass. In general, however, cumulative total production proceeded at a rate equivalent to, or slightly greater than pre-spawning production for almost all of the reproductive period.

A very different, curvilinear, pattern characterises *O.muricata* production. In general, the total production curve was almost sigmoid showing an initial period of slow growth followed by more rapid production in the late pre-spawning and early spawning periods, and declining total production rate as the reproductive period progressed. Some individuals

departed from this pattern by showing an approximately constant spawn production rate until death. In almost all cases however, the onset of spawning corresponded to the steepest section of the total production curve.

Correlation and Regression Analyses

In order to investigate further the inter-relationships between the primary reproductive characteristics outlined in Table 6.1, a correlation matrix for these variables was derived. This matrix is presented in Table 6.2. Several statistically significant associations emerged for both *A.proxima* and *O.muricata.*

In *A.proxima* individuals, total spawn output was positively correlated with the size of the first spawn mass, the length of the spawning period and the number of spawn masses produced. However, the latter two variables were also closely related to one another ($r = 0.882, p < 0.001$), indicating that auto-correlation of variables in the matrix may have resulted in spurious correlations. This problem was investigated further by deriving standardised partial regression coefficients ("beta-weights") between the variables (Sokal and Rohlf, 1981). In the above case, both the number of spawn masses produced and the length of the spawning period proved to be independently correlated with total spawn output. Surprisingly, the maximum body size was not correlated with total spawn output, ($r = 0.105, p > 0.05$, Table 6.2). Moreover, a significant negative relationship was observed between maximum body size and the number of spawn masses laid ($r = -0.399$, $p < 0.05$). Again, inspection of the beta-weights indicated that this association was independent of all other variables. However, the same analytical technique demonstrated that the observed negative correlation between maximum body size and the length of the spawning period was due entirely to the very strong relationship between spawning period and the number of spawn masses produced (Table 6.2).

The correlation matrix for *O.muricata* individuals reveals a contrasting pattern of associations to that observed in *A.proxima.* The most notable of these differences is a highly significant correlation between maximum body size and total spawn output ($r = 0.637$, $p < 0.01$). However, beta-weight analysis demonstrated that the strength of this association is

TABLE 6.2 Correlation Matrix of Reproductive Output Variables for *A.proxima* and *O.muricata*

(Figures above the diagonal are coefficients for *A.proxima* data, figures below the diagonal are for *O.muricata* data)

	(1)	(2)	(3)	(4)	(5)
(1) Total Spawn Output	-	0.606***	0.518**	0.415*	0.105
(2) No. of Spawn Masses	0.300	-	0.882***	-0.157	-0.399*
(3) Length of Sp. Period	0.462*	0.614**	-	-0.314	-0.430*
(4) Size of 1st Spawn Mass	0.594*	-0.069	-0.113	-	0.419*
(5) Maximum Body Size	0.637***	-0.093	0.005	0.690***	-

Note: Asterisks indicate statistical significance for $p \leq 0.05$ (*); $p \leq 0.01$ (**) and $p \leq 0.001$ (***).

reduced if correlations with the other variables are accounted for. (Maximum body size is significantly correlated with the size of the first spawn mass which is in turn correlated with total spawn production). Nonetheless, the overall relationship is very different to that seen for *A.proxima* individuals.

In a manner similar to that already seen in *A.proxima*, *O.muricata* individuals displayed a strong correlation between the number of spawn masses produced and the length of the spawning period ($r = 0.614$, $p < 0.01$). However, unlike *A.proxima*, *O.muricata* individuals show no significant association between total spawn output and the number of spawn masses produced. Furthermore, inspection of the beta-weights reveals that what little correlation does exist between these two variables ($r = 0.300$) is almost entirely due to the strong association between the length of the spawning period and the total spawn output ($r = 0.462$, $p < 0.05$). A final contrast between the two nudibranch species was the absence of a significant (negative) correlation between the maximum body size and the number of spawn masses produced by *O.muricata* individuals. Again, analysis of the beta-weights confirmed that this was a true reflection of the relationships between the variables.

Multivariate Analyses

In order to summarise the data in Table 6.2 and to further investigate associations within the correlation matrices, the two data sets were each subjected to Principal Components Analysis (PCA). Two separate analyses were completed for each data set. The first, an R-mode PCA, partitions the variation in the data set with respect to the original variables and describes the relationships between these variables and the new (derived) Factors. The second analysis, a Q-mode PCA, uses the same methodology to partition the variation in the data set with respect to the individual cases (*i.e.* animals) and describes the relationships between the cases and the Factors. These two analyses both derive the same Factors from a given data set, but differ in the way the results are presented. Statistics relevant to the Factors and to the R-mode analysis are summarised in Table 6.3.

In the PCA's for both data sets, the third Factor extracted from the correlation matrix had an eigenvalue of less than one. Consequently this, and all subsequent Factors, were

TABLE 6.3 Principal Components Analysis of Reproductive Output Variables for *A.proxima* and *O.muricata*

		A.proxima	*O.muricata*
Variable No.	Variable	Communality (First two Factors)	
(1)	Total Spawn Output	0.913	0.870
(2)	No. of Spawn Masses	0.920	0.760
(3)	Length of Sp. Period	0.910	0.831
(4)	Size of 1st Spawn Mass	0.787	0.813
(5)	Maximum Body Size	0.650	0.824

	A.proxima		*O.muricata*	
Factor No.	Eigenvalue	Cumulative % of Variance	Eigenvalue	Cumulative % of Variance
1	2.536	50.7	2.348	47.0
2	1.645	83.6	1.749	81.9
3	1.549	94.6	0.446	90.9
4	0.168	98.0	0.277	96.4
5	0.102	100.0	0.180	100.0

excluded from the analysis and hence only the first two Factors were considered (Johnston, 1980). These combined Factors accounted for 83.6% and 81.9% of the total variation present in the *A.proxima* and *O.muricata* correlation matrices respectively.

The proportion of the observed variation for each variable "explained" by the first two Factors (the communality) is given in Table 6.3. These data show that the majority of the variation in all the variables is represented by the first two Factors of both analyses. However, whilst variables (1)-(4) (total spawn output, number of spawn masses, length of spawning period and first spawn mass size respectively) are well represented in the *A.proxima* PCA, variable (5) (maximum body size), is not so well explained. (Only 65.0% of the observed variation in maximum body size is accounted for by the first two Factors).

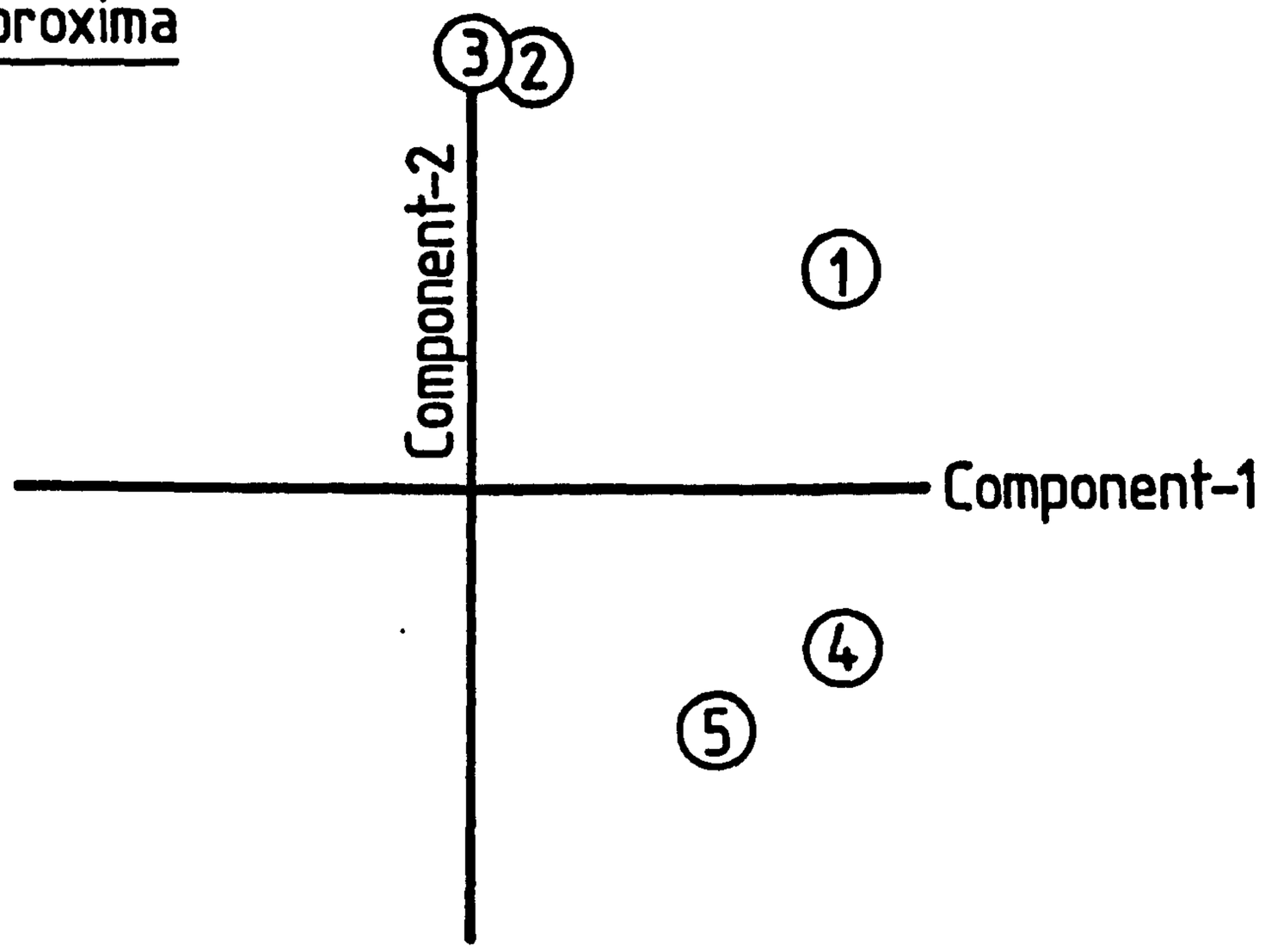
Since only two Factors were extracted in the PCA's, the results may be presented graphically. Figure 6.3 shows the variables in the R-mode PCA's for *A.proxima* and *O.muricata* plotted in two-dimensional Factor space. Figure 6.4 shows the individual cases in the Q-mode PCA's plotted in the same two-dimensional Factor space.

The associations between the variables represented in Table 6.2 can also be seen in Figure 6.3. For both *A.proxima* and *O.muricata*, the number of spawn masses produced (2) and the length of the spawning period (3) lie close together in the Factor space indicating strong positive correlation. The same also applies to maximum body size (5) and size of the first spawn mass (4), although this is less clear in *A.proxima* than in *O.muricata* (*cf.* Table 6.2). Further inter-specific differences are apparent in Figure 6.3. For *O.muricata*, total spawn output (1) and maximum body size (5) lie close together while for *A.proxima*, these variables are almost orthogonal - indicating virtually no correlation. Moreover, the maximum body size of *A.proxima* has a negative weighting on Factor 2 while the number of spawn masses and the length of the spawning period have strong positive weightings on the same Factor. This implies negative correlations between these variables (*cf.* Table 6.2).

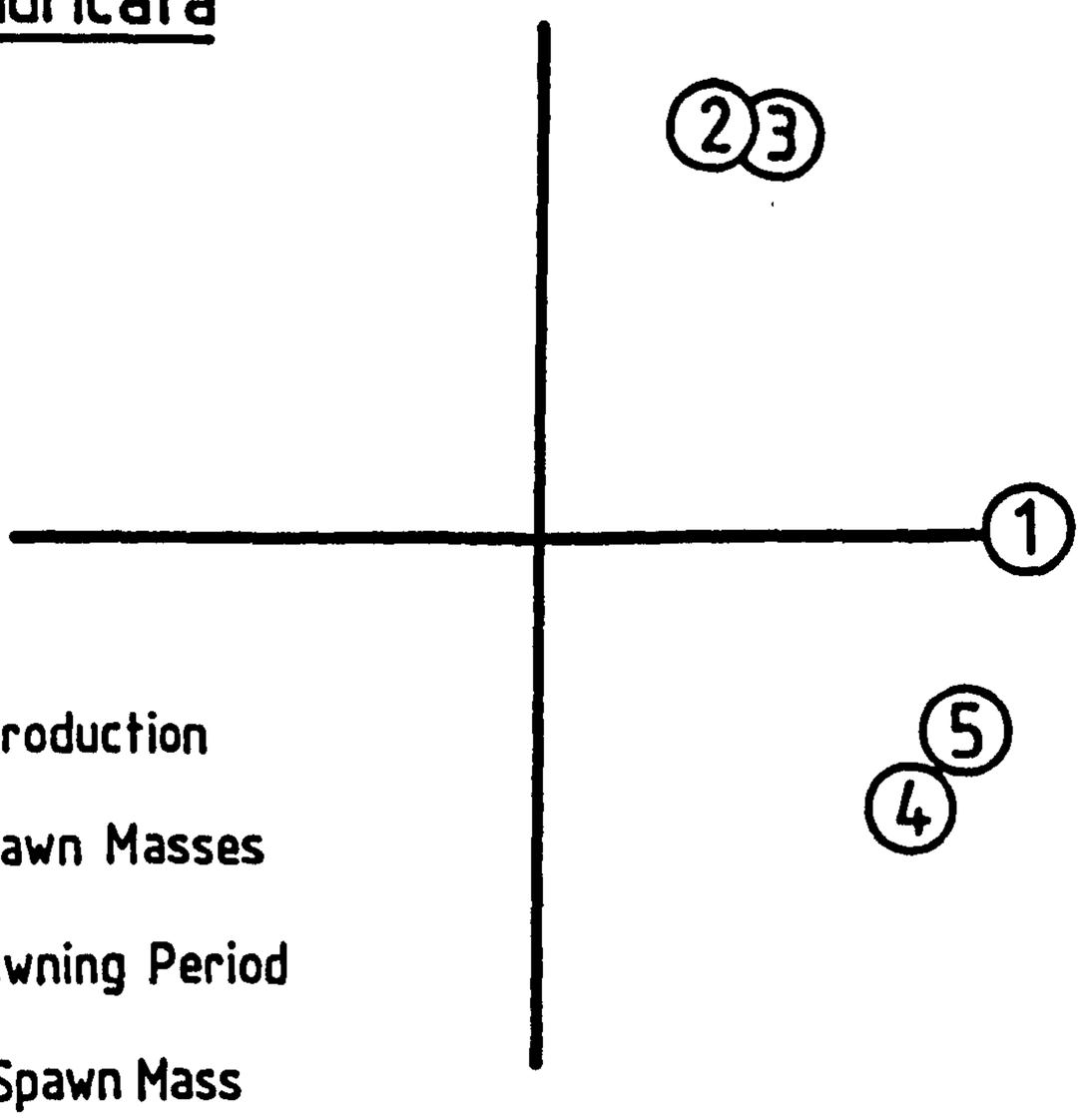
In addition to providing a convenient summary of the major trends in a correlation matrix, an R-mode PCA also permits inspection of the loadings of each original variable on

FIGURE 6.3 R - mode Principal Components Analysis for *Adalaria proxima* and *Onchidoris muricata* (first two components only)

A.proxima



O.muricata



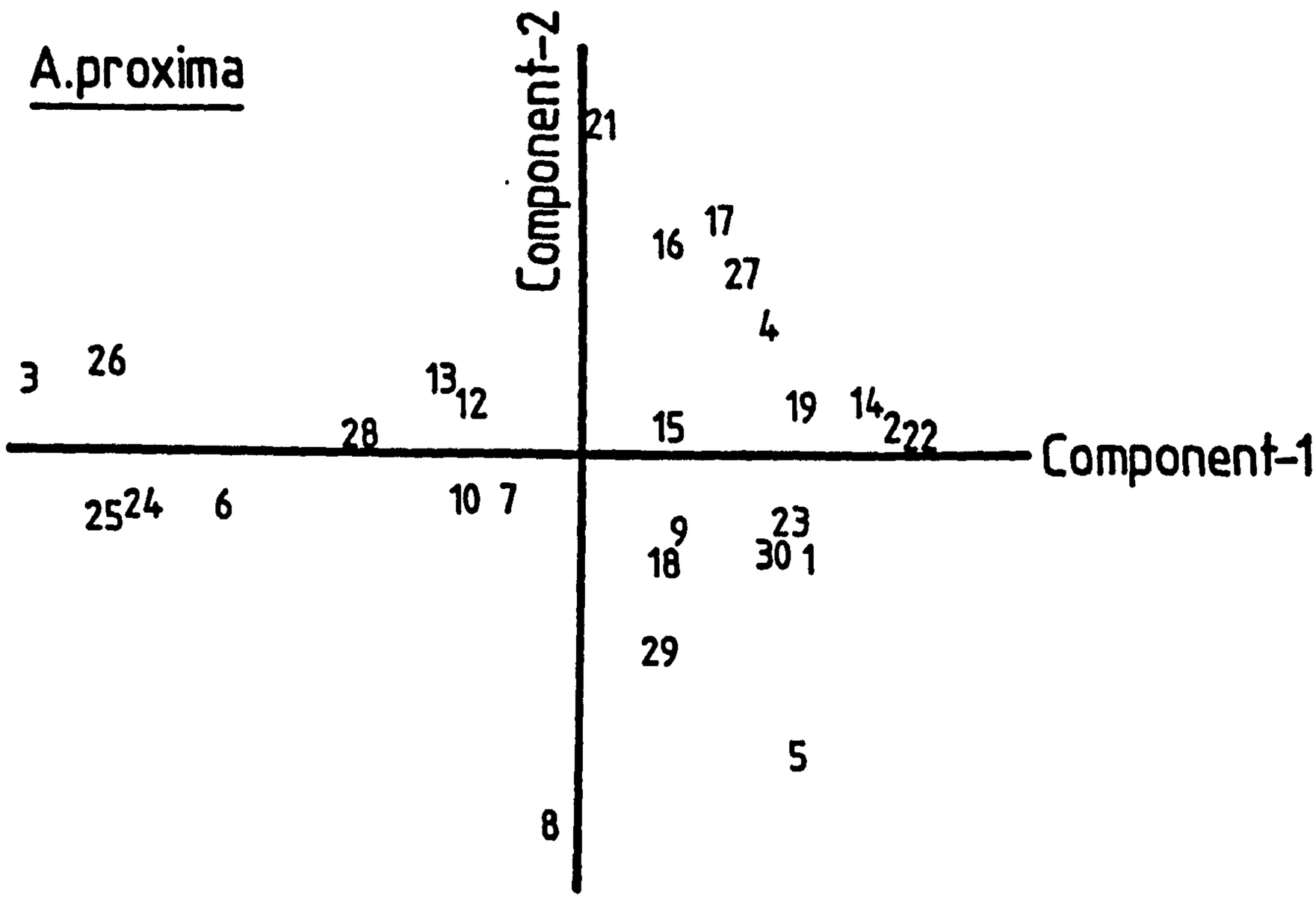
KEY:

1. Total Spawn Production
2. Number of Spawn Masses
3. Length of Spawning Period
4. Size of First Spawn Mass
5. Maximum Body Size

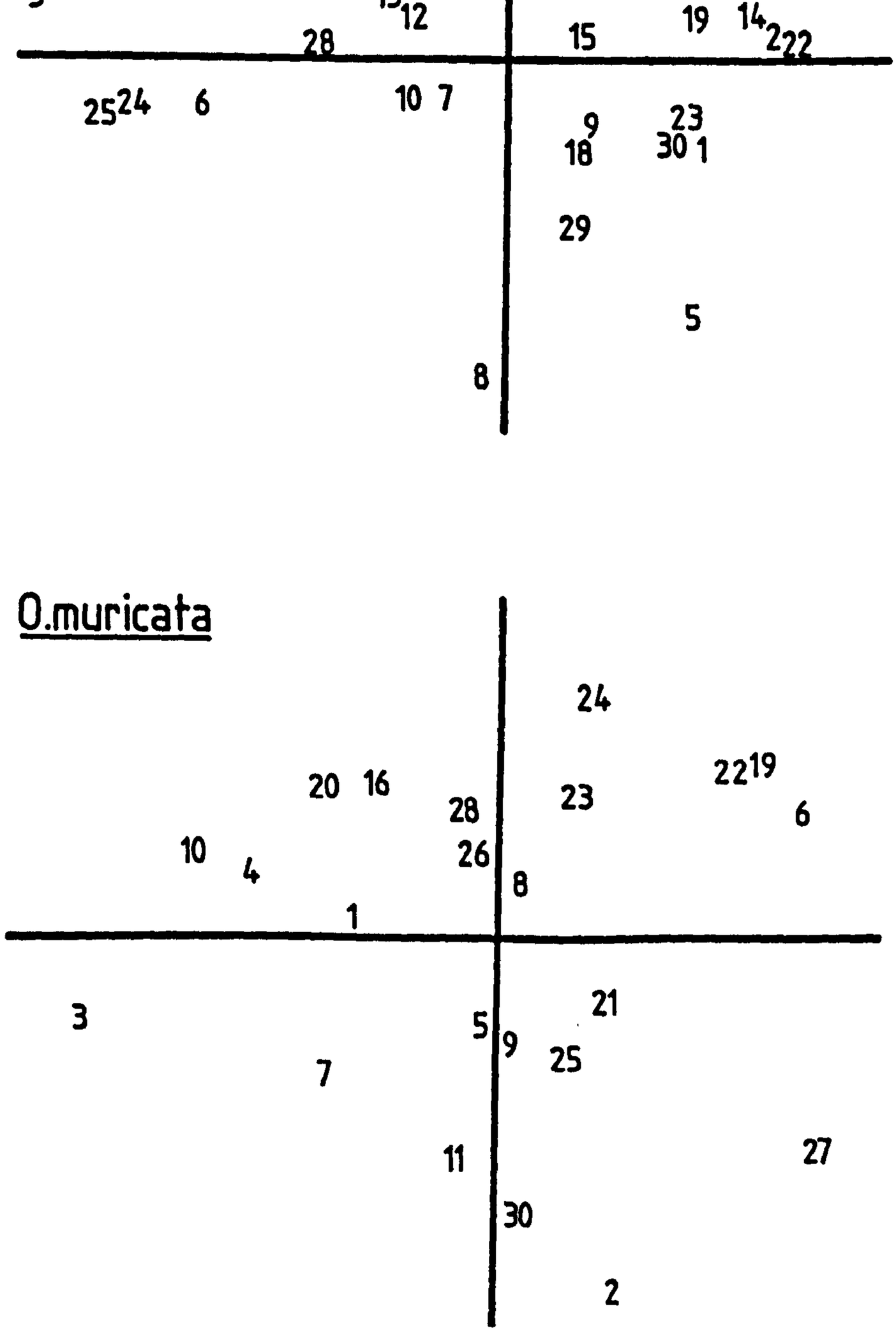
FIGURE 6.4 Q - mode Principal Components Analysis for *Adalaria proxima* and *Onchidoris muricata*

(Numbers identify individual nudibranchs)

A.proxima



O.muricata



the new Factors. This pattern can then be used when inspecting a Q-mode PCA in order to evaluate individual behaviour. By cross-referencing between Figures 6.3 and 6.4, several important features can be detected. For example, *A.proxima* individuals numbers 2, 4, 14, 16, 17 and 27 all lie very close to the position of total spawn output in two-dimensional Factor space, therefore suggesting that the most notable feature of these individuals was their production of relatively large quantities of spawn (*cf.* Figure 6.1). The precise position of these individuals in Factor space is, however, influenced by <u>all</u> the variables in the analysis. Thus, for example spawning periods for individuals 16 and 17 were long (68 and 75 days respectively) and more spawn masses were produced (10 and 12 respectively) than the overall means for the species (Table 6.1).

For both *A.proxima* and *O.muricata*, individuals which produced large quantities of spawn (*i.e.* lie in the same area of Factor space as variable (1) in Figure 6.3) showed close affinity for either variables (2) and (3) (no. of spawn masses laid and length of spawning period) or for variables (4) and (5) (size of first spawn mass and maximum body size), but no individuals display both of these tendencies. (*i.e.* no individuals had a strong positive weighting on <u>both</u> Factors). In a manner analogous to the above, individuals which produced relatively little spawn (and were therefore at the opposite side of Factor space to total spawn output in Figure 6.4), also showed associations with the above two variable groups. This pattern was however, less marked in *O.muricata* than in *A.proxima*.

6.4 <u>DISCUSSION</u>

<u>Spawning Characteristics</u>

Observations similar to those described here, have been reported by Todd (1979a) for both *Adalaria proxima* and *Onchidoris muricata*. The results presented here concur with those of Todd (1979a) in most respects.

Several aspects of the present results relate directly to the different larval strategies of *A.proxima* and *O.muricata*. Some of these concern overall energy partitioning and are

therefore discussed further in the following chapter. The remainder, which are of more immediate interest will be considered here.

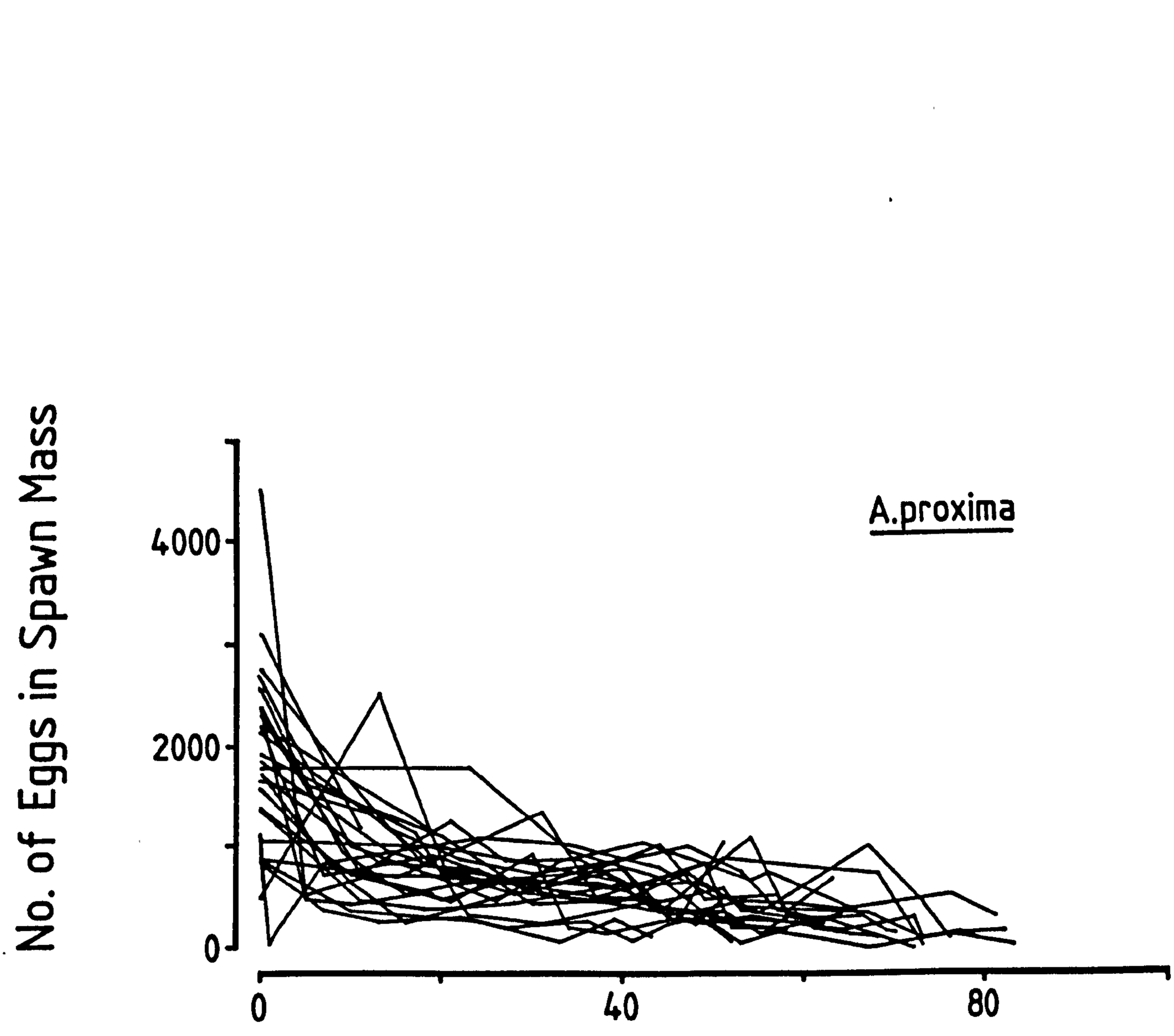
Todd (1979a) cites data for the energy invested in each (lecithotrophic) *A.proxima* embryo and each (planktotrophic) *O.muricata* embryo. In summary, he found that the energy required to produce an *A.proxima* embryo plus attendant gel, was 20 times that for an *O.muricata* embryo plus gel. He ascribed this difference to the greater size of *A.proxima* ova and also to the greater quantity of gel matrix produced (per ovum) in *A.proxima* spawn. More recent evidence exists to suggest that the energetic 'cost' of producing sufficient gel matrix to support and protect only one *A.proxima* embryo is considerable (Todd, unpubl. results). Indeed, this same characteristic also applies (on a reduced scale) to *O.muricata* spawn masses. The result is that the energy required to produce each additional embryo in a spawn mass, decreases with increasing spawn mass size (Todd, 1986a). This relationship is markedly asymptotic in both species, such that little or no increase in "efficiency" (embryos produced per unit energy invested) can be obtained above spawn mass sizes of ~ 630 (for *A.proxima*) and ~ 2300 (for *O.muricata*). (It is at, or above these sizes that the 20 times difference noted by Todd (1979a) applies). It is interesting to note, therefore, that the size of the average spawn mass of the *A.proxima* individuals studied here is very similar to the above value (675 ova), while that of the *O.muricata* individuals (~3300 ova) is considerably in excess of its respective value.

Clearly, the average spawn mass size is a necessarily imprecise measure (see Results, Section 6.3) if only because it includes the larger than usual first spawn mass (Table 6.1). In fact, although there is much individual variation, the size of spawn masses produced by both species generally declined throughout the spawning period. This decline was especially noticeable toward the end of the spawning period, but was the more pronounced in *A.proxima*. Figure 6.5 illustrates this point for the whole *A.proxima* and *O.muricata* data sets.

This pattern of spawning raises several questions relating to the possible adaptive advantage of such behaviour. Before these questions can be considered in detail however, a

FIGURE 6.5 Variation in spawn mass size with time for *Adalaria proxima* and *Onchidoris muricata*

(raw data for all individuals)


No. of Eggs in Spawn Mass
4000
2000
0
A.proxima
0
40
80
16000
8000
0
O.muricata
0
40
80
120
Days

discussion of the weight loss observed in all individuals of both species is required.

Degrowth

Several authors have noted either declining weight or length of various nudibranch species during the spawning period. For example, Clark (1975), Todd (1979a,b) and Eyster & Stancyk (1981) all noted body size reductions throughout the spawning period. By contrast, Hall (1983), Smith & Sebens (1983) and Todd & Havenhand (1983) all reported declining weight for only the latter part of the spawning period in the nudibranchs they studied.

Russell-Hunter *et al.* (1984) have emphasised the difference between catabolism of energy reserves and catabolism of structural and functional proteins, classifying only the latter as "degrowth". Russell-Hunter *et al.* (1984) further stress the ecological and evolutionary importance of degrowth, both with respect to perhaps surviving periods of temporary starvation and to maximising reproductive output. While such an ability is clearly advantageous to iteroparous organisms such as the *Lymnaea* species studies by Russell-Hunter *et al.* (1984), it is surely of even greater significance to semelparous organisms which cannot "afford" reproductive failure in their one reproductive period.

Relatively few reports of either starvation degrowth or reproductive degrowth in other mollusc species exist. O'Dor & Wells (1978) describe the hormonal control of degrowth in *Octopus vulgaris*, while Bayne & Worrall (1980) report both starvation and reproductive degrowth in field populations of the bivalve *Mytilus edulis*. With reference to nudibranchs, Clark (1975) has suggested that the observed size reductions of various nudibranch species during the spawning period may be a result of the catabolism of the somatic tissue to provide additional gamete material. Indeed, Todd (1978) histologically observed the total catabolism of the digestive gland and (subsequently) the ovotestis during the spawning period of *Onchidoris muricata* in the field. Thompson (1958) also noted a similar degeneration of the viscera and body wall in post-spawning *Adalaria proxima*. Thus it is evident that the tissue losses observed during the present study constitute true reproductive degrowth *sensu* Russell-Hunter *et al.* (1984).

The degrowth rates observed here compare favourably with other published estimates of reproductive weight loss in nudibranchs. For example, Smith & Sebens (1983) observed *Onchidoris aspera* to lose weight at the rate of - 0.13 mg dwt.d^{-1}. Applying the energetic conversions for *O.muricata* outlined in Chapter 4 (Section 4.3), this value becomes - 0.83 J.d^{-1}, which lies intermediate to the mean values obtained here for *A.proxima* (- 1.66 J.d^{-1}) and *O.muricata* (- 0.25 J.d^{-1}). Todd (1979a) does not provide figures for the degrowth of *O.muricata* but presents data for *A.proxima* roughly equivalent to - 0.3 mg dwt.d^{-1} (= - 2.4 J.d^{-1}). This value is significantly different from the mean value obtained in the present study ($p < 0.001$), but nonetheless lies within the range of the present observations (- 0.78 J.d^{-1} to - 4.24 J.d^{-1}). Despite the variability of degrowth rate observed for both species, the overall difference between the rapid degrowth rate of *A.proxima* and the much slower degrowth of *O.muricata* is significant (Mann-Whitney, $p < 0.001$). However, because the spawning period of *A.proxima* is shorter than that of *O.muricata* (Table 6.1), the overall proportion of the maximum body weight which is lost by degrowth is roughly equal in *A.proxima* (mean ± s.e. = 32.8 ± 1.6%) and *O.muricata* (32.5 ± 2.3%). Indeed, there is no significant difference between these data, (Mann-Whitney, $p = 0.828$). This suggests that degrowth imposes a constraint on these organisms whereby vital function is lost after more than ~ 33% of the maximum body weight has been catabolised. However, it has already been shown (Chapter 4; Todd 1977) that approximately 45 - 50% of the body weight of both *A.proxima* and *O.muricata* is accounted for by inorganic ash, the majority of which forms the calcareous spicules. It is unlikely that degrowth would markedly reduce the quantity of this ash in any given individual and therefore the observed degrowth losses may actually constitute as much as ~ 73% and 67% of the organic matter present in *A.proxima* and *O.muricata* respectively. Clearly, this has important consequences on the interpretation of degrowth within the wider context of spawning behaviour.

Adaptive Aspects of Production Patterns

Degrowth may cause a progressive decrease in the vitality of any organism, hastening senescence and death (Calow, 1979). In the cephalopod mollusc *Octopus vulgaris*, post-reproductive death results from an inability to surpress the hormonal mechanism which

governs degrowth throughout the reproductive period, (O'Dor & Wells, 1978). The existence of a similar, or equivalent mechanism in nudibranchs has not been investigated.

Since the onset of spawning in both *Adalaria proxima* and *Onchidoris muricata* is characterised by the onset of degrowth, it follows that current reproduction may incur some form of 'cost' which is met (at least temporarily) by degrowth. Degrowth is, in turn, necessarily associated with a finite duration of the spawning period. Degrowth rate of *A.proxima* was highly correlated with the length of the spawning period ($r = 0.669$, $p < 0.001$), such that rapid degrowth was associated with a short spawning period. The data for *O.muricata* showed greater variability and therefore the equivalent correlation for this species was not significant. However this was still markedly positive ($r = 0.321$, $p > 0.05$).

In this context, a large first spawn mass may be seen as an adaptation to maximise reproductive output at a time of declining life-expectancy. Classical life-history theory predicts sequentially greater investment in reproduction as life expectancy (and residual reproductive value) declines (Fisher, 1930; Williams, 1966; Pianka & Parker, 1975). Although this theory was originally developed for iteroparous species, no *a priori* reason exists to preclude its application to semelparous species which have an extended reproductive period. Nonetheless, the theory is clearly not applicable in this instance. The data obtained here cannot resolve this discrepancy. However, an adaptationist scenario can be proposed to explain the observed behaviour; let us assume that the onset of reproduction incurs a substantial reduction in life-expectancy which is largely (although not entirely) independent of initial spawn production and degrowth rates. Therefore, life-expectancy may decline quite markedly at the onset of spawning, and thereafter be directly related to the rapidity of degrowth, until the latter begins to affect vital function (after which time death rapidly ensues).

The less pronounced spawning behaviour of *O.muricata* (smaller first spawn mass, less rapid degrowth), may have an equally reduced effect on life-expectancy both initially and throughout the spawning period. Thus, within the context of the above hypothesis, the relatively small first spawn mass and lack of significant correlation between degrowth rate

and spawning period, imply that life-expectancy of *O.muricata* is not so greatly affected by reproduction as it is for *A.proxima*.

That *A.proxima* individuals do not produce their entire reproductive output in this one, first spawn mass may be interpreted as 'bet-hedging'. Certainly, the evolutionary pressure to reduce the potential for reproductive failure and therefore produce more than one spawn mass, must be strong. Such behaviour would undoubtedly go some way to overcoming the inherent variability of embryonic and larval survivorship in the field. However, other possibilities exist; for example, the observed behaviour may be a result of physical or physiological limitation (*i.e.* the animal is simply not capable of producing its entire reproductive allocation in one spawn mass). The sample evidence in Figure 6.5 suggests that a trade-off between reduced life-expectancy and the maximisation of the number of spawn masses produced, has resulted in a substantial energetic investment in the first spawn mass followed by the production of spawn masses which are as small as possible without significantly compromising their energetic efficiency. This behaviour ceases with the onset of senescence, at which time spawn mass size decreases and spawning frequency increases. Given the relatively short spawning period observed in the laboratory, and the probability that extrinsic mortality factors may curtail this still further in the field (Todd, 1979a), the observed behaviour may constitute the only consistently successful reproductive behaviour for *A.proxima*.

It has already been postulated that the relatively slow degrowth rate of *O.muricata* results in accordingly greater life-expectancy. Under such circumstances, therefore, the maximisation of successful reproduction is perhaps best achieved by moderate investment in several temporally and spatially separate spawn masses. (Again, Figure 6.5 indicates that this is achieved with energetically efficient spawn masses for the majority of the spawning period). This behaviour may be especially relevant with respect to the greater variability of offspring survivorship which is generally accepted to be an inevitable consequence of having long-term planktonic larvae, (see, for example, Palmer & Strathmann, 1981; Strathmann, 1985). The probability nonetheless remains that life-expectancy in the field will be shorter than in relatively protected conditions of the laboratory. It is likely that the observed,

approximately sigmoid pattern of total production in *O.muricata* will be equally abbreviated in the field. This would result in the loss of the final plateau in total production, leaving a pattern similar to that seen for *O.muricata* individual number 9 (Figure 6.2) for example.

Comparative Aspects of Production Patterns

Hall (1983) and Hall & Todd (1986) report considerable variation of individual growth and spawn production in the aeolid nudibranch *Aeolidia papillosa*. The between individual variation they observed was considerably greater than that observed here, and unlike Hall (1983) and Hall & Todd (1986), none of the individuals studied here failed to spawn, (although several individuals did produce only small quantities of spawn). The data of Hall & Todd (1986) are indeed so variable as to make any generalisations regarding the linearity or curvilinearity of production impossible. However, Todd (1979a) reports sigmoid cumulative spawn production for pairs of *Adalaria proxima* which contrasts markedly with the generally linear pattern observed here. He further observed curvilinear cumulative spawn production in *Onchidoris muricata* which, although highly variable, was of the same general pattern to that observed here. A similar pattern of cumulative spawn production was noted for *Onchidoris bilamellata*, by Todd (1979a).

Thus, in summary, it would appear that previous studies of production patterns in nudibranchs have noted more inter- and intra-specific variation than has been observed here. One feature which has shown little variation in almost all of these (and other) studies of invertebrate reproduction however, is the allometry of spawn production. Even in the highly variable data set of Hall & Todd (1986), the correlation between maximum body size and reproductive output was highly significant ($r = 0.71$, $p < 0.001$). It is perhaps surprising therefore, that no such relationship was observed for the *A.proxima* individuals in the present study ($r = 0.105$, $p >> 0.05$). A similar result was obtained by Todd (1979a) who observed a non-significant correlation between maximum body size and total spawn output for *A.proxima*, but a very significant correlation for *O.muricata*. This was further confirmed for both "pairs" and "isolated" *A.proxima* and *O.muricata* individuals by Todd & Havenhand (1983). This phenomenon has also been demonstrated in the PCA for both species. However, the PCA does suggest that although the general case may be that

maximum body size of *A.proxima* has little or no association with total spawn output, some individuals do show both high spawn output and large body size (Figures 6.3, 6.4). Indeed, it has been shown (see Results, Section 6.3) that the general case for both species is that individuals which show a high spawn output do so either in association with a larger body size and a larger first spawn mass, or with a longer spawning period and a higher number of spawn masses than the overall means for the species. The latter strategy tends to characterise relatively fecund *O.muricata* while either strategy was apparent for fecund *A.proxima*.

Although the above analyses are necessarily generalised summaries of large data sets, the lack of allometry of spawn production in *A.proxima*, (especially when compared to *O.muricata*) is readily apparent. The resulting assumption must therefore be that fecundity of *A.proxima* is largely determined by factors other than maximum body size. The potential importance of the major components of the energy budget will be considered within this context in the following chapter.

CHAPTER 7

ENERGY BUDGET ANALYSES

7.1 THE ENERGY BUDGETS

The energy budgets for individual juvenile (pre-spawning) and adult (spawning) *Adalaria proxima* and *Onchidoris muricata* are summarised in Table 7.1. The data for juveniles of both species were obtained over the same four-week period in September / October 1983. Comparable data for spawning adults were collected from mid-February 1984 until post-reproductive death in May 1984 (*O.muricata*) and from April 1984 to late June 1984 (*A.proxima*). Because of differences in the timing of the onset of reproduction between the two species, these periods include the beginning of the ovipositional period in *A.proxima* but not that of *O.muricata* (which had commenced spawning prior to mid-February). Since the first spawn mass produced by individuals of both species is significantly larger than subsequent spawn masses (see Chapter 6, Table 6.1), the inclusion of such data in the *A.proxima* data set could introduce systematic bias. Therefore, the data summarised in Table 7.1 are for the first five weeks after the onset of reproduction, (or part thereof in cases where the total reproductive period was less than five weeks). The data in Table 7.1 are also presented graphically in Figure 7.1, which includes a representation of the entire adult data set for each species. The solid portions of the histograms in Figure 7.1 represent mean daily allocation to growth (P_g), the hatched area represents mean daily allocation to respiration (R) and the open area represents mean daily allocation to spawn production (P_r) where appropriate. The bars arising from the top margin of each component block are standard errors with the exception of the bottom-most error bars in the "ADULT" data which are standard errors for the degrowth rate. Since degrowth is a net energy acquisition rather than loss, the mean daily degrowth rate is represented by the displacement of each histogram below the baseline. Total daily energy expenditure is therefore represented by the height of each histogram from the baseline. The bracket to the top-left of each histogram represents the mean total daily expenditure plus or minus one standard error.

TABLE 7.1 Summary Energy Budget Data for *Adalaria proxima* and *Onchidoris muricata*

Data are means ± one std. error for 7 individuals of each species.

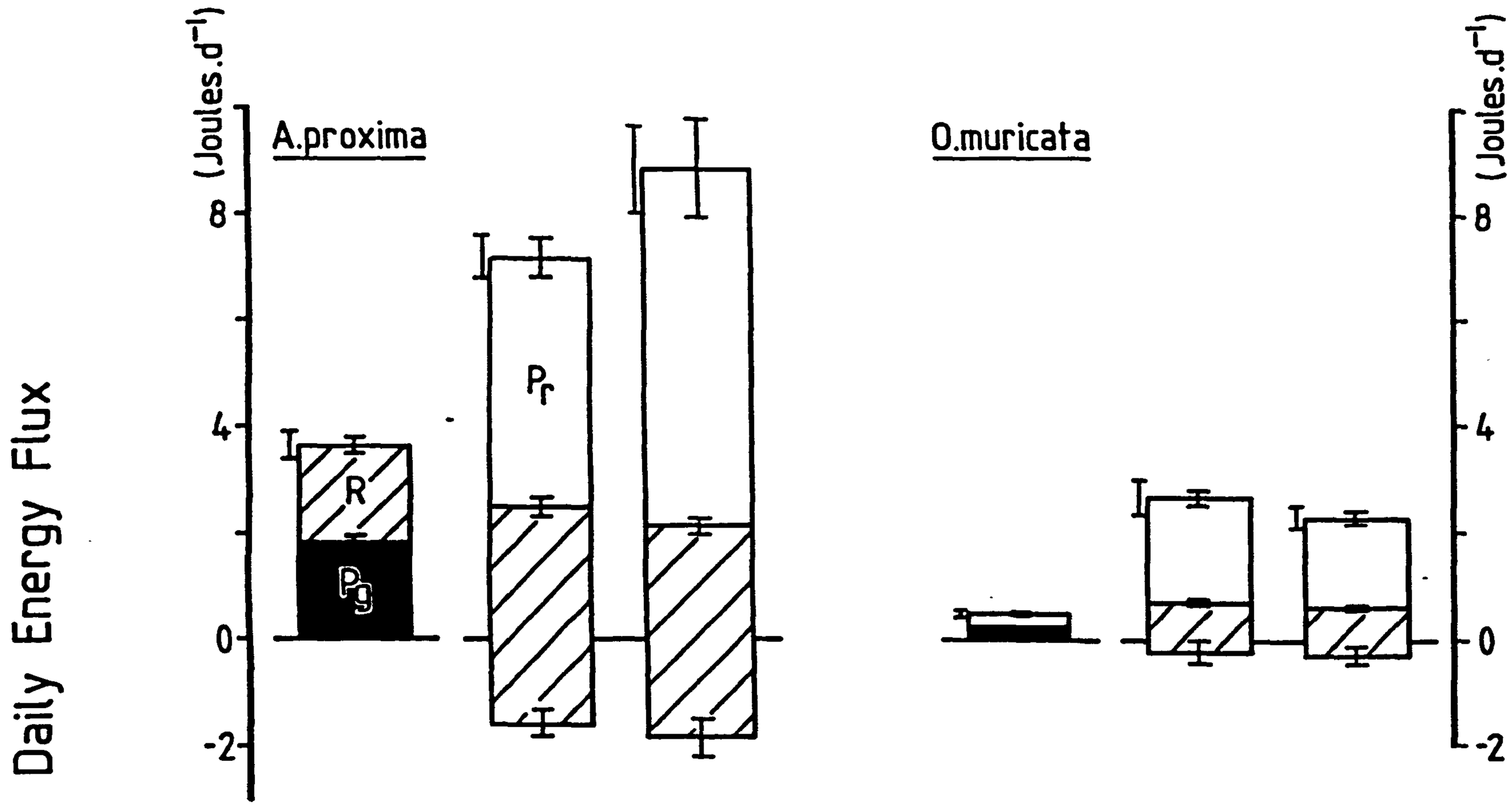
Units are $J.d^{-1}$ unless stated otherwise

	A.proxima		*O.muricata*	
	Juv.	Adult	Juv.	Adult
Mean Body Wt. (mg)	12.5 ± 1.0	33.1 ± 1.1	2.21 ± 0.13	9.71 ± 0.29
Mean Body Wt. (J)	104	275	14.1	62.0
R	1.83 ± 0.15	4.10 ± 0.18	0.227 ± 0.017	0.992 ± 0.053
P_g	1.81 ± 0.13	-1.63 ± 0.28	0.272 ± 0.041	-0.236 ± 0.220
P_r	-	4.70 ± 0.34	-	1.96 ± 0.18
C^*	14.4	18.5	2.52	8.27

* estimated from equations given in Chapter 3; Section 3.3.

FIGURE 7.1 Overall mean daily Energy Budgets for Juvenile (pre-spawning) and Adult (spawning) *Adalaria proxima* and *Onchidoris muricata*

(see text for explanation)



	Juv.	Adult	Adult (all)	Juv.	Adult	Adult (all)
Mean size (mg Dwt.)	12.5	33.3	36.0	2.21	9.71	9.20
" " (Joules)	104	275	298	14.1	62.0	58.8

Several aspects of Figure 7.1 are worthy of note. Perhaps the most striking of these is the similarity of energy partitioning in juveniles of *A.proxima* and *O.muricata*. Despite a considerable difference in total daily energy expenditure between the two species, both *A.proxima* and *O.muricata* utilise approximately half of this total flux in respiration. The consistency of this pattern is reflected by the very small error terms for both the individual components and the overall daily totals (Table 7.1 and Figure 7.1). This pattern does not continue into the reproductive period however, largely because of differences in the observed degrowth rates of the two species. As for the "juvenile" data, the low degree of variation in daily metabolic energy requirement is reflected in the small error-term for this component, and of the three components measured here (P_g, P_r and R), respiration is the least variable.

It has already been noted that degrowth in *A.proxima* is considerably more rapid than that in *O.muricata* (Chapter 6). It can be calculated from the data in Table 7.1 that the energy supplied by degrowth contributes almost 23% of the total daily energy flux in *A.proxima*, but only 9% in *O.muricata*. Moreover, spawn production accounted for only 53% of the total daily energy expenditure in adult *A.proxima* while the equivalent value for adult *O.muricata* was 66%. In the (hypothetical) absence of degrowth, and assuming that metabolic energy requirements remained constant, the energy available for reproduction in *A.proxima* would fall from 53% to 43% of total daily expenditure (*i.e.* from 4.1 $J.d^{-1}$ to 3.1 $J.d^{-1}$). For *O.muricata*, the energy available for reproduction would fall from 66% to 64% of total daily expenditure (1.96 $J.d^{-1}$ to 1.7 $J.d^{-1}$). Clearly therefore, on a daily basis, the energy acquired from degrowth is of considerably greater importance in maximising the reproductive output of *A.proxima* individuals than it is in *O.muricata* individuals.

The influence of the first spawning data when included in the *A.proxima* data set can be seen in Figure 7.1 by comparing the "ADULT" and the "ADULT (ALL)" histograms. As one would expect, inclusion of the first spawn data increases the overall mean daily energy allocation to spawn, and also increases the mean daily degrowth rate. Similarly, for *O.muricata*, the "ADULT (ALL)" histogram for the entire study period differs from the five-week "ADULT" histogram. Although this difference is small, it is in the opposite

direction to that between the *A.proxima* histograms, supporting the prior expectation of systematic bias. The cause of these differences can be seen by examining the individual energy budgets for each animal over the entire study period, (Figures 7.2 and 7.3).

Both Figures 7.2 and 7.3 share the same format as Figure 7.1 with the exception that the ordinate now represents time, and the scale on the abscissa is expanded two-fold in Figure 7.3.

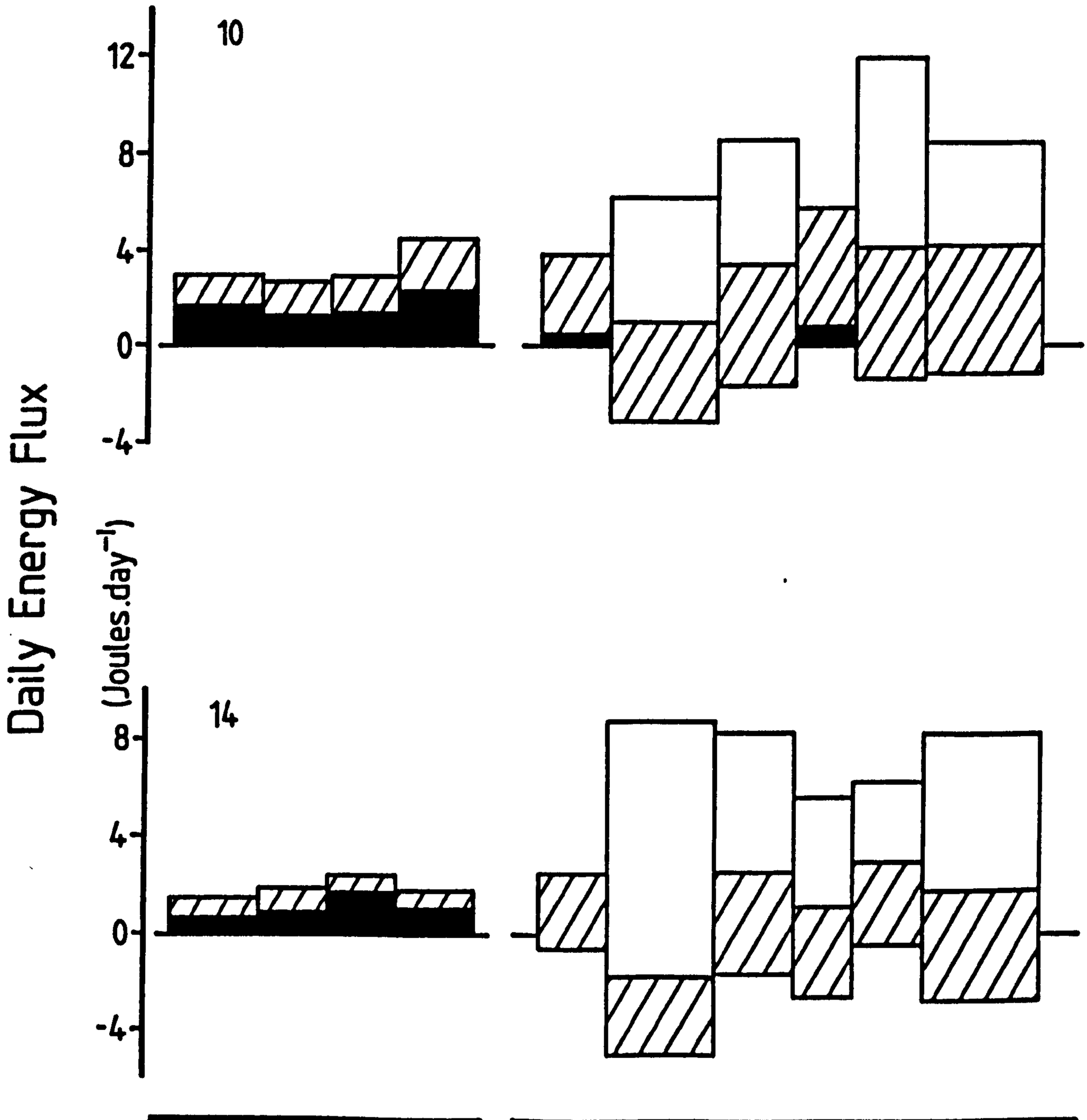
The marked contrast between data from the period in which the first spawn mass was produced, and later (spawning) periods can be seen in almost all the *A.proxima* individuals studies (Figure 7.2). In a few instances (such as *O.muricata* individual number 7), the pronounced variation seen in the (de)growth rate may have been caused by experimental error during underwater weighing (an unseen air bubble adhering to the individual in the weighing chamber, for example). However, such errors are invariably large, and therefore the majority of the variation in (de)growth rate seen in Figures 7.2 and 7.3 is almost certainly a real result.

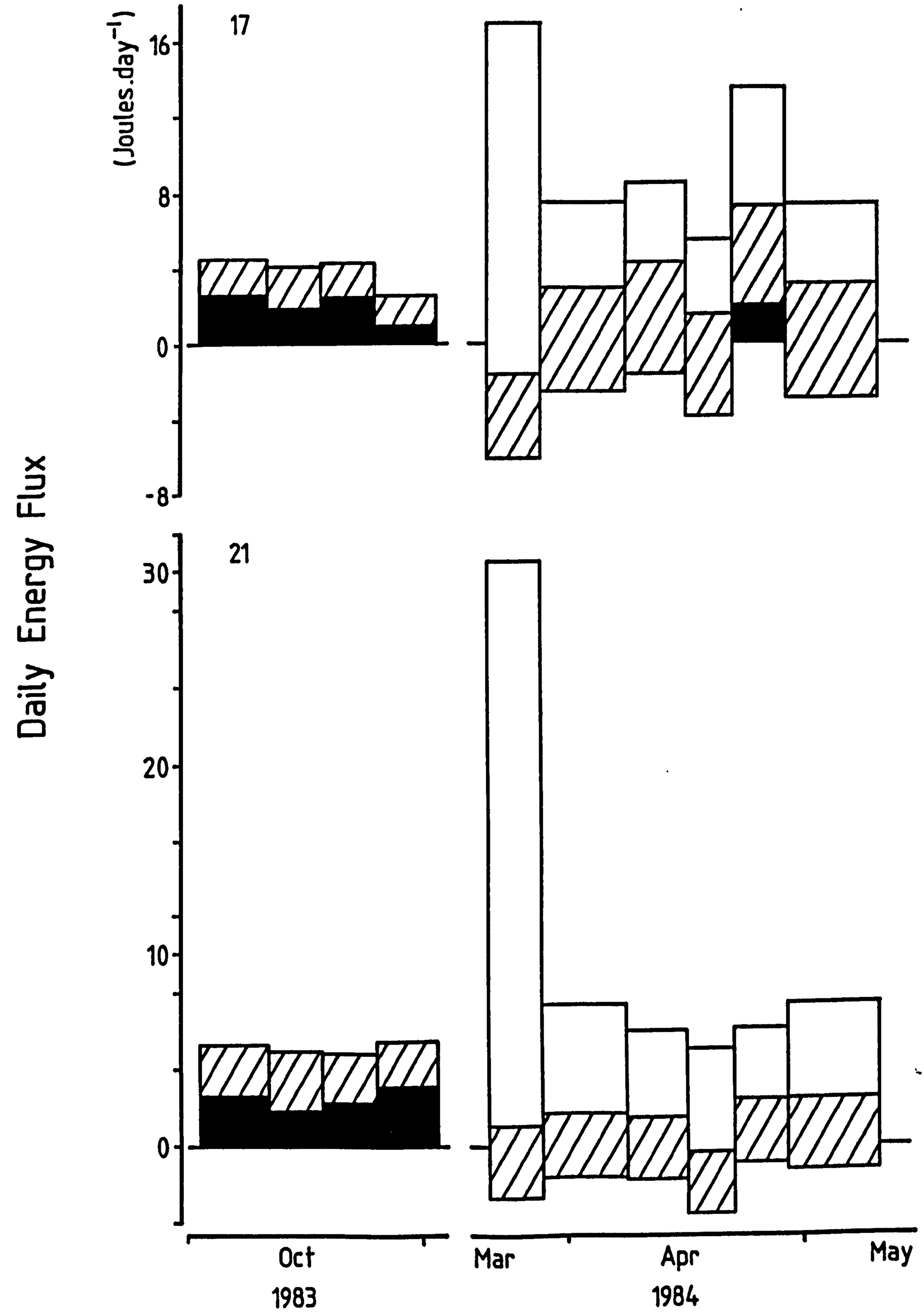
It is apparent from both Figures 7.2 and 7.3 that metabolic energy demands remained relatively constant for any given juvenile or spawning adult. This result reflects those obtained by analysis of covariance on the allometry of respiration (Chapter 5, Section 5.3). Thus, observed variation in total daily energy expenditure was largely caused by variation in (de)growth and spawn production rates. The extent to which such variation was caused, or mediated, by differential energy fluxes between the individual components is important in any analysis of energy partitioning. Potential interactions between the budget components of individuals of each species were therefore investigated by multivariate techniques. The results of these analysis are presented and discussed later in this chapter.

In order to further investigate the present results, the generalised model of Sebens (1979) was tested against these data. Sebens' model attempts to predict the optimum adult size of an organism from allometric equations describing the individual energy budget components for that organism. Essentially, the predicted optimal size is that at which the

FIGURE 7.2 Energy Budgets for seven individual *Adalaria proxima* over the pre-spawning and spawning periods

(See text for explanation)


10
12
8
4
0
-4
14
8
4
0
-4
Daily Energy Flux
(Joules.day⁻¹)
Oct
1983
Mar
Apr
May
1984


Daily Energy Flux (Joules.day^{-1})
17
16
8
0
-8
21
30
20
10
0
Oct
1983
Mar
Apr
May
1984

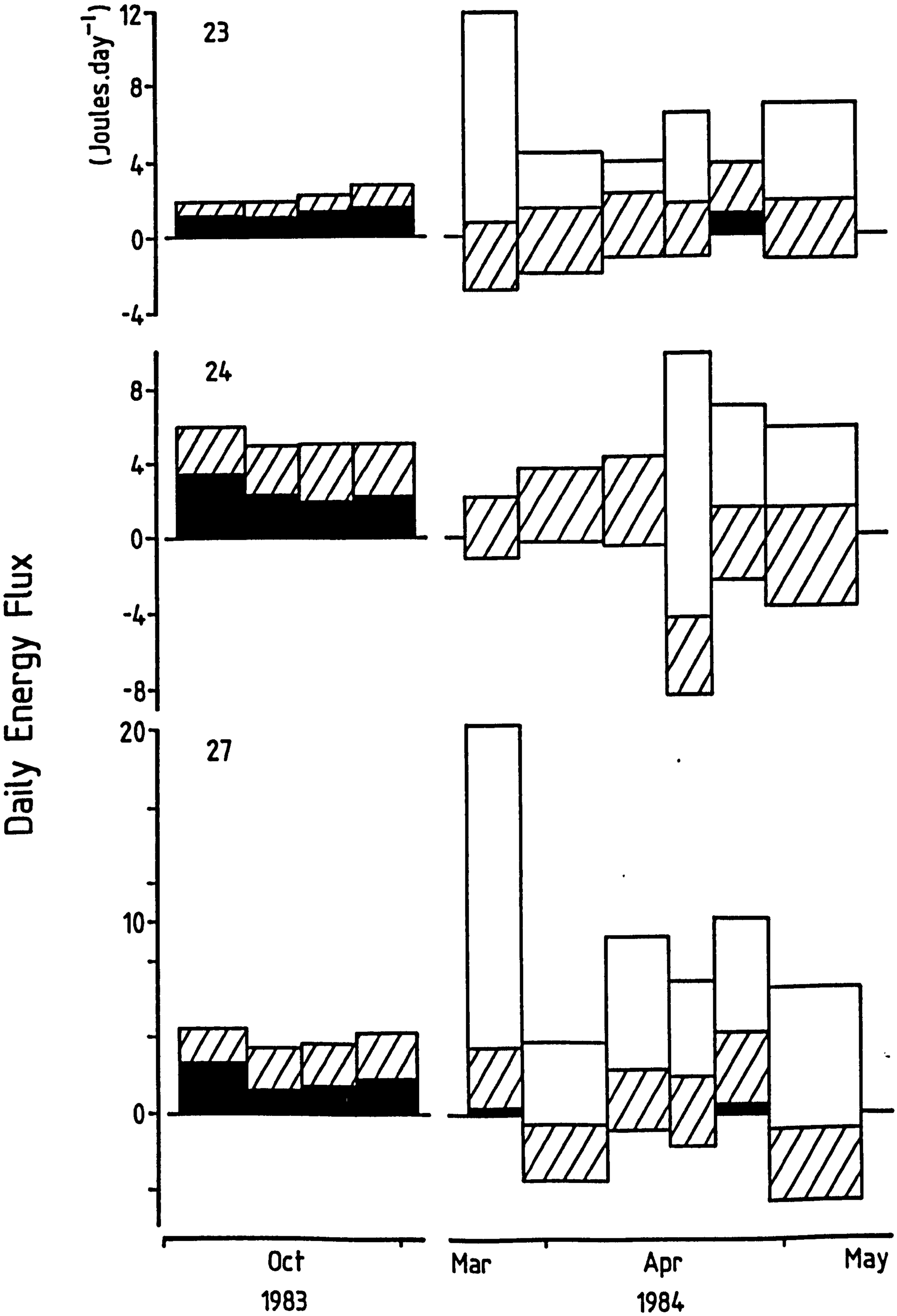
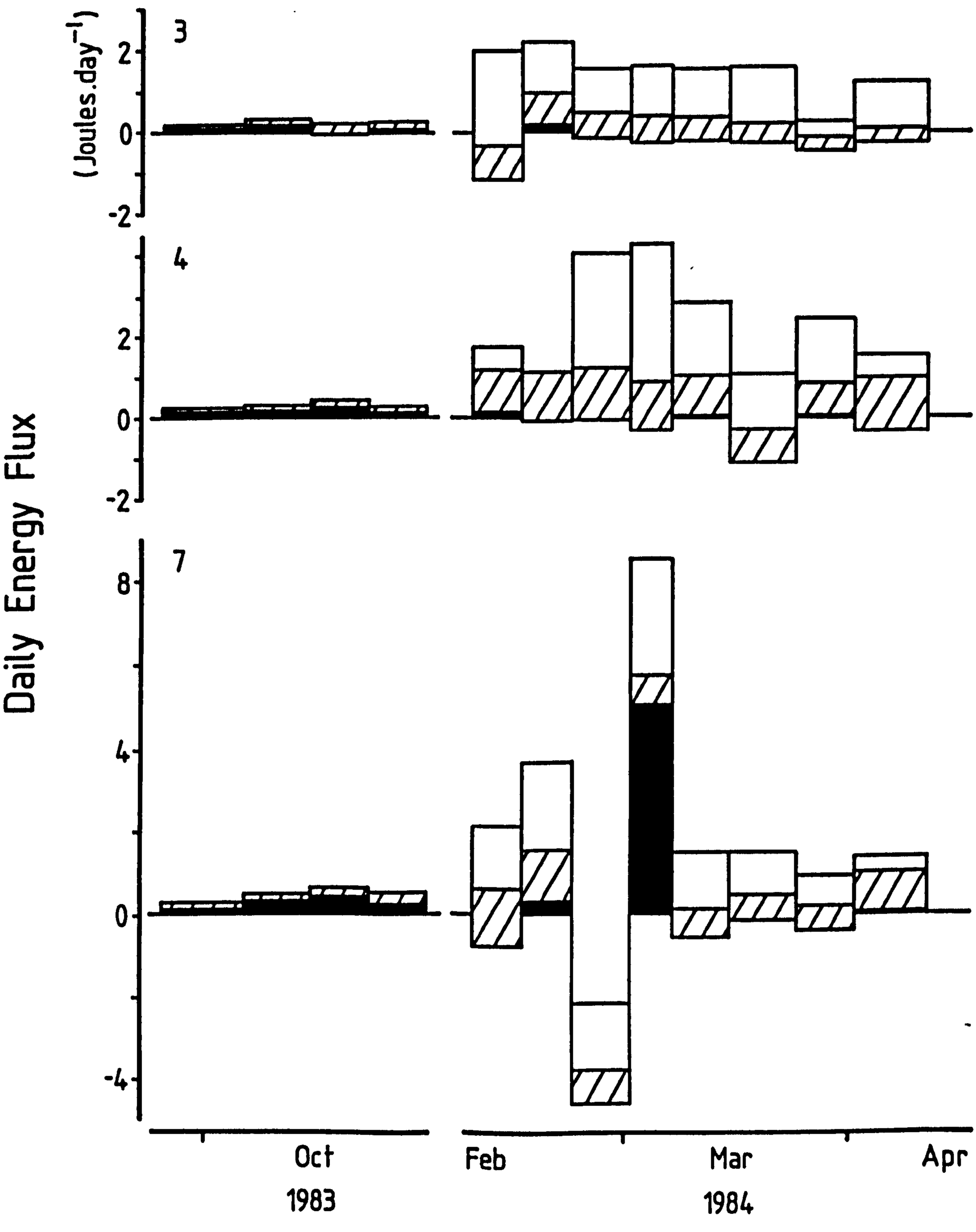
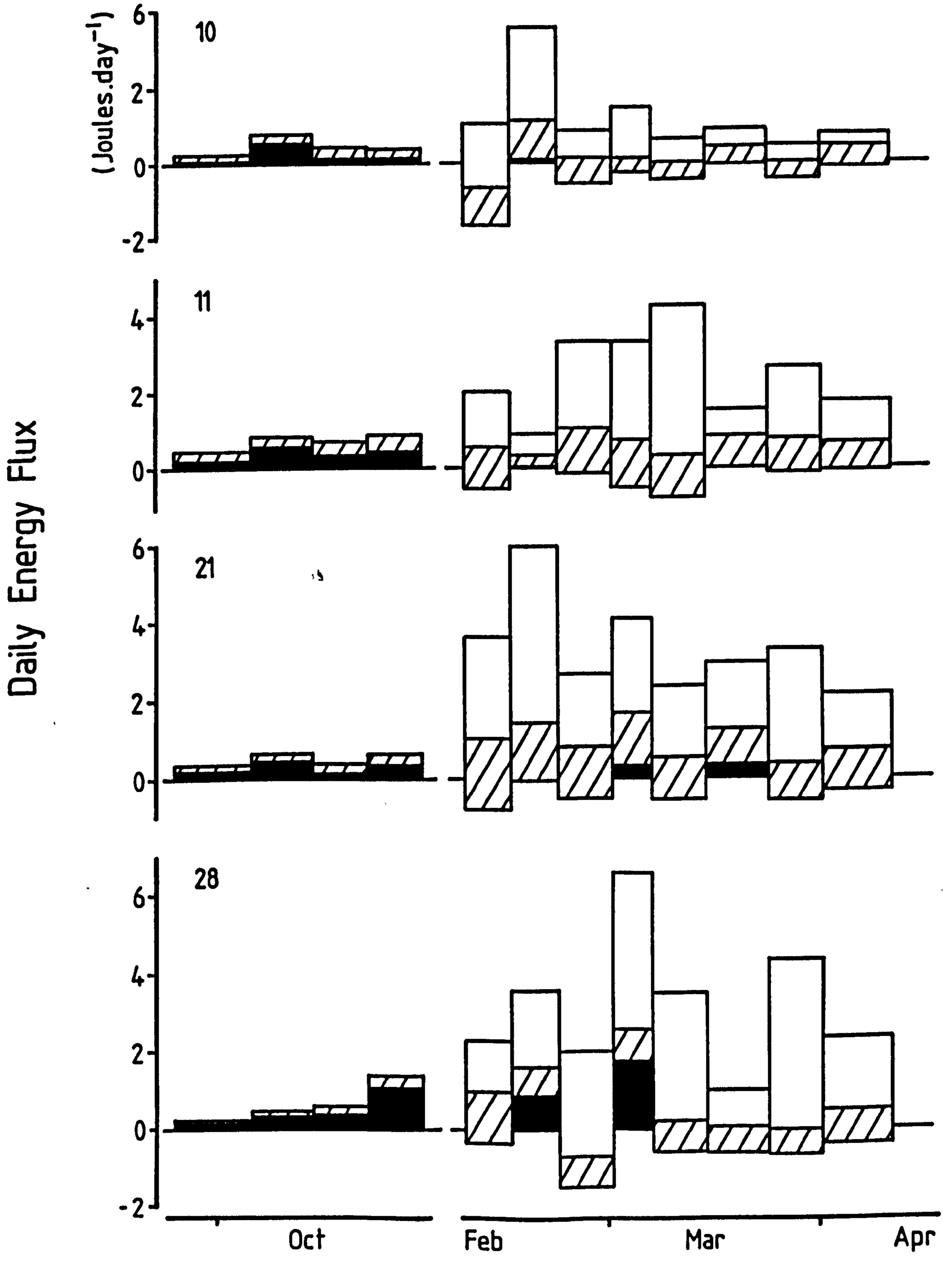

Daily Energy Flux (Joules.day^{-1})
23
24
27
12
8
4
0
-4
8
4
0
-4
-8
20
10
0
Oct
1983
Mar
Apr
May
1984

FIGURE 7.3 Energy Budgets for seven individual *Onchidoris muricata* over the pre-spawning and spawning periods

(See text for explanation)


Daily Energy Flux (Joules.day^{-1})
3
2
0
-2
4
2
0
-2
7
8
4
0
-4
Oct
1983
Feb
Mar
Apr
1984


Daily Energy Flux (Joules.day⁻¹)
10
11
21
28
6
4
2
0
-2
Oct
Feb
Mar
Apr

difference between "intake" and "cost" (the "scope for growth" *sensu* Warren & Davis, 1967) is greatest. This is, therefore, the size at which either growth (P_g) or reproduction (P_r) may be maximised.

Due to the nature of the allometric relationships for the *A.proxima* energy budget components, no size optimum exists since the function "intake minus cost" increases with increasing body size. Thus a strong selective pressure to increase body size should pertain for *A.proxima*. (However, many factors are involved here, not least of which are the possible sources of error discussed below. A more thorough discussion of this subject is therefore reserved for the end of this chapter).

A size optimum can be derived for *O.muricata* (at ~ 28 mg dry weight equivalent), however this is considerably in excess of the maximum body size of *O.muricata* recorded here (= 20.9 mg dry wt.) (although larger individuals are found at other localities (Todd, pers. comm.)). Both the *A.proxima* and the *O.muricata* data used here are shown in Figure 7.4.

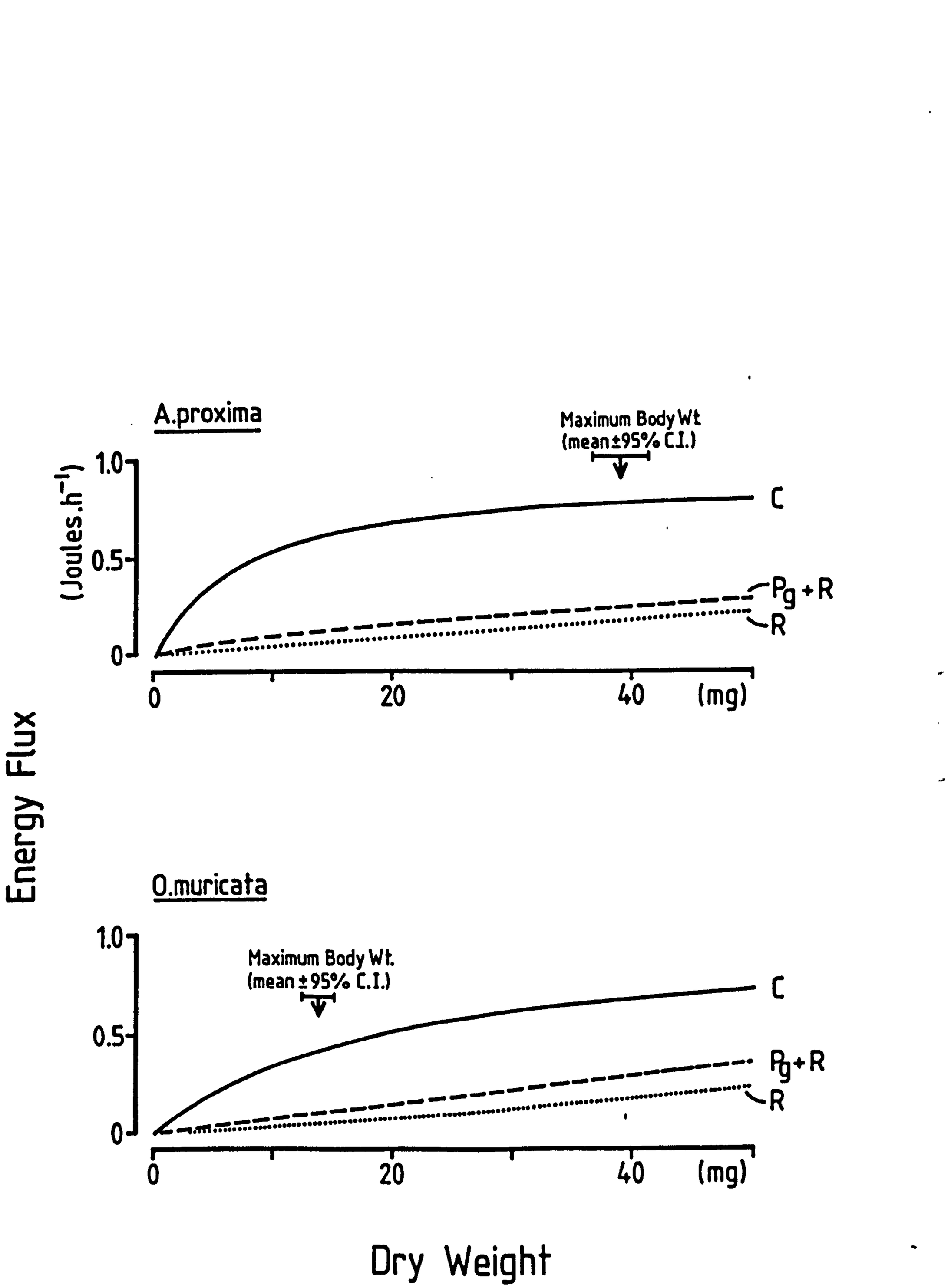
It must be noted that the failure of Sebens' model to predict the body sizes of *A.proxima* and *O.muricata* does not necessarily reflect a shortcoming in the model. Although all the allometric relationships used here have highly significant correlation coefficients, the scatter in the data is still considerable. Moreover, "costs" have not been fully quantified since neither excretion nor defaecation have been measured here.

7.2 ENERGETIC EFFICIENCIES

Any attempt to describe the physiological energetics of an organism must do so not only in terms of the magnitude of the individual energy budget components (see previous section), but also in terms of the energy flux between these components. This flux may be represented as the ratio of energy expenditure in one (or more) component(s) to that in any

FIGURE 7.4 Allometry of Energy Budget components for *Adalaria proxima* and *Onchidoris muricata*

(see text for explanation)


A.proxima
Maximum Body Wt
(mean ±95% C.I.)
1.0
0.5
0
(Joules.h⁻¹)
C
Pg + R
R
0
20
40
(mg)
Energy Flux
O.muricata
Maximum Body Wt.
(mean ± 95% C.I.)
1.0
0.5
0
C
Pg + R
R
0
20
40
(mg)
Dry Weight

other component(s).

These ratios (or "efficiencies"), and their respective values for juvenile and adult *Adalaria proxima* and *Onchidoris muricata*, are outlined in Table 7.2. Due to the requirement for estimates of consumption, the Assimilation Efficiencies and the Gross Growth and Reproductive Efficiencies have been calculated from the data in Table 7.1. The other values in Table 7.2 have been calculated from the raw data shown in Figures 7.2 and 7.3 for the standardised 5-week comparison period.

Assimilation Efficiency (AE)

The Assimilation Efficiency (AE) is the proportion of the ingested ration which is taken up and utilised in both metabolic processes and production (growth and / or reproduction). Assimilation therefore excludes that part of the ingested ration which is lost as excreta or voided as faeces (Crisp, 1971).

The AE's obtained in the present study are roughly comparable between the two species, although *A.proxima* is clearly more efficient than *O.muricata*. Interestingly, both *A.proxima* and *O.muricata* display a 50 - 60% increase in AE between the juvenile (non-spawning) and adult (spawning) stages. A similar result was obtained by Ansell (1982) for the gastropod drill *Polinices* (= *Natica*) *alderi*, and in a review of the physiological ecology of marine molluscs, Bayne & Newell (1983) suggest that although AE is largely unaffected by body size or temperature, reproductive activity may result in substantial (though not necessarily significant) changes in AE.

AE's have been derived for a number of opisthobranch molluscs. For example, Hall (1983) calculated the AE of *Aeolidia papillosa* to be 70 - 90% when feeding on a range of anemone species. Carefoot (1967) calculated AE's for one aplysiomorph and two nudibranch species which ranged from 52 to 86% while Paine (1965) found a mean AE of 62% in the bullomorph *Navanax inermis*. It must be noted that in all of the above studies AE was calculated differently to the methods used here and probably represents Absorption Efficiency rather than Assimilation Efficiency (Crisp, 1971). In a wider context, Ansell

TABLE 7.2 Energetic Efficiencies of *Adalaria proxima* and *Onchidoris muricata*

(Mean values ± one std. error)†

	A.proxima		*O.muricata*	
	Juv.	Adult	Juv.	Adult
Assimilation Efficiency $\frac{(R + P_g + P_r)}{C}$	25.3%	38.8%	19.8%	32.8%
Net Respiratory Cost $(R / A)^*$	49.7% (± 1.65%)	49.2% (± 3.0%)	51.1% (± 3.8%)	37.8% (± 3.1%)
Gross Growth Efficiency (P_g / C)	12.6%	-	10.8%	-
Net Growth Efficiency $(P_g / A)^*$	50.3% (± 1.65%)	-	48.9% (± 3.8%)	-
Gross Reproductive Efficiency $\frac{P_r}{(C - P_g)}$	-	23.3%	-	23.0%
Net Reproductive Efficiency $(P_r / A)^*$	-	50.8% (± 3.0%)	-	62.2% (± 3.1%)

* A = Assimilation *i.e.* the total assimilated energy available for partitioning. In adult animals, this therefore includes the net energy gain from degrowth.

† In cases where Efficiency was calculated using an estimate of Consumption (C), only the overall mean values for the other components were used. Therefore no error term is available for these data.

(1982), Huebner & Edwards (1981) and Bayne & Newell (1983) have found AE's of various carnivorous gastropod molluscs to vary between 27% and 87%. The AE's obtained here are therefore quite low in comparison both to other published estimates and to theoretical expectations (Welch, 1968).

Respiratory Cost

The proportion of the assimilated ration which is expanded metabolically is termed the Net Respiratory Cost (NRC). Typical molluscan values for NRC range from 33% in the prosobranch *Hydrobia ventrosa* up to 85% in the bivalve *Aulacomya ater* (Bayne & Newell, 1983). Values for carnivorous gastropods are typically in the region of 45% (Paine, 1965; Huebner & Edwards, 1981; Ansell, 1982). The values obtained here (Table 7.2) are therefore similar to other published estimates. Table 7.2 further shows NRC in adult *O.muricata* to be lower than that in adult *A.proxima*. This difference is statistically significant (Mann-Whitney test, $p < 0.001$), emphasising the point that NRC for *O.muricata* is only 75% of that for *A.proxima*, thereby releasing a greater proportion of the assimilated ration to reproduction.

Growth Efficiencies

Two different Growth Efficiencies are routinely calculated in bioenergetic studies. The Gross Growth Efficiency (GGE) is that proportion of the ingested ration which is converted to somatic growth. The Net Growth Efficiency (NGE) is the proportion of the assimilated ration which is represented by somatic growth. Since no somatic growth occurred during the reproductive period, these efficiencies have been derived for juveniles only.

Clearly, at all times GGE will be lower than NGE, and the difference between these two will be attributable to the AE and the Net Respiratory Cost (Table 7.2). Nonetheless, NGE for *A.proxima* is remarkably similar to, and not significantly different from, that for *O.muricata* (Mann-Whitney, $p > 0.05$). Russell-Hunter & Burky (1983), suggest that NGE is somewhat insensitive to body size and environmental effects and is therefore of considerable value in comparative studies. Huebner & Edwards (1981) calculated the NGE

of *Polinices duplicatus* to lie in the range of 22 - 59%, while Ansell (1982) obtained NGE's of 8 - 26% in reproductively active *P.alderi* . Paine (1965) provides data for *Navanax inermis* which yield a NGE of 28%. All of these values are low in comparison to the NGE's obtained here. However, in all of the above studies, estimates of NGE have been made for iteroparous organisms over extended periods which included reproductive activity. No strictly comparable data for non-reproductive molluscs are available. Therefore, it is perhaps more relevant to compare the NGE's derived here with Net Total (growth plus reproduction) Production Efficiencies (NPE's) derived elsewhere. Bayne & Newell (1983) summarise NPE's for more than 20 different studies of molluscs. These range from 5% in 15 year-old *Chlamys islandica* to 71% in *Archidoris pseudoargus*, the mean NPE for carnivorous gastropods being 56%. In addition to the above, Ansell (1982) found the mean NPE of *Polinices alderi* to be 60% (range = 42 - 79%), over a variety of environmental temperatures. These data are clearly more comparable to the values obtained here and suggest that production efficiency in (non-reproducing) nudibranchs is, in general, similar to that in other carnivorous gastropods.

Reproductive Efficiencies

In the same way that GGE and NGE respectively represent the proportion of ingestion and assimilation which is allocated to growth, so the Gross Reproductive Efficiency (GRE) and Net Reproductive Efficiency (NRE) represent the respective proportions of ingestion and assimilation which are allocated to reproduction. However, a notable difference exists between the GGE and GRE and also between the NGE and NRE calculated here; in the juvenile (non-reproducing) period, somatic growth (P_g) was positive and therefore represents an energetic drain. During the adult, (reproductive) period, P_g was negative and therefore constitutes an energy supply. Consequently, the GRE has been calculated as the proportion of total throughput energy (*i.e.* ingestion, (C), plus the energy gained from degrowth, (P_g)) which was allocated to reproduction. In a similar manner, NRE was calculated as the proportion of total available energy (*i.e.* assimilation (A), including P_g) allocated to reproduction (see Table 7.2). These measures are therefore not strictly

comparable to either the GGE & NGE derived here or to the NPE's cited earlier. The GRE's & NRE's do however, constitute the best possible measures equivalent to GGE & NGE.

Due to the methods of calculation, it is to be expected that GRE will exceed GGE because a portion of the energy available for reproduction (P_r) has already been assimilated (*i.e.* P_g). Nonetheless, the observed two-fold difference between GRE and GGE in both *A.proxima* and *O.muricata* indicates a substantial increase in the efficiency of allocating ingested energy to reproduction (*cf.* AE's for juveniles & adults). Despite this apparent increase, the NRE and NGE for *A.proxima* are very similar. By contrast the NRE for *O.muricata* is considerably greater than the NGE; this is largely due to a reduction in the NRC when compared to the juvenile period, and results in the NRE of *O.muricata* being markedly in excess of that for *A.proxima.* All the latter three differences are highly significant (Mann-Whitney test, $p < 0.001$), and confirm the earlier observation (Figure 7.1) that *O.muricata* partition a greater proportion of available energy to reproduction than do *A.proxima.*

7.3 GENERAL DISCUSSION

Sources of Error

The energy budgets and associated efficiencies presented in Tables 7.1 and 7.2 show that between 60% and 80% of the energy consumed by *Adalaria proxima* and *Onchidoris muricata* has not been accounted for. For the reasons outlined earlier (Chapter 2, Section 2.1), the present study has measured only respiration, growth and reproduction, leaving the two remaining budget components, (excretion and defaecation), unquantified. Some proportion of this shortfall will, therefore, be attributable to these two components.

Many energy budget studies ignore the losses due to excretion (U) and defaecation (F), or alternatively calculate the combined losses (FU) by difference. Clearly, either of these approaches is inadequate here in view of the magnitude of the unaccounted losses.

Evidence from studies which have actually quantified these fluxes suggests that they may be considerable. Thus, for example, faecal losses in *Thais lapillus* may be as high as 34% of consumption (Bayne & Scullard, 1978). Reported values for opisthobranchs range from 11% (Hall, 1983) to 48% (Carefoot, 1967) of ingested energy, with a mean of 27% (Paine, 1965; Carefoot, 1967; Hall, 1983).

Excretion of Nitrogenous compounds such as Urea and Ammonia also represent losses of ingested energy. Bayne and Newell (1983) cite several studies which have measured Nitrogenous excretion and conclude that the energy lost in this manner can vary both inter-specifically and with regard to the protein content of the ingested food (also see Kersting, 1972). Typically, approximately 10% of ingested energy may be lost this way.

An additional aspect of excretion which is of particular importance in crawling molluscs is that of mucus production. Calow (1974, 1977) calculated that mucus production by the freshwater gastropod *Ancylus fluviatilis* accounted for approximately 16% of ingested energy, while Branch (1982) found mucus production to be equivalent to 40% of total production in the limpet *Patella longicosta.* Other published values are equally high; for example Carefoot (1967) estimated total excretory losses to be ~ 15% of absorbed energy (≈ 29% of ingested energy) in the dorid nudibranch *Archidoris pseudoargus.* From these data, a conservative estimate of the cost of mucus production in *A.proxima* and *O.muricata* might be ~ 15% of ingested energy. If this estimate and those outlined above for faecal and Nitrogenous losses are applied to the energy budgets outlined in Table 7.1, the total daily energy flux which can be accounted for in adult *A.proxima* and *O.muricata* is 18.43 J and 7.25 J respectively. These figures represent a shortfall of 8% (*A.proxima*) and 15% (*O.muricata*) with respect to the estimated daily energy intake. Clearly, causes of this remaining shortfall may be attributed to the inaccuracy of the estimates of faecal and excretory losses discussed above, or to an overestimation of the mean feeding rate. Likely errors, inherent to the methodology of feeding rate determination are discussed below. Nonetheless, a difference of 10 - 15% between energy gain and loss is frequently encountered in studies such as this and is probably within the limits of biological "noise" created by the adopted methodology.

Feeding Rates

Without doubt, the greatest limitation imposed by estimating (rather than measuring) the feeding rates of *A.proxima* and *O.muricata* is that of generalisation. Variation in rates of energy acquisition both within and between individuals is of considerable value in assessing the constraints under which a given organism functions, and is essential to any analysis of the proximate causes of changing patterns of energy partitioning. Moreover, mean feeding rates may be inherently misleading (Bayne & Newell, 1983). However, for reasons outlined earlier (Chapter 3, Section 3.4), the adoption of the methods used here was unavoidable, and has perhaps restricted the scope of this analysis.

Despite this restriction, overall comparability remains and the similarity between interspecific ratios of feeding rates (*A.proxima* : *O.muricata* ≈ 2.2 : 1) and daily energy fluxes (*A.proxima* : *O.muricata* ≈ 2.6 : 1) indicates that the estimates obtained are of value in analysing overall energy expenditure. Whether or not this similarity of ratios implies that feeding rate imposes a constraint on energy expenditure, is unclear. It is self-evident that energy expenditure cannot exceed energy acquisition (*i.e.* consumption and/or degrowth); but the results presented in Chapter 3 (Section 3.3) suggest that gut absorption rate rather than actual feeding rate places the greatest limitations on assimilation in both *A.proxima* and *O.muricata*.

Growth

The data presented in Chapter 4 indicate that whatever the limitations on assimilation rate might be, they only have a significant effect on growth rate in *A.proxima*. Certainly, the almost exponential growth of *O.muricata* suggests little, if any shortage of energy supply. By contrast, the initially curvilinear and subsequently linear growth of *A.proxima* is, perhaps, indicative of exponential growth encountering a regulating or constraining influence. The validity of such a model cannot be adequately tested without measuring both feeding and growth rates for a variety of individuals over several time periods. Such a test is beyond the scope of this study and therefore this model cannot be evaluated. However, the intra-specific consistency of growth patterns does provide substantial, albeit indirect, evidence in support of such a model.

Despite the above noted differences in growth pattern between *A.proxima* and *O.muricata*, the Gross Growth Efficiencies (GGE's) and Net Growth Efficiencies (NGE's) between the two species proved to be very similar. The GGE's obtained here are comparatively low for carnivorous molluscs and show relatively low ecological efficiency (Bayne & Newell, 1983; Huebner & Edwards, 1981). However, this may be due in part to the possible overestimation of consumption rate discussed above. Given that metabolic costs are likely to be relatively constant and inflexible (Chapter 5, Section 5.4), the observed similarity between the NGE's of *A.proxima* and *O.muricata* may simply be a reflection of similar respiratory costs in these two species. Nonetheless, the implication remains that the relative efficiencies of growth processes in *A.proxima* and *O.muricata* are largely unaffected by the potentially different constraints which may operate upon those processes.

Respiration

The observed allometric exponents of the respiration rate : body weight relationships (Chapter 5, Section 5.3) have important implications for energy partitioning. Clearly, *A.proxima* individuals (overall weight exponent = 0.83) should become more metabolically "efficient" as they increase in size, while the reverse is true for *O.muricata* individuals (overall weight exponent = 1.1). This difference largely explains why a size optimum was obtained for *O.muricata* but not for *A.proxima* in Sebens' (1977) energetics model (see Section 7.1). However, it has already been shown that such changes in metabolic "efficiency" are not reflected in the energetic costs of respiration in the individuals studied here. The Net Respiratory Costs (NRC's) in juvenile *A.proxima* were virtually identical to those for adult *A.proxima*, while the NRC's of juvenile *O.muricata* are higher than those in adult *O.muricata* (Table 7.2).

Thompson & Bayne (1974) have demonstrated that vitellogenesis in *Mytilus edulis* results in an elevation of the metabolic rate, increasing the NRC and therefore causing a decrease in the overall growth (*i.e.* growth plus reproduction) efficiency. Since no such increase was observed in either metabolic rates (Chapter 5, Section 5.3) or NRC (Table 7.2), it seems likely that if such a process also operates in nudibranchs, it has been masked by other factors affecting respiratory rates and costs. For example, changes in NRC may have

been caused by changing feeding rates during the reproductive period. Although no discernible difference between feeding rates of reproductive and non-reproductive nudibranchs could be found, ingestion rates of the individuals studied here were not quantified directly and therefore this remains a possibility.

A further possible cause of these changes in NRC is the different environmental temperatures experienced throughout the animals' lifetime. These effects will be discussed later in this chapter.

The metabolic costs of degrowth have been demonstrated, at least in part, during several investigations into the effect of ration level on respiration rate (see for example Calow, 1974). Such measures represent "starvation" respiration rates and have been taken as measures of maintenance metabolism (Bayne & Newell, 1983). In this respect they perhaps bear little relation to the true metabolic costs of "active" degrowth as exemplified by the reproducing *A.proxima* studied here. Despite the expectation that degrowth would incur metabolic cost, no significant correlation was found between individual degrowth rate and respiration rate in either *A.proxima* ($r^2 = 0.14$, $p >> 0.05$) or *O.muricata* ($r^2 = 0.11$, $p >> 0.05$). The possibility that the more rapid degrowth of *A.proxima* individuals resulted in the higher NRC's of (adult) *A.proxima* therefore seems unlikely.

A further unresolved relationship is the observed temperature sensitivity of respiration rate in adult *A.proxima* (Chapter 5, Section 5.3). Analysis of the energy budget components using multivariate techniques yielded no additional information with which to clarify this situation, and no firm conclusions can be drawn. Clearly, the cause of this temperature sensitivity is effectively absent in *O.muricata* and in juvenile *A.proxima*. The only other characteristic measured here which displays a similar pattern is degrowth. Although both species degrew, this phenomenon was far more rapid in adult *A.proxima* than in adult *O.muricata*. The lack of association between degrowth rate and respiration rate has already been demonstrated (see above), but this does not preclude the possibility that the observed temperature sensitivity is in some way related to stress associated with degrowth. In the absence of a more thorough understanding of the mechanisms controlling both

respiration and degrowth, such an interpretation must remain speculative. The effects of temperature on respiratory losses have already been considered in some detail in Chapter 5. The wider implications of this relationship are discussed later in this chapter.

Reproduction

The importance and energetic costs of the male role in reproduction have not been quantified here. From a practical standpoint, the energetic costs of sperm production in such small, hermaphrodite molluscs would be very difficult to ascertain. Maynard Smith (1978) argues that these could theoretically be as high as 50% of the energy partitioned to reproduction, (and Grahame (1973) presents evidence from a study of *Littorina littorea* in support of this). However, Calow (1981) has emphasised the potential (energetic) benefits which accrue from internal fertilization in hermaphrodites, and concludes that net expenditure of energy on the male reproductive system may in fact be very low. This conclusion is supported by anatomical evidence that opisthobranchs are capable of catabolizing both mature autosperm and allosperm (Thompson, 1976), and by histological observations that spawned-out (and therefore energetically stressed) adults are still capable of spermatogenesis (Todd, 1978). It seems likely therefore, that these costs will represent a minor proportion of the overall reproductive costs. That is not to say that they may not be significant and this (unavoidable) shortcoming cannot be overlooked.

A further consideration not investigated here is the possibility of a given individual acting as a functional male or female (*i.e.* non-reciprocal exchange of gametes during copulation). This phenomenon has been observed for other opisthobranchs (for example *Aplysia dactylomela;* Lederhendler & Tobach, 1977) and may occur in *O.muricata* (Todd, 1983), although no obvious signs of this behaviour were observed in either the *A.proxima* or *O.muricata* individuals studied here. Nonetheless, the possibility remains that some individuals may have consistently maximised either their male or female roles and this may account for some of the observed variation in (female) reproductive output.

Calow (1981) asserts that the conversion of ingested energy into gametes is a more efficient process than conversion of ingested energy into somatic tissues. The data given in

Table 7.2 essentially support this contention and it is clear that the Gross Reproductive Efficiency (GRE) is approximately twice as great as the Gross Growth Efficiency (GGE) in both *A.proxima* and *O.muricata*. Despite the potential complications caused by inclusion of degrowth in calculating GRE's, and by estimating rather than measuring ingestion, the conclusion remains that overall conversion of ingested energy into gametes is a more efficient process than conversion into somatic tissues. Nonetheless, reproduction in *A.proxima* is limited to utilizing only 50% of assimilated energy because of relatively high respiratory costs, while such is not the case for *O.muricata*.

Temperature Effects

Throughout, this study has quantified the major energy budget components at ambient field seawater temperatures. The effects of these variations in environmental temperature on processes such as feeding, growth and reproduction were not assessed. However, all these processes involve metabolic (*i.e.* respiratory) energy losses, and it has already been shown (Chapter 5, Section 5.3) that respiratory losses are largely unaffected by slow changes in environmental temperature. It is perhaps reasonable therefore to expect that feeding, growth and reproduction may be equally unaffected by such changes in ambient temperature.

An exception to the above generalisation exists however, in that the respiration rates of reproductively active *A.proxima* appear to be highly sensitive to environmental temperature (Chapter 5, Section 5.3). The causes of this sensitivity are not clear and a possible explanation has been proposed (see discussion of respiration in this chapter). Nevertheless, the question remains as to what effect this temperature sensitivity may have had on the evolution of the observed reproductive characteristics of *A.proxima*. This question could be answered in part by conducting an energy budget analysis at a constant (rather than ambient) temperature. However, the results of such a study may be confounded by (ecologically unrealistic) interactions between the budget components brought about by the artificially constant conditions. Under the present circumstances, it is not possible to reach a conclusion with regard to the above question.

Difficulties clearly arise when attempting to consider any budget component in

isolation. Therefore a comprehensive analysis of the interactions between the different components has been made.

Interactions

For each individual of each species, the juvenile and adult energy budget components were combined and analysed by Principal Components Analysis (PCA). Given the small sample size (n = 7) such an analysis could only provide an indication of the underlying inter-relationships. Nonetheless, several interesting features were extracted.

The observed variation in *A.proxima* juvenile respiration rate (Figure 7.2) was strongly correlated with juvenile growth rate. This result is perhaps unsurprising since rapidly growing individuals were generally larger than their slower growing counterparts and therefore may be expected to show higher (absolute) respiration rates for allometric reasons (Chapter 5). What is surprising however, is that no other significant, or even strong, associations were found between adult respiration rates and the other energy budget components for *A.proxima*. Indeed, in two dimensional factor space, adult respiration rate lies almost orthogonal to all the other budget components indicating little (if any) association.

Several other strong associations also emerged. For example, spawn production rate was closely related to juvenile growth and respiration rates, and all three of these budget components were negatively correlated with rate of degrowth. Inevitably, some degree of autocorrelation will be involved here, but the association between rapid degrowth and high spawn production rate is nevertheless unequivocal.

For *O.muricata* overall patterns were less clear. As for *A.proxima*, spawn production rate of adult *O.muricata* was closely correlated with the juvenile growth rate. This was the only significant correlation, ($r = 0.84$, $p < 0.05$). However, unlike *A.proxima*, juvenile respiration rate was not associated with either juvenile growth rate or (adult) spawning rate in *O.muricata*. High juvenile respiration rates were generally associated with rapid adult degrowth rates, but this association was not significant. Adult respiration rates showed no clear relationship with any other components.

Despite a lack of overall clarity, the PCA's, when combined with the results presented earlier, do indicate the general constraints and inter-relationships which govern energy partitioning in *A.proxima* and *O.muricata.*

Although the generalised data used to test Sebens' (1979) model showed no size optimum (and therefore no decrease in scope for growth with increasing body size) in *A.proxima*, the analysis of unaccounted losses and the growth pattern presented earlier suggest that growth rates of *A.proxima* may be limited by rates of energy supply from ingestion. Given the form of the estimated mean feeding rate curve (Figure 3.3), this may be especially so at large adult body sizes. Therefore, remembering the earlier arguments regarding efficient spawn mass production and longevity (Section 6.4), the observed association between degrowth rate and spawn production rate may be interpreted as an adaptation to maximise reproductive output (as fewer "efficient" spawn masses) at a time when ingested energy supply may be limiting. Certainly, it is clear that the Net Reproductive Efficiency of *A.proxima* individuals would be much reduced in the absence of energy supply from degrowth. However, in order to obtain adequate fluxes of energy from degrowth, any individual must have first accreted a sufficiently large soma. In relative terms, *A.proxima* individuals are in a position to do this since, despite the above noted constraints, feeding rate (Chapter 3) and growth rate (Chapter 4) are both high when compared to those of *O.muricata*, and pre-reproductive body sizes of *A.proxima* are accordingly greater (Chapter 6, Table 6.1). Although they are of undoubted importance, differences in feeding and growth rates may not be the sole cause of the observed differences between adult body sizes of *A.proxima* and *O.muricata* . For example, Todd & Doyle (1981) present estimates of egg-to-juvenile periods for *Adalaria proxima* and *Onchidoris muricata.* Because of the extended planktonic development of *O.muricata*, the total egg-to-juvenile period for this species is considerably in excess of that for *A.proxima*, (86 days and 51 days respectively; Todd & Doyle, 1981). This has the effect of allowing less time (in a strictly annual cycle) for growth of the benthic post-metamorph. If we assume a generation time of exactly 365 days, an initial post-metamorphic size of 200 µg (S.C. Kempf, pers. comm.) and a constant size-specific growth rate of 1.5% d^{-1} (the overall mean of the values given in Chapter 4, Section 4.3), then on the basis of the above egg-to-juvenile

periods, an immediately pre-reproductive adult *A.proxima* would weigh approximately 22 mg while an equivalent *O.muricata* would weigh approximately 13 mg. Clearly the above assumptions are simplistic, but it is still apparent that an abbreviated egg-to-juvenile period may confer more advantages than simply those related to larval type *per se* (see Chapter 9).

In contrast to *A.proxima*, *O.muricata* grows to a relatively small adult size. This is the case despite a generally higher weight-specific growth rate (Section 4.3). (Therefore lending further support to the contention that egg-to-juvenile period plays an important role in determining adult body size). Moreover, growth of *O.muricata* appears to be relatively free of the energetic constraints discussed earlier. It is interesting to note, therefore, that a close association between juvenile growth rate and spawn production rate in *O.muricata* has been indicated by the PCA. Taking this, and the observed production patterns in Figure 6.2 into account, it appears that *O.muricata* may simply switch resource partitioning from P_g to P_r at the onset of reproduction. The absence of such a strong association in the *A.proxima* PCA suggests that more complex processes may be involved (such as the additional onset of rapid degrowth) in providing energy for gametogenesis. This further implies that feeding rates of *O.muricata* are sufficient to offset the costs of reproduction but not so for *A.proxima*. Although degrowth does occur in reproductively active *O.muricata*, it is considerably less rapid than in *A.proxima*. This is an important point in view of the observation that both *A.proxima* and *O.muricata* die when ~70% of the body (ash-free) dry weight has been catabolised (Chapter 6, Section 6.4). The result is that *O.muricata* individuals have a relatively longer spawning period (Table 6.1) and therefore have more time available to ingest energy, convert that energy to gametes and maximise reproductive output. This is evidenced by the observations of "Reproductive Effort" (R.E.) (Chapter 6, Table 6.1) which are considerably higher in *O.muricata* than in *A.proxima*. Given the similarity of the Gross Reproductive Efficiencies (calculated on a daily basis) for the two species (Table 7.2), the greater RE's of *O.muricata* must primarily be attributable to the longer spawning period in this species.

In summary, it is postulated that a combination of constraints imposed by rates of feeding and digestion and also by larval strategies has brought about the evolution of very different energy partitioning strategies in *A.proxima* and *O.muricata*. *A.proxima* grows to a relatively large body size before the onset of reproduction. The reproductive period is relatively short primarily because a substantial proportion of the energy utilised in reproduction is derived from degrowth of the body tissues, and this rapid degrowth leads to a correspondingly rapid onset of senescence and death.

In contrast, *O.muricata* attains only a small body size. However, reproductive costs can be met largely by recurrent energy supply from ingestion. Therefore, although degrowth does occur, it is far less rapid than in *A.proxima* and consequently the spawning period is relatively longer. This results in greater total spawn production than could be achieved by an animal the size of *O.muricata* with the energy partitioning strategy of *A.proxima*.

Clearly, energetics is only one of a number of constraints which exert selective pressure on any marine invertebrate (see Grahame & Branch, 1985 for a review). However, an organism can only adapt to such pressures whilst remaining within the limits set by energetic constraints (Todd 1983). In this respect energetics comes at (or close to) the top of a hierarchical set of diverse factors which determine the selective pressures operating at any one time. For this reason the functional aspects of reproductive allocation have received much attention in models of life-history evolution (Vance, 1973a,b; Schaffer, 1974; Tinkle & Hadley, 1975; Christiansen & Fenchel, 1979). Many studies have utilised simple measures of reproductive allocation (e.g. Grahame, 1977; Todd 1979a; Hughes & Roberts, 1980; reviewed by Stearns 1977), in attempting to analyse the evolution of differing reproductive strategies. However, such spawn : soma ratios provide a relatively poor measure of true reproductive effort *sensu* Tinkle & Hadley (1975), (Hart & Begon, 1982; Todd & Havenhand, 1983). The utility of these, and more complex measures of reproductive effort are analysed further in the light of the results discussed here in the following chapter.

CHAPTER 8

MEASURES OF REPRODUCTIVE "EFFORT"

8.1 INTRODUCTION

Reproductive Effort (RE) (*i.e.* "that proportion of metabolic resources devoted to reproduction" (Lincoln *et al.*, 1982)) as distinct from numerical fecundity, was first recognised by Fisher (1930) to be an adaptive trait. More recent workers have developed this (see, for example, Williams, 1966; Tinkle, 1969; Pianka, 1970; Schaffer, 1974) such that RE has become central to ecological theory relating physiological processes to life-history strategies (reviewed by Stearns, 1976). The utility of RE lies in its dimensionless representation of the comparative 'costs' of reproduction. This notion of a 'cost' rests on the premise that for any given organism the diversion of (finite) resources from growth and maintenance to reproduction will cause a decrease in life-expectancy and/or body size and will result in a consequent decrease in residual reproductive value, (Fisher, 1930; Williams 1966). This argument has been extended to consider the evolution of semelparity and iteroparity with respect to varying levels of RE (Schaffer, 1974, 1979; Calow & Woollhead, 1977; Calow, 1979, 1983; Aldridge, 1982).

In addition to the above considerations, marine invertebrates also display a variety of larval types (Thorson, 1946). The magnitude of RE associated with a given larval type has been the subject of much recent study (reviewed by Day & McEdward, 1984; Strathmann, 1985; Grahame & Branch, 1985). Consequently, the ability to measure RE in an accurate and reliable manner is essential if progress is to be made.

A variety of indices have been proposed and used as measures of RE. Perhaps the most important distinction which should be made is whether a given measure is instantaneous or whether it is an estimate of RE for one reproductive season, one year, or a lifetime. Clearly in some organisms, these different periods will yield very different estimates of RE. In the nudibranchs studied here however, these periods are essentially

synonymous. Consequently all measures given here are estimates of lifetime RE (with two noted exceptions).

Perhaps the simplest measure of reproductive allocation is the number of eggs produced (the numerical fecundity). However, the number of eggs produced from a given quantity of energy will depend on the larval type (Todd & Doyle, 1981) and therefore the total weight, or energy content of eggs produced (*i.e.* P_r) may be regarded as a better measure of RE. The primary disadvantage of this measure is that fecundity generally displays an allometric relationship with body size. Therefore a more frequently employed measure is a simple spawn : soma ratio (*i.e.* P_r / W, where W = energy content of the body tissues). This measure is relatively easy to obtain and consequently its use has been widespread (*e.g.* Grahame 1973, 1977; Menge 1974; Todd, 1979a,b; Hughes & Roberts, 1980; Perron, 1982). However, this measure has also been widely criticised for its simplistic properties (Browne & Russell-Hunter, 1978; Hughes & Roberts, 1980; Grahame, 1982; Todd & Havenhand, 1983), and not least because of its underlying assumption that reproductive allocation is a linear (isometric or allometric) function of body size (Calow, 1979, 1983).

Browne & Russell-Hunter (1978) have suggested the use of the proportion of non-respired assimilation which is diverted to reproduction (*i.e.* $P_r / (P_r + P_g)$) as a suitable measure of RE. This measure has been used by Hughes & Roberts (1980), Ansell (1982), Perron (1982), Bayne *et al.*, (1983) and Thompson (1983). Again, this measure has been criticised by Calow (1979, 1983) on the grounds that it does not account for potential changes in respiratory costs (R). A derivative of the above measure has been suggested by Russell-Hunter & Romano (1981) who proposed that the ratio between reproductive production rate and pre-reproductive growth rate (P_{gj}) would provide a useful dynamic measure of reproductive effort. To date however, this measure has only been used by Smith & Sebens (1983).

Perhaps the most widely cited measure of RE is that suggested by Tinkle & Hadley (1975); namely that fraction of the total (assimilated) energy budget which is diverted to reproduction. This will include energy used in additional foraging behaviour, any territorial or mating behaviour, and energy expended in carrying and/or protecting a brood. Clearly, in many species of marine invertebrate, some of these latter functions will be of minimal or no importance. Consequently, most studies have approximated this measure as P_r/A for "the period in question" (Stearns, 1976). Grahame (1973, 1982), Calow (1978, 1979), Hart & Begon (1982) and Thompson (1983) have all used this technique. However, Calow (1983) again criticises this method for ignoring potential contributions from stored energy reserves. In so doing, Calow (1979, 1983) suggested the use of "C" as a measure of reproductive "recklessness" or "restraint", where;

$$C = 1 - [(A - P_r) / (P_g + R)^*]$$

In this formulation, $(P_g + R)^*$ must be measured in pre-reproductive individuals. This measure has not only been used by Calow (1979, 1983) but also by Bayne *et al.* (1983), and is claimed to have the advantage that it accounts for the actual "cost" of reproduction (Calow, 1983).

Since some of the above measures require quantification of the major energy budget components, all of the measures considered above have been calculated only for the seven *Adalaria proxima* and *Onchidoris muricata* individuals for which such data are available. While it is acknowledged that these sample sizes are small, it is nevertheless held that sufficient data are available to provide an interpretable comparison of these measures.

In addition to the above, a further estimate of RE, the Reproductive Index, has been devised. This measure avoids the drawbacks of simple spawn : soma ratios without necessitating intensive energetic analyses. Nonetheless, this measure does require data acquisition for each individual following each spawning event.

8.2 METHODS & RESULTS

All the measures considered here have been calculated from the data in Table 8.1. The formulae used, and the results obtained are shown in Table 8.2. The Σ prefix to the energy budget components indicates a lifetime value rather than a daily rate (as used in Chapter 7 for example). Strictly, this prefix should be written $\sum_{i=1}^{n}$, indicating that it is the sum of all i cases from the first (i = 1) to the last (i = n), where each case would be a daily rate (for example). However, for convenience and simplicity, the full notation will not be used although its meaning is implied throughout. Thus it can be seen that the measures of Russell-Hunter & Romano (1981) (*i.e.* P_r / P_{gj}) and Calow (1979, 1983) ("C") are the exceptions to the earlier statement that the measures presented are estimates of lifetime reproductive effort (RE). Both of these measures are based on mean daily (rather than total lifetime) energy fluxes.

The proportion of non-respired assimilation which is allocated to reproduction (Browne & Russell-Hunter, 1978) requires a value for somatic production (P_g). Since a proportion of somatic production is catabolised during the reproductive period (see below), the effective lifetime somatic production (ΣP_g) has been taken as equal to the final (immediate pre-mortality) body size. This value has also been used in calculating the RE measure of Tinkle & Hadley (1975).

The Reproductive Index, Σ RI, given in Table 8.2 is a weight-specific measure of the balance between somatic production (or degrowth) and reproductive production. The Reproductive Index (RI) is calculated as:

$$RI = \frac{P_r + P_g}{W_{to}}$$

for any given time period 't' where W_{to} is the energy equivalent of the body weight at the

TABLE 8.1 Parameters used in calculating measures of Reproductive "Effort" of *A.proxima* (top) and *O.muricata* (bottom)

(All figures are in Joules unless otherwise stated)

Animal Number	ΣR^*	ΣP_r	$W_{max}^\dagger$	$W_{final}^\dagger$ ($=\Sigma P_g$)	$P_{gj}^{\dagger\dagger}$ ($J.d^{-1}$)	$R_j^{\dagger\dagger}$ ($J.d^{-1}$)
10	933.2	277.5	381.0	290.7	1.26	4.68
14	735.9	366.5	302.0	165.1	0.87	4.07
17	1162.3	449.3	391.6	233.4	1.43	5.56
21	888.2	448.8	345.0	175.7	1.34	3.69
23	722.1	277.3	305.2	206.3	2.16	3.30
24	1003.5	176.9	441.7	313.4	1.87	4.52
27	845.5	476.7	320.8	205.1	1.14	3.71
3	90.01	104.0	34.99	18.41	0.0951	0.771
4	158.3	139.5	76.09	60.38	0.198	1.18
7	158.5	140.3	79.20	64.35	0.326	1.03
10	128.2	85.87	77.30	41.73	0.345	0.740
11	200.9	199.2	70.57	51.05	0.391	1.12
21	211.3	262.7	77.65	62.62	0.373	1.33
28	146.2	208.6	90.60	56.58	0.434	0.949

* ΣR was estimated from the individual regressions given in Chapter 5 (Section 5.3) using the weights presented in Figures 6.1 & 6.2.

† W_{max} and W_{final} are the equivalent energy contents of the maximum and final (immediate pre-mortality) body weights respectively.

†† P_{gj} and R_j are the juvenile (pre-reproductive) growth and respiration rates respectively.

TABLE 8.2 Different measures of Reproductive "Effort" for *A.proxima* (top) and *O.muricata* (bottom)

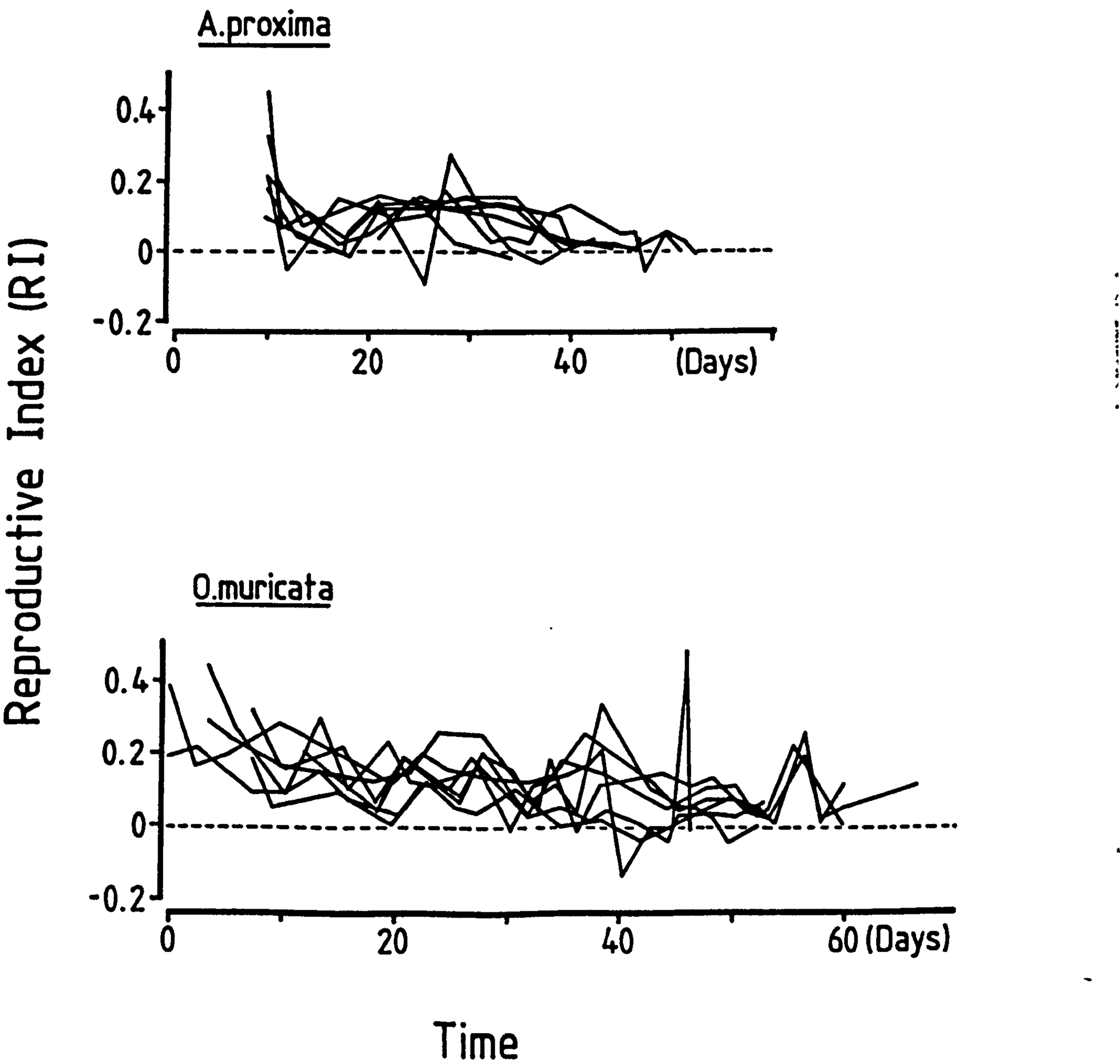
Animal No.	$\Sigma P_r/W_{max}$	$\Sigma P_r/(\Sigma P_r+\Sigma P_g)$	P_r/P_{gj}	$\Sigma P_r/(\Sigma P_r+\Sigma P_g+\Sigma R)$	"C"	Σ RI
10	72.8	48.8	400	18.5	0.500	0.615
14	121.4	68.9	611	29.9	0.714	1.136
17	114.7	65.8	419	24.4	0.584	1.065
21	130.1	71.9	577	29.7	0.704	1.102
23	90.9	57.3	151	23.0	0.639	0.760
24	40.1	36.1	364	11.8	0.778	0.215
28	148.6	69.9	581	31.2	0.629	1.390
mean	**102.6**	**59.8**	**444.3**	**24.1**	**0.650**	**0.898**
s.e.	**14.1**	**5.0**	**57.0**	**2.7**	**0.036**	**0.149**
3	297.2	84.9	1302	49.0	0.723	3.268
4	183.3	69.8	819	39.0	0.358	1.980
7	177.2	68.6	399	38.6	0.440	1.816
10	111.1	67.3	265	33.6	0.810	0.926
11	282.3	79.6	392	44.2	0.472	3.235
21	338.3	80.8	597	49.0	0.415	3.666
28	230.2	78.7	546	50.7	0.552	2.452
mean	**231.4**	**75.7**	**617.1**	**43.4**	**0.539**	**2.478**
s.e.	**30.1**	**2.6**	**122.7**	**2.5**	**0.064**	**0.369**

beginning of that time period. In the case of the nudibranchs studied here, P_r and W (and hence, P_g) were measured at each spawning and therefore an RI value can be obtained for each spawning interval of each individual nudibranch. Thus, the RI will exceed zero at all times when P_g is positive and when the amount of energy allocated to reproduction exceeds that gained from degrowth (*i.e.* $P_r > |P_g|$). If the RI equals zero, then the energy allocated to reproduction exactly balances that obtained through degrowth. Only when degrowth provides more energy than the individual produces as spawn will the RI fall below zero. The change in balance (*i.e.* the dynamics) of energy partitioning can be expressed by plotting RI as a trajectory over time for a given individual or group of individuals. This has been done for the seven individuals of each species (Figure 8.1). Here, it can be seen that despite a considerable degree of variation, the RI generally declines throughout the reproductive period, but only occasionally falls below zero. It is also clear that the RI's of the *A.proxima* individuals are generally lower than those of the *O.muricata* individuals, reflecting the greater importance of degrowth in the reproductive output of *A.proxima* individuals (Chapters 6 & 7, Sections 6.4 & 7.3).

By summing the RI's for any given animal, a total reproductive index (Σ RI) is obtained. This value represents the overall 'performance' of the individual and has the same properties as its component measure, RI. The Σ RI's for the seven *A.proxima* and *O.muricata* individuals are shown in Table 8.2.

It is clear from Table 8.2 that the different measures of RE yield very different values. Thus, of the ratio measures, Tinkle & Hadley's (1975) method yields the lowest estimates of RE (means of 24% and 43% for *A.proxima* and *O.muricata* respectively), whilst that of Russell-Hunter & Romano (1981) yields the highest estimates (444% and 617% for *A.proxima* and *O.muricata* respectively). Calow's "C" and Σ RI give very different values for computational reasons and these will be discussed later. However, a general pattern of relatively high reproductive effort in *O.muricata* with respect to that of *A.proxima* is apparent. Mann-Whitney tests of rank order confirm this general pattern; the

FIGURE 8.1 Reproductive Index trajectories over time for the seven *Adalaria proxima* and *Onchidoris muricata* Energy Budget individuals


A.proxima
0.4
0.2
0
-0.2
0
20
40
(Days)
Index (RI)
O.muricata
0.4
0.2
0
-0.2
0
20
40
60 (Days)
Reproductive
Time

RE's of *O.muricata* individuals were significantly higher than those of *A.proxima* individuals ($p < 0.05$) for all measures of effort except those of Russell-Hunter & Romano (1981) (*i.e.* P_r / P_{gj}) and Calow's "C" (Calow, 1979, 1983). It is perhaps pertinent to note that these two are the only measures of RE based on rate values rather than on lifetime totals. Of the remaining four measures, intraspecific variation is greatest in the spawn : soma ratio and in Σ RI (Table 8.2). The variation in the former is perhaps to be expected in the light of the poor correlation between the component variables (Chapter 6, Section 6.3). These relationships (between total spawn output and maximum body size for all the individuals studied, including the seven 'energy budget' animals) are also shown in Figure 8.2 (*A.proxima*) and Figure 8.3 (*O.muricata*). The lack of allometry of spawn production in *A.proxima* is readily apparent, and although the data set for *O.muricata* shows a tighter relationship, the degree of scatter is still considerable. Thus it would appear that maximum body size alone is not a reliable predictor of total spawn production in either of the species considered here.

The measure Σ RI was specifically designed to overcome some of the limitations of spawn : soma ratios based on a single estimator of body size, by measuring body weight after each spawning. In this way the best available 'gonad-free' estimate of somatic weight was obtained. It is therefore perhaps surprising to record so much intraspecific variation in this measure (see Table 8.2). However, given the computational method and the observed association between degrowth and spawn production (Chapter 6, Section 6.4), it seems probable that this variation is a real biological result; *i.e.* that the observed variation in Σ RI is a true reflection of the variation in RE or reproductive 'costs' between individuals. Figure 8.4 shows the total spawn output and Σ RI for both *A.proxima* and *O.muricata*. It is clear from this Figure that a given level of spawn production is achieved with a higher (and more variable) Σ RI in *O.muricata* than in *A.proxima*. Once again this reflects the relative importance of degrowth to reproductive output in *A.proxima* individuals (*q.v.* Chapter 6, Section 6.4).

Despite the above noted variation, Σ RI and the spawn : soma ratio are very closely

FIGURE 8.2 Total spawn output (ΣP_r) of *Adalaria proxima* in relation to the maximum body size observed after the onset of spawning

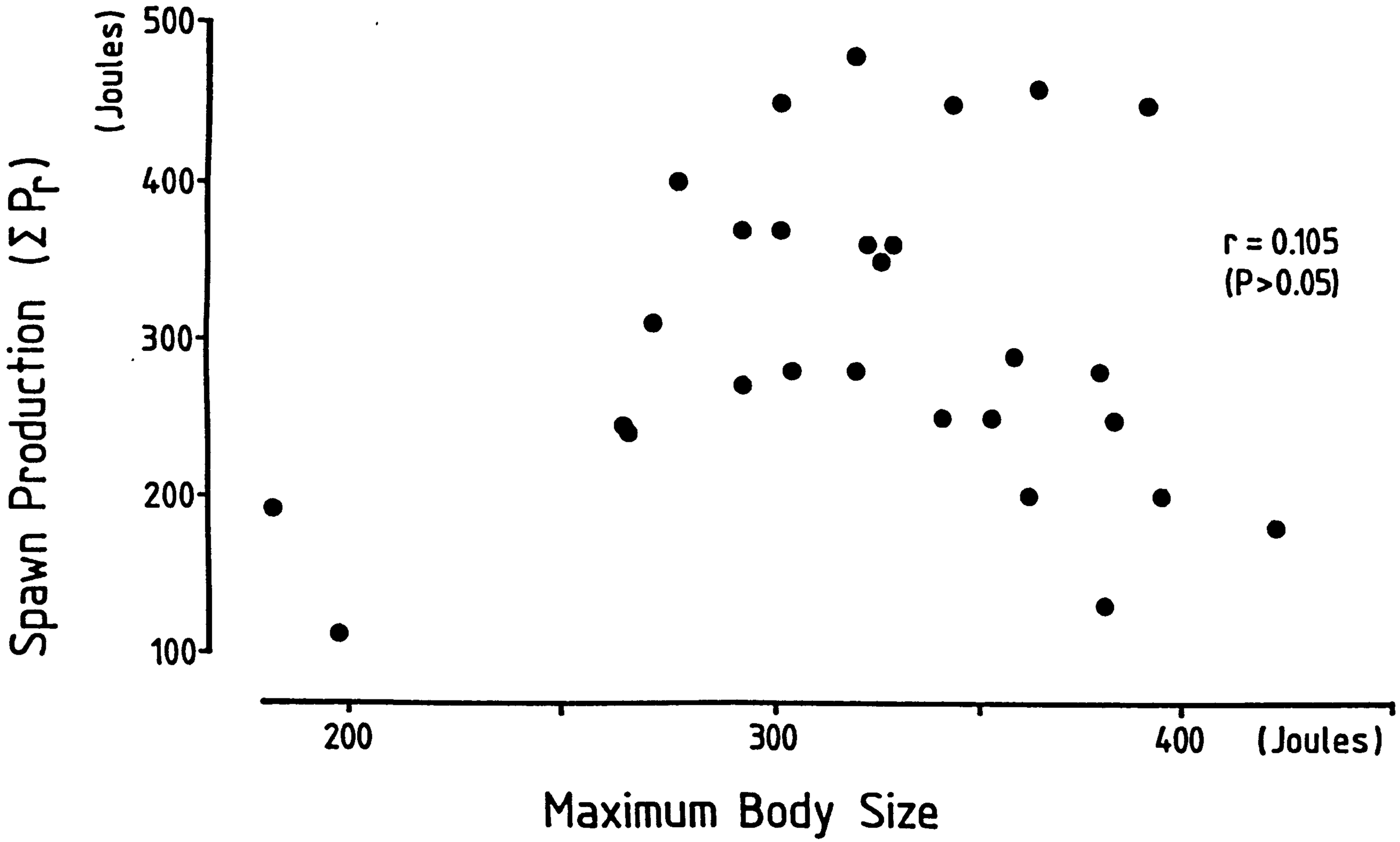

Spawn Production (ΣP_r)
(Joules)
500
400
300
200
100
200
300
400
(Joules)
Maximum Body Size
r = 0.105
(P>0.05)

FIGURE 8.3 Total spawn output (ΣP_r) of *Onchidoris muricata* in relation to the maximum body size observed after the onset of spawning

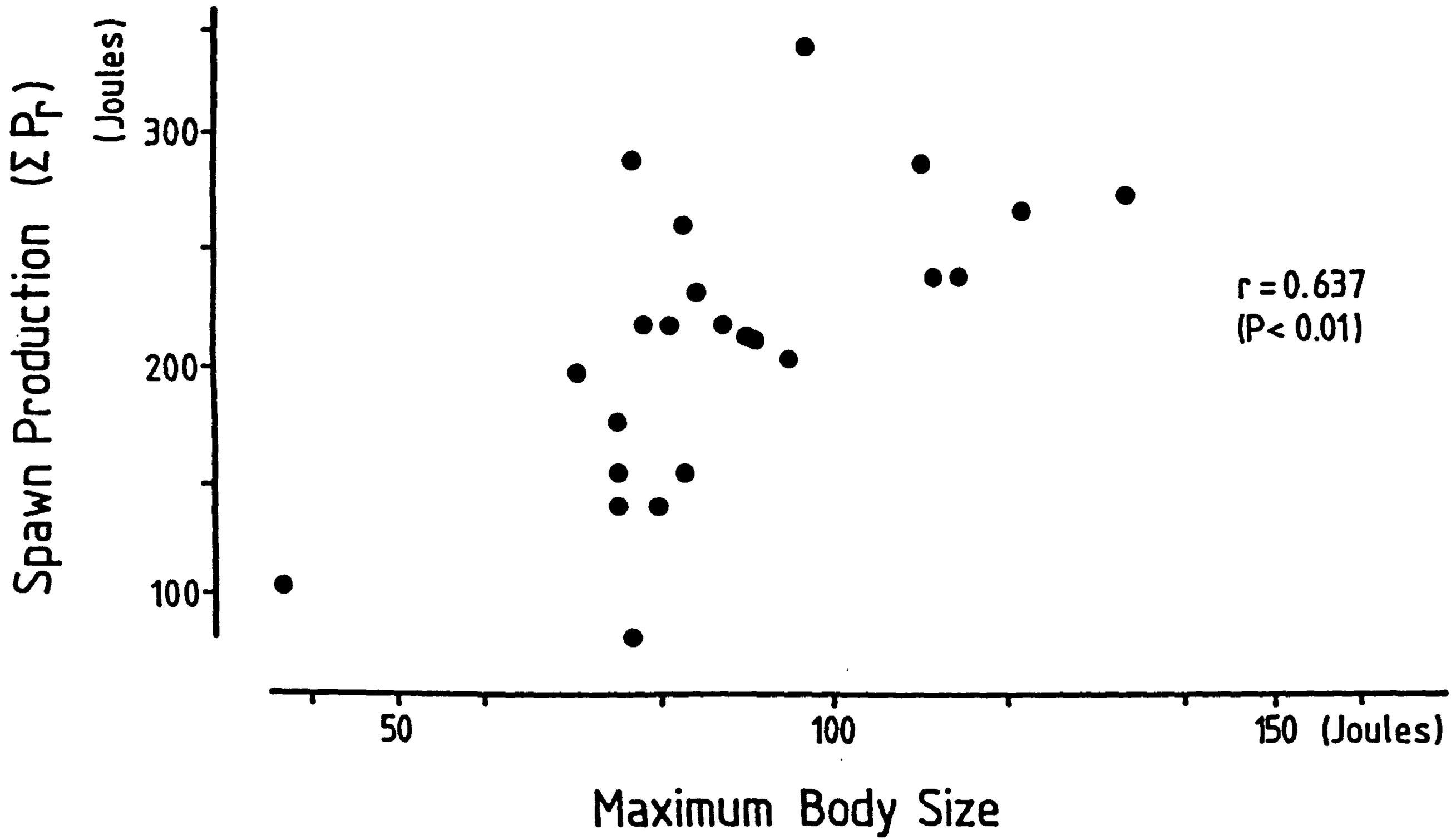
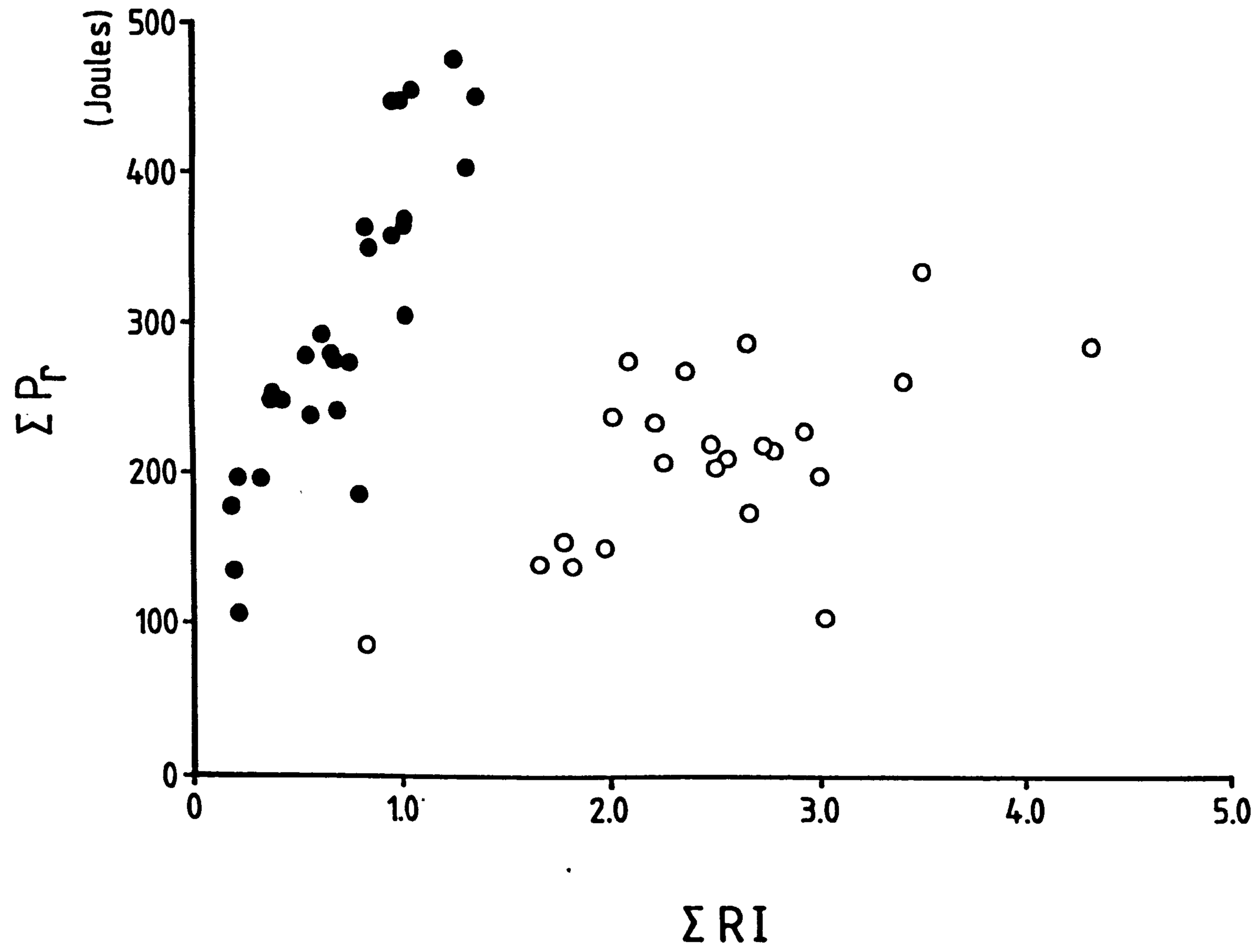

Spawn Production (ΣP_r) (Joules)
300
200
100
50
100
150 (Joules)
Maximum Body Size
r = 0.637
(P< 0.01)

FIGURE 8.4 Total spawn output (ΣP_r) in relation to total lifetime Reproductive Index (Σ RI) for *Adalaria proxima* (closed circles) and *Onchidoris muricata* (open circles)


(Joules)
500
400
300
200
100
0
ΣPr
0
1.0
2.0
3.0
4.0
5.0
ΣRI

correlated in both *A.proxima* and *O.muricata*. Table 8.3 provides the correlation matrix of all the different measures of RE used here, for both species. From this it can be seen that the four 'lifetime' measures of RE are all closely correlated ($p < 0.01$), with the associations between the spawn : soma ratio and Σ RI, and between the measures of Browne & Russell-Hunter (1978) (*i.e.* $\Sigma P_r / (\Sigma P_r + \Sigma P_g)$) and Tinkle & Hadley (1975) (*i.e.* $\Sigma P_r / (\Sigma P_r + \Sigma P_g + \Sigma R)$) being the strongest. No statistically significant correlations were obtained between any one of these four measures and either of the two 'rate' - based measures (Calow, 1979, 1983 ("C"); and Russell-Hunter & Romano, 1981 (P_r / P_{gj})), nor indeed were significant correlations obtained between the two 'rate' - based measures themselves. This pattern applies to the data sets for both species.

8.3 DISCUSSION

Several reports of Reproductive Effort (RE) in semelparous molluscs are available for comparison with the present data. Of these the majority concern the spawn : soma ratio as an estimate of RE. Todd (1979a) found caloric spawn : soma ratios of 25 - 55% for *Adalaria proxima* and 82 - 143% for *Onchidoris muricata*, while Todd & Havenhand (1983) report values of 10 - 88% and 6 - 172% respectively for the same two species. Clearly, much variation exists, but the present results are generally higher than the values cited above (see Table 8.2). Although the precise reasons are unclear, this difference is probably attributable (at least in part) to differences in husbandry techniques. Hall (1983) gives caloric spawn : soma ratios for the nudibranch *Aeolidia papillosa*. These values range from 36% to 244% with a mean value of 137%, clearly within the range of observations recorded here. De Freese & Clark (1983) give mean spawn : soma ratios for a variety of Floridian opisthobranch species. These range from 6.3% to 66% with values for nudibranchs between 9% and 37%. These RE's are considerably lower than those discussed above but are not comparable: their values are for single spawn masses only and therefore may grossly underestimate the RE of some (but not necessarily all) species.

TABLE 8.3 Correlation matrix for measures of Reproductive "Effort"

(top right = *O.muricata* , lower left = *A.proxima*)

	$\Sigma P_r / W_{max}$	$\Sigma P_r / (\Sigma P_r + \Sigma P_g)$	P_r / P_{gj}	$\Sigma P_r / (\Sigma P_r + \Sigma P_g + \Sigma R)$	"C"	Σ RI
$\Sigma P_r / W_{max}$	-	0.908^{**}	0.454	0.849^{*}	-0.287	0.996^{***}
$\frac{\Sigma P_r}{(\Sigma P_r + \Sigma P_g)}$	0.972^{***}	-	0.578	0.906^{**}	-0.050	0.905^{**}
P_r / P_{gj}	0.620	0.576	-	0.482	0.091	0.458
$\frac{\Sigma P_r}{(\Sigma P_g + \Sigma P_g + \Sigma R)}$	0.978^{***}	0.977^{***}	0.614	-	-0.153	0.839^{*}
"C"	-0.121	-0.101	0.180	-0.053	-	-0.323
Σ RI	0.993^{***}	0.963^{***}	0.616	0.969^{***}	-0.177	-

(Asterisks indicate significance level: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$)

Spawn : soma ratios have also been reported for other gastropod species. Thus, for example, Grahame (1977, 1982) found mean values of 231% (1977) and 285% (1982) for the prosobranch *Lacuna pallidula* and 501% (1977) and 427% (1982) for *Lacuna vincta.* Browne & Russell-Hunter (1978) review spawn : soma ratios from five studies of freshwater semelparous gastropods. Estimates of RE varied there from 15% to 243% with an overall mean value of 91%. Thus it is clear that estimates of RE by spawn : soma ratios are highly variable both between species and between studies, in addition to the intraspecific variation noted earlier. The estimates obtained here are typical of those observed elsewhere.

Of the remaining methods of estimating RE, that of Russell-Hunter & Romano (1981) (*i.e.* P_r / P_{gj}) is probably the least used. Smith & Sebens (1983) employed this measure to estimate RE in the nudibranch *Onchidoris aspera*, obtaining values of 41 - 108% with a mean value of 63%. This is substantially lower than the mean values obtained here for *A.proxima* and *O.muricata* (444% and 617% respectively, Table 8.2). However, Smith & Sebens (1983) used the immediate pre-spawning growth rate as a measure of P_g. An unknown proportion of this 'growth' rate will have been due to the build up of reproductive tissues prior to spawning. Consequently P_g will have been overestimated and RE correspondingly underestimated.

The measure $P_r / (P_r + P_g)$ proposed by Browne & Russell-Hunter (1978) has also been little used in studies of RE in semelparous molluscs. Browne & Russell-Hunter (1978) in their review (*loc. cit.*) found a mean value of 30% for freshwater gastropods. Aldridge (1982) obtained similar values for the freshwater prosobranch *Leptoxis carinata.* No estimates of RE by this method were found for semelparous marine molluscs, although approximate values of 74% and 81% for *Lacuna pallidula* and *Lacuna vincta* (respectively) can be derived from the data given by Grahame (1977, 1982). These values are similar to those obtained here for *A.proxima* (60%) and *O.muricata* (76%) (Table 8.2). Despite the apparent dichotomy between the RE's of freshwater and marine species when this method is used, the relatively small number of data sets available for comparison do not permit a firm

conclusion to be reached.

Grahame (1982) has used the measure suggested by Tinkle & Hadley (1975) (*i.e.* P_r / A) to estimate the RE of *Lacuna pallidula* and *Lacuna vincta*. He found mean values of 59% and 63% respectively for the two species. These values are considerably in excess of those obtained here (*A.proxima*, 24%; *O.muricata*, 43%; Table 8.2). However, the values cited by Grahame (1982) are for mean weekly energy allocation during the reproductive period rather than lifetime totals (as used here). Strictly, therefore, Grahame's (1982) values should be compared with the Net Reproductive Efficiencies (NRE's) given in Table 7.2. The mean NRE's obtained here for *A.proxima* and *O.muricata* were 51% and 62% respectively indicating remarkable similarity in energy partitioning between the *Lacuna* spp. and the nudibranchs studied here.

No data for semelparous molluscs were available from which values of Calow's "C" (Calow, 1979, 1983) could be derived. However, since the "C" values obtained here are in excess of zero, both *A.proxima* and *O.muricata* can be regarded as being reproductively "reckless" (Calow, 1978).

The different measures of RE considered above have been applied more frequently in studies of iteroparous mollusc species. Thus, for example, spawn : soma ratios have been calculated for four *Littorina* species (Grahame, 1973; Hughes & Roberts, 1980) and four species of *Conus* (Perron, 1982). $P_r / (P_r + P_g)$ has been calculated for littorinids (Hughes & Roberts, 1980), a species of *Polinices* (Ansell, 1982), four *Conus* spp. (Perron, 1982), and *Mytilus edulis* (Thompson, 1979; Bayne *et al.*, 1983). P_r / A has been estimated for *Littorina littorea* (Grahame, 1973) and Calow's "C" has been quantified for scallops (Vahl, 1981) and for *Mytilus edulis* (Bayne *et al.*, 1983). [More complete reviews of different RE measures are given by Bayne & Newell (1983), Calow (1983) and Grahame & Branch (1985).]

In many of the above studies, a lack of precision in defining the terms and quantities

used has resulted in severe limitations to the number of (valid) comparisons which can be made. This point applies to semelparous as well as to iteroparous species. However, the complications of generation time and lifetime fecundity schedules in iteroparous species can create added confusion in these circumstances (Thompson, 1983).

Hughes & Roberts (1980) attempted to overcome some of these complications by standardising their data with respect to generation time. This technique has also been applied with some degree of success by Bayne & Newell (1983) to data for *Mytilus edulis*. However, to obtain useful (*i.e.* comparable) data, such techniques must also be accompanied by an analysis of the lifetime fecundity schedule (Pianka & Parker, 1975). This in itself can be difficult since such a schedule requires an accurate estimate of the longevity of the individual(s) under scrutiny. In the absence of such estimates, the utility of any statement regarding RE per year or per generation is necessarily restricted. The latter argument applies as readily to semelparous species which lay more than one spawn mass as it does to iteroparous species because the former also display a fecundity schedule, albeit on a reduced scale (*cf.* Figures 6.5 & 8.2; Grahame, 1977, (his Figures 1 & 2); Todd, 1979a, (his Figure 2)). Perhaps most importantly, this can lead to a misinterpretation of estimates of RE when these are based on rate measurements (*e.g.* P_r / P_{gj} (Russell-Hunter & Romano, 1981) and Calow's "C" (Calow, 1979, 1983)). Clearly, if the balance of energy partitioning changes through the reproductive period, then the estimate of RE will depend on when the constituent parameters were obtained within that period. Moreover, since the different methods of estimating RE often use different parameters, this effect may be more pronounced in some measures than in others.

To an extent, this problem arises from the lack of accurate definitions which was discussed earlier. However, in semelparous species and (partially) in iteroparous species this can be avoided by standardising on measures of RE which are based on lifetime totals rather than on mean rates. This is not to deny the utility of rate-based measures of RE since they can yield valuable information relating to the dynamics of reproductive allocation within any one individual or species. However, rate-based measures do not accurately represent the

'cost' of reproduction over the entire lifetime of an organism and it is this 'cost' upon which major selective pressures are presumed to operate.

Given the above considerations, a question arises as to which of the measures of RE can be regarded as the 'best'. Several criticisms of the various measures of RE have already been presented (see Introduction, Section 8.1). The present results however, permit further evaluations to be made.

It is apparent from Table 8.3 that the spawn : soma ratio, $P_r / (P_r + P_g)$, P_r / A and $\sum RI$ are all very closely correlated. The implication is therefore, that although the magnitude of RE may vary with respect to which measure is used, the variation observed between individuals of any one species will not; *i.e.* all of these measures should be equally effective in reflecting variations in individual "effort". However, some measures are more widely applicable than others, whilst some are inherently unsound. For example, Calow's (1979) criticism of spawn : soma ratios (on the grounds that the underlying assumption of isometry or allometry of fecundity with body size was invalid), is supported by the data presented in Figures 8.2 and 8.3.

Both $P_r / (P_g + P_r)$ and P_r / A provide estimates of RE comparable with those of other studies. The former has the disadvantage of not accounting for possible fluctuations in respiration during the reproductive period (Calow, 1979, 1983). However, given the relative constancy of respiration rate (Chapter 5, Section 5.3), this criticism has no bearing here. More importantly, $P_r / (P_r + P_g)$ does not require a (time-consuming) estimate of respiration rate, unlike P_r / A. Perhaps the greatest advantage of using $P_r / (P_r + P_g)$ as an estimate of RE, however, is the relative ease with which the necessary parameters can be acquired. Thompson (1983) in reviewing a variety of measures of RE concluded that if the time required to obtain the component parameters was taken into account, $P_r / (P_g + P_r)$ gave the best results. Moreover, he demonstrated that more complex measures such as $P_r / (P_r + P_g + R)$ yielded only marginally better results. The utility of this measure is

further enhanced if it is only applied to lifetime parameters since under these conditions, criticisms of the non-linear properties of the measure which are apparent when P_g is negative (*i.e.* degrowth) (Thompson, 1983), do not apply.

The three measures of RE discussed above, and the measure P_r / P_{gj} of Russell-Hunter & Romano (1981) are essentially similar in that they all estimate the reproductive effort of an individual *sensu* Tinkle & Hadley (1975). However, the measure of Russell-Hunter & Romano (*loc. cit.*) is highly variable (Table 8.2), time-consuming to obtain, and shows neither a clear relationship to other measures of RE (Table 8.3), nor comparability between studies (*cf.* Russell-Hunter & Romano 1981; Smith & Sebens, 1983). Its utility under the present circumstances therefore seems questionable.

In contrast to the above measures, $\sum$ RI and Calow's "C" attempt to measure not reproductive "effort" *per se* but rather the consequent 'cost' of reproduction to the parental organism.

Calow's "C" is a potentially powerful tool for determining the response of an organism to selective pressure (see, for example, Bayne *et al.*, 1983). Moreover, it does not suffer from the mathematical limitations seen in some other measures (Thompson, 1983). However, because it is a rate-based measure, it can be expected to suffer from some of the restrictions discussed earlier. It is interesting to note, therefore, that although *A.proxima* appears to be more "reckless" than *O.muricata* (mean "C" values of 0.65 and 0.54 respectively, Table 8.2), this difference was not significant (Mann-Whitney test, $p > 0.20$). In view of the markedly higher degrowth rate of *A.proxima* (Chapter 6, Section 6.3) this lack of significance is perhaps surprising, and suggests that the cost of reproduction on a daily basis may be approximately equivalent in *A.proxima* and *O.muricata.*

A very different result is obtained if reproductive cost is estimated as $\sum$ RI. The mean $\sum$ RI values for *A.proxima* and *O.muricata* are 0.90 and 2.48 respectively (Table 8.2). These values imply that although overall costs of reproduction do not have to be

wholly met by degrowth (both values are greater than zero), the 'cost' of reproduction in *A.proxima* is considerably greater than in *O.muricata*. A limitation in using this measure arises, however, when total P_r is estimated by the total spawn output since the latter is almost certain to be an underestimate of the former. This will result in an overestimation of each RI and therefore a consequent overestimation of $\sum$ RI. More importantly, this error will be greater in individuals which spawn many times since the error in RI will be summed a correspondingly greater number of times. In the absence of this limitation the mean $\sum$ RI for *O.muricata* might be expected to be lower, and the mean $\sum$ RI for *A.proxima* may approach zero.

Clearly, $\sum$ RI is a more appropriate and informative measure than simple spawn : soma ratios and, in organisms which undergo significant degrowth during reproduction, may prove to be of considerable value. In the context of the present work, the measure clearly highlights the greater dependence of *A.proxima* on energy acquired from degrowth to support reproduction. Graphical representation of total spawn production on $\sum$ RI (*e.g.* Figure 8.4) can therefore reveal much information relating to the energetic costs of degrowth. However, this measure is essentially a weight-specific measure of production and therefore will not resolve the relative 'costs' of reproduction in species which do not exhibit degrowth. Under such circumstances, the measure of Browne & Russell-Hunter (1978) (*i.e.* $P_r / (P_r + P_g)$), or Calow's "C" may yield more useful results.

Herein lies one of the major difficulties in reliably estimating (or measuring) RE in its broadest sense: the most appropriate measure may vary according to the biology of the species under study. Moreover, logistic constraints may impose further restrictions. Undoubtedly such considerations have contributed to the development of the great variety of procedures and methods used in published studies. Nonetheless, measures which are readily obtained (such as $P_r / (P_g + P_r)$) could still be calculated whilst noting any possible causes of deviations or inaccuracies.

Some authors (*e.g.* Slobodkin, 1959; Christiansen & Fenchel, 1979) have assumed

RE to be effectively constant both between species and between different life-history strategies. Given the difficulties of defining and measuring RE whilst taking sufficient account of fecundity schedules, longevity, and the applicability of different measures, such assumptions seem tempting (indeed, for theoretical applications they may be the most justifiable). It must be remembered however, that RE is a component measure which is of use only in a comparative sense. Although selection may operate to increase fecundity, for example, the corresponding increase in RE (assuming other factors remain constant) is a result rather than a cause of that selective pressure. Thus RE is a tool which is used to study differences in life-history traits, it is not a trait itself and therefore cannot be directly affected by natural selection. This distinction is important when formulating and analysing life-history theory.

Notwithstanding the above, the requirement for well defined and documented data against which life-history theory and different measures of RE can be tested remains considerable. Certainly, in the absence of such data, little if any progress will be made.

CHAPTER 9

EMBRYONIC AND LARVAL CULTURE

9.1 INTRODUCTION

The first comprehensive ecological study of the larvae of benthic marine invertebrates was that of Thorson (1946). He identified three major functional groupings according to the parental zygotic investment and the trophic requirements of the larva. The first of these groups, long-term planktotrophy, included larvae which require to feed in the plankton in order to complete development and attain morphological and physiological competence to settle and metamorphose. These larvae, by virtue of their long pelagic life and their "enormous numbers" were deduced to be able to disperse over considerable distances. The second category he identified were short-term planktotrophic larvae for which "dispersal is the main object", although these larvae can and do feed in the plankton. Thirdly were lecithotrophic larvae which subsist on stored yolk reserves and for which the sole purpose of a pelagic phase is dispersal. Thorson (1946) failed to observe lecithotrophic larvae in any opisthobranch mollusc. More recent studies have resulted in slight changes in classification such that very long-term planktotrophic larvae, potentially capable of crossing ocean basins are referred to as "teleplanic" (Scheltema, 1978, & refs. therein), and non-pelagic, lecithotrophic larvae are sometimes referred to as "direct" developers (although, strictly, this is different to non-pelagic lecithotrophy, see *e.g.* Jablonski & Lutz, 1983).

Many authors have considered the relative advantages and disadvantages of these different larval types and their associated effects on (for example), energetics, gene-flow, fecundity, trophic relationships, dispersal (and, therefore, delay of metamorphosis) and total pre-juvenile development time. Available data have recently been reviewed by Day & McEdward (1984), Grahame & Branch (1985), Strathmann (1985) and Todd (1985). Much of this work has involved theoretical modelling (*e.g.* Vance, 1973a,b, 1980, 1984; Christiansen & Fenchel, 1979; Obrebski, 1979; Pechenik, 1979; Caswell, 1981; Jackson & Strathmann, 1981; Palmer & Strathmann, 1981; Grant, 1983), and comparatively few field

and experimental observations exist.

Bayne (1983) reviews the physiological ecology of mollusc larvae, and notes that the great majority of work to date has concerned commercially important species of bivalve, (but see, for example, Pechenik, 1980).

The ontogeny and culture of nudibranch larvae has been comprehensively reviewed by Todd (1981) and by Hadfield & Switzer-Dunlap (1984). Both of these reviews include some discussion of the ecological implications of the observed developmental patterns. (For a more general treatment of this subject, see Strathmann, 1985). The present study has developed the methods previously found to be successful for rearing *Onchidoris bilamellata* (Todd, 1981) with the aim of elucidating the major parameters affecting larval growth and longevity in *Adalaria proxima* and *Onchidoris muricata*. In this respect, the present study is the first report of growth and metamorphosis of *O.muricata* larvae in laboratory cultures.

9.2 MATERIALS & METHODS

Algal Culture

Three species of unicellular algae were cultured as larval food. These were *Isochrysis galbana* (Parke), *Pavlova* (= *Monochrysis*) *lutheri* (Droop), and *Rhodomonas* sp.. All three species are naked flagellates. *I.galbana* and *P.lutheri* are both approximately 3 - 4 µm in diameter whereas *Rhodomonas* is an ellipsoid with a maximum dimension of ~ 15 µm. Innocula of all three species were obtained from the Culture Centre for Algae & Protozoa, Cambridge.

All the glassware used for algal (and larval) culture was previously unused. At all times glassware was cleaned in hot water only, and autoclaved prior to use (detergents were never used).

Algal suspensions were batch cultured in 1 l and 2 l flasks held in constant light at

room temperature. All cultures were constantly aerated with 0.22 µm filtered compressed air. Cultures were maintained in Provasoli's E.S. medium (Provasoli 1968) which had been passed through a sterile 0.22 µm filter prior to use. New batch cultures were started approximately weekly and two or three cultures of each species were maintained simultaneously. This methodology allowed algal cells which were in log-phase growth to be harvested at any time. Only these 'log-phase' algal cells were used in larval cultures.

In addition to the batch cultures, stock cultures of each algal species were maintained in parallel. These cultures were kept in ~ 100 ml of E.S. medium in screw-top 250 ml conical flasks. Stock cultures were not aerated although the screw cap was always loose to permit the passage of gases. Batch cultures were set up using innocula of 2 ml of stock culture suspension. New stock cultures were set up approximately every 2 months.

When algae were required for larval culture experiments, an aliquot of algal culture suspension was drawn off from a batch culture using a sterile pipette. This aliquot was centrifuged at either 3000 rpm (*I.galbana* and *P.lutheri*) or 1500 rpm (*Rhodomonas* sp.) for four minutes. The supernatant was decanted and the pellet was resuspended in 0.22 µm filtered sea-water (FSW). This process of centrifugation and resuspension was repeated once prior to counting and use.

Cell densities in algal suspensions were counted on a Coulter Counter Model D fitted with a 50 µm aperture tube. At all times, mean densities of five repeat counts were used.

Spawn Mass Culture

A number of *Adalaria proxima* and *Onchidoris muricata* individuals were maintained in the manner previously described (Chapter 4, Section 4.2) solely for the production of spawn masses. Newly laid spawn masses were carefully excised from the substratum (usually a "Teaboy" container) using a scalpel and fine forceps. Spawn masses were examined for cleavage using a Wild M8 stereomicroscope. Only spawn masses in which the embryos had not yet begun to divide were used for experimental purposes.

Uncleaved spawn masses were incubated in air-saturated FSW in a test-tube placed in a constant temperature water-bath. Each test-tube was agitated daily and the FSW was replaced with new, air-saturated FSW every alternate day. In initial experiments the test-tubes were constantly aerated. However, it was found that this procedure frequently led to rapid disintegration of the spawn mass, and for this reason constant aeration was discontinued. All spawn masses were examined periodically in order to record the developmental stage of the embryos.

"Hatching" was defined as the first emergence of swimming larvae. This is especially important when considering lecithotrophic larvae which, in contrast to planktotrophic forms, do not appear to hatch within only a short period.

Larval Culture

Newly hatched larvae were concentrated using a "Tri-pour" filter (Plate 9.1). This filter consisted of a polyethylene "Tri-pour" 100 ml beaker from which the bottom had been removed and replaced with 41 μm mesh gauze. This filter was nested in a glass 100 ml beaker filled with FSW such that the edges of the "Tri-pour" vessel rested on the mouth of the glass beaker. This assembly permitted the concentration of any volume of larval suspension by pouring this through the Tri-pour filter, (Plate 9.2). Moreover, in this way, larval contact with the air-water interface was minimised. The latter is important because the shells of larval nudibranchs are hydrophobic and readily become trapped in the surface film, because of this many larvae may become 'rafted' together at the surface.

After initially concentrating the newly hatched larvae, they were rinsed by pouring FSW through the filter assembly. Larvae were further concentrated within the filter assembly by spinning the "Tri-pour" within the glass beaker. The vortex thus created caused larvae to collect at the centre of the filter. By gently raising the filter within the beaker larvae became trapped and temporarily immobilised on the filter gauze. These larvae could be briefly resuspended (and yet remain concentrated) by lowering the filter a few millimeters. Again these larvae could be temporarily immobilised by raising the filter within the glass

PLATE 9.1 A "Tri-pour" filter (see text for explanation)

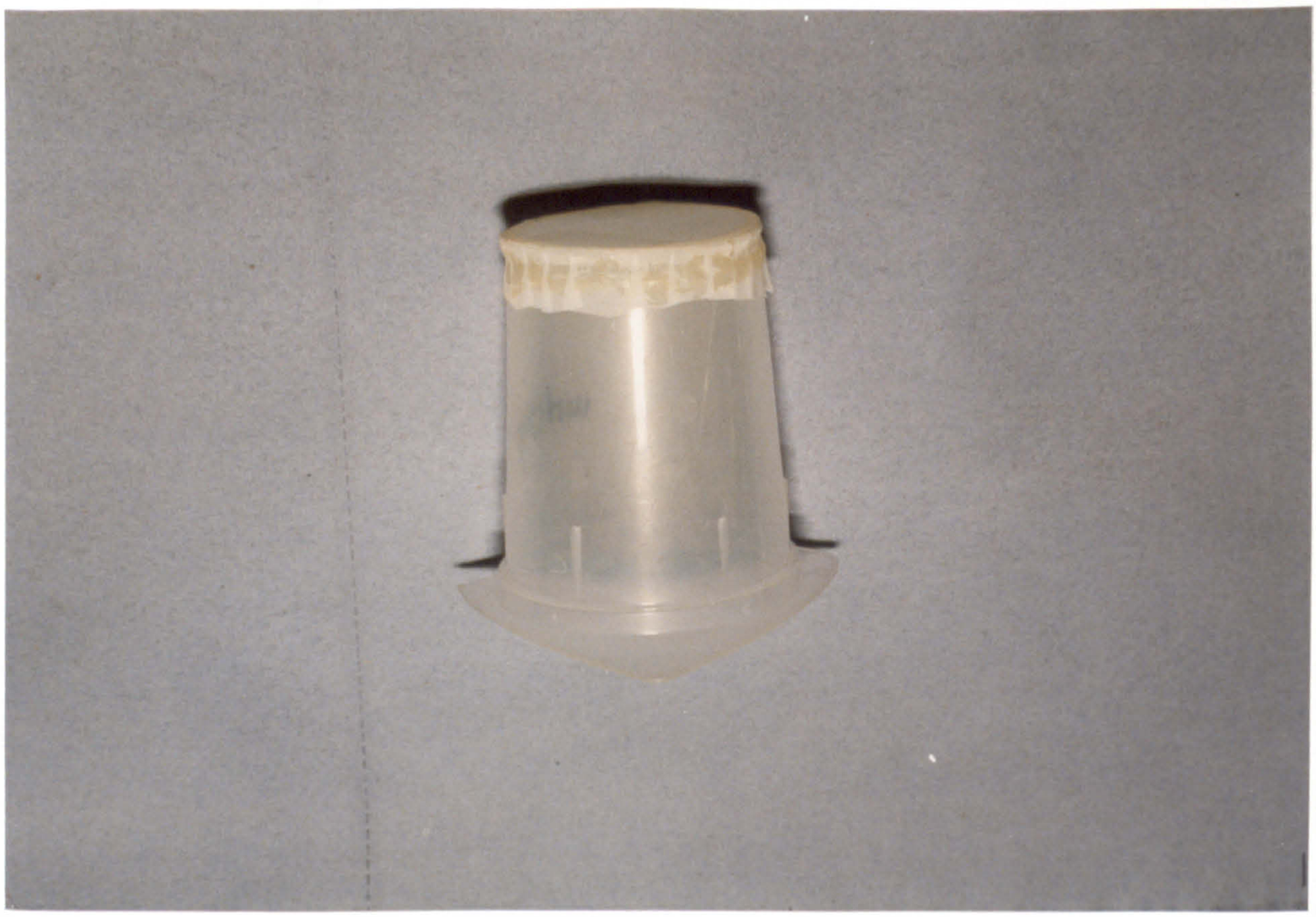
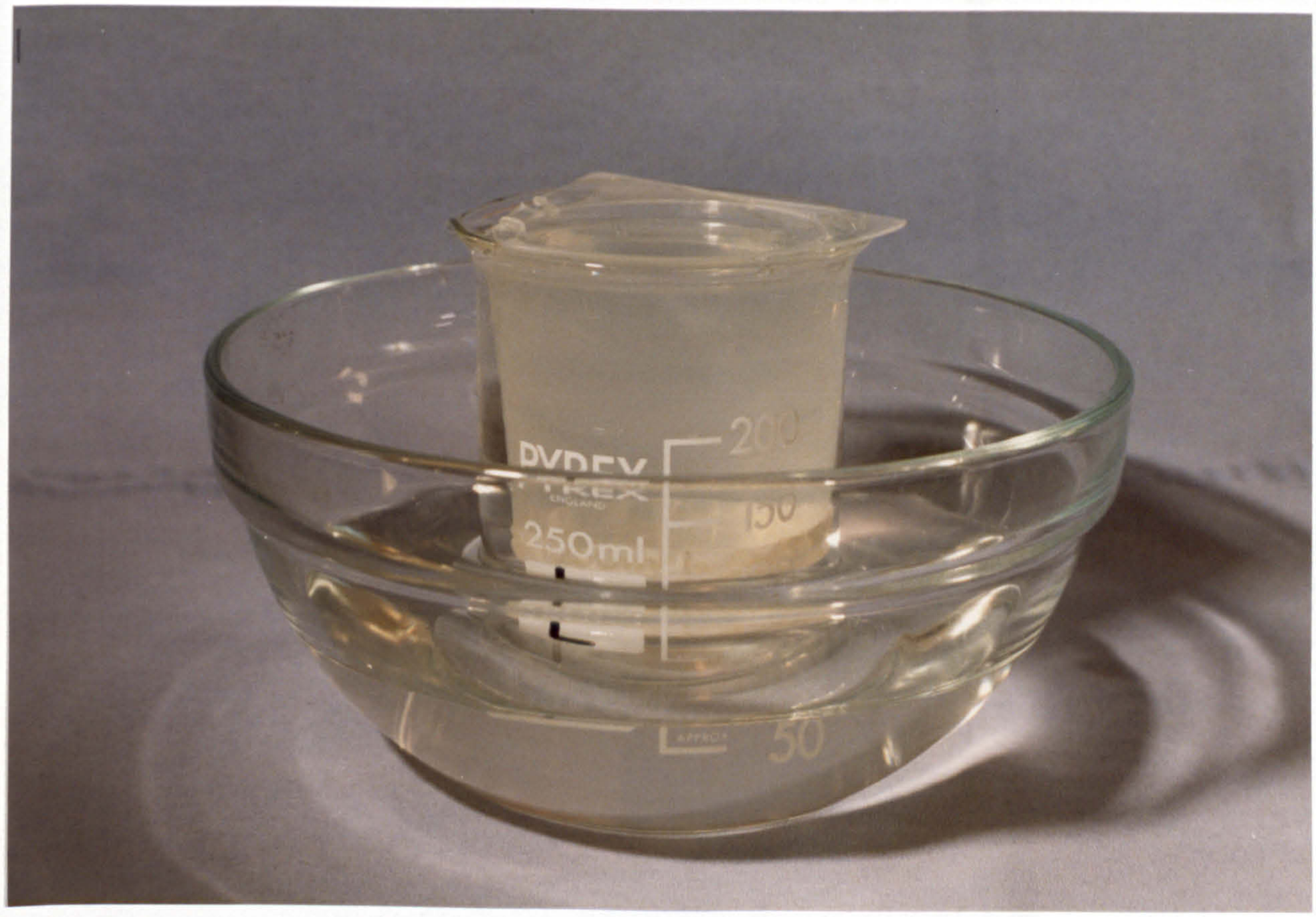


PLATE 9.2 Larval filtration assembly (see text for explanation)


PYREX
250ml
200
150
100
50

beaker. Larvae which had been concentrated in this way were then removed using a sterile Pasteur pipette and introduced to the larval culture vessel. At all times during this process, the filter gauze remained below the water surface.

Larvae were cultured in FSW at an approximate density of 2 larvae.ml^{-1}. Algal suspensions were added to the cultures to provide a total algal concentration of 50 cells.µl^{-1} (in both monocultures and 'mixtures'). Bacterial and ciliate infestations of cultures were controlled by adding a mixture of Streptomycin Sulphate and Penicillin G at final concentrations of 50 µg.ml^{-1} and 60 µg.ml^{-1} respectively. The size of larval culture vessel used varied from 400 ml to 100 ml depending upon the number of larvae. All culture beakers were covered with "Parafilm" and incubated in constant temperature water baths at one of a range of temperatures. All cultures were maintained in constant light.

Larval culture media were changed approximately every four days. Procedures for changing culture media were the same as those described above for setting up cultures of newly hatched larvae. In any one culture, the same species (or mixtures of species) of algal food were used throughout. After changing the culture media any larvae which had become rafted at the water surface were re-suspended by gently dripping FSW onto them from a Pasteur pipette.

At hatching and at each subsequent change, a sample of larvae (n = 12) was removed from each culture and the maximum shell dimension (from the tip of the shell aperture) was measured using an eyepiece graticule fitted to a transmission light microscope.

When larvae had progressed to the stage where a fully developed propodium was visible, a sample of larvae was removed to test these for metamorphic competence. In these tests, a known number of larvae (approximately 10) were placed in clean filtered seawater in a small evaporating dish or embryo dish. For the evaluation of competence to metamorphose, a colony of *Electra pilosa* encrusting *Fucus serratus* was added. However, in some experiments, *F.serratus* only (no *E.pilosa*) was added. In the latter, FSW-only controls were also run. These competence trials were inspected two or three times daily until

all the visible larvae had either metamorphosed or died.

9.3 RESULTS

Spawn Mass Culture

Attempts were made to culture a total of 149 spawn masses of *A.proxima* and *O.muricata*, the results of which are summarised in Table 9.1. In all cases "Days to Hatch" (Table 9.1) are the days from oviposition to the first observed hatch. In *O.muricata* all larvae hatched from a given spawn mass within a few hours. In *A.proxima* hatching occurred over ~ 3 days (1 - 2 days if agitated).

Perhaps the most important outcome was the observation that for all the temperatures where comparisons can be made, the embryonic development time (*i.e.* the time from egg deposition to hatching) for *A.proxima* was approximately twice that for *O.muricata*, (Table 9.1).

Surprisingly, the embryonic development time of *A.proxima* at 6 °C was in excess of that at 4 °C (68 and 65.5 days respectively, Table 9.1). However, this difference was not significant (Mann-Whitney test, $p = 0.165$), and given the high mortality rate in 6 °C cultures (only 2 out of 16 spawn masses survived to hatch), this result must be treated with caution. Overall, spawn mass survivorship was low. This result may be representative of field conditions, although it is likely that a considerable degree of microbial infestation occurred through the cut surface of the spawn mass (where the mass had originally been adherent to the substratum).

The Q_{10} values for *O.muricata* (Table 9.1) show that development time was most affected by temperature in the range 5 -10 °C. Above 10 °C, further increases in temperature did not elicit such a rapid decrease in development time. Surprisingly, this effect also appears to apply between 4 and 5 °C. Although temperature control was precise

TABLE 9.1 Summary of spawn mass culture data for *A.proxima* (top) and *O.muricata* (bottom)

Temperature (°C)	Number of Masses Studied	Number of Masses Hatched	Days to Hatch (mean ± 1 s.e.)	Q_{10}'s	
4.0	9	4	65.5 ± 0.56	0.83	3.61
6.0	16	2	68.0 ± 0.71	7.55	
10.0	19	6	30.3 ± 0.99		
16.0	16	0	-		
			Geometric mean Q_{10} = 2.50		
4.0	9	2	34.0 ± 0.0	1.57	3.06
5.0	9	2	32.5 ± 0.35	5.32	
6.0	22	4	27.5 ± 0.25	5.61	
10.0	30	17	13.8 ± 0.22	2.08	
16.0	19	10	8.9 ± 0.22		
			Geometric mean Q_{10} = 3.14		

(temperature variations were kept below 0.1 °C), the sample sizes involved at 4 ° and 5 °C were comparatively small and the possibility of sampling error therefore arises. In general, however, the Q_{10}'s for *A.proxima* and *O.muricata* embryonic development time were similar.

The size of uncleaved ova was not determined in either *A.proxima* or *O.muricata* spawn masses.

Larval Culture & Metamorphosis - *O.muricata*

A total of twelve separate spawn masses of *Onchidoris muricata* were hatched and the larvae cultured. Mean shell size of hatching larvae was found to vary considerably between spawn masses, (Kruskal-Wallis test, $H_{adj} = 83.77$, $p << 0.001$), ranging between 120 µm and 157 µm. The overall mean size (± one standard error) of all hatching larvae measured was 143.7 ± 0.85 µm. Of the twelve initial spawn masses (separated into 23 individual cultures), only eight cultures survived to reach metamorphic competence. Mortality of cultures showed no systematic bias with regard to algal diet, with the exception that none of the four cultures fed on *P.lutheri* alone survived to metamorphosis. Summary characteristics of these eight cultures are given in Table 9.2, and plots of mean size (± two standard errors) and range of sizes over time, are shown in Figures 9.1 & 9.2.

It can be seen from Table 9.2 that mean growth rates at 5 °C were lower than at 10 °C. Although much variation exists (Figures 9.1 & 9.2), this difference is significant (Mann-Whitney test, $p = 0.037$). The difference between maximum growth rates at these temperatures is also significant at the same level. However, no significant difference was found between mean growth rates at 10 °C and maximum growth rates at 5 °C (Mann-Whitney test, $p >> 0.05$). These contrasts in growth rates are reflected in the lengths of the larval period (Table 9.2). Mean length of the larval period at 10 °C was 42 days, almost exactly half that observed at 5 °C (= 87 Days). A similar comparison can be drawn between the time taken for the eyespots to first appear (mean times of 62 and 29.4 days at 5 °C and 10 °C respectively). However, no significant differences could be detected

TABLE 9.2 Summary of larval culture data for *Onchidoris muricata*

Temperature (°C)	Algal Food*	Appearance of Eyespots (days)	(μm)	Growth Rate (μm.d^{-1}) mean	max.	First Metamorph observed (days)	Shell Size at Metamorphosis (μm)
5	*Rh.*	66	273	1.85	2.39	80	304
5	*I.g.*	63	281	1.76	2.52	> 92†	≥ 304†
5	*I.g.*	57	279	2.00	3.29	94	306
10	*Rh.*	26	279	3.53	6.61	38	312
10	*Rh.*	32	229	3.06	4.60	45	304
10	*I.g.*	26	248	2.92	5.59	43	295
10	*P.l.*	31	221	2.18	4.10	> 46†	≥ 298†
10	MIX	32	287	3.42	6.08	42	307

* *Rh.* = *Rhodomonas* sp.; *I.g.* = *Isochrysis galbana* ; *P.l.* = *Pavlova lutheri* ; MIX = 1 : 1 : 1 mixture of the above three species at the same (total) concentration as used in the monospecific diets

† No metamorphosis was observed in these cultures

FIGURE 9.1 Growth of *Onchidoris muricata* veligers on different algal diets at 5 °C.
Different time axes apply to different sibling groups

(means ± 1 std. error)

Solid line & squares = *Isochrysis galbana*

Broken line & circles = *Rhodomonas sp.*

M at arrow indicates date of first metamorph

- at arrow indicates no metamorphosis observed

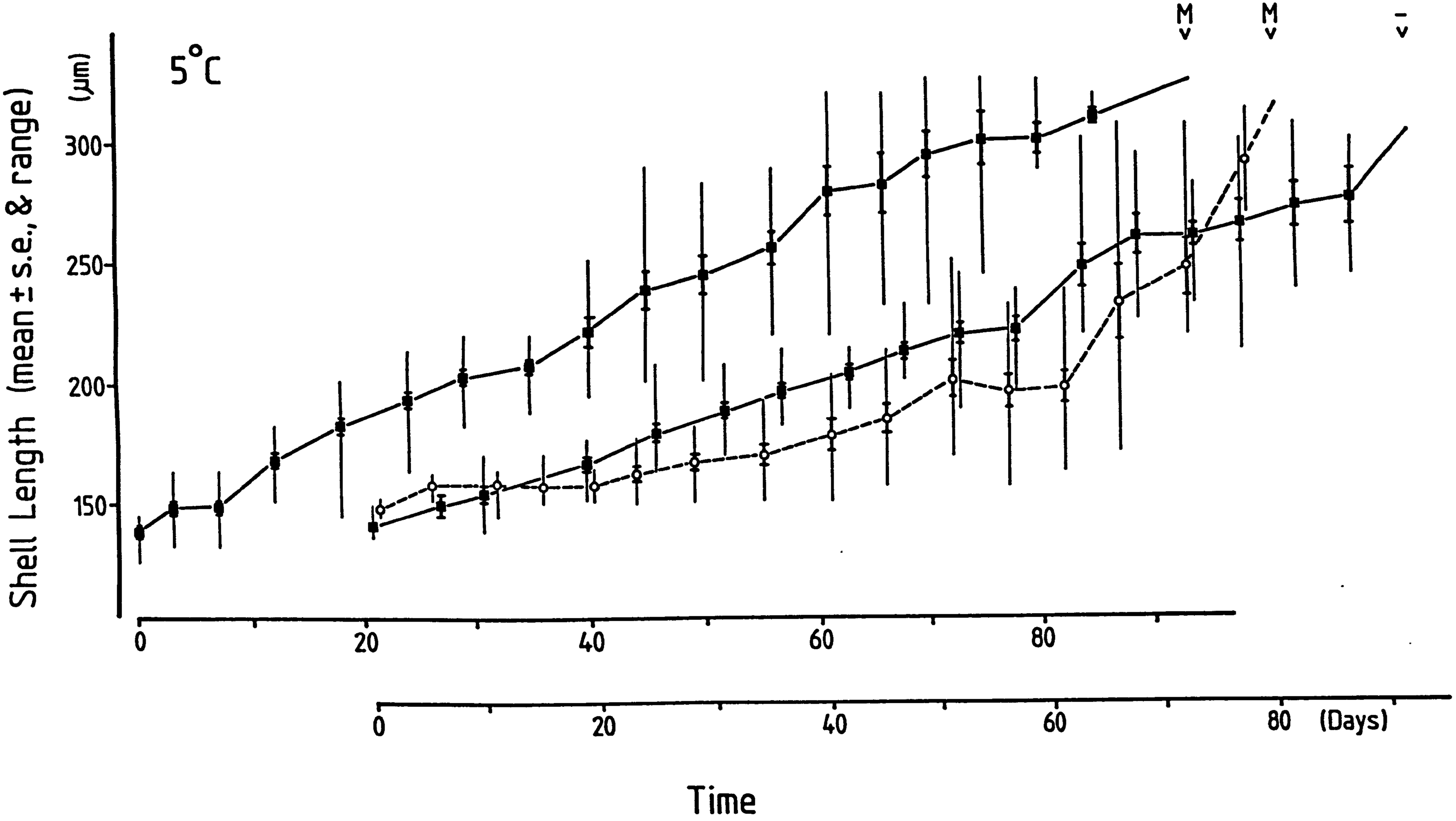
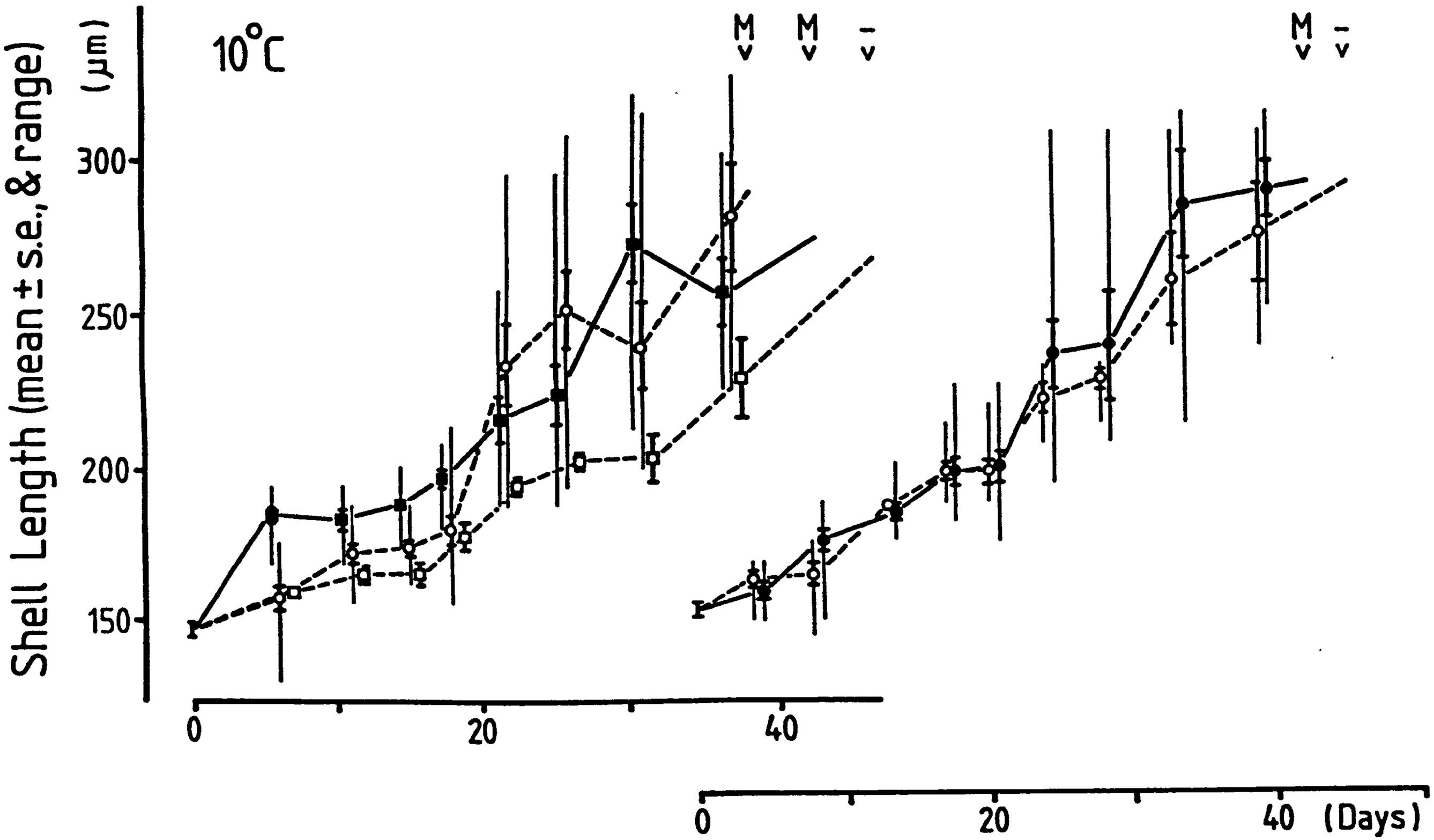

5°C
M
M
Shell Length (mean ± s.e., & range)
(µm)
300
250
200
150
0
20
40
60
80
0
20
40
60
80
(Days)
Time

FIGURE 9.2 Growth of *Onchidoris muricata* veligers on different algal diets at 10 °C.

(notation as for Figure 9.1)

Solid line & squares = 1 : 1 : 1 Mixture of
Pavlova lutheri, I.galbana & *Rhodomonas sp.*
Broken line & circles = *Pavlova lutheri*


10°C
M
M
Shell Length (mean ± s.e., & range) (μm)
300
250
200
150
0
20
40
0
20
40 (Days)
Time

between shell sizes at the different temperatures when measured either at the appearance of eyespots (Mann-Whitney test, $p = 0.456$) or at metamorphosis (Mann-Whitney test, $p = 1.000$).

The results presented in Table 9.2 suggest that algal diet may have had some influence on development. At either of the two temperatures used, the time taken for the appearance of eyespots was generally longer when the larvae were fed on *Rhodomonas* sp. than when fed on *I.galbana*. However, total larval development time ("First metamorph observed", Table 9.2) showed no such pattern. Indeed the suggestion is that this period was generally shorter in cultures which were fed on *Rhodomonas* sp.. This pattern may be explained in part by the shape of the growth curves for larvae cultured on *Rhodomonas* and *I.galbana* (Figure 9.1). At 5 °C, growth of *O.muricata* larvae is virtually linear when fed *I.galbana*, however curvilinear, almost exponential, growth was observed in the culture which was fed *Rhodomonas*. It is perhaps relevant to note at this point that whilst variation in larval size and growth rate was generally high in all the cultures studied, this variation was greater in the 5 °C *Rhodomonas* culture than in either of the 5 °C *I.galbana* cultures.

The overall Q_{10} for mean larval development time is 4.291 which compares favourably with a Q_{10} of 3.897 calculated from (the means of) the maximum growth rates. However, both of these figures represent upper bound estimates since the data used in their derivation are from the fastest growing larvae. Perhaps the more realistic Q_{10} value is that of 2.612 obtained from the mean growth rate data given in Table 9.1.

All larvae of *O.muricata* which survived to attain competence were provided with *Electra pilosa* encrusting *Fucus serratus* to stimulate metamorphosis. Some were observed to metamorphose within 24 hours of contact with this stimulus, although many larvae (of similar size and gross morphology) failed to metamorphose at all. Metamorphosis was characterised by a cessation of swimming, the pediveliger preferring to crawl across the substratum. This crawling was generally followed by the casting of the velar cilia, resorption of the velar lobes and casting of the shell and operculum prior to visceropedal

fusion resulting in the formation of the post-metamorph. In some individuals, the shell was cast (either with or without the operculum) prior to the cessation of swimming and casting of the velar cilia. Although the majority of such individuals died soon afterwards, some did, in fact, metamorphose successfully.

No direct observations of post-metamorphs preying upon *E.pilosa* were made. However, several post-metamorphs were observed adhering to the walls of newly formed *E.pilosa* zooids. The frontal membranes of these zooids (which were initially intact) had been ruptured. Moreover, the viscera (visible through the dorsum) were of an opaque cream colouration in contrast to the mid-brown colour of the viscera in one- and two-day old post-metamorphs. These juveniles had metamorphosed approximately five days prior to the above observation, and were fully formed with a complete mantle fold, tubercle rudiments on the dorsum and visible rhinophore rudiments. The ontogeny of these features was similar to that described for *Onchidoris bilamellata* by Todd (1981), however the time-course of these changes was not recorded here.

Larval Culture & Metamorphosis - *A.proxima*

The results of metamorphosis experiments undertaken using larvae of *A.proxima* are summarised in Table 9.3. Mean shell size at hatching was 277.9 µm ± 3.73 (standard error), (n = 20). No measurements of between-spawn mass variation in shell size at hatching were made. All cultures were maintained at 10 °C throughout. At all times for all larvae observed, the mantle fold was retracted from the margin of the larval shell and no evidence of shell growth was found.

It is clear from Table 9.3 that successful metamorphosis of larvae occurred more frequently in the presence of *Electra pilosa* than when this stimulus was absent. However, the successful (spontaneous) metamorphosis of one of the larvae maintained in FSW only, indicates that such settlement cues are not essential for development to proceed. Larvae of *A.proxima* were also observed to settle and metamorphose in the presence of the gelatinous bryozoan *Alcyonidium hirsutum* (Fleming), (although no time course data were recorded for this observation).

TABLE 9.3 Metamorphosis of *Adalaria proxima* pediveligers using different metamorphosis cues.

Culture No.	Cue	Microalgal Food	Initial no. of larvae	Final No. of metamorphs	First metamorph seen*	Last veliger seen†
	None	None	10	0	-	194h (~8d)
	"	"	5	0	-	146h (~6d)
	"	"	11	0	-	144h (6d)
#1	"	"	5	1	137h (~6d)	165h (~7d)
#2	"	"	5	0	-	165h (~7d)
	"	*Rhodomonas*	11	0	-	218h (~9d)
	"	*Rhodomonas*	10	0	-	168h (7d)
	E.pilosa	None	8	2	43.5h	122h (~5d)
	E.pilosa	"	10	4	48.5h	145h (~6d)
#3	*E.pilosa*	"	6	6	66.5h	90h (~4d)
#4	*E.pilosa*	"	5	3	71.0h	90h (~4d)

* Time since "hatching"

† see text for explanation

Because of the experimental technique employed, it was not always possible to locate all the individuals within a given experimental culture simultaneously. Moreover, the presence of an empty larval shell did not necessarily indicate that a larva had metamorphosed since many individuals died before the onset of metamorphosis. Therefore the time when the last swimming veliger was observed (Table 9.3) is an estimate of the (maximum) longevity of larvae prior to metamorphosis or death. (Clearly, in cultures where no metamorphs were seen, only the latter applies). The data presented in Table 9.3 indicate that the length of the larval period was extended in the absence of a settlement cue. Perhaps of more interest is the observation that the presence of microalgal food further extended this longevity, although this effect was not substantial. An analysis of the length of the larval periods indicated that significant variation exists, (Kruskal-Wallis test, $H_{adj} = 7.271$, $p < 0.05$) between the different treatments. Further analysis of these data showed that although (in the absence of a settlement cue) larval period was longest when microalgal food was present, there was no significant difference between this and either of the other two treatments (Mann-Whitney tests, $p > 0.05$). However, a significant difference was noted between the length of the larval period in the presence of a settlement cue, and that when no cue was presented, (Mann-Whitney test, $p = 0.037$).

In addition to the above observations, the time-course of metamorphosis in FSW-only treatments was less rapid than when *E.pilosa* was presented (Table 9.4). As in the larval cultures, the data given in Table 9.4 apply to the most rapidly developing larvae seen in any one culture. In a manner analogous to that seen in metamorphosing *O.muricata*, some *A.proxima* larvae were observed to evacuate their shells before casting the velar cilia and subsequent regression of the velar lobes. Only a very small proportion of these individuals survived to metamorphose successfully.

TABLE 9.4 Time-course of metamorphosis in *Adalaria proxima*

All larvae hatched at t - 48h. Culture numbers indicate cultures in Table 9.3 (n.r. = not recorded)

	Larvae in FSW only		Larvae + *E.pilosa*	
Culture no.	#1	#2	#3	#4
Shell Cast	23h	<18.5h	<18.5h	<18.5h
Operculum Cast	23h	23h	<18.5h	<23h
Velum Regressed	42h	23h	18.5h	<23h
Viscero-pedal Fusion	3 days	-	42h	42h
Full mantle fold	n.r.	-	n.r.	3 days
Rhinophore rudiments	n.r.	-	11 days	12 days

9.4 DISCUSSION

The embryonic development and larval biology of *Adalaria proxima* has been detailed comprehensively by Thompson (1958a). He reports the diameter of uncleaved *A.proxima* ova to be 180 µm, although other workers (Todd, 1979a; Todd & Havenhand, 1985) have found egg diameters somewhat smaller than this (165 µm, Todd & Havenhand, 1985; Todd, 1986a). Egg sizes were not recorded here and therefore no comparisons can be made. However, from the above data it appears that some variation in egg diameter may exist.

Thompson (1958a) also reports an embryonic period of 36 - 39 days at 10 °C for *A.proxima*. This figure is greater than that observed here (30.3 days, Table 9.1), and again indicates that some degree of variation may be present.

The data presented in Table 9.3 lend support to Thompson's (1958a) observation that *A.proxima* undergo an obligatory, pre-competent pelagic phase of approximately 48 hours duration prior to metamorphosis. However, more recent observations (C.D.Todd, unpubl. results) indicate that this may not be the case, with some larvae settling almost immediately after hatching (see also Chapter 10, Section 10.4). Inevitably, some degree of confusion and variability will be present because a larva which hatched from its egg capsule may have remained within the egg mass for some considerable time before emerging (*i.e.* "hatching" in the sense implied here). The existence, therefore, of an obligatory pre-competent pelagic phase has yet to be conclusively demonstrated. Perhaps of greater interest is the observed variation in the length of the pelagic phase in *A.proxima* larvae (columns 6 & 7, Table 9.3). Clearly, this variability may result in corresponding variation in larval survivorship and dispersal. Thompson (1958a) reported that both pre-competent and competent *A.proxima* veligers would immediately cease swimming, settle, and begin to crawl upon contact with the adult prey (and settlement cue) *Electra pilosa*. No evidence of such behaviour was observed here, although most veligers did eventually settle on the *E.pilosa* colony surfaces.

Although Thompson (1958a) describes the metamorphosis of *A.proxima* larvae in considerable detail, he provides no information with respect to the time taken for the completion of the various processes involved. Therefore, no data are available for comparison with the results presented in Table 9.4. Nonetheless, the time at which post-metamorphic *A.proxima* juveniles first appeared to feed (4 - 5 days) accords well with the results of other studies (Perron & Turner, 1977; Kempf & Willows; 1977; Harrigan & Alkon, 1978; but see Todd, 1986b).

Miller (1958) found the diameter of uncleaved *Onchidoris muricata* ova to be in the range 78 - 85 μm. This value is in agreement with data obtained by Todd (1979a) and Todd & Havenhand (1985) although, as for *A.proxima* , some degree of variability is indicated.

Miller (1958) additionally observed that the embryonic phase of *O.muricata* was approximately 14 days at 9 - 10 ^{o}C. This value is remarkably similar to that obtained here (13.8 days, Table 9.1). Unfortunately, no data concerning the duration of the larval period are available for comparison with those presented here. However, Todd & Doyle (1981) estimated the larval period of *O.muricata* to be approximately 60 days at 7 ^{o}C. The data presented in Table 9.2 indicate that this is probably a very good approximation given the variability in larval period observed here.

From Tables 9.1, 9.2 & 9.3 it can be calculated that the overall egg-to-juvenile periods for *A.proxima* and *O.muricata* were approximately 33 days and 56 days respectively at 10^{o} C, and 69 days and 110 days respectively at 5^{o} C. Applying the Q_{10} values given in Table 9.1, yields egg-to-juvenile periods of 57 days (*A.proxima*) and 88 days (*O.muricata*) at 7^{o} C. These estimates compare very favourably with those given by Todd & Doyle (1981) for these same two species at this temperature (51 d & 86 d respectively), and show that the benthic growth model presented earlier (Chapter 7, Section 7.3) is based on biologically realistic data.

Several important qualifications to the data shown in Table 9.2 and Figures 9.1 & 9.2 must be made before any conclusions can be drawn. Firstly, these data are necessarily imprecise because only sub-samples of larvae were observed and measured every 4 to 5 days. Secondly, this was done with a maximum resolution of only 6.2 μm (the calibration limit of the eyepiece graticule in the microscope). Thirdly, the curvilinearity of growth in the 5 °C *O.muricata* culture which was fed on *Rhodomonas* (Figure 9.1) may be an artifact of larval and algal swimming rates: *Rhodomonas* is quite a large flagellate and swims poorly in comparison to *Isochrysis galbana* and *Pavlova lutheri,* such that a suspension of *Rhodomonas* will slowly settle (over a number of days) to the bottom of the container. Since *O.muricata* veligers initially swam close to the water surface, (some, indeed, becoming rafted in the surface film), the total concentration of algae available for ingestion would have been lower than that farther down the the culture vessel. As the larvae grew older, the amount of swimming activity decreased, causing them to sink to the bottom of the culture vessel and therefore to enter a zone of denser algal food. This may also account for the relatively high variation in growth rates noted earlier. That a similar effect was not generally seen in the 10 °C *Rhodomonas* cultures (but see Figure 9.2), may be due to increased swimming of this alga at higher temperatures.

An interesting feature of the data presented in both Figure 9.1 and Figure 9.2 is the change in the size-range of larvae in the cultures. In almost all cases, the maximum larval length was attained some time before the first pediveliger was seen to metamorphose. This characteristic has been observed for other nudibranch species (Perron & Turner, 1977; Kempf & Willows, 1977; Switzer-Dunlap & Hadfield, 1977; Harrigan & Alkon, 1978) and has been attributed to the retraction of the mantle fold from the shell margin some 2 - 6 days prior to metamorphosis (see refs. above). It is likely, therefore, that *O.muricata* demonstrated similar behaviour, although no direct observations are available with which to confirm this.

As for *A.proxima*, post-metamorph *O.muricata* were observed to feed on the adult prey *Electra pilosa* some four to five days after metamorphosis. However, unlike *A.proxima*, the gut of immediate post-metamorph *O.muricata* was observed to take on a

brown colouration identical to that of the detritus adhering to the *E.pilosa* colony surface. Pilkington & Fretter (1970) suggest that organic detritus may be an important food source for gastropod veligers, and recently an intermediate diet has been demonstrated for *Onchidoris bilamellata* (Todd, 1986b). Whilst no direct evidence of the ingestion of detritus by post-metamorphic *O.muricata* was collected, the implication remains that detritus does constitute an intermediate diet (*sensu* Todd, 1981).

The observed variation in larval development rates of *O.muricata* (Table 9.2, Figures 9.1 & 9.2) may be at least partially attributable to sampling errors. Although the number of culture replicates used here is too small to permit rigorous analysis, the possibility exists that some degree of this variation may have been caused by the algal diet. The potential inadequacy of monospecific algal diets has been stressed by many authors (*e.g.* Walne, 1963; Franz, 1975; Castanga & Vogel (cited in Franz, 1975); Kempf & Willows, 1977; Todd, 1981, 1983). In many cases it has been assumed that this is because algal monocultures cannot provide all the essential (micro)nutrients for successful growth and metamorphosis (Shiraishi & Provasoli, 1959; Pilkington & Fretter, 1970; Pechenik & Fisher, 1979). However, it may also prove that the composition of the algal culture medium itself has as significant an effect on the food value of those algae as as the particular species of alga used. The observation that some algal species may be toxic to gastropod veligers (Pilkington & Fretter, 1970; Kempf & Willows, 1977) further complicates the picture and stresses the potential inadequacies of laboratory cultures (Kempf & Willows, 1977; Todd,1981; and see also Pechenik & Fisher, 1979). Moreover Pilkington & Fretter (1970) noted that the food value (and toxicity) of various algae to veligers differed from one gastropod species to another. Thus, generalisations with regard to the food value of different species of algae are, at best, of limited value.

Despite the criticisms noted above, the results obtained here, and elsewhere, are of some comparative value. For example, the Q_{10} values obtained here are broadly similar between *A.proxima* and *O.muricata* (Table 9.1), and generally approximate those seen in the adult animals (Chapter 5, Section 5.3). However, unlike the adult animals, no plateau in

temperature sensitivity was observed so that as temperature increases, development rate will accelerate. Values similar to those obtained here have been found in *Onchidoris bilamellata* embryos by Todd & Doyle (1981) (Q_{10} = 2.34) and for *Mytilus edulis* veligers by Sprung (1984a) (mean Q_{10} = 2.62).

Todd & Doyle (1981) have suggested that such Q_{10}'s will "telescope" the development of embryos and larvae so that eggs which are laid later in the spring will experience higher temperatures and therefore develop more rapidly. Consequently they predict that larvae hatching from these later spawn masses will settle at approximately the same time as those hatching from spawn masses laid much earlier in colder conditions. Whilst this prediction is theoretically tenable, several complicating factors exist which will cause an increase in the degree of variation in larval period such that this "telescoping" may not be seen in the field.

The first of these is the apparent variation between spawn masses. Because of the geometric properties of a sphere, even a small change in diameter would result in a substantial change in overall volume and, therefore, in energy content. Given the variation in hatching sizes of *O.muricata* larvae noted earlier, and the observation of a relatively constant shell size at metamorphosis (Table 9.2), it can be concluded that if growth rates were constant, then different larval periods would result, (although it must be noted that rates and sizes of shell growth may not accurately reflect somatic growth).

In addition to the above are variations in hatching time caused by morphometric restrictions on gaseous diffusion rates (and, therefore, on development rates) within individual spawn masses (*e.g.* Strathmann & Chaffee, 1984; Hunter & Vogel, 1986; see also Kempf & Willows, 1977). Further variations in embryonic period could be brought about by variations in air temperature differentially affecting either spatially or temporally separate spawn masses during periods of tidal emersion (Miller, 1958).

Differences in the spatial and temporal distribution of phytoplankton food sources may also increase the variation in the length of the pelagic period for planktotrophic larvae. Finally, the possibility exists that some degree of variation in growth rates, and therefore in length of larval period, is genetically determined (*e.g.* Newkirk & Haley, 1982).

Some of the above factors will have contributed to the variation in growth rates of *O.muricata* larvae observed here (Figures 9.1 & 9.2), although part of this variation will also be attributable to the laboratory conditions discussed earlier. Certainly, variations in larval growth rates similar those seen here have been observed in cohorts of larvae of the bivalve *Heteranomia squamula* (L.) in the field (pers. obs.).

Thus, the implication is that for any species possessing planktotrophic larvae (such as *O.muricata*) the length of the larval period may depend upon a number of interacting factors such that some degree of variation in that period is to be expected. Indeed, many of the above considerations will also apply (albeit on a reduced scale), to species possessing a pelagic lecithotrophic larva (*e.g. A.proxima*).

A probable consequence of the variability of larval period is that dispersal will vary in a similar manner. This will have concomitant effects on gene-flow and colonisation ability. Clearly, dispersal should be greater for *O.muricata* larvae than for *A.proxima* larvae. However, *per capita* mortality of *O.muricata* larvae is likely to be higher due to the increased possibility of predation or offshore mixing associated with a longer pelagic period (Palmer & Strathmann, 1981). In this respect, it is the "effective larval dispersal" (Scheltema, 1978) which is the most important factor. This is the distance over which the larva of a species can successfully disperse, settle and metamorphose whilst still encountering sufficient numbers of conspecifics to permit reproduction and thereby maintain populations. Several factors in addition to the duration of the pelagic period can influence effective larval dispersal: predation and offshore mixing are two extrinsic factors which can have substantial effects. Intrinsic factors which can affect the effective larval dispersal include both behavioural and physiological adaptations.

Phototaxis is an example of a behavioural adaptation which can markedly affect dispersal. Active swimming (which was invariably observed to be upward) would enable the larva to escape an environment containing benthic suspension feeders, into the surface waters. From there, the direction and degree of dispersal would depend on the various current patterns at different depths and the the swimming activity of the larva. Positive phototaxes (and negative geotaxes) have been found in other species of nudibranch (*e.g.* Thompson, 1958a; Chia & Koss, 1978; Todd, 1981;).

The delay of metamorphosis (*i.e.* the extension of the competent period) is another (physiological) adaptation which can influence larval dispersal. The results obtained here for *A.proxima* larvae (Table 9.3) indicate that metamorphosis may be delayed by a factor of two (or more) in the absence of a suitable settlement cue. However, this period may be extended still further if microalgal food is available (Table 9.2), although more comprehensive studies have not observed this effect (Kempf & Todd, in prep.). Other studies have observed delay of metamorphosis both in nudibranchs (Perron & Turner, 1977; Bickell & Chia, 1979; Kempf, 1981; Kempf & Hadfield, 1985) and in other gastropod species (Scheltema, 1978; Pechenik & Fisher, 1979; Pechenik, 1980, 1984; Pechenik & Lima, 1984; Pechenik *et al.*, 1984). Such a capability is clearly of value when suitable settlement sites are infrequently encountered, but recent evidence (Miller & Hadfield, 1986) suggests that settlement may also be further delayed if settlement cues are encountered frequently during both pre-competent and competent periods.

In this respect it is perhaps interesting to note that although the embryonic period of *A.proxima* at 10 °C (30.3 days, Table 9.1) is equal to that of the dorid nudibranch *Archidoris pseudoargus* Rapp. at the same temperature (29.8 days, pers. unpubl.obs.), *A.proxima* veligers are lecithotrophic and pelagic for perhaps a week, whilst those of *Archidoris pseudoargus* are planktotrophic, requiring a further 37 days to complete development in the plankton prior to metamorphosis (Todd & Havenhand, 1985). Strathmann (1977) concluded that although increasing energy allocation per ova generally correlates with a progression of larval type from planktotrophy to non-pelagic lecithotrophy (see, for example, Todd & Doyle, 1981), in some instances, this extra energy may be used

to produce a larger planktotrophic veliger which is better adapted to a planktonic existence. Theoretical reasons exist for expecting higher survivorship of larger planktonic larvae (Christiansen & Fenchel, 1979), and in the present context, the mode of larval development in *Archidoris pseudoargus* can be interpreted as a means of minimising planktonic mortality whilst retaining the capability for dispersal (*e.g.* Pechenik, 1979).

Theoretical considerations predict that only the extremes of true planktotrophy and non-pelagic lecithotrophy are evolutionarily stable (Vance, 1973a,b). However, setting aside the possibility of these being transitional phases, the existence of intermediate modes of development refutes this hypothesis. Indeed, several authors have criticised both this and more recent theoretical approaches on a variety of grounds, (Pechenik, 1979; Caswell, 1981; Perron & Carrier, 1981; Grant, 1983; and see Grahame & Branch, 1985, and Todd, 1985 for reviews). The factors which have dictated the evolution of a given larval type are almost certain to be many and complex. They include subjects discussed here as well as those considered in other chapters. A discussion of the overall interactions and implications of these factors is outwith the scope of the present chapter and is consequently reserved for Chapter 11.

CHAPTER 10

BIOCHEMICAL GENETICS

10.1 INTRODUCTION

Evolutionary theory predicts that genetic divergence between populations of the same species will be positively correlated with inter-population distance and environmental difference. A corollary of this argument is that genetic divergence between populations will be inversely correlated with the degree of inter-population mixing brought about by dispersal. Moreover, dispersal alone will not dictate gene flow; it is the number of dispersants which survive to reproduce in a new population (the effective dispersal) which will ultimately affect divergence (Endler, 1977).

The nudibranchs studied here present a ready opportunity to study the genetic consequences of variation in effective dispersal due to the different larval types displayed (see Chapter 9). Especially in small, genetically isolated populations, increased fixation by random genetic drift could lead to more rapid (genetic) divergence (Kimura, 1983), and in a similar manner, more rapid local adaptation of sub-populations may occur. However, processes such as these will inevitably be mediated by immigration (*i.e.* dispersal), and, as a consequence, migration may become of overriding importance in the evolution of genetically sub-divided populations (Wright, 1978).

It is therefore logical to predict that marine invertebrate species which have relatively long-lived pelagic larvae (such as *O.muricata*) will show less (spatial) genetic heterogeneity than species such as *A.proxima* which display a more abbreviated larval existence. Indeed, species with non-pelagic development ought, perhaps, to show the highest degree of inter-population variation (*e.g.* Scheltema, 1971; Crisp, 1978; Jablonski & Lutz, 1983).

An additional topic, of broader relevance to the evolution of larval development types, is whether *A.proxima* and *O.muricata* hold a common ancestry and, if so, how

recently have they diverged? Alternatively, the apparent phenotypic similarity of these two species may simply be an example of convergent evolution. The two species are currently placed in distinct genera (Thompson & Brown, 1984), on the basis of differences in radular structure and the form of the rhinophores. Indeed, as Thompson & Brown (1976) point out, some (especially juvenile) specimens can only be distinguished unequivocally by dissecting out the radula.

There is an extensive literature on the general uses of biochemical genetics in systematics (*e.g.* Avise, 1974; Thorpe, 1979, 1982; Ferguson, 1980; Oxford & Rollinson, 1983). Levels of biochemical genetic divergence between populations or species are routinely reduced to a single figure by a variety of statistical procedures (see Thorpe, 1982). Commonly used measures are the genetic identity I and its converse, genetic distance, D ($= -\log_e I$). The use of such measures in systematics has been reviewed by Thorpe, (1979, 1982, 1983) and Avise & Aquadro (1982). The basis of this technique rests on the hypothesis that (genetically determined) amino acid substitution in protein molecules is a stochastic, approximately regular process and that consequently the number of substitutions in a given protein may be related to evolutionary time (Thorpe, 1982). Thus, measures of genetic identity can be used to estimate (approximate) evolutionary time since the divergence of a common lineage (Thorpe, 1982; but see also Jukes & Holmquist, 1972; Lessios, 1979; Korey, 1981). Moreover, the relationship between biochemical genetic identity and taxonomic separation seems to hold over a wide range of vertebrate, invertebrate, and plant taxa (Thorpe, 1983).

A variety of techniques are available to investigate the biochemical genetics of a species (see *e.g.* Thorpe, 1982). The most straightforward and inexpensive of these is enzyme electrophoresis. This method has therefore been used throughout this study.

10.2 MATERIALS & METHODS

Electrophoresis

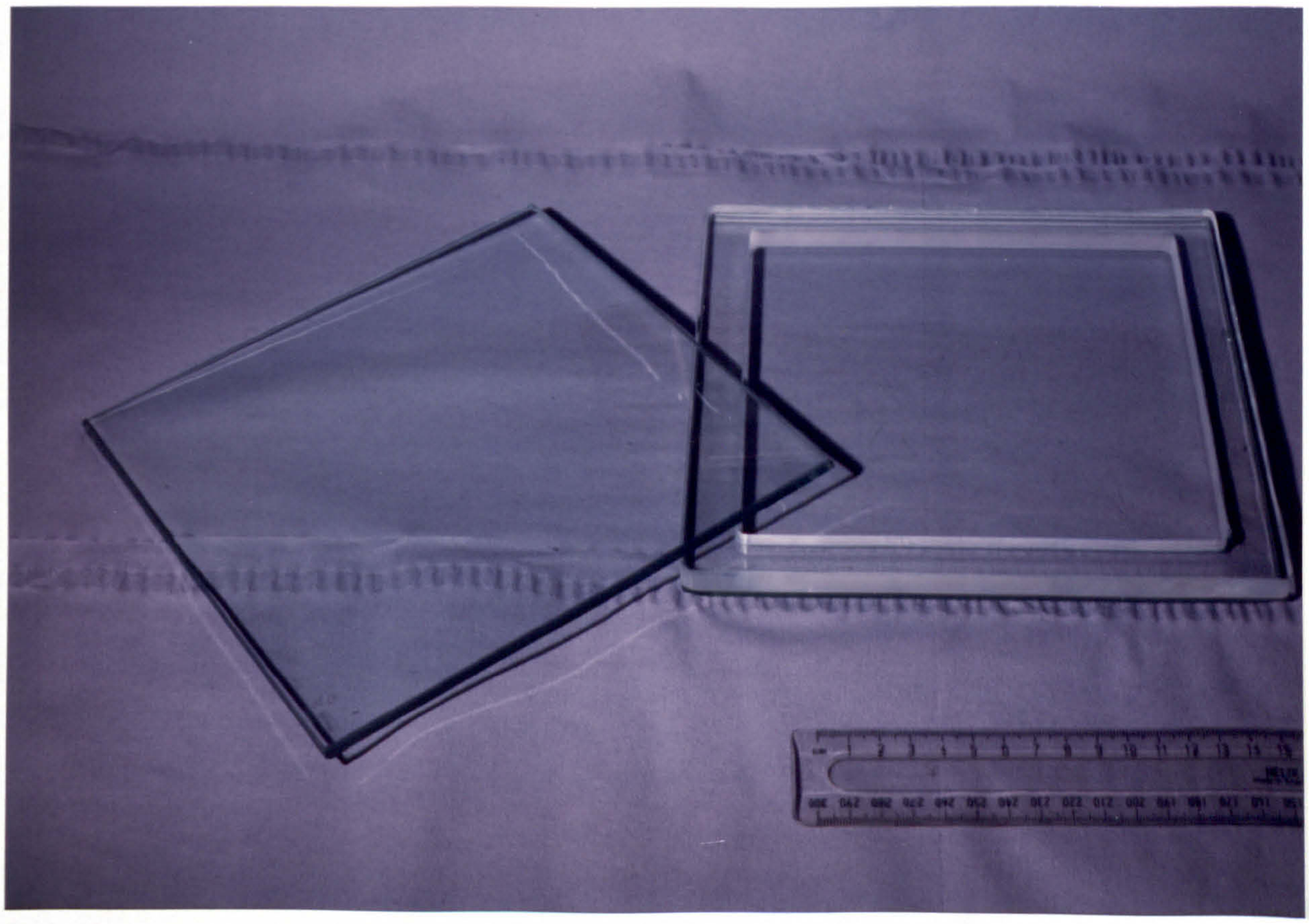
The electrophoresis undertaken in the present study used the conventional horizontal starch gel technique, (reviews by *e.g.* Sargent & George, 1975; Harris & Hopkinson, 1977; Ferguson, 1980).

Initial attempts at starch gel electrophoresis using a discontinuous Tris-Citric acid and Sodium Hydroxide - Boric Acid buffer system (Poulik, 1957) proved unsuccessful. Subsequent experimentation using a continuous Tris-Citrate pH 8.0 buffer system (Ward & Beardmore, 1977) yielded consistently interpretable results and therefore this system was used throughout the remainder of the study.

Gels were prepared using 30 g of hydrolysed electrophoresis starch (Sigma Chemical Co.) added to 250 ml of buffer in a 1 litre side-arm flask. This mixture was shaken thoroughly to ensure an even consistency and was swirled continuously whilst heating over a flame. When the suspension became viscous, the flask was removed from the heat and 'degassed' under vacuum for approximately 30 seconds. The hot, molten starch was poured into a 160 x 160 x 6 mm glass mould (Plate 10.1) and after removing any air bubbles with a Pasteur pipette, was covered with a glass sheet. This sheet was pressed down to meet the top surface of the mould, thus expelling any excess starch solution and ensuring that the resulting gel was of uniform thickness. The gel was then left to cool to room temperature before the upper glass sheet was removed. The gel and mould were covered in "Clingfilm" and placed in a refrigerator for approximately two hours.

Whole fresh nudibranchs were blotted dry on tissue paper and homogenised in ice-cold 0.1 M Tris-Citrate buffer (pH 8.0). [Trials using either digestive gland or muscle foot tissues only did not significantly improve the results over those obtained using whole animals]. The resulting crude extract was absorbed onto 6 x 6 mm squares of filter paper (Whatmann, No.3). These were then placed in individual 6 x 6 x 0.5 mm sample wells which had been cut (vertically) into the gel approximately 40 mm from the cathodic end,

PLATE 10.1 Starch electrophoresis gel mould and glass cover plate



using a "Perspex" former. The gel was then placed on the gel tray and the electrode wicks (surgical lint) were positioned on the ends of the gel, creating a continuous bridge between the gel and the electrodes. This assembly was covered in "Clingfilm" and placed in a refrigerator (Plate 10.2). The power supply was connected and set to run at a constant current of 30 - 35 mA for between four and five hours. At the end of this period, the power was switched off and disconnected before the gel was removed from the tray. The filter paper sample applicators were removed from the gel using fine forceps. The gel was then removed from its mould by inverting it over a glass sheet and gently easing off the mould. The gel was reinverted (to regain its original orientation) and the margins (about 10 mm in from the edges, and the portions which had supported the electrode wicks) were trimmed off. The top right-hand corner of the gel was marked by a cut. The gel was sliced horizontally using a length of monofilament nylon which was held tight. The thickness of each slice was dictated and maintained by "Perspex" spacers of known thickness laid along each side of the gel, upon which the monofilament rested. During the slicing procedure, a glass sheet was placed on top of the gel in order to ensure uniform thickness in the slices. The sliced gel was then placed in a tray of cold water and the individual slices floated off into staining trays.

Enzyme staining methods were based upon those of Shaw & Prasad (1970) and Harris & Hopkinson (1977) with modifications according to Schaal & Anderson (1974). Agar overlays were used only when staining for Peptidases.

<u>Samples</u>

The majority of the nudibranchs used in this study were obtained from three sites on the Argyllshire coast of Scotland (Figure 10.1). However, *Adalaria proxima* and *Onchidoris muricata* were also collected from Robin-Hoods Bay, North Yorkshire, and from Kingsbarns and Kinkell Braes (St Andrews) in Fife.

PLATE 10.2 Assembled starch gel and gel tray ready for electrophoresis

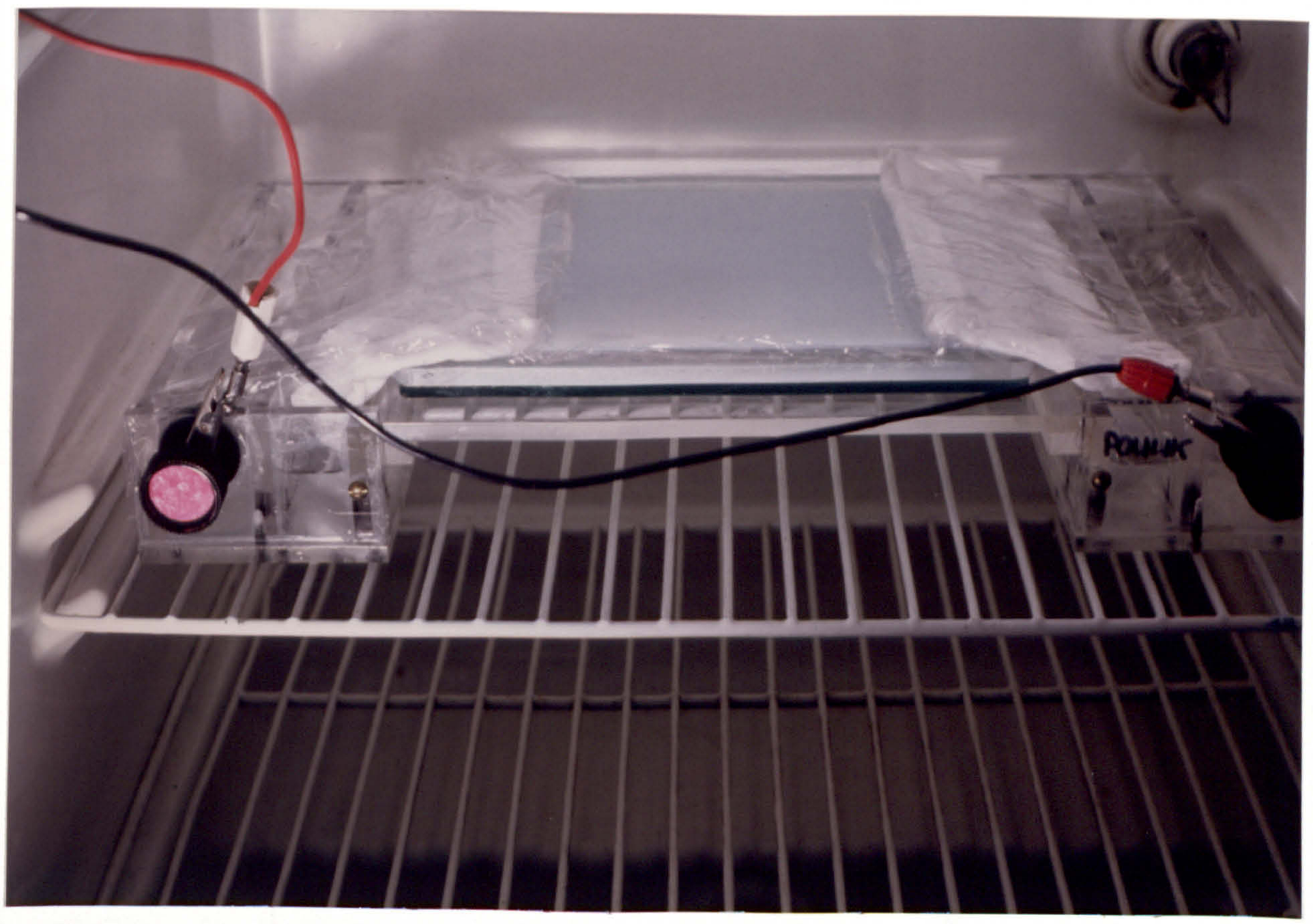
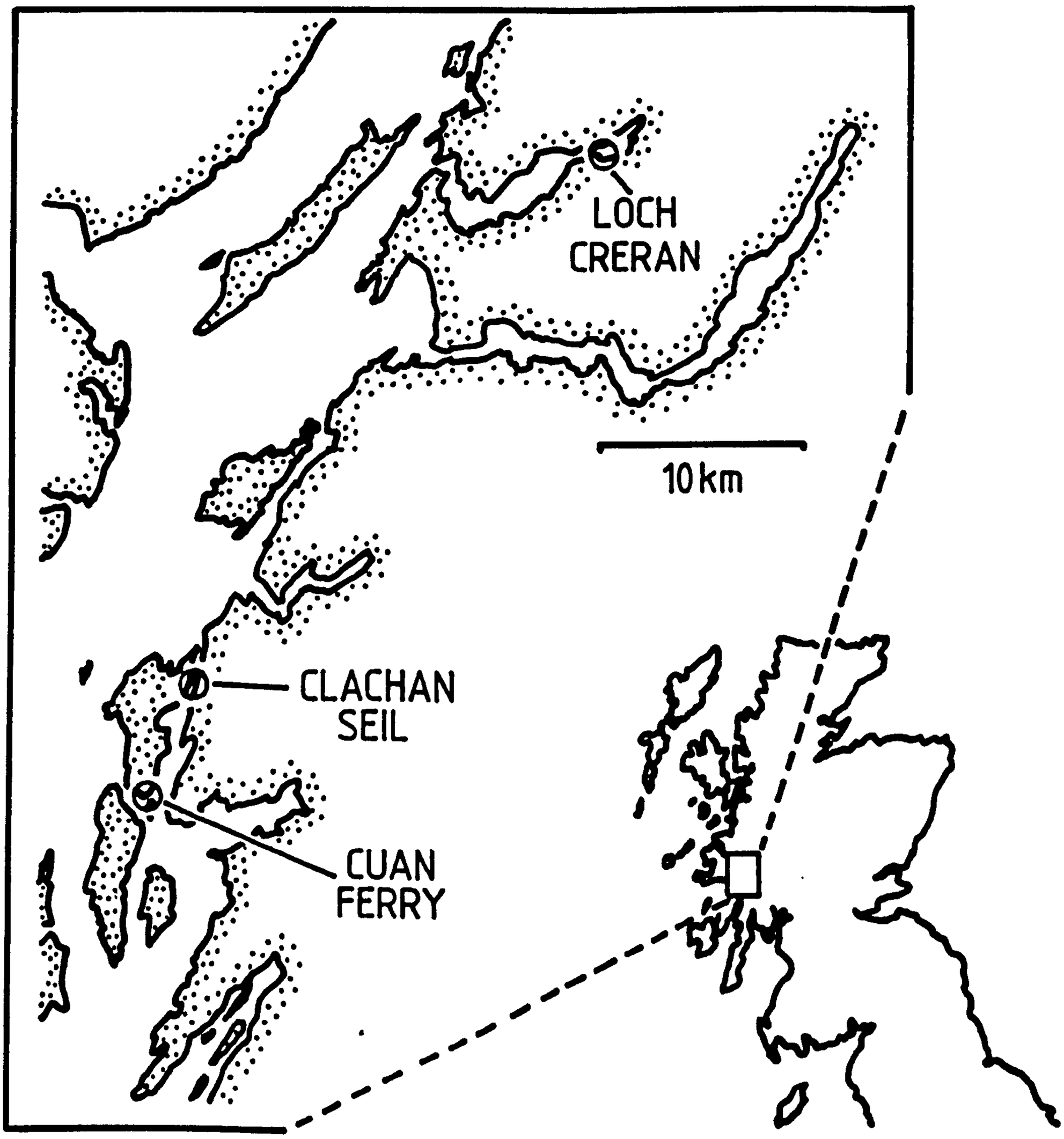

POWNIK

FIGURE 10.1 Location of *Adalaria proxima* populations used in biochemical genetic studies


LOCH
CRERAN
10 km
CLACHAN
SEIL
CUAN
FERRY

10.3 RESULTS

This work comprised two, essentially separate, studies:

The first of these utilised animals collected from spawning adult populations in May 1985. Samples of *Adalaria proxima* were taken from Loch Creran (Figure 10.1) and *Onchidoris muricata* samples were taken from Kinkell Braes (St Andrews). The aim was to screen a substantial number of loci in order to search for polymorphisms (which could be used later for inter-population analyses), and thereby to obtain data from which the genetic identity of these two species could be estimated.

Up to 15 *Onchidoris muricata* and 45 *Adalaria proxima* were stained for a total of 27 enzymes. Of these, 13 enzymes (14 loci) gave useful results. These enzymes, the numbers of animals used, and the respective allele frequencies are given in Table 10.1. Several other enzymes (Sorbitol dehydrogenase [E.C. 1.1.1.14], Phosphofructokinase [E.C. 2.7.1.11], Malate dehydrogenase-2 [E.C. 1.1.1.37], and Mannose phosphate isomerase [E.C. 5.3.1.8]) were apparently monomorphic in *A.proxima* but were too weak to score in *O.muricata*. This difference between the species was probably caused by the smaller body size and, therefore, presumably smaller total amounts of enzyme available in *O.muricata*. (It was also noticeable that some other enzymes which gave useful results in both species stained more rapidly and/or with greater intensity in *A.proxima*). The remaining enzymes (Malic enzyme [E.C. 1.1.1.40], Octopine dehydrogenase [E.C. 1.5.1.11], Adenosine deaminase [E.C. 3.5.4.4], Adenylate kinase [E.C. 2.7.4.3], Octanol dehydrogenase [E.C. 1.1.1.73], alcohol dehydrogenase [E.C. 1.1.1.1], Glutamate dehydrogenase [E.C. 1.4.1.3], Nucleoside phosphorylase [E.C. 2.4.2.1], Aconitase [E.C. 4.2.1.3], Aldolase [E.C. 4.2.1.13] and 6-phosphogluconate dehydrogenase [E.C. 1.1.1.44]) showed inadequate, or no activity, and therefore gave no useful results.

From the data summarised in Table 10.1, the genetic identity (*I*) (Nei, 1972) between the two species was calculated to be 0.432, and the genetic distance (*D*) was 0.839. The genetic similarity (*S*) and genetic distance (*D*) values (Thorpe, 1979) were 0.403 and

TABLE 10.1 Allele frequencies at 13 enzyme loci in *Adalaria proxima* and *Onchidoris muricata*

n = sample size (number of animals used); enzymes used: *Lap* (leucine aminopeptidase, EC 3.4.1.1); *Pgi* (phospho-glucose isomerase, EC 5.3.1.9); *Pgm* (phosphoglucomutase, EC 2.7.5.1); *G6pdh* (glucose-6-phosphate dehydrogenase, EC 1.1.1.49); *Idh* (isocitrate dehydrogenase, EC 1.1.1.42); *Mdh* (malate dehydrogenase, EC 1.1.1.37); *Pep* (peptidase, EC 3.4.11-13); *α-Gpdh* (α-glycerophosphate dehydrogenase, EC 1.1.1.8); *Got* (glutamate oxalo-acetate transaminase, EC 2.6.1.1); *To* (tetrazolium oxidase, EC 1.15.1.1); *Hk* (hexokinase, EC 2.7.1.1); *Fum* (fumarase, EC 4.2.1.2)

Locus	Allele	*Adalaria proxima*	*n*	*Onchidoris muricata*	*n*
Lap	1	1.00	3	1.00	4
Pgi-1	1	1.00	3	0.00	4
	2	0.00		1.00	
Pgm	1	0.00	45	0.06	15
	2	0.00		0.94	
	3	0.44		0.00	
	4	0.49		0.00	
	5	0.07		0.00	
G6pdh	1	0.00	5	1.00	11
	2	1.00		0.00	
Idh	1	1.00	3	1.00	4
Mdh-1	1	0.00	3	1.00	4
	2	1.00		0.00	
Pep-1	1	0.00	8	1.00	11
	2	1.00		0.00	
Pep-2	1	0.00	8	0.05	11
	2	0.00		0.05	
	3	0.00		0.32	
	4	1.00		0.55	
	5	0.00		0.05	
α-Gpdh	1	1.00	3	1.00	4
Got	1	1.00	3	0.00	4
	2	0.00		1.00	
To	1	1.00	20	1.00	15
Hk	1	1.00	3	0.00	4
	2	0.00		1.00	
Fum	1	0.13	8	0.00	4
	2	0.69		1.00	
	3	0.13		0.00	
	4	0.06		0.00	

0.597 respectively.

Since no published estimates of natural levels of genetic variability of nudibranch species exist, these are given here. Mean observed and expected heterozygosities per locus for *A.proxima* were 0.082 and 0.085 respectively. Equivalent values for *O.muricata* were 0.059 and 0.054 respectively. No locus in either species displayed any significant deviation from Hardy-Weinberg expectations. However, tests of fit to Hardy-Weinberg frequencies are notoriously weak unless deviations are substantial or sample sizes very large (Lewontin, 1958; Fairbairn & Roth, 1980).

The second study in investigated intraspecific, variation in allele frequencies at polymorphic loci. This study centred on samples taken from *Adalaria proxima* populations at the three locations shown in Figure 10.1. Samples of *O.muricata* were also taken from these, and other sites, but the number of animals collected was generally low and the results obtained were inferior to those obtained from *A.proxima*. Indeed, in the majority of cases, not all individuals from any one sample could be scored reliably. Since, under such circumstances, the chances of genotyping errors are considerable (J.P.Thorpe, pers. comm.), these data were excluded from any further analysis.

Three polymorphic loci were studied in samples of *A.proxima*. Samples were taken from all sites in October 1985 and in February 1986. A further sample was taken from Clachan Seil only in March 1986. The allele frequencies for the three loci at each of these three sites are given in Table 10.2. Typical gels for these loci are shown in Figure 10.2.

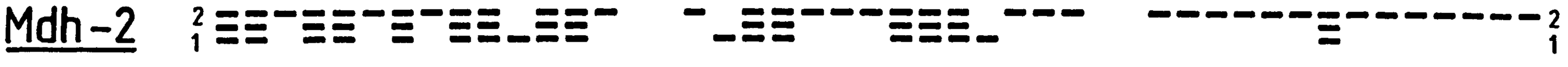
In general, for any particular site, allele frequencies of samples collected on different dates were statistically similar, (Table 10.2). Although some degree of (temporal) within-site variation was observed, the only significant difference (*G*-tests, adjusted where necessary for a 2 x 2 contingency table, $p < 0.05$, Sokal & Rohlf, 1981) was for *Pgm* in the samples from Clachan. In this case, the October sample differed from the February sample. A third sample was therefore taken at this site (in March) to ascertain the consistency of this trend. The March sample was not significantly different from the

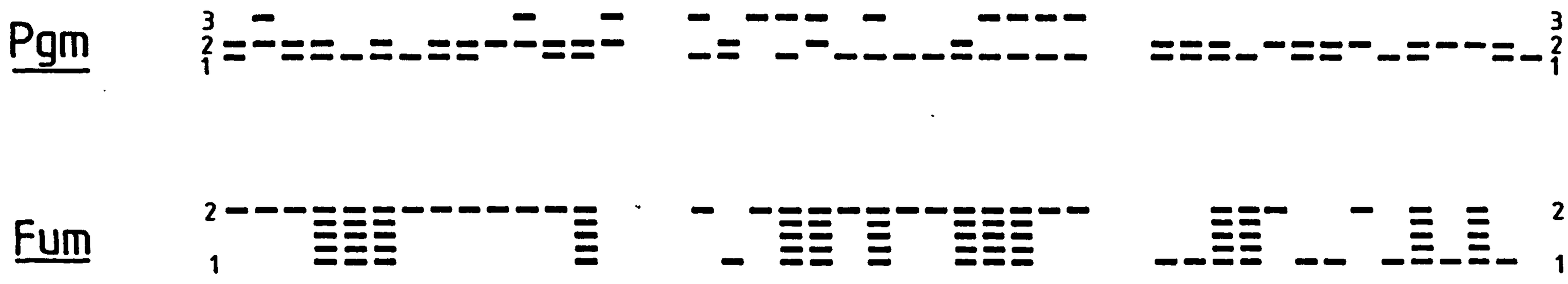
TABLE 10.2 Allele frequencies for *Fum, Mdh-2 & Pgm* at Loch Creran, Cuan Ferry and Clachan Seil for October 1985, February 1986 and (for Clachan Seil only) March 1986.

	Allele	Oct. 1985	Feb. 1985	Mar. 1986	**OVERALL**
Fum					
CRERAN	1	0.721	0.714	-	**0.719**
	2	0.279	0.286	-	**0.281**
	n	86	42		**128**
CUAN	1	0.212	0.375	-	**0.259**
	2	0.788	0.625	-	**0.741**
	n	80	32		**112**
CLACHAN	1	0.192	0.158	0.122	**0.145**
	2	0.808	0.842	0.878	**0.855**
	n	26	38	74	**138**
Mdh-2					
CRERAN	1	0.286	0.250	-	**0.273**
	2	0.714	0.750	-	**0.727**
	n	84	48		**132**
CUAN	1	0.071	0.143	-	**0.086**
	2	0.929	0.857	-	**0.914**
	n	56	14		**70**
CLACHAN	1	0.339	0.400	0.317	**0.345**
	2	0.661	0.600	0.683	**0.655**
	n	62	50	82	**194**
Pgm					
CRERAN	1	0.456	0.333	-	**0.418**
	2	0.441	0.433	-	**0.439**
	3	0.103	0.233	-	**0.143**
	n	68	30		**98**
CUAN	1	0.488	0.450	-	**0.475**
	2	0.512	0.550	-	**0.525**
	3	0.000	0.000	-	**0.000**
	n	82	40		**122**
CLACHAN	1	0.534	0.219	0.294	**0.367**
	2	0.086	0.656	0.544	**0.399**
	3	0.379	0.125	0.162	**0.234**
	n	58	32	68	**158**

FIGURE 10.2 Diagrammatic representation of typical gels for each of the three loci studied

(Alleles are numbered in order of increasing mobility)





CRERAN CLACHAN CUAN

February sample, but did differ ($p < 0.05$) from the October sample, confirming the initial observation. The possibility that this difference arose as a result of the relatively small sample sizes cannot be overlooked, although further investigations of such changes are necessary before any firm conclusion can be reached.

As allele frequencies were otherwise consistent, data for all sample dates were amalgamated to increase the sample sizes for inter-site analyses of genetic diversity. For each locus, comparisons were made between sites using *G*-tests (Sokal & Rohlf, 1981). The outcomes of these tests are given in Table 10.3. Although no significant differences were found between Creran and Clachan at either *Mdh-2* or *Pgm* (Table 10.3), the overall result is one of surprisingly high genetic differentiation between the three *A.proxima* populations. Genetic differences of this magnitude over such small geographical scales are particularly surprising in view of the fact that this species possesses a pelagic larva (see Berger, 1983).

10.4 DISCUSSION

The biochemical genetic data presented here are the first reported for any species of nudibranch mollusc. Therefore, the genetic similarity and distance values derived therefrom are of general comparative interest.

The similarity (I (Nei, 1972), and S (Thorpe, 1979)) values between *Adalaria proxima* and *Onchidoris muricata* (0.432 and 0.403, respectively) are higher than expected between genera: estimates of I or S between confamilial genera are usually below 0.35 (87% of such values reviewed by Thorpe, 1982, fell below 0.40) with a mean value of 0.27 (Thorpe, 1982). Between congeneric species, most estimates of I or S lie between 0.35 and 0.8, with a mean value of 0.54, (Thorpe, 1982). The present work therefore indicates the genetic divergence between *A.proxima* and *O.muricata* to be more typical of estimates between congeneric species rather than between distinct genera. Nevertheless, it must be stressed that intergeneric I and S values in excess of those observed above have been found

TABLE 10.3 Between-site *G*- test comparisons of allele frequencies for *Fum, Mdh-2 & Pgm*

(G_{adj} = *G* adjusted for a 2 x 2 contingency table)

	CRERAN : CUAN	CUAN : CLACHAN	CLACHAN : CRERAN
Fum	$G_{adj} = 49.345$ df = 1 $p < 10^{-10}$	$G_{adj} = 6.624$ df = 1 $p = 1.01 \times 10^{-2}$	$G_{adj} = 95.595$ df = 1 $p < 10^{-10}$
Mdh-2	$G_{adj} = 10.845$ df = 1 $p = 9.91 \times 10^{-4}$	$G_{adj} = 20.237$ df = 1 $p = 9.94 \times 10^{-6}$	$G_{adj} = 1.932$ df = 1 p = 0.16
Pgm	$G = 23.867$ df = 2 $p = 6.57 \times 10^{-6}$	$G = 46.659$ df = 2 $p < 10^{-10}$	$G = 3.292$ df = 2 p = 0.19

in other taxa.

A variety of calibrations of Nei's (1972) D (= $-\log_e I$) with evolutionary time suggest that a D value of 1.0 is equivalent to a divergence time of ~ 18 million years (see Yang *et al.*, 1974; Sarich, 1977; Wilson *et al.*, 1977; Thorpe, 1982). If stochastically similar evolutionary rates of enzyme molecules in nudibranch species are assumed, then the D value obtained here (0.839) would indicate an approximate divergence time between *A.proxima* and *O.muricata* of ~ 15 million years ago. However, sampling and other errors of I or S are invariably substantial (see Nei & Roychoudhury, 1974; Li & Nei, 1975; Nei, 1978; Thorpe, 1979, 1982) and for most practical purposes are effectively a function of the number of enzyme loci sampled. In the absence of corroborative evidence, therefore, it would be unwise to draw any firm conclusions on the basis of the present results alone.

Although a shortage of animals resulted in relatively small sample sizes in the initial study (Table 10.1), this should not have introduced any systematic bias into the estimates of overall heterozygosity. For both species, the levels of heterozygosity are well within the range found in natural outbreeding populations of many animal species, (see Nevo, 1978).

The (theoretically predicted) effects of larval dispersal on gene-flow (and hence, inter-population genetic heterogeneity) have been outlined in the Introduction (Section 10.1). In this respect, the absence of a study on the population genetics of *O.muricata* to complement that of *A.proxima* is regrettable. However, the results obtained from the three Argyllshire *A.proxima* populations present some interesting contrasts to theoretical expectations.

In view of the relatively brief pelagic phase of *A.proxima*, it had been predicted that closely separated sites (such as Cuan Ferry and Clachan Seil, Figure 10.3) would show relatively little genetic heterogeneity, whereas the converse would apply to more distant sites (such as between Loch Creran and the above two sites). However, the results obtained here (Table 10.3) do not generally accord with the above expectation.

The absence of significant genetic heterogeneity at two loci between Loch Creran and Clachan Seil may be a result of the (chance) coincidence of a finite number of allele frequencies. Certainly, significant differences in *Fumarase* allele frequencies between these sites were found, and similar differences between Loch Creran and Cuan Ferry were found at all three loci. However, the explanation of the observed differences between Cuan Ferry and Clachan Seil (where none were predicted) is more problematic. In the latter case, several possibilities exist.

The first of these is that there is a common genetic (*i.e.* larval) input to both sites and marked post-settlement selection. The second is that considerable reciprocal gene-flow (*i.e.* larval transport) exists between the two sites, but that considerable post-settlement selection still operates. Despite the small sample sizes, these two possibilities are considered to be unlikely due to the magnitude of observed genetic heterogeneity (especially with respect to the proximity and environmental similarity of the sites) (*cf.* Koehn *et al.* 1980; Hilbish, 1985). The third possibility is that larval transport between these sites is non-reciprocal or asymmetrical, and that larvae from one site are swept past the other during the 1 - 2 day obligatory pelagic period which was reported by Thompson, (1958a). Certainly such effects may occur; larvae hatching at times of peak tidal currents at these sites could be transported several kilometers in a matter of hours. However, the potential variability of actual obligatory pelagic periods, and potential errors in Thompson's (1958a) estimate thereof, have already been discussed (Chapter 9, Section 9.4). Therefore it would seem that the most probable explanation for the observed patterns is that there is limited gene-flow between these sites due to the relatively rapid settlement of pelagic larvae after emerging from the spawn mass (see Chapter 9, Section 9.4; Kempf & Todd, unpubl. obs.). Given the inter-population genetic heterogeneity observed here, it should be possible (by extensive field sampling) to determine more accurately the effective dispersal capacity of this species and therefore test the validity of this hypothesis.

Although no comparable data for *O.muricata* from these sites are available. Studies of *O.muricata* populations on the coasts of Fife (Eastern Scotland) and North Yorkshire

(N.E. England) (Todd, unpubl. obs.) indicate that small scale spatial genetic heterogeneity (as seen here for *A.proxima*) does not exist, although allele frequency differences between the two study areas (a distance of ~ 350 km) may be present.

Other studies of genetic heterogeneity with respect to larval dispersal capacity have provided conflicting results (see Berger, 1983, for a review). Thus, for example Snyder & Gooch (1973) found substantial genetic heterogeneity between populations of *Littorina saxatilis* separated by as little as 2 km. However, Berger (1973), whilst recording a greater degree of spatial genetic heterogeneity in non-pelagic "direct" developers (including *L.saxatilis*) than in species possessing planktotrophic larvae (*e.g. L.littorea*), did not observe such marked variation over such short distances. These studies may have been confounded by genetic variation due to (at that time) undetected cryptic species of the *L.saxatilis / rudis* complex. However, other studies, (*e.g.* Ward & Warwick, 1980) have observed similar variation whilst being aware of such taxonomic complications. The converse prediction, that species with planktotrophic larvae would show spatial genetic homogeneity is supported by the studies of (for example) Gooch *et al.*, (1972), Beaumont, (1982), Meehan, (1985), and Grassle & Grassle (1978) but contradicted by work on *Siphonaria* spp. (Johnson & Black, 1982, 1984a,b, and see also Hoagland, 1984).

It is apparent from the above that no consistent patterns of spatial genetic heterogeneity can be found with respect to larval development type alone, [although the underlying trends accord with theoretical expectations (see Berger, 1983)]. The causes of this lack of correlation are certainly many and varied. Two factors which may prove to be of major significance are the degree of generation overlap, and individual longevity in the population under study. Clearly, if individuals are long-lived then any immigrants to that population will merely dilute (rather than replace) the current gene-pool. Under such circumstances, the rate of genetic divergence between adjacent populations will depend on a variety of factors including the degree of generation overlap, the genotypes of the immigrants, the size of the breeding population (Kimura, 1983) (which is itself an indirect function of longevity and generation time), and perhaps most importantly, the numbers of immigrants themselves (Endler, 1977; Wright, 1978). If, for comparative purposes, these

various factors are assumed to be constant, the genetic structure of populations of long-lived individuals is likely to be less spatially heterogeneous than that of populations comprising shorter-lived animals. At its ultimate conclusion, this effect may become most noticeable in semelparous organisms where the entire breeding population is replaced at each generation. In reality, the above noted factors are unlikely to remain constant, and therefore such effects will be mediated by a complex network of interactions. However, individual longevity may prove to be an important factor affecting the relationship between genetic heterogeneity and larval type.

Not all of the limitations of this study are biological however. Undoubtedly, more extensive sampling would clarify some of the problems outlined here. Additional improvements to experimental techniques could also yield considerably more information from each sample. For example, it has been estimated that only 20 - 30% of single amino acid substitutions in a given enzyme may be detectable by conventional electrophoretic techniques (Shaw, 1965; Lewontin, 1974; King & Wilson, 1975), and use of different electrophoretic methods can frequently yield contrasting or conflicting results (*e.g.* Coyne, 1976; Bonhomme & Selander, 1978; Gill, 1978; Ramshaw *et al.*, 1979; Racine & Langley, 1980). The increased resolution obtained by Iso-Electric Focussing in polyacrylamide gels is a case in point. However, the relatively high costs of such techniques is likely to prove prohibitive for all but the smallest scale of work. Despite the apparent inadequacies of conventional starch gel electrophoretic techniques, little (if any) systematic bias is included by such methods, and comparability remains good (Thorpe, 1982).

CHAPTER 11

SUMMARY & CONCLUSIONS

Significance of observed variability

In many aspects of ecology, the trend has been to overlook or ignore variation at the level of the individual in deference to using the more 'convenient' mean values from a population (for example), as a basis for the generation and testing of hypotheses. This trend has applied as readily to the ecology of marine invertebrates as to any other group of organisms. Theoretical considerations of the variance around such mean values have generated a number of hypotheses relating to the evolution of both specific life-history traits (*e.g.* Slatkin, 1974; Gillespie, 1977; Ekbohm *et al.*, 1980) and to overall fitness (*e.g.* Real, 1980; Lacey *et al.*, 1983). However, it is only recently that emphasis has been placed on investigating the ecological significance of variation within individual organisms (*e.g.* Bayne & Newell, 1983).

In this respect the substantial variation which was frequently observed both between and within individuals in the present study, is of particular interest. Rather than dismissing this as biological "noise", the emphasis has actually been placed on such characteristics. For example, considerable variation between individuals was observed in almost every aspect of larval ecology (Chapter 9). Indeed, the results of larval culture experiments have suggested that the duration of the pelagic phase of both *Adalaria proxima* and (especially) *Onchidoris muricata* may be highly variable in the field leading to corresponding variation in the degree of dispersal of sibling larvae. In direct contrast to this, population genetic evidence (Chapter 10) has shown that despite the noted variation in pelagic period, genetic differentiation between adjacent populations of *A.proxima* is substantial. Although unequivocal interpretation is not yet possible, the suggestion is that actual pelagic dispersal may be considerably abbreviated from that which might be expected on the basis of larval culture data.

Within the energy budget study, substantial inter- (but not intra-) individual

variation was generally observed in the respiration rates of both species (Chapter 5). Additional intra-individual variation was observed in rates of spawn production (Chapter 6, Section 6.3) and daily energy fluxes (Chapter 7, Section 7.1). Bayne & Newell (1983) suggest that such variation within individuals may be compensatory with the increase in any one energy budget component being balanced by changes in the others. The analyses presented in Chapter 7 have indicated that this is generally not the case for the energy budget components measured here, although the possibility of an association between these fluxes and variations in individual feeding rates (which were not quantified) remains.

Because fecundity is probably the single most important fitness component, the observed variability of total reproductive allocation ($\sum P_r$) in *O.muricata* and (especially) in *A.proxima* is noteworthy. Multivariate analyses of the relationships between $\sum P_r$ and the primary energy budget components outlined in Chapter 7 (*i.e.* growth and respiration of juveniles and adults) indicate that for *A.proxima*, none of the observed variation in $\sum P_r$ is explained by these components (multiple r^2 (adjusted for degrees of freedom) = 0.0%). Conversely, the equivalent value for *O.muricata* is 54.0%. Indeed the great majority of the variation in $\sum P_r$ of *O.muricata* was explained by only two of these variables, namely juvenile growth rate and adult respiration rate (multiple r^2 (adjusted for df) = 76.1%). This lends some support both to Bayne & Newell's (1983) hypothesis, and to the earlier contention (Chapter 7) that *O.muricata* individuals simply switch energy partitioning from somatic growth to reproduction at the onset of spawning.

It is apparent, therefore, that the variation in $\sum P_r$ of *A.proxima* cannot be explained in terms of compensatory costs in other energy budget components. The suggestion of Todd (1986a) that variations in respiratory costs may account for the variation in $\sum P_r$ which was observed both here and elsewhere (Todd, 1979a), is interesting in view of the observed inter-individual variation in respiration rate of *A.proxima* (Chapter 5, Section 5.3). However, although adult respiration rates displayed the strongest of all the

correlations with $\sum P_r$, this relationship was positive (*i.e.* non-compensatory) and was not statistically significant (simple $r = 0.361$, $df = 5$, $p >> 0.05$).

Larval types and Reproductive Strategies

It has been shown (Chapter 9, Section 9.4) that *A.proxima* has a short egg-to-juvenile period relative to that of *O.muricata*. Therefore, by difference, the benthic phase of *A.proxima* must be relatively longer, allowing more time for the juvenile to grow before commencing reproduction. This effect will be augmented by the relatively short spawning period of *A.proxima*. Since *A.proxima* has a more rapid feeding rate, the combination of the latter and the duration of the benthic period allow individual *A.proxima* to grow to a comparatively large pre-reproductive body size. It was observed that in adult *A.proxima* the rate of spawning (Chapters 6 & 7) was considerably in excess of that supportable by energy acquisition through feeding alone (Chapter 3, Section 3.3), and that the resulting energy deficit was met by rapid degrowth of the body tissues (see Section 6.3). An hypothesis has been presented (Chapter 7, Section 7.3) relating the rapidity of somatic degrowth to the reduction in life-expectancy which is assumed to be a consequence of that degrowth.

In contrast, *O.muricata* has a shorter benthic period and lower feeding rates than *A.proxima*. However, the energetic costs of reproduction in the (correspondingly smaller) adult can be met by recurrent energy acquisition through feeding (Chapter 7, Section 7.3). As a result, the reproductive period is long, degrowth is slow, and total reproductive output is almost certainly greater than would be achieved through adopting the reproductive "strategy" (*sensu* Grahame & Branch, 1985) of *A.proxima*.

Thus, although the possibility exists that there is a continuum of strategies trading-off the egg-to-juvenile period against the maximum body size (and hence energy allocation to reproduction), other extrinsic and intrinsic factors such as feeding rate may have imposed additional constraints which have ultimately shaped the evolution of the different reproductive strategies observed.

Possible causes of the evolution of different larval types

The question which was posed at the outset still remains: "Why, given such notable ecological similarities have these two species evolved different larval types?"

On the basis of the results obtained here this question cannot be answered directly. However, several relevant observations can be made; the evidence presented in Chapter 10 (Section 10.4) suggests that *A.proxima* is an evolutionary derivative of of an ancestral *"O.muricata"* stock. Certainly, in the opisthobranchs, planktotrophy is assumed to be the ancestral developmental pattern (Hadfield & Switzer-Dunlap, 1984). The answer to the above question must therefore lie in the selective pressures which caused *A.proxima* to relinquish that pattern. Although phylogenetic constraints can often confound such issues (Grahame & Branch, 1985), pelagic lecithotrophy is almost certainly not a fixed trait in the genus *Adalaria* (*cf. Leptasterias*). However, planktotrophy may indeed be fixed within the genus *Onchidoris* (data in Miller, 1958; Todd, 1977; Thompson & Brown, 1984) and therefore such constraints should not be overlooked.

Todd (1979a) suggested that the evolution of a pelagic lecithotrophic larva in *A.proxima* may have been an adaptation to overcome the variability of individual reproductive success which, he assumed, would accrue from the observed variation in individual $\sum P_r$. As evidence for this he noted the lack of allometry between maximum body size and $\sum P_r$ in *A.proxima* (whereas a strong allometric relationship was found in *O.muricata*). These observations have been corroborated by Todd & Havenhand (1983) and the data given here (Chapter 6, section 6.3; Chapter 8, Figures 8.2 & 8.3). However, as Todd (1979a) noted, even if this variation is taken into account, the overall $\sum P_r$ for *A.proxima* individuals was generally in excess of that for *O.muricata* individuals. Therefore, *A.proxima* should still be able to reproduce successfully by means of a planktotrophic larva. This argument is not evolutionarily valid however, because pelagic lecithotrophy (if it is energetically supportable) is likely to be even more successful.

Several theoretical considerations relating larval type to adult energy resources have been presented (*e.g.* Vance, 1973a,b; Chia, 1974; Underwood, 1979; Day & McEdward, 1984; and see Grahame & Branch, 1985 and Todd, 1985 for reviews). Although such considerations may have been important in restricting the evolutionary options of *O.muricata* (Todd, 1979a), they have generally failed to provide a consistent explanation for the evolution of 'safer' developmental types such as pelagic lecithotrophy across the range of marine invertebrate phyla. Whilst it is clearly unrealistic to expect generalised 'rules' to apply to all the members of a diverse array of marine invertebrate species, the cause of these failures may not lie in the inadequacies of the models themselves, but rather in the methods used to test them. Tests of such models have generally utilised a variety of measures of reproductive effort (RE) (see Chapter 8). The lack of support for a general association between either larval type and RE (reviewed by Grahame & Branch, 1985) or indeed between life-history strategy and RE (see Todd, 1985), may therefore be attributable to the adoption of inappropriate or inadequate measures of RE. Given the paucity of data available for comparison with the results presented in Chapter 8, it is not as yet possible to evaluate this hypothesis.

A final scenario which may answer the stated question is that the shorter egg-to-juvenile period which is associated with pelagic lecithotrophy results in a generally larger reproductive adult (for the reasons outlined earlier). This in turn may result in a higher ΣP_r (despite the possibly poor allometric relationship; Chapter 8, Section 8.2), and therefore permit an increase in fecundity and/or (given the necessary mutations) in egg size. This is clearly a circular argument since a given individual must first risk reduced fecundity by increasing egg-size (and therefore reducing planktonic period) before gaining the benefits of increased development time. However, this risk may be reduced if such a transition was gradual rather than saltatory.

Conclusions

In any study of evolutionary ecology difficulties will necessarily arise when attempting to determine the selective pressures and evolutionary mechanisms which resulted in the 'end-product' we observe. Although the intensive investigations into the physiological and larval ecology of *Adalaria proxima* and *Onchidoris muricata* described here have permitted an analysis of the proximate causes and effects of the reproductive patterns of these two species, it is not yet possible to conclude the nature of the selective pressures which resulted in the evolution of pelagic lecithotrophic larvae in *A.proxima* alone. In all such studies it must be remembered that rather than requiring a maximal or optimal solution, "...natural selection works with what is available to do only the best necessary job" (Grahame & Branch, 1985).

ALDER, J. & A. HANCOCK (1845-55) *"A monograph of the British nudibranchiate Mollusca"* Ray Soc., London.

ALDRIDGE, D.W. (1982) "Reproductive tactics in relation to life-cycle bioenergetics in three natural populations of the freshwater snail, *Leptoxis carinata*." *Ecology*, **63**: 196-208.

ALTMAN, P.L. & D.S. DITTMER (1968) *"Biology Data Book Vol. III"* Biological Handbooks, Bethesda, Md.

ANSELL, A. (1982) "Experimental studies of a benthic Predator-Prey relationship. II. Energetics of Growth and Reproduction, and food conversion efficiencies, in long-term cultures of the Gastropod drill *Polinices alderi* feeding on the bivalve *Tellina tenuis*." *J. exp. mar. Biol. Ecol.*, **61**: 1-29.

AVISE, J.C. (1974) "Systematic value of electrophoretic data." *Syst. Zool.*, **23**: 465-481.

AVISE, J.C. & C.F. AQUADRO (1982) "A comparative summary of genetic distances in vertebrates: patterns and correlations." *Evol. Biol.*, **15**: 151-185.

BAYNE, B.L. (1983) "Physiological ecology of marine molluscan larvae." *In:* VERDONK, N.H., J.A.M. van den BIGGELAAR & A.S. TOMPA (eds.) *"The Mollusca Vol. III: Development."*, Acad. Press, N.Y., pp. 299-343.

BAYNE, B.L. & R.C. NEWELL (1983) "Physiological Energetics of Marine Molluscs" *In:* SALEUDDIN, A.S.W. & K.M. WILBUR (eds.) *"The Mollusca Vol. IV: Physiology, Part 1"*, Acad. Press, N.Y., pp.499-515.

BAYNE, B.L. & C. SCULLARD (1978) "Rates of feeding by *Thais (= Nucella) lapillus* (L.)." *J. exp. mar. Biol. Ecol.*, **32**: 113-129.

BAYNE, B.L. & C.M. WORRALL (1980) "Growth and production of mussels *Mytilus edulis* from two populations." *Mar. Ecol. - Prog. Ser.*, **3**: 317-328.

BAYNE, B.L., J. WIDDOWS & R.I.E. NEWELL (1977) "Physiological measurements on estuarine bivalve molluscs in the field." *In:* KEEGAN, B.F., P. O'CEIDIGH & P.J.S. BOADEN (eds.) *"Biology of benthic organisms"* Pergamon Press, Oxford, pp. 57-68.

BAYNE, B.L., P.N. SALKELD & C.M. WORRALL (1983) "Reproductive effort and reproductive value in different populations of *Mytilus edulis* L." *Mar. Biol. Lett.*, **3**: 89-105

BEAUMONT, A.R. (1982) "Geographic variation in allele frequencies at three loci in *Chlamys opercularis* from Norway to the Brittany coast." *J. Mar. Biol. Ass. U.K.*, **62**: 243-261.

BEHRENTZ, A. (1931) "Trekk av *Lamellidoris muricatas* biologi av dens generationsorganers bygning." *Nyt. Mag. Naturvidensk*, **70**: 1-26.

BERGER, E.M. (1973) "Gene-enzyme variation in three sympatric species of *Littorina*." *Biol. Bull.*, **145**: 83-90.

BERGER, E.M. (1983) "Population genetics of marine gastropods and bivalves." *In:* RUSSELL-HUNTER, W.D. (ed.) *"The Mollusca Vol VI: Ecology"* Acad. Press, N.Y., pp. 563-596.

BEST, B.A. & J.E. WINSTON (1984) "Skeletal strength of encrusting cheilostome bryozoans." *Biol. Bull.*, **167**: 390-409.

BICKELL, L.R. & F.-S. CHIA (1979) "Organogenesis and histogenesis in the planktonic veliger of *Doridella steinbergae* (Opisthobranchia, Nudibranchia)." *Mar. Biol.*, **52**: 291-313.

BONHOMME, F. & R.K. SELANDER (1978) "Estimating total genic diversity in the house mouse." *Biochem. Genet.*, **16**: 287-297.

BOUCHET, P. & J. TARDY (1976) "Faunistique et biogeographie des nudibranches des cotes françaises de l'Atlantique et de la Manche." *Ann. Inst. oceanogr.*, **52**: 205-213.

BRANCH, G.M. (1982) "The biology of limpets: physical factors, energy flow and ecological interactions." *Oceanogr. Mar. Biol. Ann. Rev.*, **19**: 235-380.

BROWNE, R.A. & W.D. RUSSELL-HUNTER (1978) "Reproductive effort in molluscs." *Oecologia*, **37**: 23-27.

BROWN, A.C. & F.M. da SILVA (1979) "The effects of temperature on Oxygen consumption in *Bullia digitalis* Meuschen (Gastropoda: Nassaridae)." *Comp. Biochem. Physiol.*, **62A**: 573-576.

CALOW, P. (1974) "Some observations on locomotory strategies and their metabolic effects in two species of freshwater gastropods, *Ancylus fluviatilis* Mull. and *Planorbis contortus* Linn.." *Oecologia*, **16**: 149-161.

CALOW, P. (1978) "The evolution of life-cycle strategies in freshwater gastropods." *Malacologia*, **17**: 351-364.

CALOW, P. (1979) "The cost of reproduction - a physiological approach." *Biol. Rev.*, **54**: 23-40.

CALOW, P. (1981) "Resource utilization in reproduction." *In:* TOWNSEND, C.R. & P. CALOW (eds.) *"Physiological ecology"* Blackwell, Oxford, pp. 245-270.

CALOW, P. (1983) "Life-Cycle Patterns and Evolution" *In:* RUSSELL-HUNTER, W.D. (ed.) *"The Mollusca Vol. VI: Ecology"*. Acad. Press, N.Y., pp. 649-678.

CALOW, P. & A.S. WOOLLHEAD (1977) "The relationship between ration, reproductive effort and age-specific mortality in the evolution of life-history strategies - some observations on freshwater triclads." *J. Anim. Ecol.*, **46**: 765-781.

CAREFOOT, T.H. (1967) "Growth and nutrition of three species of Opisthobranch Molluscs." *Comp. Biochem. Physiol.*, **21**: 627-652.

CASWELL, H. (1981) "The evolution of "mixed" life-histories in marine invertebrates and elsewhere." *Am. Nat.*, **117**: 529-536.

CHADWICK, S.A. & J.P. THORPE (1981) "An investigation of some aspects of predation by dorid nudibranchs (Mollusca: Opisthobranchia)." *In:* LARWOOD, G.P. & C. NIELSEN (eds.) *"Recent and Fossil Bryozoa"* Olsen & Olsen, Denmark, pp. 51-58.

CHIA, F.-S. (1974) "Classification and adaptive significance of developmental patterns in marine invertebrates." *Thalassia Jugosl.*, **10**: 121-130.

CHIA, F.-S. & M. SKEEL (1973) "The effect of food consumption on growth, fecundity and mortality in a sacoglossan opisthobranch, *Olea hansineensis*." *Veliger*, **16**: 153-158.

CHIA, F.-S. & R. KOSS (1978) "Development and metamorphosis of the planktotrophic larvae of *Rostanga pulchra* (Mollusca: Nudibranchia)." *Mar. Biol.*, **46**: 109-119.

CHRISTIANSEN, F.B. & T.M. FENCHEL (1979) "Evolution of marine invertebrate reproductive patterns." *Theoret. Pop. Biol.*, **16**: 267-282.

CLARK, K.B. (1975) "Nudibranch life-cycles in the North-West Atlantic and their relationship to the ecology of fouling communities." *Helgol. Wiss. Meeresunters.*, **27**: 28-69.

CLARKE, A. (1979) "On living in cold water: *K*- strategies in Antarctic benthos." *Mar. Biol.*, **55**: 111-119.

CLARKE, A. (1983) "Life in cold water: The physiological ecology of Polar marine ectotherms." *Oceanogr. Mar. Biol. Ann. Rev.*, **21**: 341-453.

CONOVER, R.J. (1966) "Assimilation of organic matter by zooplankton." *Limnol. Oceanogr.*, **11**: 338-345.

COYNE, J.A. (1976) "Lack of genic similarity between two sibling species of *Drosophila* as revealed by varied techniques." *Genetics*, **84**: 593-807.

CRISP, D.J. (1971) Energy flow measurements. *In:* HOLME, N.A. & A.D. McINTYRE (eds.) *"Methods for the Study of Marine Benthos"* IBP Handbook No. 16, pp. 197-279.

CRISP, D.J. (1978) "Genetic consequences of different reproductive strategies in marine invertebrates." *In:* BATTAGLIA, B. & J.A. BEARDMORE (eds.)*"Marine Organisms: Genetics, Ecology and Evolution"* Plenum Press, N.Y., pp. 257-274.

CRISP, M., J. DAVENPORT & S.E. SHUMWAY (1978) "Effects of feeding and of chemical stimulation on the Oxygen uptake of *Nassarius reticulatus* (Gastropoda: Prosobranchia)." *J. Mar. Biol. Ass. U.K.*, **58**: 387-399.

DAVENPORT, J. (1976) "A technique for measuring Oxygen consumption in small aquatic organisms." *Laboratory Practice*, **25**: 693-695.

DAY, R. & L. McEDWARD (1984) "Aspects of the physiology and ecology of pelagic larvae of marine benthic invertebrates" *In:* STEIDINGER, K.A. & L.M. WALKER (eds.) *"Marine Plankton Life Cycle Strategies"* CRC Press, Florida, pp. 93-120.

De FREESE, D.E. & K.B .CLARK (1983) "Analysis of Reproductive energetics of Florida Opisthobranchia (Mollusca: Gastropoda)." *Int. J. Inv. Repr.*, **6**: 1-10.

DUVAL, C. (1963) *"Inorganic thermogravimetric analysis"* Acad. Press, N.Y..

EKBOHM, G., T. FAGERSTRÖM & G.I. AGREN (1980) "Natural selection for variation in offspring numbers: comments on a paper by J.H. Gillespie." *Am. Nat.*, **115**: 445-447.

ELLIOTT, J.M. & W. DAVIDSON (1975) "Energy equivalents of Oxygen consumption in animal energetics." *Oecologia,* **19**: 195-201.

ENDLER, J.A. (1977) *"Geographic variation, speciation and clines"* Princeton Univ. Press, Princeton, N.J..

EYSTER, L.S. & S.E. STANCYK (1981) "Reproduction, growth and trophic interactions of *Doriopsilla pharpa* Marcus, in South Carolina." *Bull. Mar. Sci.,* **31**: 72-82.

FAIBAIRN, D.J. & D.A. ROTH (1980) "Testing genetic models of isozyme variability without breeding data: can we rely on the chi-squared?" *Can J. Fish. Aquat. Sci.,* 37: 1149-1159.

FERGUSON, A. (1980) *"Biochemical systematics and evolution"* Blackie, Glasgow.

FISHER, R.A. (1930) *"The genetical theory of natural selection"* Oxford Univ. Press, Oxford.

FRANZ, D.R. (1970) "Zoogeography of northwest Atlantic opisthobranch molluscs." *Mar. Biol.,* **7**: 171-180.

FRANZ, D.R. (1975) "An ecological interpretation of nudibranch distribution in the Northwest Atlantic." *Veliger,* **18**: 79-83.

GADGIL, M. & W.H. BOSSERT (1970) "Life history consequences of natural selection." *Am. Nat.,* **106**: 14-31.

GARSTANG, W. (1894) "Faunistic notes at Plymouth during 1893-4." *J. Mar. Biol. Ass. U.K.,* **3**: 210-235.

GILL, P.D. (1978) "Survey of isoenzymes in the snail *Cepaea nemoralis* using different buffer/gel systems in polyacrylamide disc gel electrophoresis: validity of comparisons and effect of 'nothing dehydrogenase' activity." *Biochem. Genet.,* **16**: 531-540.

GILLESPIE, J.H. (1977) "Natural selection for variance in offspring numbers: a new evolutionary principle." *Am. Nat.,* **111**: 1010-1014.

GNAIGER, E. & H. FORSTNER (1983) *"Polarographic Oxygen sensors: aquatic and physiological applications"* Springer-Verlag, Berlin.

GOOCH, J.L., B.S. SMITH & D. KNAPP (1972) "Regional survey of gene frequencies in the mud snail *Nassarius obsoletus*." *Biol. Bull,* **142**: 36-48.

GRAHAME, J. (1973) "Assimilation efficiency of *Littorina littorea* (L.) (Gastropoda: Prosobranchiata)." *J. Anim. Ecol.*, **42**: 383-398.

GRAHAME, J. (1977) "Reproductive Effort and *r*- and *K*- selection in two species of *Lacuna* (Gastropoda: Prosobranchia)." *Mar. Biol.*, **40**: 217-224.

GRAHAME, J. (1982) "Energy flow and breeding in two species of *Lacuna*: comparative costs of egg production and maintenance." *Int. J. Inv. Repr.*, **5**: 91-99.

GRAHAME, J. & G.M. BRANCH (1985) "Reproductive patterns of marine invertebrates." *Oceanogr. Mar. Biol. Ann. Rev.*, **23**: 373-398.

GRANT, A. (1983) "On the evolution of brood protection in marine benthic invertebrates." *Am. Nat.*, **122**: 549-555.

GRASSLE, J.F. & J.P. GRASSLE (1978) "Life histories and genetic variation in marine invertebrates." *In:* BATTAGLIA, B. & J.A. BEARDMORE (eds.) *"Marine Organisms: Genetics, Ecology and Evolution"* Plenum Press, N.Y., pp. 347-364.

GRIFFITHS, R.J. (1980) "Filtration, respiration and assimilation in the Black Mussel *Choromytilus meridionalis*." *Mar. Ecol.- Prog. Ser.*, **3**: 63-70.

GRIFFITHS,R.J. (1981) "Population dynamics and growth of the bivalve *Choromytilus meridionalis* (Kr.) at different tidal levels." *Est. Coast. & Shelf Sci.*, **12**: 101-118.

HADFIELD, M.G. & M. SWITZER-DUNLAP (1984) "Opisthobranchs" *In:* TOMPA, A.S., N.H. VERDONK & J.A.M. van den BIGGELAAR (eds.) *"The Mollusca Vol. VII: Reproduction"* Acad. Press, N.Y., pp. 209-350.

HALL, S.J. (1983) *"Aspects of the Biology and Ecology of the nudibranch mollusc Aeolidia papillosa* (L.)." Unpubl. Ph.D. Thesis, Univ. of St Andrews, U.K..

HALL, S.J. & C.D. TODD (1986) "Growth and reproduction in the aeolid nudibranch *Aeolidia papillosa* (L.)." *J. Moll. Stud.*, (in press).

HAMMEN, C.S. (1979) "Metabolic rates of marine bivalve molluscs determined by calorimetry." *Comp. Biochem. Physiol.*, **62A**: 955-959.

HARRIGAN, J.F. & D.L. ALKON (1978) "Larval rearing, metamorphosis, growth and reproduction of the aeolid nudibranch *Hermissenda crassicornis* (Eschscholtz, 1831) (Gastropoda: Opisthobranchia)." *Biol. Bull.*, **154**: 430-439.

HARRIS, H. & D.A. HOPKINSON (1978) *"Handbook of enzyme electrophoresis in human genetics"* North-Holland, Amsterdam.

HARRIS, L.G. (1973) "Nudibranch associations." *Curr. Topics comp. Pathobiol.*, **2**: 213-315.

HART, A. & M. BEGON (1982) "The status of general reproductive strategy theories, illustrated in winkles." *Oecologia*, **52**: 37-42.

HARVELL, D. (1984) "Why nudibranchs are partial predators: Intracolonial variation in bryozoan palatibility." *Ecology*, **65**: 716-724.

HIRSHFIELD, M.F. & D.W. TINKLE (1975) "Natural selection and the evolution of reproductive effort." *Proc. Nat. Acad. Sci. U.S.A.*, **72**: 2227-2231.

HILBISH, T.J. (1985) "Demographic and temporal structure of an allele frequency cline in the mussel *Mytilus edulis*." *Mar. Biol.*, **86**: 163-171.

HITCHMAN, M.R. (1978) *"Measurement of dissolved Oxygen. Chemical Analysis Vol. 49"*, Wiley, London.

HOAGLAND, K.E. (1984) "The use of molecular genetics to distinguish species of the gastropod genus *Crepidula* (Prosobranchia: Calyptraeidae)." *Malacologia*, **25**: 607-628.

HUEBNER, J.D. & D.C. EDWARDS (1981) "Energy budget of the predatory marine gastropod *Polinices duplicatus*." *Mar. Biol.*, **61**: 221-226.

HUGHES, R.N. (1971) "Ecological energetics of *Nerita* (Archaeogastropoda: Neritacea) populations on Barbados, West Indies." *Mar. Biol.*, **11**: 12-22.

HUGHES, R.N. (1972) "Annual production of two Nova Scotian populations of *Nucella lapillus*." *Oecologia*, **8**: 356-370.

HUGHES, R.N. & D.J. ROBERTS (1980) "Reproductive effort of winkles (*Littorina* spp.) with contrasted methods of reproduction." *Oecologia*, **47**: 130-136.

HUNTER, T & S. VOGEL (1986) "Spinning embryos enhance diffusion through gelatinous egg masses." *J. exp. mar. Biol. Ecol.*, **96**: 303-308.

HURST, A. (1967) "The egg masses and veligers of thirty northeast Pacific opisthobranchs." *Veliger*, **9**: 255-288.

JABLONSKI, D. & R.A. LUTZ (1983) "Larval ecology of marine benthic invertebrates: palaeobiological implications." *Biol. Rev.*, **58**: 21-89.

JACKSON, G.A. & R.R. STRATHMANN (1981) "Larval mortality from offshore mixing as a link between precompetent and competent periods of development." *Am. Nat.*, **118**: 16-26.

JARDINE, I.W. (1985) " Height on the shore as a factor influencing growth rate and reproduction of the Top-shell *Gibbula cineraria* (L.)." *In:* MOORE, P.G. & R. SEED (eds.) *"The Ecology of Rocky Coasts"* Hodder & Stoughton, Kent, pp.225-259.

JOHNSON, M.S. & R. BLACK (1982) "Chaotic genetic patchiness in an intertidal limpet, *Siphonaria* sp.." *Mar. Biol.*, **70**: 157-164.

JOHNSON, M.S. & R. BLACK (1984a) "Pattern beneath the chaos: the effect of recruitment on genetic patchiness in an intertidal limpet." *Evolution*, **38**: 1371-1383.

JOHNSON, M.S. & R. BLACK (1984b) "The Wahlund effect and the geographical scale of variation in the intertidal limpet *Siphonaria* sp.." *Mar. Biol.*, **79**: 295-302.

JOHNSTON, R.J. (1980) *"Multivariate statistical analysis in geography"* Longman, London.

JUKES, T.H. & R. HOLMQUIST (1972) "Evolutionary clock: non-constancy of rate in different species." *Science*, **177**: 530-532.

KAUFMAN, K.W. (1981) "Fitting and using growth curves." *Oecologia*, **49**: 293-299.

KERSTING, K. (1972) "A Nitrogen correction for caloric values." *Limnol. Oceanogr.*, **17**: 643-644.

KEMPF, S.C. (1981) "Long-lived larvae of the gastropod *Aplysia juliana:* Do they disperse and metamorphose or just slowly fade away?" *Mar. Ecol. - Prog. Ser.*, **6**: 55-61.

KEMPF, S.C. & A.O.D. WILLOWS (1977) "Laboratory culture of the nudibranch *Tritonia diomedea* Bergh (Tritoniidae, Opisthobranchia) and some aspects of its behavioural development." *J. exp. mar. Biol. Ecol.*, **30**: 261-276.

KEMPF, S.C. & M.G. HADFIELD (1985) "Planktotrophy by the lecithotrophic larvae of a nudibranch, *Phestilla sibogae* (Gastropoda)." *Biol. Bull,* **169**: 119-130.

KIMURA, M. (1983) *"The neutral theory of molecular evolution"* Cambridge Univ. Press, Cambridge.

KING, M.C. & A.C. WILSON (1975) "Evolution at two levels. Molecular similarities and biological differences between humans and chimpanzees." *Science,* **188**: 107-116.

KOEHN, R.K., R. NEWELL & F. IMMERMANN (1980) "Maintenance of an aminopeptidase allele frequency cline by natural selection." *Proc. Nat. Acad. Sci. U.S.A.,* **77**: 5385-5389.

KOREY, K.A. (1981) "Species number, generation length and the molecular clock." *Evolution,* **35**: 139-147.

LACEY, E.P., L.A. REAL, J. ANTONOVICS & D.G. HECKEL (1983) "Variance models in the study of life histories." *Am. Nat.,* **122**: 114-131.

LEDERHENDLER, I.I. & E. TOBACH (1977) "Reproductive roles in the simultaneous hermaphrodite *Aplysia dactylomela.*" *Nature,* **270**: 238-239.

LESSIOS, H.A. (1979) "Use of Panamanian sea urchins to test the molecular clock." *Nature,* **280**: 599-601.

LEWONTIN, R.C. (1958) "A general method for estimating the equilibrium of gene frequency in a population." *Genetics,* **43**: 419-434.

LEWONTIN, R.C. (1974) *"The Genetic Basis of Evolutionary Change"* Columbia Univ. Press, N.Y..

LI, W. & M. NEI (1975) "Drift variances of heterozygosity and genetic distance in transient states." *Genet. Res.,* **25**: 229-248.

LINCOLN, R.J., G.A. BOXSHALL & P.F. CLARKE (1982) *"A Dictionary of Ecology, Evolution and Systematics"* Cambridge University Press, Cambridge.

MAYNARD-SMITH, J. (1978) *"The evolution of sex"* Cambridge University Press, Cambridge.

MEEHAN, B.W. (1985) "Genetic comparison of *Macoma balthica* (Bivalvia, Telinidae) from the eastern and western North Atlantic Ocean." *Mar. Ecol. - Prog. Ser.*, **22**: 69-76.

MENGE, B. (1974) "Effect of wave action and competition on brooding and reproductive effort in the sea-star *Leptasterias hexactis*." *Ecology*, **55**: 84-93.

MENGE, B. (1975) "Brood or broadcast? The adaptive significance of different reproductive strategies in the two intertidal sea stars *Leptasterias hexactis* and *Pisaster ochraceus*." *Mar. Biol.*, **31**: 87-100.

MILLEN, S. (1983) "Range extensions of opisthobranchs in the northeastern Pacific." *Veliger*, **25**: 383-386.

MILLER, M.C. (1958) *"Studies on the nudibranchiate Mollusca of the Isle of Man"* Unpubl. Ph.D. Thesis, Univ. Liverpool, U.K..

MILLER, M.C. (1961) "Distribution and food of the nudibranchiate Mollusca of the south of the Isle of Man." *J. Anim. Ecol.*, **30**: 95-116.

MILLER, M.C. (1962) "Annual cycles of some Manx nudibranchs with a discussion of the problem of migration." *J. Anim. Ecol.*, **31**: 545-569.

MILLER, S. & M.G. HADFIELD (1986) "Ontogeny of phototaxis and metamorphic comptetence in larvae of the nudibranch *Phestilla sibogae* Bergh (Gastropoda: Opisthobranchia)." *J. exp. mar. Biol. Ecol.*, **97**: 95-112.

NEI, M. (1972) "Genetic distance between populations." *Am. Nat.*, **106**: 283-292.

NEI, M. (1978) "Estimation of average heterozygosity and genetic distance from a small number of individuals." *Genetics*, **89**: 583-590.

NEI, M. & A.K. ROYCHOUDHURY (1974) "Sampling variances of heterozygosity and genetic distance." *Genetics*, **76**: 379-390.

NEWELL, R.C. & H.R. NORTHCROFT (1967) "A re-interpretation of the effect of temperature on the metabolism of certain marine invertebrates." *J. Zool. Lond.*, **151**: 277-298.

NEWKIRK, G.F. & L.E. HALEY (1982) "Phenotypic analysis of the european oyster *Ostrea edulis:* Relationship between length of larval period and postsetting growth rate." *J. exp. mar. Biol. Ecol.*, **59**: 177-184.

OBREBSKI, S. (1979) "Larval colonizing strategies in marine benthic invertebrates." *Mar. Ecol. - Prog. Ser.*, **1**: 293-300.

O'DOR, R.K. & M.J. WELLS (1978) "Reproduction versus somatic growth: hormonal control in *Octopus vulgaris*." *J. Exp. Biol.*, **77**: 15-31.

ODUM, H.T. (1951) "Nudibranch spicules made of amorphous Calcium Carbonate." *Science*, **114**: 395.

OXFORD, G.S. & D. ROLLINSON (1983) (eds.) *"Protein polymorphism: Adaptive and taxonomic significance"* Acad. Press, London.

PAINE, R.T. (1965) "Natural history, limiting factors and energetics of the opisthobranch *Navanax inermis*." *Ecology*, **46**: 603-619.

PAINE, R.T. (1966) "Endothermy in bomb calorimetry." *Limnol. Oceanogr.*, **11**: 126-129.

PALMER, A.R. & R.R. STRATHMANN (1981) "Scale of dispersal in varying environments and its implications for life histories of marine invertebrates." *Oecologia*, **48**: 308-318.

PEARSON, M.P., M.D. BURNS & P. SPENCER-DAVIES (1984) "An underwater respirometer and programmable data logger for *in situ* energy budget studies." *J. exp. mar. Biol. Ecol.*, **74**: 231-239.

PECHENIK, J.A. (1979) "Role of encapsulation in invertebrate life-histories." *Am. Nat.*, **114**: 859-870.

PECHENIK, J.A. (1980) "Growth and energy balance during the larval lives of three prosobranch gastropods." *J. exp. mar. Biol. Ecol.*, **44**: 1-28.

PECHENIK, J.A. (1984) "The relationship between temperature, growth rate and duration of planktonic life for larvae of the gastropod *Crepidula fornicata*." *J. exp. mar. Biol. Ecol.*, **74**: 241-257.

PECHENIK, J.A. & N.S. FISHER (1979) "Feeding, assimilation and growth of mud snail larvae, *Nassarius obsoletus* (Say), on three different algal diets." *J. exp. mar. Biol. Ecol.*, **38**: 57-80.

PECHENIK, J.A. & G.M. LIMA (1984) "Relationship between growth, differentiation, and length of larval life for individually reared larvae of the marine gastropod *Crepidula fornicata*." *Biol. Bull.*, **166**: 537-549

PECHENIK, J.A., R.S. SCHELTEMA & L.S. EYSTER (1984) "Growth stasis and limited shell calcification in larvae of *Cymatium parthenopeum* during trans-Atlantic transport." *Science*, **224**: 1097-1099.

PERRON, F.E. (1982) "Inter- and intra-specific patterns of reproductive effort in four species of cone shells (*Conus* spp.)." *Mar. Biol.*, **68**: 161-167.

PERRON, F.E. & R.D. TURNER (1977) "Development, metamorphosis, and natural history of the nudibranch *Doridella obscura* Verrill (Corambidae: Opisthobranchia)." *J. exp. mar. Biol. Ecol.*, **27**: 171-185.

PERRON, F.E. & R.H. CARRIER (1981) "Egg size distributions among closely related marine invertebrate species: are they bimodal or unimodal." *Am. Nat.*, **118**: 749-755.

PETERS, R.H. (1983) *"The ecological implications of body size"* Cambridge Univ. Press, Cambridge.

PIANKA, E.R (1970) "On *r*- & *K*- selection." *Am. Nat.*, **100**: 592-597.

PIANKA, E.R. & W.S. PARKER (1975) "Age-specific reproductive tactics." *Am. Nat.*, **109**: 453-464.

PILKINGTON, M.C. & V. FRETTER (1970) "Some factors affecting the growth of prosobranch veligers." *Helgol. Wiss. Meeresunters.*, **20**: 576-593.

POTTS, G.W. (1970) "The ecology of *Onchidoris fusca* (Nudibranchia)." *J. Mar. Biol. Ass. U.K.*, **50**: 269-292.

POULIK, M.D. (1957) "Starch gel electrophoresis in a discontinuous system of buffers." *Nature*, **180**: 1477-1479.

PROPP, M.V., M.R. GARBER & V.I. RYABUSCKO (1982) "Unstable processes in the metabolic rate measurements in flow-through systems." *Mar. Biol.*, **67**: 47-49.

PROVASOLI, L. (1968) "Media and prospects for the cultivation of marine algae." *In:* WATANABE, A. & A. HATTORI (eds.) *"Culture and Collections of Algae" Proc. U.S. Japan Conf. Hakone, Jap. Soc. Plant Physiol.*, pp. 63-75.

RACINE, R.R. & C.H. LANGLEY (1980) "Genetic analysis of protein variations in *Mus musculus* using two-dimensional electrophoresis." *Biochem. Genet.*, **18**: 185-198.

RAMSHAW, J.A.M., J.A. COYNE & R.C. LEWONTIN (1979) "The sensitivity of gel electrophoresis as a detector of genetic variation." *Genetics*, **93**: 1019-1037.

REAL, L.A. (1980) "Fitness, uncertainty, and the role of diversification in evolution and behaviour." *Am. Nat.*, **115**: 623-638.

RICKER, W.E.(1973) "Linear Regressions in Fishery Research." *J. Fish. Res. Bd. Can.*, **30**: 409-434.

RODHOUSE, P.G., C.M. RODEN, G.M. BURNELL, M.P. HENSEY, T. McMAHON, B. OTTWAY & T.H. RYAN (1984) "Food resource, gametogenesis and growth of *Mytilus edulis* on the shore and in suspended culture: Killary Harbour, Ireland." *J. Mar. Biol. Ass. U.K.*, **64**: 513-529.

RUSSELL-HUNTER, W.D. & D.E. BUCKLEY (1983) "Actuarial Bioenergetics of non-marine Molluscan productivity" *In:* RUSSELL-HUNTER, W.D. (ed.) *"The Mollusca, Vol. VI: Ecology"* Acad. Press, N.Y., pp.463-503.

RUSSELL-HUNTER, W.D. & F.A. ROMANO (1981) "Reproductive effort of molluscs in bioenergetic terms: some computational methods." *Biol. Bull.*, **161**: 316.

RUSSELL-HUNTER, W.D., R.A. BROWNE & D.W. ALDRIDGE (1984) "Overwinter tissue degrowth in natural populations of freshwater pulmonate snails (*Helisoma trivolvis* and *Lymnaea palustris*)." *Ecology*, **65**: 223-229.

SARGENT, J.R. & S.G. GEORGE (1975) *"Methods in zone electrophoresis"* BDH Chemicals, Poole, Dorset.

SARICH, V.M. (1977) "Rates, sample sizes and the neutrality hypothesis for electrophoresis in evolutionary studies." *Nature*, **265**: 24-28.

SCHAAL, B.A. & W.W. ANDERSON (1974) *"An outline of techniques for starch gel electrophoresis of enzymes from the American oyster Crassostrea virginica (Gmelin)."* Tech. Rep. **74** (3), Georgia Mar. Sci. Center, Georgia.

SCHAFFER, W.M. (1974) "Selection for optimal life histories: The effects of age structure." *Ecology*, **55**: 291-303.

SCHAFFER, W.M. (1979) "Equivalence of maximising reproductive value and fitness in the case of reproductive strategies." *Proc. Nat. Acad. Sci. U.S.A.*, **76**: 3567-3569.

SCHELTEMA, R.S. (1971) "Larval dispersal as a means of genetic exchange between geographically separated populations of shoal-water benthic marine gastropods." *Biol Bull.*, **140**: 284-322.

SCHELTEMA, R.S. (1978) "On the relationship between dispersal of pelagic veliger larvae and the evolution of marine prosobranch gastropods." *In:* BATTAGLIA, B. & J.A. BEARDMORE (eds.) *"Marine Organisms: Genetics, Ecology and Evolution"* Plenum Press, N.Y., pp. 303-322.

SCHMIDT-NIELSEN, K. (1984) *"Scaling: Why is animal size so important?"* Cambridge Univ. Press, Cambridge.

SEBENS, K.P. (1979) "The energetics of asexual reproduction and colony formation in benthic marine invertebrates." *Am. Zool.*, **19**: 683-697.

SEED, R. (1976) "Observations on the ecology of *Membranipora* (Bryozoa) and a major predator *Doridella steinbergae* (Nudibranchiata) along the fronds of *Laminaria saccharina* at Friday Harbor,Washington." *J. exp. mar. Biol. Ecol.*, **24**: 1-17.

SHAW, C.R. (1965) "Electrophoretic variation in enzymes." *Science*, **149**: 936-943.

SHAW, P.R. & R. PRASAD (1970) "Starch gel electrophoresis of enzymes - a compilation of recipes." *Biochem. Genet.*, **4**: 297-320.

SHIRAISHI, K. & L. PROVASOLI (1959) "Growth factors as supplements to inadequate algal foods for *Tigriopus japonicus*." *Tohoku J. Agric. Res.*, **10**: 89-96.

SLATKIN, M. (1974) "Hedging one's evolutionary bets." *Nature*, **250**: 704-705.

SLOBODKIN, L.B. (1959) "Energetics in *Daphnia pulex* populations." *Ecology*, **40**: 232-243.

SMITH, D.A. & K.P. SEBENS (1983) "The physiological ecology of growth and reproduction in *Onchidoris aspera* (A&H) (Gastropoda: Nudibranchia)." *J. exp. mar. Biol. Ecol.*, **72**: 287-304.

SNYDER, T.P. & J.L. GOOCH (1973) "Genetic differentiation in *Littorina saxatilis*." *Mar. Biol.*, **22**: 177-182.

SOKAL, R.R. & F.J. ROHLF (1981) *"Biometry"* Freeman, San Francisco.

STEARNS, S.C. (1976) "Life history tactics: a review of the ideas." *Q. Rev. Biol.*, **51**: 3-47.

STEARNS, S.C. (1977) "The evolution of life history traits: a critique of the theory and a review of the data." *Ann. Rev. Ecol. Syst.*, **8**: 145-171.

STICKLE, W.B. (1973) "The reproductive physiology of the intertidal prosobranch *Thais lamellosa* (Gmelin). I: Seasonal changes in the rate of oxygen consumption and body component indexes." *Biol. Bull.*, **144**: 511-524.

STRATHMANN, R.R. (1978a) "The evolution and loss of feeding larval stages of marine invertebrates." *Evolution*, **32**: 894-906.

STRATHMANN, R.R (1978b) "Progressive vacating of adaptive types during the Phanerozoic." *Evolution*, **32**: 907-914.

STRATHMANN, R.R (1985) "Feeding and non-feeding larval development and life-history evolution in marine invertebrates." *Ann. Rev. Ecol. Syst.*, **16**: 339-361.

STRATHMANN, R.R. & C. CHAFFEE (1984) "Constraints on egg masses II: Effect of spacing, size and number of eggs on ventilation of masses of embryos in jelly, adherent groups or thin-walled capsules." *J. exp. mar. Biol. Ecol.*, **84**: 85-93.

SWENNEN, C. (1961) "Data on distribution, reproduction and ecology of the nudibranchiate molluscs occurring in the Netherlands." *Neth. J. Sea. Res.*, **1**: 191-240.

SWITZER-DUNLAP, M. & M.G. HADFIELD (1977) "Observations on development, larval growth, and metamorphosis of four species of Aplysiidae (Gastropoda: Opisthobranchia) in laboratory culture." *J. exp mar. Biol. Ecol.*, **29**: 245-261.

THOMPSON, R.J (1983) "The relationship between food ration and reproductive effort in the green sea-urchin *Strongylocentrotus droebachiensis*." *Oecologia*, **56**: 50-57.

THOMPSON, R.J. & B.L .BAYNE (1974) "Some relationships between growth, metabolism and food in the mussel *Mytilus edulis*." *Mar. Biol.*, **27**: 317-326.

THOMPSON, T.E. (1958a) "The natural history, embryology, larval biology and post-larval development of *Adalaria proxima*." *Phil. Trans. R. Soc. Ser. B.*, **242**: 1-58.

THOMPSON, T.E. (1958b) "Observations on the radula of *Adalaria proxima* (A & H) (Gastropoda: Opisthobranchia)." *Proc. Malac. Soc. Lond.*, **33**: 49-56.

THOMPSON, T.E. (1961) "Observations on the life history of the nudibranch *Onchidoris muricata* (Müller)." *Proc. Malac. Soc. Lond.*, **34**: 239-242.

THOMPSON, T.E. (1964) "Grazing and the life cycles of British nudibranchs." *In:* CRISP, D.J. (ed.) *"Grazing in Marine and Terrestrial environments"* Blackwell, Oxford.

THOMPSON, T.E. (1976) *"Biology of opisthobranch molluscs. Vol I"* Ray Society, London.

THOMPSON, T.E. & G.H. BROWN (1976) *"British opisthobranch molluscs"* Linn. Soc. & Acad. Press, London.

THOMPSON, T.E. & G.H. BROWN (1984) *"Biology of opisthobranch molluscs. Vol II"* Ray Society, London.

THORPE, J.P. (1979) "Enzyme variation and taxonomy: the estimation of sampling errors in measures of interspecific genetic similarity." *Biol. J. Linn. Soc.*, **11**: 369-386.

THORPE, J.P. (1982) "The molecular clock hypothesis: Biochemical evolution, genetic differentiation and systematics." *Ann. Rev. Ecol. Syst.*, **13**: 139-168.

THORPE, J.P. (1983) "Enzyme variation, genetic distance and evolutionary divergence in relation to levels of taxonomic separation." *In:* OXFORD, G.S. & D. ROLLINSON (eds.) *"Protein polymorphism: Adaptive and taxonomic significance"* Acad. Press, London, pp. 131-152.

THORSON, G. (1946) "Reproduction and larval development of Danish marine bottom invertebrates, with special reference to the planktonic larvae in the Sound (Øresund)." *Meddr Kommn Danm. Fisk. -og Havunders., Serie: Plankton,* **4**: 1-523.

TINKLE, D.W. (1969) "The concept of reproductive effort and its relation to the life-histories of lizards." *Am. Nat.*, **103**: 501-516.

TINKLE, D.W. & N.F. HADLEY (1975) "Lizard reproductive effort: caloric estimates and comments on its evolution." *Ecology*, **56**: 427-434.

TODD, C.D. (1977) *"The ecology of nudibranch molluscs"* Unpubl. Ph.D. Thesis, Univ. Leeds, U.K..

TODD, C.D. (1978a) "Gonad development of *Onchidoris muricata* (Müller) in relation to size, age and spawning (Gastropoda: Opisthobranchia)." *J. Moll. Stud.*, **44**: 190-199.

TODD, C.D. (1978b) "Changes in spatial pattern of an intertidal population of the nudibranch mollusc *Onchidoris muricata* in relation to spatial pattern and environmental heterogeneity." *J. Anim. Ecol.*, **47**: 189-203.

TODD, C.D. (1979a) "Reproductive energetics of two species of dorid nudibranchs with planktotrophic and lecithotrophic larval strategies." *Mar. Biol.*, **53**: 57-68.

TODD, C.D. (1979b) "The population ecology of *Onchidoris bilamellata* (Gastropoda: Nudibranchia)." *J. exp. mar. Biol. Ecol.*, **41**: 213-255.

TODD, C.D. (1979c) "The annual cycles of two species of *Onchidoris* (Opisthobranchia: Nudibranchia)." *In:* NAYLOR, E. & R.G. HARTNOLL (eds.) *"Cyclic Phenomena in Marine Plants and Animals"*, Proc. 13th European Marine Biological Symposium, Pergamon Press, Oxford, pp. 65-72.

TODD, C.D. (1981) "The Ecology of Nudibranch Molluscs." *Oceanogr. Mar. Biol. Ann. Rev.*, **19**: 141-234.

TODD, C.D. (1983) "Reproductive and trophic ecology of nudibranch molluscs." *In:* RUSSELL-HUNTER, W.D. (ed.) *"The Mollusca Vol.VI: Ecology"* Acad. Press, N.Y., pp.225-259.

TODD, C.D. (1985) "Reproductive Strategies of North Temperate Rocky Shore Invertebrates." *In:* MOORE, P.G. & R. SEED (eds.) *"The Ecology of Rocky Coasts."* Hodder & Stoughton, Kent, pp. 203-219.

TODD, C.D. (1986a) "Reproductive energetics and larval strategies of nudibranch molluscs: effects of ration level during the spawning period in *Onchidoris muricata* (Müller) and *Adalaria proxima* (A & H)." *Malacological Reviews*, (in press).

TODD, C.D. (1986b) "Larval strategies of nudibranch molluscs: similar means to the same end?" *In:* EDMUNDS, M. (ed.) *Proc. 9th Int. Malac. Congress, Edinburgh, 1986*, (in press).

TODD, C.D. & R.W. DOYLE (1981) "Reproductive strategies of marine benthic invertebrates: A settlement - timing hypothesis." *Mar. Ecol. - Prog. Ser.* **4**: 75-83.

TODD, C.D. & J.N. HAVENHAND (1983) "Reproductive effort: Its definition, measurement and interpretation in relation to molluscan life-history strategies." *J. Moll. Stud. Suppt.* **12A**: 203-208.

TODD, C.D. & J.N. HAVENHAND (1985) "Preliminary observations on the embryonic and larval development of three dorid nudibranchs." *J. Moll. Stud.*, **51**: 97-99.

TODD, C.D. & J.N. HAVENHAND (1986) "Inter-relationships of Life-Cycle, Life-History and larval adaptations of Nudibranch Molluscs." *Am. Malac. Bull.*, **4**: 103-104.

UNDERWOOD, A.J. (1979) "The ecology of intertidal gastropods." *Adv. Mar. Biol.*, **16**: 111-210.

VAHL, O. (1980) "Seasonal variations in seston and in the growth rate of the Iceland scallop *Chlamys islandica* (O.F.Müller) from Balsfjord,." *J. exp. mar. Biol. Ecol.*, **48**: 195-204.

VAHL, O. (1981) "Energy transformations by the Iceland scallop, *Chlamys islandica* (O.F.Müller) from 70° N. I: The age-specific energy budget and net growth efficiency." *J. exp. mar. Biol. Ecol.*, **53**: 281-296.

VANCE, R.R. (1973a) "On reproductive strategies in marine benthic invertebrates." *Am. Nat.*, **107**: 339-352.

VANCE, R.R. (1973b) "More on reproductive strategies in marine benthic invertebrates." *Am. Nat.*, **107**: 353-361.

VANCE, R.R. (1980) "The effect of dispersal on population size in a temporally varying environment." *Theor. Pop. Biol.*, **18**: 343-362.

VANCE, R.R. (1984) "The effect of dispersal on population stability in one-species, discrete-space population growth models." *Am. Nat.*, **123**: 230-254.

WALNE, P.R. (1963) "Observations on the food value of seven species of algae to the larvae of *Ostrea edulis* L.. Feeding experiments." *J. Mar. Biol. Ass. U.K.*, **43**: 767-784.

WARD, R.D. & J.A. BEARDMORE (1977) "Protein variation in the plaice (*Pleuronectes platessa*)." *Genet. Res.*, **30**: 45-62.

WARD, R.D. & T. WARWICK (1980) "Genetic differentiation in the molluscan species *Littorina rudis* and *Littorina arcana* (Prosobranchia: Littorinidae)." *Biol. J. Linn. Soc.*, **14**: 417-428.

WARREN, C.E. & G.E. DAVIS (1967) "Laboratory studies on the feeding, bioenergetics and growth of fish." *In*: GERKING, S.D. (ed.) *"The biological basis of fish production"* Blackwell, Oxford, pp. 175-214.

WELCH, M.E. (1968) "Relationships between assimilation efficiencies and growth efficiencies for aquatic consumers." *Ecology*, **49**: 755-759.

de WILDE, P.A.W.J. (1973) "A continuous flow apparatus for long-term recording of Oxygen uptake in burrowing invertebrates, with some remarks on the uptake in *Macoma balthica*." *Neth. J. Sea. Res.*, **6**: 157-162.

WILLIAMS, G.C. (1966) "Natural selection, the costs of reproduction, and a refinement of Lack's principles." *Am. Nat.*, **100**: 687-690.

WILSON, A.C., S.S. CARLSON & T.J. WHITE (1977) "Biochemical evolution." *Ann. Rev. Biochem.*, **46**: 573-569.

WRIGHT, J.R. & R.G. HARTNOLL (1981) "An energy budget for a population of the limpet *Patella vulgata*." *J. Mar. Biol. Ass. U.K.*, **61**: 627-646.

WRIGHT, S. (1978) *"Evolution and the genetics of populations. Vol. IV: Variability within and among populations"* Chicago Univ. Press, Chicago.

YANG, S.Y., M. SOULÉ & G.C. GORMAN (1974) "*Anolis* lizards of the eastern Caribbean: a case study in evolution. I. Genetic relationships, phylogeny, and colonisation sequence of the *roquet* group." *Syst. Zool.*, **23**: 387-399.

ZEUTHEN, E. (1953) "Oxygen uptake as related to body size in organisms." *Q. Rev. Biol.*, **28**: 1-12.