ASPECTS OF THE LIFE HISTORY AND PHYSIOLOGICAL ECOLOGY OF LONG-LIVED NUDIBRANCH MOLLUSCS

Jonathan Davies

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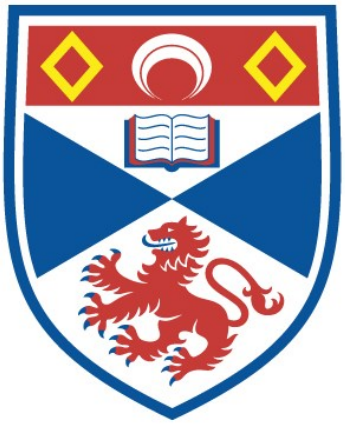

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ASPECTS OF THE LIFE HISTORY AND PHYSIOLOGICAL ECOLOGY OF LONG-LIVED NUDIBRANCH MOLLUSCS

BY

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Jonathan Davies March 25, 1992.

I was admitted to the Faculty of Science of the University of St. Andrews under Ordinance General Nº 12 in October 1985 and as a candidate for the degree of Ph.D. in October 1986.

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CONTENTS

ABSTRACT

Nudibranchia is the major Order of the gastropod Sub-class Opisthobranchia. Nudibranchs demonstrate a considerable evolutionary departure from the typical shelled gastropod and have achieved wide morphological and ecological diversity. Most species are recorded from nearshore rocky habitats where they are primary macrobenthic predators; most species are stenophagous limited to a single, or a few, prey species. Previous investigations had shown that most nudibranch species have evolved to an annual semelparous life history strategy and adopt a planktotrophic larval development strategy which would appear to be the ancestral conditions. Reviews of published data had drawn attention to some species which demonstrated an evolutionary departure from these ancestral modes and had evolved extended life history strategies combined with more advanced larval development strategies. *Archidoris pseudoargus* and *Cadlina laevis* are sponge-grazing dorid nudibranchs, and *Tritonia hombergi* , which feeds on an alyconarean soft coral, appeared to have extended life cycles. The aim of the present study was to investigate the life history and larval development strategies of these species and, via a study their physiological ecology, attempt to determine the selective forces which may have driven any evolutionary departure away from the ancestral condition.

Archidoris pseudoargus was found to have a biennial semelparous life history strategy and a planktotrophic larval development strategy; the planktotrophic larvae were found to have some characteristics of the more advanced lecithotrophic larval form. *Cadlina laevis* was found to have a perennial iteroparous life history strategy and a direct mode of larval development; this was the first observation of perennial iteroparity in the Order Nudibranchia. *Archidoris pseudoargus* and *Cadlina laevis* had markedly seasonal growth patterns with a high growth rate in spring and early summer, followed by a period of reduced growth, or even degrowth in late summer and over the winter. An allometric relationship was determined between body size and feeding rate for *A. pseudoargus*; the exponent was less than unity. For both species, a significant allometric relationship was determined between body size and respiration rate with an exponent less than unity indicating an increase in metabolic efficiency with body size.

Both species displayed an acclimatization of respiration rate with increasing temperature; the acclimatization was more pronounced in *C. laevis*. Fecundity of both species was shown to have a significant allometric relationship body size with an exponent less than unity. Insufficient numbers of *T. hombergi* were collected and kept alive in the laboratory to undertake any experiments to generate meaningful data to answer these principal questions for this species.

Results of the present study together with data published from previous investigations have been interpreted to suggest that the extension of the life cycle is a consequence of seasonal variations in prey quality and quantity. Rather than being an exception to the rule, it is suggested that extended life cycles could be the ancestral condition and the more common annual/sub annual life cycles would be a more adapted condition. These conclusions are based on the results obtained from laboratory investigations which are discussed in the their application to the field situation.

CHAPTER 1

GENERAL INTRODUCTION

The gastropod Sub-class Opisthobranchia consists of nine Orders: Nudibranchia, Anaspidea, Cephalaspidea, Sacoglossa, Notaspidea, Gymnosomata, Thecosomata, Acochlidiacea and Pyramidellacea of which Order Nudibranchia is the major order (Hadfield & Switzer-Dunlap 1984). Nudibranchs demonstrate a considerable evolutionary departure from a typical shelled gastropod, having undergone complete detorsion of the visceral mass and lost the shell and operculum, the mantle cavity and the gill. Consequently they have achieved a very wide morphological and ecological diversity, with species recorded in habitats ranging from interstitial spaces in sediments (Swedmark 1964) through to pelagic oceanic habitats (Cheng 1975). Most species are recorded from nearshore rocky habitats where nudibranchs are primary macrobenthic predators (Todd 1981, 1983). In general, most species are specific predators on a single, or a few, prey species: Todd (1981) found that, for species where nutritional information was available, approximately 50% of species are associated with a single, or perhaps two, prey species. In view of their wide variety of body form, diverse 'flower-like' egg masses and their importance within benthic communities, nudibranchs have attracted the attention of biologists since the mid-nineteenth century. Alder & Hancock (1845-1855) produced a monograph of British nudibranchs which included excellent colour illustrations of the main species. In the early part of the twentieth century, investigators concentrated on aspects of the physiology of nudibranchs (for review: Hyman 1967; Franc 1968). In the 1950's, attention began to focus on the embryonic development and, more recently, reproductive ecology; reproductive ecology will be discussed in more detail later in this introduction. Although specific predators, and hence potentially important structuring elements of marine benthic communities, nudibranchs were generally overlooked in many of early ecological studies. Harris (1973) reviewed much of the early literature which concentrated on the basic biology and taxonomy of nudibranchs, with Todd (1981, 1983) producing the first comprehensive reviews of nudibranch ecology.

Todd (1981, 1983) noted that the majority of nudibranch species for which data are available, undergo simple annual life cycles with total post-spawning mortality and thus

no generation overlap. In recent times, nudibranchs have received considerably more attention in the scientific literature, investigations concentrating on most aspects of their biology and ecology but for annual species; references to specific aspects of nudibranch biology/ecology are reviewed in the *Introduction* to each Chapter. Not all nudibranch species have annual life cycles, biennial life cycles (*i.e.* two years) have been recorded (Miller 1962; Todd 1977, 1981) which, on the basis of the few records of biennialism, would appear to be a departure from the ancestral annual life cycle. Initially, the broad objectives of the present study were to undertake a rationalization of contrasting life cycle, life history and larval reproductive strategies, with contemporary ecological theory for nudibranch species with extended life cycles, while highlighting the functional aspects of physiological ecology. After preliminary investigations, some of these objectives were amended but, nevertheless, the broad intention to concentrate on aspects of physiological ecology remained throughout.

Sibly & Calow (1986) published a volume entitled *Physiological ecology of animals: an evolutionary approach* which emphasizes the importance of a detailed understanding of physiology and highlights the links between an organism's physiology, the environment and neo-Darwinian principles of natural selection. Whilst it is beyond the scope of the present introduction to cover all the principles discussed in this volume, the following summary provides an introduction to the general philosophies considered during the present study. The unseen genetic component of an organsim, the genotype, is expressed as the structure and function which we observe, the phenotype. Why certain phenotypic traits have evolved in association with specific ecological conditions is the study of adaptation. Physiological processes (phenotypes) which control the recurrent functions of an organism cannot act in isolation and have to operate within the context of each other, hence there are constraints within organisms. Genotypes determine phenotypes, but the physiology of phenotypes constrains what association of genes (genotypes) can evolve; physiological ecology aims to bring these two aspects together (Sibly & Calow 1986).

Organisms function via a series of physiological processes which depend on energy: energy is therefore a common resource between all living organisms. Organisms must obtain energy from the environment, either directly, or indirectly from the breakdown of food material. Organisms are in a constant state of energetic flux which can be termed their energy budget. In the application of the *Law of conservation of energy*, the energy input to an organism must match the energy output. For animals, the energy input derives ultimately from food, the energy output is ultimately heat and excreta, although for growing organisms, the production of tissue acts as a temporary energy store. A more detailed introduction to energetics is presented in Chapter 3 and the foregoing text only serves as a brief introduction to the main principles. Energy budget models have been used to study animal production and to describe the economy of ecological systems. Patterns of resource use are controlled by enzymes and hence ultimately by genes, so following neo-Darwinian principles, patterns of resource use which best promote the spread of genes will be favoured. In this sense, phenotypes are adapted and the study of adaptation investigates the occurrence of, and explanation for, traits within a population (Sibly & Calow 1986). The core neo-Darwinian hypothesis usually adopted is that successful phenotypic traits will be those which *maximize* fitness: ecological *fitness* of an individual may be defined as the number of offspring produced by that individual which themselves survive to reproduce and contribute to subsequent generations (Lincoln *et al.* 1982). As stated earlier, a physiological process cannot act in isolation and in practice, it will not be possible to *maximize* components of fitness simultaneously. Ultimately there will be compromise between competing components, or a trade-off, which will result in the *optimization* rather than *maximization.*

Stable populations might simplistically be viewed as being attributable to each individual replacing itself by successful reproduction. Reproductive success in an evolutionary context is termed *fitness* (see above). In order to be able to reproduce, an organism must survive until maturity where survival is dependent on the successful adaptation of the organism to its environment; if an organism is highly adapted to its' environment, the greater its prospects for survival. Williams (1966) stated "the central

biological problem is not survival as such, but the design for survival." Thus the process of refining the design of an organism for adaptation to the environment is central to increasing the fitness of an organism. An organism's life history may be defined as the entire sequence of changes through which that organism passes in its development from conception through to death (Lande 1982). Considerations of design that constrain biological structures set bounds to the variety of life histories possible: within these bounds, nature selects according to reproductive success, or fitness. There is a need to apply the precautionary principle when investigating life history theory, particularly with respect to the perfectibility of organisms (Grahame & Branch 1985). An organism's life history is a complex, interlinked series of physiological processes which are each subject to natural selection. Nevertheless the number of genotypic variants upon which selection can act are not infinite, and will be determined by ancestral phylogenetic constraints. For example, planktotrophic larval development is considered the ancestral condition and the loss of a pelagic feeding larval stage is considered an evolutionary adaptation (Strathmann 1985). Loss of feeding is an absorbing state, any evolutionary period in which non-feeding larvae were strongly favoured could permanently remove this trait from a lineage. For an organism which has lost the feeding larval stage, selection acting on other physiological mechanisms may render a non-feeding larval development mode less appropriate. If a feeding larval stage has been lost from a lineage, reacquisition of this trait would be impossible and therefore selection will be limited to acting on genotypic variants of a less appropriate phenotype. Grahame & Branch (1985) concluded that "whilst devising ingenious adaptive explanations for observed features, we must bear in mind that natural selection works with what is available to do only the best necessary job."

Evolution of life history strategies and, in particular, 'trade-offs' between life history traits, have been a central theme of recent ecological theory (Gadgil & Bossert 1970; Schaffer 1974; Stearns 1976, 1977, 1989; Hodgkin & Barnes 1991; Ricklefs 1991). Two main theories have been proposed to account for evolution of life history strategies: *r*-K selection theory (MacArthur & Wilson 1967) and bet-hedging theory (Murphy 1968; Schaffer 1974; Stearns 1976); these two theories are discussed in

Chapter 5 and the discussion will not be repeated here. Trade-offs represent the cost paid in the currency of fitness when a beneficial change in one trait is linked to a detrimental change in another (Stearns 1989). Arguably the most prominent life history trade-off involves the cost of reproduction, which itself has two main components: how current reproduction affects future survival and how current reproduction affects future reproduction. It has been assumed that a negative causal relationship exists between the effort invested in reproduction at one time and the residual reproductive value (RRV: Fisher 1930) of the parent.

Costs of reproduction have been the subject of intense debate during recent years. Calow (1979) reviewed the physiological aspects of reproductive cost and investigated the basis for the supposed negative causal relationship outlined above. Calow (1979) concluded that, in general, a negative causal relationship does exist but with a number of exceptions, for instance entoparasites, and emphasised the need to consider costs in terms of the energy budgets of organisms. However, Bell (1984a&b) found no evidence of this negative causal relationship in five species of freshwater invertebrate although these species all reproduced asexually. In fact, Bell's experiments produced the paradoxical result that there appeared to be a benefit associated with reproduction. However these experiments and their conclusions were criticised by Reznick *et al.* (1986) on the basis that the experimental design could not adequately address the primary question of the role of costs in life history theory. In a reply to these criticisms, Bell (1986) warned that life history theory, and the discussion of the cost of reproduction, was developing in a manner that would make tests of the theory difficult to measure. Reznick (1985) reviewed theoretical studies of life history theory in relation to the cost of reproduction and found that these studies conformed to one of four different types, and concluded that the most effective studies are those which seek to determine the genetic basis for these trade-offs. A controversy remains over the best method to determine the cost of reproduction (Reznick 1992; Partridge 1992), although many recent methods involve contrived experimental simulations of the natural habitat which raises a serious question of realism.

Trade-offs in life history theory are discussed more fully in Chapter 6 and only a brief introduction will be presented here. One of the main trade-offs is between the investment in current reproduction and the post-reproductive survival of the parents. Organisms may reproduce sexually once and then die - *semelparous reproduction* - or reproduce more than once, or continually, over a protracted period - *iteroparous reproduction.* These terms are defined in the context of the *life history* of the organism whereas the *life cycle* of an organism is defined in the context of time - *ephemeral, annual, biennial or perennial.* Explaining the likely selection pressures favouring the evolution of semelparous or iteroparous life histories has been the subject of intense study (Cole 1954; Murphy 1968; Charnov & Schaffer 1973; Stearns 1976; Bulmer 1985). Whether semelparity or iteroparity is the more advantageous depends on the ratio of juvenile to adult survivorship: semelparity relies on the survival of the young to maturity, whereas iteroparity relies more on the survival of the adult.

On the basis of the foregoing discussions, it is apparent that any investigation into the life history and life cycle of an organism needs to address the trade-offs between the life history parameters. Comparisons require the use of a 'common currency' which is energy (Calow 1979; Sibly & Calow 1986). A study of the energy budget of an organism will provide the information necessary to determine the magnitude of parental investment to reproduction, and clues to the evolution of the selective mechanisms to optimize the fitness of an organism. Thus energy budget investigations are central to studies of the life history theory of nudibranch molluscs.

Ecology of nudibranch reproduction has been extensively investigated (for review: Hadfield & Switzer-Dunlap 1984; Havenhand 1986; Hadfield & Miller 1987; Havenhand & Todd 1988b). All nudibranchs are hermaphrodite and oviparous with internal fertilization and mutual, probably simultaneous, exchange of spermatozoa (Hadfield & Switzer-Dunlap 1984). Knowledge of opisthobranch reproduction was considerably advanced by the pioneering investigations of T. E. Thompson (Thompson 1958, 1959, 1960, 1961, 1962, 1966, 1967) which culminated in the 1976 appearance of Volume 1 of *Biology of Opisthobranch Molluscs* (Thompson 1976). More recently, Thompson (1988)

produced a revised synopsis of British benthic opisthobranchs. Ghiselin (1965) and Beeman (1977) presented detailed anatomical descriptions of the structure and function of nudibranch reproductive systems. Nudibranchs lay benthic spawn masses with the eggs set into a gel matrix (stroma). Egg size is clearly correlated with the mode of larval development and the rate of intracapsular development, although there is considerable overlap of egg size between the modes of development (Hadfield & Miller 1987). Considerable recent debate has centred on the link between egg size and reproduction (McEdward & Carson 1987; McEdward & Coulter 1987; Clarke *et al.* 1991). In general, the larger the egg and the greater the yolk content, the slower the rate of development. Thompson (1967) reported that three distinct development modes could be recognised within the Sub-class Opisthobranchia: pelagic planktotrophy (Type 1), pelagic lecithotrophy (Type 2) and non-pelagic direct development (Type 3). These three modes form an evolutionary continuum with type 1 the most primitive and type 3 the most advanced (Strathmann 1985).

For marine invertebrates, the mode of larval development is a pivotal part of their life history. Attempts to elucidate the selective forces determining the evolution of the mode of larval development have been the subject of considerable research and debate. Vance (1973a&b) and Christiansen & Fenchel (1979) proposed that only the extremes of the range of developmental strategies available were evolutionarily stable *i.e.* obligate planktotrophy and obligate lecithotrophy (direct development). Since its inception, this model has been subject to intense debate (for review see Jablonski & Lutz 1983; Day & McEdward 1984; Grahame & Branch 1985; Strathmann 1985) and at present, there remains no clear model to explain the selective forces determining the evolution of the mode of larval development. Todd (1991) reviews the larval strategies of nudibranch molluscs and proposes the three fundamental larval strategies are not similar means to the same end. Todd (1991) emphasises the importance of total egg to juvenile periods and detritivoral feeding of post-metamorphic juveniles. During the evolution of life history strategies of marine invertebrates, selection can act on the mode of larval development in addition to the energetic trade-off leading to semelparity or iteroparity.

Miller (1962) concluded that *Archidoris pseudoargus* (Rapp) (Sub order Doridoidea) had a life span of 2-2$^1/_2$ years, although Thompson (1966) presented data which he interpreted as this species having an annual life cycle. Todd (1977) found that *A. pseudoargus* spawns in the second year of post-larval life and restated the notion that *A. pseudoargus* has a biennial life cycle, as does *Tritonia hombergi* Cuvier (Sub order Dendronotoidea). *A. pseudoargus* and *Tritonia hombergi* have semelparous life histories. Casual laboratory observations (C. Todd pers. comm.) suggested that *Cadlina laevis* (L.) (Sub order Doridoidea) had a perennial life cycle combined with an iteroparous life history. *A. pseudoargus* larvae are planktotrophic, *T. hombergi* larvae are lecithotrophic, and *C. laevis* embryos undergo complete development within the benthic spawn mass (*i.e.* direct development). Observations for these three species therefore indicate a departure from the 'normal' nudibranch life cycle, and these observations form the basis of the present study. To provide a comparison between these 'atypical' species and nudibranch species with a more 'typical' life cycles, some experiments incorporated two annual, semelparous species: the dorids *Adalaria proxima* (Alder & Hancock) and *Onchidoris bilamellata* (L.).

The order Nudibranchia has four sub-orders: Dendronotoidea, Doridoidea, Arminoidea and Aeolidoidea. *Archidoris pseudoargus, Cadlina laevis, Adalaria proxima* and *Onchidoris bilamellata* are attributed to the Doridoidea and *Tritonia hombergi* is attributed to the Dendronotoidea. The Dendronotoidea is the most primitive order and individuals are typically long and slender, with retractile sheathed rhinophores, and arborescent secondary processes along the length of the body. Dendrontids are exclusively predators on cnidarians. Species within the Doridoidea are, in general, flattened with a broad, rounded foot and oval body outline. Secondary gills form a circlet around a mid-dorsal posterior anus, and these gills are often retractile into a protective gill pocket. The mantle is invariably heavily impregnated by endoskeletal calcareous spicules, with the dorsum being drawn up into many short papillae. More primitive species (*e.g. Gnathodoris*) possess jaws in addition to the radula, although most species lack jaws and rely on the radula for feeding. Some species (*e.g. Onchidoris bilamellata*) has a well

developed 'buccal pump' to facilitate the suctorial method of feeding (Crampton 1977). Dorids as a group are somewhat catholic, preying on a wide range of encrusting invertebrates, particularly sponges, bryozoans and ascidians. There is an evolutionary tendency towards reducing the mantle and elongation of the body (*e.g.* Chromodorididae and Goniodorididae), and in some cases, the development of dorsal processes resembling cerata (*e.g.* Polyceridídae).

Archidoris pseudoargus is the largest and most commonly found dorid on British shores (Thompson 1988), although during the present study, it was not so abundant on Scottish shores. *A. pseudoargus* occurs in a wide variety of colour forms from uniform white, through all shades to red, plus blotchy markings of yellow, brown, pink and green; these colour combinations produce a disruptive coloration and render the individual less conspicuous within its habitat. In contrast, juveniles are always pale (grey to yellow) with a single purple blotch anterior to the gills. Specimens may attain a length of 120 mm although adults are typically 40-80 mm in length. Body shape is generally oval, with 8-10 tripinnate gills which retract into a deep gill pocket.

Archidoris pseudoargus has a 'boreo-arctic' distribution with specimens recorded from the Faeroes, Iceland, Norway and the Baltic in the north, to the Atlantic coasts of France, Spain and Portugal in the south; specimens have also been recorded in the Mediterranean (Thompson 1988). Its depth range extends from the the lower mid-shore to 300 m below chart datum (Thompson 1988). *A. pseudoargus* is a specific predator on encrusting sponges primarily the siliceous species *Halichondria panicea* (Pallas) although Todd (1981) lists five other prey species recorded in the literature.

Cadlina laevis is translucent white or cream, with white or 'acid-yellow' sub-epidermal markings in the mantle skirt; a rare northern variant had an overall yellow body tinge (Thompson 1988). Rudman (1984) reviewed the genus *Cadlina* and noted that these sub-epidermal markings were large multi-cellular glands which form a sub-marginal row around the mantle. García-Gómez *et al.* (1991) investigated the function of mantle glands, and concluded that they probably serve a defensive function, secretions render the individual less palatable to potential predators. *C. laevis* individuals are oval, flattened

dorso-ventrally and may attain a body length of 32 mm. General body form consists of five, or rarely seven, tripinnate gills, lamellate rhinophores issuing from low crenellate pallial sheaths, and short broad and flattened oral tentacles.

Cadlina laevis is a predominantly northern species recorded most commonly in north-east Britain. Barbour (1979) considers *C. laevis* to have a 'boreo-subarctic, amphi-atlantic' distribution with specimens recorded from Greenland, several locations in the Arctic Sea, Faeroes, Norway, the Mediterranean (although questioned by Bouchet & Moreteau 1976) and from both seaboards of the USA (Thompson 1988). Lemche (1938) reported specimens from the Gulf of Mexico, although Franz (1970) considers this to be an error and reports Cape Cod, Massachusetts to be the southern distributional limit. *C. laevis* is recorded from the lower intertidal to subtidal habitats to a depth of 800 m below chart datum (Thompson 1988). In the intertidal, specimens are generally recorded on the underside of boulders in close association with its prey species, but in subtidal habitats, specimens are often recorded on open rock surfaces. *C. laevis* is a specific predator on encrusting slime sponges especially *Halisarca dujardini* Johnston; Todd (1981) lists *Stylotella columella* as another reported prey species.

Adalaria proxima is a small (up to 17 mm), yellow-orange dorid with an ample, heavily tuberculate mantle; tubercles are spiculose and become taller and more slender towards the edge of the mantle. It is predominantly an intertidal species although individuals have been recorded at depths up to 60 m below chart datum (Thompson 1988). *A. proxima* has a boreo-arctic distribution and is more common in northern parts of Britain. It has been recorded from East Greenland and the White Sea, the Baltic and Massachusetts (Thompson 1988). *A. proxima* feeds preferentially on the intertidal cheilostome bryozoan *Electra pilosa*. *A. proxima* employs a lecithotrophic mode of larval development (Thompson 1958).

Onchidoris bilamellata is a common intertidal dorid attaining a length up to 40 mm. Individuals generally have a pale ground colour with blotchy brown markings over the dorsal surface. *O. bilamellata* has a wide geographic range with records from the Pacific and Atlantic coast of America, Greenland, Iceland and the Atlantic coast of France; it

occurs all around the coast of the British Isles (Thompson 1988). It is a specialist predator on acorn barnacles: the radula is used to prise apart the barnacles' opercula plates and then ingest the soft parts of the body, the cirri and other remains of the chitonous exoskeleton are left behind. *O. bilamellata* individuals produce large benthic spawn masses where the eggs hatch to release planktotrophic larvae (Todd 1979a).

Tritonia hombergi is the largest British nudibranch and may attain a length of 200 mm. Body colour ranges from white or yellow to dark purplish brown, with a lighter ventral surface. The mantle bears numerous soft tubercles and dorso-lateral pallial gills which are much divided becoming arborescent in adults. The largest gills are pedunculate and reflected inwards, the smaller gills project out laterally; the number of gills increases with age. *T. hombergi* has a distinctive bi-lobed oral veil which is present even in the smallest juveniles; each lobe has up to 40 finger-like processes (Thompson 1988). Specimens have been recorded from all around the British Isles and elsewhere from the Faeroes, Norway, Brittany, Portugal and the Mediterranean coasts of Spain and France (Thompson 1988). It is almost exclusively a subtidal species recorded to a depth of 80 m below chart datum. *T. hombergi* is a specific predator on the alcyonarean soft coral *Alcyonium digitatum*, commonly known as 'dead-man's fingers'.

Thompson (1966) studied the anatomy of the reproductive apparatus, embryonic development, larval development and the life history of *Archidoris pseudoargus*, and reviewed the early literature pertaining to this species. On the basis of field observations and histological studies, Thompson concluded that *A. pseudoargus* had an annual life cycle. It would appear that Thompson (1966) did not take account of the possibility that this species does not undergo gametogenesis in its first year, and a interpretation of the data on this basis would lead to the conclusion that *A. pseudoargus* had a biennial life cycle. But, despite its large size, it has not been subject to further detailed investigation in recent years. Carefoot (1967) considered *A. pseudoargus* in a study of the growth and nutrition of opisthobranchs. Todd (1977) presented notes on the spawning cycle and Todd & Havenhand (1984) present observations on larval growth. Potts (1981, 1983)

investigated respiration and the structure and function of the respiratory apparatus of *A. pseudoargus* and *Onchidoris bilamellata.*

The ecology and life history of *Cadlina laevis* has not been investigated in any detail. Roginsky (1962) reported that *C. laevis* had direct development which led Thompson (1967) to publish a detailed account of the embryonic development of this species, and a more general discussion of the larval development within the Order Opisthobranchia. Barbour (1979) published notes on the distribution and food preference of *C. laevis*. A number of investigations have considered other species within the genus *Cadlina* although most studies have considered aspects of taxonomy. Rudman (1984) published the results of a detailed investigation into the taxonomy of the family Chromodorididae of the Indo-West Pacific, which included a general description of the genus *Cadlina* and an account of the full anatomy of *C. laevis*. Subsequently, Rudman (1985, 1990) published additional accounts of other species within the genus *Cadlina.* Dehnel & Kong (1979) published one of the few physiological studies within this genus where they investigated the effect of temperature on developments rates in *C. luteomarginata.*

Thompson (1960, 1961, 1962) published accounts of the defensive adaptations, the structure and functioning of the reproductive organs, and the ontogeny of *Tritonia hombergi* . More recently, Kempf & Todd (1989) have investigated the feeding potential of *T. hombergi* and *Adalaria proxima* larvae.

Aspects of the physiological ecology of *Adalaria proxima* have been more thoroughly investigated. Havenhand (1986) followed by Havenhand & Todd (1988a & b) and Todd & Havenhand (1988) published the results of a detailed study into the physiological ecology of *A. proxima* (and *Onchidoris muricata*). Earlier studies concentrated on the reproductive ecology of this species: Thompson (1958) published an account of the natural history, larval biology and post-larval development; Todd (1979b, 1987) considered reproductive energetics. More recently, Havenhand (In press) used data for *A. proxima* to support a model describing the importance of the egg to juvenile period in the evolution of larval reproductive strategies, and Todd *et al.* (1991) studied artificial

(chemical) induction of metamorphosis of *A. proxima* larvae. Havenhand *et al.* (1986) and Todd *et al.* (1988) investigated the genetic structure of geographically distinct populations of *A. proxima* and noted that, even for species with pelagic larvae, populations at sites only a few kilometres apart could have significantly different genetic structures.

Despite being a one of the commonest intertidal species, few investigations have considered the life history of *Onchidoris bilamellata.* Potts (1970) and Todd (1979a) published accounts of the population ecology of this species. Hadfield (1963) and more recently, Todd & Doyle (1981) and Todd (1991) have studied the larval ecology of *O. bilamellata.* Todd & Doyle (1981) proposed the 'settlement-timing hypothesis' in an attempt to explain how long term planktotrophy was the only larval development mode that could bridge the gap between an empirically predicted optimum time for adults to spawn and the observed optimum time for larvae to settle and metamorphose. This hypothesis has been the subject of debate (Strathmann 1985) and criticism (Grant & Williamson 1985) and whilst at first defended (Todd 1985a), the hypothesis has now been withdrawn (Todd 1991) on the basis of new information. The 'settlement-timing hypothesis' assumed that newly metamorphosed *O. bilamellata* juveniles preyed on barnacle cyprids to overcome likely prey-size constraints imposed by adult barnacles. Juvenile nudibranchs must therefore metamorphose at a time when cyprids settle onto hard substrata. Todd (1991) observed newly metamorphosed juveniles grazing detritus, and inferred that juveniles depend on this intermediate diet until they attain a size at which they are able to switch to the adult diet. Because of this dietary switch, there is no requirement to 'fine tune' juvenile metamorphosis with cyprid settlement and thus the 'settlement-timing hypothesis' becomes void. In recent years, many studies have investigated physio-chemical cues for settlement and metamorphosis of nudibranch larvae. One of the most important investigations have been undertaken by Chia & Koss (1988, 1989) and Arkett *et al.* (1989) who have identified putative chemoreceptors and a possible neurophysiological mechanism for the initiation of metamorphosis between competent *O. bilamellata* veligers and a chemical cue from the adult barnacle prey.

By and large, nudibranch molluscs have annual semelparous life cycles (Todd 1981, 1983). Observations for *Archidoris pseudoargus*, *Cadlina laevis* and *Tritonia hombergi* suggest that these species have evolved extended life cycles and therefore show an evolutionary departure from the 'normal' nudibranch life cycle. Therefore, the central purpose of the present study was to investigate these observations and attempt to answer the question "Why do these species live longer than a year?"; and for *C. laevis*, "Is this species perennial and iteroparous and if so, why?"

Larval development within the Nudibranchia can follow one of three development modes: planktotrophic, lecithotrophic or direct development; planktotrophic development is considered the ancestral condition (Strathmann 1985). A further aim of the present study has been to study the modes of larval development displayed by *Archidoris pseudoargus*, *Cadlina laevis* and *Tritonia hombergi* in an attempt to determine the evolution of life history strategies of these species.

CHAPTER 2

GENERAL METHODOLOGY

2.1 Introduction

General procedures and standard techniques which were employed at all stages of the project are described below; techniques specific to each facet of the study are described in the relevant chapters.

2.2 Animal husbandry

Animals and their prey for the present study were collected from a number of locations around Great Britain. Specimens of Archidoris pseudoargus were collected from the shores at Robin Hood's Bay (North Yorkshire), East Sands - St Andrews - and Kingsbarns (Fife), Loch Creran and Clachan Seil, (Argyll). Cadlina laevis were collected from the shores at East Sands, Kingsbarns and Clachan Seil. Tritonia hombergi were collected by beam trawls in the Firth of Forth, off the Isle of Man and off the coast of North Wales, with a small number of individuals collected by SCUBA diving at the Isle of May (Firth of Forth) and the Isle of Cumbrae (Firth of Clyde). In addition, small numbers of all three species were collected during opportunist visits to other sites in Fife and west Scotland.

Small colonies of *Halichondria panicea* and *Halisarca dujardini* were collected from East Sands and Kingsbarns (on small stones) or Loch Creran (epiphytic on *Fucus serratus* and *Ascophyllum nodosum*). Occasionally when these sources were unavailable, colonies were scraped from large stones or bedrock. However, this was an unsatisfactory method because damage to sponge colonies during collection reduces their survivorship in the laboratory. Sponge colonies growing epiphytically on algae were the preferred option: collection did not damage the sponge colonies and cut pieces of algae survived for many weeks in the laboratory. Colonies of *Alcyonium digitatum* were retained from beam trawls or collected by SCUBA diving at the Isle of May and the Isle of Cumbrae.

All animals were maintained in the aquarium of the Gatty Marine Laboratory, University of St Andrews. Animals were kept in 2.5 l plastic containers with minimum water depth of 4 cm. All containers were stored in racks, either in a 'cascade' system or

on the surface of large (2 m x 1 m x 1.5 m) holding tanks. All containers were provided with a constant flow of unfiltered, non-recirculated sea-water at near-ambient temperatures. Small specimens were kept in individual mesh containers (Toby 'Teaboys', Aldridge Plastics Ltd) within the 2.5 l containers. At all times, animals were fed *ad libitum* with their preferred prey. Animals were examined daily to monitor their condition and to maintain an adequate supply of fresh prey material.

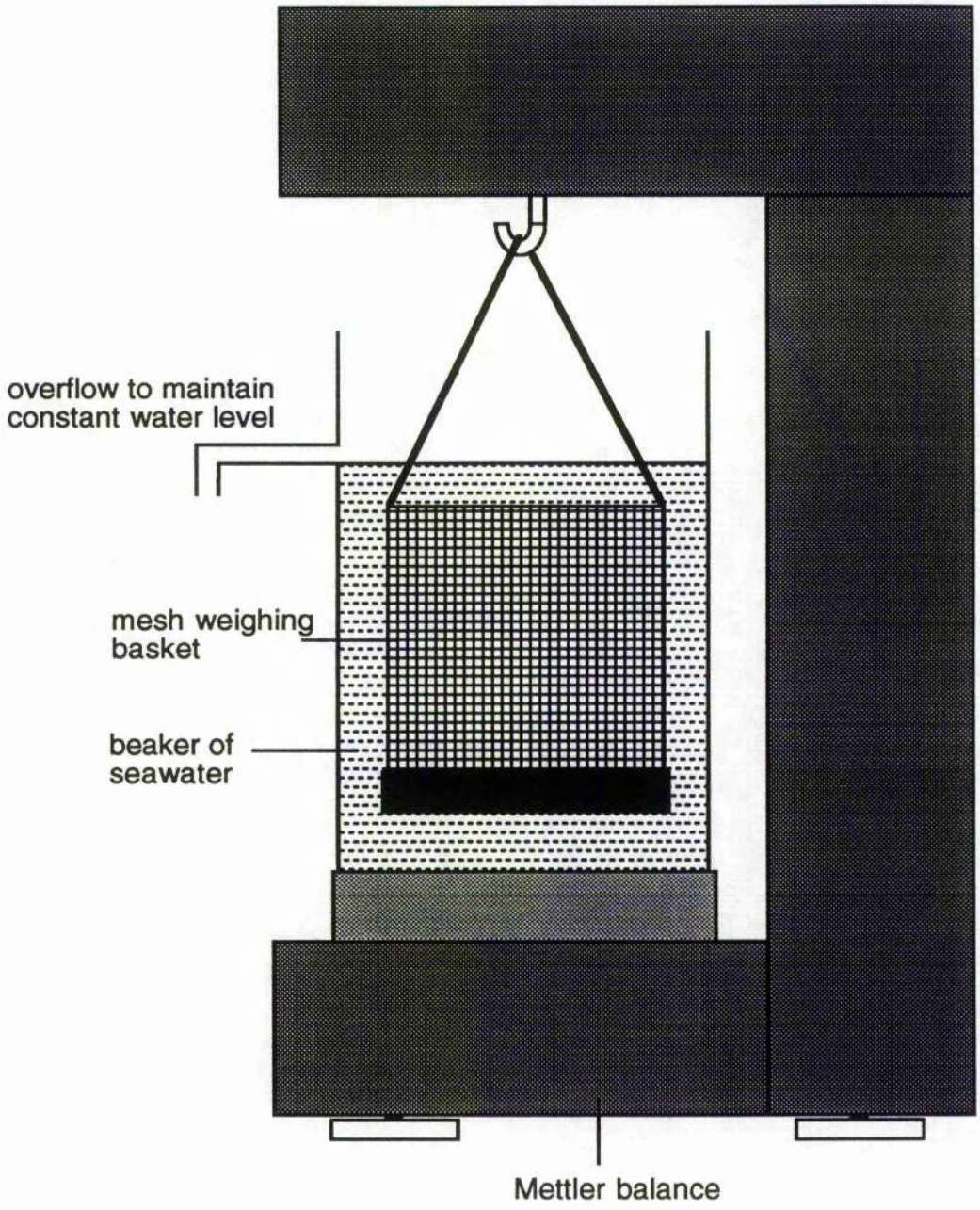
2.3 Weight determinations

Many of the procedures followed during this study required accurate measurement of the weight of live and dead material. Weight determinations of small quantities of dry material for gravimetric and calorimetric analysis were made on a Mettler ME22 analytical microbalance. Weight determinations on live material were performed either immersed in water ('weight under water') or in air ('wet weight') using a Mettler laboratory balance. All weights were recorded as milligrams and converted to other units as required.

In general, live material was weighed whilst immersed in water to minimize stress on the organism and to minimise errors derived from variations in the internal water content of the material. Palmer (1982) discusses procedures for weighing under water and their advantages over other techniques. The apparatus developed for weighing under water is shown in Figure 2.1. A mesh basket, retained in a large beaker of filtered sea-water at ambient temperature, was suspended from the weighing arm of the balance. Trial determinations had shown that changes in the volume of the sea-water in the beaker gave rise to differing weights for a given object. Therefore a constant volume of 900 ml was maintained at all times by adding water to raise the level above the over-flow and allowing excess water to drain to waste prior to each determination. When the material was added to the basket, care was taken to ensure no air bubbles were introduced to the system, either attached to the material or to the basket.

Variations in salinity (daily or seasonal) can introduce an error to the accurate replication of weights under water. Daily salinity measurements for the seawater entering the Laboratory revealed no significant change over an annual cycle; salinity is constant at

Figure 2.1 Apparatus for determining the weight under-water of live material. A mesh basket, immersed in a beaker of seawater at ambient temperature, was attached to the arm of a Mettler laboratory balance. Constant volume was maintained by allowing the water displaced by the live material to flow to waste.


overflow to maintain
constant water level
mesh weighing
basket
beaker of
seawater
Mettler balance

30 mosmols · kg $^{-1}$ (Dr Neil Hazon, pers. comm.). Therefore it has been assumed that the density of seawater is constant throughout the year and will not introduce an error to the determination of weight under water.

Prior to the development of weighing under water, live material was wet weighed in air. Material was carefully blotted dry in a standardised manner using clean absorbent laboratory wipes, and then weighed in air. All the organisms used in this study had external body cavities of varying dimensions and the complete removal of all water within these cavities was very difficult and may introduce an error into a determination of weight. Despite these problems, wet weighing in air provided a quick, repeatable and reliable estimate of body weight, although it is a stressful process for the organism. Consequently, weighing under water was the preferred method for determining the body size of live material.

2.4 Live weight to dry weight conversions

All living material used in experimental procedures was either weighed under water or damp weighed. To convert these live weights to dry weights, a representative sample of living material covering the 'normal' range of sizes encountered in natural populations was weighed, dried and then reweighed. Data were fitted to GM regression models (see later) to generate a conversion equation for live weight to dry weight (Ricker 1973). Following the procedures outlined by Sokal & Rohlf (1981), confidence limits for the regression intercepts were calculated and where these limits incorporated zero, the regression model was constrained to pass through the origin. The latter procedure is based on the assumption that an organism of zero live weight will have zero dry weight. Where the confidence intervals of an intercept did not include zero, presumably caused by errors in the weighing/drying procedure, it was assumed that these errors were unbiased and similar for all determinations.

2.5 Drying

To overcome the problem of variations in the water content of living material, all live weights were converted to dry weights by freeze-drying living material in a vacuum freeze-dryer (Chemlab Instruments). Living material was rinsed in 0.9% (w/v) ammonium formate (HCO_2NH_4) and then frozen. Ammonium formate is isotonic with sea-water and replaces the sea-water on the surface of living material. Ammonium formate sublimes during the freeze drying process and therefore, unlike sea-water, does not leave any salt residue on the dry material. After freezing, material was transferred to the freeze dryer for a minimum of 24 hours although some larger nudibranchs required up to 48 hours. Freeze dried material is strongly hygroscopic and therefore after drying, material was quickly transferred to a desiccator for storage under anhydrous conditions. Dry material was stored at room temperature to overcome any problems of condensation upon removal from the desiccator.

2.6 Inorganic ash content

Living material contains both inorganic and organic constituents, the inorganic compounds generally forming skeletal components. In general, inorganic compounds are biochemically inactive. Not surprisingly, the proportion of inorganic material varies between species and so any interspecific comparisons of the biochemical constituents of body tissue have to be corrected for their inorganic ash content.

To determine the inorganic ash content, living material was dried (as above), weighed and incinerated in a muffle furnace at 520 °C for 4 hours. The time and temperature are important to ensure complete combustion of organic material without any volatilization of the inorganic salts. At temperatures greater than 600 °C calcium carbonate, a major constituent of invertebrate skeletons, decomposes to calcium oxide with a loss of carbon dioxide. Paine (1964, 1966) and Crisp (1984) provide more detailed descriptions of the determination of inorganic ash content and discuss the problems and sources of error associated with the procedure. After incineration, the

material was reweighed and the relative proportions of inorganic material calculated by subtraction from the dry weight.

2.7 Calorimetry

Accurate estimations of the calorific content of biological material is a necessary procedure in studies of the energy flow through living organisms. Energy and more importantly, energy flux is a 'common currency' between all organisms and is most commonly used for inter- and intraspecific comparisons. The calorific content of living material was measured directly using a Phillipson microbomb calorimeter (Gentry Instruments). A stainless steel bomb, filled with oxygen under pressure, rests in thermal contact with a copper ring with electrically insulated thermocouple junctions. Temperature changes resulting from each firing were recorded by a Vitatron 2001 series flat-bed chart recorder.

Material for combustion was dried, homogenized, pelleted, re-dried, and the pellets weighed to within ± 0.01 mg. Pellets were placed in the bomb in contact with a platinum wire through which an electrical charge is passed to effect ignition. Ignition is a violent process which often results in material adhering to the wire or spluttering on the walls of the bomb, and to avoid any cross contamination between samples, the platinum wire was changed and the bomb cleaned with 70% alcohol after each firing. A small quantity of water was placed in the base of the bomb (to standardise humidity) and then the bomb was charged with oxygen to a pressure of 25 bar. The charging process raises its temperature and so prior to firing, the bomb was cooled in water. After cooling, the bomb was placed on the thermocouple and the temperature between the two components allowed to equilibriate (a flat trace on the chart). A successful firing is shown by an immediate rapid rise in the chart trace. If firing was not successful the current through the wire was increased gradually until ignition was achieved, although too large a current burns the wire rather than effecting ignition. Following ignition, the bomb remains in contact with the thermocouple until a cooling curve is attained.

To calibrate the chart recorder, three freeze-dried pills of benzoic acid (calorific content of 26.44 $J \cdot mg^{-1}$) were fired at the beginning and repeated at the end of each day's firings and, if necessary, in the interim following any changes to the recording apparatus. The calorific content of each experimental pill was calculated following the methods described by Crisp (1984). Inorganic ash content was calculated by the process described earlier rather than weighing the residue after firing (as suggested by Crisp 1984).

During ignition, temperatures attained within the bomb are sufficient for some inorganic materials to dissociate as a result of endothermic reactions. In particular, calcium and magnesium carbonates (major constituents of invertebrate skeletons) dissociate to their oxides releasing carbon dioxide and consuming 1.78 $J \cdot mg\ CaCO_3^{-1}$. Energy consumed by these reactions is 'lost' to the bomb walls and will not be recorded by the chart recorder. To correct for this lost energy, the ash was assumed to be entirely $CaCO_3^{-1}$ (except for *Halichondria panicea*) and thus a correction could be applied to the calculations of the calorific content. Endothermy and the influence of water of hydration of salts in bomb calorimetry is discussed by Paine (1964; 1966) who suggests possible corrective measures. From these calculations, the total calorific content of biological material could be determined.

2.8 Data storage and analysis

Data collected during the course of these investigations were stored and analysed by computer. Initially, all data storage and analyses were undertaken using Minitab™(Minitab Inc.) on a DEC VAX 11/785 mainframe computer at the University of St Andrews. In the latter stages of the study, data were stored and analysed with Statworks™ (Cricket Software) and Minitab on an Macintosh™ personal computer (Apple Computers Ltd).

Statistical procedures throughout have followed those outlined by Sokal & Rohlf (1981). Many statistical analyses are based on the assumption that samples approximate to a normal distribution. All data were tested for normality using a Kolmogorov-Smirnov

test which reports the probability of obtaining the observed deviation from a theoretical normal distribution. Non-normal data were either transformed to achieve normality, or analysed by non-parametric procedures which make no underlying assumptions about the distribution of samples. All mean data are quoted ±1 standard error (± 1 se); probability estimates from significance tests for each statistic are represented as follows:

$$* = 0.05 \geq P > 0.01 \qquad ** = 0.01 \geq P > 0.001 \qquad *** = P < 0.001$$

A number of the procedures followed during these studies required a conversion from a measured variable to an estimate of a second variable via a regression equation. 'Normal' model I regression analysis (that is, y on x) assumes that the values of the independent (measured) variable (x) are determined without error. In all cases during the present study, the independent variable was subject to measurement error and variable pairs were frequently inter-dependent (*e.g.* live weight to dry weight). Where both variables are subject to random variation, a model II or geometric mean (GM) regression model will best explain the data (Sokal & Rohlf 1981). Consequently GM regression procedures have been used throughout the present study. A discussion of the use of GM regressions is presented by Ricker (1973) and Havenhand & Todd (1988a).

Analysis of covariance (ANCOVA) is an important statistical procedure to test a dependent variable for homogeneity among group means when subject to different treatment effects. ANCOVA includes the same assumptions as model I regression and, in particular, that the independent variable is measured without error. ANCOVA procedures based on model II are not available and therefore their use during the present study is questionable; these problems are discussed later.

CHAPTER 3

FEEDING & GROWTH

3.1 Introduction

A central tenet of ecological theory has been that the ecological fitness of an organism or species is determined by the number of offspring which survive to reproduce in the subsequent generation (although see Hodgkin & Barnes 1991). For marine invertebrates in general, the number of offspring produced during reproduction (fecundity) is, to some extent at least, a function of body size; therefore for an organism to maximize its fitness, selection should favour mechanisms to increase body size. Similarly, if generation time is an key life history feature, mechanisms which facilitate completion of the generation will determine the fitness of an organism.

All such mechanisms come into the general category of *growth* which in turn, requires the synthesis of new body material from basic components, plus the energy to drive the synthetic reactions. These basic components and the necessary energy are derived from the environment via the process of *feeding*.

3.1.1 General principles of energetics

Processes to increase the body mass of an organism are termed *production*. The most comprehensive form that investigations of production may take is the measurement of the flow of organic matter and energy through an organism. *Production* is also used in a population context as a measure of energy flux through populations (Crisp 1984). In general, energy flow refers to rates of change of biomass and is divisible into a number of separate processes which together constitute the flow of energy into and out of an organism. Assuming the conservation of energy:

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

where C = consumption (total intake of food), P = production (food assimilated into body mass), R = respiration (food converted into heat), G = gonadal output (food converted to gametes), U = excreta which includes both urine and exudates such as mucus, and F = Egesta (food not absorbed and voided as faeces) (Crisp 1984). Consumption is the

most important component since it sets the limit to energy which will be available for all other processes. *R* is similarly important as it sets the limit to the fraction of *C* available to *G, U* and *P*. But the energy balance equation includes terms which represent both gains and losses of energy, and for an organsim to grow, gains must exceed losses. The amount of energy available to an organism for growth and reproduction is:

$$A - (R + U) = P + G$$

where *A* = assimilation or the energy actually absorbed from the food (*i.e.* $C - F$). When $[A - (R + U)]$ is positive, there is a net gain of energy and there is 'scope for growth and reproduction' (Bayne *et al.* 1973, 1976); when $[A - (R + U)]$ is negative, body reserves must be utilized to maintain the organism in a viable condition. When the losses $(R + U)$ exactly balance the absorbed ration, the value of *A* is the 'maintenance ration'. Clearly, except for short periods, an organism must maintain a positive energy balance to grow and reproduce in order to maximize its fitness and permit the survival of the population.

To maximize the scope for growth, an organism should minimize the 'losses' due to respiration and excretion. Adaptation of respiration rates is discussed in chapter 4. Losses due to excretion, largely nitrogenous urine and mucus, have usually been ignored or calculated by difference when calculating energy budgets, usually because their determination is difficult . Where measurements have been made, there is evidence that *U* may represent a significant energy loss (Bayne & Newell 1983; Horn 1986).

In most marine molluscs, ammonia is assumed to be the dominant end product of protein metabolism; among bivalves, ammonia comprised between 60 and 90% of the total measured nitrogen excretion (Bayne 1976). Nitrogenous excretion in molluscs is reviewed by Bayne & Newell (1983). Horn (1986) calculated the energy budgets for low and high shore chitons and determined the losses due to excretion *U* to be only 0.04% of the overall energy budget.

Mucus production is particularly relevant to marine molluscs where it is produced for locomotion, feeding, and in the production of faeces, but has been largely ignored in studies of energetics (Hughes 1970, 1971; Huebner & Edwards 1981; Davis & Wilson

1985). Estimates of the proportion of mucus production within an energy budget vary from 9% in the gastropod *Hydrobia ventrosa* (Kofed 1975) to ~40% in a limpet (Branch 1982). More recently, the energetics of mucus production were considered by Horn (1986) and reviewed by Kideys & Hartnoll (1991).

In the present study, nitrogenous excretion and mucus production were not quantified, although both *Archidoris pseudoargus* and *Cadlina laevis* have the ability to produce copious quantities of mucus. McCance & Masters (1937) noted that 80 g of fresh *A. pseudoargus* tissue produced 24 g of mucus in 1.5 hours. Therefore, at least for *A. pseudoargus*, mucus production may be a significant loss function to the overall energy budget.

Energy budgeting of marine invertebrates has been the subject of a very large number of investigations during the past twenty years. It is beyond the scope of the current study to provide a detailed literature appraisal of these investigations and only a few important investigations relating to molluscs will be documented. Crisp (1984) provided detailed information on the experimental procedures necessary to calculate energy flux through individuals and populations. Bayne & Newell (1983) reviewed the physiological energetics of molluscs and observed that most studies had concentrated on bivalves. This trend has continued during the past decade with many comprehensive studies calculating the energy budgets for bivalves (*e.g.* Hummel 1985; MacDonald & Bourne 1987; Harvey & Vincent 1989; Iglesias & Navarro 1991). Studies on gastropods, and opisthobranchs in particular, have been much less widespread: Smith & Sebens (1983) studied *Onchidoris aspera*, Hall & Todd (1986) studied *Aeolidia papillosa*, Todd & Havenhand (1988) studied *O. muricata* and *Adalaria proxima*, and Carefoot (1989) studied *Aplysia dactylomela*.

3.1.2 Feeding

Marine invertebrates require a source of energy to fuel basal metabolism (Chapter 4), and for growth and reproduction. Invertebrates are unable to synthesize energy from basic elements and must obtain chemical compounds from the external environment.

Acquisition and ingestion of energy (or food) from an external source are referred to as *feeding*. Food material generally consists of complex organic compounds which have to be broken down into simpler compounds prior to absorption into the body; the breakdown and absorption of food are termed *digestion* and *assimilation* respectively.

The quantification of feeding is far from a simple measure of energy uptake. Feeding represents the most apparent, and probably the most significant, interaction between organisms in an ecological community. Acquisition of food is the interface between behavioural ecology and physiological ecology and has been extensively studied (Krebs & McCleery 1984). Acquisition includes predator-prey interactions, where changes in prey abundance or prey quality potentially have a significant impact on the energy balance of the predator, and the actual mechanics of ingestion, which includes physical 'handling' of the prey. MacArthur (1972) considered the feeding of a predator to comprise a period of search (during which the predator senses the prey), and pursuit (during which the prey item is pursued, captured and eaten). Pursuit and prey capture are usually considered together as 'handling time'. All activities associated with search and handling time represent a cost to the predator which must be set against the energetic benefit accrued from consuming each prey item. In addition, natural variation in prey quality will compound the cost of prey capture.

Predator-prey interactions have been extensively studied and a number of models developed to interpret these relationships. In particular, *optimal foraging theory* is concerned with how a predator overcomes the inherent patchiness of prey populations. Optimal foraging theory is concerned with the order in which a predator tackles prey patches, and the length of time which should be spent in each patch to maximize the net rate of energy acquisition (Sibly & Calow 1986). Foraging and prey handling were not investigated during the present study. Optimal foraging theory was reviewed by Hughes (1980) and a summary in relation to the Mollusca presented by Bayne & Newell (1983).

Predation is an important structuring element of marine benthic communities, and mollusc predation in particular can have a profound structuring effect. On rocky shores, gastropods are one of the main predatory taxa and many investigations have considered

the predatory effects of gastropods in relation to rocky-shore community structure (*e.g.* Hughes 1985; Fairweather 1988). Similar predatory influences occur in subtidal habitats: Allmon & Sebens (1988) studied the impact of the introduction of the nudibranch *Tritonia plebia* on the population of the soft coral *Alcyonium siderium* in New England, USA. They concluded that *T. plebia* was responsible for the disappearance of the soft coral at two sites, and a sharp reduction in population numbers at a further two sites. Predator-prey interactions were not considered in detail during the present study, although there are indications that variation in prey value/abundance could be a primary factor determining the life cycles of the species under consideration.

Acquisition of food, or the ingestion rate, is the basis for calculations to determine the efficiency with which an organism converts food to new tissue or to metabolic energy. Efficiencies are an important consideration because they normalise differences in absolute rates between individuals and between species (Peters 1983). Within an organism, energetic efficiencies can vary as a result of environmental factors, and endogenous factors such as age and reproductive condition. Therefore it is important to quantify ingestion rate and assimilation efficiency to determine the upper limit of the 'scope for growth'.

Most nudibranchs are specific predators on a single or a limited range of prey species; dorid nudibranchs primarily graze on encrusting sponges and bryozoans (Todd 1981). Nudibranchs are potentially important epibenthic predators - especially those species which specialise on dominant epifaunal species - and consequently there is a large volume of data on the dietary preference and feeding behaviour of these organisms (for review: Thompson 1964; Harris 1973; Todd 1981, 1983). Many studies have concentrated on nudibranch species feeding on bryozoans (Seed 1976; Yoshioka 1982, 1984; Harvell 1984; Havenhand & Todd 1988a; Todd & Havenhand 1989). Hall & Todd (1986) investigated the influence of prey quality on growth and reproduction of *Aeolidia papillosa.* In contrast, few studies have investigated sponge grazing species. In a preliminary report of a world-wide review of the food of nudibranchs, McDonald & Nybakken (1991) state that 15 out of 57 nudibranch families, or 191 out of 651 species,

were sponge grazers. Harris (1973) provides notes on sponge grazing nudibranchs in an early review of nudibranch associations, and Wägele (1989) provides notes on sponge grazing species from Antarctica. Bloom (1981) used dorid nudibranchs to investigate the evolution of resource partitioning and resource gradients.

Archidoris pseudoargus grazes on encrusting siliceous sponges, chiefly *Halichondria panicea* and *Hymeniacidon perleve* (Thompson 1988), although four other sponge species have been recorded as prey for this dorid: *Mycale aegagrophillia, Grantia compressa, Myxilla incrustans* and *Tethya aurantia* (Todd 1981). Nevertheless, *A. pseudoargus* is generally considered to be a specific predator to *Halichondria panicea* (pers obs.). Just & Tendal (1983) dissected out the stomach caecum and identified the sponge spicules from twenty *A. pseudoargus* individuals originating from localities ranging from Iceland to Plymouth, at depths ranging from 20-280 m. In addition to *Halichondria panicea*, spicules from *Suberites ficus/S. luetkeni, Leucosolenia* sp., *T. aurantia, Corybas ovulum, Myxilla rosacea, Myxilla* sp., *Lissodendoryx* sp. (?), *Jophon piceus* and *Hymedesmia* sp. (?) were recorded. Just & Tendal (1983) consider *A. pseudoargus* to be a '*Halichondria* specialist' at intertidal and upper subtidal depths, but a 'sponge opportunist' in the deeper subtidal. Carefoot (1967) determined the ingestion rates of *A. pseudoargus,* and Bloom (1981) studied the patterns of ingestion of the congeneric species *Archidoris montereyensis* in north-west USA; the latter study included a re-evaluation of the feeding data of Carefoot (1967).

Information on the feeding of *Cadlina laevis* is restricted to field notes on its food preference. *C. laevis* feeds on encrusting slime sponges, especially *Halisarca dujardini* (Thompson 1988), although Thompson (1964) notes that in north-eastern USA, *Stylotella columella* was consumed. Barbour (1979) observed *C. laevis* feeding on *Halisarca dujardini* in the field, but when deprived of food in the laboratory, a 'brown material', assumed to be algal detritus, was present in the stomach.

In the present study, experiments to determine the rate of consumption were only undertaken for *Archidoris pseudoargus* (not for *Cadlina laevis*) with the aim of quantifying the calorific input and the rate of egestion. From these determinations, it

would be possible to calculate the assimilation efficiency of this species. No experiments were undertaken to study the feeding behaviour of *A. pseudoargus,* although some observations were made on the periodicity of feeding bouts. It was not the intention to study any aspects of predator-prey interactions.

3.1.3 Growth

Metabolic constraints determine that growth can only occur when energy acquisition exceeds energy expenditure (on respiration, egestion and excretion). With reference to the balanced energy equation, production and gonadal output are often summed to provide an overall measure of *production*, or the mass of living tissue elaborated by an organism:

$$P = P_g + P_r$$

where P_r is the gonadal production, and P_g is the somatic production. For an organism to maximize its fitness, it should maximize P_r but P_r is, at least to some extent, dependent upon body size *i.e.* P_g. Therefore during the life cycle of an organism, energetic resources are first invested in somatic production and then reproductive biomass. When this switch occurs, and how it is effected, are pivotal factors in the life cycle of a species. Short-lived species are dependent on an adequate supply of food early in their life cycle to effect the switch to reproductive allocation at an early stage. If food is not available to a juvenile, early development will be slow and time to maturity will increase, possibly extending the life cycle.

All factors in the balanced energy equation are rate processes which are dependent on ambient conditions. The environment acts, not directly on growth, but on the mechanisms of energy supply and demand that influence the scope for growth (Brett 1979). Therefore, when considering the physiological effects of growth, seasonal factors are important and the different demands of somatic growth and gametogenic production, set against environmental changes in food availability, must be understood (Bayne & Newell 1983). Seasonal growth is the result of complex interactions between temperature, food, reproductive activity and energy balance. Bayne & Newell (1983) considered that it is the combined effects of ration and temperature that has the most

profound influence on physiological energetics in the natural habitat. An understanding of the mechanisms of growth and their integration with the environment, is therefore central to the interpretation of the ecological fitness of an organism.

Growth patterns of invertebrates have been extensively studied, both from a theoretical and practical stand-point. Growth is a central part of investigations to determine the energy budget of a species, and those investigations of energy budgets listed earlier, all consider growth. Study of molluscan growth patterns have followed this general trend although most effort has been directed towards bivalve molluscs, especially species which have commercial importance (*e.g.* Bayne & Worrall 1980; Vahl 1980, 1981; MacDonald & Thompson 1985a&b). Growth of opisthobranchs has received less attention although a number of comprehensive studies have been undertaken (for summary: Havenhand 1986). Energetics of growth in opisthobranchs were investigated by Paine (1965), Carefoot (1967, 1987, 1989), Chia & Skeel (1973), Bloom (1981) Smith & Sebens (1983), Hall & Todd (1986), and Havenhand & Todd (1988a). Growth of *Archidoris pseudoargus* has been investigated both in the field (Miller 1962) and in the laboratory (Carefoot 1967). Growth of *C. laevis* had not been reported prior to the present study.

Todd (1983) postulated that a species surviving longer than a year must either exploit a temporally stable food source, or be able to withstand periods of starvation when the preferred food is not available. *Halichondria panicea* and *Halisarca dujardini* are available year-round and do not exhibit any marked seasonal availability (although see Barthel 1986): they are therefore considered to be temporally stable and so *Archidoris pseudoargus* and *Cadlina laevis* should have a regular food supply. A central aim of the present study was to investigate the growth of *Archidoris pseudoargus* and *Cadlina laevis* to determine if growth was constant with time, or if there were any seasonal patterns. Any seasonal variation in growth would have potentially important constraints on life cycles or life history strategies of these species. Measurement of absolute growth would also permit interspecific comparisons with other opisthobranch species for which data are available.

3.2 Methods

Studies of feeding and growth require a reliable measure of body size. Throughout the present study, weight was used as an indicator of body size. Live weights, both of molluscs and their prey, were determined following the methods outlined in Chapter 2. A representative sample of animals (spanning the size range encountered in the field) and prey were live weighed (under water for *Archidoris pseudoargus* and *Halichondria panicea*; wet for *Cadlina laevis*), dried and reweighed; dry material was then used to determine the inorganic ash content and calorific value. Live weights could then be converted to dry weights and thence to joule equivalents (Chapter 2).

3.2.1 Feeding

Archidoris pseudoargus individuals were collected from the shores of Fife and maintained in the laboratory at ambient temperature (see Chapter 2). Individuals for feeding experiments were chosen to span the range of body sizes observed in the field at the time of the experiment. General observations during day-to-day animal husbandry suggested that damaged colonies of *Halichondria panicea* tended to degrade rapidly, and thus probably lose their prey value, over a period of less than 7 days. Therefore intact colonies of *H. panicea* were used for the feeding determinations to reduce the problem of prey survival.

Prior to the start of each determination, individuals were deprived of food for three days to allow previously ingested material to be voided from their guts. Colonies of *Halichondria panicea* were weighed (under water) and placed in the centre of clean 2.5 l plastic tubs. *Archidoris pseudoargus* were weighed (under water) and placed in contact with the prey material, one animal per tub. Preliminary observations had shown that this species feeds sporadically and therefore to calculate a realistic consumption rate, each determination lasted five days. During that period, further observations were recorded on feeding behaviour and faecal production. At the end of the determination, the animal and the remains of its prey were reweighed (under water) and any change in weight of the

H. panicea was assumed to be a result of grazing by the nudibranch. To control for natural changes in the live weight of the prey material, colonies of *H. panicea* were weighed (under water), left for five days in plastic tubs and reweighed. No changes in the weight of *H. panicea* colonies were observed during this control process.

All values for live weight were converted to dry weight (see above). To permit intra- and interspecific comparisons, absolute feeding rates were corrected for the body size of the nudibranch by converting the feeding rate to a size specific feeding rate (SSFR).

Spearman rank correlation coefficients were calculated to determine whether there is a significant statistical correlation between the weight of prey consumed and the size of the nudibranch. Data were fitted to GM regression models to obtain a 'best fit' to determine any relationship between body size and feeding rate.

A determination of the inorganic ash content of faeces was undertaken in conjunction with the feeding experiments. During the course of each feeding determination, all faeces produced by the nudibranch were carefully siphoned out of the plastic tubs onto pre-ashed, pre-weighed glass-fibre filter discs. These discs, plus the faeces, were frozen, freeze-dried, weighed, ashed and reweighed to calculate the organic ash content of the faeces [glass-fibre filter discs withstand the ashing procedure without any change of weight].

3.2.2 Growth

3.2.2.1 *Archidoris pseudoargus*

In autumn 1985, seventeen *Archidoris pseudoargus* were collected from East Sands, St Andrews. Upon return to the laboratory, they were weighed under water, established in 2.5 l plastic containers and maintained at ambient temperature according to the methods outlined in Chapter 2. To monitor growth over the winter, these animals were weighed (under water) approximately every fourteen days until the following spring when *A. pseudoargus* enters its' spawning season. All measures of live weight were

converted to dry weight prior to calculating growth rates. Growth rates were calculated for each individual according to the following formula:

$$\text{Growth Rate} = \frac{\text{Size at } T_2 - \text{Size at } T_1}{T_2 - T_1}$$

Where T_1 = time (in days) at start of the growth period, and T_2 = time at the end of the growth period; *size* = dry weight of the nudibranch.

To undertake inter- and intraspecific comparisons, growth rates were scaled to body size by calculating size specific growth rates (SSGR) where:

$$\text{SSGR} = \frac{\text{Growth Rate}}{\text{Size}}$$

where *Size* is the geometric the mean of body sizes (dry weights) at T_1 and T_2. All individual SSGRs were pooled to calculate a 'species' SSGR for the period over the winter.

To investigate the size distribution of individuals at a single site, an exhaustive search of the shore at Robin Hood's Bay, North Yorkshire was undertaken over a period of three days in the spring of 1986 and again in the spring of 1987. All individuals collected were returned to the laboratory to determine their body size. Animals were placed in a flat-bottomed glass bowl resting on a sheet of graph paper and, when the animal began to crawl (and hence attain its full length), its body length was recorded to the nearest millimetre. A size/frequency distribution was constructed from these data to examine the structure of the population at the beginning of the spawning season.

3.2.2.2 *Cadlina laevis*

A laboratory population of *Cadlina laevis* had been established in October 1982 with individuals collected predominantly from the shores of Fife, with additional individuals collected from Argyll. Individuals were constantly added to the population during the period preceding and during the present study to maintain a large laboratory population. Juveniles were reared from egg masses laid in the laboratory, and

supplemented with field-collected individuals. All individuals were maintained in 2.5 l plastic tubs in a constant flow cascade system; water (at ambient field temperatures) was flowed to waste through the cascade. Aeration was essentially provided by the action of the cascade and the relatively high surface to volume ratios of the tubs. Small adults and juveniles were held in 'Teaboys' within the larger tubs. To monitor growth rates, all individuals were wet weighed (see Chapter 2) on a monthly basis from the time of collection until their death and, when necessary, immediately post spawning.

All measures of live weight were converted to dry weight prior to calculating growth rates. For all individuals, growth rates were calculated from first weighing until death using the method outlined earlier (Section 3.2.2.1). Growth rates were converted to size specific growth rates (SSGR). To investigate any patterns or trends, a mean 'species' SSGR was calculated for each growth increment.

3.3 Results

3.3.1 Live weight to dry weight calibrations

The conversion equations for all live to dry weights are given below, and the raw data, together with a fitted GM model and its' accompanying statistics, are shown in Figures 3.1 to 3.3.

***Archidoris pseudoargus*:**

$$\text{Log}_e \text{ dry weight} = 1.076 \cdot \text{Log}_e \text{ live weight} + 0.979$$
$$F = 295.71^{***};\ r = 0.97;\ n = 17$$

***Cadlina laevis*:**

$$\text{Dry weight} = 0.211 \cdot \text{live weight} - 3.126$$
$$F = 357.77^{***};\ r = 0.98;\ n = 11$$

***Halichondria panicea*:**

$$\text{Dry weight} = 3.826 \cdot \text{live weight}$$
$$F = 820.61^{***};\ r = 0.98;\ n = 34$$

Figure 3.1 Relationship between dry weight and live weight (weight under water: see Chapter 2) for *Archidoris pseudoargus*. A total of 17 individuals were used in the experiment which covered a live weight range from 20 to 872 mg. The line represents a 'best-fit' geometric mean regression when the data were transformed to natural logarithms.

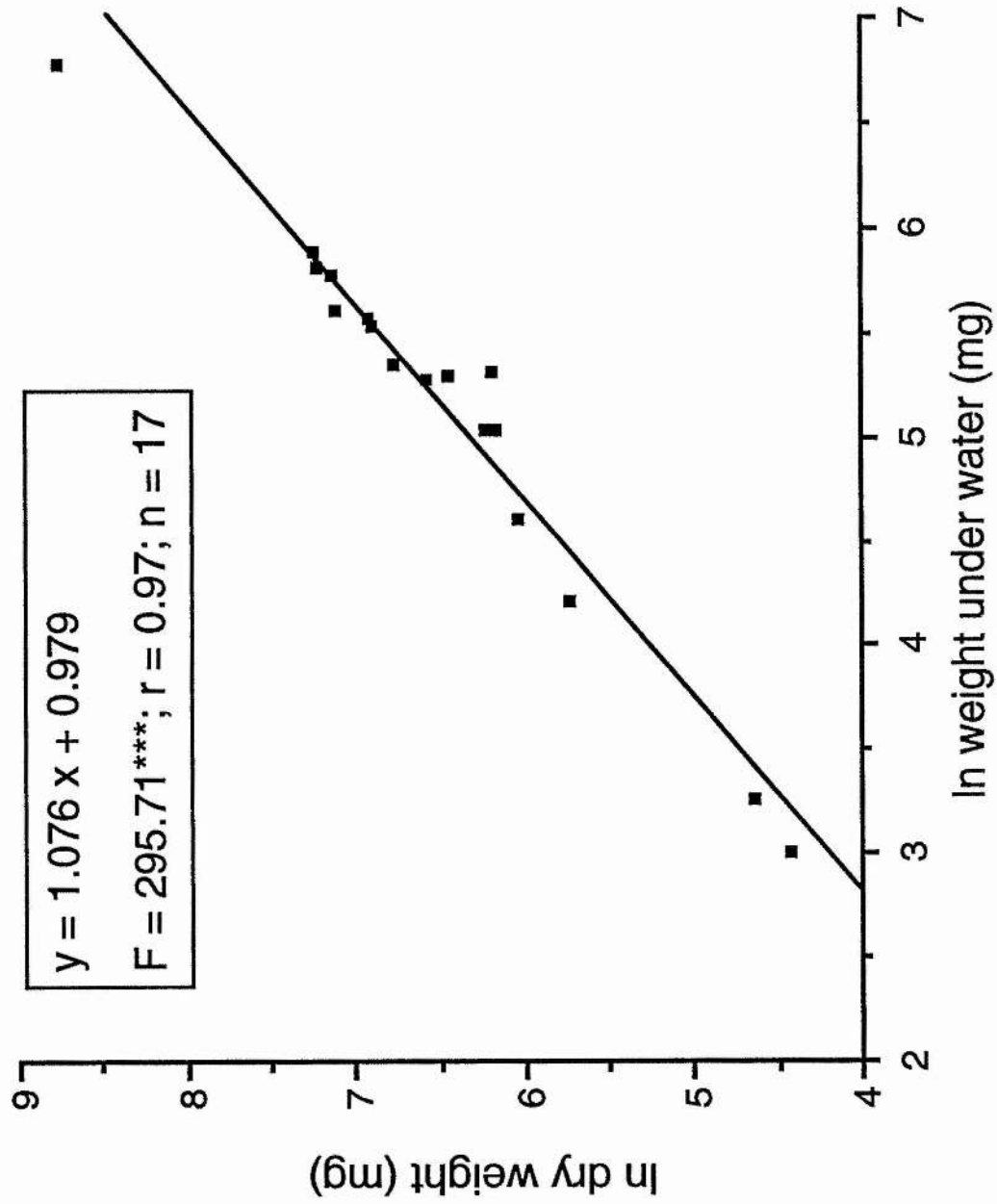

y = 1.076 x + 0.979
F = 295.71***; r = 0.97; n = 17
ln dry weight (mg)
ln weight under water (mg)
9
8
7
6
5
4
2
3
4
5
6
7

Figure 3.2 Relationship between dry weight and live weight (wet weight in air; see Chapter 2) for *Cadlina laevis*. A total of 11 individuals were used in the experiment which covered a live weight range from 95 to 1142.6 mg. The line represents a 'best-fit' geometric mean regression.

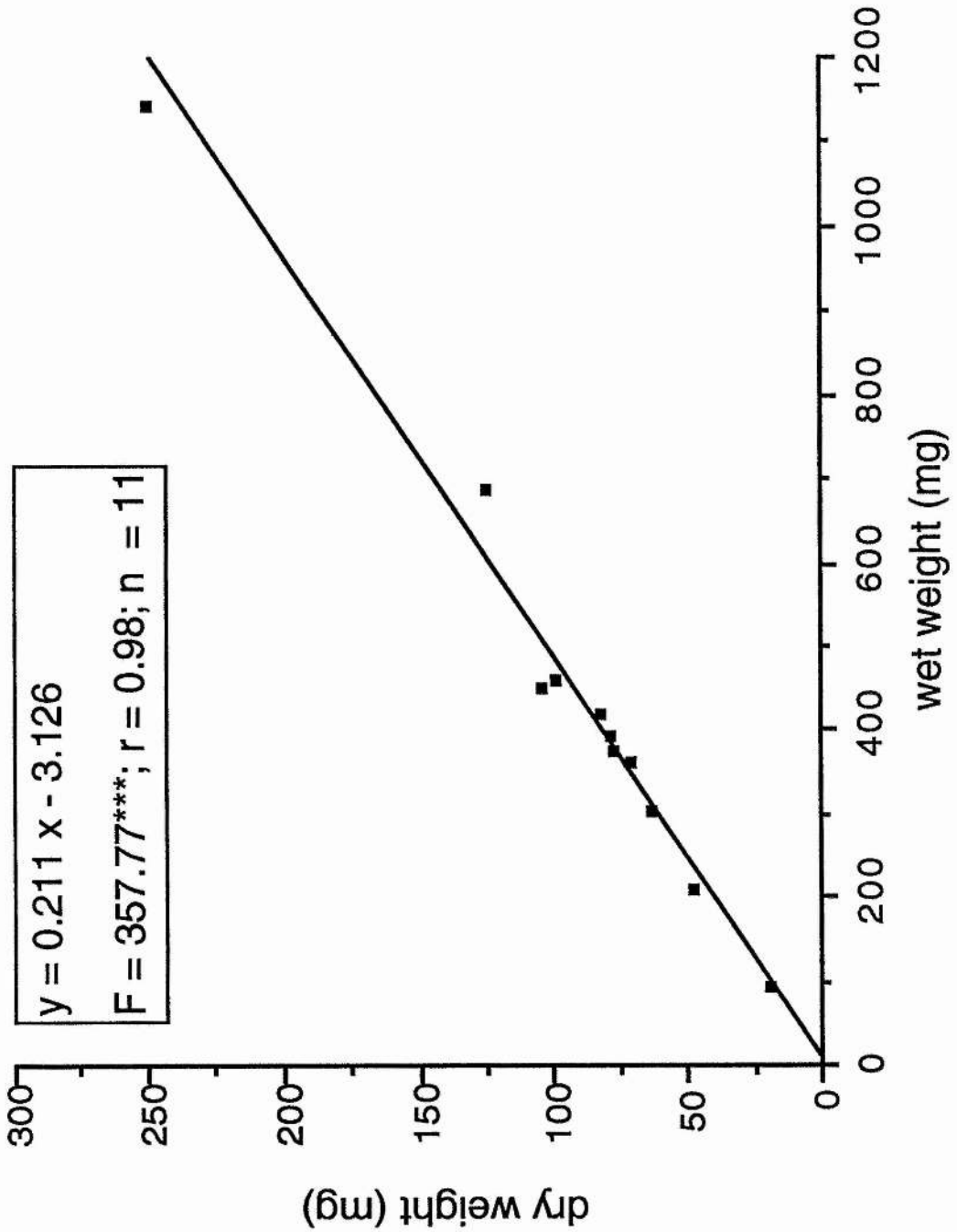
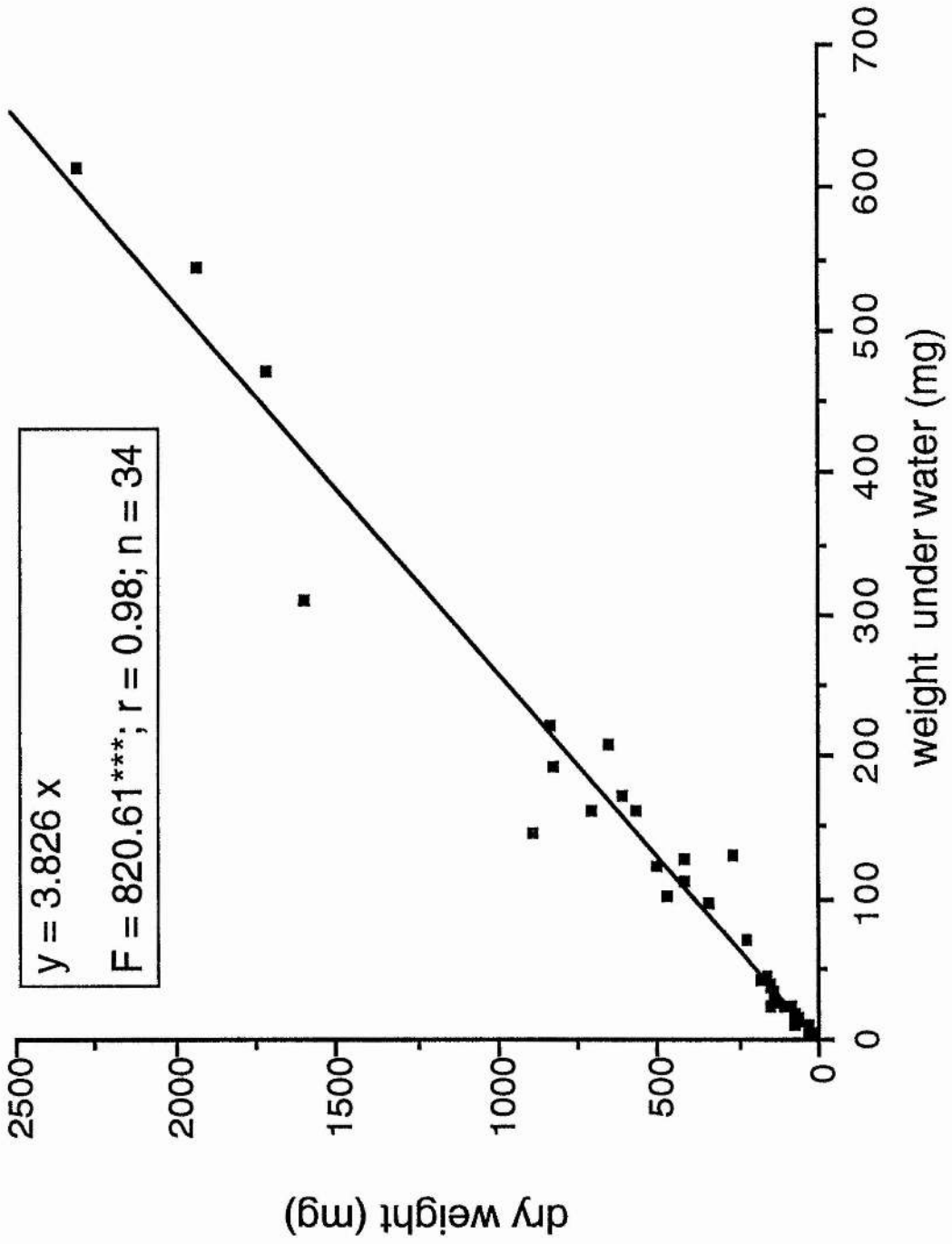

y = 0.211 x - 3.126
F = 357.77***; r = 0.98; n = 11
dry weight (mg)
wet weight (mg)
0
50
100
150
200
250
300
0
200
400
600
800
1000
1200

Figure 3.3 Relationship between dry weight and live weight (weight under water; see Chapter 2) for *Halichondria panicea.* A total of 34 determinations were undertaken with pieces of *Halichondria panicea* covering a range of live weights from 5.5 to 614.5 mg. The line represents a 'best-fit' geometric mean regression; the regression was constrained to pass through the origin.


y = 3.826 x
F = 820.61***; r = 0.98; n = 34
dry weight (mg)
weight under water (mg)
0
500
1000
1500
2000
2500
0
100
200
300
400
500
600
700

3.3.2 Calorific conversions

The results of calorimetric analysis and inorganic ash determinations for the species studied are presented in Table 3.1. Data presented in Table 3.1 have been used throughout the project to convert dry weights to energy equivalents which permit intra- and interspecific comparisons within the present study, and with previously published data.

3.3.3 Feeding

A total of 21 feeding rate determinations were undertaken, of which only 18 produced a positive result; no change in the weight of the prey material was recorded for three determinations. For these three determinations, one animal was not observed in contact with the prey at any time, whilst the other two determinations concerned very small molluscs (only 28 and 63 mg dry weight): these were, nevertheless, observed in contact with the prey material during the determination. It is possible that these small individuals were not able to prey directly on the sponge due to prey/size limitations, rather they graze detritus and/or microflora on the surface of the sponge. Small stock animals in the laboratory, and small individuals in the field, were generally observed in persistent and close contact with their prey, whereas larger individuals appear to lack such a close affinity to their prey.

Archidoris pseudoargus fed sporadically rather than grazed constantly. It appears that these animals feed until satiated and then move away from the prey, presumably to digest the recently ingested material. Faecal production mirrors the feeding behaviour, with faecal mounds produced sporadically after the animal has departed its prey. In the field, *A. pseudoargus* individuals, and juveniles in particular, were often observed 'buried' in sponge colonies.Wave action in the intertidal environment may dislodge individuals from the substrata and this 'burying' behaviour may be an adaptation to retain contact with the prey. Similarly, such behaviour may be a mechanism to reduce the likelihood of predation, *i.e.* a cryptic adaptation.

Table 3.1 Data from the calorimetric and inorganic ash determinations for *Archidoris pseudoargus*, *Cadlina laevis* and *Halichondria panicea*. Values for the *Calorific content* of the two nudibranch species have been corrected for endothermic dissociation of calcium carbonate. The *corrected calorific content* is the *calorific content* corrected for the percentage ash (*i.e.* the calorific content of the organic fraction of the tissue). *N* is the number of determinations.

Species	Ash content (%)	Calorific content ($J \cdot mg^{-1}$)	Corrected calorific content ($J \cdot mg^{-1}$ ash free)	N
Archidoris pseudoargus	36.86 ± 0.32	11.519 ± 0.39	18.26 ± 0.62	18
Cadlina laevis	31.62 ± 0.40	12.64 ± 0.49	18.48 ± 0.70	25
Halichondria panicea	64.26 ± 1.01	7.313 ± 0.14	20.46 ± 0.40	51

Feeding rates were variable with body size (Figure 3.4), paired observations were strongly correlated (Spearman rank coefficient = 0.657**). Size specific feeding rates (SSFR) ranged from 0.012 to 0.148 mg · mg $^{-1}$ · day $^{-1}$ with a mean SSFR of 0.049 ± 0.007 mg · mg^{-1} · day $^{-1}$. When the data are converted to energy equivalents (corrected for the ash content of the sponge), the SSFRs ranged from 0.021 to 0.264 J · J $^{-1}$ · day $^{-1}$ with a mean SSFR of 0.087 ± 0.013 J · J $^{-1}$ · day $^{-1}$.

Feeding rate is generally considered to display an allometric relationship with body size and therefore when the data were transformed to natural logarithms, the relationship became:

$$\text{Log}_e \text{ Feeding rate} = 0.902 \cdot \text{Log}_e \text{ body size} - 2.404$$

or

$$\text{Feeding rate} = 0.09 \cdot \text{Body size}^{0.902}$$

$$F = 23.03^{***};\ r = 0.75;\ n = 18$$

When these data are converted to energy equivalents, these relationships became:

$$\text{Log}_e \text{ Feeding rate} = 0.902 \cdot \text{Log}_e \text{ body size} - 1.589$$

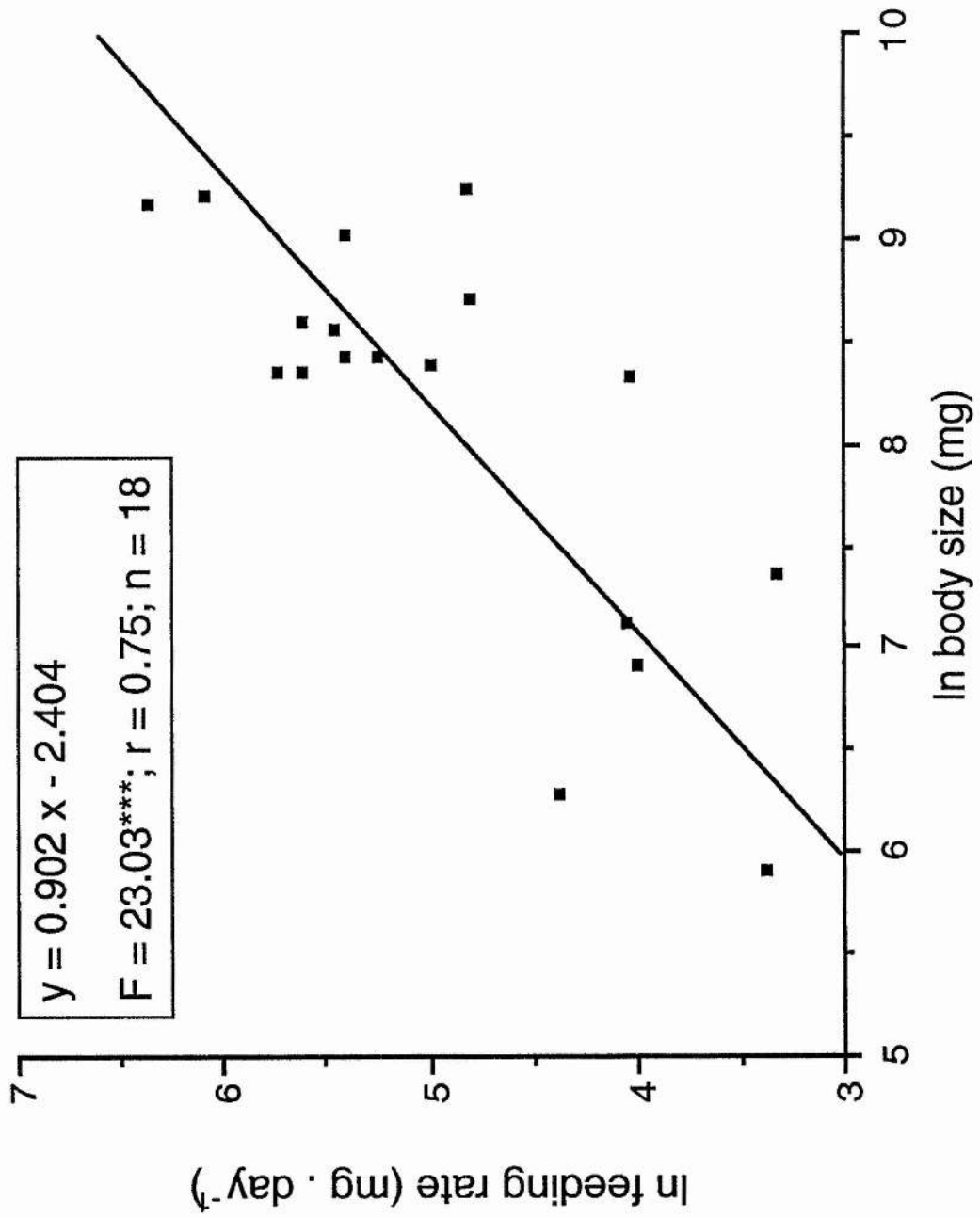
or

$$\text{Feeding rate} = 0.204 \cdot \text{Body size}^{0.902}$$

$$F = 23.04^{***};\ r = 0.77;\ n = 18$$

Faecal mounds would often be broken by the movements of the animal or the water within the tubs prior to their removal. Despite the care taken during the siphoning procedure, it was not possible to gather all the faecal material in a manner that would permit the weight of the faecal material to be used as a direct measure of egestion rates. The inorganic ash content of the faeces ranged between 65.1% and 83.06% with a mean of 71.9 ± 8.24%; therefore the organic content of the faeces is 28.1% (this may be an overestimate due to the loss of the water of hydration from the silica oxide within the sponge spicules [Carefoot 1967]).

Figure 3.4 Feeding rate (dry weight of *Halichondria panicea* consumed per day) plotted against body size (dry weight) for *Archidoris pseudoargus*. A total of 18 determinations were undertaken on individuals covering a range of body sizes from $3.71 \cdot 10^2$ to $10.21 \cdot 10^3$ mg (mean: $4.73 \cdot 10^3 \pm 7.41 \cdot 10^2$ mg dry weight); consumption rate ranged from 28.1 to 585.5 mg $\cdot$ day $^{-1}$ (mean: 191.1 ± 35.3 mg $\cdot$ day $^{-1}$). A significant 'best-fit' geometric mean regression was obtained when the data were transformed to natural logarithms.


y = 0.902 x - 2.404
F = 23.03***; r = 0.75; n = 18
ln feeding rate (mg · day⁻¹)
ln body size (mg)
7
6
5
4
3
5
6
7
8
9
10

An estimate of the efficiency of digestion can be calculated using the equation given by Bloom (1981):

$$E = 100 \text{ x } \frac{\left(\frac{O \text{ x } S}{P} - \text{R}\right)}{\left(\frac{O \text{ x } S}{P}\right)}$$

where E is the efficiency of digestion, O & P are the digestible organic and spicule fractions respectively of the sponge, and R & S are the digestible organic and spicule fractions respectively, of the faeces. Bloom (1981) was able to make the distinction between the 'free' organic fraction and the skeletal organic fraction and assumed that the latter is not digested by the nudibranch. These components were not determined during the present study and both organic fractions are combined to a single 'organic fraction'. The organic fraction from the faecal ash determination will be mainly undigested material, non-digested organic material, and mucus. If one assumes that there is no differential absorption of organic material, the organic fraction voided in faeces is energy 'lost' to the organism. If the organic fraction of the faeces is 28.1% and the organic fraction of the food is 35.7%, the efficiency of digestion E for *Archidoris pseudoargus* is 29.6%. If one extends the assumption that there is no differential absorption of the food material, the mean SSFR can be corrected for the efficiency of digestion to give a 'corrected' assimilation rate of 0.026 $J \cdot J^{-1} \cdot day^{-1}$.

3.3.4 Growth

3.3.4.1 *Archidoris pseudoargus*

Of the seventeen animals maintained over the winter, nine spawned the following spring, but the remaining eight animals did not spawn. These animals were all fed *ad libitum* and assumed to feed normally, as indicated by the regular disappearance of the prey *Halichondria panicea*, and the volume of faeces produced by each animal. Table 3.2 presents a summary of the data for growth over the winter and indicates which animals spawned the following spring. Rather unexpectedly, the change in body size over the

Table 3.2 A summary of the overwinter growth of *Archidoris pseudoargus*. (N° SSGRs indicates the number of size specific growth rate determinations (*i.e.* one fewer than the total number of size determinations) during the experiment; *Date of spawn* gives the date when the the animal spawned during the growth experiment.

Animal	Starting date	Initial size (mg)	Final size (mg)	Mean SSGR (± 1 se) (day $^{-1}$)	N° SSGR	Date of Spawn
1	5:11:85	3419.8	3004.5	-0.0002 ± 0.0034	8	5:05:86
2	5:11:85	14818.0	3032.8	-0.011 ± 0.0069	8	3:05:86
4	5:11:85	10607.6	12201.5	0.0009 ± 0.002	8	15:04:86
5	5:11:85	2888.9	2076.1	-0.0042 ± 0.0077	7	*
6	5:11:85	1076.7	1177.2	0.0009 ± 0.0028	7	*
7	5:11:85	621.7	611.9	-0.0004 ± 0.0039	7	*
8	5:11:85	29.3	12.8	-0.0067 ± 0.0032	7	*
9	5:11:85	715.7	582.7	-0.0015 ± 0.0055	7	*
10	5:11:85	6547.7	7216.2	0.0007 ± 0.0031	8	28:03:86
11	5:11:85	379.8	310.2	-0.0015 ± 0.0036	7	*
12	19:11:85	639.9	514.8	-0.0008 ± 0.0074	6	*
13	19:11:85	5131.3	4859.2	-0.0009 ± 0.0026	7	28:03:86
14	19:11:85	3004.3	3392.1	0.0011 ± 0.0042	7	6:04:86
15	19:12:85	13916.8	10928.3	-0.0034 ± 0.0046	5	23:03:86
16	19:12:85	12044.1	7196.1	-0.0056 ± 0.0027	5	6:04:86
17	19:12:85	8584.9	9079.0	0.0003 ± 0.0081	5	25:03:86

winter fluctuated about a mid point and the overall change was, on average, very low. Correspondingly, mean size specific growth rate was also low, or even negative, indicating a decline in body size over the winter. These results differ markedly from previous investigations of nudibranch growth patterns which indicated the period during the winter is the primary growth phase. Changes of body size for spawning individuals over the winter period are shown in Figure 3.5, and non-spawning individuals in Figure 3.6.

From these data, it appears that non-spawning individuals did not increase in size, rather that body size remained relatively constant for the whole period. Spawning individuals appeared to show an increase in body size during the immediate pre-spawning period; spawning individuals died within two weeks post spawning and thus no further growth was recorded. When the data are converted to size specific growth rates (SSGR), these patterns become more apparent. SSGRs for spawning and non-spawning individuals are shown in Figures 3.7 and 3.8.

To summarise the above data, a mean SSGR (± 1 se) was calculated for spawning and non-spawning individuals for the overwinter period; these data are presented in Figure 3.9. The large increase on the third data point for non-spawning individuals is probably an experimental error cause an inaccurate weight determination of two individuals. The live weight (and hence dry weight) of these individuals changed sharply at a single weighing, but note that the weight recorded at the preceding and subsequent weighing were very similar. In general, SSGR was constant, but negative, for the overwinter period indicating a slow decline in body size. In the spring, the sharp increase in the growth rate was predominantly a result of an increase in the growth rate of the spawning individuals. These data suggest that the main growth period for *Archidoris pseudoargus* is not during the winter, individuals generally maintaining size in winter with growth increasing in the spring.

During the surveys at Robin Hood's Bay, a total of 51 animals were collected, ranging in length from 7-43 mm; a size frequency distribution for these animals is shown in Figure 3.10. *Archidoris pseudoargus* begin spawning in late March and continue until

Figure 3.5 Change in body size (as dry weight) over the winter for 9 *Archidoris pseudoargus;* these individuals spawned in the following spring; date of spawning is given in Table 3.2. Animals 1-10 were started on November 11, animals 13-14 were started on November 19, and animals 15-17 were started on December 12. The final body size is the immediate pre-spawning size. Summary data for these individuals are presented in Table 3.2.

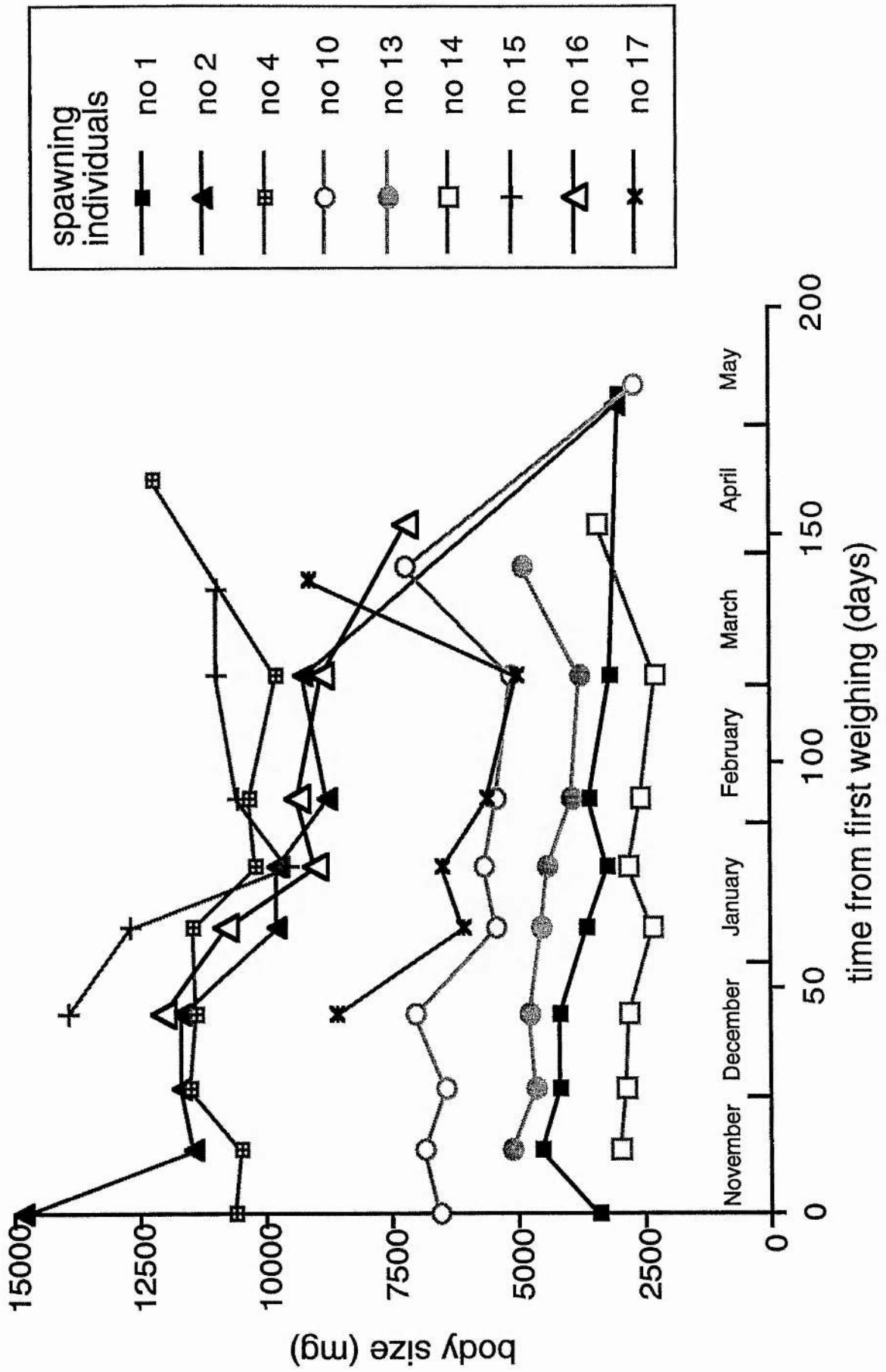

spawning individuals
no 1
no 2
no 4
no 10
no 13
no 14
no 15
no 16
no 17
body size (mg)
15000
12500
10000
7500
5000
2500
0
50
100
150
200
November
December
January
February
March
April
May
time from first weighing (days)

Figure 3.6 Change in body size (as dry weight) over the winter for 7 *Archidoris pseudoargus;* these individuals did not spawn in the following spring. Animals 5-9 and 11 were started on November 5, and animal 12 was started on November 19. Summary data for these individuals are presented in Table 3.2.

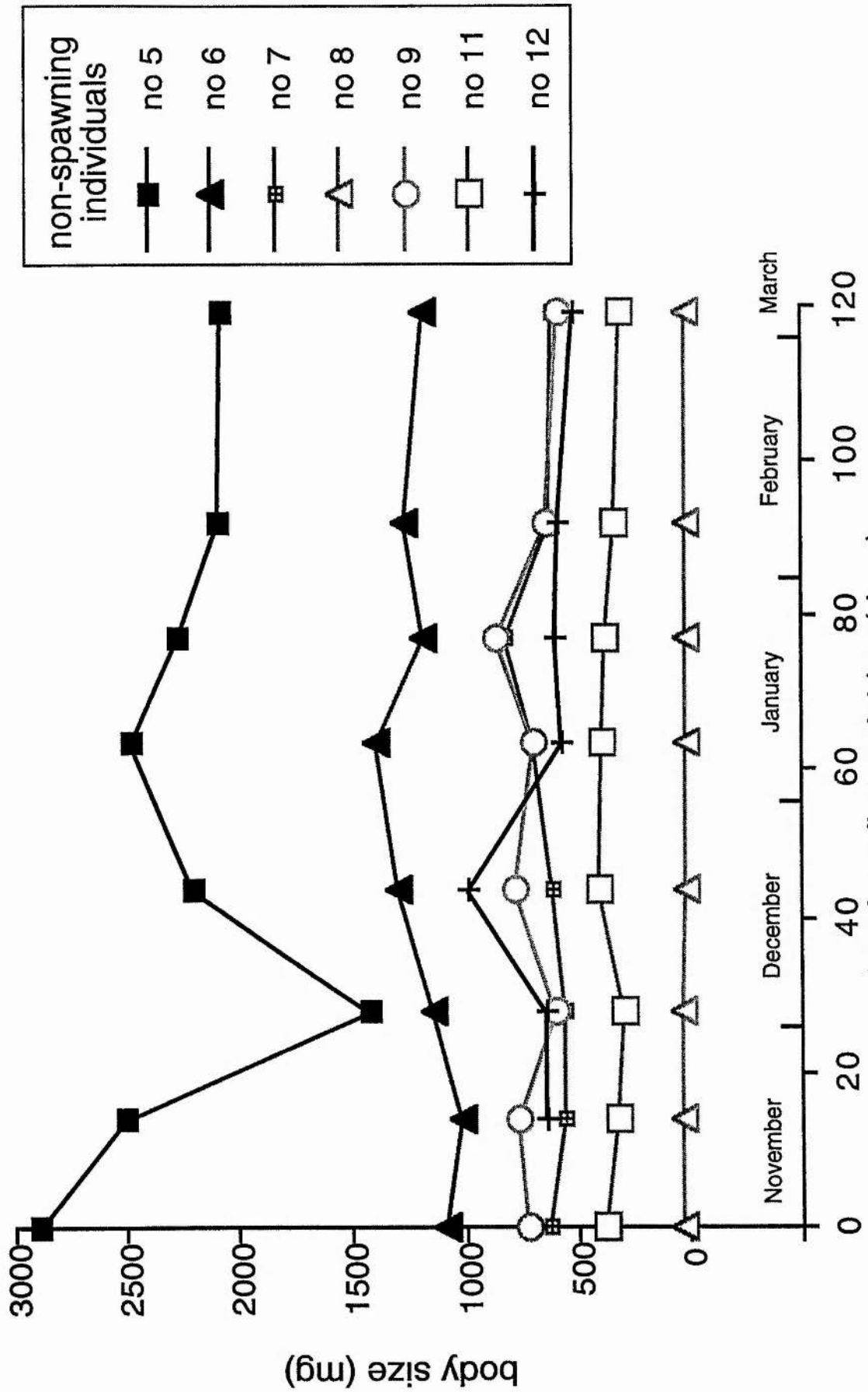

non-spawning individuals
no 5
no 6
no 7
no 8
no 9
no 11
no 12
body size (mg)
3000
2500
2000
1500
1000
500
0
November
December
January
February
March
0
20
40
60
80
100
120
time from first weighing (days)

Figure 3.7 Size specific growth rate (SSGR) over the winter for the 9 *Archidoris pseudoargus* which spawned at the end of the observation period (see Figure 5). Mean SSGR for these 9 individuals is presented in Figure 9. SSGR is the growth rate (change in size per day) divided by the geometric mean of body size at the beginning and the end of the growth period. Summary data for these individuals are presented in Table 3.2.

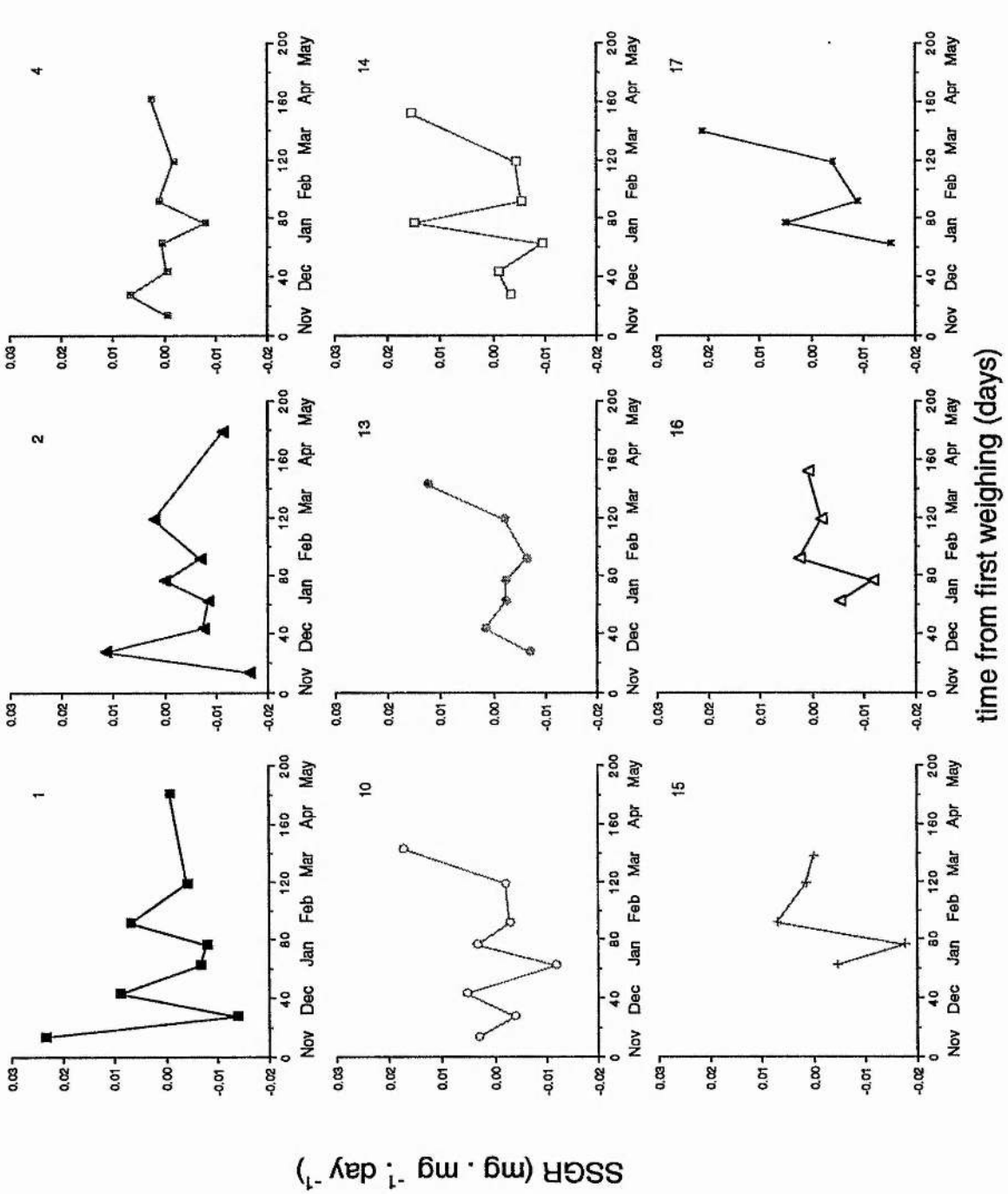

SSGR (mg · mg -1 day -1)
time from first weighing (days)
1
2
4
10
13
14
15
16
17
0.03
0.02
0.01
0.00
-0.01
-0.02
0
40
80
120
160
200
Nov
Dec
Jan
Feb
Mar
Apr
May

Figure 3.8 Size specific growth rate (SSGR) over the winter for the 7 *Archidoris pseudoargus* which did not spawn at the end of the observation period (see Figure 6). Mean SSGR for these 7 individuals is presented in Figure 9. SSGR is the growth rate (change in body size per day) divided by the geometric mean of body size at the beginning and the end of the growth period. Summary data for these individuals are presented in Table 3.2.

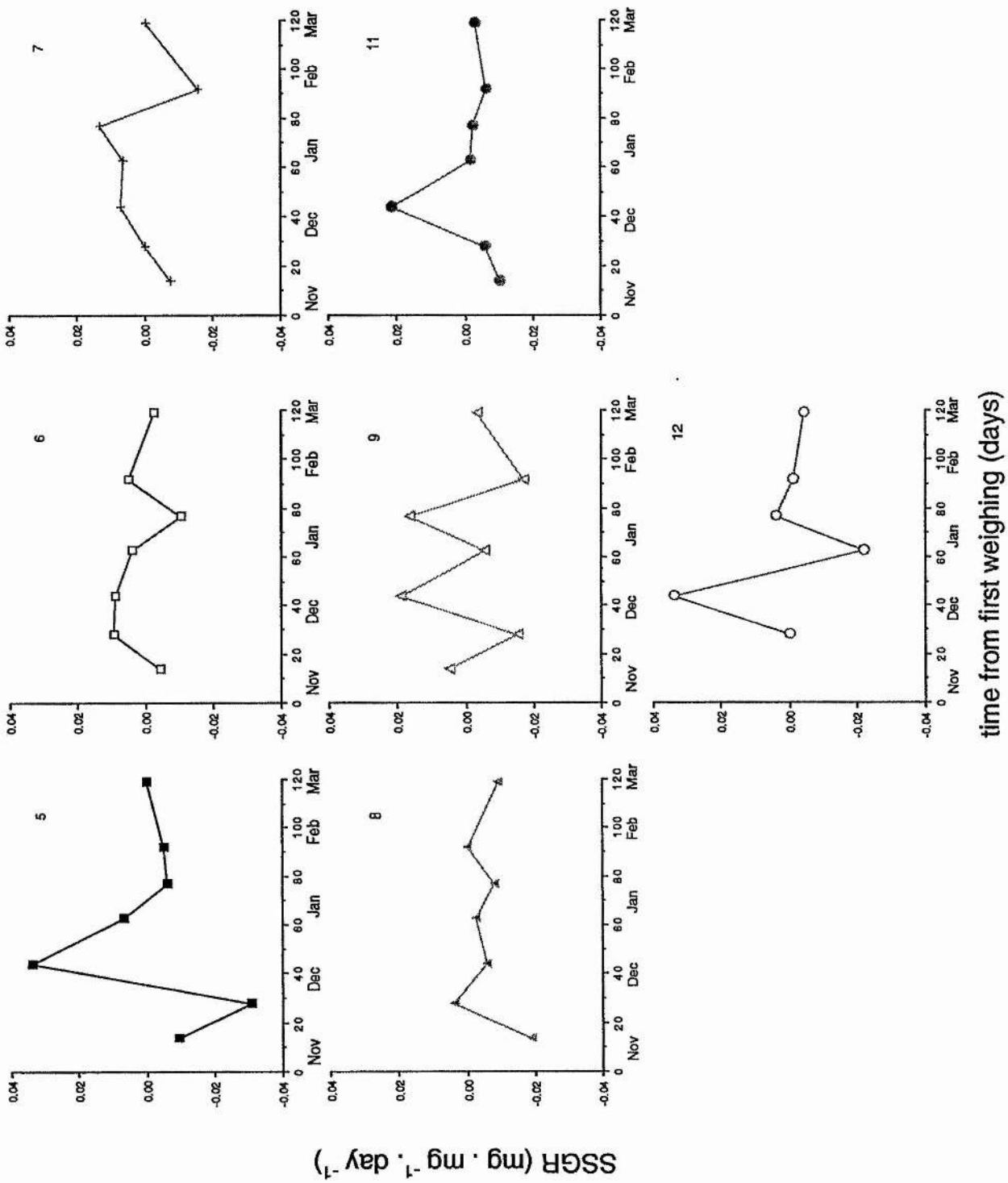
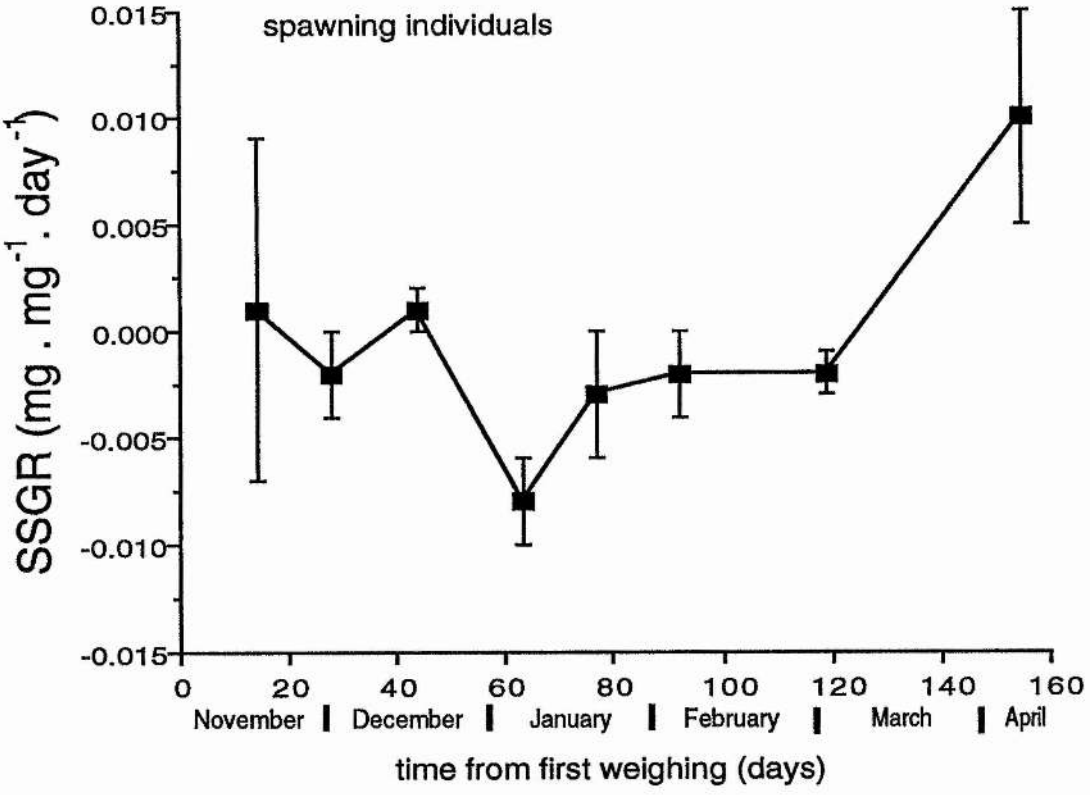

SSGR (mg · mg⁻¹· day⁻¹)
time from first weighing (days)
5
6
7
8
9
11
12
0.04
0.02
0.00
-0.02
-0.04
0
20
40
60
80
100
120
Nov
Dec
Jan
Feb
Mar

Figure 3.9 Mean size specific growth rate (SSGR) (± 1 se) over the winter for *Archidoris pseudoargus*. This figure summarises the data for individual nudibranchs presented in Figures 3.7 and 3.8. Upper diagram represents the data for those individuals which spawned at the end of the observation period (see Table 3.2); the lower diagram represents individuals which did not spawn.


spawning individuals
SSGR (mg . mg-1 . day-1)
0.015
0.010
0.005
0.000
-0.005
-0.010
-0.015
0
20
40
60
80
100
120
140
160
November
December
January
February
March
April
time from first weighing (days)

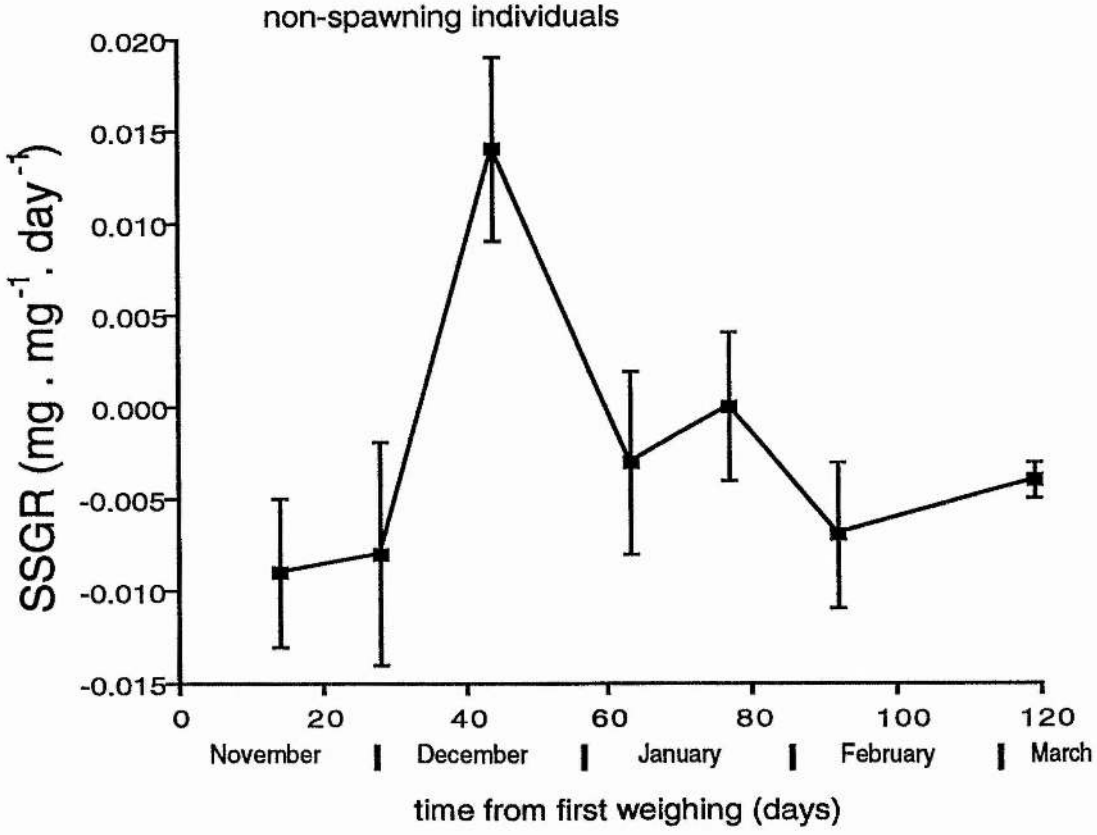
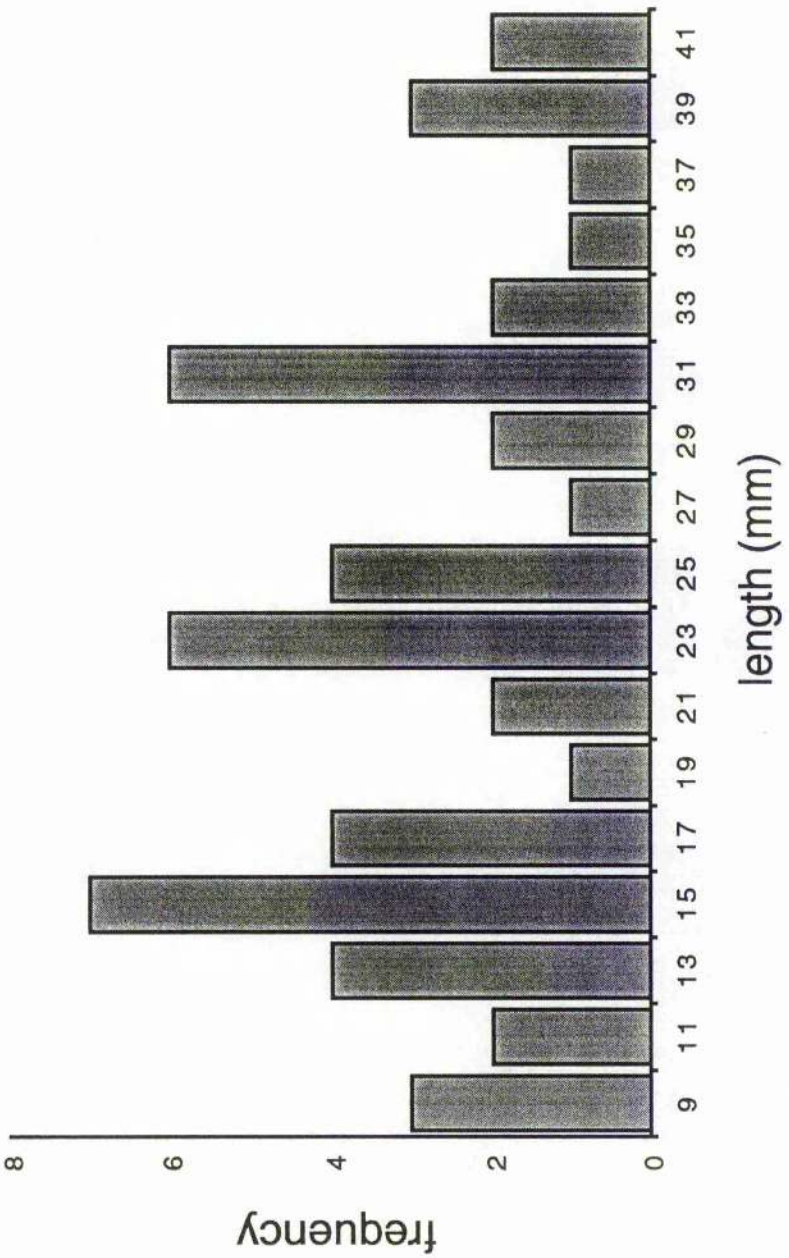

non-spawning individuals
SSGR (mg . mg-1 . day-1)
0.020
0.015
0.010
0.005
0.000
-0.005
-0.010
-0.015
0
20
40
60
80
100
120
November
December
January
February
March
time from first weighing (days)

Figure 3.10 Size-frequency distribution for 51 *Archidoris pseudoargus* collected from two field visits (April 1986 & 1987) to Robin Hood's Bay, North Yorkshire. Although no obvious age structure can be detected, it is certain that individuals of, for example, 7 and 43 mm length, are of markedly differing ages.

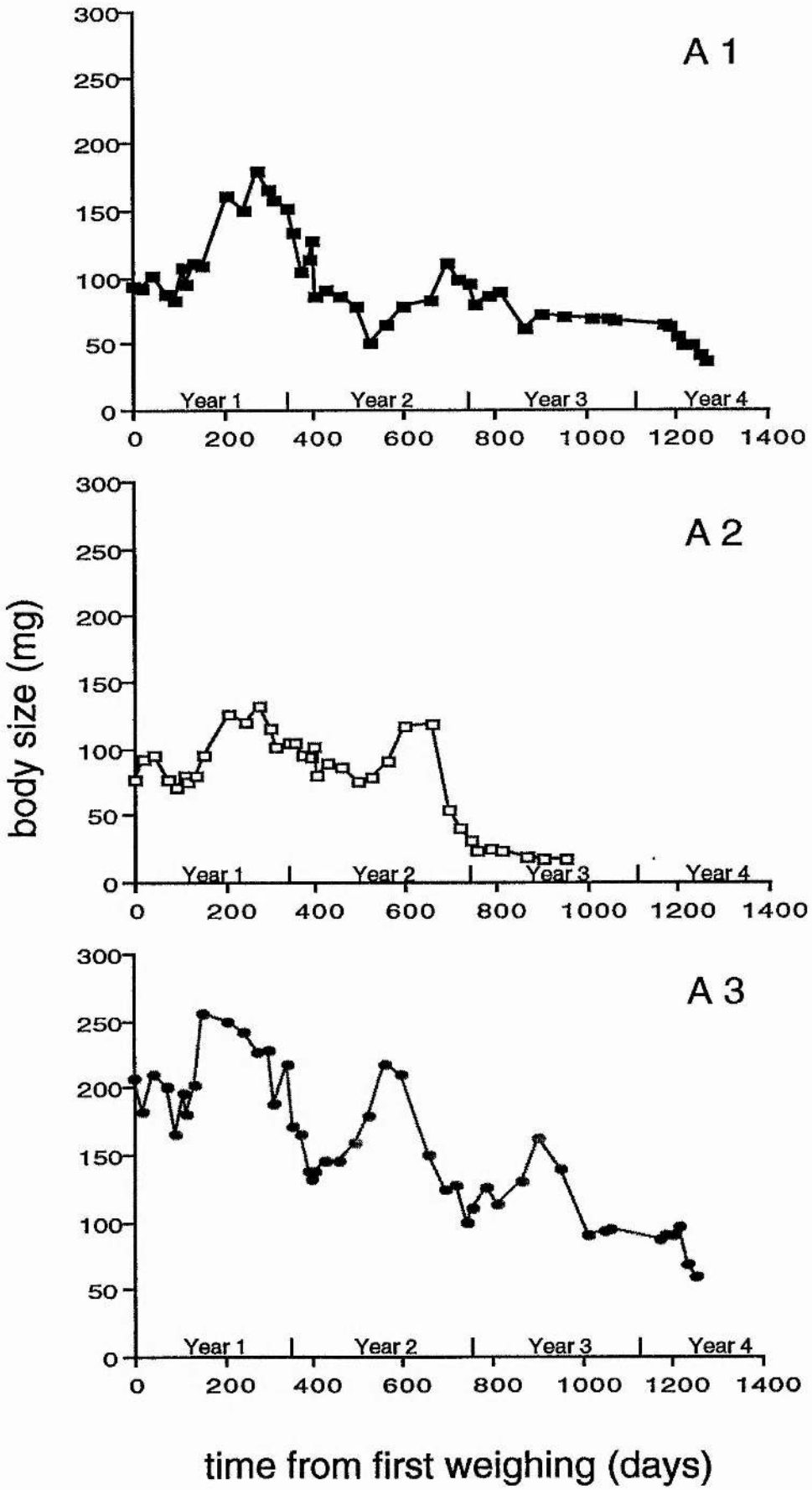

frequency
0
2
4
6
8
9
11
13
15
17
19
21
23
25
27
29
31
33
35
37
39
41
length (mm)

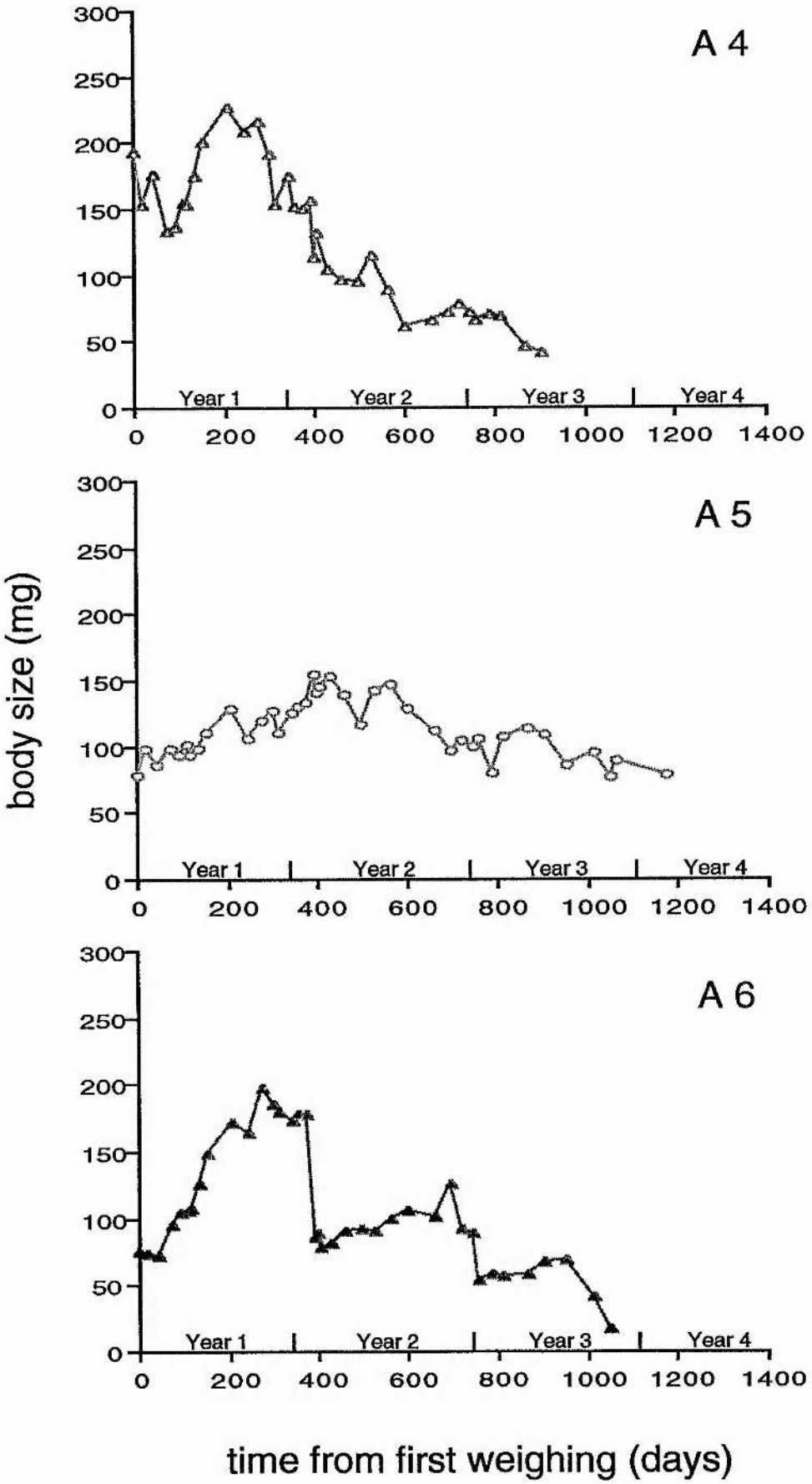
June. If this species has an annual life cycle, one would expect all individuals to be at or near spawning size at the beginning of their spawning season. These data from Robin Hood's Bay show a very broad range of sizes and these, combined with the growth data presented above, demonstrate that there is more than one age class present in the population. All animals collected at Robin Hood's Bay were returned to the laboratory to monitor their further development. Only the larger individuals (>30 mm) spawned during that spawning season, the remainder continued to grow but did not spawn. These growth data, in addition to the size frequency distribution in Figure 3.10, all support the conclusion that *Archidoris pseudoargus* has at least a biennial life cycle - all individuals die post spawning.

3.3.4.2 *Cadlina laevis*

In October 1982, twenty eight individuals had been collected to establish a laboratory population of *Cadlina laevis*. Most nudibranch species have an annual life cycle but, of the original laboratory population, six individuals had survived ≈ 2.5 years until May 1985 and two had survived for ≈ 3.5 years until January 1986. When the laboratory population was established, it was assumed that *C. laevis* had an annual life cycle and therefore all field-collected individuals would have hatched from egg masses laid that year. With the knowledge that the species has a perennial life cycle, it is impossible to determine the age of field-collected adults. Based on the data collected for the growth of laboratory-hatched juveniles, the age of field collected juveniles could be more reliably determined. Figure 3.11 displays the changes in body size for field collected adults from the time of collection until their death (only data for the largest and longest surviving individuals are shown). From these data, it is quite apparent that there are distinct seasonal patterns to the growth of adult *C. laevis*, and these patterns are repeated annually. In particular, individuals A1-3 indicate that there are periods of rapid growth each year followed by an extended period of a slow decline in weight; very rapid decreases in body weight during December-February, are attributable to the individual spawning. For the laboratory population in general, peak body weight was achieved 120 ± 17 days pre-spawning in 1983/4 and 138 ± 30 days in 1984/5; that is peak weight is

Figure 3.11 Change in body size (measured as dry weight) over time for 6 adult *Cadlina laevis;* the animals shown were the longest surviving individuals. The observation period began on the November 11, 1982 and the final measurements (A1 and A 3) were taken on March 4, 1986. There appears to be a cyclical growth pattern with individuals increasing in size during the summer and followed by an decline over the winter . These data are summarised in Figure 12.


A 1
A 2
A 3
body size (mg)
time from first weighing (days)
Year 1
Year 2
Year 3
Year 4
0
50
100
150
200
250
300
0
200
400
600
800
1000
1200
1400


A 4
A 5
A 6
body size (mg)
time from first weighing (days)
Year 1
Year 2
Year 3
Year 4
0
50
100
150
200
250
300
0
200
400
600
800
1000
1200
1400

achieved in mid-summer. At spawning, body weight represents a 25% reduction of peak body size for that spawning season.

Size specific growth rates were calculated for all animals shown in Figure 3.11 and a mean SSGR (±1 se) calculated for each month of the study (Figure 3.12). An annual growth cycle is more apparent from these data: growth increases sharply in the spring (post spawning) and remains positive until early summer; SSGRs are relatively constant, but negative in late summer and autumn with a further burst of growth in early winter prior to the spawning season. Over the whole period, the SSGR is negative indicating a gradual decline in body size with time with, in general, animals dying post-spawning, usually within a month.

Juveniles hatch in the spring and early summer at a body length of 0.8 -1 mm. Juveniles (identified as such by their small size and the absence of a developing gonad visible through the dorsum) were collected from the field in the autumn at a length of 4-5 mm and maintained in the laboratory. There were no records of these juveniles spawning in their first year. Juvenile growth and mean monthly size specific growth rate (SSGR) are shown in Figures 3.13 and 3.14 respectively. Juvenile growth over the winter was approximately linear (note declining SSGR) followed by a rapid increase during the following spring and early summer (constant and increasing SSGR). In general juvenile growth was somewhat variable although for some individuals at least, appeared exponential during the spring and summer period. There was a further burst of rapid growth during the early winter (immediately pre-spawning) when five of these juveniles spawned for the first time in the winter of their second year, when they were approximately 22 months old. Rather surprisingly, five individuals did not spawn despite attaining the minimum spawning size (approximately 100 mg live weight: see Chapter 5).

Not all juveniles showed this rapid increase in size during their second year. In Figure 3.13, *J6* and *J8-10* grew very slowly for the duration of the experiment, although these individuals were maintained during a different calender year when environmental conditions and/or food quality may have been less favourable. *J6* on the other hand, was observed during the same calender year as all other individuals in Figure 3.13 yet

Figure 3.12 Mean size specific growth rates (SSGR) (± 1 se) for the 6 adult *Cadlina laevis* shown in Figure 3.11. SSGR is the growth rate (change in body size per day) divided by the geometric mean of body size at the beginning and the end of the growth period. Not all animals survived the full duration of the study and the number at the top represents the number of animals used to calculate the mean SSGR. There appears to be a marked cyclical pattern where the SSGR increases rapidly in the late spring and to a lesser extent in the autumn. At other times SSGR is constant but negative indicating a decline in body size with time.

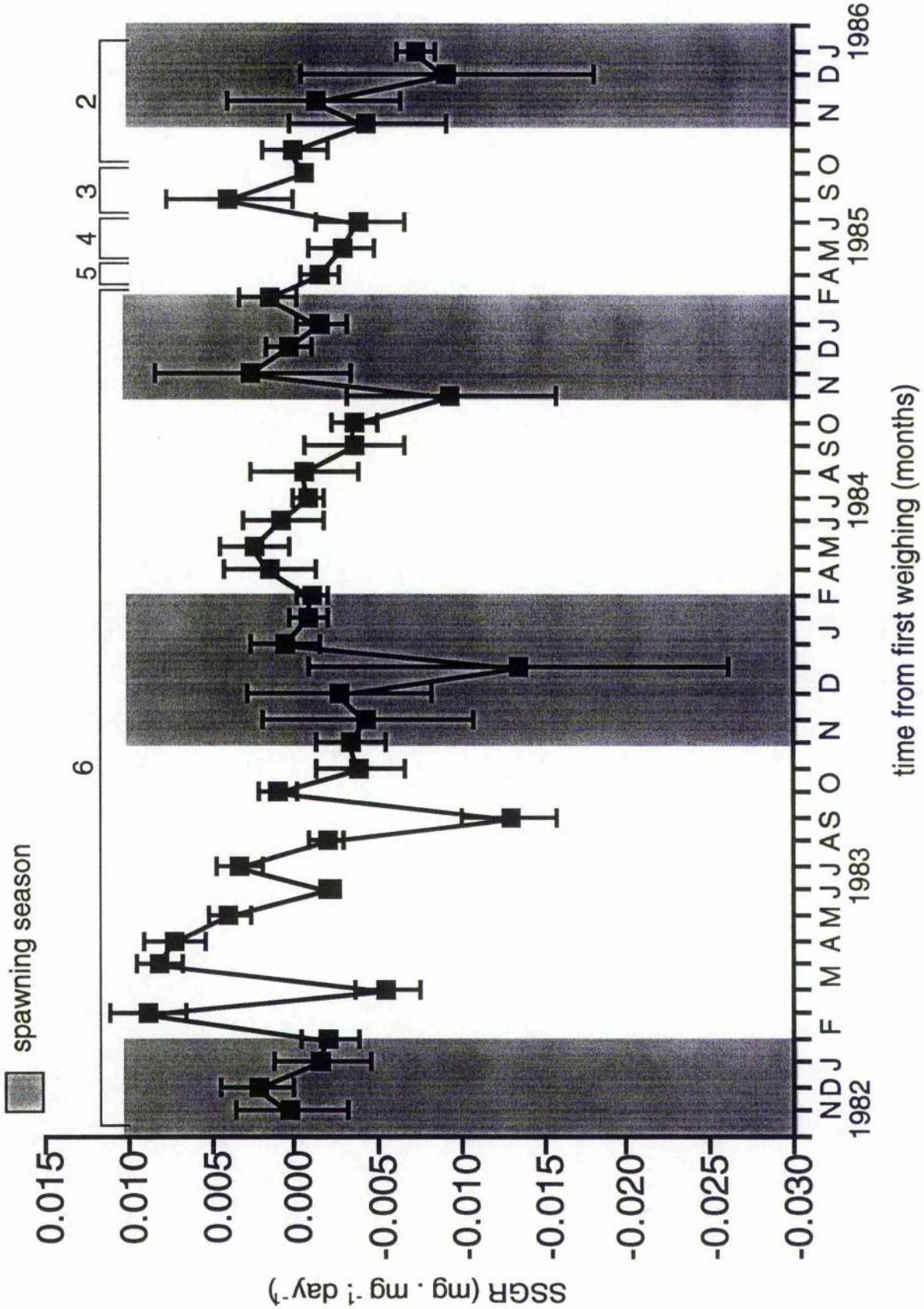

spawning season
SSGR (mg · mg⁻¹ day⁻¹)
0.015
0.010
0.005
0.000
-0.005
-0.010
-0.015
-0.020
-0.025
-0.030
6
5
4
3
2
N D J F M A M J J A S O N D J F A M J J A S O N D J F A M J S O N D J
1982
1983
1984
1985
1986
time from first weighing (months)

Figure 3.13 Change in body size for 10 juvenile *Cadlina laevis* from the field. Individuals J 1-J 7 were observed during the period November 17, 1983 to March 9, 1985; individuals J 8-10 were observed for the period October 10, 1984 to November 19, 1985. Individuals J 1, J 3, J 4 and J 5 spawned for the first time during December-February, at the end of the observation period. These data are summarised in Figure 3.14.

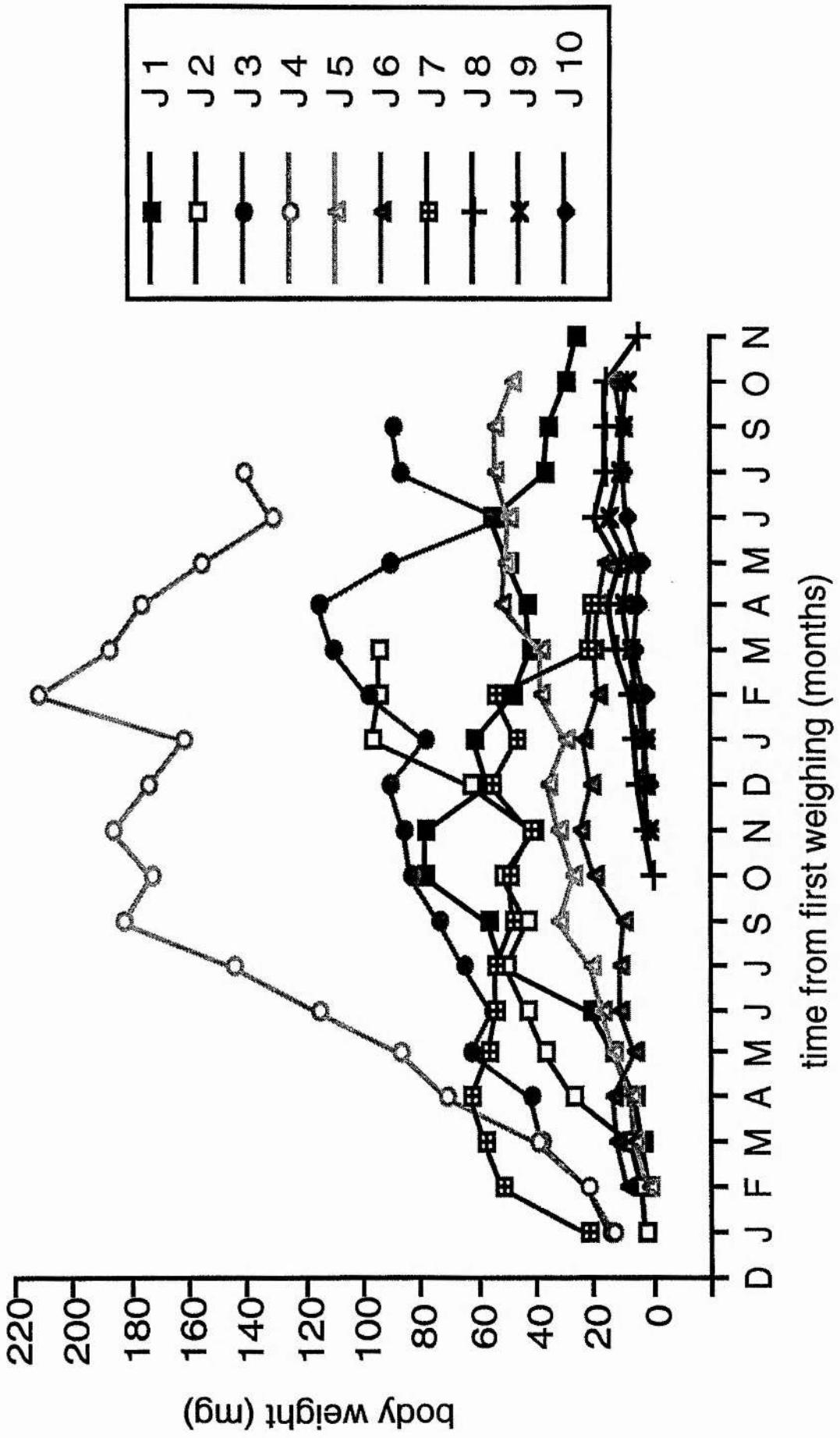
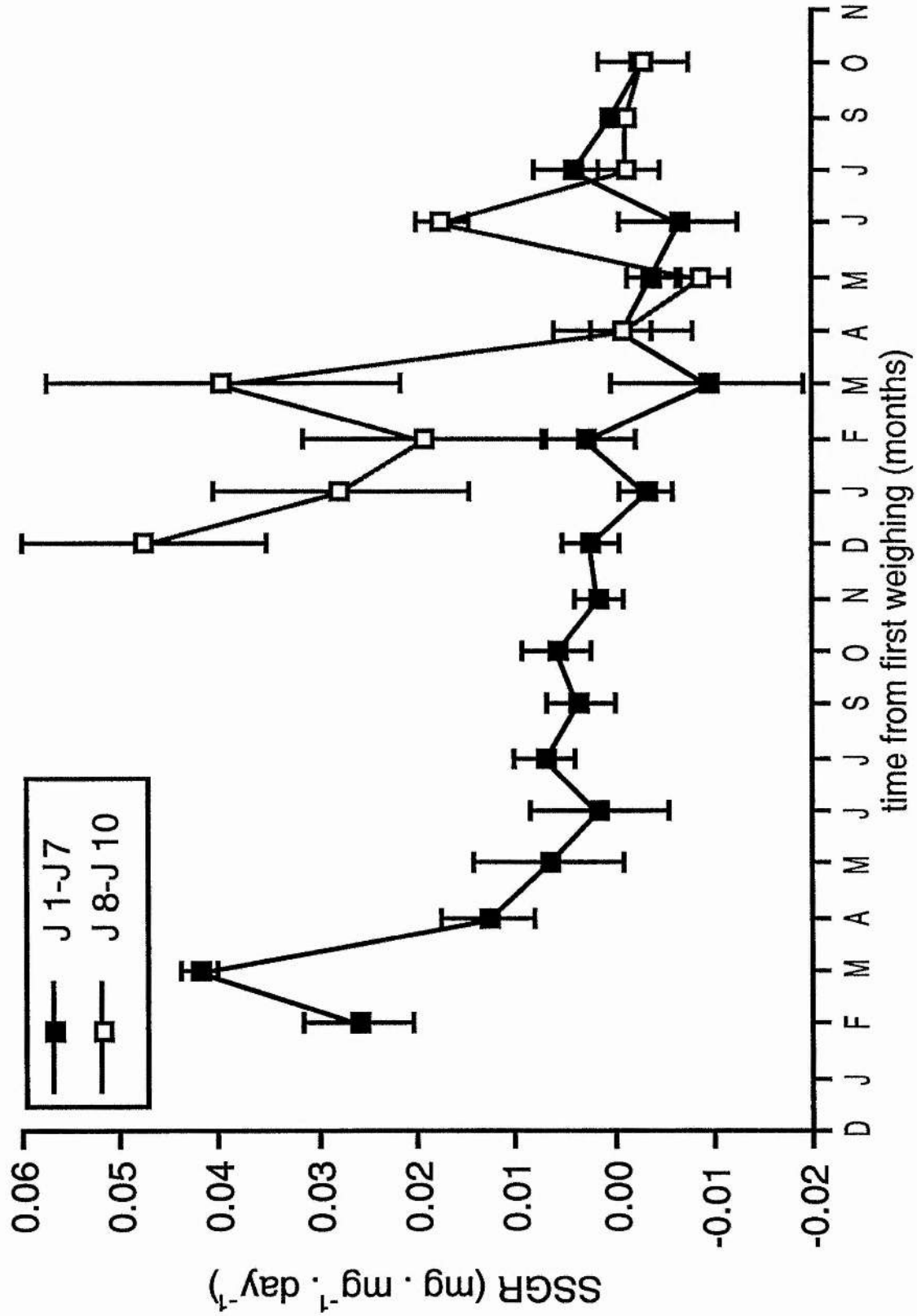

J 1
J 2
J 3
J 4
J 5
J 6
J 7
J 8
J 9
J 10
body weight (mg)
220
200
180
160
140
120
100
80
60
40
20
0
D J F M A M J J S O N D J F M A M J J S O N
time from first weighing (months)

Figure 3.14 Mean size specific growth rate (SSGR) (± 1 se) for the 10 juvenile *Cadlina laevis* shown in Figure 3.13. Data for individuals J 8-10 and data for individuals J 1-J7 are differentiated due to the different observation period. SSGR is the growth rate (change in body size per day) divided by the geometric mean of the body size at the beginning and the end of the growth period. Initially SSGR is high and declines with time although there appear to be two rapid increases of growth, a large increase in late spring and a smaller increase in the Autumn.


SSGR (mg · mg⁻¹ · day⁻¹)
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
-0.02
J 1-J7
J 8-J 10
D J F M A M J J S O N D J F M A M J J S O N
time from first weighing (months)

displayed a low growth rate equivalent to *J8-10*. Rather than as a result of environmental conditions, the slow growth of these four individuals may demonstrate the lower limit of the natural variability in growth for this species. A slow growing genotype is unlikely to survive in the field, whereas in the more benign conditions of the laboratory, these genotypes can survive for an extended period.

From these data for adult and juvenile *Cadlina laevis*, a summary life cycle may be constructed. Juveniles hatch from benthic egg masses during the spring and early summer, growing steadily until late autumn. Over the winter, juveniles appear to maintain a constant body size, or even de-grow, and then resume growth the following spring, maintaining positive growth through to the following winter. After an early winter increase in body size, individuals spawn for the first time in their second winter. This cyclical pattern to growth appears to continue throughout an individual's life cycle, culminating in a final winter increase and spawning, followed by closely by death. Some individuals appear to survive at least to an age of five years.

3.4 Discussion

3.4.1 Calorimetric and gravimetric analysis

Brey *et al.* (1988) reviewed the available literature on the energy content of benthic macro-invertebrates and presented mean calorific values for the primary marine phyla. For the phyla considered during the present study:

Porifera: Range = 21.94 - 27.92 $J \cdot mg^{-1}$ ash free dry wt; Mean = 22.87

Gastropoda: Range = 18.75 - 30.64 $J \cdot mg^{-1}$ ash free dry wt; Mean = 23.27

Values determined for the species considered in the present study (Table 3.1) lie towards the lower end of these ranges. Carefoot (1967) recorded a calorific content of 11.96 $J \cdot mg^{-1}$ with an ash content of 37% for *Archidoris pseudoargus* (= 18.98 $J \cdot mg^{-1}$ ash free) which concur with the values determined in the present study. McCance & Masters (1937) reported an ash content of 33% of the dry weight for the viscera of *A. pseudoargus*. Calorimetric or gravimetric data for *Cadlina laevis* are not

available in the literature although the values determined in the present study are within the range of values reported for other nudibranch species. Paine (1964) determined the calorific content of 13 species of opisthobranch, recording values from 20.68 to 27.93 $J \cdot mg^{-1}$ ash free dry wt; the ash content of these species varied from 25-43%. Hall & Todd (1986) recorded a calorific value of 20.8 $J \cdot mg^{-1}$ ash free dry wt, and an ash content of 16.94%, for the nudibranch *Aeolidia papillosa*. Havenhand & Todd (1988a) reported somatic calorific values of 18.37 and 13.06 $J \cdot mg^{-1}$ ash free dry wt, with ash contents of 45.03% and 48.9%, for *Adalaria proxima* and *Onchidoris muricata* respectively.

Paine (1966) drew attention to the problems of endothermy in bomb calorimetry as a result of the dissociation of calcium carbonate into calcium oxide and water. Odum (1951) found the dermal spicules of nudibranchs to be composed of $CaCO_3$, therefore, bomb calorimetric determination of nudibranch tissues with a large percentage ash are likely to under estimate the true calorific value. During the present study, all calorific values for nudibranch tissue have been corrected for endothermy assuming the ash is composed of 100% $CaCO_3$. Havenhand & Todd (1988a) investigated the elemental composition of nudibranch ash by dispersive X-ray analysis (EDAX) and X-ray ion probe analysis. Havenhand & Todd (1988a) found that the Ca^{2+} content of ash to be 27-32%, and in addition, the Mg^{2+} content to be 10-13%; $MgCO_3$ also dissociates via an endothermic reaction but consumes less energy than $CaCO_3$ (1.34 $J \cdot mg^{-1}$ and 1.79 $J \cdot mg^{-1}$ respectively). Thus, the assumption that the nudibranch ash is composed of 100% $CaCO_3$ is an over estimate and the calorific values quoted in the present study will also over estimate the true value. EDAX analysis of *Archidoris pseudoargus* ash indicated that both Mg^{2+} and Ca^{2+} ions were present, with Mg^{2+} comprising approximately half the amount of the latter. EDAX is semi-quantitative and no quantitative analyses were undertaken. Consequently the ash was assumed to be all $CaCO_3$ but noting the foregoing discussion on the potential error associated with this assumption.

Carefoot (1967) recorded caloric values of 10.119 $J \cdot mg^{-1}$ for *Halichondria panicea*, which is rather higher than the value recorded in the present study

(7.313 $J \cdot mg^{-1}$), and an ash content of 50% which is 17% lower than the present study. Bloom (1981) reported an ash content of 42.55 % also for *H. panicea*. Paine (1964) drew attention to the potential source of error created by the water of hydration of the silicon salts in the sponge tissue. When material is oven-dried, this water of hydration is not fully removed and thus the oven-dry weight will be an over estimate of the true weight; water of hydration is removed when the material is ashed in a muffle furnace. Paine (1964) calculated up to a 65% underestimate of the true ash content of tissue if material has been oven-dried. In the present study, material was vacuum freeze-dried which will remove this hydrated water and thus permit more accurate estimates of the dry weight and ash content of tissue. In addition, Carefoot (1967) and Bloom (1981) recorded their values in the early summer whereas in the present study, the determinations were undertaken in the autumn. These differences could be attributed to seasonal variations in the biochemical composition of *H. panicea*; a phenomenon recorded by Barthel (1986).

3.4.2 Feeding

Feeding, or more specifically assimilation, sets the upper limit to the energy available for growth and reproduction, and is the pivotal component of an organism's energy budget. Although the feeding rates of *Archidoris pseudoargus* were not fully investigated during the present study, preliminary results indicate a number of patterns which may have some ecological significance. Considerations of feeding rates have two important aspects: the relationship between feeding rate and body size, and the overall size specific feeding rate.

Peters (1983) suggested that feeding rate should increase allometrically with body size according to the relationship:

$$\text{Feeding Rate} = a \cdot \text{body size}^{0.75}$$

where a is a fitted parameter representing the feeding rate of an individual of unit size. If this relationship is proven, an exponent less than unity will result in larger individuals consuming proportionally less food than small individuals; a phenomenon which is

generally attributed to the increased metabolic 'cost' of feeding, in simple mechanical terms, for larger organisms. For nudibranch molluscs, feeding rate will be a function of the buccal and radula size, their structure, the volume of the digestive system, and the constraints imposed by prey handling. As the predator increases in size, the size of the buccal/radula/digestive apparatus will become less of a limitation to overcoming prey handling problems, and prey handling times will decrease, or set to a lower limit. It should be noted that the simple model outlined above does not take account of these factors, prey handling times in particular. Todd & Havenhand (1989a) discussed these limiting factors and proposed a series of models to explain the feeding rate/body size relationship for *Adalaria proxima* and *Onchidoris muricata.* Using an iterative least squares regression procedure to determine the 'best fit', they concluded that feeding rate was dependent on radula size, and the surface area of the gut; the data were best explained by a rectangular hyperbola rather than a simple power relationship. Thus feeding rate will scale in an asymptotic manner with body size.

For the investigations of feeding rate undertaken during the present study, it was not the intention to follow the curve fitting procedures outlined by Todd & Havenhand (1989a), rather to gain a understanding of the basic relationship between feeding rate and body size and to calculate the rate of energy acquisition. A value of 0.902 was recorded for the exponent (of the expression of Peters (1983)) which falls within the range for invertebrates in general, but is slightly higher than values previously recorded for gastropod molluscs. Published values of the exponent for carnivorous molluscs vary between 0.37-0.78 (Bayne & Newell 1983); Havenhand (1986) recorded values of 0.329 for *Adalaria proxima* and 0.847 for *Onchidoris muricata*, and Peters (1983) noted values between 0.4-1.2 for a variety of invertebrate taxa. Perhaps of greater significance is that the exponent for feeding (*i.e.* 0.902) is slightly higher than the exponent for respiration recorded in Chapter 4 ($b = 0.881$), indicating a reduction in the unit metabolic costs of feeding for larger individuals. Therefore for large individuals, reduced metabolic costs may permit a greater allocation of energy to production, albeit somatic or gonadal.

Carefoot (1967) completed a detailed examination of the energy budget of *Archidoris pseudoargus* and recorded a size specific feeding rate of 0.076 mg · mg $^{-1}$ · day $^{-1}$ (0.085 J · J^{-1} · day $^{-1}$): in the present study, the mean feeding rate was 0.049 mg · mg $^{-1}$ · day $^{-1}$ (0.055 J · J^{-1} · day $^{-1}$). Comparative data for other sponge grazing nudibranchs are rather scarce. Data are available for other species of opisthobranch, although comparisons are difficult due to the varying form in which the rates are quoted. Paine (1965) determined that *Navanax inermis* consumed ≈ 11.5 % of its body weight (in calorific terms) per day. Carefoot (1989) recorded a feeding rate of 0.096-0.113 J · mg body wt $^{-1}$ · day $^{-1}$ for *Aplysia dactylomela* grazing on red algae. From Figure 1 of Havenhand & Todd (1988a), maximal feeding rates of *Adalaria proxima* and *Onchidoris muricata* are 0.083 and 0.14 J · mg body wt $^{-1}$ · day $^{-1}$ respectively. For predatory gastropods in general, Conover (1978) estimated that feeding rates vary between 1.5% and 44% of body size per day (which is equivalent to 0.015-0.44 mg · mg $^{-1}$ · day $^{-1}$). Values for *A. pseudoargus* are therefore at the lower end of this range.

Reported values for the assimilation efficiency of *Archidoris pseudoargus* vary from 52% (Carefoot 1967) to an approximate assimilation efficiency of 29% in the present study. Bloom (1981) analysed the feeding behaviour of *A. montereyensis* recording an 'extraction efficiency' of 93%, although these calculations take account of the non-digestible organic fraction of the sponge tissue, namely the skeletal spongin fibres. If these data are recalculated using a total organic fraction, the extraction efficiency is reduced to ≈ 80%. Published values for other opisthobranchs also vary: Paine (1965) recorded assimilation efficiencies from 22-70% for *Navanax inermis*. Todd & Havenhand (1988) recorded assimilation efficiencies of 25.3-38.8% and 19.8-32.8% for *Adalaria proxima* and *Onchidoris muricata* respectively. Published assimilation efficiencies for carnivorous gastropods vary between 38 and 95% (Bayne & Newell 1983). Carefoot (1967) extended his analysis to consider absorption of the primary dietary components (proteins & carbohydrates), and noted that *A. pseudoargus* absorbs 93% of available nitrogen (= protein) and 53% of carbohydrate. Earlier calculations were based on the

assumption that the organic content of faeces is equivalent to that of the prey material, clearly this assumption is doubtful and any estimate of assimilation efficiency, based on the percentage organic content of faeces, will only approximate the true value. Nevertheless, endogenous control of assimilation efficiency could provide a powerful means to adapt to a variable food source. Assimilation efficiency is functionally interrelated with gut capacity, the residence time for food in the gut and the ingestion rate. Changing these parameters can maximize the rate of energy acquisition in terms of short term changes in food supply. Newell & Branch (1980) proposed that species could be broadly grouped into 'exploiters' (high food abundance, low assimilation efficiency) and 'conservers' (low abundance, high efficiency). If *A. pseudoargus* is able to adapt its assimilation efficiency, perhaps by varying the differential uptake of dietary components, it may switch between these modes. Laboratory maintained animals fed to excess may adopt an exploitative strategy with time, whereas in the field, organisms may encounter a more variable food supply and, via differential absorption, adopt a 'conservationist' strategy.

As outlined earlier, Carefoot (1967) measured feeding rates of *Archidoris pseudoargus* at the beginning of the summer compared to early autumn in the present study. Seasonal variation in feeding rates have been widely observed within the Mollusca. As feeding rates are inextricably linked to the availability of prey, many of the reported seasonal variations in feeding rates have been attributed to seasonal changes in prey abundance and/or prey quality. Bayne & Newell (1983) provide a comprehensive review of feeding in the mollusca which will not be repeated in the present discussion. Gomez (1973) reported that *Tritonia festiva* changes its diet between *Ptilosarcus guerneyi* (Pennatularia) and *Lophogorgia chilensis* (Gorgonaria) depending on prey availability. Bayne & Scullard (1978) recorded a seasonally variable feeding rate in the dogwhelk *Nucella lapillus* from 3-15% of body size per day in October, to 1.5-6% in March. Many studies have attributed seasonal variations in the growth of bivalves to seasonal prey abundance. In particular, filter feeding species which rely on phytoplankton for their energy are susceptible to seasonal variation in energy supply (*e.g.* MacDonald & Thompson 1985a&b; Iglesias & Navarro 1991). During the present study, feeding rates

were not determined on a seasonal basis, and differences between the present rates and published values for *A. pseudoargus* cannot be fully explained. It should be noted that the feeding rates presented above were measured in the laboratory when food was available to excess. These rates may not extend to the field where spatial variations in the abundance of the prey material may reduce the daily food intake, or where individuals locate a very large sponge colony from which they never need move (except to locate a mate). Barthel (1986) observed a seasonal variation in the biochemical composition of *Halichondria panicea* which may affect the seasonal rate of energy acquisition for *A. pseudoargus*; this issue will be discussed later.

Mean feeding rates can be misleading, and it is the variability of feeding rates caused by a complex of exogenous (temperature, prey size and abundance) and endogenous (predator size, reproductive condition, basal metabolic rate) factors that is of interest in assessing the energetics of an individual organism (Bayne & Newell 1983). Feeding behaviour, comprising of 'search' and 'pursuit' (which includes 'handling time') (MacArthur 1972), is of central importance to the overall assimilation rate. Sponges can be ranked according to the degree of organisation of their skeletons (Bloom 1976): (i) sponges with loose packed spicules and spongin, and (ii) sponges with highly organised skeletons with spicules bound into spongin ropes. *Halichondria panicea* is in the former category. Dorids consuming this type of sponge have a radula with fine teeth, a caecum in the floor of the stomach which packs spicules into spicule-mucus 'ropes', and a narrow bore intestine which moves faecal material by ciliary means (Millot 1937; Forrest 1953). But, these caecate dorids must stop feeding periodically to allow the digestive wastes to pass through the digestive gland and be voided as faeces. Such quiescent periods form an important part of the prey handling time and reduce the overall mean feeding rate in comparison to nudibranch species which prey on organisms with a reduced inorganic skeletal structure, for instance anthozoan and hydroid 'polyps', which pose fewer prey-handling/digestive problems.

Prey-handling problems may be more significant for small juvenile *Archidoris pseudoargus* and the negative results for the two determinations involving juveniles may

in fact have some ecological significance. *Halichondria panicea* is a heavily spiculose sponge and the spicules themselves provide a handling size constraint to a potential predator: the diameter of a predators alimentary system must be sufficient to admit spicules. Juvenile dorids may not be able to consume the adult prey until their guts exceed this minimum diameter. In the interim, juveniles may graze detritus on the surface of the sponge. Todd (1991) observed similar prey size constraints to juvenile *Onchidoris bilamellata* attempting to feed on barnacles: for the first few weeks of benthic life, juvenile nudibranchs graze detritus on the barnacle test until they attain a body size which permits preying on the barnacle.

Observations from the present study suggest *Archidoris pseudoargus* feeds in a sporadic manner with these quiescent phases forming an important part of the total feeding time. Carefoot (1967) noted that irregularities in the growth pattern of this species were attributable to an interrupted feeding pattern. To overcome this 'lost' feeding time, caecate dorids must adapt their feeding to maximize food intake. It would appear that *A. pseudoargus* may select the most necessary dietary components, have a high assimilation rate (>60% Carefoot 1967) and have evolved a feeding rate/body size relationship with a reduced unit metabolic rate for large individuals. These adaptations may permit *A. pseudoargus* to maximize assimilation and to optimize the energy available to growth and reproduction.

3.4.3 Growth

Studies on the growth of *Archidoris pseudoargus* and *Cadlina laevis* in the laboratory contribute information to help elucidate the life cycle of these species. Early field investigations into the size of individuals at different times of the year led Cuénot (1903) and Renouf (1915) to conclude that *A. pseudoargus* has an annual life cycle, possibly with two generations. Miller (1958, 1962) examined the size distribution within populations around the Isle of Man and concluded that *A. pseudoargus* has a life-span of 2-2$^1/_4$ years. Thompson (1966) studied the reproductive condition of *A. pseudoargus* populations in North Wales and concluded, on the strength of the apparent absence of a

'resting' phase of gametogenesis, that this species has an annual life cycle. Todd (1977, 1981) studied the size-frequency distribution of *A. pseudoargus* from north Yorkshire and concluded that this species has a biennial life cycle. Based on data for the over-winter growth, the minimum size at spawning (Chapter 5) and the size frequency distribution of individuals collected from Robin Hood's Bay collected during the present study, it would appear to confirm that *A. pseudoargus* has a biennial life cycle. It has always been considered that nudibranchs have, at most, a biennial life cycle (Todd 1981), whereas the data collected for the growth of *Cadlina laevis* clearly demonstrates that this species has a perennial life cycle, individuals living at least four years. It would appear that this is the first record of a long-lived nudibranch species and one which demonstrates *iteroparity*. That is not to say it is unique, just that data for other iteroparous species are lacking. Todd (1981) observed that all the largest British Doridoidea for which data are available are sponge grazers although precise details of the preferred prey-species are lacking for many of these species (see Todd 1981: Table 1). These large species are very rare, possibly due to a restricted dietary preference for rare sponge species (Todd 1981), and data are not available for their growth patterns or their life cycle. Data are available for *Jorunna tomentosa,* a specialist grazer on *Halichondria panicea*, which indicate that this species also has a biennial life cycle (Todd 1981). Biennial or perennial life cycles may be common amongst these large, sponge grazing dorids; further investigations are necessary to elucidate these ideas.

Previous studies into the growth patterns of nudibranch molluscs have concentrated on annual species. During the present study, the maximum SSGR for *Archidoris pseudoargus* was 0.01 ± 0.05 $\cdot day^{-1}$, recorded for the period immediately pre-spawning in March-April. For *Cadlina laevis*, maximum adult SSGR was 0.087 ± 0.0023 $\cdot day^{-1}$, recorded for February. For juveniles *J1-7,* the maximum SSGR was 0.0429 ± 0.0028 $\cdot day^{-1}$, recorded for February-March; while for *J8-10* the maximum SSGR was 0.0477 ± 0.0124 $\cdot day^{-1}$, recorded for December, although a rate of 0.0396 ± 0.0178 $\cdot day^{-1}$ was recorded for the following February-March. For these sponge grazing dorids, maximal growth occurs in late winter/spring. Recorded values for the SSGR of other opisthobranch are: 0.013-0.034 $\cdot day^{-1}$ for *Navanax*

inermis during December-April (Paine 1965); 0.025 · day $^{-1}$ for *Aplysia punctata*, 0.033 · day $^{-1}$ *A. pseudoargus*, 0.002 · day $^{-1}$ for *Dendronotus frondosus*, all for the period June to September (Carefoot 1967); 0.016 · day $^{-1}$ for *Onchidoris aspera* during October-December (Smith & Sebens 1983); 0.005-0.415 · day $^{-1}$ for *Aeolidia papillosa* during October-June (Hall & Todd 1986); ≈0.038 and ≈0.042 · day $^{-1}$ for *Adalaria proxima* and *Onchidoris muricata* respectively for August (Havenhand & Todd 1988a). Thus the values recorded during the present study are equivalent to those recorded for other nudibranch species. In general, temperate nudibranchs spawn in the spring/early summer resulting in juveniles recruiting in mid/late summer. These juveniles have to undergo rapid growth to gain adult size by the following spring; that is, most growth occurs during the autumn and winter periods (Hall & Todd 1986; Havenhand & Todd 1988a).

Growth patterns of *Archidoris pseudoargus* in the present study showed a marked departure from such a typical 'annual pattern'. Overall, growth during the winter was negative, resulting in a slow decline in body size, followed by a spring increase for spawning individuals. This trend was recorded for both adults and, perhaps more surprisingly in view of past studies (see above), for juveniles. Maximal growth must therefore occur during the summer periods when the juvenile first metamorphoses and then in its second summer. Carefoot (1967) recorded rapid growth in *A. pseudoargus* during the summer period: a 100 mg (dry weight equivalent) individual increased to >2000 mg dry weight over a period of 75 days at a temperature of 15 °C (mean summer temperature). Using the data for respiration from Chapter 4, and the feeding data presented above (including data from Carefoot 1967), it is possible to calculate the scope for growth in the summer and winter: in the summer *A. pseudoargus* has a positive scope for growth of 0.857 J · mg $^{-1}$ · day $^{-1}$; in autumn, it has a negative scope for growth of -0.0142 J · mg $^{-1}$ · day $^{-1}$. From these observations, it would appear that the growth pattern of *A. pseudoargus* is the reverse of that observed for most other temperate annual nudibranch molluscs, *i.e.* maximal growth occurs during the summer period. An inability to maintain positive growth in the autumn and winter will result in a small body size the following spring. Fecundity is positively related to body size, and the body size attained

by individuals in their first spring may be insufficient to ensure successful reproduction. Growth is maximal for the summer period to attain a body size sufficient to survive over the winter and undergo successful reproduction the following spring.

Why does *Archidoris pseudoargus* depart from this more typical annual life cycle when its prey, *Halichondria panicea*, appears to be available throughout the year? It is possible that the cause may be attributable to a seasonal variation in prey value rather than prey abundance. Barthel (1986) published the results of a detailed investigation into the seasonal growth of *H. panicea* in the Western Baltic. Data showed that this sponge has markedly seasonal growth and seasonal variations in its biochemical composition: sponge growth was rapid in the spring and early summer preceding spawning. Post-spawning, adults degenerated in the autumn and winter. Values for a 'condition index' (simply the ratio of organic: inorganic body fractions) mirrors this seasonal pattern, where the proportion of organic material in the sponge declines in autumn and over the winter. Spring growth of the sponge is achieved via protein metabolism: the protein content increases rapidly in the spring. Barthel (1986) found seasonal growth of the sponge to be strongly dependent on seasonal temperature changes and less correlated to seasonal food availability. If this phenomena is common throughout the geographical range of this sponge, *A. pseudoargus* would be subject to seasonal variations in its food quality. Carefoot (1967) observed that *A. pseudoargus* selectively absorb protein from its prey which, in view of the sponge biochemistry, may be an adaptation to maximize energy intake. Juvenile *A. pseudoargus* will be faced with declining prey quality in autumn and during the winter which may explain their apparent lack of growth during the winter period. In the following spring when the prey quality increases, adults are able to assimilate energy (= protein) to increase body size immediately prior to spawning, and juveniles can begin their primary growth phase. Thus, the extended life cycle of *A. pseudoargus* may be attributable to seasonal variations in prey quality.

Cadlina laevis has been shown to have an extended life cycle over periods up to five years with marked seasonal growth patterns. Juveniles appear to maintain a slow linear growth pattern during their first autumn and early winter, followed by a rapid increase

during the following spring. Positive growth is maintained until mid-summer, followed by a decline into early autumn. An early winter burst precedes the first spawning season at an age of approximately 22 months. A similar seasonal pattern was observed in adults: a rapid increase in growth in spring, a summer decline, an autumn burst prior to spawning, and an decline over the winter. Body size declines post-spawning until the following spring. SSGRs are negative other than during the short spring/autumn bursts of growth, suggesting that body reserves are employed to maintain basal metabolism to slow the decline in body size. Body reserves could be replaced during the spring and late summer bursts: a spring burst would support gametogenesis and summer growth, and the autumn burst would provide sufficient body reserves to survive over the winter.

Seasonal growth is the result of complex interactions among temperature, food, reproductive activity and energy balance, and therefore it is difficult to fully elucidate the precise reasons for the seasonal patterns observed in *Archidoris pseudoargus* and *Cadlina laevis,* although seasonal variations in food supply may be a major factor. Seasonal growth is common in the marine environment and is generally attributed to fluctuations in food availability and temperature; Elvin & Gonor (1979) considered that food level explained 96% of the seasonal variance in the scope for growth in the Pacific mussel *Mytilus californianus*. Seasonal growth patterns are well documented for the Mollusca, albeit mainly for bivalves (*e.g.* Vahl 1981; MacDonald & Thompson 1985a&b; Iglesias & Navarro 1991; for review: Bayne & Newell 1983). For carnivorous species, the problem of fluctuating food quality is particularly relevant where the energetic costs of search and pursuit may be high relative to the energetic value of the meal *i.e.* for prey of low profitability. For spiculate sponges, fluctuations in the prey value combined with the requirement to feed sporadically due to prey handling problems has important implications on the scope for growth and the time available for growth.

Feeding and growth are rate processes which will vary with changes to the ambient temperature. Seasonal changes in ambient temperature will therefore influence both feeding and growth rates. Perhaps more importantly, it is the influence of temperature on the basal metabolic rate which ultimately sets the upper limit for the energy available for

growth. For carnivorous species, confronted with a non-continuous food supply, changing the ingestion rate may not be viable and therefore metabolic compensation, and/or acclimatization, may be vital to increasing the energy supply to production mechanisms during the summer period. *Archidoris pseudoargus* and *Cadlina laevis* have lower size specific respiration rates than annual species (see Chapter 4), and demonstrate some adaptation of respiration rates to changes in ambient temperature (acclimatization is more pronounced in *C. laevis*). If these laboratory-based observations extend to the natural environment, longer lived nudibranchs may be adapted to achieve a high scope for growth during the summer period when food supplies appear maximal.

Finally, seasonal changes in the food quality, and/or its energy content, may similarly influence growth rates. Carefoot (1967) observed that *Archidoris pseudoargus* has a higher assimilation efficiency for protein than carbohydrate, and proteins are used directly for growth which may be an adaptation to seasonal variations in prey quality. Sundet & Vahl (1981) found that during the summer, immature Iceland scallops *Chlamys islandica* invest in rapid growth via protein absorption rather than laying down carbohydrate reserves to overcome energy shortages during the winter. Bayne & Newell (1983), whist concluding that, amongst molluscs, the role of protein metabolism as an energy substrate during starvation requires further research, provided a number of examples where energy demands during the winter were met by protein catabolism; in larger individuals of *Mytilus edulis,* protein catabolism provided 75% of the energy demand during the winter period (Gabbott & Bayne 1973). Selective absorption for protein may be an adaptation to increase growth rate and hence body size, whilst also providing an energy reserve both for *A. pseudoargus* and *C. laevis*; larger individuals are metabolically more efficient and will therefore have a lower basal energy requirement during the winter period. The gradual decline in body size during winter for these nudibranch species may represent the use of body reserves for maintenance metabolism, albeit at a reduced rate.

Todd (1983) discussed the potential impact of prey stability/availability on the duration of nudibranch life cycles. He concluded that, where a prey organism is

temporally and spatially stable and presents no prey size constraints to a juvenile, an annual or biennial life cycle will be supported. Expressed most crudely and simplistically, selection will favour prolongation from the simple annual life cycle if the consequent increase in (size/age-specific) fecundity over compensates the decreased (size/age-specific) survivorship. Where a prey is unavailable for a short period or exerts prey-size constraints on settling veligers, an annual life cycle is most plausible or an extended life cycle if the year-old nudibranchs can survive a period without a supply of prey. In principle, Todd (1983) was proposing that it was the year-round availability of the prey which led to selection for the longer life cycle. From the data collected during the present study, it would appear that prey seasonality (*i.e.* temporally unavailable) may be the selective force which led to the prolongation of the life cycle for *Archidoris pseudoargus* and *Cadlina laevis*.

Halichondria panicea imposes considerable prey-size constraints on newly hatched veligers of *Archidoris pseudoargus* (and *Jorunna tomentosa*?). Similarly newly hatched veligers of *Tritonia hombergi* (also a biennial) feeding on the soft coral *Alcyonium digitatum*, are unable to bite into the prey and appear to graze the surface detritus for some months (C Todd pers. comm.; pers. obs.). As a consequence of these prey size constraints, juvenile nudibranchs are unable to feed on the adult prey and may therefore have a reduced energy input during early life. When these juveniles attain a size capable of consuming the adult prey, if the prey quality has seasonal variation (with a winter decline), their energy supply will remain low. A low energy supply over the winter will result in a small body size the following season which, depending on the specific body size/fecundity relationship, may be below the threshold necessary to produce sufficient gametes for successful reproduction. Increasing prey quality during the following summer, combined with a favourable differential interaction of age-specific fecundity and age-specific survivorship, may provide the selective force to prolong the life cycle to the following year. One method to overcome periods of low resource availability is to reduce the energy 'lost' to basal metabolism: both *A. pseudoargus* and *Cadlina laevis* have low (relative to annual species) basal metabolic rates (see Chapter 4).

Todd's (1983) hypothesis of the conditions that will favour prolongation of a life cycle - namely a temporally and spatially stable prey which imposes no size constraints to newly hatched veligers - may not explain the biennial life cycle of *Archidoris pseudoargus* or *Tritonia hombergi*. It would appear from the preceding discussion, that the contrary - namely seasonally variable prey which imposes considerable prey handling constraints to newly hatched veligers - may explain biennial, and possible perennial, life cycles of nudibranch molluscs.

CHAPTER 4

RESPIRATION

4.1 Introduction

Physiological processes which ensure the continued survival of living organisms require energy, usually derived from external resources. Animals satisfy their energy requirements by the production of ATP from the catabolism of assimilated food material. ATP production is maximal when this catabolism follows an aerobic pathway utilising oxygen, although anaerobic pathways are important when oxygen is not available. The uptake of oxygen for these oxidative processes and the subsequent release of waste end products, usually carbon dioxide, is termed respiration (Schmidt-Nielson 1979). A requirement for oxygen to fuel the basic metabolic processes appears to be universal in multicellular organisms although the contribution of anaerobic metabolism is largely unknown. Respiration may be separated into a two stage process: the exchange of gaseous products with the external environment and internal biochemical processes.

4.1.1 Gaseous exchange

Aquatic organisms must obtain oxygen from, and release carbon dioxide into, the surrounding water. Solubility of gases in seawater is dependent on many factors although temperature is the principal determinant factor. Oxygen solubility is markedly dependent on temperature and saturated seawater at 0 °C contains 1.6 times as much oxygen as seawater at 20 °C. Absorption and release of gases relies on the process of diffusion. Small organisms are able to absorb sufficient oxygen directly through the skin but, as the size of an organism increases, its surface area in relation to its volume decreases and surface diffusion of oxygen becomes insufficient to meet metabolic requirements. Most aquatic animals have evolved specialised extensions of the body, or gills, to increase the surface area to satisfy these higher gaseous requirements of larger body sizes. The rate of diffusion is proportional to the concentration gradient and, to maintain a high concentration gradient across the surface of gills, water is constantly moved across the gills. This movement is effected by a plethora of mechanisms ranging from passive flow

to active pumping using body muscles. Many marine invertebrates employ cilia to create a respiratory water flow.

Molluscan gills are termed ctenidia and are extensions of the mantle epithelium. Opisthobranchs lose these ctenidia during the ontogenetic process of detorsion. Nudibranch molluscs have a large mantle area which is used for gaseous exchange (*e.g.* Hancock & Embleton 1852; Graham 1957; Thompson 1976; Potts 1983). Surface area to volume constraints have led to most dorid nudibranchs evolving secondary pinnate gills as outgrowths from the dorsal mantle surface. These secondary gills are generally located as a circlet around the anus. In addition, complex blood vascular systems have evolved to carry respiratory gases around the body. Potts (1981) investigated the anatomy of the gills and vascular system of *Archidoris pseudoargus* and *Onchidoris bilamellata*. *A. pseudoargus* has complex cilliated, tri-pinnate gills which are contractile and retractile into a deep branchial pocket surrounding the anus. *A. pseudoargus* has a complex blood vascular system which shows modifications to permit blood to return directly to the heart rather than to the gills for oxygenation. Such complex mantle vascularisation may be an adaptation to increase gaseous exchange when the gills are contracted into the branchial cavity, for instance during tidal emersion.

Anatomical or histological studies of the vascular system were not undertaken for those nudibranch species currently under investigation. Current investigations concentrated on the adaptive ecological significance of the metabolic respiratory process and the foregoing text aims to provide a general introduction to the gaseous exchange part of the respiratory process.

4.1.2 Biochemical respiratory processes

Mechanisms which generate ATP are surprisingly uniform throughout the animal kingdom (Sibly & Calow 1986). In the absence of oxygen, glucose molecules pass along a glycolytic pathway which generates two ATP molecules. Invertebrates that experience long term or continuous anoxia, use alternatives to this glycolytic pathway which increase the ATP per glucose-unit input from 2 to between 4 and 6. In the presence of oxygen, the

glycolytic pathway leads directly into the tricarboxylic acid (TCA) cycle which generates reduced nicotinamide adenine dinucleotide (NAD_r). NAD_r is then oxidised to generate ATP via an electron transport system. Aerobic oxidation of glucose releases 36 ATP molecules per glucose-unit input, and hence represents a significant improvement in respiratory efficiency over anaerobic mechanisms.

Clarke (1991) defined respiration as "simply a measure of instantaneous demand for ATP" and it therefore represents a cost to an organism, not a benefit. Most of the energy allocated to respiration is ultimately dissipated as heat. This concept of respiratory loss is an important consideration when attempting to elucidate any evolutionary adaptation to fluctuating or strongly seasonal environmental conditions. Energy released from metabolic activities may be partitioned into that required for the main life history processes of feeding, growth and reproduction and the energy required to keep the organism alive; metabolic processes keeping an organism alive are termed the *basal metabolism*. Basal metabolism is defined as the sum of those activities which maintain the internal environment of an organism in the face of external environmental constraints (Clarke 1980). Adjustment and adaptation of basal metabolic processes to meet environmental constraints is central to an organism's fitness. Assuming resources are finite at ambient environmental conditions, an estimate of the minimal energy requirement to sustain life sets a base to determine the energy available to production (somatic and gametic). Measurement of the rate of these metabolic processes is an important aspect of any study investigating the evolution of components of the life history and life cycle of a species.

Basal metabolism is usually estimated by measuring the basal oxygen consumption although the latter includes the oxygen consumption associated with the processes of digestion, feeding, movement, growth and gametogenesis. It is impossible to exclude all these processes and therefore intractable to measure basal metabolism *per se*. Measuring the oxygen consumption of a quiescent organism in a respiration chamber can minimize the contribution to the total oxygen consumption from the processes of activity, feeding and digestion, but will nonetheless also include an instantaneous measure of growth and

gametogenesis. Methods which follow a standard procedure to minimize the contribution of these processes measure the *standard metabolic rate* (Peters 1983). Absolute values of total oxygen consumption measured in respiration chambers tend to vary between species, reducing the validity of interspecific comparisons, although intraspecific measurements provide a good approximation of the standard metabolic rate and are generally comparable (Clarke 1983). To overcome these problems, regression analysis of oxygen consumption against body size is used to determine the standard metabolic rate of an organism of unit size. These values are reliable and can be used for interspecific comparisons (Peters 1983). Rates of oxygen consumption are readily determined in the laboratory and thus, in the present study, the rate of oxygen consumption was chosen as a measure of metabolic costs.

Biochemical respiratory mechanisms are rate reactions which are influenced by a wide variety of external environmental factors (mainly temperature, salinity, humidity and the partial pressure of oxygen), endogenous factors (such as body size, activity, gametogenic status and sex), and by interaction between these and time-dependent variables such as season. When ranked in order of their relative effect on the oxygen consumption of an organism, body size and exposure temperature are the most important variables (Newell & Roy 1973; Widdows 1978; Bayne & Newell 1983). In view of the importance of respiration in the life cycle of an organism, studies of the respiration of marine invertebrates have been widespread. Within the phylum Mollusca, there is a large volume of literature pertaining to this subject although the majority of studies have investigated the class Pelecypoda (= Bivalvia) (for review see Bayne & Newell 1983). Studies generally can be divided into those which have investigated the influence of environmental and endogenous factors on the respiration rate, and those which have measured respiration as part of a study to determine the energy budget of an organism. An exhaustive review of the literature pertaining to respiration would form a considerable text on its own and is impossible within the limits of the current study. Consequently, attention is drawn to some major review articles with only a small number of additional references quoted.

Investigations of environmental and endogenous influences on respiration have concentrated on the influence of temperature, predominantly in relation to metabolic cold adaptation in polar organisms (for review: Clarke 1980, 1983, 1987a, 1991). Classically, changes in oxygen consumption with temperature have been interpreted in the context of an organism's ability to compensate for changes in temperature. Clarke (1991) challenges this view and emphasizes the need to consider respiration as a cellular process involving the generation and utilization of ATP, which is itself linked via a feedback mechanism to oxygen consumption. Brown *et al.* (1978) and Brown & Da Silva (1984) attributed changes in the rate of oxygen consumption to changes in temperature to explain the geographic distribution of two species of the whelk *Bullia*. Iglesias & Navarro (1991) integrated the rate of oxygen consumption of the bivalve *Cerastoderma edule* with measurements of body size, temperature and reproductive condition. The regression exponent *b* increased from 0.646 to 0.746 when individuals became sexually active. Carefoot (1987) investigated the influence of diet on the oxygen consumption of the opisthobranch *Aplysia dactylomela*. A 150-200% increase in oxygen consumption above a baseline level was recorded when food was presented to *Aplysia*. Specific dynamic action effects caused oxygen consumption to increase by up to 300% which persisted for up to 14 hours. Carefoot (1989) observed a decline in oxygen consumption during copulation of *Aplysia*, although this decline was not statistically significant.

Intertidal organisms are subject to wider extremes of environmental variation than those organisms which are subject to the more consistent subtidal environment. In particular, air exposure can result in desiccation of the respiratory surfaces which will reduce the diffusion of oxygen. Interruptions to the oxygen supply will reduce aerobic respiration and therefore lower respiratory efficiency. Widdows & Shick (1985) noted that air exposure causes a marked reduction in the scope for growth and the growth efficiency of *Cerastoderma edule*, due to a high rate of energy expenditure during periods of tidal emersion. Horn (1986) observed that aerial respiration accounted for 11.6% and 41.1% of the annual energy loss of low and high shore chitons respectively. Shell-less

opisthobranchs will be subject to increased desiccation and hence to potentially greater interruptions to oxygen supply.

Studies on the oxygen consumption of opisthobranchs are not so widespread and generally form part of a study of energy budgeting. Paine (1965) studied the energetics of *Navanax inermis*; Carefoot (1987, 1989) evaluated the energy budgets of *Aplysia*; Havenhand & Todd (1988a) investigated the energy budgeting of *Adalaria proxima* and *Onchidoris muricata*, and provided a discussion of the life history implications of the relationship between oxygen consumption and body size and temperature. Carefoot (1967) calculated respiration rates of *Archidoris pseudoargus* as part of a study of the energetics of this species. Potts (1983) measured the oxygen consumption of *A. pseudoargus* and *Onchidoris bilamellata*, and ligatured the branchial pocket to determine the oxygen uptake via the body surface rather than the gills. Smith & Sebens (1981) considered the relationship between respiration rate and temperature during a study of growth and reproduction of *Onchidoris aspera* (see below).

Fitness is dependent on body size which itself has been shown to have a marked influence on oxygen consumption: increasing body size increases oxygen consumption (Bayne & Newell 1983). Therefore, any adaptation of respiration rate to reduce the impact of increasing in body size could have a profound influence on the fitness of an organsim. *Archidoris pseudoargus* is one of the largest British nudibranchs; *Cadlina laevis*, whilst considerably smaller than *Archidoris pseudoargus*, can nevertheless attain a modest body size. It is therefore appropriate to investigate the relationship, if any, between body size and metabolic rate (= oxygen consumption). Any adaptation of the oxygen consumption in relation to body size may affect other life cycle and life history parameters of these species.

Adult individuals of these species are present throughout the year and are therefore exposed to the annual range (approximately 4-17 °C) of ambient temperatures. This is in marked contrast to annual species with a pelagic larval phase where adults will be exposed to only a limited (seasonal) range of temperatures. Smith & Sebens (1983) investigated the response of individual *Onchidoris aspera,* acclimated to a temperature of

8 °C, to acute changes in ambient temperature covering the range 0 to 25 °C. Temperatures >15 °C exceeded the physiological upper thermal limit of this species. *O. aspera* is an annual species and Smith & Sebens (1983) concluded that temperature plays a large role in delimiting the life cycle of this species: summer temperatures were >20 °C which reduce the possibility of adult individuals persisting throughout the summer months and thereby extending their life cycle. For annual species, it is likely that there will be a lesser selective pressure for adaptation to varying environmental temperature. For an organism to extend its life cycle, it must be able to withstand the full annual range of ambient temperatures. Determining the relationship between temperature and metabolic rate for these longer-lived nudibranch species was considered an important additional aim to the study.

Within the normal temperature range that an animal can physiologically tolerate, the rate of oxygen consumption generally increases with increasing temperature. In general, a rise of 10 °C might be expected to result in a two to three fold rise in the rate of oxygen consumption. A measure of the change in respiration rate with a 10 °C rise in temperature is the Q_{10} (Schmidt-Nielson 1979). Although Johnston *et al.* (1974) consider that there is no rational basis for the use of Q_{10} in biology or medicine, Q_{10}'s do provide a descriptive statistic to aid the interpretation of changes in the magnitude of rate reactions (oxygen consumption in the present case). Q_{10} values were determined for both *Archidoris pseudoargus* and *Cadlina laevis* and compared with the annual species *Adalaria proxima.*

4.2 Materials and methods

Measurement of the respiration rate of nudibranchs was determined in saturated seawater with the animals constrained within an appropriately small, closed respiratory chamber.

4.2.1 Apparatus

Several methods exist for measuring the rate of oxygen consumption of an organism immersed in water. These methods are described by Hitchman (1978) and

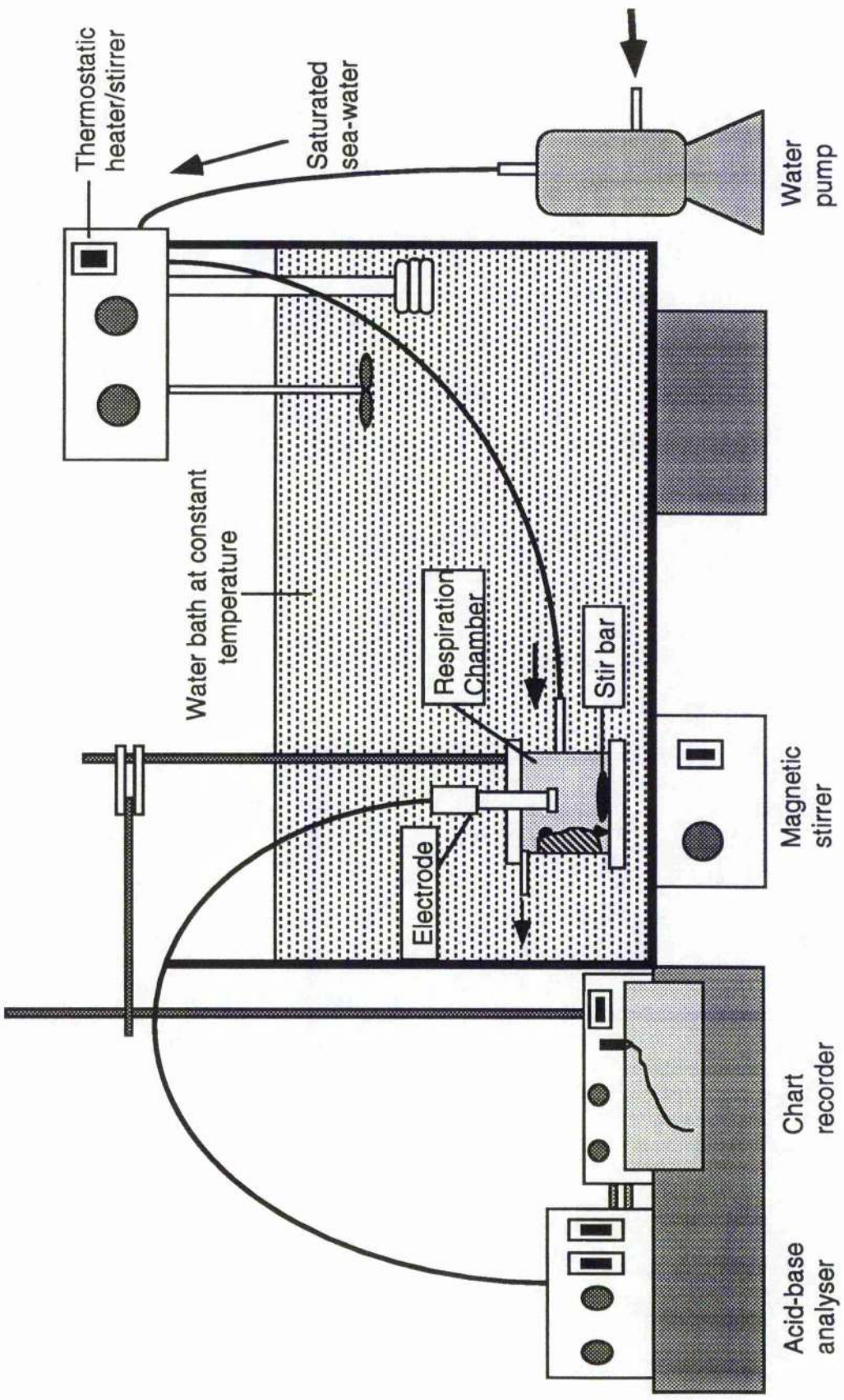
Crisp (1984), and reviewed by Gnaiger & Forster (1983). For the present study, a polarographic method using membrane polarising oxygen detectors, or 'oxygen electrodes', was chosen for two main reasons. Firstly, the method is straightforward and provides reliable, consistent results; and secondly, the necessary apparatus and experience of the methodology were already well established in the laboratory (Havenhand 1986).

All measurements were made using a Radiometer™ PHM 71 Mk II amplifier fitted with a pO_2 module and a Radiometer E5406 oxygen electrode; output recordings were made on a Vitatron 2001 series flat-bed pen recorder. All determinations were made in a constant temperature room maintained at ambient seawater temperature. To correct for minor changes in air temperature within the room, the electrode and respiration chamber were immersed in a water bath maintained at constant temperature (± 0.5°C) independent of the room, using a combined Grant™ thermostatic heater/stirrer and flow cooler. A diagram of the apparatus is shown in Figure 4.1.

All determinations were carried out in a sealed respiratory chamber fitted with an electrode, a seawater inflow and a capillary outflow. The inflow and outflow permitted water to be pumped through the chamber prior to an experimental determination and during assembly, which avoided the introduction of air bubbles to the chamber. Pumping continued during the initial stabilisation which avoided the premature depletion of oxygen by the animal. Havenhand (1986) investigated the oxygen diffusion through the inflow and outflow and concluded that it was negligible in relation to the changes resulting from the animal's oxygen consumption. Therefore the chamber could effectively be closed by stopping the water flow.

To reduce measurement errors resulting from oxygen consumption by any micro-organisms present in seawater, all seawater used in respiration experiments was filtered through a 0.22 µm Flow Laboratories™ filter to remove micro-organisms. Concentrations of oxygen in seawater are strongly dependent on temperature and may vary considerably with minor (<1 °C) temperature fluctuations, especially at low temperatures. Errors due to the change in oxygen concentration with temperature were

Figure 4.1 Apparatus to measure the oxygen consumption of nudibranchs. The animal is constrained within a respiration chamber fitted with a polarographic oxygen electrode, a inflow and outflow and a magnetic stir bar. The whole assembly is immersed in a water bath maintained at constant temperature (± 0.5 °C). Changes in the oxygen concentration within the respiration chamber are recorded by an acid-base analyser and displayed via a chart recorder.


Thermostatic heater/stirrer
Saturated sea-water
Water pump
Water bath at constant temperature
Respiration Chamber
Stir bar
Electrode
Magnetic stirrer
Chart recorder
Acid-base analyser

reduced by storing carboys of filtered seawater within the constant temperature room. Air (filtered through a 0.22 μm Millipore™ filter) was constantly bubbled through the seawater to maintain oxygen saturation.

4.2.2 Methods

At the beginning and the end of each day, the apparatus was calibrated by determining a zero pO_2 value from a sodium sulphite/sodium tetraborate solution (Radiometer Product N° S4156:'zero solution'), and a saturation value from air-saturated seawater at ambient temperature. Polarographic electrodes consume oxygen during the measurement process. Therefore during each measurement of saturation, seawater was constantly pumped through the chamber at an adjustable flow rate between 0.5 and 3.0 ml · min^{-1} to ensure rapid replacement of the oxygen consumed by the electrode. Oxygen content of the seawater, corrected for chlorinity, temperature and barometric pressure (recorded at the beginning and end of each day), was calculated following the methods of Hitchman (1978).

Prior to each determination involving a nudibranch, the respiratory chamber and electrode assembly were sterilized with 10% (v/v) 'Milton' solution, rinsed thoroughly with filtered seawater (FSW) and filled with saturated FSW. An animal and a magnetic stir bar were placed in the chamber and the chamber sealed with the electrode assembly, while at all times avoiding the introduction of any air bubbles. The pump was started to ensure that the internal pressure of the chamber was slightly greater than the external pressure to avoid air or freshwater from the water bath being sucked into the sealed chamber. The whole assembly was placed in the water bath above the magnetic stirrer. To allow the system to stabilise, FSW was pumped through the chamber for approximately five minutes and then the inflow clamped and the pump turned off to seal the chamber and start the determination. A determination was terminated when a smooth decline was recorded on the chart trace, but at no time was the oxygen content of the chamber allowed to fall below 70% of saturation; below 60% of saturation, an animal is likely to become stressed and its oxygen consumption will be altered (Crisp 1984). At the end of the

determination, the electrode assembly was carefully removed and the volume of water in the chamber accurately measured to the nearest 5 µl with an 'Alga' micrometer syringe. After each determination the animal was weighed (under water) to permit the calculation of a size specific respiration rate.

To correct for the oxygen consumption of the electrode and of micro-organisms adhering to the animal's mucus, the chamber was refilled without cleaning and a blank determination obtained. Blank determinations were recorded for the first and every alternate animal during each day, and the adjacent 'blank' oxygen consumption subtracted from the experimental animal's oxygen consumption.

For *Archidoris pseudoargus* and *Cadlina laevis*, studies were undertaken to investigate how the respiration rate varies with body size and temperature. Animals were chosen to embrace the normal range of sizes present throughout the life cycle of each species. Determinations were undertaken throughout the year at ambient temperature.

Data were fitted to geometric mean regression models to explain the relationship between body size and respiration rate at each temperature; data were pooled and a model fitted to the complete data set. Multiple regression analysis was undertaken on the pooled data to determine the influence of temperature in addition to body size. Respiration rates were converted to size specific respiration rates by dividing the measured oxygen consumption by the body size (dry weight). Measurements of body size were converted to Joule equivalents using the conversions in Chapter 3; oxygen consumption was converted to Joule equivalent heat loss using a conversion of 13.66 $J \cdot mg\ O_2^{-1}$ (Elliot & Davidson 1975) where 1 mg O_2 by weight = 0.7 ml O_2 at normal temperature and pressure (Crisp 1984).

4.2.3 Q_{10} determinations

Q_{10} values were calculated using the equation proposed by Schmidt-Nielson (1979):

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\frac{10}{T_2 - T_1}}$$

where: T = temperature and R = size specific respiration rate.

Mean (± 1 se) size specific respiration rates were calculated at each temperature for both species (from the data collected during the body size/O_2 investigation at different temperatures). These values were substituted into the above equation to calculate Q_{10}'s to investigate how each species adapts to changes in ambient temperature. These mean data would permit interspecific comparisons within the present study, and with data published from previous studies.

In addition to the determinations outlined above, respiration rates were measured at 2, 6 & 10 °C for three individuals of *Adalaria proxima*. Three determinations were undertaken, on successive days, at each temperature; individuals were acclimated to the experimental temperature for five days prior to measurement of their respiration rate. These data would permit a comparison of the relative ability of annual and biennial/perennial species to adapt to changes in temperature.

4.3 Results

Data for each species will be presented separately followed by interspecific comparisons of the relationship between body size and respiration rate, and Q_{10} values.

4.4.1 *Archidoris pseudoargus*

A total of 114 respiration rate determinations were made for *Archidoris pseudoargus* with a mean size specific respiration rate (±1 se) of

0.195 ± 0.018 µl $O_2 \cdot$ mg dry weight $^{-1} \cdot h^{-1}$. A 'best fit' geometric mean (GM) regression model was obtained when the data were transformed to natural logarithms ($\log_e$), and the data explained by the expression:

$$\text{Log}_e \text{ Respiration rate} = 0.881 \cdot \log_e \text{ body size} - 1.128$$

$$F = 236.73^{***}; r = 0.82; n = 114.$$

If this expression is converted to base 10, it becomes

$$\text{Respiration rate} = 0.324 \cdot \text{body size}^{0.881}$$

When the data are converted to joule equivalents (Chapters 2 & 3), the expressions become:

$$\text{Log}_e \text{ Rate of energy loss} = 0.881 \cdot \log_e \text{ J body size equivalent} - 7.223$$

or $$\text{Rate of energy loss} = 0.00073 \cdot \text{J body size equivalent}^{0.881}$$

Figure 4.2 shows the relationship between respiration rate (as rate of energy loss) and body size (energy equivalent).

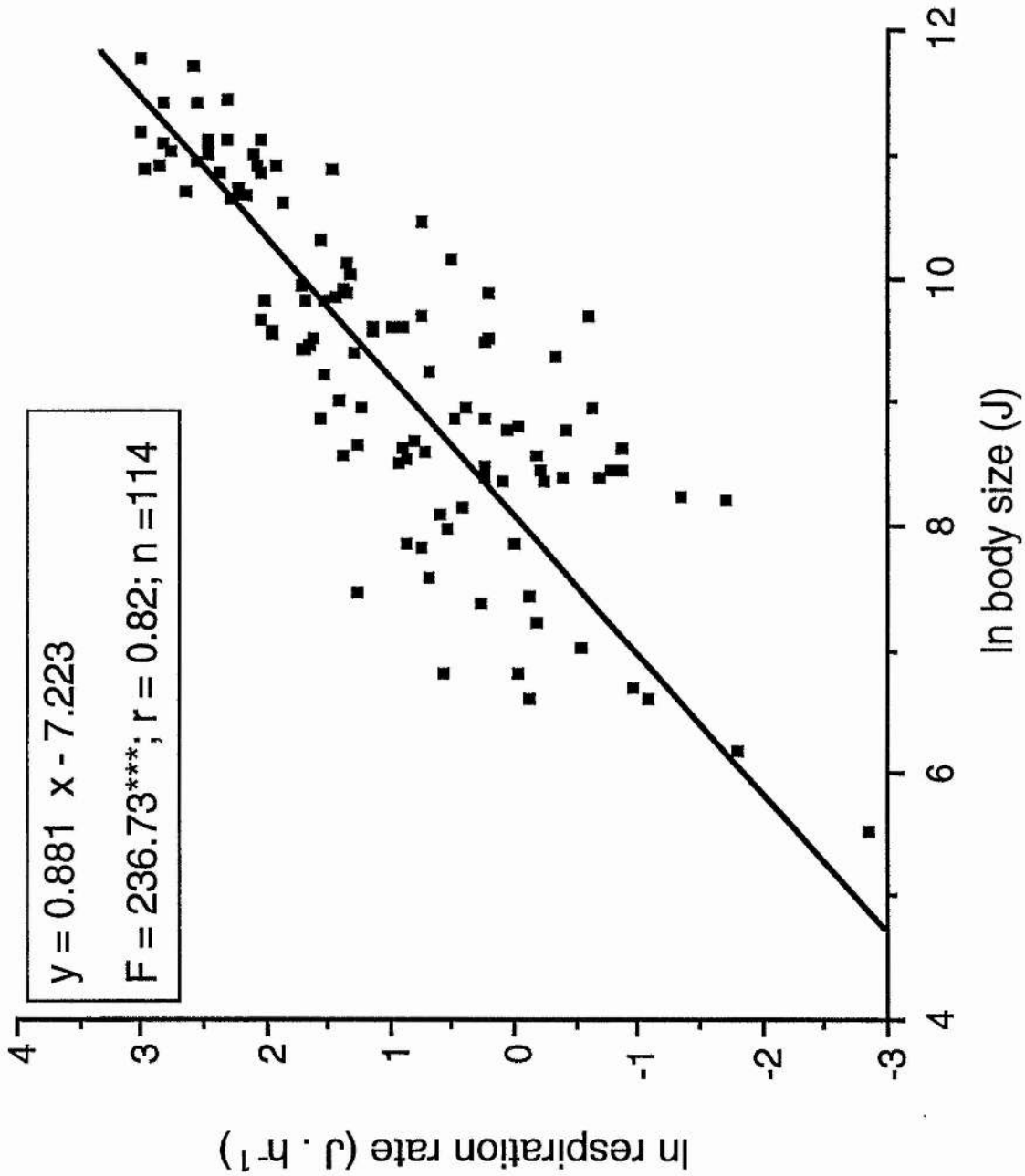
Determinations were undertaken over an ambient temperature range of 5-14.5 °C. In order to assess whether temperature has an influence on the rate of oxygen consumption, multiple regression analysis was undertaken on the pooled data incorporating temperature as an independent variable:

$$\text{Log}_e \text{ Respiration rate} = 0.188 \text{ temperature} + 0.836 \cdot \log_e \text{ body size} - 2.676$$

$$F = 237.88^{***}; r = 0.90; n = 114.$$

To determine the relative magnitudes of changes in body size or temperature on respiration rate, standard partial regression coefficients (b') were calculated (Sokal & Rohlf 1981). For body size, $b' = 0.949$ and for temperature $b' = 0.383$ and therefore changes in body size will have the greater relative effect on respiration rates. Nevertheless, changes in temperature will have a significant effect on respiration rate. It should be noted that the multiple regression analysis follows a model I regression procedure and therefore assumes that the independent variables are measured without

Figure 4.2 The relationship between respiration rate (as heat loss: joules per hour) and body size (as energy equivalent) for *Archidoris pseudoargus*. The line represents the 'best-fit' geometric mean regression when the data are transformed to natural logarithms. A total of 114 determinations were made at ambient temperature which ranged from 5 to 14.5 °C. Individuals used for this experiment spanned a size range of $2.49 \cdot 10^2$ to $13.08 \cdot 10^3$ J ($2.16 \cdot 10^1$ to $11.26 \cdot 10^3$ mg dry weight); respiration rates ranged from 0.06 to 20.31 $J \cdot h^{-1}$ (2.97 to $10.41 \cdot 10^2$ µl $O_2 \cdot h^{-1}$).


y = 0.881 x - 7.223
F = 236.73***; r = 0.82; n =114
ln respiration rate (J . h⁻¹)
ln body size (J)
4
3
2
1
0
-1
-2
-3
4
6
8
10
12

error. Measurement of temperature will satisfy this criteria although measurement of body size will not, and therefore the regression model is partially violated. Consequently no firm conclusions can be drawn from these analyses, although clearly the influence of temperature on respiration rate cannot be discounted. The validity of fitting a single model to the pooled respirometric determinations at different ambient temperatures is questionable and required further investigation.

A visual comparison of the relationship between respiration rate and body size at the different ambient temperatures (Figure 4.3 & Table 4.1) suggests that these relationships may be slightly different. At 5 °C no significant relationship was found between respiration rate and body size although the number of determinations is probably inadequate to resolve the relationship. As temperature increases, there appears to be a general decline in the slope and hence in the exponent of the power equation, although the outcome at 11 °C is an exception to this trend. These differences were investigated by an analysis of covariance (ANCOVA) on the data from Table 4.1. To make any meaningful comparisons between the means of the dependent variable, it must be assumed that the regression slopes are parallel. Homogeneity of slope is fundamental to ANCOVA and its test must precede any further tests (Sokal & Rohlf 1981). To investigate homogeneity of slope an analysis of variance (AOV) was undertaken for the model:

Log_e respiration rate = $\log_e$ body size + temperature + ($\log_e$ body size · temperature)

The AOV revealed the interaction term to be highly significant (F = 4.019**) and therefore the slopes of the individual regression equations are not homogeneous and will intersect: ANCOVA could not be undertaken on these data. Therefore, for *Archidoris pseudoargus,* the relationship between oxygen consumption and body size varies with ambient temperature.

To further investigate changes in the oxygen consumption with temperature, a mean size specific respiration rate (R) was calculated for each temperature. These mean R values were used to calculate Q_{10} values (Table 4.2 & Figure 4.4). *Archidoris pseudoargus* shows an increase in R with increasing temperature, although the rate of

Table 4.1 Relationship between respiration rate and body size at different ambient temperatures for *Archidoris pseudoargus*. T = temperature (°C); y = $\log_e$ respiration rate ($\mu l\ O_2 \cdot h^{-1}$); x = $\log_e$ body size (mg dry weight); VO_2 = respiration rate.

T	GM regression		F	r	n
5	$y = 1.644\ x - 6.691$	or $VO_2 = 0.0002\ x^{1.931}$	0.414^{ns}	0.28	7
8	$y = 1.114\ x - 3.472$	or $VO_2 = 0.031\ x^{1.114}$	19.6***	0.79	14
9	$y = 0.873\ x - 1.259$	or $VO_2 = 0.112\ x^{0.974}$	374.8***	0.99	13
11	$y = 1.251\ x - 4.262$	or $VO_2 = 0.014\ x^{1.251}$	118.4***	0.89	29
12	$y = 0.986\ x - 1.856$	or $VO_2 = 0.156\ x^{0.936}$	183.4***	0.97	14
14.5	$y = 0.713\ x + 0.621$	or $VO_2 = 1.86\ x^{0.713}$	111.5***	0.88	36

Figure 4.3 The relationships between respiration rate and body size (as dry weight) for *Archidoris pseudoargus* at temperatures (T) between 5 to 14.5 °C. Data for each temperature are given in Table 4.1. Each line represents the 'best-fit' geometric mean regression where the data are transformed to natural logarithms; no line is fitted to T = 5 because regression analysis produced a non significant relationship. For each plot: ordinate = $\log_e$ respiration rate ($\mu l\ O_2 \cdot h^{-1}$) and abscissa = $\log_e$ body size (mg dry weight); all axes have the same scale.

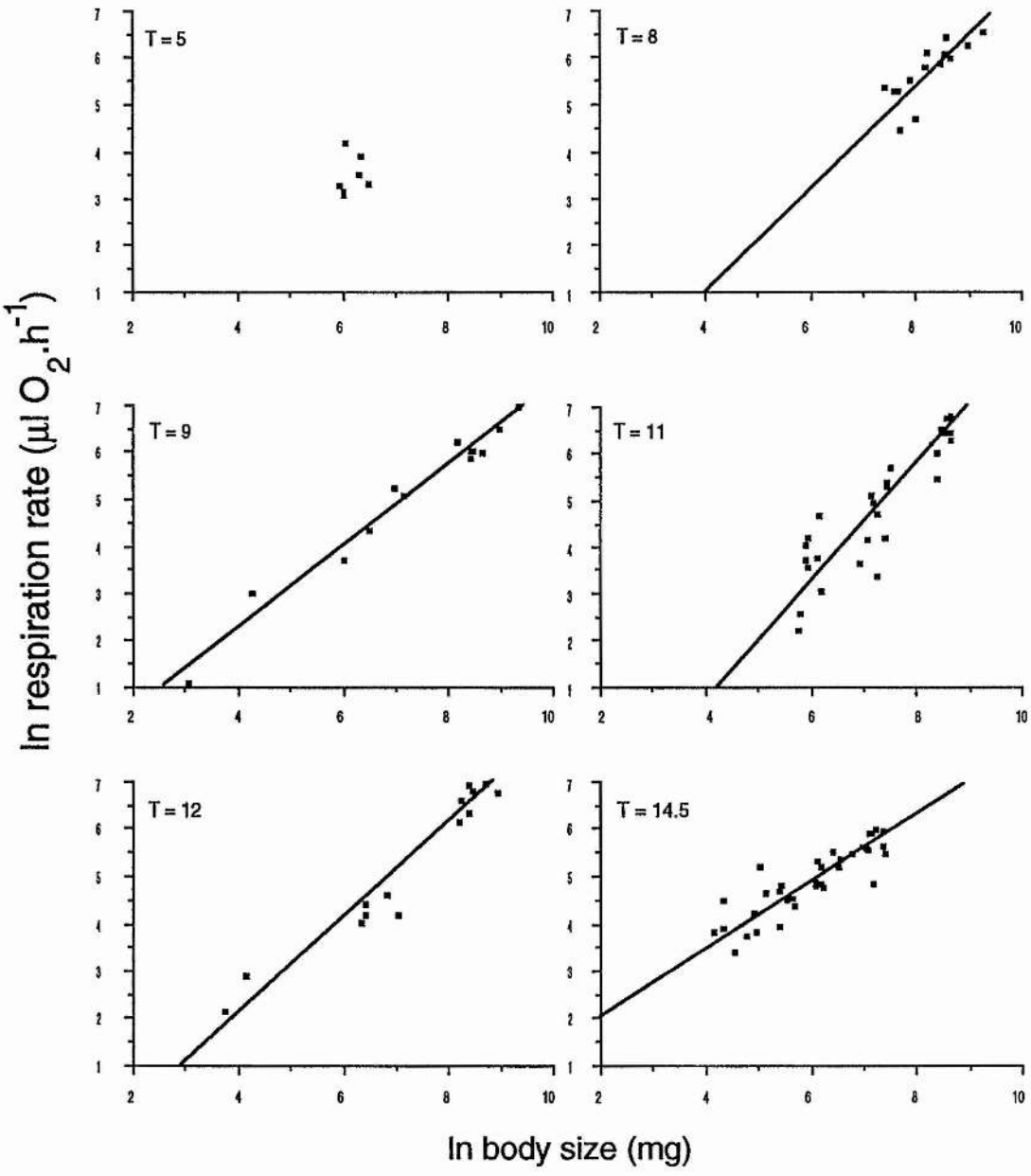
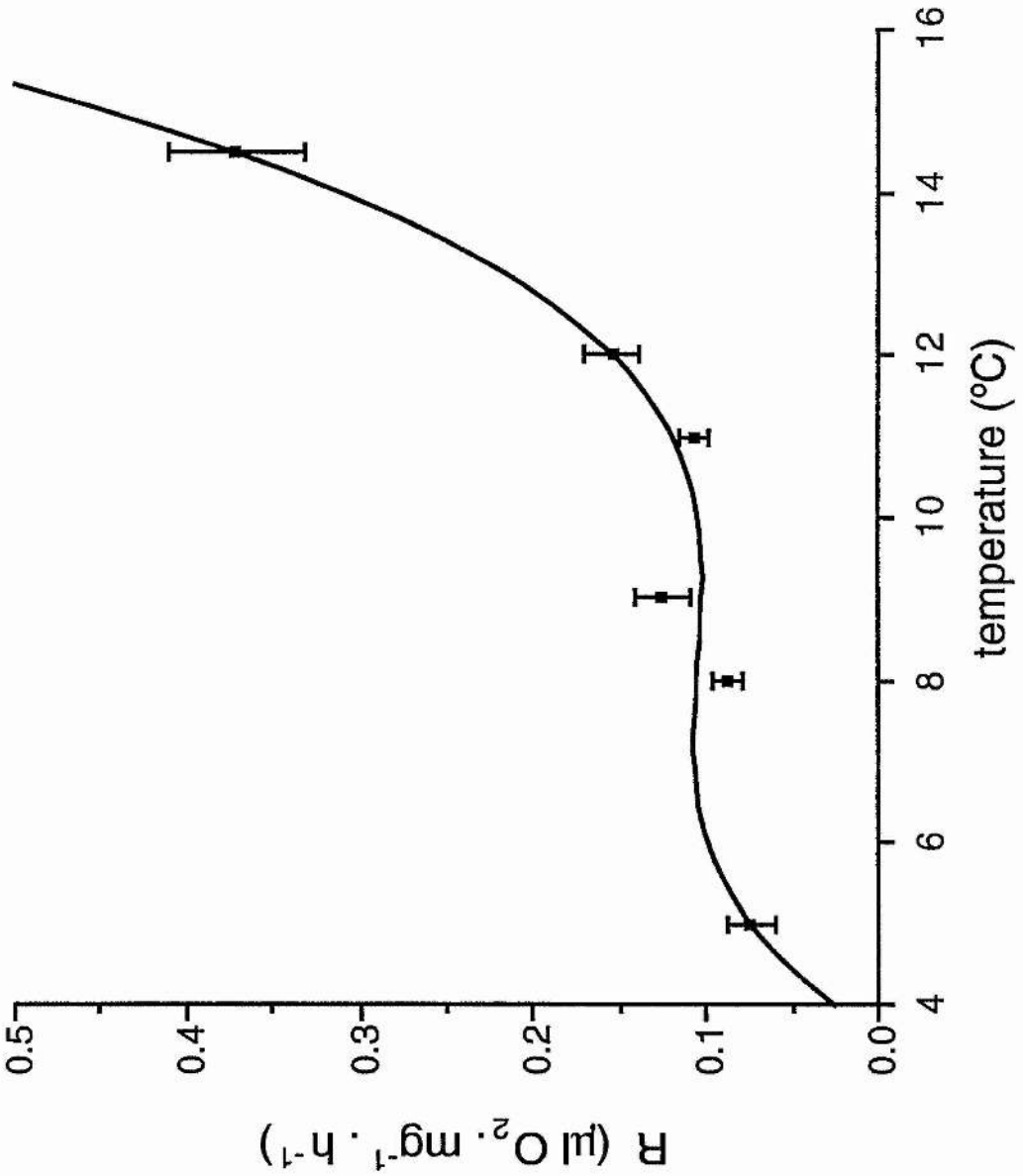

T = 5
T = 8
T = 9
T = 11
T = 12
T = 14.5
ln respiration rate (µl O_2.h^{-1})
ln body size (mg)

Table 4.2 Mean size specific respiration rate (R) with an associated Q10 value for Archidoris pseudoargus at different ambient temperatures. The number of individual determinations for the derivation of R is shown as 'n'.

Temperature (°C)	n	R ($\mu l\ O_2 .mg^{-1} h^{-1}$)	Q_{10}	Q_{10}
5	7	0.074 ±0.014	1.5	5.6
8	14	0.088 ±0.008	1.9	
11	29	0.107 ±0.009	42.2	
14.5	36	0.371 ±0.038		

Figure 4.4 The relationship between mean size specific respiration rate R (± 1 se) and temperature for *Archidoris pseudoargus*. Data for the number of determinations at each temperature are given in Table 4.1. Data are fitted to a third order polynomial model ($F = 41.2^*$; $r = 0.992$; $n = 5$).


R (µl O2 . mg-1 . h-1)
0.0
0.1
0.2
0.3
0.4
0.5
4
6
8
10
12
14
16
temperature (°C)

increase is dependent on the temperature. Empirical models were fitted to the data and, applying Occam's Razor, the data were best explained by a third order polynomial ($F = 41.2^*$; $r = 0.992$; $n = 5$). At lower temperatures up to approximately 11 °C, R values increase slowly with temperature, but then there is a sharp increase beyond 12 °C; there is a 'plateau' in the lower temperature range. These temperatures approximate to the annual range recorded in the field. Q_{10} values increase gradually with increasing temperature from 5-11 °C and rise sharply between 11-14.5 °C, suggesting an acceleration of R at temperatures approximating the upper end of the annual range. *A. pseudoargus* may be less able to acclimatize its respiration rate to these higher temperatures, or this increase is a reflection of increased demand for ATP to fuel growth and gametogenesis: the species 'opens up' at these higher temperatures.

4.4.2 *Cadlina laevis*

A total of 111 respiration rate determinations were undertaken for *Cadlina laevis* which displayed a mean size specific respiration rate (±1 se) of 0.138 (± 0.011) $\mu l\ O_2 \cdot mg\ dry\ wt^{-1} \cdot h^{-1}$. A 'best fit' geometric mean (GM) regression model was obtained when the data were transformed to natural logarithms ($\log_e$); the data were explained by the expression:

$$\text{Log}_e \text{ Respiration rate} = 0.848 \cdot \log_e \text{ body size} - 1.692$$

$$F = 333.7^{***};\ r = 0.87;\ n = 111.$$

If this expression is converted to base 10, it becomes

$$\text{Respiration rate} = 0.184 \cdot \text{body size}^{0.848}$$

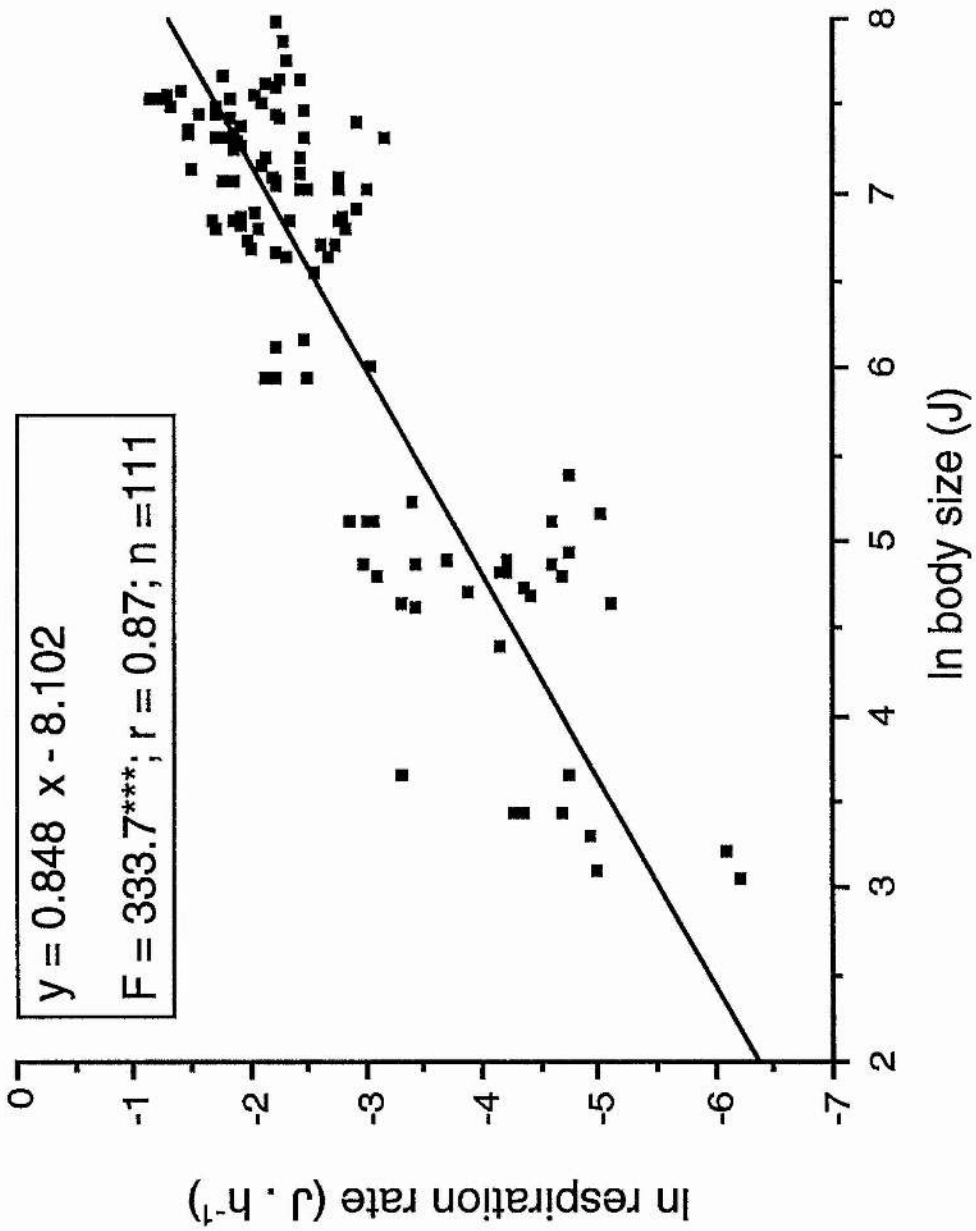
When the data are converted to joule equivalents, the expressions become:

$$\text{Log}_e \text{ Rate of energy loss} = 0.848 \cdot \log_e \text{ J body size equivalent} - 8.102$$

$$\text{Rate of energy loss} = 0.0003 \cdot \text{J body size equivalent}^{0.848}$$

Figure 4.5 shows the relationship between respiration rate (as rate of energy loss) and body size (energy equivalent).

Figure 4.5 The relationship between respiration rate (as heat loss: joules per hour) and body size (as energy equivalent) for *Cadlina laevis;* data are transformed to natural logarithms. The line represents the 'best-fit' geometric mean regression ($r = 0.87$). A total of 111 determinations were made at ambient temperature which ranged from 2 to 18 °C. Individuals used for this experiment spanned a size range of 21 to $2.9 \cdot 10^3$ J; respiration rates ranged from 0.002 to 0.32 $J \cdot h^{-1}$.


y = 0.848 x - 8.102
F = 333.7***; r = 0.87; n =111
ln respiration rate (J . h-1)
ln body size (J)
0
-1
-2
-3
-4
-5
-6
-7
2
3
4
5
6
7
8

Determinations were made over a temperature range of 2-18 °C. In order to assess the possible influence of temperature on the rate of oxygen consumption, multiple regression analysis was undertaken on the pooled data:

$$\text{Log}_e \text{ Respiration rate} = 0.063 \cdot \text{temperature} + 0.809 \cdot \log_e \text{body size} - 2.14$$

$$F = 223.8^{***};\ r = 0.90;\ n = 111.$$

Calculation of the standardised partial regression coefficients realises $b' = 0.241$ for temperature and $b' = 0.951$ for body size. Although changes in body size have a relatively greater effect on respiration rate than changes in temperature, the effect of temperature cannot be ignored. Earlier comments regarding the validity of these multiple regression analyses cast some doubt on these conclusions and therefore the influence of temperature on respiration rate was investigated further.

Expressions explaining the relationship between respiration rate and body size at the different ambient temperatures are given in Table 4.3; the data are displayed graphically in Figure 4.6. Differences in these relationships between body size and oxygen consumption with changes in temperature were investigated by an analysis of covariance. To investigate homogeneity of slope, an analysis of variance (AOV) was undertaken for the model:

Log_e respiration rate = $\log_e$ body size + temperature + ($\log_e$ body size * temperature)

The AOV revealed the interaction term to be highly significant ($F = 5.413^{**}$) and therefore the slopes of the individual regression equations are not homogeneous and will intersect: ANCOVA could not be undertaken on these data. From these results, no clear pattern emerges to determine the influence of temperature on the relationship between respiration rate and body size.

For each temperature, a mean size specific respiration rate (R) was calculated and a 'species Q_{10}' value determined for each pair of temperatures; these data are presented in Table 4.4 and Figure 4.7. Empirical models were fitted to the data and, applying Occam's Razor, the data were best explained by a second order polynomial ($F = 143.1^{***}$;

Table 4.3 Relationship between respiration rate and body size at different ambient temperatures for *Cadlina laevis*. T = temperature (°C); y = $\log_e$ respiration rate ($\mu l\ O_2 \cdot h^{-1}$); x = $\log_e$ body size (mg dry weight); VO_2 = respiration rate.

T	GM regression		F	r	n
2	$y = 1.299\ x - 4.343$	or $VO_2 = 0.013\ x^{1.299}$	4.9*	0.53	15
3	$y = 0.5\ x - 0.789$	or $VO_2 = 0.454\ x^{0.5}$	131.6***	0.97	9
6	$y = 0.987\ x - 2.54$	or $VO_2 = 0.079\ x^{0.987}$	11.7**	0.70	14
10	$y = 1.019\ x - 2.388$	or $VO_2 = 0.092\ x^{1.019}$	8.3*	0.66	13
12	$y = 1.054\ x - 2.181$	or $VO_2 = 0.113\ x^{1.054}$	124.4***	0.88	39
18	$y = 0.73\ x - 0.605$	or $VO_2 = 0.547\ x^{0.73}$	279.9***	0.99	9

Figure 4.6 The relationships between respiration rate and body size (as dry weight) for *Cadlina laevis* at temperatures (T) between 2 to 18 °C. Each line represents the 'best-fit' geometric mean regression in which the data are transformed to natural logarithms. Data for each temperature are given in Table 4.3. For each plot: ordinate = $\log_e$ respiration rate ($\mu l\ O_2 \cdot h^{-1)}$ and abscissa = $\log_e$ body size (mg dry weight); all axes have the same scale.

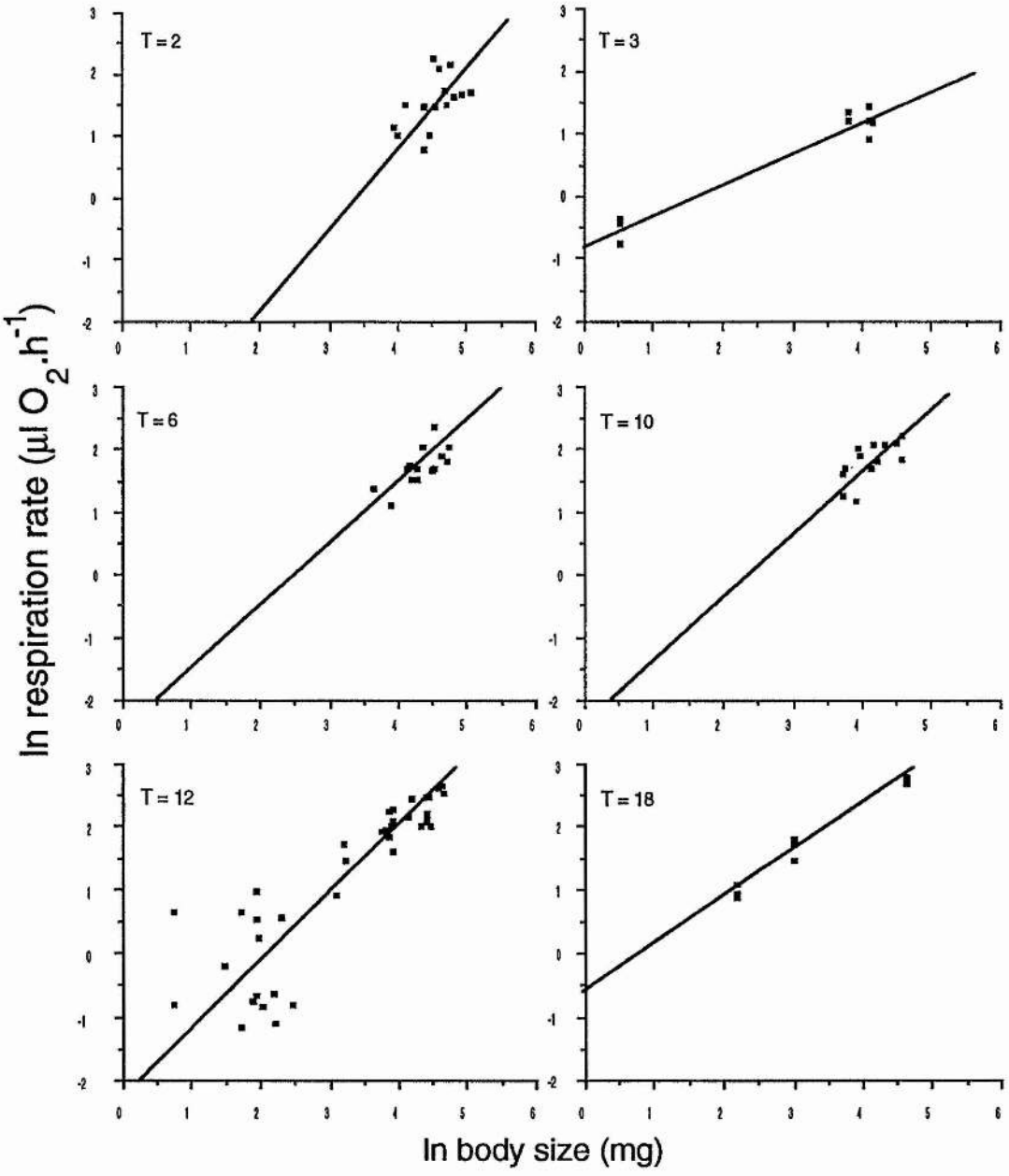
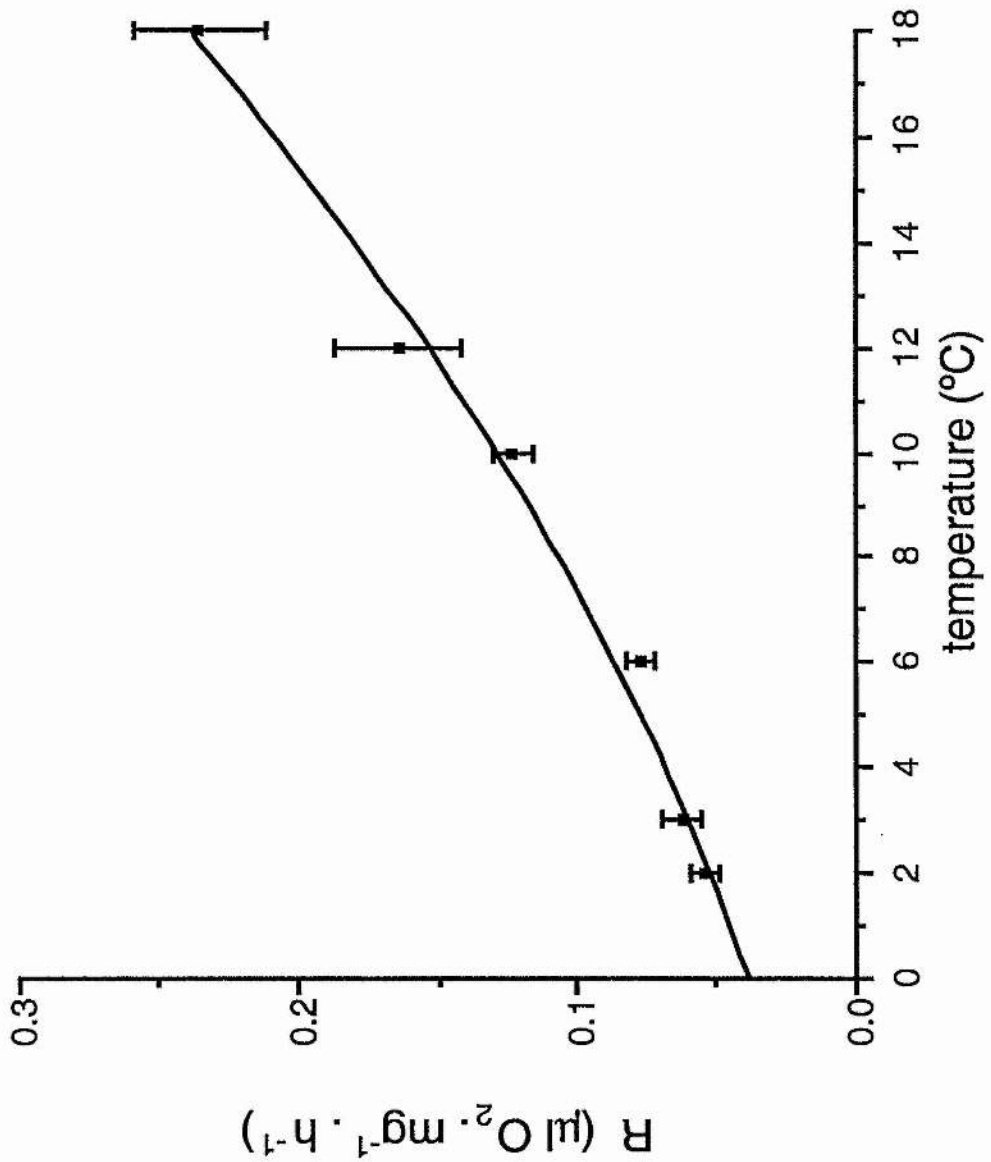

T = 2
T = 3
T = 6
T = 10
T = 12
T = 18
ln respiration rate (µl O_2.h^{-1})
ln body size (mg)

Table 4.4 Mean size specific respiration rate (R) with an associated Q_{10} value for *Cadlina laevis* at different ambient temperatures. The number of individual determinations for the derivation of R is shown as 'n'.

Temperature (°C)	n	R ($\mu l\ O_2 . mg^{-1} . h^{-1}$)	Q_{10}	Q_{10}
2	15	0.054 ±0.005	2.4	2.5
6	14	0.077 ±0.003	2.1	
10	13	0.103 ±0.007	10.5	
12	39	0.164 ±0.023	1.8	
18	9	0.235 ±0.024		

Figure 4.7 The relationship between mean size specific respiration rate R (± 1 se) and temperature for *Cadlina laevis*. Data for each temperature are given in Table 4.3. Data are fitted to a second order polynomial model ($F = 143.1^{***}$; $r = 0.995$; $n = 6$).


R (µl O2. mg-1 . h-1)
0.0
0.1
0.2
0.3
0
2
4
6
8
10
12
14
16
18
temperature (°C)

r = 0.995; n = 6). For *Cadlina laevis*, mean R increases steadily with temperature and, unlike *Archidoris pseudoargus*, there does not appear to be a plateau in the mid-range of temperatures. Q_{10} values remain relatively constant across a range of temperatures, which suggests that R for *C. laevis* was not strongly influenced by temperatures which approximate an annual seasonal range. In comparison to *Archidoris pseudoargus*, *C. laevis* was more able to acclimatize its respiration rate to changing ambient temperature, or demand for ATP shows less seasonality.

4.3.3 Q_{10} investigations

Table 4.5 and Figure 4.8 present the results of the Q_{10} investigations comparing *Cadlina laevis* with *Adalaria proxima*. At all temperatures, *A. proxima* has a higher size specific respiration rate (R) than *C. laevis*. Q_{10} values for *C. laevis* are less variable than values for *A. proxima*, suggesting that R for *A. proxima* is more strongly influenced by changes in temperature, which may be a reflection of a reduced ability to acclimate respiration rate. Although these data for respiration rates were standardised to size specific respiration rates, data presented earlier indicate that the relationship between respiration rate and body size is complex and influenced by many factors. Individuals used for the Q_{10} investigation had different body sizes both within and between species and therefore these interspecific differences may be attributable to differences in body size, rather than evolutionary differences in an ability to acclimate respiration rate to changes in ambient temperature.

4.4 Discussion

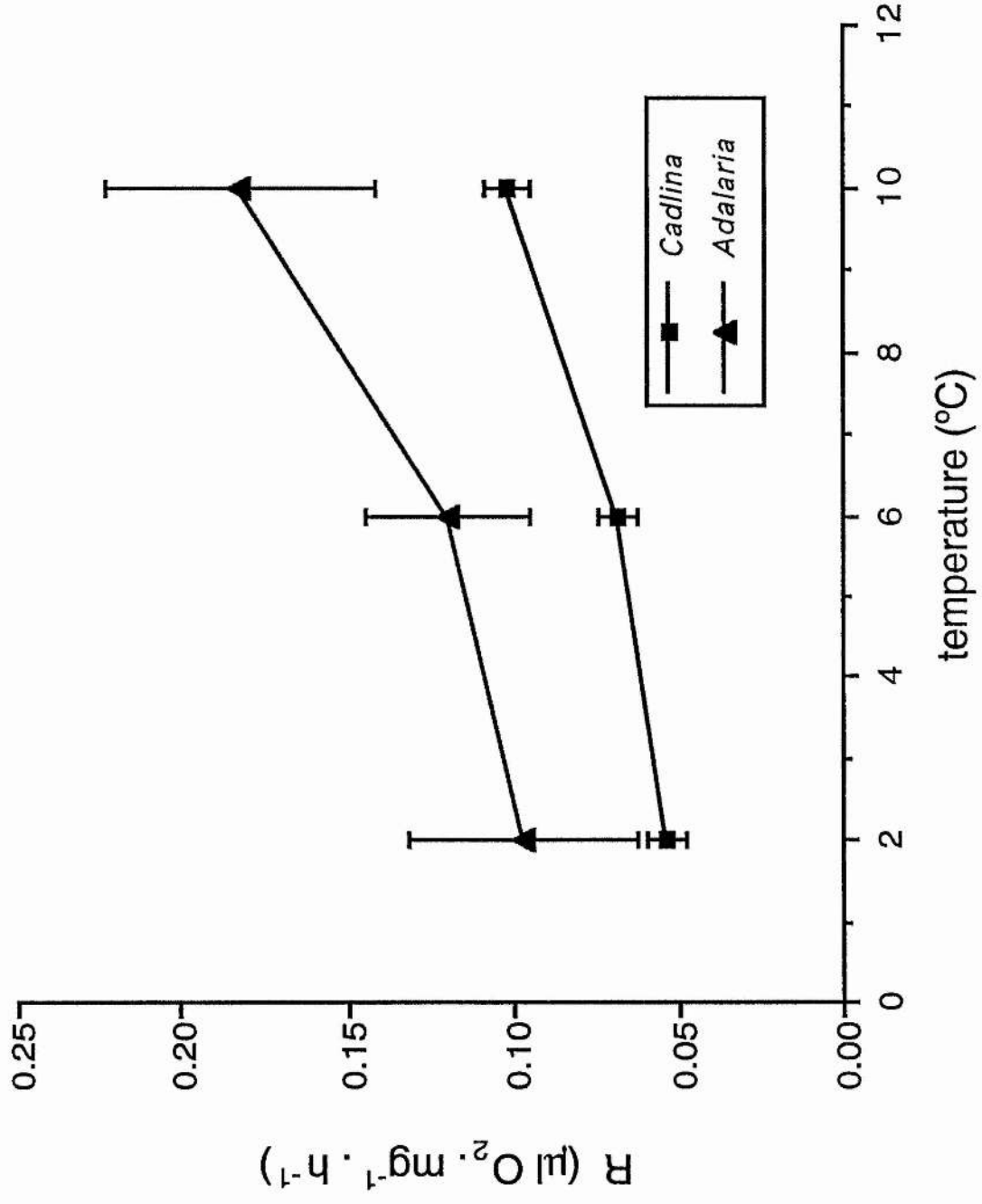
4.4.1 Introduction

The ability to sustain an energetic gain from the environment and to channel energy into growth and reproduction, is of central importance in controlling the ability of an individual to compete under conditions where environmental resources are limiting. Compensatory adjustments of the components of energy gain and expenditure are involved in this process. When the effects of both components are combined, an insight

Table 4.5 A comparison of the mean size specific respiration rate R (expressed as $\mu l\ O_2 \cdot mg^{-1} \cdot h^{-1}$) with an associated Q_{10} value for *Cadlina laevis* and *Adalaria proxima* at different ambient temperatures (°C). Each value of R is the mean of 3 determinations for 3 animals (*i.e.* 9 observations).

	Cadlina laevis			*Adalaria proxima*		
Temperature	**R**	**Q_{10}**	**Q_{10}**	**R**	**Q_{10}**	**Q_{10}**
2	0.0538	2.341	2.186	0.0970	1.716	2.204
6	0.0756	2.043		0.1204	2.829	
10	0.1006			0.1825		

Figure 4.8 The relationship between size specific respiration rate R (± 1 se) and temperature for *Cadlina laevis* and *Adalaria proxima.* Each value of R is the mean of three determinations on three animals (n = 9). Animals were acclimated for five days at each temperature and then the three determinations obtained on successive days. Data points are joined for clarity, the line does not imply a continuous relationship between the data points.


R (µl O2. mg-1 . h-1)
0.25
0.20
0.15
0.10
0.05
0.00
0
2
4
6
8
10
12
temperature (°C)
Cadlina
Adalaria

can be gained into the endogenous and environmental factors that interact to control conditions under which an individual can realise an energetic gain and therefore growth and reproduction can occur. Metabolic costs, or energy lost to the environment, may represent up to 80% of the energy assimilated from ingested material (Bayne & Newell 1983). Adaptations to reduce these metabolic costs, or methods which effect energy conservation, are central to the survival of an organism under conditions where the energy resource (= food) may be limiting, or the quality of the resource is variable. Body size and environmental temperature have a profound influence on the standard metabolic rate and therefore the relationship between these variables dictates the amount of energy available to an organism to complete its life history.

4.4.2 Relationship between body size and oxygen consumption

For *Archidoris pseudoargus* and *Cadlina laevis*, the rate of oxygen consumption is strongly dependent on body size. Allometric relationships were recorded for both species: statistically significant correlation coefficients were noted for both species, despite there being considerable 'noise' about the respective regression lines. Such variations could be a consequence of measuring the *standard respiration rate*, with its associated instantaneous contribution to the total oxygen consumption by growth, activity, digestion and gametogenesis. For instance, the phenomenon of specific dynamic action (SDA), the post-ingestive effect of consumption on respiration rate, can last from less that 14 hours in *Aplysia dactylomela* (Carefoot 1987) to 42 days in *Asterias rubens* (although the animal had been starved for 13 months prior to being fed; Vahl 1984). During the present study animals were fed *ad libitum*, therefore one might expect all individuals to have a similar nutritional status and SDA effects to be minimal. Sporadic feeding of *Archidoris pseudoargus* (Chapter 3) may give rise to varying degrees of SDA between individuals which may account for some of the variation in the respiration rate/body size relationship. Although a standard procedure was maintained throughout, it is impossible to account for these possible error factors. Nevertheless, the procedures followed during the current investigation were standardised as much as possible to eliminate possible sources of

error. The results obtained over the course of these investigations were consistent, without any suggestion of excessive intra- or inter-individual variation for either species.

Measurement of oxygen consumption in a respiratory chamber cannot account for the contribution of anaerobic processes to total metabolism. Anaerobic metabolism may form a substantial part of the total metabolic output, particularly during aerial exposure for intertidal organisms. Pamatamat (1980) found that anaerobic heat production (= respiration), for two species of bivalve mollusc, may rise to >20% of the total heat production during periods of starvation; Shick *et al.* (1983) found that anaerobic heat loss accounted for >72% of the total aerial respiration for *Mytilus edulis*. Clarke (1983) considered that anaerobic metabolism is an adaptation to short term hypoxic conditions, and an organism will revert to aerobic metabolism when oxygen concentrations return to normal. Bayne & Newell (1983) concluded that anaerobic respiration represents 5-7% of the total heat loss under 'normoxic' conditions. During the present study, experimental organisms were always subject to oxygen concentration at or near saturation (= 'normoxic'?) and therefore the contribution of anaerobic respiration to basal metabolism is assumed to be minimal. If this assumption is not valid, and individuals vary in the degree to which they rely on anaerobic respiration for energy production, anaerobic respiration may account for some of the variance in the experimental determinations of the present study. Nevertheless, an ability to generate energy via anaerobic respiration will be an important adaptation for intertidal organisms, and both *Cadlina laevis* and *Archidoris pseudoargus* occur in intertidal habitats. Potts (1983) found that aerial respiration of *A. pseudoargus* was approximately 25% lower than aquatic respiration (at 10 °C). If an individual is to maintain a constant basal metabolic rate in both aerial and aquatic habitats, anaerobic mechanisms would comprise 25% of aerial respiration.

Peters (1983), in a review of the ecological implications of body size, concluded that body size relations have a 'central role' in animal ecology, and further stated that 'most body size relations' take the form:

$$Y = a \cdot W^b$$

where Y is the biological characteristic in question (here, oxygen consumption), W is the body size, the constant a denotes the level of metabolic rate of an organism of unit body size, and b is the exponent. The value of a varies according to a wide variety of factors including activity and temperature. For most allometric relationships, the value of b has been found to vary from 0.67 to 0.75 (Zeuthen 1953: Hemmingsen 1960). Bayne & Newell (1983) reviewed the allometry of respiration rate for molluscs and recorded a mean exponent of 0.7 for molluscs in general, and 0.73 for predatory gastropods, although these data do not include nudibranchs. Clark (1975), in a review of 18 nudibranch species, recorded values of b ranging from 0.1 to >1. Carefoot (1967) recorded a value of $b = 0.6$ for *Archidoris pseudoargus* whilst Potts (1983), for the same species, recorded a value of $b = 0.38$ at 4 °C, 0.42 at 10 °C and 0.77 at 18 °C. Havenhand & Todd (1988a) recorded values of $b = 0.83$ for *Adalaria proxima* and $b = 1.08$ for *Onchidoris muricata,* using the same apparatus as the present study. Havenhand & Todd (1988a) found considerable intraspecific variation and note that, under those circumstances, 'species' exponents derived from pooled data may conceal ecologically important variation. Sufficient replicate data for individual nudibranchs were not collected during the present study to draw any conclusions on the degree on intra-individual variation. Nevertheless, it is an important principle to be borne in mind when 'species' statistics derived from pooled data.

If metabolic rate is expressed per unit body size, the exponent in the allometric relationship becomes $(b - 1)$ and therefore values of $b < 1$ result in an overall negative exponent. A negative exponent indicates that large organisms consume proportionally less oxygen than small organisms and, by implication, larger organisms are metabolically more efficient. Thus in energetic terms, the metabolic cost of maintaining a given biomass of a large organism is less than that required to maintain a small organism. Where energy supply is finite, large organisms may be able to allocate proportionally more energy to the basic life history processes of growth and reproduction. Both *Archidoris pseudoargus* and *Cadlina laevis* show $b < 1$ and therefore increasing body size can result in proportionally more energy available for growth and reproduction. *Archidoris*

pseudoargus has a marginally higher exponent ($b = 0.881 \pm 0.047$ against $b = 0.848 \pm 0.040$ for *Cadlina laevis*), although this difference is not statistically significant. An important aspect of these rates of energy loss is the ecological efficiency of these species. Larger individuals are proportionally more efficient which may be important under conditions where resources are limiting, perhaps due to localised food depletion as a population cohort matures. Under such circumstances, greater ecological efficiency may permit these species to maintain the energetic allocation to growth and reproduction.

Differences in the value of the constant a represent changes in the respiration rate of a 'standard sized' organism. In the present study, $a = 0.324 \pm 0.333$ for *Archidoris pseudoargus* and $a = 0.184 \pm 0.147$ for *Cadlina laevis*. Whilst these values are not significantly different, there is an indication that the respiration rate for a 'standard sized' *Archidoris pseudoargus* is slightly greater than for a 'standard sized' *Cadlina laevis*. Such differences have important ecological considerations. Pough (1980) (referenced in Peters 1983) considered that low values of a were indicative of an organism better suited to withstanding low resource levels. Bayne & Newell (1983) recorded a mean value of $a = 1.63$ for predatory gastropods, and Potts (1983) recorded values of $a = 1.79$ - 2.07 for *Archidoris pseudoargus* at temperatures between 4 and 18 °C. Values of a recorded in the present study are thus somewhat higher than those previously recorded for gastropods, although error terms were not quoted in these earlier studies. It is not possible to determine whether the methodology itself (*i.e.* a polarographic technique versus chemical techniques) results in these higher values of a, or if these are real results and therefore have some ecological significance. A further confounding factor is that respiration rates are affected by a wide variety of environmental factors, endogenous factors and other time dependent variables such as season (Bayne & Newell 1983); anaerobic respiration may also be a contributory factor (see above).

When the data were converted to joule equivalents (*Archidoris pseudoargus*: $a = 0.0007 \pm 0.0003$; *Cadlina laevis*: $a = 0.0003 \pm 0.0001$) the values were similar to those recorded by Havenhand & Todd (1988a) for the annual species *Adalaria proxima*

($a = 0.0014$) and *Onchidoris muricata* ($a = 0.0005$). Thus *Archidoris pseudoargus* and *Cadlina laevis* have a lower 'standard respiration rate' than *Adalaria proxima*, but a marginally higher value of the exponent *b* (see above); while *a* for *Onchidoris muricata* is intermediate between *A. pseudoargus* and *C. laevis*, but has a much greater exponent and may therefore be less efficient with increasing body size. No error terms are available for the data in Havenhand & Todd (1988a) and therefore it is not possible to determine the statistical significance of these apparent differences between the two studies. Equivalence of data between the present study and Havenhand & Todd (1988a) suggests that the experimental techniques employed by the present study were valid.

Assuming that these values are realistic, what are their possible ecological significance? For a finite quantity of energy, a low 'standard metabolic rate' results in more energy available for other body functions, in particular growth and reproduction. Similarly, should the food supply be interrupted and the organism have to rely on endogenous reserves to meet basal metabolic requirements, a low standard metabolic rate will prolong the period over which the organism can survive the interruption. Gilfillan *et al.* (1977) observed that the bivalve *Mya arenaria* reduced its respiratory costs when environmental temperatures and food availability were low. Branch (1979) and Newell & Branch (1980) studied intertidal limpets and noted that metabolic rate increased with increasing food availability. Both *Archidoris pseudoargus* and *Cadlina laevis* have a life cycle longer than a year and low basal metabolic rates may be an adaptation to overcome interruptions in food supply, or fluctuations in food quality.

To maintain constant biomass, the energy lost through respiration must balance the rate of energy supply. If energy supply is variable, or even interrupted, the rate of energy turnover may be crucial to the continued survival of the organism. Following the calculations of Peters (1983), it is possible to calculate the turnover time for the energy content of an organism. Turnover time is defined as the the time required to metabolize an amount of energy equal to the energy content of the body tissues. Based on the calorific conversions in Chapter 3, the turnover time for *Archidoris pseudoargus* is $\approx$ 8.6 days per mg dry weight, and for *Cadlina laevis* the turnover time is $\approx$ 3.3 days per mg dry weight.

For an 'average' poikilotherm, the turnover time is ≈1.1 days per mg dry (body) weight (Peters 1983). Thus *A. pseudoargus* and *C. laevis* have long turnover times relative to an 'average' poikilotherm.

Respiration is a 'cost of living' (Sibly & Calow 1986) and, where resources are finite, this cost may compromise the fitness of an organism. From data collected during the current study, it would appear that both *Archidoris pseudoargus* and *Cadlina laevis* have adapted to reduce the rate of energy loss through basal metabolism. In particular, these losses are proportionally lower for large individuals, which may permit a greater allocation of energy to the primary life history processes of growth and reproduction. In addition, improving respiratory efficiency may improve the organism's ability to withstand any fluctuations in food supply or food quality. In general, *Cadlina laevis* appears to have the lower 'standard respiration rate', improved efficiency with increasing body size and a relatively long metabolic turnover rate. These adaptations all contribute to improving the prospects of survival for a longer lived species when, by chance alone, there may be more interruptions to its energy supply.

4.4.3 Influence of temperature on oxygen consumption

At a cellular level, respiration is a series of enzymatically controlled chemical reactions. In general, an increase in temperature will increase the rate of chemical reactions and therefore it is intuitive that the rate of respiration to depend, to some extent, on ambient temperature. A parallel increase of respiration rate and temperature in the short term is an *acute* response. If, after a period of time, respiration rates stabilise at an intermediate value between the original and the acute value, the response is termed *acclimation*. A seasonal adjustment of respiration rate in parallel with seasonal changes in temperature is termed *acclimatization* (Clarke 1983). Clarke (1991) makes clear distinctions between these effects and their relationship with laboratory experiments: most experiments consider acute or acclimated responses, rather than considering acclimatization. However during the present study, the general relationship between respiration rate and temperature was determined from experiments at different times of the

year, and hence the response measured approximates acclimatization. Q_{10} investigations on *Cadlina laevis* and *Adalaria proxima* involved more short term changes and the response determined approximates acclimation.

It was noted earlier (4.4.2) that there was considerable 'noise' around the regression line for the relationship between respiration rate and body size. These determinations span a temperature range of 2-18 °C for *Cadlina laevis* and 5-14.5 °C for *Archidoris pseudoargus*, and thus temperature may account for some of the observed variation. Multiple regression analysis on respiration rate against body size and temperature improved the 'goodness of fit' of the model; temperature accounted for a 12% elevation in the correlation for *Archidoris pseudoargus* and a 6% elevation in *Cadlina laevis*. Analysis of the standard partial regression coefficients shows an increase in temperature of one standard deviation increases the respiration rate by one third of a standard deviation for *Archidoris pseudoargus* and one quarter of a standard deviation for *Cadlina laevis*. These interpretations should be viewed with caution because the independent variable 'body size' was subject to a measurement error, and therefore violates one of the assumptions of the multiple regression model; temperature was measured without error. Type II geometric mean multiple regression procedures are not available. Nevertheless, a very high correlation coefficient for type I regression indicates that the type I and geometric mean regression lines will be similar. Interpretations based on type I regression analysis will indicate broad trends rather than statistically significant relationships. Multiple regression analyses on these data for *A. pseudoargus* and *C. laevis* indicates that temperature will affect the respiration rate for these species although the effect may be less for *C. laevis*.

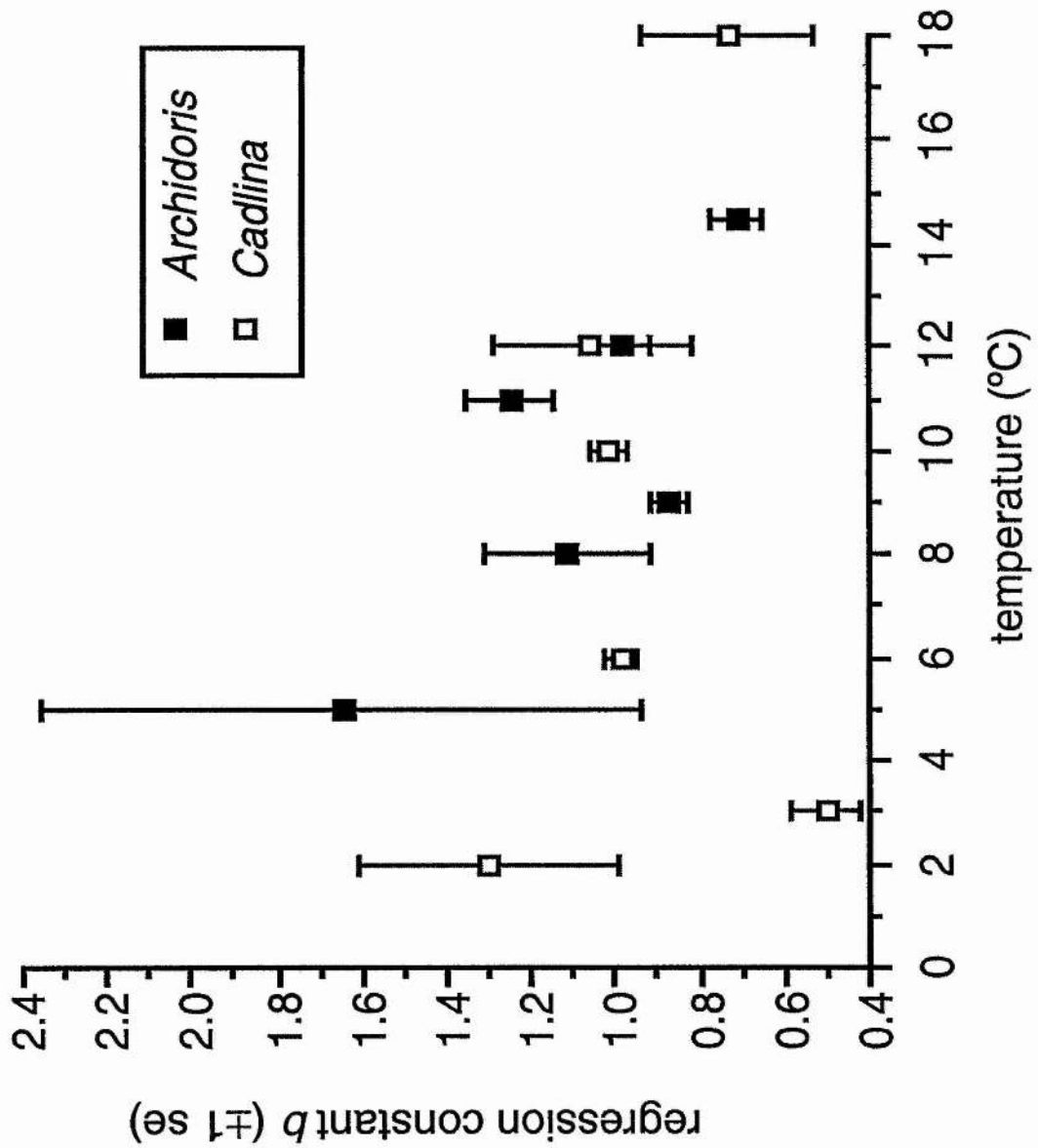
For *Archidoris pseudoargus,* at all experimental temperatures other than at 5 °C, statistically significant relationships were determined between respiration rate and body size. At 5 °C only 7 determinations were undertaken and the level of 'noise', perhaps due to a wide range of body size for the individuals investigated, may occlude any allometric relationship. For *Cadlina laevis*, statistically significant relationships between respiration rate and body size were determined at all experimental temperatures although, on average,

the 'goodness of fit' was poorer than for *A. pseudoargus*. An analysis of variance indicated that the slopes of the individual regression equations of respiration rate against body size at different ambient temperatures were not homogeneous. Therefore, it may be inferred that the relationship between body size and respiration rate will vary with ambient temperature, which may have considerable ecological significance.

There was considerable variation in the value of the exponent *b* (*A. pseudoargus*; $b = 1.251$ ($\pm$ 0.108) to 0.713 ($\pm$ 0.06); *C. laevis*: $b = 1.299$ ($\pm$ 0.306) to 0.5 ($\pm$ 0.042)) but it would appear that there is a general decline in *b* with increasing temperature (Figure 4.9). For both species, over the mid-range of ambient temperatures, there are no significant differences between the values of the exponent *b*. For *C. laevis*, the values of the exponent are significantly different at the extremes of temperature. Similarly for *A. pseudoargus* the value of *b* at 14.5 °C is significantly lower than the value at intermediate temperatures; in Figure 4.9, the value for *b* at 5 °C is included for completeness despite the regression not being statistically significant. For both species, there appears to be a significant trend in a decreasing value of the exponent *b* with increasing temperature. From the discussion in the preceding section (4.3.2), a value of the exponent *b* of <1 indicates that respiration rates are proportionally lower with increasing body size. Combining these latter observations with the preceding discussion, respiration rates are proportionally lower for larger individuals at higher ambient temperatures. For species with a life cycle longer than one year, individuals, and larger individuals in particular, will be exposed to the full range of ambient temperatures and reducing respiration rates will reduce the proportion of an individual's energy budget 'lost' to the environment. In situations where resources are limited, reducing respiratory losses will permit a greater energetic allocation to growth and reproduction.

A decrease in the exponent *b* with increasing temperature has been recorded for other taxa: Ikeda (1970, 1974: referenced in Clarke 1983) recorded an exponent *b* of 0.78-0.83 for boreal species and 0.54-0.60 for tropical species. Clarke (1983) noted that this variation of *b* with habitat temperature, disappears when the exponent is calculated by geometric mean regression rather than type 1 regression. Horn (1986) measured the

Figure 4.9 A graphical representation of the regression coefficient b ($\pm$ 1 se) from the individual GM regression equations relating oxygen consumption to body size against the ambient experimental temperature. Full regression equations and their associated statistics are given in Table 4.1 (*Archidoris pseudoargus*) and Table 4.3 (*Cadlina laevis*).

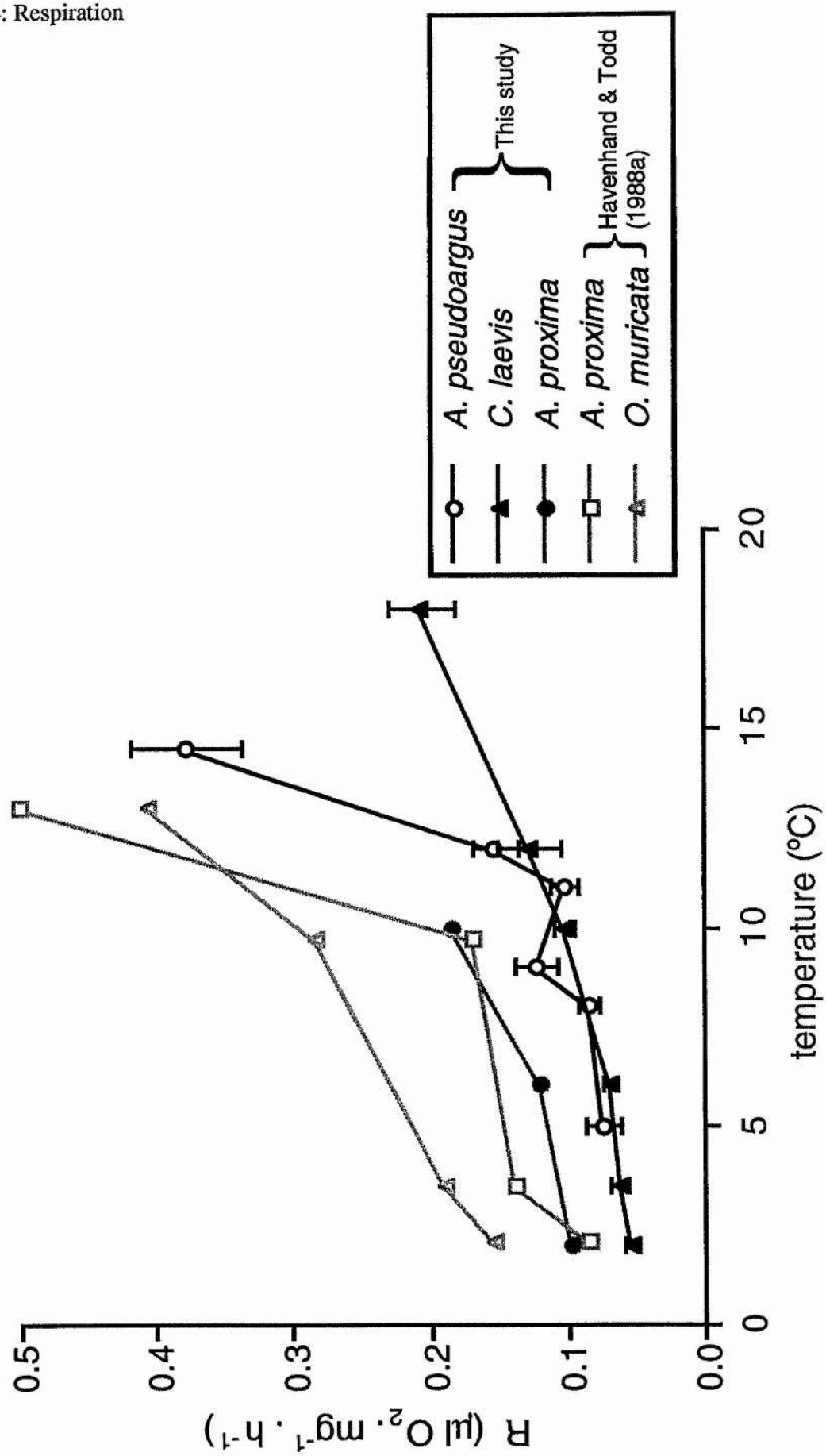

Archidoris
Cadlina
regression constant b (±1 se)
temperature (°C)
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0
2
4
6
8
10
12
14
16
18

respiration rate of the chiton *Chiton pelliserpentis* in air and in water at temperatures between 5 and 27 °C. As experimental temperature increased, the value of the exponent *b* decreased for low shore individuals, although no standard errors were given to determine whether the decline was statistically significant. In the same investigation, no decline in *b* was observed for high shore individuals. From the available information, it is impossible to determine whether a decline in the weight exponent has ecological significance or is simply an experimental artifact. More detailed investigations are required to elucidate any evolutionary significance from this relationship.

Values of *a,* the respiration rate of a standard sized organism, recorded during the present study increased with increasing temperature: for *A. pseudoargus* $a = 0.031$ (± 1 se: 0.006 - 0.159) at 8 °C to 1.86 (± 1 se: 1.296 - 2.672) at 14.5 °C; for *C. laevis*: $a = 0.013$ (± 1 se: 0.0032 - 0.0052) at 5°C to 0.547 (± 1 se: 0.471 - 0.633) at 18 °C. These values suggest, as one would expect, an increase in the standard metabolic rate with an increase in ambient temperature; the increase is lower for *C. laevis* than for *A. pseudoargus*. In seawater at 0 °C, the partial pressure of oxygen is 1.6 times greater than the partial pressure at 20 °C. Thus as temperature increases and the rate of oxygen consumption rises, there is less oxygen available. Lower basal metabolism with increasing temperature may be an adaptation to overcome a lower ambient partial pressure of oxygen. These conclusions are somewhat tenuous because in general, the interpretation of the effects of temperature on the level of metabolic energy expenditure is complicated by many endogenous and environmental factors which interact with one another under natural conditions (Newell & Branch 1980).

Analysis of the size specific respiration rates (R) and Q_{10} values for *Archidoris pseudoargus* and *Cadlina laevis* suggests that respiration rates for the latter species are less strongly influenced by changes in ambient temperature. When the data for *A. pseudoargus*, *C. laevis*, and *Adalaria proxima* from the present study are compared with the data presented in Havenhand & Todd (1988a) for *A. proxima* and *Onchidoris muricata,* differences in the absolute value of R, and the response of R to increasing temperature are observed (Figure 4.10). Each of these nudibranch species exhibits, to

Figure 4.10 The relationship between size specific respiration rate R (± 1 se) and temperature for four species of nudibranch mollusc. Data for *Onchidoris muricata* and *Adalaria proxima* were obtained from Table II in Havenhand & Todd (1988a). Data for *Archidoris pseudoargus*, *Cadlina laevis* and *Adalaria proxima* from the present study are given in Table 4.1, 4.3 & 4.5. Data points are joined for clarity, the line does not imply a continuous relationship between the data points.


R (µl O2. mg-1 . h-1)
0.0
0.1
0.2
0.3
0.4
0.5
0
5
10
15
20
temperature (°C)
A. pseudoargus
C. laevis
A. proxima
A. proxima
O. muricata
This study
Havenhand & Todd (1988a)

varying degrees, a 'plateau' effect over the mid-range of temperatures as described by Havenhand & Todd (1988a). *C. laevis* has the lowest R value of the four species. R values for *A. pseudoargus* are similar to *C. laevis* at temperatures up to 11 °C, but increase sharply at higher temperatures. R values for *A. proxima* and *O. muricata* are consistently higher than the other two species, demonstrate a 'plateau' in the mid-range, and a sharp increase in R at temperatures greater than 10 °C: the latter response is analogous to that observed for *A. pseudoargus*. It is perhaps significant that the temperatures within the 'plateau' represent the ambient temperature for winter, spring and early summer, ambient seawater temperatures rising beyond the 'plateau' only in late summer and early autumn. *A. proxima* and *O. muricata* are annual species and, for the period when temperatures exceed the 'plateau', are either in the larval or early juvenile phase of their life cycle, when the absolute rate of energy turnover will be lower and resources less likely to be limiting. These observations may be interpreted in a number of ways. A classical interpretation relates the ability of an organism to acclimatize to changes in temperature. Adults of longer-lived species have to overcome these higher temperatures to prolong their survival and therefore it is critical that R values acclimatize to higher temperatures to minimize the energy lost to basal metabolism. Of the four species studied, *C. laevis* has the lowest value of R and appears to have greater acclimatization to changes in temperature. *A. pseudoargus* has a lower value of R than the annual species, and appears to acclimatize to changes in temperature at the lower end of the ambient range. Only at the upper end of the range does the R value increase sharply, suggesting that *A. pseudoargus* is less able to acclimatize to higher temperatures. Nevertheless, at the higher temperatures, the overall value of R for *A. pseudoargus* is lower than the rate for annual species. These factors may be an adaptation to improve survival for individuals of the longer-lived species. Smith & Sebens (1983) concluded that, for *Onchidoris aspera,* temperature plays a large role in delimiting the life cycle of this species to an annual life cycle. Summer temperatures in the adult habitat rise to greater than the upper physiological limit of thermal tolerance, which reduces the possibility of a population persisting over the summer months. Therefore, an ability to acclimatize to higher temperatures is central to an organsim extending its life cycle.

Clarke (1991), in a review of cold adaptation of marine organisms, concluded that "simple measures of the rate of complex physiological processes such as growth and respiration do not necessarily give any firm indication of whether the underlying process shows compensation for temperature." Clarke (1991) proposed that future studies should avoid using respiration as a tool to investigate thermal (= cold) adaptation. Whilst accepting these comments in principle, the investigations in the present study were not based on acclimation experiments, rather on nudibranchs collected directly from the field at different times of the year, or animals maintained at near-ambient field temperature in the laboratory. These individuals will therefore be acclimatized to ambient temperatures and the data for oxygen consumption will reflect the ability of a species to acclimatize to seasonal changes in ambient temperature.

Respiration represents a 'cost' to the organism and therefore there is no selective advantage in elevating respiration itself. Increasing ATP production will fuel protein synthesis for growth and/or gametogenesis, but at the expense of food reserves or increased consumption. Clarke (1991) emphasizes that increased ATP production to simply 'compensate' for increasing temperature is selectively disadvantageous as it requires an increase in food intake. ATP is utilized in all metabolic processes including growth, gametogenesis, neural activity, muscular activity and excretion. A change in temperature may influence any one (or all) of these processes, which will change the rate of ATP utilization and hence the oxygen consumption. If resources are limiting, the organism may compromise its future survival by elevating ATP production. A seasonal variation in resource availability may itself impose restrictions on respiratory activity and, therefore, low oxygen consumption may be a reflection of reduced ATP demand as a consequence of a lack of a respiratory substrate - the organism 'shuts down'. When resources are more freely available, ATP synthesis can increase to fuel metabolic processes and thus oxygen consumption will rise accordingly - the organism 'opens up'. Thus at low and intermediate temperatures, low oxygen consumption may be a feature of the organism 'shutting down' rather than temperature acting directly to depress these rate reactions. For *Archidoris pseudoargus* in the present study, R at 14.5 °C was determined

on 'adult' individuals in August/September. It is likely that these individuals will be in their maximal growth phase during a period when resources are not limiting. Elevated R at higher temperatures may therefore be a consequence of increased cellular activity in relation to growth and gametogenesis, rather than a simple thermodynamic increase in the rate reactions associated with basal metabolism. At intermediate and low temperatures, resource availability may be low and therefore the organism 'shuts down'.

From Figure 4.4 which displays the relationship between R and temperature for *Archidoris pseudoargus*, although the curve represents an empirical model (*i.e.* a third order polynomial), the shape of the curve may have an important biological interpretation. Organisms have upper and lower lethal limits to temperature (Schmidt-Nielson 1979) above and below which, the organism cannot survive. Figure 4.4 appears to indicate that at temperatures less than 5 °C, R rapidly declines towards 0 - death of the organism. At temperatures above 12 °C, R increases very rapidly which is likely to result in the demand for ATP to exceed supply, eventually leading to death - the organism 'burns out'. In contrast Figure 4.7, which displays the relationship between R and temperature for *Cadlina laevis*, indicates that R is less strongly influenced by temperatures within the 'normal' ambient range.

An organism's ability to adapt to fluctuations in ambient temperature has a considerable bearing on its local and geographical distribution. Between *Cadlina laevis* and *Archidoris pseudoargus* , *C. laevis* has a more northern geographical distribution which may be reflected in the differing response of R at the lowest ambient experimental temperatures. It would appear that *C. laevis* is able to withstand lower ambient temperatures than *A. pseudoargus*. Unfortunately, this hypothesis is not supported by the data at the higher ambient temperatures: if *A. pseudoargus* has a more southern geographical distribution, the species should be better able to withstand higher temperatures. If these arguments are extended to a local scale, a clearer pattern may emerge. Whilst *C. laevis* has been recorded to depths of 800 m below chart datum (Thompson 1988), in Britain this species appears to be more common in intertidal habitats, whereas *A. pseudoargus* is commonly recorded in subtidal habitats (pers. obs.).

Intertidal habitats are subject to more frequent, and a wider range of, ambient temperatures in comparison with subtidal habitats. Intertidal organisms should therefore be more able to withstand fluctuating temperatures than subtidal species. This hypothesis would appear to be supported by the data for *C. laevis* and *A. pseudoargus* presented in Figures 4.4 and 4.7, where the intertidal species displays a more controlled response to temperature.

Newell & Branch (1980) postulated that the metabolic responses of animals to changes in temperature may ultimately depend on the availability of food, both in terms of absolute amounts and predictability, and considered two different physiological responses were possible. When food supply is abundant and predictable, an organism may not be required to control its metabolic rate and can adopt an *exploitative* strategy. If food supply is limiting or unpredictable, an organism may need to adopt a *conservationist* strategy to reduce metabolic losses. Energy conservation could be effected by reducing oxygen consumption, reducing its acute temperature dependence, or by acclimatization. Branch & Newell (1978) and Branch (1979) found that for the gastropod genus *Patella*, respiratory adjustments were most important for species experiencing food shortage. Brown & Da Silva (1984) found the respiration rate of *Bullia digitalis* to be relatively independent of temperature between 10 to 25 °C and considered this species to be an *energy conservationist*. A 'plateau' in the centre of the respiration rate/temperature relationship, indicating acclimatization to increasing temperature, was estimated to represent a 75% saving at 20 °C. Iglesias & Navarro (1991) found no evidence of temperature compensation in the intertidal bivalve *Cerastoderma edule* which resulted in reduced growth over summer months. Similarly, Widdows & Shick (1985) observed reduced growth in the same species due to relatively high respiration during periods of tidal emersion. Thus, compensation of R with increasing temperature may be an important adaptation to maintain an energetic allocation to production (somatic and/or gametic), during periods of tidal emersion.

For a species to survive longer than one season, it must be able to withstand the full range of ambient environmental conditions. If resources are finite, perhaps due to

seasonality of prey abundance or prey quality, reducing respiratory costs will permit a continued allocation of energy to growth and reproduction. In addition, by chance alone, the longer an individual survives the more likely it is that it will encounter interruptions to its energy supply. If an individual is unable to overcome interruptions to its energy supply, it will not survive. If death occurs during the pre-reproductive phase, the individual will not contribute to the subsequent generation and hence its fitness will be zero. For the nudibranch species discussed above, *Cadlina laevis* and *Archidoris pseudoargus* live longer than one year and appear to have the necessary adaptations to adopt a 'conservationist strategy' toward respiratory costs. Both species exhibit acclimatization of respiration rate with increasing temperature in comparison to annual species; *C. laevis*, the longer lived species, exhibits the greater independence between respiration rate and temperature.

CHAPTER 5

REPRODUCTION

5.1 Introduction

Reproduction is defined in the *Oxford English Dictionary* to be 'the act of generating offspring'. It has been repeatedly stated that natural selection acts to maximize the fitness of an organism, where fitness is defined as the number of descendants produced by an organism which themselves survive to reproduce in subsequent generations. Therefore the fundamental components of fitness are *reproduction* and *survival*. The number of offspring produced, the number of offspring fathered and the proportion of these offspring which survive to maturity are the factors that determine how many descendants are left by a genotype expressing a particular life history pattern. The life history of an organism is the schedule of events from birth through to death via growth and reproduction. Therefore those life history events which maximize the number of offspring under ambient environmental conditions may be defined as the optimum for those conditions (although see Hodgkin & Barnes 1991: discussed later). But what are the life history events, and how can selection act on these events to enable an organism to evolve an optimum life history and to maximize its fitness?

Fitness of the average individual ought to rise in proportion to the number of offspring produced but in reality, reproduction incurs a cost in terms of growth and survival (for review: Calow 1979; Reznick 1985). Organisms have only finite resources available to complete their life cycle which have to be partitioned between the competing demands of growth, metabolism and reproduction. Sibly & Calow (1986) considered two models to account for the costs of reproduction: *absorption costs* which are only realized after gamete release, and *direct costs* which form part of the organisms immediate energy budget. Selection ought to favour delaying the cost of reproduction for excess *direct costs* could lead to no viable offspring being produced. Sibly & Calow (1986) considered reproductive cost, and the optimum combination of life history variables to maximize fitness. To maximize fitness there will be trade-offs between life history variables. Four fundamental trade-offs can be identified prior to reproduction (after Sibly & Calow 1986):

i Trade-off between fecundity and the time to first breeding.

ii Trade-off between investment in reproduction and post-reproductive survival of the parents.

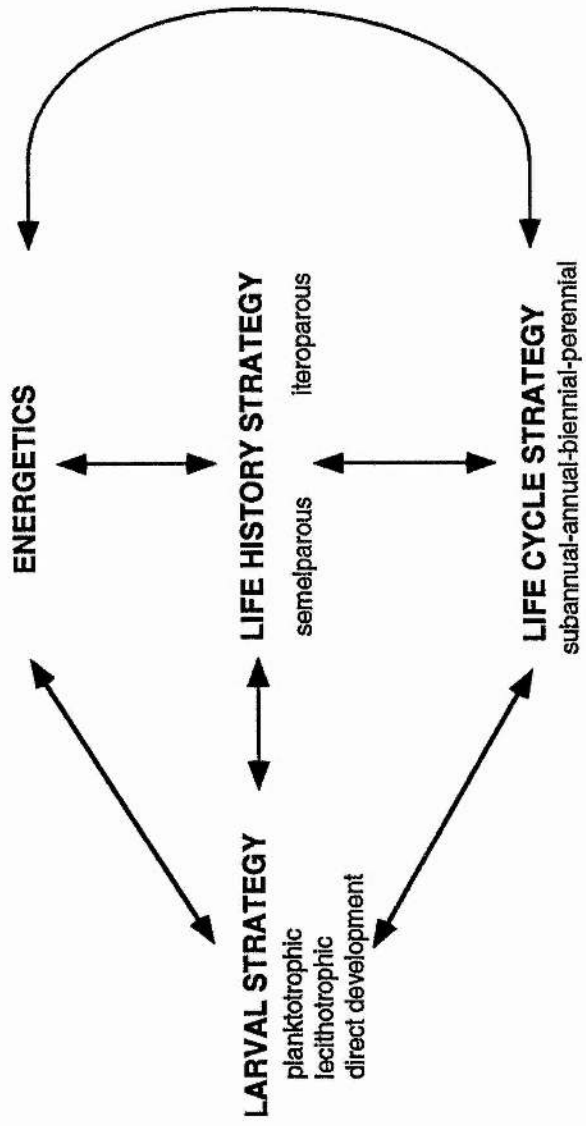
iii Trade-off between fecundity and the survival of the offspring.

iv Costs of parental care.

The outcome of these trade offs, whether in isolation, or in combination, can have a profound effect on the fitness of the organism. For marine invertebrates, these trade-offs can be interpreted as follows. Time of first reproduction relates to the *life cycle* of an organism which is usually defined in terms of years, namely sub-annual (< one year), annual (one year), biennial (two years) or perennial (>two years). A trade-off between investment in current reproduction and the post-reproductive survival relates to the dichotomous reproductive modes of *semelparity* - a single massive reproductive event followed by death, or *iteroparity* - repeated reproduction, usually over a number of years. A trade-off between fecundity and survival of the offspring relates to the mode of larval development: for a finite energetic allocation, is this energy partitioned into many small eggs or fewer large eggs. Marine invertebrate embryos/larvae develop via three basic modes: planktotrophic, lecithotrophic and direct development (a discussion of trade-offs in relation to larval biology is given in Chapter 6, and will not be considered in the current Chapter). Parental care is a rather broad area which can be interpreted at a number of levels from the cost of protecting the gametes (*e.g.* internal fertilization, egg cases), through to parents actively protecting offspring (*e.g.* brooding). The terms *reproductive strategy* or *life history strategy* (Todd 1985b) have been differentiated to describe the suite of trade-offs made by an organism. Figure 5.1 (after Todd 1985b) summarises these reproductive strategies, and stresses the inevitable inter-relationships between these criteria.

During the past twenty years, a considerable amount of research effort has been directed towards identifying a unifying theory of life history strategy, to explain the myriad of combinations of reproductive traits adopted by organisms (for review: Stearns 1976, 1977, 1989; Sibly 1991). Life history strategies of marine invertebrates have

Figure 5.1 The inter-relationships between the traits comprising a reproductive strategy; terms in lower case represent the evolutionary options available for that trait (after Todd 1985b).


ENERGETICS
LIFE HISTORY STRATEGY
semelparous
iteroparous
LIFE CYCLE STRATEGY
subannual-annual-biennial-perennial
LARVAL STRATEGY
planktotrophic
lecithotrophic
direct development

featured prominently in this context and many detailed investigations have been undertaken (for review: Jablonski & Lutz 1983; Day & McEdward 1984; Grahame & Branch 1985; Strathmann 1985; Todd 1985b). Two main theories have been proposed to account for life history evolution: *r*-K selection theory (MacArthur & Wilson 1967), and bet-hedging theory (Murphy 1968; Schaffer 1974; reviewed by Stearns 1976). *r*-K theory is based on determinate schedules of mortality and fecundity and emphasizes the importance of environmental predictability and abiotic factors on populations. It predicts that for populations dominated by density-independent mortality and subject to fluctuating environmental conditions, there will be *r* selection for early maturity, high reproductive effort (RE) and large numbers of small offspring. At the other extreme, the connections between environmental stability, density-dependent mortality and and biotic factors predict that 'K' selection will favour delayed maturity, low (periodic) RE and small numbers of 'quality' offspring. *r*-K theory has been the subject of a considerable number of investigations although no satisfactory conclusion has been reached (Grahame 1977; Hughes & Roberts 1980; Suchanek 1981; Hart & Begon 1982; Atkinson & Newbury 1984).

Bet-hedging theory is based on the differential rates of juvenile and adult mortality and variation thereof. It predicts that an environment which confers constant predictability of juvenile survivorship (relative to the adult) should favour species of short life and high RE, which reproduce at an early age. Conversely, an environment which confers more variable juvenile survivorship (in contrast to the adult) should favour long lived, large bodied species which make a small, but repeated and temporally extended allocations to reproduction. Both theories have been subject to intense debate without any satisfactory conclusion. At present the consensus is in favour of bet-hedging, on the grounds its predictions are based on selection at the level of the individual rather than population effects, and its predictions are falsifiable: K cannot realistically be expressed as a function of life history traits (Stearns 1977).

Many aspects of the reproductive strategies of marine molluscs have been investigated (for review: Grahame & Branch 1985; Todd 1985b), and reproduction and

reproductive strategies of opisthobranchs, and nudibranchs in particular, have received considerable attention (for reviews: Todd 1981, 1983; Hadfield & Switzer-Dunlap 1984; Hadfield & Miller 1987). Investigators have undertaken studies on each of the fundamental trade-offs in isolation and in combination, although few studies have addressed the whole issue. Based on the premise that selection of life history strategies is linked to energy budgets (Ebert 1982), Havenhand (1986) completed a detailed investigation into the life history strategies of the nudibranchs *Adalaria proxima* and *Onchidoris muricata* (see also: Havenhand & Todd 1988a&b, 1989b; Todd & Havenhand 1988). But all investigations of nudibranch reproduction have, to date, considered annual (or sub-annual) semelparous species.

Archidoris pseudoargus, *Tritonia hombergi* and *Cadlina laevis* all live longer than one year, and *Cadlina laevis* has further adopted an iteroparous mode of reproduction. The central aim of the present investigation was to investigate the reproduction of these species to attempt to elucidate why they have adopted different fundamental trade-offs. Prior to describing the investigations in detail, I will describe some of the theory relating to reproduction.

In Chapter 3 it was noted that the energy available to production is partitioned between somatic growth and gonadal production. Towards maturation, resources are diverted away from somatic growth and therefore reproduction has a cost. Calow (1979) stated that in general there was a negative correlation between current reproductive investment and future growth, survival and reproduction. Thus the proportion of an organism's resources invested in current reproduction is critical: *Reproductive effort* (RE) is defined as that proportion of metabolic resources devoted to reproduction (Lincoln *et al.* 1982), and was recognized by Fisher (1930) to be an adaptive trait. RE is central to ecological theory relating physiological processes to life history strategies (*e.g.* Williams 1966a&b; Tinkle 1969; Pianka 1970; Schaffer 1974; Stearns 1976, 1977), and has been used in attempts to explain the evolution of semelparity or iteroparity (*e.g.* Schaffer 1974, 1979; Calow & Woollhead 1977; Browne & Russell-Hunter 1978; Calow 1979, 1983; Aldridge 1982). For marine invertebrates, RE has been linked to the

evolution of larval development modes (Day & McEdward 1984; Grahame & Branch 1985; Strathmann 1985 Clarke *et al.* 1991), and intraspecific variation in reproductive output with latitudinal gradients (Clarke 1987b). Many of the problems related to the interpretation of RE in the context of differences in life history strategies have been attributed to the method employed to calculate the statistic. Havenhand & Todd (1989b) reviewed these ideas and discuss RE in relation to nudibranch molluscs. They propose a new statistic, the *reproductive index* which is a weight specific measure of the balance between somatic and gonadal production (although see Grant 1990; Willows 1990; and Todd & Havenhand 1990: for a reply to Grant).

If resources are limiting, should an organism invest all its energy into a single reproductive event - semelparous or 'big-bang' reproduction - or spread its investment over a number of reproductive events - iteroparous reproduction? Cole (1954) investigated the fecundity schedules of semelparous and iteroparous organisms to determine the conditions which might favour each schedule. 'Cole's result' suggests an ideal semelparous organism could attain the same fitness as an iteroparous organism merely by elevating its fecundity by one, but Cole (1954) assumed that there is no adult or juvenile mortality. Charnov & Schaffer (1973) incorporated juvenile and adult survivorship into the model and developed a more realistic formulation, although 'Cole's result' could still occur if the probability of juvenile survivorship to maturity (c) is equal to the probability of an adult surviving between reproductive seasons (p). Charnov & Schaffer (1973) concluded that:

$$B_s = B_i + p/c$$

where B_s and B_i are the fecundity of semelparous and iteroparous organisms respectively. Therefore when $p < c$ a semelparous strategy is favoured, and conversely when $p > c$ an iteroparous strategy is favoured.

From the preceding arguments it would appear that the evolution of a reproductive mode is relatively straightforward and depends on age-specific mortality. A proportion of species live longer than one year and yet remain semelparous. Anadromous and

catadromous fish may spend many years in the adult habitat prior to making a migration to the juvenile habitat for a single, and fatal, massive reproductive event. A similar phenomenon is recorded in the plant kingdom where species may live >100 years prior to a single massive reproductive event (Young & Augspurger 1991). Of greatest interest in the context of the present study is the question of biennialism, whereby every year, a lineage of strict facultative biennials 'misses' an opportunity to breed. A biennial organism must produce more offspring than an annual or a perennial iteroparous organism to maintain itself in a selective contest.

Biennialism has been most thoroughly discussed using examples drawn from the plant kingdom. Hart (1977) identified the incidence of biennialism within the North American flora to be only 1.4% and posed the question 'Why are biennials so few?'. Hart (1977) extended 'Cole's result' to identify when a biennial life history might be favoured, and proposed that:

$$\lambda_b = (C_1 C_2 S_b)^{1/2}$$

where C_1 and C_2 represent adult and juvenile survivorship respectively, S_b the fecundity of the biennial and λ_b the rate of population growth. When C_1 and C_2 are the optimum for the biennial, for λ_b to equal that of annual/perennial, S_b has to be twice that of an annual and four times that of a perennial. These conclusions depend on optimum conditions, which will presumably occur only within a narrow environmental range. Hart (1977) suggested that a biennial strategy is ideally suited to exploiting a resource that is seasonal or only intermittently available. Silvertown (1983) re-evaluated the data for biennial plants and concluded that biennialism is more common than was at first thought, especially in species that utilise resources that are spatially and temporally variable but where available, are stable for a period of time: for instance gaps in forest canopies created by the death of a canopy individual. De Jong *et al.* (1987) reviewed the factors favouring a biennial life cycle in plants and concluded that biennials are favoured where the growing season is short; this idea was supported by the observation of Lacey (1986), that biennials are more common with increasing latitude and/or altitude. If *Archidoris pseudoargus* and *Tritonia hombergi* are to maintain their fitness, they will have to

maximize their life history strategies to ensure that sufficient juveniles are produced to offset the cost of 'missing' a reproductive opportunity.

For iteroparous reproduction, selection ought to favour traits which maximize the lifetime fecundity rather than each reproductive event. Nonetheless, each reproductive event will still have a cost, which may compromise the prospects for future survival; as age increases, the prospects for future survival decline and therefore at some point, an organism should adopt the 'big-bang' approach and invest everything in one final reproductive event. Williams (1966) proposed the idea of 'residual reproductive value' (RRV) which is the sum of the current reproductive effort (RE), combined with an estimate of all the future reproductive episodes. In general, it is considered that RE should increase with age or $^{1}/_{RRV}$ (Williams 1966; Pianka 1970). Perron (1982) recorded an increase in RE with age in the gastropod genus *Conus*, but Vahl (1981) could find no relationship between RE and RRV in the scallop *Chlamys islandica*. For *Cadlina laevis* undergoing iteroparous reproduction, is there evidence that individuals allocate more energy to reproduction as they get older?

For marine invertebrates, the trade off between fecundity and the survival of offspring is very complex as it encompasses the evolution of the mode of larval development and, within a particular mode, how egg size influences offspring survival. A thorough discussion of larval development will follow in Chapter 6 but the question of variations in egg size are pertinent to the current Chapter. Where an adult invests a finite amount of energy in reproduction, in simplistic terms, this energy can be apportioned between a large number of small eggs or a small number of large eggs. Larval development mode and egg size are linked where small eggs tend to give rise to planktotrophic larvae, and large eggs tend to give rise to lecithotrophic or directly developing larvae. Egg size can be viewed as a predictor of parental investment and the commitment of the parent to the future survival of the offspring. Vance (1973a&b) proposed a model to address selection for egg size in relation to parental investment and environmental conditions. In Vance's model, selection is assumed to maximize the number of offspring settling into the adult habitat in relation to the total energy committed

by the parent. It predicts that only the extremes of egg size to be evolutionary stable, and both extremes can coexist and be stable under some larval mortality and growth rates. To overcome one of the limitations of this model, Vance introduced a minimum egg size for feeding larvae. The model proposed by Vance (1973a&b) has been subject to considerable debate which is summarised by Strathmann (1985) and Todd (1985b). In particular, Todd (1985b) criticised the model on its fundamental assumption that selection will favour that strategy which results in the highest reproductive efficiency, concluding that selection will act on the production of absolute numbers of viable offspring, even if these are produced inefficiently. Christiansen & Fenchel (1979) proposed a further model to consider the evolution of egg size in relation to reproductive effort (RE) which was based on the premise that there is an evolutionary trade-off between fecundity and larval survivorship, and that this trade-off can be formulated with respect to RE. This model made similar predictions to Vance's earlier model in that only the extremes of egg size are evolutionarily stable. Unfortunately, Christiansen & Fenchel's model assumed particular size-growth-mortality relations without supporting data (Strathmann 1985).

Classically, life history theory has used egg size as a predictor of parental investment (see above) assuming that larger eggs contain more organic material and that there is little intraspecific variation in egg size or organic content. Strathmann & Vedder (1977) recorded a significant correlation between egg size and organic content among species with small eggs (≈50 - 200 μm diameter). Recently, investigations into variations in egg size have considered intraspecific variations. McEdward & Coulter (1987) recorded considerable intraspecific variation in egg size and organic content (with no correlation between these variables) for two species of starfish, albeit with a small sample size. McEdward & Carson (1987) reported the results of an extensive study of the variation in egg size and organic content, and the inter-relationship thereof, for the starfish *Solaster stimpsoni*. *S. stimpsoni* had considerable variation in egg size and organic content at all levels of intraspecific comparison from siblings to inter-population variation. Clarke *et al.* (1991) investigated intraspecific variations in egg size within populations of the deep water prawn *Pandalus borealis* and, in particular, whether the mode of larval development (= egg size) and reproductive effort are linked. Data

suggested that egg size and organic content increased with latitude but both egg size and reproductive effort varied from one location to another and were not correlated. Clarke *et al.* (1991) concluded that it will be very difficult to test hypotheses concerning the relationship between egg size and parental investment with field data, and therefore investigate one of the fundamental trade-offs in reproduction.

A trade-off which has attracted considerable attention is the cost of parental care, and for marine invertebrates, the question of brooding, its link with the allometry of body size and reproductive output. Larger adults can allocate, in absolute terms, more energy to reproduction and therefore by inference, have a higher potential fecundity. Menge (1975) considered that a planktotrophic mode of larval development for *Pisaster ochraceus* allows the opportunity of exploiting temporally and/or spatially unpredictable areas of high quality prey. In contrast, very much smaller adults of *Leptasterias hexactis* are incapable of producing sufficient gametes to offset planktonic mortality and therefore constrained to exploit less variable resources. Brooding is associated with small body size for many co-occurring species of marine invertebrate, although the trend is not universal. Strathmann & Strathmann (1982) and Strathmann *et al.* (1984) investigated the question of brooding and small adult size and further extended the link to brooding with simultaneous hermaphroditism. A number of hypotheses were proposed but none provided a universal explanation, and it would appear that observed trends are the result of selection for different life history traits in different taxa. All nudibranch molluscs have internal fertilization followed by the production of a benthic egg mass; there are no reported examples of extended parental care (Hadfield & Switzer-Dunlap 1984). But in the context of the present study, the question of brooding versus free spawning could be considered in its broadest context as equivalent to planktotrophy versus direct development.

Levitan (1991) questioned the use of body size as an indicator of reproductive success for free-spawning species and drew attention to the relationship between fertilization success and population density. Although there is a clear allometric relationship between body size and gamete output, a large, free spawning gonochoric

individual in isolation will have a high gamete output but low fertilization success and hence low zygote production. In contrast, small individuals at a high population density will have a relatively lower (absolute) gamete output but the high density of conspecifics will result in a greater fertilization success and higher zygote production. For free-spawning species, body size may not be a good predictor of zygote production. For species living at low densities, methods to improve fertilization success will have considerable adaptive significance to increasing fitness. Thus free spawning species, breeding aggregations, chemical attraction/location of conspecifics, and physical and/or chemical cues to synchronise gamete release are all mechanisms to improve fertilization success. A logical next step is the development of internal fertilization, together similar mechanisms to bring individuals together, which will have adaptive significance to improving fertilization success. The vast majority of opisthobranch species are functional simultaneous hermaphrodites with cross fertilization the general rule (Hadfield & Switzer-Dunlap 1984). Hermaphroditism can be viewed as a further adaptation to improving fertilization success, particularly for mobile individuals living at low density. A low density model has been proposed to explain the evolution of hermaphroditism: by being hermaphrodite, individuals living at low density overcome the risk that when two gonochoric individuals meet they are the same sex. Whilst the ideas of Levitan (1991) are important when considering the body size/fecundity relationship in the context of the life history of a free spawning species, for species with internal fertilization, body size will be an important determinant of the fitness of the individual.

One final aspect of life history strategies which has received little attention is the question of 'egg to juvenile periods' or EJPs. For short lived species, the total time spent within an egg capsule and/or as a larva can form a significant proportion of the life cycle, and reducing the EJP will increase the time available to the benthic phase. If an organism reproduces within a fixed time frame, a shorter EJP will result in a longer benthic phase and thereby maximize the opportunity to attain the final body size. If an organism reproduces at a fixed body size, reducing the EJP can reduce the total generation time. Hodgkin & Barnes (1991) noted that the 'wild type' of the nematode *Caenorhabditis elegans* gained a selective advantage over a more fecund congeneric mutant by virtue of a

shorter generation time. Havenhand (In press) has developed a model to examine the impact of changing the EJP on the evolution of life history, or more specifically the larval development strategy. Whilst important in the context of life history strategies, the concept will be considered more fully in Chapter 6.

5.2 Methods

Nudibranchs were collected from the field and maintained in the laboratory following the methods described in Chapter 2. To permit cross fertilization, animals were maintained in pairs or occasionally in larger groups, and where possible, animals with contrasting marking were kept together to permit identification and separation of individuals. Occasionally during field visits, animals would be found in the process of spawning or closely adjacent to egg masses; for the latter, it was assumed that the individual had recently completed spawning. These animals and their egg masses were collected and returned to the laboratory for further analysis. Age of each egg mass was determined from its' cleavage stage. During the present study, spawning data were collected for 33 *Archidoris pseudoargus*, 27 *Tritonia hombergi* and the laboratory population of *Cadlina laevis*.

During the spawning season, nudibranchs were examined daily to ensure spawning individuals and their egg masses were correctly identified. Animals move away from the egg mass after spawning has been completed, and in containers with more than one animal, it was important to correctly identify the spawning individual. At the earliest opportunity, the egg mass was carefully excised from the container using a sharp scalpel and fine forceps taking care to avoid damaging its integrity. Most egg masses have a thicker gelatinous border in contact with the substratum which facilitates removal without damage. Both the animal and its egg mass were weighed under water and the animal returned to the aquarium. Live weights were converted to dry weights and thence to energy equivalents following the methods described in Chapter 2.

Cadlina laevis produces an egg mass with large yolky eggs. These egg masses were squeezed gently between two microscope slides to produce a near monolayer which

permitted the number of eggs to be enumerated via a *camera lucida* attachment on a Wild™ M8 stereo microscope. A proportion of the *Cadlina laevis* egg masses were weighed (under water), rinsed in 0.9% (w/v) Ammonium formate, frozen, freeze-dried and reweighed to provide data for a live weight/dry weight conversion. Dry material was used for calorimetric and gravimetric analysis (see Chapter 2).

Developing egg masses were returned to the aquarium where they were retained in clean containers. A high water flow was maintained at all times to ensure complete aeration; additional aeration via an 'air stone' was added near the time of hatching to facilitate liberation of the larvae. Egg masses required for larval culture (see Chapter 6) were kept in beakers of aerated sterile seawater to reduce bacterial and cilliate infection of subsequent larval cultures. Developing egg masses were regularly examined under a binocular microscope to observe the development process.

Spearman rank correlation coefficients were calculated to determine an statistically significant relationship between body size and spawn output for the three nudibranch species. Data were analysed for normality, transformed if necessary and fitted to geometric mean regression models to further elucidate any relationship between the body size and spawn output.

Instantaneous measures of the reproductive effort (RE) were calculated using both a spawn to soma ratio and the equation of Browne & Russell-Hunter (1978)

$$RE = \frac{P_g}{P_g + P_s}$$

where P_g = Spawn output and P_s = Somatic production (body size).

5.3 Results

Reproductive data for *Archidoris pseudoargus*, *Tritonia hombergi* and *Cadlina laevis* are presented separately, followed by data for the reproductive effort for all three species together.

5.3.1 *Archidoris pseudoargus*

A total of 33 spawning events were recorded and these data summarised in Table 5.1. Adult *Archidoris pseudoargus* were observed copulating in October/November but did not begin to spawn in mid-March, spawning continued until early June. Occasional egg masses were produced during the winter but were probably an artifact due to laboratory conditions; winter egg masses were not observed in the field (see also Todd 1977). Copulation was an extended process with individuals remaining paired for long periods: copulation trials recorded individuals remaining paired for trials lasting nine days. Whilst not quantified, observation over the winter period suggested that individuals spent approximately 50% of the time paired, presumably copulating. Most animals produced a single egg mass, with only one animal producing two egg masses; in the latter case, the second spawn was very small, amounting to only 3%, weight for weight, of the size of the first egg mass. Post spawning mortality was 100%, animals rapidly decrease in size and generally died within 7-14 days post-spawning.

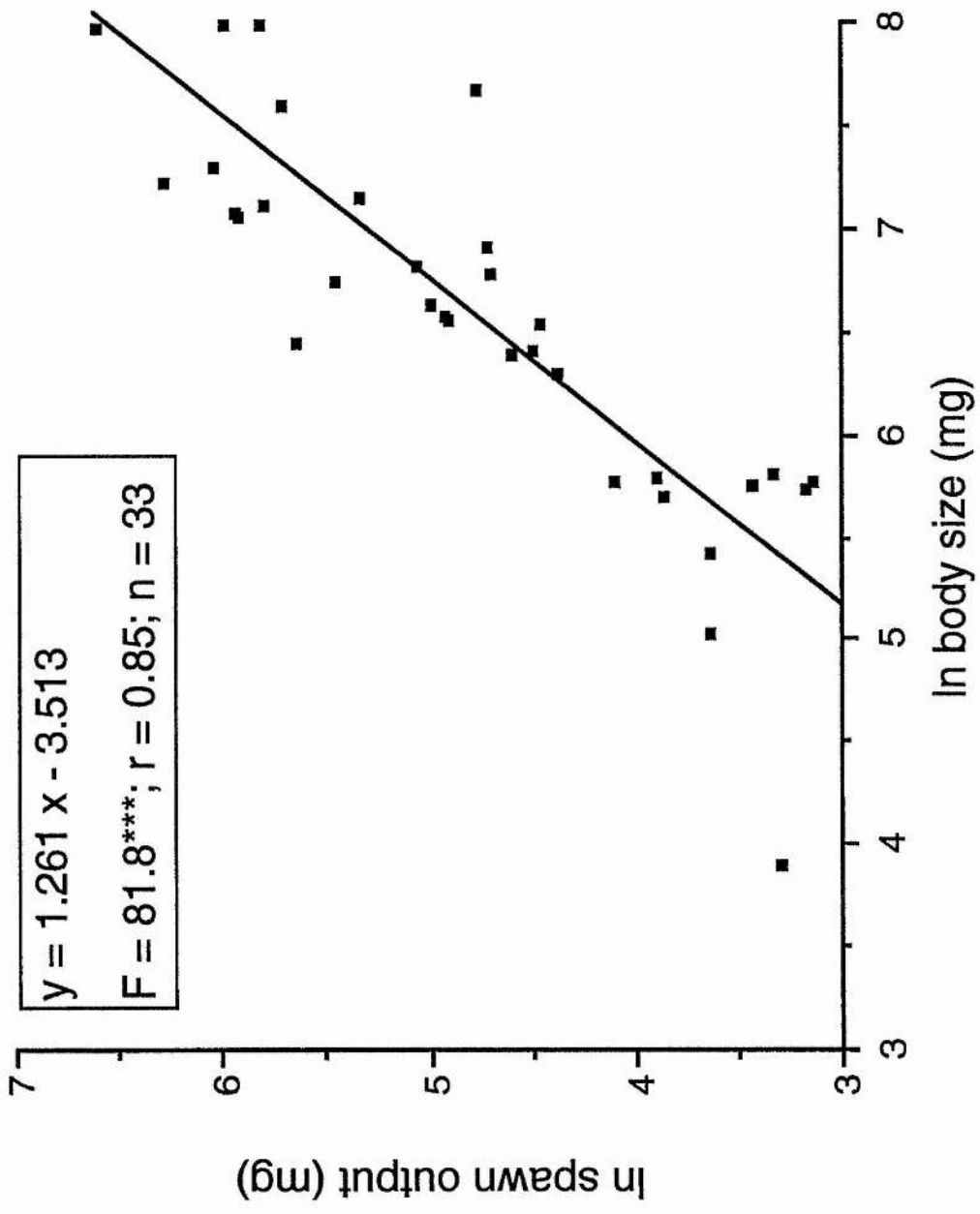
Egg masses are a creamy white colour and comprise a large, single ribbon approximately 1 cm wide and formed into a coil; a single egg mass can have up to five whorls with a total diameter of up to 8 cm. Egg number was not enumerated and published estimates vary from 50,000 (Alder & Hancock 1845-55) to 654,000 for a very large egg mass (Colgan 1914); Thompson (1966) estimated there to be 554 ova per 1 mm length of spawn ribbon. Egg diameter ranged from 150 to 175 μm, often with multiple ova within each egg capsule. Free swimming, planktotrophic veliger larvae hatch from the egg mass after 21 days at 14.5 °C.

A conversion from live weight to dry weight was not determined for *Archidoris pseudoargus* spawn and therefore to determine the relationship between body mass and spawn output, weight under water was employed (Figure 5.2). Spearman rank correlation coefficient analysis indicated a highly significant relationship between spawn output and body size. A best fit geometric mean regression was obtained when data were transformed to natural logarithms:

Table 5.1 A summary of all reproductive data for *Archidoris pseudoargus*, *Tritonia hombergi* and *Cadlina laevis*. All measurements of body size and the spawn output of *C. laevis* are dry weights; the spawn outputs for *Archidoris pseudoargus* and *Tritonia hombergi* are live weights (under water).

Species	N	Body size (mg)		Spawn output (mg)		Spearman
		Range	Mean ± 1 se	Range	Mean ± 1 se	coefficient
Archidoris pseudoargus	33	175.3-14212.7	4445.9± 681.9	23-736	186 ± 29.9	0.888***
Tritonia hombergi	31	1327.2-8291.4	4706 ± 566.9	24-222	106.8 ± 11.4	0.224^{ns}
Cadlina laevis	63	13.9-203.5	89.9 ± 5.7	1.4--71.4	19.5 ± 1.7	0.718***

Figure 5.2 The relationship between body size and spawn output (both as weight under water) for *Archidoris pseudoargus* for 33 spawning events (see summary data in Table 5.1); data are transformed to natural logarithms ($\log_e$). The line represents the 'best fit' geometric mean regression between the variables which yielded a significant allometric relationship.


y = 1.261 x - 3.513
F = 81.8***; r = 0.85; n = 33
ln spawn output (mg)
ln body size (mg)
3
4
5
6
7
8

$$\text{Log}_e \text{ spawn output} = 1.261 \cdot \log_e \text{body size} - 3.513$$
$$F = 81.8***;\ r = 0.85;\ n = 33$$

or: $$\text{Spawn output} = 0.03 \cdot \text{body size}^{1.261}$$

An exponent greater than unity suggests that larger animals produce proportionally larger egg masses in comparison to smaller individuals.

5.3.2 *Tritonia hombergi*

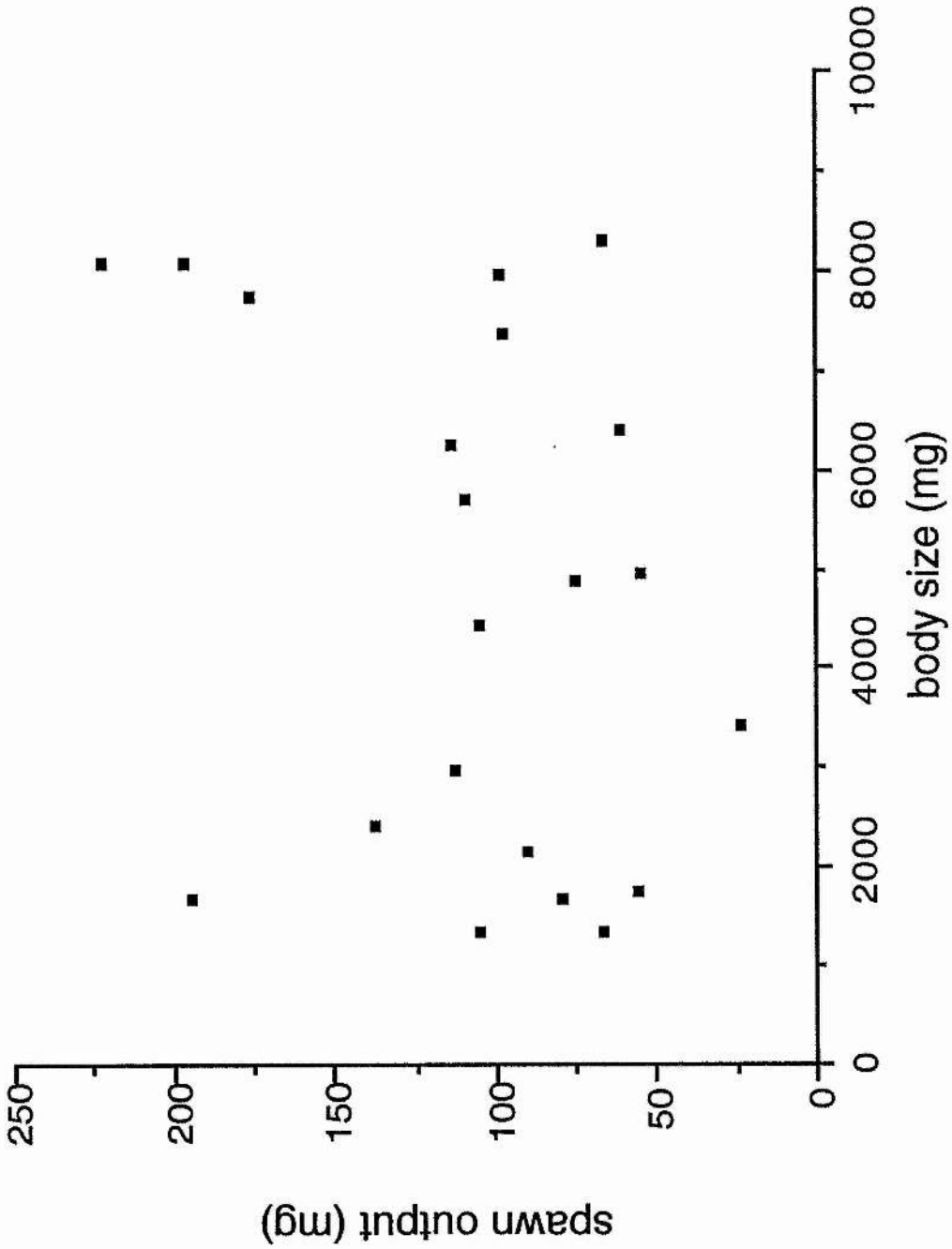
Adult *Tritonia hombergi* begin to spawn in March, continuing until early June, and individuals produced a single egg mass, or very occasionally two egg masses. Post-spawning mortality was 100%. Egg masses comprise a gel tube filled with large pink to white coloured yolky eggs; the tube is attached to the substratum along one edge. Eggs are not embedded in a gel matrix and if the gel tube is damaged, eggs can be liberated. Hatching occurred after 30 days at 14.5 °C with the release of pelagic lecithotrophic veliger larvae.

A total of 31 spawning events were recorded and these reproductive data are summarised in Table 5.1. Rather surprisingly, a Spearman rank correlation analysis indicated no significant relationship between body size and spawn output (Figure 5.3). Egg numbers were not enumerated and therefore it is not possible to determine whether there is any significant correlation between egg number and body size.

5.3.3 *Cadlina laevis*

Cadlina laevis individuals spawn between December and February (exceptionally out-with these months: <10% during present study) producing a single egg mass each spawning season. An egg mass comprises a small, solid gel tube with large (approximately 400 µm diameter) white/cream coloured, yolky eggs set into the gel matrix; the tube is formed into a coil with a diameter of up to 20 mm. Development is completed within the egg capsule, the embryos hatching directly as crawling benthic juveniles. During the course of the present study, 16 adults spawned in more than one season: two adults spawned four times, ten adults spawned three times and six adults

Figure 5.3 A graphical presentation of the data for body size (dry weight) and spawn output (weight under water) for *Tritonia hombergi*; data are summarised in Table 5.1. No significant relationship was determined between these two variables (Spearman's coefficient = 0.224^{ns}).


spawn output (mg)
250
200
150
100
50
0
0
2000
4000
6000
8000
10000
body size (mg)

spawned twice. For these 16 individuals, the mean interval between spawning events was 340 ± 19.4 days and in general, individuals spawned in the same month each year.

A total of 63 spawning events were recorded during the present study and these reproductive data are summarised in Table 5.1. Egg number within egg masses ranged from 63 to 2426 with a mean (± 1 se) 665 ± 62. Egg number is strongly correlated with the size of the egg mass (Spearman rank correlation coefficient = 0.853***; Figure 5.4). A geometric mean regression model was fitted to the data and the relationship between the size of the egg mass and egg number was explained by the expression:

$$\text{Eggs number} = 36.14 \cdot \text{size of egg mass} - 39.42$$

$$F = 345.5^{***};\ r = 0.92;\ n = 63$$

Body size and spawn output (as energy equivalents) were strongly correlated (Figure 5.5) and a best fit geometric mean regression was obtained when data were transformed to natural logarithms:

$$\text{Log}_e \text{ spawn output} = 1.495 \cdot \text{log}_e \text{ body size} - 4.765$$

$$F = 109.48^{***};\ r = 0.80;\ n = 63$$

or $$\text{Spawn output} = 0.009 \cdot \text{body size}^{1.495}$$

Body size and number of eggs were less strongly correlated (Spearman rank correlation coefficient = 0.644***; Figure 5.6) and a best fit geometric mean regression was obtained when data were transformed to natural logarithms:

$$\text{Log}_e \text{ egg number} = 1.585 \cdot \text{log}_e \text{ body energy} - 5.329$$

$$F = 51.2^{***};\ r = 0.69;\ n = 63$$

or $$\text{Egg number} = 0.0048 \cdot \text{body energy}^{1.585}$$

For both body size and total spawn output and body size and egg number, the exponent of the relationship is greater than unity, suggesting that larger individuals have an proportionally greater fecundity than smaller individuals.

Figure 5.4 The relationship between the size of a *Cadlina laevis* egg mass (dry weight) and its corresponding number of eggs. The line represents the 'best fit' geometric mean regression between the variables which yielded a significant relationship.

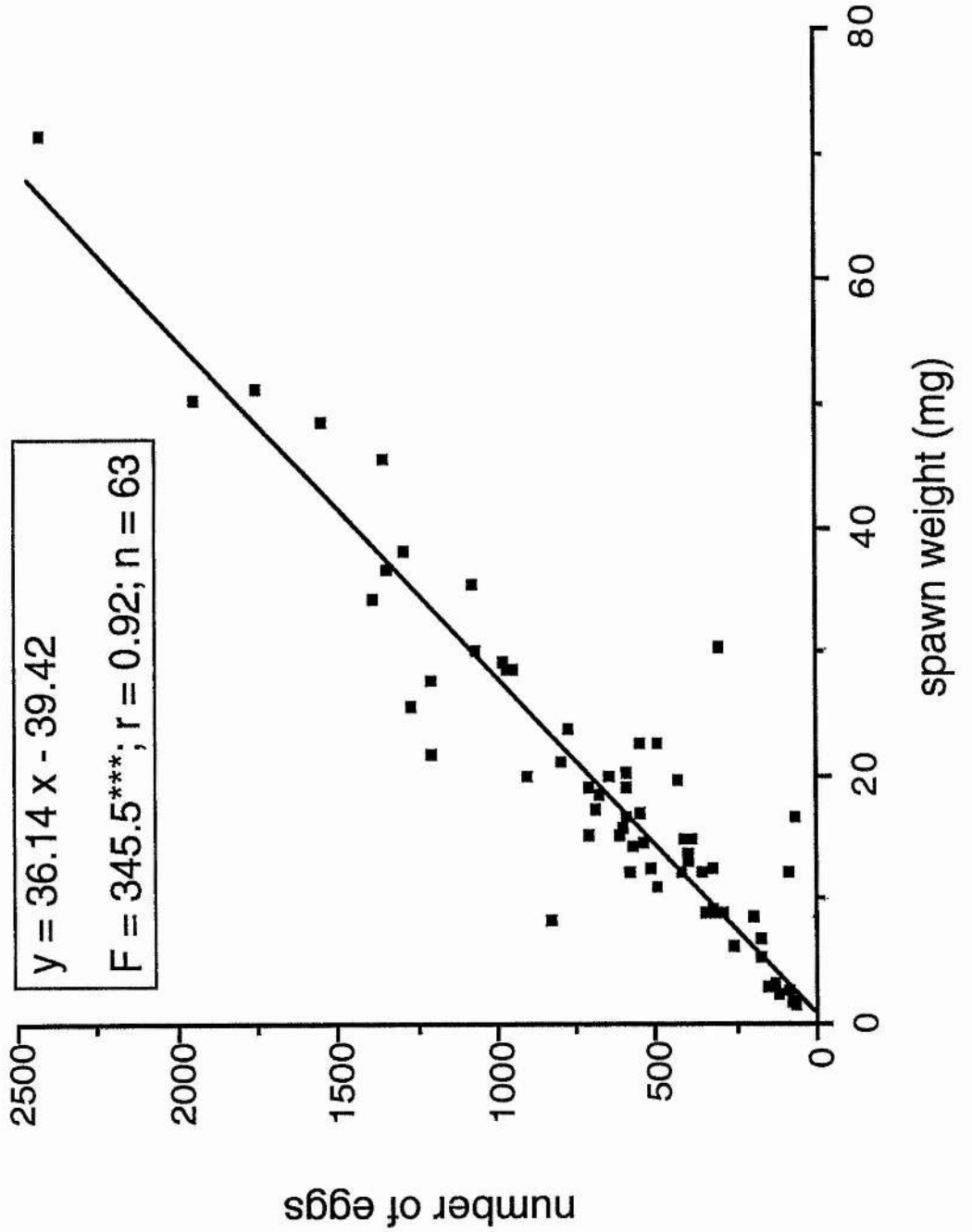

y = 36.14 x - 39.42
F = 345.5***; r = 0.92; n = 63
number of eggs
spawn weight (mg)
0
500
1000
1500
2000
2500
0
20
40
60
80

Figure 5.5 The relationship between body size and spawn output (both as energy equivalents) for *Cadlina laevis* (data are summarised in Table 5.1); data are transformed to natural logarithms ($\log_e$). The line represents the 'best fit' geometric mean regression between the variables which yielded a significant allometric relationship.

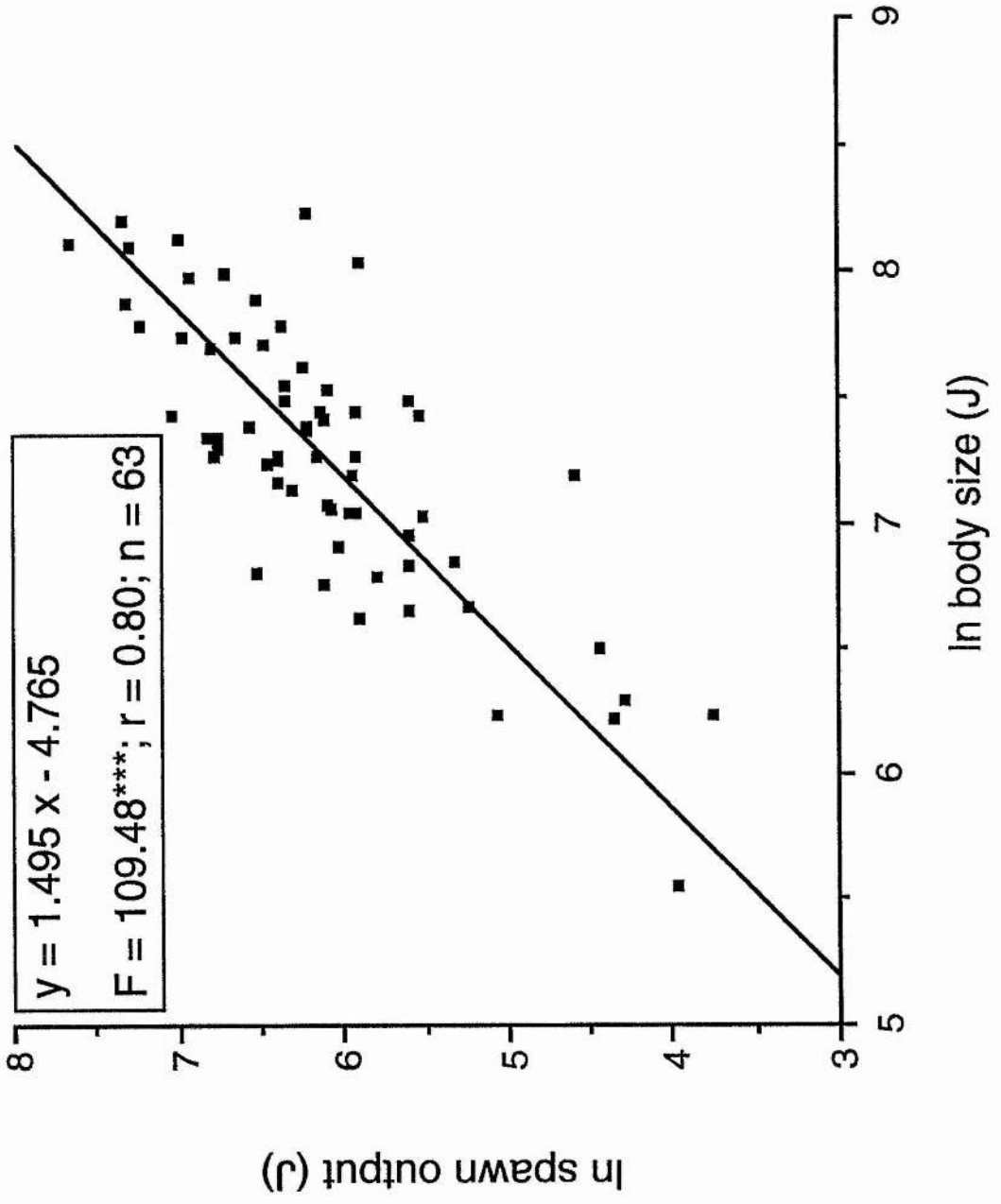
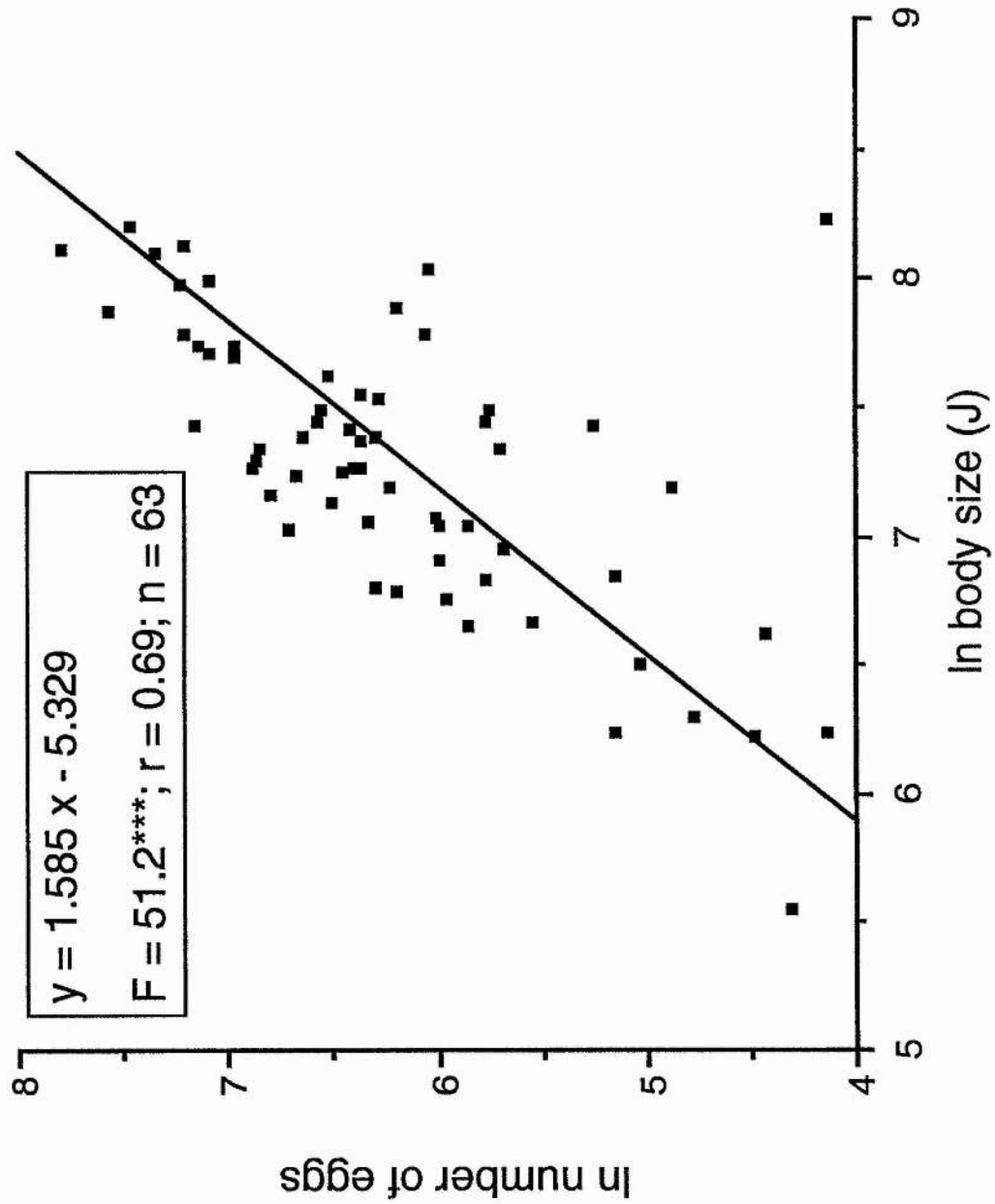

y = 1.495 x - 4.765
F = 109.48***; r = 0.80; n = 63
ln spawn output (J)
ln body size (J)
8
7
6
5
4
3
5
6
7
8
9

Figure 5.6 A graph illustrating the relationship between body size (as an energy equivalent) and the number of eggs within a egg mass for *Cadlina laevis*; data are transformed to natural logarithms ($\log_e$). The line represents the 'best fit' geometric mean regression between the variables.


y = 1.585 x - 5.329
F = 51.2***; r = 0.69; n = 63
ln number of eggs
ln body size (J)
4
5
6
7
8
5
6
7
8
9

5.3.4 Reproductive effort

Data for the instantaneous reproductive effort (RE) for *Archidoris pseudoargus*, *Tritonia hombergi* and *Cadlina laevis* are presented in Table 5.2. Data are given for spawn to soma ratio and the $P_g/(P_r + P_g)$ ratio; all data have been converted to a percentage for comparison with published data (see later). Data for *A. pseudoargus* and *T. hombergi* were calculated from live weights rather than dry weights. Determination of live weight will vary according to the water content of the material but is assumed to be consistent within a material (*i.e.* within a species, within an egg mass). On this basis, comparison of live weights between animal and spawn is questionable because the water content of body and spawn will vary independently. Because a live weight to dry weight conversion was not determined for *A. pseudoargus* or *T. hombergi*, a comparison of live weights is unavoidable and thus, any interpretation of the results of such a comparison should be viewed with caution.

All species have a similar minimum RE, *T. hombergi* and *C. laevis* have a similar maximum RE although maximum RE for *A. pseudoargus* is lower than the other two species. Rather surprisingly, the mean RE is significantly lower in the biennial semelparous species compared to the iteroparous species (Kruskal Wallis H = 25.877***); mean RE for the biennial species are not significantly different ($H = 0.34^{ns}$).

For *Cadlina laevis,* the annual RE of animals which spawned repeatedly, and the RE at first spawning, are shown in Table 5.3. There were no significant differences in the RE between the reproductive events (Kruskal Wallis $H = 6.607^{ns}$). In the foregoing discussion of adult longevity, it is clear that a 'lifetime' value could not be calculated because the age of the adults at the time of collection cannot be determined.

5.4 Discussion

Reproduction is arguably the most important event in the lifetime of an organism for if it fails to reproduce successfully, its fitness will be zero. Similarly, if a species is unsuccessful at reproduction it will become extinct. Reproduction is a complex process

Table 5.2 A summary of the data for reproductive effort as spawn/soma ratio and $P_r/(P_g+P_r)$ (converted to percentages) for *Archidoris pseudoargus*, *Tritonia hombergi* and *Cadlina laevis*. Mean values were calculated from arcsine-transformed data following the method outlined by Sokal & Rohlf (1981).

Species	Spawn to soma		$P_r/(P_g+P_r)$	
	Range	Mean (± 1 se)	Range	Mean (± 1 se)
Archidoris pseudoargus	6.5-55.1	19.0 >17.2 <20.8	5.2-35.5	15.4 >14.2 <16.6
Tritonia hombergi	5.3-74.6	21.7 >18.5 <25.2	5.0-42.7	16.8 >14.9 <18.8
Cadlina laevis	7.5-75.5	33.9 >31.9 <35.9	7.0-43.0	24.3 >23.1 <25.4

Table 5.3 A summary of the annual reproductive effort (calculated both as spawn to soma ratio and $P_r/(P_g+P_r)$) of laboratory-maintained individuals for *Cadlina laevis*. Data for *Year 1-3* are laboratory spawning years for adults and therefore *Year 1* does not necessarily represent an individual's first spawning event. *First spawning* is the RE for laboratory-reared juveniles and therefore does represent an individual's first spawning event. Mean values were calculated from arcsine-transformed data following the method described by Sokal & Rohlf (1981).

Reproductive event	Spawn to soma	$P_r/(P_g+P_r)$	n
First spawning	38.4 >33.7 <43.2	30.5 >27.9 <33.2	5
Year 1	31.2 >28.5 <34.0	23.2 >21.5 <24.9	16
Year 2	43.8 >39.3 <48.3	28.9 >26.9 <31.0	16
Year 3	32.3 >27.5 <37.2	24.8 >21.9 <27.8	10

with many different facets which are themselves fundamental to the success of the process. But, reproduction involves a cost to the organism which leads to a trade-off between life history parameters. Four fundamental trade-offs determine the primary facets of the reproductive process. It is not surprising therefore that organisms have evolved many different ways to maximize reproduction in view of these trade-offs: these trade-offs form an organism's life history strategy. Because all these processes are closely interlinked, it is very difficult to consider the selective pressures driving the evolution of each process in isolation. For the purposes of clarity, each process will be discussed separately, with the salient points drawn together in a conclusion. Larval strategies are an integral part of the life history strategy of marine organisms, but a discussion of this subject will be left for Chapter 6.

For *Archidoris pseudoargus* and *Cadlina laevis*, statistically significant allometric relationships were determined between body size and reproductive output, and for both species, the exponent of the relationship exceeded unity. These results are in agreement with the generalization in population biology that fecundity is proportional to body size. In addition, an exponent greater that unity indicates that a larger body size results in a proportionally greater fecundity. Rather surprisingly, no significant relationship between body size and fecundity was determined for *Tritonia hombergi* . Todd (1979b) and Havenhand & Todd (1988b) reported a similar result for *Adalaria proxima* which, perhaps more significantly, also lays eggs which hatch into lecithotrophic veligers. Todd & Havenhand (1988) concluded that fecundity of *A. proxima* is determined by the pattern of energetic allocation to reproduction, and this pattern underlies selection for a lecithotrophic life history strategy. Whilst detailed analyses of energetic allocation were not undertaken for *T. hombergi*, the lack of allometry between body size and fecundity would appear to lend support to this hypothesis for selection of lecithotrophic larval development.

Data for the copulation frequency and spawning frequency of *Archidoris pseudoargus*, *Tritonia hombergi* and *Cadlina laevis* highlight some interesting differences between these species and other opisthobranch species (for which data are available).

Hadfield & Switzer-Dunlap (1984) reviewed reproduction in opisthobranchs and provided data on copulation and spawning frequency. Spawning frequency has always been measured in the laboratory and so the results obtained may not reflect the natural environment. Harris (1975) reported that *Phestilla* spp. produced ≈1.5 egg masses per day for a 100 day period; Christensen (1977) reported 2-3 egg masses per month for *Precuthona peachi*; Eyster (1981) reported 2-4 egg masses over 1.5 months for *Armina tigrina*; Havenhand & Todd (1988b) reported a mean value of 8.89 ± 0.71 egg masses over 60.6 ± 3.9 days, and 19.61 ± 0.99 egg masses over 105.0 ± 4.0 days for *Adalaria proxima* and *Onchidoris muricata* respectively. Thus for the nudibranchs observed in the present study, a single, or very rarely two, egg masses for the semelparous *A. pseudoargus* and *T. hombergi*, and a single egg mass for *C. laevis* would appear to be unusual. These data are more surprising when one notes that both *A. pseudoargus* and *T. hombergi* die at a moderate body size when it would appear that a further allocation to reproduction was possible. Clearly, further studies are required to investigate the pattern of energetic allocation for these large nudibranchs before any further conclusions may be drawn on their apparently low spawning frequency.

Perhaps the most important and significant finding from the present study is the iteroparous reproduction of *Cadlina laevis*, the first recorded example of iteroparity in nudibranchs. Whilst it may be suggested that the repeated spawning of annual nudibranchs is equivalent to iteroparous reproduction, these repeated spawning events occur within a single reproductive season, albeit rather extended in some cases. None of these species undergoes a quiescence of gametogenesis between reproductive events, combined with a period of adult growth. There are a few cases of iteroparity within the order Opisthobranchia: Seager (1982) reported a 4.5 year life span for the Antarctic cephalaspidean *Philine gibba* and interpreted demographic data to show 2 spawning seasons; Lemche (1956) reported that the boreal cephalaspidean *Cylichna cylindracea* was probably perennial, maturing at two years and living until three, spawning in years two and three; Switzer-Dunlap (1980) found that the Indo-Pacific anaspidean *Dolabella auricularia* spawned every 10 days for as long as five years, after reaching sexual maturity at a post-larval age of 7-9 months. Whilst spawning over an extended period,

D. auricularia probably has a single spawning 'season' and therefore not strictly iteroparous. Therefore, *C. laevis* appears to be the first example of a perennial, iteroparous nudibranch.

From the above observation, why has *Cadlina laevis* evolved away from the presumably ancestral (for opisthobranchs) semelparous life history strategy. From the theories of bet-hedging and *r*-K selection, iteroparity is favoured when adults experience variable juvenile survivorship or environmental stability. *C. laevis* opts for the 'safest' mode of larval development (=direct) which will maximize survivorship from egg to benthic juvenile. Juveniles hatch in the spring when environmental conditions will be improving and food availability will be increasing. These conditions would appear to favour juvenile survivorship and thus not provide a selective pressure to change from a semelparous to an iteroparous life history strategy. But, a drawback of direct development will be a reduction in the dispersal of juveniles away from the benthic habitat and therefore juveniles will be in direct competition with adults for the available food resources. If these resources are temporally variable, juvenile survivorship may be unpredictable which, from the theory of bet-hedging would favour repeated or iteroparous reproduction (Willows 1990). It is apparent that the complex interlinking between the different trade-offs for the cost of reproduction do not permit these trade-off's to be considered in isolation. Consequently, a full evaluation of the evolution of an iteroparous life history in *C. laevis* requires data on juvenile survivorship and the temporal/spatial stability and/or variability the prey organism.

Hadfield & Switzer-Dunlap (1984) note that some opisthobranch species copulate prior to each spawning event: *e.g. Rostanga pulchra* (Chia & Koss 1978), some species spawn a fixed period after copulation, whilst other species show no correlation between copulation and spawning: MacGinitie (1934) noted that a field collected specimen of *Aplysia californica*, denied a mate, spawned 27 times over four months although only the first 15 egg masses were fertile! Duration of copulation varies widely from seconds (10-30 s in *Acteonia cocksi*: Hadfield & Switzer-Dunlap 1984) to several minutes (*e.g. Tenellia pallida*: Eyster 1979) to hours in large dorids. Copulation lasting >24

hours has been reported for the large dorid *Hexabranchus sanguineus* (Gohar & Soliman 1963) and *Adalaria proxima* (Todd 1979b), while copulation amongst chains of *Aplysia* have been reported to last several days (Eales 1921). Thus the long periods of copulation observed between paired *Archidoris pseudoargus* would appear rather unusual, and surprising, in view of the feeding behaviour and variability in prey quality discussed in Chapter 3. Obviously, whilst copulating, individuals cannot feed yet energy will be continually lost to basal metabolism. It is possible, in view of the allometry between respiration rate and body size, that paired animals form a 'super organism' which has a lower basal metabolism in comparison to the sum of the two individual metabolic rates. Clearly further investigation will be required to elucidate any such effect. It is also possible that sperm transfer is very slow, and in view of the large number of eggs produced, a large volume of sperm will be required. Thompson (1966) noted that *A. pseudoargus* is able to store sperm which would lend some support to this hypothesis. No clear conclusions can be drawn to explain the length of copulation within *A. pseudoargus* and further investigations are required.

An organism has a finite amount of energy which can be acquired during its life cycle, which has to be apportioned to the competing, and partially exclusive, demands of growth, metabolism and reproduction. The allocation of energy to reproduction -the reproductive effort (RE) - by an organism has a profound influence on the mode of reproduction and hence an organism's longevity: *i.e.* the dichotomy between semelparous and iteroparous life history strategies (Calow & Woollhead 1977; Calow 1979, 1983; Aldridge 1982). A massive allocation to reproduction at the expense of growth and metabolism will compromise the prospects for future survival (semelparity); a low allocation to reproduction and a higher allocation to growth and metabolism is likely to improve the prospects for future adult survival (iteroparity). *r*-K theory of life history evolution suggests that populations structured by fluctuating environmental conditions should favour high RE; stable environmental conditions should favour low RE. Bet-hedging suggests a low RE where the variability of juvenile survivorship is high relative

to that of the adult; when juvenile survivorship is constant and predictable relative to the adult, high RE is favoured (Stearns 1976).

A number of analytical indices have been proposed and used as measures of RE (for review: Havenhand & Todd 1989b), although it is often not clear whether values quoted in the literature represent an instantaneous or a lifetime measure. In the present study, the values quoted are instantaneous, although for *Archidoris pseudoargus* and *Tritonia hombergi* they also represent a lifetime measure because these species have only the single reproductive event. For a measure of RE to be useful for comparative purposes, it should incorporate the major energy budget components (Tinkle & Hadley 1975; Calow 1983). In the present study, these components have not been adequately determined and therefore the most simple index of RE, the spawn to soma ratio (expressed as a percentage), has been calculated to permit general interspecific comparisons. This measure has been widely criticised for its simplistic properties (*e.g.* Browne & Russell-Hunter 1978; Havenhand & Todd 1989b) mainly because it assumes a linear relationship between the reproductive allocation and body size. Nonetheless, spawn to soma ratios are widely quoted in the literature and provide some measure of comparison with the data recorded from the present study.

Todd (1979b) recorded spawn to soma ratios of 25-55% for *Adalaria proxima* and 82-143% for *Onchidoris muricata*; for the same species, Havenhand and Todd (1988b) subsequently recorded values of 40-149% and 111-338% respectively. Hall (1983) recorded values ranging from 36-244% with a mean of 137% for the nudibranch *Aeolidia papillosa*. De Freese & Clark (1983) gave mean spawn to soma ratios of 6.3-66% for a variety of opisthobranch species from Florida although these values are for single spawning events only and will underestimate lifetime values. Spawn to soma ratios are available for other gastropod species: for example, Grahame (1977, 1982) quoted values of 231-285% for the prosobranch *Lacuna pallidula* and 427-501% for *L. vincta*. Browne & Russell-Hunter (1978) recorded a mean value of 91% for freshwater gastropods. Thus, published spawn to soma ratios are highly variable within and between species, but it is apparent that the values recorded for the three species investigated during the present

study are at the lowest end of the overall range. In particular, the values for *Archidoris pseudoargus* and *Tritonia hombergi* are surprising considering that individuals of these species are large (and by inference have a high potential reproductive capacity), semelparous and have 'missed' a reproductive season as a consequence of a biennial life cycle. It should be noted however, these calculations are based on live weight and therefore, for the reasons outlined earlier (5.3.4), no firm conclusions can be drawn on the apparent magnitude of the RE of *A. pseudoargus* and *T hombergi.* A comparison of live weights assumes equivalence in the calorific content of body tissue and spawn. It is more likely that gametic tissue will have a higher energetic composition and therefore the spawn to soma ratio will be higher than these calculated values. For *Cadlina laevis*, each reproductive event has a low RE although on the assumption that an individual lives for 3-4 years, lifetime RE will be 75-100%, which is equivalent to published figures. In these present examples, for *Archidoris pseudoargus* and *Cadlina laevis* the relationship between spawn output and body size is allometric with an exponent greater than unity. Therefore the assumption for the spawn to soma ratios - that reproductive allocation is a linear function of body size - is clearly invalidated. More detailed investigations of the energy budgets of these species are required.

An important consideration during any discussion of the magnitude of RE is whether data are for field or laboratory spawning individuals. Energy consumed by processes associated with maintenance and survival is likely to be lower in animals maintained in 'near-perfect' conditions within a laboratory, therefore more energy may be allocated to reproduction. Todd (1979a) recorded 'field RE' of 48.7 and 63.81 against a laboratory value of 150.5 for *Onchidoris bilamellata.* All values of RE quoted in the present study are for laboratory maintained animals and may therefore overestimate the actual field RE of these species.

In general, studies investigating the link between RE and life history strategies have produced variable and conflicting results. Studies to determine the link between RE and the type of larva, or parental protection, have been examined in numerous comparisons of two or more closely related species without consistent results (Hughes & Roberts 1980;

Grahame 1982). Hadfield & Miller (1987) concluded that 'pie arguments', where the total energy available is the 'pie' which is divided to give different allocations to reproduction, are not supported by data from studies on opisthobranchs. Similarly, studies of RE in relation to *r*-K theory and bet-hedging have also proved inconclusive (Hines 1982). *Archidoris pseudoargus* has pelagic larvae (with presumably highly variable and unpredictable survivorship) and, in the laboratory at least, low adult mortality which, according to the predictions of of *r*-K theory and bet-hedging, would favour low RE. A similar argument could be extended to *Tritonia hombergi* although larval mortality will be reduced due to a shorter pelagic period. *Cadlina laevis* does not have pelagic larvae and therefore juvenile survivorship may be more predictable and hence favour a higher reproductive effort. Thus it would appear that these species follow classical theory although these results are not conclusive and, due to a lack of detailed information on individual energy budgets, more comprehensive indices of RE cannot be calculated. The conclusion of Todd & Havenhand (1989b) is pertinent to the present study: "Whilst RE can provide useful comparative indices, the possibility of even simple relationships between RE and life history parameters will not be established unless well defined and strictly comparable measures of RE are used".

In the context of the present study, the findings of Grahame (1982) are perhaps more relevant. For two species of the gastropod *Lacuna* with contrasting reproductive strategies, there was only a 4% difference between the RE (determined as a spawn to soma ratio) but in absolute terms, the overall energy turnover of *L. vincta* was considerably higher than its' congener *L. pallidula*. If size is a reflection of absolute energy flow, the very large nudibranchs in the present study will have large energy fluxes. Moreover, for *Archidoris pseudoargus* and *Cadlina laevis*, the relationship between spawn output and body size was allometric with an exponent greater than unity, suggesting that larger individuals have proportionally larger spawn outputs. Absolute size is therefore significant in determining the absolute fecundity. For *Tritonia hombergi* there was no significant relationship between body size and spawn output, but other relationships (*e.g.* body size and egg number) were not investigated. Thus, by virtue of a

large body size, RE can be low but absolute fecundity is high which may offset losses due to larval mortality.

For an organism with a biennial semelparous life cycle to achieve a similar rate of population growth as an annual species, its fecundity has to be at least double what it would be if the organism reproduced as an annual species. Using the equation for the relationship between body size and spawn output, and assuming that size specific growth rate (SSGR) is constant over the life span (*i.e.* assuming allometric growth), it is possible to calculate the likely body size and therefore spawn output at year one. Based on the data for the growth of juvenile *Cadlina laevis* presented in Chapter 4 (Figure 12), mean SSGR over the first 12 months was 0.006 ± 0.002 mg · mg $^{-1}$ · day $^{-1}$. At year one, a *C. laevis* individual would have a body size of ≈ 28 mg; at year two this individual would attain a body size of ≈ 210 mg. Clearly, spawn output at year two will be more than double the spawn output for year one. If one follows a similar calculation for *Archidoris pseudoargus* where the mean body size (weight under water) at spawning was ≈ 996 mg, even assuming a mean SSGR of 0.01 mg · mg $^{-1}$ · day $^{-1}$ only results in a body size of ≈ 160 mg at year one. Although these calculations are rather basic, they suggest that both *A. pseudoargus* and *C. laevis* exceed the minimum fecundity to justify a biennial age of first reproduction (and last for *A. pseudoargus*).

Most other nudibranch species have an annual life cycle which would suggest that this is the ancestral condition; extended life cycles have, one presumes, evolved in response to selective pressure. What factors could cause selection for a longer life cycle? If fecundity is a measure of fitness, extending the life cycle would lead to a gradual increase in body size and thereby fecundity (assuming all other factors are equal). Extending the life cycle is equivalent to increasing the egg to juvenile period (EJP) which has been shown to increase fitness according to the predictions of a model proposed by Havenhand (In press). But there are a number of problems associated with a gradual increase in development time. Prolonging adult development would both increase the risk of pre-spawning mortality, and add a corresponding delay to the release of larvae into the water column. Delaying larval release may avoid the 'spring bloom' of planktonic

predators and thereby reduce larval mortality (although the larva's prey may be similarly less abundant), and/or increase the rate of larval development due to increased water temperature. Nevertheless, larvae will metamorphose later in the year and may not attain a juvenile size capable of surviving the less favourable environmental conditions in winter. Seasonal prevailing environmental conditions and/or prey availability (or quality) would further compound the problems facing a larva metamorphosing late in the season. If an organism is required to attain a minimum size prior to reproduction, a seasonal environment may restrict juvenile growth giving rise to an adult of a size incapable of producing sufficient gametes for successful reproduction. To achieve maximal fitness, a small adult should grow during the following season attaining reproductive size and/or maturity later in the season. If this delay became progressively later, finally, a release of gametes very late in the season may result in total reproductive failure which equates to zero fitness for a semelparous organism. In this instance, selection ought to favour delaying reproduction until the following year - a biennial life cycle. In the plant kingdom, it has been recognised that an individual must attain a minimum size prior to reproduction (Werner 1975). Also in the plant kingdom, there are many documented cases where environmental conditions result in a delay in reproduction: Lacey (1986) concluded that in the wild carrot *Daucus carota*, nutrient supply determines the year of flowering. Thus a seasonal environment can result in directional selection towards an extended life cycle.

But the need to attain large body size and, by inference, a high fecundity may not be the determining selective force for a biennial life cycle. Reproductive success requires an individual to replace itself in subsequent generations, and to that end, a parent should maximize the probability of larval and juvenile survival. *Halichondria panicea* has seasonal variations in its biochemical composition and therefore its perceived prey value. For *Archidoris pseudoargus*, the timing of reproduction and/or its generation time may assume greater importance than absolute fecundity. Hodgkin & Barnes (1991) demonstrated that generation time had a greater selective value over fecundity for a nematode species. Adult *A. pseudoargus* attain a body size capable of high reproductive output by the late summer/autumn of their second year but delay reproduction (at risk of

overwinter adult mortality) until the following spring. Reproduction in early spring ensures that larvae develop during a period of increasing water temperature and planktonic food supply (spring bloom) (but more predators!), and settle and metamorphose in late spring/early summer, thus maximising the time available for growth against a seasonally variable prey. Delaying reproduction will result in an increase in body size and by inference fecundity, but it is not high fecundity *per se* that provides the primary selective pressure for the delay.

There are few documented cases of biennialism in the marine environment. Somerton & MacIntosh (1985) found that the blue king crab *Paralithoides platypus* has a biennial reproductive strategy and concluded that this was an adaptive strategy to enable individuals to expend more energy per brood and reduce the risks associated with an annual moult. *P. platypus* has a longer life cycle, in comparison to its' congener the red king crab, *P. camptschatica,* which has annual reproduction, to offset less frequent reproduction. Jenson & Armstrong (1989) considered *P. platypus* in more detail and proposed that a biennial reproductive strategy could be an *adaptive feature* (as proposed by Somerton & MacIntosh), or a *limitation* caused by physiological constraints. Jenson & Armstrong (1989) concluded that the evolution of a biennial reproductive strategy was a consequence of physiological and energetic constraints incurred by species in a 'harsh' environment. Similarly, the chaetognath *Sagitta elegans* has biennial life cycle in arctic waters, but an annual life cycle in a nearly land-locked fjord with an elevated water temperature on Baffin Island, Newfoundland (McLaren 1966). Sardà (1991) recorded biennial spawning and moult synchronism in Mediterranean populations of the Norway lobster *Nephrops norvegicus*, but was unable to satisfactorily determine the primary cause. Sardà (1991) concluded that biennial behaviour could be a consequence of endogenous natural variation and/or exogenous variation in food quality, temperature or even commercial fishing pressure.

Miller-Way & Way (1989) recorded semelparous biennial life histories in populations of the freshwater pleurocerid gastropod *Leptoxis dilatata*, and concluded that food quality and quantity were the primary cause of an extended life cycle. Similarly,

Payne (1979) attributed semelparous biennial (& triennial) life histories in the pleurocerid gastropod *Goniobasis livescens* to variations in food quality. These data suggest that a biennial reproductive strategy is favoured when the growing season is short (= reduced food supply) and/or prevailing environmental conditions are stressful.

What selective factors may have led to *Archidoris pseudoargus*, *Tritonia hombergi* and *Cadlina laevis* evolving an extended life history strategy? These organisms are subject to similar physical environmental conditions to other north Atlantic nudibranch species which have annual life cycles. It would appear unlikely that physical environmental conditions alone could have such a marked evolutionary effect, as this implies that the internal physiology of these longer lived species is very different. Nudibranchs have evolved to feed on a wide variety of prey and perhaps this is the key factor determining the life cycle. Reproduction requires energy to fuel the process of gametogenesis, and any reduction or seasonal variation in the energy supply may preclude successful reproduction. If the supply of energy is seasonal, or perhaps there are initial prey handling problems for juveniles, it may be selectively advantageous to maximize body size during the first growing season and thereby increase fecundity in the second season. A seasonal energy supply may provide the selective pressure for the evolution of a biennial or perennial life history strategy.

CHAPTER 6

LARVAL BIOLOGY

6.1 Introduction

Earlier this century, Thorson (1946, 1950) undertook a series of detailed investigations into the development and evolution of marine invertebrate larvae and proposed a classification of larval development types. Subsequent to Thorson's pioneering work, numerous classifications have been proposed for the range of larval development types observed amongst marine invertebrates (Mileikovsky 1971), with each classification depending largely on the particular interests of the investigator. For example, classifications have been based on the relative dispersal ability of larvae (Scheltema 1978), on the feeding ability of larvae (Strathmann 1978) and on palaeobiological factors (Jablonski & Lutz 1983; Jablonski 1986). Thompson (1967) discussed the general larval developmental processes in Opisthobranchia and considered that larval development followed one of three strategies:

Type 1: species with pelagic planktotrophic veliger larvae. Veligers hatch from small eggs at an early stage in their development. They have a small foot, but lack a propodium and eyes. Larvae must feed in the plankton to gain energy for growth and further development.

Type 2: species with pelagic lecithotrophic veliger larvae. Veligers hatch from intermediate sized eggs at a late stage of development, and possess a well developed foot, a propodium and eyes. Yolk reserves are present in the digestive gland to permit larvae to complete development without the requirement to feed.

Type 3: species with direct development. Large eggs permit embryos to undergo complete development within the capsule; fully developed benthic juveniles crawl away from the egg mass .

It is generally accepted that planktotrophic development is the primitive and/or ancestral mode of development, and that pelagic lecithotrophic and direct developmental modes are evolutionary derivatives (Strathmann 1985). If a period of development in a benthic egg mass /egg capsule, or in parental brood chamber precedes a pelagic phase, the

development has been termed 'mixed' (Pechenik 1979); all opisthobranch molluscs exhibit mixed development.

Investigations into the mechanisms of, and the selective pressures for, the evolution of the larval development mode of marine invertebrates have considered a wide variety of life history, environmental and palaeobiological parameters. A number of comprehensive reviews of larval ecology have been published since Thorson's early work (see: Mileikovsky 1971; Jablonski & Lutz 1983; Crisp 1984; Grahame & Branch 1985; Strathmann 1985; Young 1990). The distribution of development types displayed by marine invertebrates is not random. Patterns have emerged linking larval type with adaptive reproductive strategy - '*r*-K theory' and 'bet hedging' (*e.g.* Grahame 1977), latitude (Thorson 1946, 1950), body size (*e.g.* Strathmann *et al.* 1984), speciation and extinction (Scheltema 1978; Jablonski 1986) and the energy resources available to individual species (*e.g.* Todd 1991). Despite this large body of data, no clear explanation predominates and it is likely that the "selective regimes for such evolutionary shifts will comprise 'special cases' for many particular species" (Todd 1991).

Larval development of opisthobranch molluscs has been investigated for nearly 150 years since the pioneering work of Alder & Hancock (1845-55). Thompson (1958, 1959, 1962, 1966, 1967) published a series of thorough, and very detailed, studies of the developmental processes for nudibranch species with planktotrophic, lecithotrophic and direct modes of larval development. More recently, larval development in the Opisthobranchia has been reviewed by Hadfield & Switzer-Dunlap (1984), Hadfield & Miller (1987), Havenhand (1991) and Todd (1991). Laboratory husbandry of marine invertebrate larvae has improved since early investigations and the culture of larvae from hatching through to metamorphosis has now become almost routine; culture of nudibranch larvae has proven relatively straightforward (Kempf & Willows 1977; Chia & Koss 1978; Todd & Havenhand 1984; Todd 1987, 1991; Kempf & Todd 1989; Carroll & Kempf 1990). Consequently it is now possible to study in some detail, many aspects of the physiological ecology of marine invertebrate larvae. Bayne (1983) reviewed the mechanisms of feeding, growth and respiration of marine molluscan larvae;

Havenhand (1991) reviewed aspects of the behaviour of opisthobranch larvae; and Todd (1991) reviewed aspects of the physiological ecology of nudibranch larvae.

In recent years, attention has centred on the transition from larvae to post-metamorphic juvenile. To effect this transition, a larvae has to settle and metamorphose into the benthic habitat. It seem intuitive that post-metamorphic development should occur within the adult habitat, usually in conjunction with conspecifics. Habitat selection and, in particular, physical and chemical cues to facilitate habitat selection and subsequent metamorphosis, have attracted considerable attention. Havenhand (1991) provides a comprehensive review of investigations of these phenomena for opisthobranch larvae, and only a selection of the key studies will be mentioned in this current review.

Development from egg through to juvenile comprises a number of key stages. Initially embryos undergo development within egg capsules of benthic egg masses. Egg masses vary in their structure and composition between species and, for large egg masses, an important consideration is the effect of the egg mass itself on the rate of gas exchange to the embryos. Chaffee & Strathmann (1984) found that the development stage of embryos of the cephalaspidean *Melanochlamys diomedea* was more advanced when embryos were located near the surface of the egg mass. Mechanical strength of the egg mass appears to correlate with the development mode: the longer the benthic development, the stronger the egg mass. For example, the egg masses of the planktotrophic species *Onchidoris muricata* are thinner that the lecithotrophic species *Adalaria proxima* (Todd 1979a) which in turn are thinner than the egg mass of *Cadlina laevis* which has direct development (pers. obs.). Mechanisms for the release of larvae are poorly understood but tearing of the egg membrane by radula action, use of velar cilia, enzymatic action and action by microflora and meiofauna have been observed (*e.g.* Todd 1981; Gibson & Chia 1989; Carroll & Kempf 1990). *Haminaea callidegenita* embryos are poecilogonous (*i.e.* having both lecithotrophic veligers and crawl away juveniles from the same egg mass) and although development within the egg mass appears synchronous, there was considerable variation in the time taken for individuals to attain metamorphic competence (Gibson & Chia 1989). For the latter species, it would

appear that the effect of gas exchange rates nor mechanical retardation of hatching significantly influence development in *H. callidegenita* (Havenhand 1991).

When larvae escape from the egg mass , they begin to swim in the water column. Initially larvae appear to be negatively geotactic and swim upwards in the water column (*e.g.* Chia & Koss 1978; Hubbard 1988). As eyespots develop, larval swimming appears to decline and the larvae sink in the water mass, a process which has been linked to negative phototaxis (*e.g.* Chia & Koss 1978, 1988; although see Todd 1981 for an alternative hypothesis). Miller & Hadfield (1986) investigated phototaxis in *Phestilla sibogae* larvae which is the only study to date to have quantified the ontogenetic changes in phototactic behaviour, swimming patterns and the onset of metamorphic competence.

When pelagic development is complete and a larva is ready to settle and metamorphose, the development stage is termed competence. Competent larvae need to select a suitable habitat to settle and metamorphose, usually the adult habitat; habitat selection is generally effected via a physio-chemical cue (see below). In the absence of a suitable cue, the ability of larvae to delay metamorphosis has considerable adaptive significance (for review: Day & McEdward 1984). Delays to metamorphosis appear longer for planktotrophic than lecithotrophic species: Kempf (1981) recorded an exceptional metamorphic delay of >300 days for *Aplysia juliana*; lecithotrophic veligers of *Haminaea callidegenita* were able to delay metamorphosis for up to 20 days (Gibson & Chia 1989). Longer metamorphic delays have been observed for lecithotrophic larvae which have been observed to feed (termed facultative planktotrophs): Kempf & Hadfield (1985) observed a ≈27 day delay for unfed *Phestilla sibogae* which extended to 42 days if the larvae were fed. Kempf & Todd (1989) also recorded facultative planktotrophy for the lecithotrophic larvae of *Adalaria proxima*, although no there was no difference in the length of the delay period for fed and unfed larvae.

For species with pelagic larvae, the transition from the pelagic to the benthic phase is crucial to the continuing development of the juvenile. Usually this transition additionally involves metamorphosis from to the juvenile (and adult) body form. To maximize fitness, larvae should settle and metamorphose into a habitat conducive to their

continuing growth and survival. It has been proposed that larvae 'read' the environment via physio-chemical cues to optimize habitat selection. Settlement and metamorphosis of pelagic marine invertebrate larvae, and in particular the physio-chemical cues to metamorphic induction, have been extensively studied (for summary: Morse 1990; Pawlik 1990). Consequently, many aspects of the metamorphosis of nudibranch larvae have been investigated (Hadfield 1978, 1984; Todd *et al.* 1991). In general, species with highly specific food requirements metamorphose in response to chemical cues from the food substance; species with less specific food requirements usually settle and metamorphose in response to the general characteristics of the environment in which their prey and their conspecifics live (Hadfield & Scheuer 1985; Hadfield & Miller 1987). In the majority of cases, the biochemical analyses have not completely identified the natural compounds which induce metamorphosis. In perhaps the most complete identification, Hadfield & Scheuer (1985) reported that *Phestilla sibogae* larvae are induced to metamorphose by a molecule of 200-500 daltons, which is polar and temperature- and pH-stable. Chia & Koss (1988) investigated the cues to settlement and metamorphosis for *Onchidoris bilamellata*. Larvae of *O. bilamellata* were observed to modify their swimming behaviour when exposed to a dissolved cue emanating from live barnacles (adult prey), although the stimulus was insufficient to initiate metamorphosis. Subsequent work has identified putative chemoreceptors on the surface of the larval propodium which respond to cues for settlement (Chia & Koss 1989), and preliminary neurophysiological investigations have identified one of these receptors depolarises in response to a dissolved cue which initiates metamorphosis (Arkett *et al.* 1989). *Archidoris pseudoargus* feeds almost exclusively on *Halichondria panicea* (see Chapter 3) and therefore one would expect that *A. pseudoargus* would receive a cue to induce metamorphosis from the prey species; an aim of the present study was to test this expectation.

Notes on the development of *Archidoris pseudoargus* were first recorded by Alder & Hancock (1845-55) and then by Reid (1846), Saunders (1880), Elliot (1910) and Allen & Nelson (1911). The first detailed study of development was published by Thompson (1958, 1966) although he was unsuccessful in attempts to culture *A. pseudoargus* larvae through to metamorphosis. More recently, Todd & Havenhand (1984) published notes on

the embryonic and larval development of *A. pseudoargus*. The aim of the present study was to investigate the growth and development of *A. pseudoargus* larvae at different temperatures and under different ration levels. It was hoped that information gathered from these experiments may contribute to a discussion on why this species has evolved its particular larval development mode.

Roginsky (1962) observed that *Cadlina laevis* had direct development, and this was the first British nudibranch shown to follow this development strategy. Thompson (1967) described in detail the ontogenetic processes which comprise this direct development in *C. laevis*. Development of *C. laevis* was not studied in detail during the present study but will be discussed in the context of the evolution of larval reproductive strategies in long-lived nudibranch species.

6.2 Methods

6.2.1 Egg mass development

Egg masses of *Archidoris pseudoargus* and *Cadlina laevis*, after weighing, were either maintained in clean plastic tubs within the laboratory aquarium or in beakers of sterile filtered seawater maintained at constant temperature. At all times, the seawater was fully aerated either by maintaining a high water flow or by bubbling air into the beakers. Egg masses were monitored daily to record their stage of development.

6.2.2 Larval culture

To investigate the growth and development of the larvae of *Archidoris pseudoargus*, veligers were collected from hatching egg masses and reared through to metamorphosis. A standard methodology was employed throughout all experiments.

All larval culture methods used seawater filtered through a 0.22 µm Flow™ filter to remove all micro-organisms. Containers of filtered seawater (FSW) were maintained at ambient temperature. All glassware and ancillary equipment was used from new and

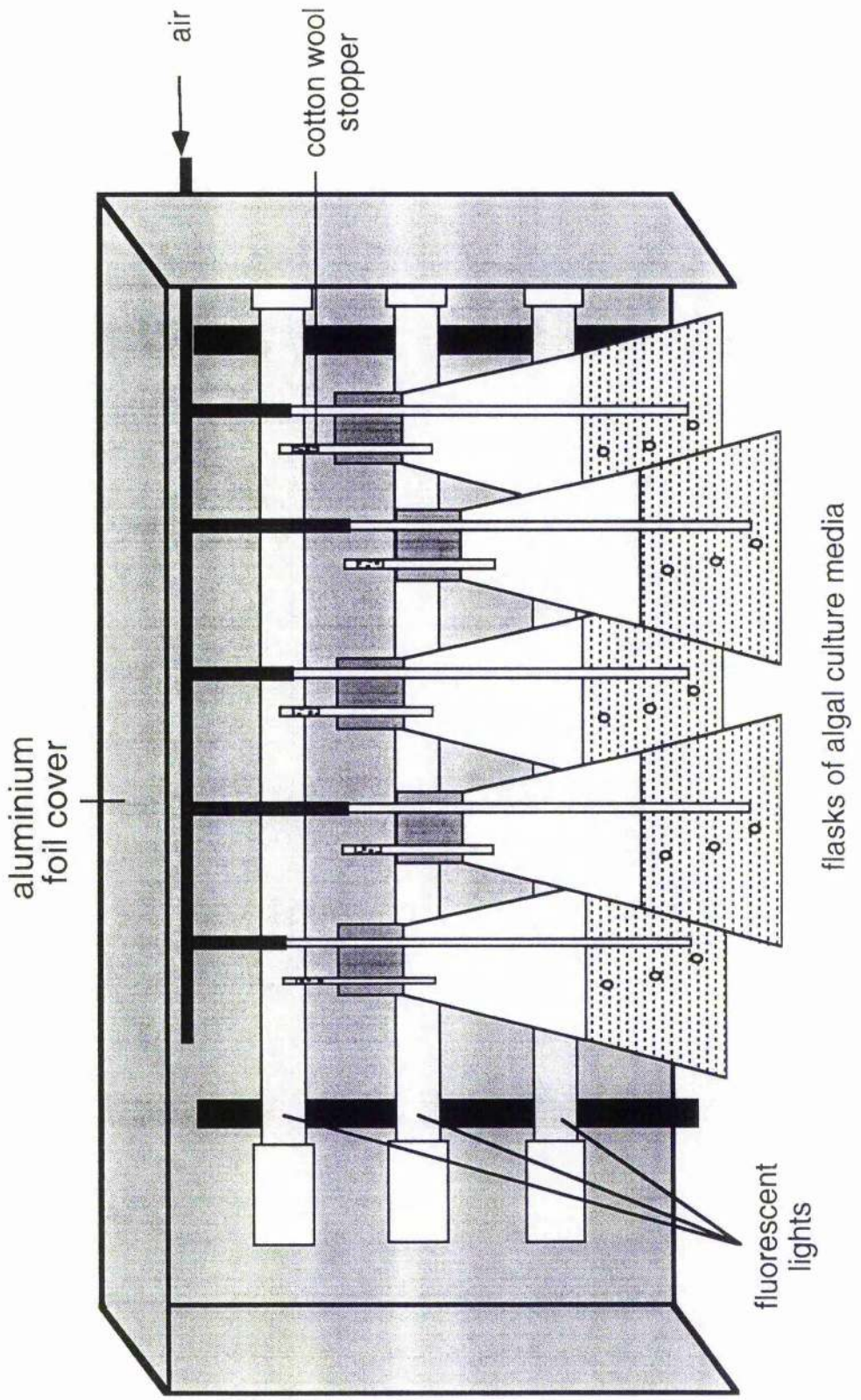
cleaned with hot water only; care was taken to avoid contamination with any detergents or laboratory chemicals. Glassware was sterilized in an autoclave prior to use.

Veliger larvae were fed on the unicellular phytoplankters *Isochrysis galbana* Parke (supplied by the Scottish Marine Biological Association, Oban) and *Rhodomonas* sp. (Cambridge Culture Collection). Phytoplankton were batch cultured in 0.22 μm filter sterilized Provasoli's E.S. media (Provasoli 1968) under constant fluorescent illumination. Algal cultures were maintained in flasks with filtered air constantly bubbled through the culture media (Figure 6.1). New cultures were initiated from separate stock cultures when required. To prepare algal cells for nudibranch larval cultures, a volume of the algal culture media was centrifuged at 2500 rpm for five minutes to concentrate the algae; the culture media were then discarded. Algae were resuspended in FSW at ambient temperature and the concentration of algal cells determined with a haemocytometer. Aliquots of this algal suspension were added to the larval cultures as required (see below).

When veligers began to move within the egg capsules, egg masses were rinsed thoroughly in FSW to remove debris and microorganisms and transferred to clean beakers of FSW until veligers hatched. To promote hatching, air was vigorously bubbled through the beakers. When veligers hatched, the fluid in the beaker was poured through a 40 μm nitex mesh filter held in a beaker of FSW (see: Switzer-Dunlap & Hadfield 1981), veligers were retained in the filter container. Veligers were flushed with FSW and pipetted into prepared larval culture media.

Larvae were reared at a concentration of approximately $5 \cdot ml^{-1}$ in beakers of larval culture media which were maintained in thermostatically controlled water baths ($\pm$ 0.5 °C). Media consisted of FSW with the antibiotics Penicillin G and Streptomycin sulphate added at concentrations of 50 and 60 $\mu g \cdot ml^{-1}$ respectively to control any bacterial and ciliate infections. Algae were added to the larval culture media at varying concentrations (see below). Media were changed every five days until the larvae attained competence to metamorphose. The contents of a culture beaker were poured through a nitex filter, larvae were flushed with FSW and pipetted into beakers with fresh larval

Figure 6.1 A diagram of the apparatus employed to culture the unicellular algae *Isochrysis galbana* and *Rhodomonas* sp. Algae were maintained in conical flasks containing 0.22 µm filtered Provasoli's E.S. media under constant illumination. Air, filtered through cotton wool, was bubbled through the culture media.


air
cotton wool stopper
aluminium foil cover
flasks of algal culture media
fluorescent lights

culture media, previously acclimated to the required culture temperature. At each change of media, a sample of the larvae was pipetted onto a microscope slide and shell length measured under a compound microscope fitted with an eyepiece graticule. Shell length (across the widest point) was determined for at least 20 larvae and a mean (± 1 se) shell length determined.

In 1986 and 1987, *Archidoris pseudoargus* larvae were cultured under different temperature and ration regimes. Initially larvae were cultured at 5, 10, 15 & 18 °C with a ration of 1:1 *Isochrysis: Rhodomonas* at a total concentration of 50 algal cells · μl^{-1}; three cultures were run at each temperature. To determine the influence of food quality and ration level on larval development, *A. pseudoargus* larvae were culture at 15 °C and fed *Isochrysis* only, *Rhodomonas* only and a 1:1 mixture, all at 50 algal cells · μl^{-1}. In addition, larvae were reared at 15 °C in culture media only without any algal cells added. For comparative purposes, veligers of *Onchidoris bilamellata* were cultured at 18 °C following the same procedures.

6.2.3 Gravimetric analysis

To determine the ratio of organic to inorganic material in *Archidoris pseudoargus* larvae, three cultures were established at 18 °C. Every five days a sample of larvae was pipetted onto a filter disc, rinsed in 0.9% (w/v) Ammonium formate, frozen and then freeze-dried. Aluminium foil pans were ashed (to remove any organic material adhering to the pan) and weighed to ± 0.0005 mg on a Mettler ME22 balance. Ten larvae were carefully transferred to each of these foil pans, and the pans plus larvae were re-dried, weighed, ashed for three hours at 550 °C and then re-weighed. Inorganic and organic fractions were calculated by subtraction. Larvae sometimes 'popped' out of the pans during the ashing procedure and so the number of larvae in each pan was recounted at the end of the experiment. Ash content 'per larva' were determined on the basis of the recounts.

6.2.4 Metamorphosis

When developing *Archidoris pseudoargus* larvae reached the pediveliger stage (determined by well developed eyes and propodium, and an ability to crawl), they were transferred to small glass dishes to undertake a preliminary investigation of metamorphosis. Ten pediveligers were placed in each dish to which were added either small, fresh pieces of *Halichondria panicea,* or choline (10^{-2}M) or potassium ions (19 mMol $\cdot$ l^{-1}). Three trials were undertaken for each treatment. Dishes were checked daily to determine the number of post-metamorphic juveniles.

6.3 Results

6.3.1 Larval development

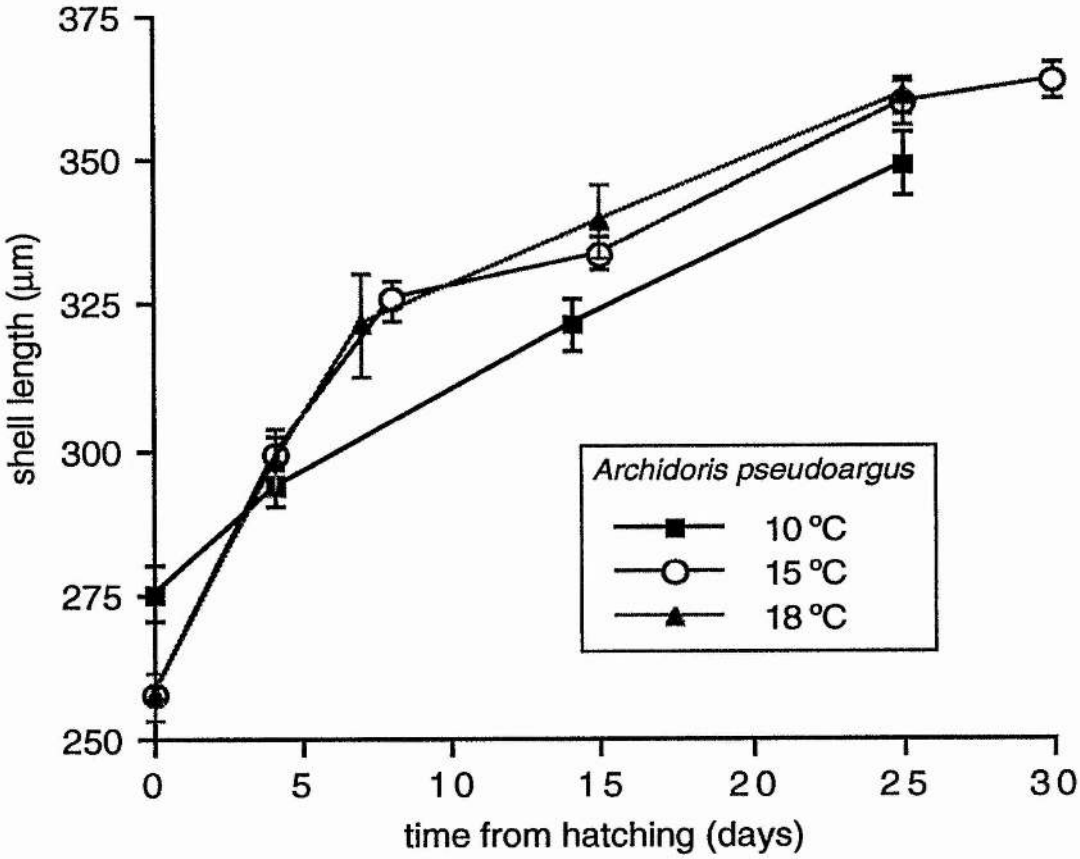
Development of *Archidoris pseudoargus* larvae within the benthic egg mass is outlined in Table 6.1. Larvae hatch after 21 days at 14.5 °C as large planktotrophic veligers with a shell length of 240-275 µm. Although shell diameter is large, the larval body is small giving the shell an 'empty' appearance at hatching. One striking feature of this larva is the size of its velum - it is proportionally very much larger than the velum of most other reported planktotrophic nudibranch larvae, but not as large as, for example, the exceptional *Aegries punctilucens* (Thiriot-Quievreux 1977). During the first few days of pelagic life, larvae are strongly negatively geotactic and swim to the surface of the culture vessels. Larvae become trapped in the surface film ('rafted') and die unless they are resuspended. Therefore during the first few days, 'rafted' larvae were regularly resuspended by pipetting FSW into the culture vessels to break the surface tension and release the larvae. Larval cultures were prone to ciliate infections which, unless controlled, would kill the larvae. A number of cultures did not run to completion because of the premature death of larvae caused by bacterial and/or ciliate infections.

Growth patterns of *Archidoris pseudoargus* larvae are independent of temperature and differ significantly from the growth pattern of *Onchidoris bilamellata* (Figure 6.2 a&b). *Archidoris pseudoargus* larvae grow rapidly during the first 10 days

Table 6.1 Time post-hatching (in days) for *Archidoris pseudoargus* embryos to attain the main stages of development within the benthic spawn mass. Temperature was maintained at 14.5 °C throughout development.

Developmental stage	Time post-hatching (days)
Oviposition	0
4/8 cell	3
Late cleavage/early blastula	4
Gastrulation	6
Mid veliger	14
Pediveliger	20
Hatching	21

Figure 6.2a Growth of *Archidoris pseudoargus* and *Onchidoris bilamellata* larvae at different temperatures; data for 1986. Larvae were cultured on a diet of 1:1 *Rhodomonas* and *Isochrysis*, at a total concentration of 50 algal cells · ml^{-1}.


375
350
325
300
275
250
shell length (µm)
0
5
10
15
20
25
30
time from hatching (days)
Archidoris pseudoargus
10 °C
15 °C
18 °C

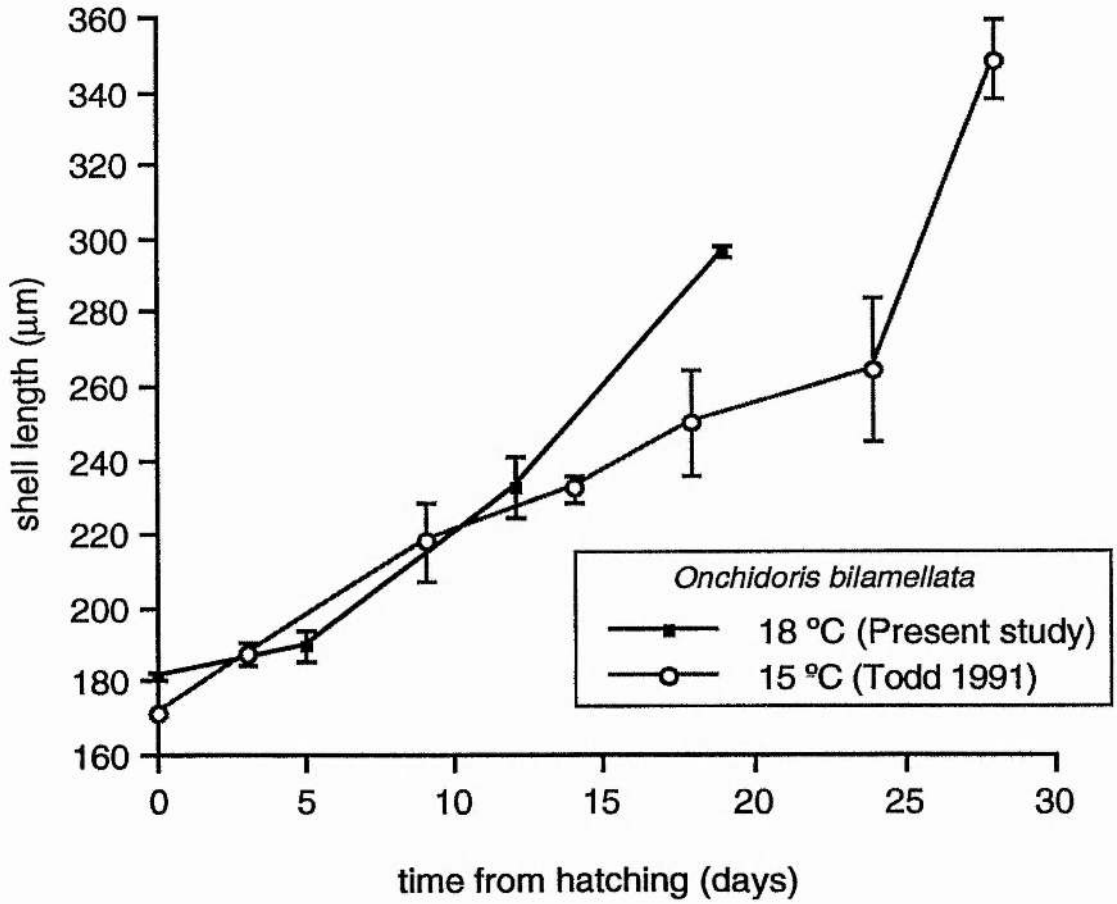
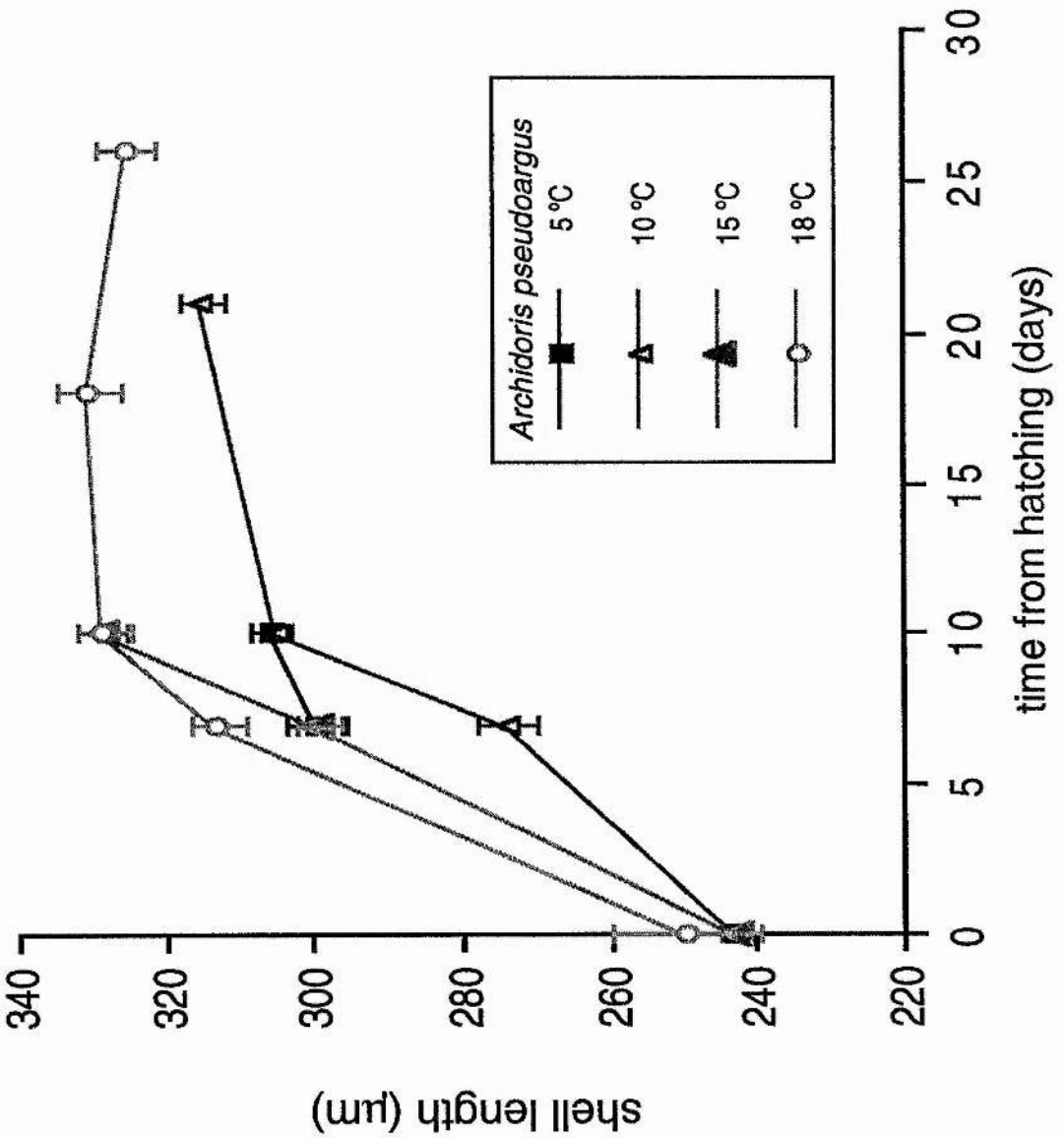

360
340
320
300
280
260
240
220
200
180
160
shell length (µm)
0
5
10
15
20
25
30
time from hatching (days)
Onchidoris bilamellata
18 °C (Present study)
15 °C (Todd 1991)

Figure 6.2b Growth of *Archidoris pseudoargus* larvae at different temperatures; data are for 1987. Larvae were cultured on a diet of 1:1 *Rhodomonas* and *Isochrysis*, at a total concentration of 50 algal cells · ml^{-1}.


Archidoris pseudoargus
5 °C
10 °C
15 °C
18 °C
shell length (µm)
340
320
300
280
260
240
220
0
5
10
15
20
25
30
time from hatching (days)

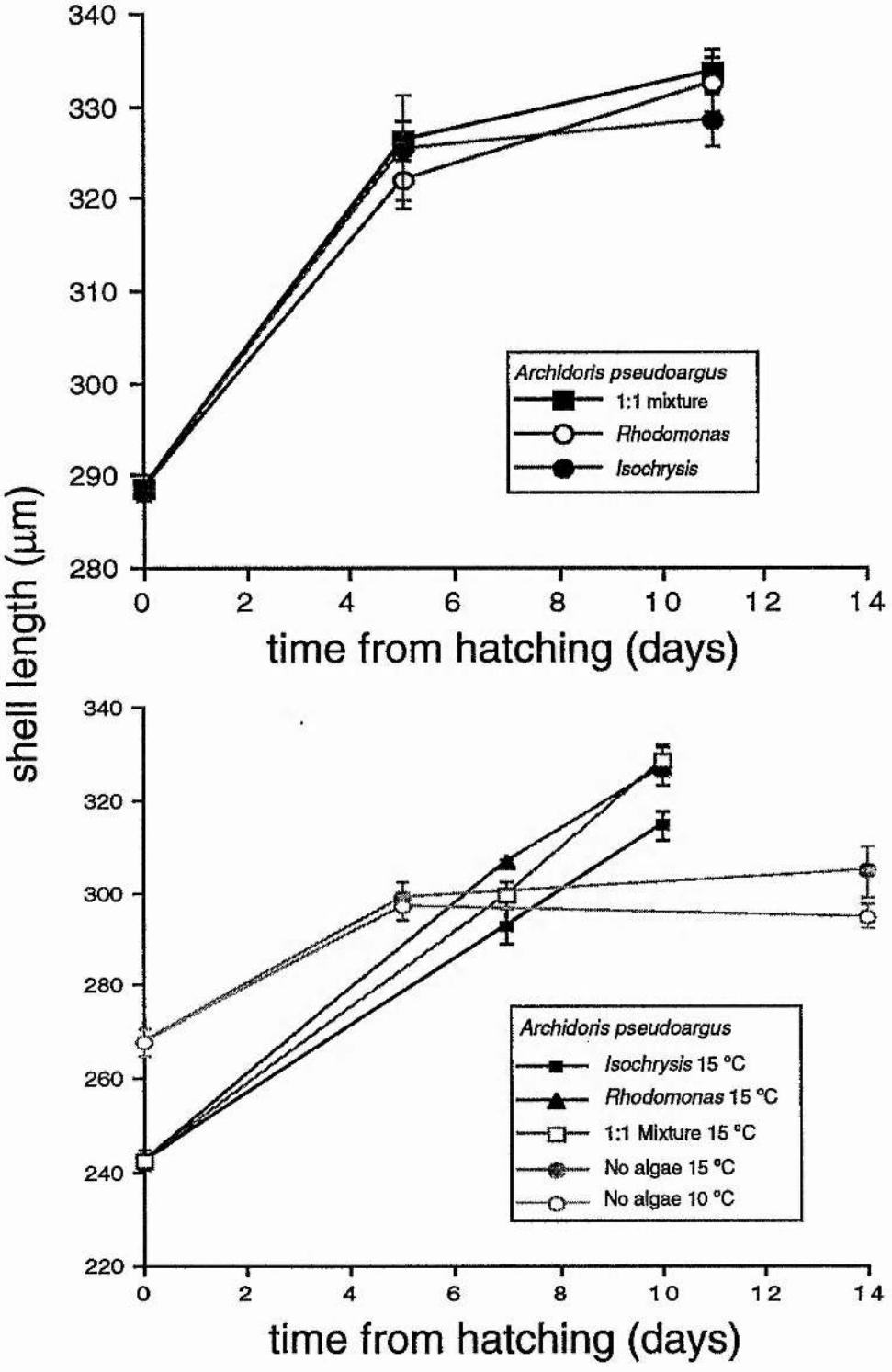
followed by a gradual decline in the growth rate towards an asymptote. In the first few days, the 'empty' appearance of *A. pseudoargus* larvae quickly declines as the larval gut fills with algae. In contrast, *O. bilamellata* appears to grow slowly at first with the growth rate increasing with age, although Todd 1991 shows *O. bilamellata* larvae to grow exponentially during the first 10 days, the growth rate declining as the larva attains competence (Todd 1991: his Figure 1). Similar growth rates and growth patterns were obtained for *A. pseudoargus* larvae in 1986 and 1987. Unfortunately in both years, cultures at 5 and 10 °C suffered from ciliate infestations and no larvae attained competence. Table 6.2 summarises the data for successful larval cultures.

During the rapid growth in the first 10 days, the digestive diverticula, especially the left diverticula, rapidly fill with algal cells. In cultures with 1:1 *Rhodomonas: Isochrysis* as the algal diet, the digestive diverticula develop a distinct red/pink coloration suggesting that *Rhodomonas* was the preferred diet. However, it should be noted that *Rhodomonas* cells have a very dense coloration and are much larger unicells than *Isochrysis.* Ingestion of only a small number of *Rhodomonas* cells could lead to the larvae developing this pink coloration. Figure 6.3 shows the growth of *Archidoris pseudoargus* larvae reared on different diets in 1986 and 1987; the figure for 1987 includes data for larvae reared in culture media only. These data suggest that *Rhodomonas* alone provides most of the dietary components necessary for normal growth; *Isochrysis* alone does not appear to support maximal growth. Kruskal-Wallis nonparametric analysis on 1987 data revealed that, at 10 days post-hatching, the shell diameter of larvae reared on *Isochrysis* only were significantly smaller than shell diameter of larvae reared on *Rhodomonas* only or the 1:1 mixture ($H = 25.94$ ***; *Isochrysis* $n = 54$; *Rhodomonas* $n = 52$; 1:1 $n = 47$). It would appear that *Isochrysis* does not provide all the necessary dietary components to maintain growth. In 'unfed' cultures, larvae displayed the rapid early growth of fully fed cultures for the first 5 days, probably supported by yolk reserves from pre-hatching phase; *Archidoris pseudoargus* larvae have considerable cytoplasmic yolk granules after hatching (Thompson 1966). Rather surprisingly, these larvae were able to maintain slight growth from 5 days through to at least 14 days at 15 °C, larvae surviving to a least 19 days at

Table 6.2 Data for the rearing of *Archidoris pseudoargus* larvae in 1986 & 1987. *Shell length* was measured across the widest part of the shell; *time to attain competence* is the time from hatching to when the first larvae within a culture begin to demonstrate 'crawling' behaviour.

Year	Temperature (°C)	Shell length at hatching (μm)	Shell length at competence (μm)	Time to attain competence (days)
1986	15	257.4 ± 4.4	363.4 ± 3.3	30
	18	257.4 ± 4.4	361.5 ± 3.1	25
1987	18	249.6 ± 10.1	330.3 ± 4.6	28

Figure 6.3 The influence of diet on the growth of *Archidoris pseudoargus* larvae. Larvae were cultured at constant temperature on different algal diets. In 1986 (upper figure), larvae were cultured on one of three dietary regimes: 1:1 *Rhodomonas* and *Isochrysis*, *Isochrysis* only and *Rhodomonas* only, all maintained at a total concentration of 50 algal cells · ml $^{-1}$ at a temperature of 15 °C. In 1987 (lower figure), these regimes were repeated and in addition, larvae were reared in the culture media only (*i.e.* no algae) at temperatures of 10 and 15 °C.


shell length (µm)
340
330
320
310
300
290
280
0
2
4
6
8
10
12
14
time from hatching (days)
Archidoris pseudoargus
1:1 mixture
Rhodomonas
Isochrysis
shell length (µm)
340
320
300
280
260
240
220
0
2
4
6
8
10
12
14
time from hatching (days)
Archidoris pseudoargus
Isochrysis 15 °C
Rhodomonas 15 °C
1:1 Mixture 15 °C
No algae 15 °C
No algae 10 °C

10 °C. After 10 days, shell diameter of larvae at 10 and 15 °C at did not differ significantly (Kruskal-Wallis: $H = 1.374^{ns}$; 10 °C n = 19; 15 °C n = 9)

6.3.2 Gravimetric analysis

Investigations into the ratio of organic to inorganic fractions in *Archidoris pseudoargus* larvae revealed a number of patterns (Table 6.3 and Figure 6.4). Initially the ash content of larvae is high but declines with age. These data are consistent with the observation that at hatching the larval shell appears 'empty' with the body tissue increasing rapidly to 'fill' the shell as the larvae age. It is likely that assimilated material is employed for the development of body tissue rather than shell growth. Body tissue will be retained post-metamorphosis whereas the shell is disgarded, and therefore any extra energy above the minimum necessary for adequate shell development will be 'lost' to the larva.

6.3.2 Metamorphosis

Investigations into the metamorphosis of *Archidoris pseudoargus* were restricted by the comparative lack of larvae attaining competence. Larvae exposed to the adult prey *Halichondria panicea* had not metamorphosed after seven days exposure; several larvae stopped crawling and shed their shells but metamorphosis did not occur. In dishes with dissolved choline or elevated potassium ion concentration, dishes with potassium had 20% metamorphosis after seven days; for choline, 40% of larvae had metamorphosed after seven days. These results could not, however, be investigated further due to a lack of available competent larvae.

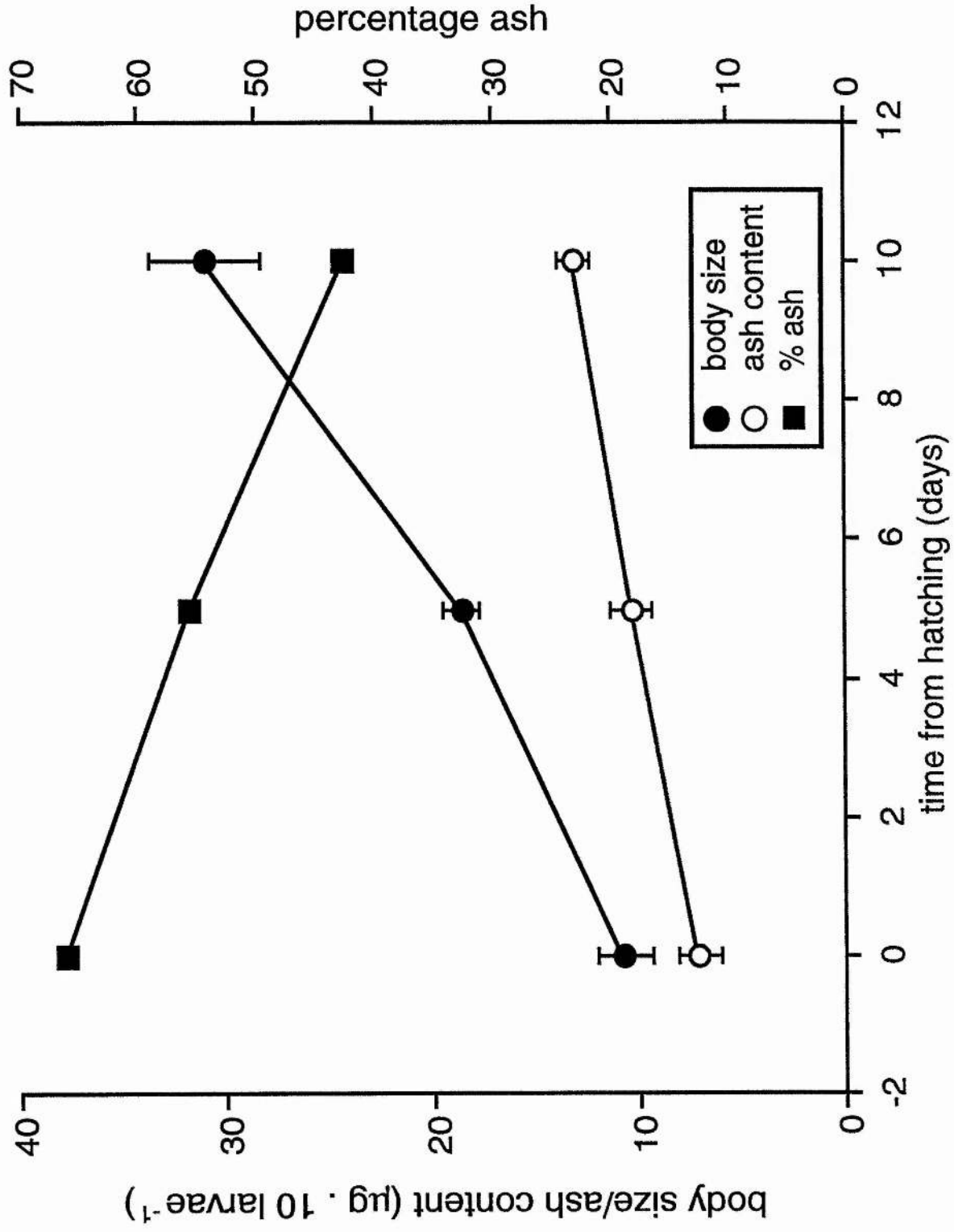
6.4 Discussion

Making provision for the successful development of its offspring from egg through to a juvenile, is the final phase of an organism's life history. Failure at any stage of the larval development process will reduce parental fitness to zero; thus selection should

Table 6.3 Body size and the inorganic ash fraction of *Archidoris pseudoargus* larvae reared at 18 °C (all measures (± 1 se) are for 10 larvae).

Time from hatching (days)	Body size (µg)	Ash content (µg)	Percentage Ash
0	10.7 ± 1.3	7.1 ± 1.0	66.2
5	18.6 ± 0.9	10.4 ± 1.0	55.6
10	31.0 ± 0.8	13.2 ± 0.8	42.6

Figure 6.4 Body size, ash content and the percentage ash of *Archidoris pseudoargus* larvae reared on a 1:1 diet of *Isochrysis* and *Rhodomonas,* at a total concentration of 50 algal cells · ml $^{-1}$, maintained at a constant temperature of 18 °C. Each data point is the mean (± 1 se) of ten determinations of ten larvae.


percentage ash
70
60
50
40
30
20
10
0
body size
ash content
% ash
body size/ash content (µg · 10 larvae⁻¹)
40
30
20
10
0
-2
0
2
4
6
8
10
12
time from hatching (days)

favour mechanisms to promote survival from egg to juvenile to increase fitness. Such mechanisms form the larval development strategy of a marine organism.

Archidoris pseudoargus has been selected for a larval development strategy comprising an initial period of intracapsular development in a benthic egg mass followed by pelagic planktotrophic development to attain a competent pediveliger which settles into the benthic habitat and metamorphoses into a crawling juvenile nudibranch. Of the nudibranch species studied previously, 67% display a similar larval strategy (Hadfield & Miller 1987), although such a high percentage is not surprising since planktotrophic development is generally considered to be the ancestral mode (Strathmann 1985). Planktotrophic development involves a lengthy period in the plankton where mortality rates, due to predation and dispersal away from suitable adult habitat, are considerable; >99% mortality has been estimated (Thorson 1950). However planktotrophic development is generally associated with high fecundity, and Brousseau (1978) estimated that only 0.0001% of the larvae produced by the bivalve *Mya arenaria* need to survive to maintain the population; Vahl (1981) estimated that only 0.00005% of the larvae produced by the scallop *Chlamys islandica* were necessary to maintain its population. On the basis of chance mortality alone, a shorter planktonic period will increase the number of larvae surviving to metamorphosis, therefore selection ought to favour mechanisms to reduce the planktonic period, but at a cost to dispersal (but see Palmer & Strathmann 1981).

Archidoris pseudoargus lays large benthic egg masses from which planktotrophic larvae hatch after 21 days at 14.5 °C. Thompson (1966) recorded a development time of 28 days at 10 °C. For other species with planktotrophic development, Todd & Havenhand (1984) recorded a time to hatching of ≈ 14 days at 10 °C decreasing to ≈ 9 days at 16 °C for *Onchidoris muricata*; Todd & Doyle (1981) recorded a time to hatching of 19 days at 10 °C and 12 days at 16 °C for *O. bilamellata*. From these data it would appear that *A. pseudoargus* has a relatively long benthic development phase for a planktotrophic species. However, *Archidoris pseudoargus* has an egg diameter of 140-170 µm (Thompson 1966; Todd & Havenhand 1984; present study) which is at the upper

limit of the range of egg diameters recorded for planktotrophic opisthobranchs (45-160 μm, mean = 84 μm: Hadfield & Miller 1987). In fact, an egg diameter of 140 μm is equivalent to the mean egg diameter of lecithotrophic opisthobranchs (143 μm: Hadfield & Miller 1987). In general, an increase in egg diameter is associated with a longer development time due to the increased mechanical constraints to cleavage of a larger egg. The ecological implications of varying egg size have been the subject of considerable debate since Vance (1973a&b) and Christiansen & Fenchel (1979) proposed models which predicted that only the extremes of egg size were evolutionarily stable (for review: Strathmann 1985). These arguments will be discussed later. Thus the longer benthic development of *A. pseudoargus* probably attributable to a larger egg size.

But why should a species be selected for a larger egg diameter when larger eggs are energetically more expensive and, from a finite allocation to reproduction, result in lower fecundity? For a planktotrophic species, lower fecundity will increase the chance of total reproductive failure. Strathmann (1985) concluded that "most species with feeding larvae have an egg size above the minimum size needed for development". Egg size is proportional to hatching size and larger eggs generally result in larger larvae (Hadfield & Miller 1987). Hatching *Archidoris pseudoargus* larvae have a shell length of 240-275 μm; Thompson (1966) recorded a hatching size of 290-300 μm, and Todd & Havenhand (1984) recorded a hatching size of 241 μm. For other planktotrophic species, hatching *Onchidoris bilamellata* larvae have a shell length of ≈160 μm (present study; Todd 1991); hatching *Onchidoris muricata* larvae have a shell length of 140 μm (Todd & Havenhand 1984). For the Nudibranchia in general, most planktotrophic species have a hatching shell length of <250 μm (Hadfield & Miller 1987). Therefore *A. pseudoargus* larvae are large in comparison to most other planktotrophic nudibranch species. If size at metamorphosis is fixed, or if there is a maximum size attainable by a pelagic larvae (500 μm: Hadfield & Miller 1987), increasing the hatching size will reduce the pelagic development time and reduce the total larval mortality, but at a cost to reduced dispersal. Perron (1981) recorded decreasing planktonic development time with increasing hatching size for six species of

the tropical gastropod *Conus*. *Archidoris pseudoargus* may be selected for increased egg size to decrease pelagic larval development time and thus decrease total larval mortality.

In general, total pelagic development time for *Archidoris pseudoargus* is less than other nudibranch species with planktotrophic development (Table 6.4). For the species listed, *A. pseudoargus* has one of the smallest benthic sizes (*i.e.* size of post-metamorphic juvenile) which may be a result of this relatively short pelagic development time. A small benthic size is perhaps counter-intuitive when one considers the hatching size (see above). *Halichondria panicea*, the adult prey species, is a heavily spiculose sponge which provides considerable handling problems to a small juvenile. Selection ought to favour a larger benthic size to enable the juvenile to tackle the adult prey. It has been generally assumed that juveniles feed on the the adult prey and larval development strategies may have evolved to overcome prey handling problems (Todd & Doyle 1981). Recent observations suggest that post metamorphic juveniles for some nudibranch species are detritivores, only switching to the adult prey when they have attained a size capable of overcoming prey-handling problems (Todd 1991). Observations of post-metamorphic *A. pseudoargus* juveniles suggest that they do not feed directly on *Halichondria panicea*, but rather graze detritus on the surface of the sponge. Thus there may not be a requirement to attain a large benthic size, and thus pelagic development time can be reduced with the concomitant benefits discussed earlier.

In general, large eggs have a greater organic content than smaller eggs (Strathmann & Vedder 1977) and for planktotrophic species, the organic content of the egg represents the total parental contribution to the offspring. Few studies have attempted to investigate this important life history parameter, while McEdward & Carson (1987) question the basic hypothesis after recording a large intraspecific variation in organic content of echinoderm eggs of similar size. Nonetheless, *Archidoris pseudoargus* produces large yolky eggs in comparison to other planktotrophic nudibranchs. Thompson (1966) observed large quantities of yolk granules in the digestive diverticula of newly-hatched *A. pseudoargus* larvae. This stored energy reserve may explain the early growth patterns of this species.

Table 6.4 A comparison of the pelagic development times for *Archidoris pseudoargus* larvae with other nudibranch species with planktotrophic larval development from Britain and other biogeographical areas.

Species	Time to competence	Benthic size (μm)	Reference
Archidoris pseudoargus	30 days at 15 °C		Present study
	25-28 days at 18 °C		ditto
	37 days at 10 °C	≈300	Todd & Havenhand (1984)
British species			
Onchidoris bilamellata	31 days at 15 °C	≈470	Todd (1991)
	40-50 days at 10 °C		Chia & Koss (1988)
O. muricata	58-59 days at 10 °C	≈300	Todd & Havenhand (1984)
Rostanga pulchra	35-40 days at 10-15 °C	≈270	Chia & Koss (1978)
Others			
Tritonia diomedea	34-41 days at ≈12 °C	≈440	Kempf & Willows (1977)
Melibe leonina	30-48 days at 12-14 °C	≈335	Bickell & Kempf (1983)
Doridella steinbergae	25-26 days at 12-15 °C	≈210	Bickell & Chia (1979)
Hypselodoris infucata	16 days at 26 °C	≈306	Hubbard (1988)

Initial growth of *Archidoris pseudoargus* larvae is very rapid with the larvae attaining near maximum shell length approximately half way through the pre-competent phase. In contrast, *Onchidoris bilamellata* larvae grow slowly at first, followed by an exponential phase attaining maximum shell length at competence. Hubbard (1988) recorded a similar pattern for the tropical dorid *Hypselodoris infucata*. In a review of the physiological ecology of molluscan larvae, Bayne (1983) stated that the normal growth curve is linear or asymptotic and occasionally sigmoidal; there is evidence of greater sigmoidicity at lower temperatures (Loosanoff *et al.* 1951; Sprung 1982). Growth of *A. pseudoargus* larvae concurs with these conclusions; at 5 °C, initial growth would suggest a sigmoidal growth curve. When *A. pseudoargus* larvae are cultured without food this early growth pattern is still observed (although larvae may absorb nutrients directly from the culture media: Shilling & Manahan (quoted by Kempf & Todd 1989). It is likely therefore, that this early growth will be supported by internal yolk reserves rather than from ingested material, although the possibility of bacterial ingestion cannot be discounted. As the yolk supply diminishes, ingested food material will gradually replace yolk as the primary source of energy. Large yolk reserves are more typical of lecithotrophic larvae which, in general, do not feed in the pelagic phase, but rely on their stored energy reserves for recurrent energy expenditure (Kempf & Todd 1989). Kempf & Todd (1989) considered the incorporation of larger yolk-derived energy reserves to be an important evolutionary step in the change from obligate planktotrophy to obligate lecithotrophy, and ultimately to direct development.

Results of the gravimetric analysis support the observation that newly hatched *Archidoris pseudoargus* larvae have an 'empty' appearance, the volume of body tissue increasing steadily during early growth. Newly-hatched larvae have an ash content of 66% which declines to 29% after 18 days. Equivalent data are not available for other planktotrophic nudibranch species although Kempf & Todd (1989) recorded an ash content of 38.5% and 50% for newly hatched larvae of *Tritonia hombergi* and *Adalaria proxima* respectively; larvae of these latter species are pelagic lecithotrophic. Holland & Spencer (1973) observed that the the inorganic proportion of bivalve veligers declines

during larval development; in the oyster *Ostrea*, tissue organic fraction increases from 21-30% to 37-40% during development (M Helm quoted by Bayne 1983). Mann & Gallager (1985) report the results of a detailed investigation of the physiological and biochemical energetics of larvae of *Teredo navalis* and *Bankia gouldi*, two species of wood boring mollusc. For larvae of *T. navalis*, the ash content declined from 84% at release to 75% after 40 days (although a value of 61.8% was recorded at 30 days); a similar result was recorded for starved *B. gouldi* but no change in the ash content was observed for fed larvae. *A. pseudoargus* larvae have a relatively high organic content at metamorphosis which may be important for provisioning the subsequent juvenile to overcome prey-handling problems during the first few weeks of benthic life (see above).

Kempf & Todd (1989) suggested that one of the changes in larval characteristics during a transition from planktotrophy to lecithotrophy is the loss of inducer-mediated metamorphosis. In general, studies have shown that the natural induction of metamorphosis for a nudibranch veliger is attributable to some component of the live adult prey material (*e.g. Adalaria proxima*: Thompson 1958; *Onchidoris bilamellata*: Todd 1981; Arkett *et al.* 1989; *Phestilla sibogae*: Hadfield & Scheuer 1985). Most nudibranchs are monophagous predators and thus prey-induced settlement and metamorphosis should perhaps be expected. Preliminary investigations by Todd & Havenhand (1984) suggested that *Archidoris pseudoargus* larvae metamorphose following contact with *Halichondria panicea*. Although only partially investigated during the present study, there was no indication that contact with *H. panicea* induced metamorphosis in competent *A. pseudoargus* pediveligers. Thompson (1966) recorded a similar result although questions remain over the developmental status of the larvae because the author stated that he was unsuccessful in rearing larvae to competence. Boucher (1986) noted that the tropical dorid *Gymodoris striata* may not have a specific metamorphic cue, and Hubbard (1988) found non-specific metamorphic induction in the the sponge grazing dorid *Hypselodoris infucata*. An inability to feed on *H. panicea* in the post-metamorphic period would obviate the necessity to cue to the adult prey. It is likely that *A. pseudoargus* larvae respond to either a habitat cue or perhaps to another sponge species normally associated with the adult prey. Recently, the influence of microbial films on settlement and

metamorphosis has been considered (Maki & Mitchell 1985; Maki *et al.* 1989). Microbial films may 'describe' the habitat to a settling larvae and indicate the suitability of that habitat for settlement and metamorphosis. Such ideas are at an early stage of investigation but may provide clues to the likely cues for non prey-mediated settlement and metamorphosis. Bloom (1981) found that adult *Anisodoris nobilis* (and possibly for *Archidoris montereyensis*, and *Diaulula sandiegenisis*) attained a larger final adult size if juveniles fed on the sponge *Myxilla incrustans* and switching to *H. panicea* having once attained a minimum size. It is possible that a similar phenomenon may be present in *A. pseudoargus* and therefore the metamorphic cue will be the intermediate prey rather than the final adult prey.

Archidoris pseudoargus clearly defies the models of Vance (1973a&b) and Christiansen & Fenchel (1979) in that it appears to have an intermediate egg size, hatching ultimately as a large planktotrophic larva which has a relatively short pelagic phase. Kempf & Todd (1989) considered it would not be unreasonable to predict that at least a few species would have 'intermediate' larval stages falling between the two extremes. "Intermediate larvae would have unusual features in the length of the larval period, growth, amount of parentally derived yolk in the larva, a dependence on feeding for nutrition and metamorphosis and the presence, absence or function of organs associated with larval feeding" (Kempf & Todd 1989). Whilst the latter authors were considering feeding in lecithotrophic larvae (as a primitive feature), the characteristics of short pelagic period, asymptotic growth, and early larval growth supported by internal yolk reserves, suggest that *A. pseudoargus* larvae are representative of an evolutionarily 'advanced' planktotrophic larvae.

On the basis of size and by inference gross energetic allocation to reproduction, *Archidoris pseudoargus* individuals are, in absolute terms, clearly capable of producing sufficient eggs which could develop by either of the three larval development modes. Individual embryo success improves from planktotrophy to lecithotrophy to direct development and therefore selective pressures should favour an evolutionary change to the 'safest' larval development mode. As *A. pseudoargus* has a semelparous biennial life

history strategy, it is imperative that it overcomes the disadvantages of both aspects of this strategy (see Chapter 5). Intuitively, one would expect directional selection for the 'safest' larval reproductive strategy. In contrast, *Cadlina laevis* has evolved the 'safest' larval strategy (= direct development) combined with an iteroparous perennial life history strategy. Nevertheless, direct development has inherent disadvantages in reduced dispersal, which could result in local extinction, and inbreeding depression. Large organisms have the potential for extremely high fecundity and are able to produce sufficient small planktotrophic eggs to offset pelagic mortality; Menge (1975) estimated that the large starfish *Pisaster ochraceus* was able to produce 4×10^7 eggs and thus replace itself despite presumably massive planktonic mortality. Smaller organisms may not be able to generate sufficient gametes to overcome planktonic mortality and therefore selection moves towards a 'safer' larval strategy. Strathmann & Strathmann (1982) and Strathmann *et al.* (1984) proposed that small animals have evolved brooding as a 'safer' larval development strategy because their small size does not permit the production of sufficient planktonic larvae. Brooding could be equated to direct development in that the adult invests additional energy in 'parental care'. *C. laevis* is a relatively small dorid which may be incapable of producing sufficient planktotrophic larvae and therefore selection has moved towards the 'safer' larval development strategy. Ultimately for a population to remain viable, an organism must replace itself in the subsequent generation and therefore if planktotrophic development satisfies this requirement there will be lower selective pressure to change the reproductive (and larval) strategy. If *A. pseudoargus* has an 'advanced' planktotrophic larva, is it evolving toward the 'safer' lecithotrophic mode, or is there selective advantage in having a planktotrophic mode and, therefore, has *A. pseudoargus* evolved mechanisms of maximising the success of the planktotrophic mode? What are the likely advantages of a planktotrophic larval strategy?

The relative advantages and disadvantages of a planktotrophic mode of larval development have been the subject of intense debate during recent years. A number of comprehensive reviews have been published (Strathmann 1980, 1985; Jablonski & Lutz 1983; Day & McEdward 1984; Grahame & Branch 1985; Todd 1985b) and I do not propose to repeat these reviews, rather to highlight a number of important points.

Dispersal away from the adult habitat has been invoked as the major benefit of pelagic development (although see Palmer & Strathmann 1981). Dispersal allows for increased gene flow between populations (although see Todd *et al.* 1988), spreading of sibling larvae, colonisation of widespread habitats, perhaps with an extension to the geographical range, and a means of overcoming density independent mortality in patchy habitats (Crisp 1974). If food supplies are temporally unstable, it would be selectively advantageous for larvae to disperse away from the adult habitat to colonise new sites. For *Archidoris pseudoargus*, adults will have been present in a habitat for two years and local food supplies may have diminished, thus it may be advantageous for larvae to spread away from the adult habitat. Strathmann (1985) concluded "though dispersal has profound evolutionary consequences, it does not appear to be the selection for dispersal that maintains a feeding larval stage in life histories." Direct development reduces dispersal and retains larvae within the adult habitat, although at the risk of local extinction if the habitat becomes unfavourable. *Cadlina laevis* feeds on the sponge *Halisarca dujardini* which, whilst locally abundant, appears spatially rare (pers. obs.). Dispersal away from an abundant food source may result in larvae unable to find another suitable habitat with an adequate food supply. In this instance, selection would favour reduced dispersal to enable larvae to exploit the local food source, populations will be regulated by density dependent selection. If *H. dujardini* has restricted distribution, selection ought to favour direct development (or extended planktotrophy but with corresponding increase in larval mortality - see above). Further investigations are required to determine the spatial and temporal stability of *H. dujardini,* and to determine the energy budget of *C. laevis,* before any firm conclusions can be drawn on the selective pressure which has led to the evolution of direct development.

A much overlooked aspect of selection for larval reproductive strategies are egg to juvenile periods (EJPs). Todd & Doyle (1981) proposed the 'settlement-timing hypothesis' to explain why *Onchidoris bilamellata* has been selected for a planktotrophic development mode when, on energetic grounds, lecithotrophic or direct development could be supported. Todd (1991) has subsequently withdrawn this hypothesis with regard to *O. bilamellata* although the principle remains important. Havenhand (In press)

has developed a model to explain how the reduction of the EJP can increase the benthic development period, or decrease the overall generation time. If, as suggested in earlier chapters, the food supply of *Archidoris pseudoargus* is seasonally variable, it will be advantageous to reduce the EJP to ensure post-metamorphic juveniles have the maximum period for growth prior to any seasonal reduction in food quality. Similarly, *Cadlina laevis* has markedly seasonal growth which could be a reflection of seasonal variations in food supply (see Chapter 3). A necessity to target the appearance of post-metamorphic/post-hatching juveniles with maximal food availability may be compounded if these juveniles are unable to feed on the adult prey during early benthic life. Todd & Doyle (1981) determined that lecithotrophic development, followed by direct development may, at least for some species, provide the shortest EJP. *A. pseudoargus* may be 'intermediate' in the evolution of a lecithotrophic mode to reduce its EJP whilst *C. laevis* have evolved direct development as a 'safer' option but with a relatively short EJP. Similarly, if a pelagic phase is advantageous (for some of the reasons outlined above), *A. pseudoargus* may have evolved mechanisms to minimize the pelagic development time whilst maintaining a dispersive pelagic phase.

From the data collected during the present study, it is not possible to determine the precise factors influencing the evolution of planktotrophic development in *Archidoris pseudoargus* or direct development in *Cadlina laevis*. Further investigations are required particularly investigating seasonal variations in prey quality and the biochemical composition of the larvae with age, and the cues to metamorphosis for *A. pseudoargus*.

CHAPTER 7

GENERAL DISCUSSION

Most species of nudibranch mollusc display annual, semelparous life history strategies whereas all dominant rocky shore species have extended life cycles and iteroparous reproduction (Todd 1985). Thus for nudibranchs an important question is whether an annual, semelparous life history strategy is the ancestral condition, or an adapted strategy as a consequence of natural selection. In conjunction, many nudibranch species employ a planktotrophic mode of larval development which is also considered to be the ancestral condition (Strathmann 1985). Previous observations had suggested that *Archidoris pseudoargus* and *Tritonia hombergi* had evolved to a biennial life history, with the latter species additionally evolving to a lecithotrophic mode of larval development, and *Cadlina laevis* had evolved to a perennial life history with a direct mode of larval development. In Chapter 1, it was stated that the central aim of this present study was to investigate these observations and to attempt to answer the questions "Why do these species live longer than a year", and for *C. laevis*: "Is this species perennial and iteroparous, and if so why?" Additional to these principal questions was the aim to investigate the larval development strategies of these species.

Data collected during the present investigation and presented in Chapters 3-6, confirm these earlier observations on the life history and the mode of larval development of these species. Archidoris pseudoargus has evolved to a semelparous, biennial (at least) life history with a planktotrophic mode of larval development; investigations on the larval biology indicate this species may have an 'advanced planktotrophic larvae'. *Cadlina laevis* has evolved to a perennial life cycle with an iteroparous life history, which was the first observation of this life history strategy within the order Nudibranchia. Insufficient numbers of *T. hombergi* were collected and kept alive in the laboratory to undertake any experiments to generate meaningful data to answer these principal questions for this species. Consequently, the remainder of this discussion will concentrate on *A. pseudoargus* and *C. laevis*. Confirming early observations on the longevity of these species was only part of the present study, it remains to propose reasons why there is this apparent departure from the more common reproductive strategy observed within the Nudibranchia.

In reality, it is improbable and perhaps unrealistic to expect that within the time frame imposed on these studies one will be able to make meaningful conclusions on the evolutionary forces which mould an organism's life history and larval development strategy. Stearns & Koella (1986) considered that strong tests of life history theory are impossible because so many predictions require a response to selection to be observed. Life history and larval development strategies have evolved over millennia and therefore significant changes are unlikely to be observed within the time frame of an investigation. Thus all conclusions based on the data collected during the present study provide some pointers to these evolutionary forces rather that definitive conclusions. To support these pointers, ideas and conclusions from earlier published studies will be incorporated into the following discussion.

Organisms function via a series of physiological processes which depend on energy: energy is therefore a common resource between all living organisms. Organisms must obtain energy from the environment, either directly, or indirectly from the breakdown of food material. In the application of the *Law of conservation of energy*, the energy input to an organism must match the energy output. For animals, the energy input derives ultimately from food, the energy output is ultimately heat and excreta, although for growing organisms, the production of tissue acts as a temporary energy store. Patterns of production are controlled by enzymes and hence ultimately by genes, so following neo-Darwinian principles, patterns of production which best promote the spread of genes will be favoured. In this sense, phenotypes are adapted and the study of adaptation investigates the occurrence of, and explanation for, traits within a population (Sibly & Calow 1986). The core neo-Darwinian hypothesis usually adopted is that successful phenotypic traits will be those which *maximize* fitness: ecological *fitness* of an individual may be defined as the number of offspring produced by that individual which themselves survive to reproduce and contribute to subsequent generations. That is, fitness of the individual is dependent on its reproductive success and the reproductive success of its offspring. Physiological processes cannot act in isolation and in practice, it will not be possible to *maximize* all components of fitness simultaneously. Ultimately there will be

compromise between competing components, or a trade-off, which will result in the *optimization* of each component to *maximize* total fitness. In a complex life history strategy there will therefore be multiple routes to reproductive success. From the foregoing discussion, energy flux may be considered a central tenet of all life history events. Processes to effect the acquisition of energy may be considered the primary adaptive life history parameters, other life history parameters adapt to maximize fitness from the baseline energy supply set by the processes of acquisition.

Carnivorous organisms gain energy by consumption of a prey material. For prey, predation will reduce its future fitness to zero and therefore predator-prey interactions may provide profound selective pressures for the evolution of life history parameters. Predators which evolve more effective means of prey capture are likely to increase their fitness at the expense of a loss of fitness to the prey; prey which develop effective anti-predator tactics will increase their fitness at the expense of the predator's fitness. Most nudibranch molluscs are specific predators on a single, or restricted range of, prey species (Todd 1981, 1983), and any evolutionary changes to their prey are likely have a profound influence on the fitness of the nudibranch. Could changes to the availability of resource energy (*i.e.* prey) provide the selective pressure to extend an organism's life cycle? Data collected from investigations in the plant kingdom, together with some examples from the marine environment, suggest that resource availability could lead to extended life cycles.

What adaptations would be necessary to contend with an extension to the life cycle? *Archidoris pseudoargus* and *Cadlina laevis* are temperate species and are therefore subject to seasonal variations in the environment. Adult and juvenile survivorship is dependent on adaptations which overcome these seasonal variations - variations in temperature in particular. In addition, it is likely that there will be seasonal variations in prey supply and prey quality, and thus long lived species should be adapted to overcome seasonal changes in energy supply. Organisms should be metabolically efficient to ensure that energy is not 'wasted' - particularly when the energy supply is variable. Ultimately to maximise fitness, organisms should evolve mechanisms to effect successful reproduction.

Following classical theory, it will be necessary for biennial semelparous species, and iteroparous species which first reproduce in their second year, to achieve sufficient fecundity, or ensure offspring survival to reproduction, to offset the 'missed' reproductive event.

Increasing the duration of the life cycle will increase the probability of pre-spawning mortality and therefore mechanisms to increase adult survivorship assume greatest importance. For *Archidoris pseudoargus*, there is a positive relationship between feeding rate and body size, although the relationship is allometric with an exponent less than unity suggesting a mechanical constraint in larger individuals (Chapter 3: Figure 3.4). Carefoot (1967) recorded an overall assimilation efficiency of 52% but a protein assimilation efficiency of 93%. From the data presented by Barthel (1986), the organic component of *Halichondria panicea* is predominantly protein, and thus a high protein extraction/assimilation efficiency will maximize the energetic gain from ingested material. Phylogenetic adaptation to a prey resource gradient has determined that caecate dorids must feed intermittently, the non-feeding periods are required to process the waste food material. A high extraction efficiency, combined with selective absorption, will offset the 'lost' feeding time. Thus for *A. pseudoargus*, high assimilation rate ensures energy is available for growth and reproduction. *Cadlina laevis* is an aceacate dorid evolved to the opposite extreme of a prey resource gradient - the slime sponge *Halisarca dujardini* . Based on the conclusions of Bloom (1981), it is likely that *C. laevis* is evolved to maximise the energetic gain from this prey.

To ensure that energy gained from feeding can be utilised for production (somatic growth and gametogenesis), energy losses from respiration, mucus production and excretion should be minimised. Whilst mucus production and excretion were not quantified, size specific respiration rates (R) for *Archidoris pseudoargus* and *Cadlina laevis* were lower than R values for annual species (Chapter 4: Table 4.5; Figures 4.8 & 4.10). For both species, a statistically significant allometric relationship was determined between oxygen consumption and body size with an exponent less than unity (Chapter 4: Figures 4.2 & 4.5), indicating that larger organisms have proportionally lower respiration

rates - *i.e.* reduced energetic losses. For *A. pseudoargus,* this respiratory exponent was less than the feeding rate/body size exponent, further indicating improved metabolic efficiency with increasing body size; increasing body size can have considerable ecological benefits. Considering the feeding pattern of *A. pseudoargus*, a low value for R will be important to reduce losses to respiration during 'resting' periods. Preliminary investigations suggest that *C. laevis* is able to withstand long interruptions (months) to its food supply, and a low value of R will be an important contributory adaptation to maintain an individual's viability.

In a seasonal environment, an increase in temperature will increase the rate of physiological processes and therefore acclimatization of loss functions (particularly respiration) will maximize the energy available to growth and gametogenesis. Similarly for a seasonal food supply, improved metabolic efficiency when food is available will lessen the demand on body reserves when food is limiting and thus maintain body size and/or allocation to production (somatic or gametic). A low basal metabolic rate will reduce the requirement to synthesize energy reserves to overcome a reduced supply at other times of the year. An increase in temperature will increase the rate of chemical reactions which may permit increased feeding, growth and gametogenesis but at the expense of increased energy loss due to respiration. This will be particularly important at the beginning of the growing season when endogenous reserves will be low. Acclimatization to increasing temperature during this critical period will be important to avoid an excessive drain on endogenous reserves which could lead to the death of the individual. Additionally, acclimatization will maximize energy available to production as other rate reactions increase. Both *Archidoris pseudoargus* and *Cadlina laevis* show acclimatization of their respiration rates with increasing temperature although for *A. pseudoargus*, mean size specific respiration rate increase rapidly at temperatures approaching the mean summer temperatures (Chapter 4: Figures 4.4 & 4.7). Nevertheless, acclimatization of R to increasing temperature at the lower end of the annual range will be important to overcome an increase in R during the spring when endogenous reserves may be low. For both species, there appears to be a trend, whilst not statistically

significant, of a decline in the weight exponent *b* with increasing temperature (Chapter 4: Figure 4.9), indicating that larger individuals are more efficient at higher temperature. This may be an important additional adaptation to reduce respiratory losses and maximize energy to production during summer periods, possibly when prey abundance and/or prey quality is at its peak. *A. pseudoargus* and *C. laevis* demonstrate important adaptations to an extended life cycle - low R and acclimatization to seasonal changes in temperature - which combine to maximize the energy available to production.

Fitness is a function of the number of offspring and their quality (= survivorship). Assuming equivalent larval/juvenile survivorship, species with a biennial and semelparous life cycle must have increased fecundity to offset the 'missed' reproductive event. For both *Archidoris pseudoargus* and *Cadlina laevis*, a significant allometric relationship exists between body size and fecundity suggesting larger individuals have a proportionally higher fecundity (Chapter 5: Figures 5.2 & 5.5). *A. pseudoargus* has massive fecundity which would, from simplistic calculations (Chapter 5), more than offset the 'missed' reproductive event. This massive fecundity is complemented by a relatively short egg to juvenile period to reduce the loss of larvae due to planktonic mortality. For *C. laevis*, simplistic calculations indicate that fecundity at first reproduction offsets the 'missed' reproductive event caused by low juvenile growth during their first winter. Furthermore, *C. laevis* has evolved to an iteroparous life history strategy which will lead to higher lifetime fecundity. It could be further postulated that a direct mode of larval development is equivalent to an increase in fecundity by reducing larval mortality and thereby increasing the number of juveniles recruiting to the adult habitat. Although direct development results in an increase in egg size and the direct trade off between fecundity and egg size may reduce the total number of recruiting juveniles. Increasing larval and/or juvenile survivorship will increase fitness of longer-lived species. The planktotrophic larval development strategy of *A. pseudoargus* has some of the characteristics of the 'safer' lecithotrophic mode of larval development presumably resulting in increased larval survivorship. *Cadlina laevis* has evolved to the 'safest' direct mode of larval development which will increase juvenile survivorship - but at the expense

of a loss of dispersal and reduced gene flow with the associated risk of inbreeding depression.

Increasing body size would appear to confer a significant selective advantage to a individual by increasing metabolic efficiency and increasing fecundity. But rather surprisingly, peak body size of *Cadlina laevis* occurs approximately four months prior to spawning, and pre-spawning body size is 25% less than peak body size; a rather surprising observation as intuitively, the proliferation of tissue in gametogenesis should lead to an increase in body size. Body size does increase immediately prior to spawning for *A. pseudoargus*. This opens the question as to when does gametogenesis occur: is it rapid process prior to spawning? Thompson (1966) reported, on the basis of an extensive histological study, that *A. pseudoargus* undergoes gametogenesis throughout the winter period. From other studies, extended gametogenesis would appear to be the norm in nudibranchs and thus it is likely that gametogenesis in *C. laevis* follows a similar pattern. It is possible that the decline in weight of *C. laevis* is a consequence of the organism converting carbohydrate and protein reserves to lipid (lipids are less dense than carbohydrates and proteins), but the magnitude of the decline would appear too great for this to be the only explanation. Energy for gametogenesis in *A. pseudoargus* and *C. laevis* may be derived from the catabolism of endogenous reserves; available energy is maximized by a low size specific respiration rate (R). In the spring when prey quality increases, adults increase the energy intake prior to spawning to maximize spawn output; acclimatization of R at this stage will facilitate the allocation of resources to gametic production. Havenhand & Todd (1988b) reported that *Adalaria proxima* diverts body reserves to gametogenesis. If *A. pseudoargus* and *C. laevis* adopt this allocation pattern, it may explain the observation that adults only produce a single spawn mass. If body reserves are utilized in gametogenesis, the limits of body metabolism may be exceeded and if the post-spawning rate of energy acquisition is insufficient to offset losses, the organism will expire - *i.e.* semelparous reproduction as observed in *A. pseudoargus*. Similarly, it could be postulated that spawning in these species is linked a pre-programmed end-point, perhaps related to prey availability *sensu* the settlement timing hypothesis (Todd & Doyle 1981), and thus the organism maximizes output at the

optimum time. Further investigations are required to determine the patterns of resource allocation to resolve these issues.

In summary, it would appear that *Archidoris pseudoargus* and *Cadlina laevis* have evolved a series of adaptations to the basic life history processes to offset the potential selective disadvantages associated with a biennial first (and last for *A. pseudoargus*) age of first reproduction and extended life cycle: a positive allometric relationship between body size and feeding rate; low size specific respiration rate and increased metabolic efficiency with body size; an ability to acclimatize to seasonal changes in temperature; proportionally higher fecundity with increasing body size; adaptations to reduce larval mortality and increase juvenile survivorship. It is possible that the selective pressure to extend the life cycle was derived from co-evolutionary predator-prey adaptations. Phylogenetic constraints, perhaps combined with interspecific competition within the guild, prevented prey-switching when prey evolved towards seasonal growth patterns. As a consequence, reduced energy acquisition may prevent the predator attaining a minimum size necessary for successful reproduction in its first season, and thus provide the selective pressure towards delaying reproduction to the following season.

Returning to the central aim of the present study, namely to determine the selective pressures leading to the prolongation of a life cycle, it has been implicitly assumed that extended life cycles are an evolutionary *departure* from the ancestral condition. The foregoing discussions have highlighted the physiological adaptations necessary for an extended life cycle. In a review of the reproductive strategies of rocky shore invertebrates, Todd (1985) noted that with a few exceptions, all dominant rocky shore species have extended life cycles and iteroparous life history strategies. Most species attributed to the principal taxonomic groups: Anthozoans, Polychaetes, Crustacea, Gastropods (except Opisthobranchs), Molluscs and Echinoderms live longer than one year. On hard substrata, competition for space is important and the fundamental requirement of a reproductive strategy is to maximise occupation and retention of space. Todd (1985) argued that this requirement could only be met via a perennial iteroparous life history strategy.

Carnivorous species do not have this requirement for space occupation but have a requirement to exploit a dependable prey source. For a stable and predictable prey, an organism can increase fitness by increasing larval and/or juvenile survivorship by evolving to an iteroparous life history combined with non-pelagic larval development. A temporally unstable prey would give rise to selective pressure for shorter life cycle (to match that of the prey); similarly, spatially unstable prey would provide the selective pressure to evolve to pelagic larval development.

Based on the life cycles of most other marine invertebrates, extended life cycles could be the ancestral condition within the order Nudibranchia and shorter life cycles would thus be the more adapted/evolved strategy. To complete their life cycle, short-lived species usually require a high growth rate (Clark 1975). From a finite energy supply, high growth rates require a high energy allocation which can be provided by increasing assimilation and/or increasing metabolic rate (Clark 1975). Results from the present study would support this hypothesis. Increasing the respiration rate increases the rate of ATP production which provides the energy for growth and gametogenesis. Clark (1975) suggested that the respiration rates for species with cerata (mainly the more evolved aeolids) were higher that for non-ceratal species (dorids). *Archidoris pseudoargus* and *Cadlina laevis* have lower respiration rates than annual species. Acclimatization of respiration rate to increasing temperature may limit the supply of ATP to growth and gametogenesis. For *A. pseudoargus*, the rapid increase to the respiration rate at higher temperatures may have a selective advantage by increasing its metabolic rate when food is abundant; higher metabolic rates may lead to a larger body size with a corresponding increase in fecundity. Improved assimilation efficiency can be achieved by gut and dietary specialization. Dorids have primitive unbranched guts whilst aeolids and some dendronotids have evolutionarily more advanced branched guts. Branched guts have a higher surface area/volume ratio and therefore permit higher assimilation rates. *A. pseudoargus* also has a low feeding rate and extraction efficiency in comparison to annual species. But, a high growth rate is dependent upon an adequate supply of energy in the form of the prey species.

Selection for life history and life cycle strategies could be a function of prey availability. To date, those nudibranch species with extended life cycles have temporally and spatially stable prey in the form of sponges and alcyonarean soft corals. Bloom (1981) concluded that dietary specialization could provide the selective pressure to evolve toward the extremes of an evolutionary gradient. Todd (1983) suggested that temporally unstable prey may provide the selective pressure for the evolution of shorter life cycles. *Cadlina laevis* may demonstrate the ancestral life cycle and life history strategy - perennial iteroparous reproduction. Evolution of direct development is an adaptation to improve juvenile survivorship to maximise fitness. But for *Archidoris pseudoargus* (and other biennial species), the results from the present study pose another fundamental question: 'Why die after first reproduction?' Post-reproductive adult *A. pseudoargus* have a considerable body size in comparison to most other nudibranch species and, on the basis of size alone, would appear able to survive to reproduce in future seasons. What are the selective pressures leading to a reduction in the life cycle? Aeolids generally prey on hydroids which tend to have temporal fluctuations in population density. Prey availability may provide the selective pressure to evolve towards a shorter life cycle (*sensu* Todd 1983). *Halichondria panicea* has been shown to have seasonal variations in its biochemical composition (= prey value) and such variations may have provided the selective pressure for *A. pseudoargus* to *reduce* its life cycle from the ancestral condition.

At the beginning of the present study, suggestions of extended life cycles and iteroparous reproduction in the order Nudibranchia were based on casual observations and considered a departure from the ancestral condition. Subsequently, the results obtained during the study have demonstrated these conditions exist, and further provided data to support the postulated adaptations necessary to support these life cycle and life history strategies. A perennial iteroparous reproductive strategy may be the ancestral condition and an annual semelparous reproductive strategy an evolutionary derivative; biennial semelparous strategy being perhaps an evolutionary intermediate. Clearly further studies are required to investigate these ideas and in particular, attempt to answer the question: 'Why die after first reproduction?'

CHAPTER 8

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