THE BRACHIOPOD GENOME

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"Our classifications will come to be, as far as they can be so made, genealogies . . . we have to discover and trace the many diverging lines of descent in our natural genealogies by characters . . . which have long been inherited" (DARWIN, 1859a, p. 486).

STRUCTURE, COMPOSITION, AND ORGANIZATION OF THE NUCLEAR AND MITOCHONDRIAL GENOMES

INTRODUCTION

The genome is the complement of genetic material that an organism inherits from its ancestors; it is embodied chemically in deoxyribose nucleic acid (DNA). In most metazoans the genome consists of two components, nuclear and mitochondrial, that have separate evolutionary origins and distinct modes of inheritance. The nuclear genome, packaged in the chromosomes, typical of eukaryotes and of still-mysterious origin (GOLDING & GUPTA, 1994), is by far the largest component, being involved in the storage, transmission, recombination, and controlled expression of most of the genetic information. The mitochondrial genome (mtDNA), a relatively small element within the mitochondrion, is a relic of the genome of a prokaryotic endosymbiont (KARLIN & CAMPBELL, 1994) and is now responsible for only a limited number of essential functions, having lost most of its genes either to the chromosomes or completely. In metazoans, the mitochondrial genome is usually transmitted asexually by the maternal parent.

Investigation of genome functional anatomy may be thought of as proceeding at four levels characterized by use of different analytical approaches. At the organismal and population level, genetic markers (identifiable phenotype differences determined by allelic, homologous genes) are used to trace the transmission of the genome between individuals of successive generations or between populations. This approach may either clarify the mechanism of heredity itself (e.g., show how a character is transmitted in a controlled mating), clarify the mechanism of heredity in relation to the life cycle (e.g., tell whether the organisms are haploid or diploid), or illuminate the genetic structure of populations (e.g., show whether there is gene flow between them). At the cellular and subcellular levels, microscopic techniques may be used to characterize the structural organization of the genome, especially to determine the size and number of chromosomes and the existence of special features such as sex chromosomes. Microscopy may also be used to characterize chromosome behavior in somatic and germinal cell divisions. At the third level, biochemical analyses of deoxyribose nucleic acid (DNA) or ribose nucleic acid (RNA) extracted in bulk enables some properties of the genome to be recognized and measured, such as the total quantity of DNA per nucleus, the genome complexity, or the existence of genome fractions composed of repeated sequences (BRITTEN & KOHNE, 1968). The fourth level of analysis involves determination of the organization, sequence, and modes of expression of individual genes and gene products, whether RNAs or proteins, using the techniques of molecular biology. Such sequence information provides the primary evidence for the reconstruction of phylogenetic history and for the identification of evolutionarily homologous structures and developmental functions (ZUCKERKANDL & PAULING, 1965).

Unhappily, no coherent program of research to characterize any brachiopod genome at any analytical level has been undertaken. All that exists are scattered observations at each level, together with the

beginnings of an attempt to use DNA sequence data to provide a molecular framework for brachiopod phylogeny (B. COHEN & A. GAWTHROP, unpublished work, 1995; COHEN, GAWTHROP, & CAVALIER-SMITH, in preparation) and work in progress on the complete DNA sequence of *Lingula* mtDNA (K. ENDO, personal communication, 1994). This chapter will briefly review the available, scattered information and will provide an account of results from the ongoing work on molecular phylogeny.

THE GENOME IN RELATION TO LIFE CYCLE AND POPULATION DYNAMICS

The organization of the brachiopod nuclear genome in relation to the life cycle is firmly established. Like most sexually reproducing metazoans, brachiopods are normally diploid (AYALA & others, 1975; VALENTINE & AYALA, 1975; HAMMOND & POINER, 1984; BALAKIREV & MANCHENKO, 1985; COHEN, BALFE, & CURRY, 1986), and gametes are produced by meiosis. Although brachiopod meiosis has not been fully described, evidence for its existence can be found in the pioneer cytological work of YATSU (1902a) and in recent studies of gametogenesis (JAMES & others, 1992). As would be expected of marine organisms that broadcast gametes of at least one sex, genetic markers indicate that sampled populations are large (*sensu* population genetics) and random mating (AYALA & others, 1975; VALENTINE & AYALA, 1975; HAMMOND & POINER, 1984; BALAKIREV & MANCHENKO, 1985; COHEN, BALFE, & CURRY, 1986). The limited available evidence is consistent with the anticipated matrilineal transmission of the brachiopod mitochondrial genome (COHEN & others, 1991; COHEN & others, 1993).

GENOME SIZE AND CHROMOSOME NUMBER

Despite the vast scope of animal cytology, there appears to be only a single report of brachiopod chromosomes: YATSU (1902a) clearly figured eight small, paired chromosomes in oogenesis of *Lingula anatina.* The range of variation in number, size, and architecture of brachiopod chromosomes remains unknown.

The DNA content of brachiopod genomes (nuclear plus mitochondrial) has been measured by HINEGARDNER (cited by BRITTEN and DAVIDSON, 1971). Using a fluorometric assay on DNA extracted from *Glottidia pyramidata* and *Lingula* sp., he estimated the haploid DNA content as 0.43 and 0.38 picograms, corresponding respectively to 4.15 and 3.67 x 10^8 nucleotide pairs per haploid genome. Since the estimated measurement error was 5 percent, the two species probably have different genome sizes (R. HINEGARDNER, personal communication, 1993). These genome sizes are comparable with the lowest values recorded for other metazoans and are about one-tenth the average sizes of molluscan, echinoderm, or mammalian genomes, implying that the brachiopod genome is probably not rich in repetitive sequences. If so, then these estimates of genome size are also good estimates of genome complexity, i.e., of the overall coding capacity. However, some evidence suggestive of repetitive nuclear sequence has been obtained (P. BALFE & B. COHEN, unpublished work, 1985).

The mitochondrial genomes of two species of *Terebratulina* are within the size range typical for metazoans (14 to 16 kb), but the mtDNA of *T. septentrionalis,* like that of several other organisms, contains a region that varies slightly in size in different individuals and perhaps also within individuals (COHEN & others, 1991). Although a quasi-complete mitochondrial genome of *T. retusa* has been cloned, the clone has not been sequenced, and only very preliminary evidence of gene order has been published (JACOBS & others, 1988). A mitochondrial genome of *Lingula anatina* has been cloned and is currently being sequenced; preliminary data indicate that this genome is atypically large, around 27 to 28 Kb (K. ENDO, personal communication, 1994). Sporadic examples of oversize mtDNAs have been observed in other phyla

(summarized by RAND, 1993), but the phenomenon appears to have no general significance. Determination of gene order in the mtDNA of a selection of brachiopods and other lophophorates is highly desirable, since the rate of rearrangement, except of tRNA (transfer RNA) genes, is low, and this character can strongly establish high-level phylogeny (SMITH & others, 1993; BOORE & BROWN, 1994).

GENOME BULK COMPOSITION

The overall base composition and nearest-neighbor base doublet frequencies of total DNAs from *Terebratulina retusa* and *Crania (Neocrania) anomala* have been determined. Base composition was unremarkable, around 33 percent G + C, and no density satellites were detected, suggesting that if repetitive DNA sequences exist they are not highly distinctive in base composition. Nearest-neighbor base doublet frequencies were not informative (RUSSELL & SUBAK-SHARPE, 1977). The base composition of *Lingula* DNA was not very different (SHIMIZU & MIURA, 1972).

TOWARD A GENEALOGICAL CLASSIFICATION OF THE BRACHIOPODA

INTRODUCTION

Publication of the first edition of Part H of the *Treatise* (MOORE, 1965) coincided with a watershed in genome studies when ZUCKERKANDL and PAULING laid the foundations for the study of molecular evolution by pointing out that the information-bearing molecules of the genome comprise the "documents of evolutionary history" (ZUCKERKANDL & PAULING, 1965, p. 357). At first, these documents could be read only indirectly and laboriously through the amino-acid sequencing of proteins. More recently, with the development of nucleic acid sequencing and especially of DNA sequencing based on the polymerase chain reaction (PCR), genomic sequences are more readily obtained, and our knowledge and understanding of the genealogical relationships of many organisms has been revolutionized, although homoplasy remains a problem (HILLIS & MORITZ, 1990).

By pointing to the potential for genomic information to be used for the reconstruction of evolutionary history, ZUCKERKANDL and PAULING (1965) identified the key to satisfying DARWIN's prescient advice that the aim of taxonomists should be to create classifications that are "as far as they can be so made, genealogies" (DARWIN, 1859a, p. 486). Genealogy requires the genome; and, to the extent that the genomes of living brachiopods contain or retain phylogenetically useful information and that funds can be obtained to support the considerable work required to extract and analyze it, brachiopod taxonomists can look forward for the first time to being able to justify their classifications on genealogical grounds independent of morphology and of the fossil record. By determining genealogically validated sister groups it should become possible to confirm the evolutionary polarity of at least some morphological character-state transformations and hence to provide an independent, phylogenetically valid framework for classification. Unfortunately, the concerted application of DNA sequencing to the brachiopod genome started only in 1991, so relatively few results are yet available. Before we present a preliminary account of these new data, we shall first briefly review the scanty existing knowledge of the brachiopod genome obtained by inference from analyses of proteins and of RNA.

STUDIES OF PROTEINS

Properties of whole proteins may be treated as homologous characters and used for population genetic or phylogenetic studies. Alternatively, the amino-acid sequence of part or all of a protein may be determined chemically and used in phylogenetic comparison either directly or as a palimpsest of the genomic coding sequence. The comparison of brachiopod protein sizes and partial amino-acid sequences was pioneered by JOPE (1986), but such comparisons have yet to

prove phylogenetically useful. Similarly, the use of allelic variants of whole proteins (allozymes) is informative mainly in a population genetics context, and its few reported applications to brachiopods have already been noted (Ayala & others, 1975; Valentine & Ayala, 1975; Hammond & Poiner, 1984; Balakirev & Manchenko, 1985; Cohen, Balfe, & Curry, 1986).

Protein sequencing, both partial and complete, is still relatively cumbersome because of the need for purification. And because many expressed genes are represented in the genome by gene families resulting from duplication, the phylogenetic utility of amino-acid sequences may be limited unless parallel genomic studies establish that paralogy has been avoided. Nevertheless, studies of protein sequences are attractive because of the possibility that such knowledge might permit useful information to be recovered from residues in fossil or empty shells and for the light that might be shed on processes such as biomineralization (Tuross & Fisher, 1989; Cusack & others, 1992; Cohen, 1994; Williams, Cusack, & Mackay, 1994).

In an alternative approach, the taxonomic distribution of specific protein functions may be studied in the hope that phylogenetically useful markers will be discovered (Livingstone & others, 1983; Hammen & Bullock, 1991). This approach, however, has not been generally fruitful because of the universality of most biochemical processes and because it is not known whether the differential distribution of particular enzyme activities is due to differential gene expression or differential distribution of the corresponding genes. A similar difficulty applies to the oxygen-binding protein hemerythrin. The hemerythrin of *Lingula* appears to be the only brachiopod protein whose complete amino-acid sequence has been determined. It is a heteropolymeric protein comprising distinct alpha and beta polypeptides and it binds oxygen cooperatively, unlike the homopolymeric hemerythrins of polychaetes, priapulans, and sipunculans (Klippenstein, 1980; Satake & others, 1990; Yano, Satake, Ueno, Kondo, & Tsugita, 1991; Yano, Satake, Ueno, & Tsugita, 1991; Zhang & Kurtz, 1991). The alpha and beta polypeptides show 65 percent amino-acid identity, while the corresponding subunits of *L. reevii* and *L. unguis* (= *anatina*) show 95 percent and 87 percent identity respectively (Negri & others, 1994). The existence of two distinct hemerythrin polypeptides in *Lingula* but not in polychaetes, priapulans, and sipunculans provides indirect evidence for a gene duplication event in brachiopod evolution, while recent evidence for two hemerythrins in a discinid suggests that this duplication antedated divergence of the lingulid and discinid lineages (M. Cusack, personal communication, 1995). The distribution of hemerythrins in protostomes appears to be sporadic and apparently unrelated to phylogeny (Fig. 180–185). This probably indicates that one copy of this coding sequence is plesiomorphic in metazoans and that it is expressed only where the function of the hemerythrin gene product contributes to fitness. If so, the presence or absence of hemerythrin protein cannot be phylogenetically informative.

STUDIES OF NUCLEIC ACID SEQUENCES

Molecular Characteristics of RNAs

Because capability for the analysis, especially sequencing, of RNA developed before that for sequencing DNA, the earliest studies of brachiopod nucleic acids were on RNAs. Standing alone is the early work of Ishikawa (1977), who combined thermal dissociation with gel electrophoresis to compare the subunit sizes of ribosomal RNA (rRNA) in an explicitly phylogenetic study of all three phyla of lophophorates, including both an articulated and an inarticulated brachiopod. He concluded that their rRNAs were of the protostome type (Ishikawa, 1977).

RNA Sequences and Sequences of Genes Coding for RNAs

The smallest rRNA subunit, 5S rRNA (S = Svedberg unit, an indirect measure of mo-

lecular mass), was also the first to be extensively sequenced and many of these short sequences are available for comparison, including one from *Lingula* (Komiya & others, 1980; Erdmann & others, 1985; Hori & others, 1985; Hori & Osawa, 1986). It has become clear, however, that 5S rRNA sequences provide little useful phylogenetic information (Halanych, 1991; Hillis & Dixon, 1991; Steele & others, 1991).

The first attempt at a molecular phylogeny of the animal kingdom, using partial sequences of the nuclear-encoded 18S or small subunit (SSU) rRNAs, again employed *Lingula* to represent all brachiopods (Field & others, 1988). Despite some controversy over interpretation of the results, there was again the clear conclusion: "the lophophorate lineage represented . . . by a brachiopod, is solidly affiliated with the protostome group . . ." (Field & others, 1988, p. 749)(Patterson, 1985, 1989; Ghiselin, 1988; Adoutte & Philippe, 1993). Extension of this work to other brachiopod lineages is described below, but, instead, sequencing quasicomplete SSU genes rather than parts of the RNA transcribed from them and aiming to build a comprehensive molecular framework for brachiopod systematics (Cohen, Gawthrop, & Cavalier-Smith, in preparation). In addition to the SSU gene, the large nuclear-encoded rRNA subunit (28S or LSU) is also useful for phylogenetic comparison, and a number of brachiopod and other lophophorate sequences have been obtained, either from the gene or from the RNA transcript (A. Chenuil, personal communication, 1994; K. Halanych, personal communication, 1994; B. Cohen & M. Burke, unpublished work, 1995), but no publications using these sequences have yet appeared.

In all rRNAs and their genes, the number of accumulated sequence changes is at least roughly proportional to time (the molecular-clock hypothesis), and these sequences combine blocks that are highly conserved (because they are functionally constrained) interspersed with less strongly conserved regions. Also, the SSU (18S) gene generally changes more slowly than the LSU gene (Hillis & Dixon, 1991). Thus, both overall and regional rates of divergence differ. Moreover, since metazoan mtDNA generally accumulates base substitutions severalfold faster than nuclear DNA, the corresponding mitochondrial SSU (12S) and LSU (16S) genes further increase the divergence-time range over which useful results may be obtained. Thus, particular sequencing targets can be chosen to match expected divergence levels (Hillis & Dixon, 1991). For example, the 18S sequence may not retain enough signal to resolve unambiguously events close to the protostome radiation (Adoutte & Philippe, 1993; Philippe, Chenuil, & Adoutte, 1994), but it can be informative about more recent events in brachiopod evolution. Similarly, resolution of the most recent divergences may be limited by lack of signal in nuclear SSU or LSU, but adequate resolution of such events (e.g., those giving rise to taxa below the family level) may be obtained from comparison of mitochondrial sequences. Preliminary evidence indicates that the overall rate of brachiopod mtDNA evolution may be relatively low compared to other metazoans (Cohen & others, 1993), and this seems also to be true for the nuclear-encoded SSU gene (see below and Cohen, Gawthrop, & Cavalier-Smith, in preparation).

Gene Sequence Comparisons: Work in Progress

The results to be presented here reflect the state of the literature and of work in progress in the authors' laboratory in May 1995. Brief details of materials and methods are given in the text and figure captions, and full details will be published elsewhere (Cohen, Gawthrop, & Cavalier-Smith, in preparation). Nuclear-encoded SSU rRNA gene sequences, each approximately 1,790 nucleotides long, were obtained by the direct sequencing of DNA amplification products obtained by PCR (polymerase chain reaction) using oligonucleotide primers matching highly conserved terminal regions of eukaryotic rRNA genes. A notable advantage of

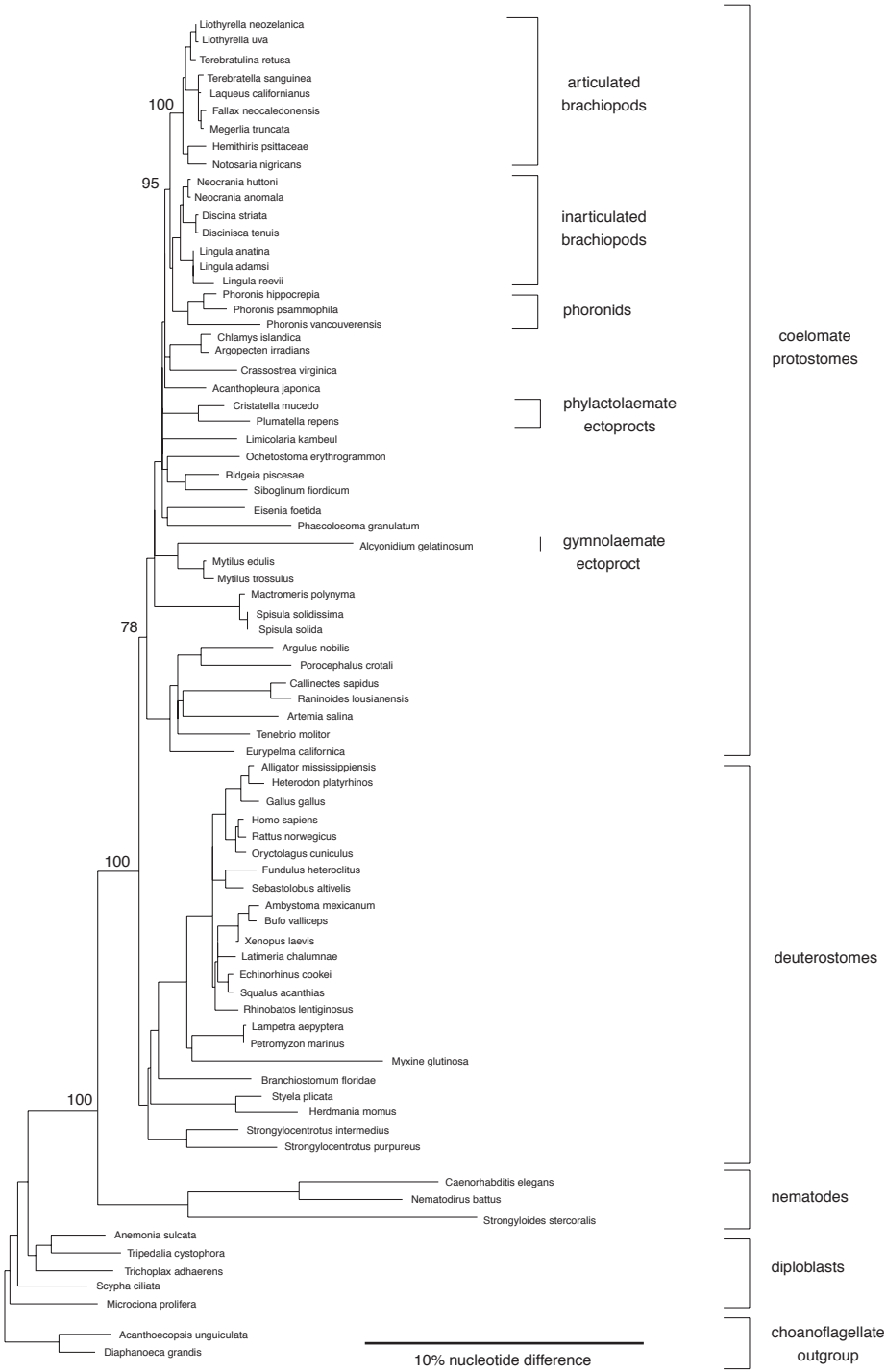

FIG. 180. *For explanation, see facing page.*

this approach is that the resulting sequence is a consensus, undistorted by divergence between the multiple genomic copies of this gene family or by gene cloning or PCR artifacts. Using this method we have determined sequences from 23 species of articulated brachiopods, 6 inarticulated brachiopods, 2 phoronids, and 1 ectoproct, while sequences from 1 articulated brachiopod, 2 inarticulated brachiopods (1 a partial sequence), 1 phoronid, and 2 ectoprocts are available from other sources (FIELD & others, 1988; B. WINNEPENNINCKX & R. DE WACHTER, personal communication, 1994; HALANYCH & others, 1995). The sequences analyzed are complete except for regions corresponding to the terminal primers (all cases), up to 18 undetermined nucleotides adjacent to 1 terminal primer (3 cases), and 2 large internal sections that are missing from the first brachiopod sequence to be determined (*Lingula reevii,* FIELD & others, 1988). The sequences were aligned manually with one another and subsequently with many protostome and other outgroup sequences obtained from databases (BENSON & others, 1994; MAIDAK & others, 1994) and other sources (B. WINNEPENNINCKX & R. DE WACHTER, personal communication, 1994; RUNNEGAR & others, in preparation; WINNEPENNINCKX, BACKELJAU, & DE WACHTER, 1995). In addition to the data and analyses described here, mitochondrial SSU (12S rRNA) and cytochrome oxidase subunit I (COI) partial sequences are being obtained from selected taxa (S. STARK, C. THAYER, & B. COHEN, unpublished observations, 1993; B. COHEN & A. GAWTHROP, unpublished work, 1995) using primers that yield amplified fragments of approximately 400 and approximately 700 nucleotides respectively. These data will provide genetically independent tests of the nuclear gene phylogeny and should clarify relationships among relatively recently diverged brachiopods for which the nuclear SSU gene provides insufficient resolution. The COI gene may, in addition, help clarify unresolved deep branches (HILLIS & DIXON, 1991; FOLMER & others, 1994).

DNA was extracted from animals that had been freshly collected or had been preserved in alcohol (without prior formalin fixation) during the last 10 years. No full-length PCR amplification product has been obtained from DNA extracted from specimens preserved over 20 years ago. No attempt was made to amplify from fossils or dried specimens, except in the case of one sample vial that was crushed in transit and from which the alcohol had recently evaporated. These specimens of *Platidia anomioides* were recovered from the packaging using acid-washed forceps and an SSU gene of distinctive

FIG. 180. High-level phylogenetic relationships of phoronids, ectoprocts, and brachiopods. Neighbor-joining tree based on an alignment of conserved nucleotide sites. Representative ingroup and unpublished outgroup sequences were aligned manually with published sequences selected from the Ribosomal Database Project (release 3). The GCG program PLOTSIMILARITY (DEVEREAUX, HAEBERLI, & SMITHIES, 1984) was used to identify sites showing less than 60 percent similarity (as defined by the program), and these were then excluded, leaving 1,632 sites of which 1,361 were variable. The Kimura 2-parameter algorithm implemented in the program DNADIST (FELSENSTEIN, 1993) was used to obtain a distance matrix corrected for unseen multiple events, and the tree was constructed from this matrix using the program NEIGHBOR 81 (FELSENSTEIN, 1993). Alternative correction algorithms and taxon-addition orders did not alter tree topology. Bootstrap frequencies (%) based on 500 replicates obtained with SEQBOOT (FELSENSTEIN, 1993) are given for key nodes.

Key to nonlophophorate, coelomate protostome taxa: Annelida, Oligochaeta: *Eisenia foetida;* Annelida, Polychaeta: *Glycera americana, Lanice conchilega;* Arthropoda, Arachnida: *Androctonus australis, Eurypelma californica;* Arthropoda, Crustacea: *Argulus nobilis, Artemia salina, Branchinecta packardi, Berndtia purpurea, Callinectes sapidus, Philyra pisum, Porocephalus crotali, Pugettia quadridens, Raninoides lousianensis, Stenocypris major, Trypetasa lampas, Ulophysema oeresundense;* Arthropoda, Insecta: *Tenebrio molitor;* Echiura: *Ochetostoma erythrogrammon;* Mollusca, Gastropoda: *Limicolaria kambeul, Onchidella celtica;* Mollusca, Aplacophora: *Epimenia australis;* Mollusca, Polyplacophora: *Acanthopleura japonica;* Mollusca, Bivalvia (filibranchs): *Argopecten irradians, Chlamys islandica; Crassostrea virginica, Placopecten magellanicus;* Mollusca, Bivalvia (eulamellibranchs): *Mactromeris polynyma, Mulinia lateralis, Mytilus edulis, Mytilus trossulus, Spisula solida, Spisula solidissima, Tresus capax;* Pogonophora: *Siboglinum fiordicum;* Priapulida: *Priapulus caudatus, Priapulus caudatus* 2; Sipuncula: *Phascolosoma granulatum;* Vestimentifera: *Ridgeia piscesae* (new).

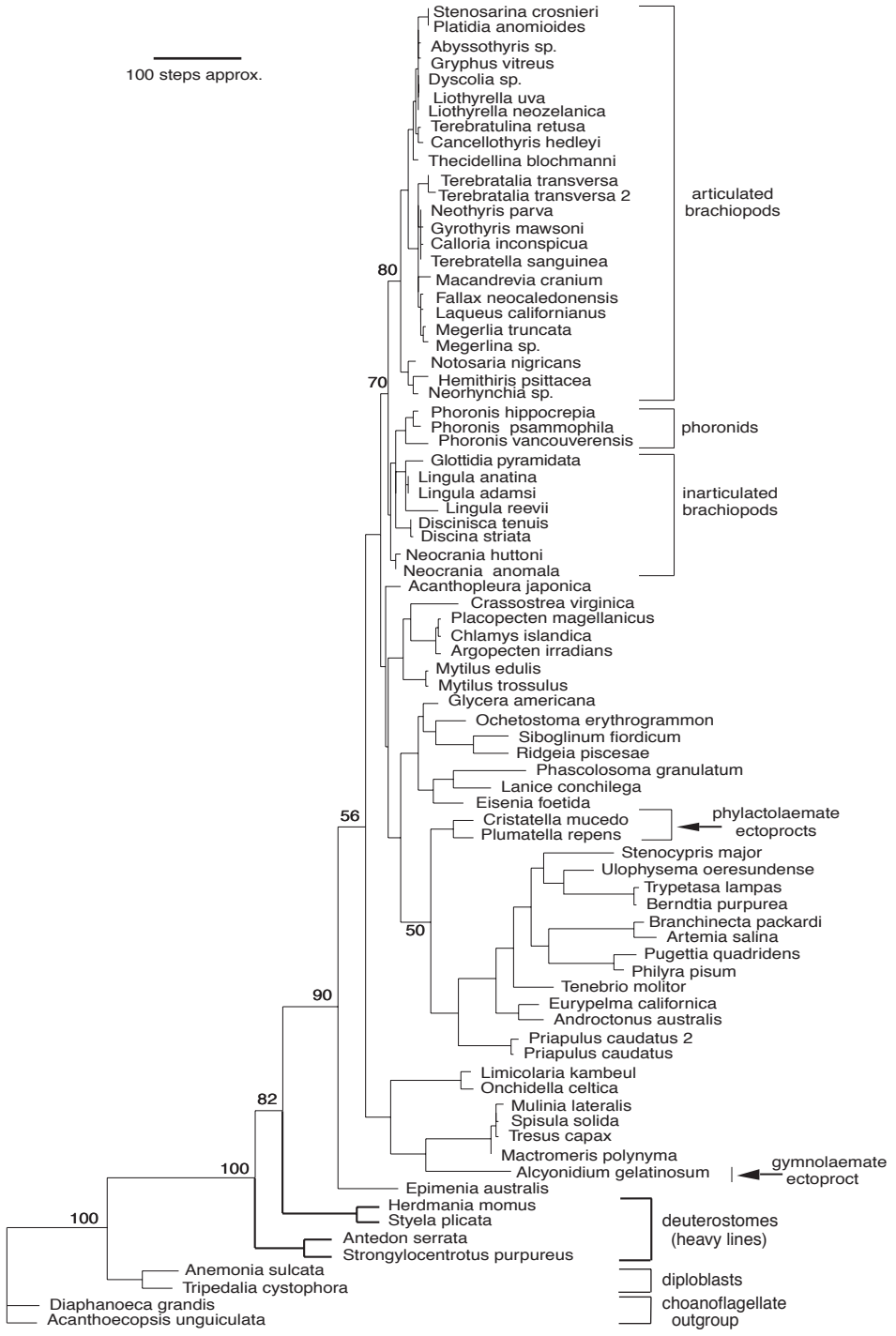

FIG. 181. *For explanation, see facing page.*

sequence was amplified from the resulting DNA preparations. Confirmation of the unexpected result obtained from these specimens should be sought.

Subject to obvious logistical constraints, we aimed to obtain nuclear-encoded SSU sequences from at least two taxa from every major, extant brachiopod lineage and from as many family-level taxa (*sensu* MOORE, 1965) as possible. Phoronids and an ectoproct were included so as to address the long-standing question of lophophorate relationships. We have used and will compare the results of three methods for phylogenetic reconstruction that have been reported to be among the most consistent, efficient, and robust under test conditions (HILLIS & MORITZ, 1990; HUELSENBECK, 1995). The parsimony method, with equally weighted (EP) or *a posteriori* weighted characters (WP), follows cladistic principles, permits the exclusion of plesiomorphic similarity by including only parsimony-informative sites, and lends itself to measurement of the support for each node. With *a posteriori* weighting based on the rescaled consistency index, homoplastic characters are proportionately downweighted and the number of equally most parsimonious trees reduced. WP will therefore be treated as the method of choice. The maximum likelihood method (ML) is less sensitive to artifacts due to base-composition and evolutionary rate differences but is difficult to apply to large data sets because of computational intensiveness. Since parsimony and likelihood analyses use somewhat different models of the evolutionary processes, any result that is supported by both methods is likely to be a good estimate of the phylogeny represented by the data. The distance matrix method, particularly with the neighbor-joining tree construction program (NJ) is fast and convenient but possibly less reliable. Following common practice, we will generally treat the gene trees obtained as species trees; i.e., we shall describe conclusions in terms of the relationships of taxa.

Several well-known problems complicate the reconstruction of deep-branch protostome phylogeny from SSU rRNA gene sequence data. These problems, and strategies to deal with them, are as follows. The first arises from the great age and relative rapidity of the Cambrian or late Precambrian diversification that gave rise to the major lineages. Because most parts of the SSU gene evolve slowly, branches around deep divergence points are expected to be short, and nodes connecting high-level taxa of early origin may not (indeed, often do not) receive strong support. One solution to this problem is to add sequence data from other genes, but the total needed may be impractically high (PHILIPPE, CHENUIL, & ADOUTTE, 1994).

FIG. 181. High-level phylogenetic relationships of phoronids, ectoprocts, and brachiopods. Weighted parsimony tree based on an alignment of 80 complete SSU sequences from all available brachiopods, phoronids, and ectoprocts together with selected protostome and other outgroups. For list of organisms representing nonlophophorate, coelomate protostome taxa, see caption to Figure 180. Reliability of the alignment was maximized by starting with sequences from unarguably closely related brachiopod taxa, by minimizing the number of introduced gaps, and by nucleating alignment of the four most variable regions on conserved secondary structures (i.e., implied functional homologies of the transcribed rRNA molecules) inferred by means of a free energy minimization RNA folding program (JAEGER, TURNER, & ZUKER, 1989a, 1989b; ZUKER, 1989). The alignment contained 2,114 sites, of which 804 were parsimony-informative. The following heuristic search options (using PAUP; SWOFFORD, 1993) were invoked: collapse zero-length branches, no topological constraints, outgroup rooting, closest addition, no steepest descent, TBR, MULPARS, ACCTRAN. In trial analyses random addition, steepest descent, and DELTRAN did not alter tree topology. An initial search with equally weighted characters found 48 equally most parsimonious trees of length = 4,711 steps, RI = 0.604. Following three cycles of reweighting according to the worst fit of the rescaled consistency index (RCI, baseweight = 100) followed by heuristic search, the number of trees reduced to 6 of 86,565 weighted steps, RI = 0.717. These six trees differed only in the arrangement of the articulated brachiopod terminal taxa on the shortest branches, i.e., those whose relationships are most weakly resolved, and one of these trees is shown. Bootstrap % are given for key nodes based on 50 heuristic search replicates with reweighted characters. The limited number of replicates was dictated by computational constraints, which also precluded calculation of support indices (BREMER, 1988; KÄLLERSJÖ & others, 1992) (new).

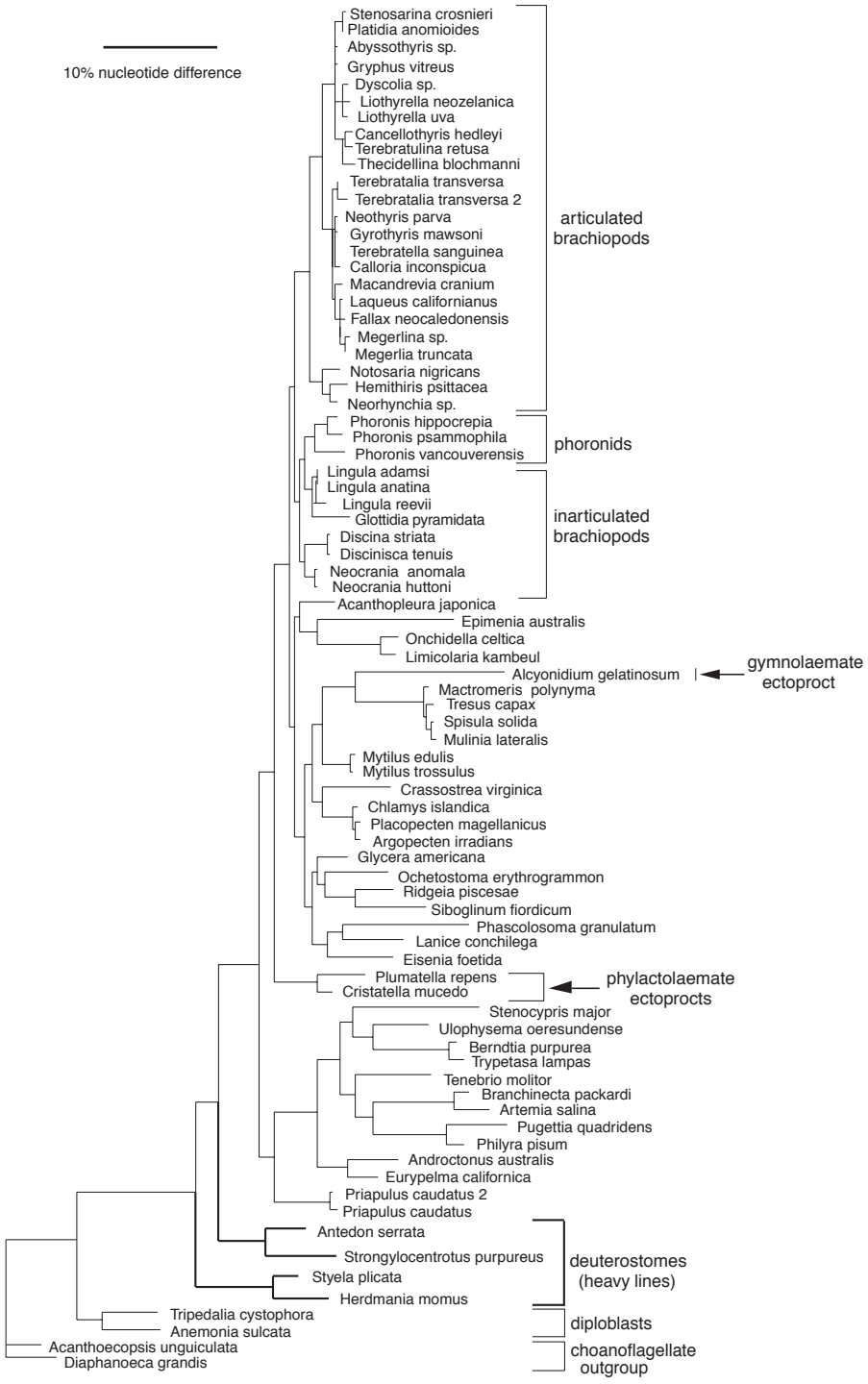

FIG. 182. *For explanation, see facing page.*

Another solution is to seek genomic synapomorphies such as mitochondrial genome rearrangements. Faster-evolving sequences cannot be used because they introduce excessive homoplasy and reach saturation. The second problem is that long branches may artificially cluster together. This can be alleviated by not relying completely on reconstruction methods most sensitive to this effect and by increasing the number of related taxa studied, thus subdividing long branches. The third problem is that ingroup phylogeny is not independent of the outgroup used. For the lophophorate phyla this is particularly awkward since there is no certain, independent basis on which to identify the most appropriate outgroup, which therefore has to be identified recursively from preliminary analysis of the same sequence data that will be used to determine phylogeny. This problem will be addressed below. Finally, all the reconstruction methods used here may also cluster sequences according to similarity of nucleotide composition, independently of sequence similarity. Since the SSU genes of brachiopods and phoronids are very similar in sequence and composition, this base composition effect is unlikely to mislead, but we have no objective assessment of its influence. The LogDet transformation (Huson & Wetzel, 1994; Lockhart & others, 1994) may eliminate compositional effects, but our results with this method are too preliminary to be described.

The earlier conclusion that lophophorates are protostomes (Ishikawa, 1977; Field & others, 1988) was recently confirmed by analysis of SSU sequences from single articulated and inarticulated brachiopods, a phoronid, and an ectoproct (Halanych & others, 1995). Since analyses of our more extensive data agree entirely with this conclusion (Fig. 180–182), we will take this point as established. In contrast, the latter report (Halanych & others, 1995) also claimed that phoronids are the sister group of articulated brachiopods and that ectoprocts are the sister group of a clade comprising brachiopods, molluscs, and annelids. Our analyses disagree strongly with the former assignment; we find phoronids to be contained within a clade comprising inarticulated brachiopods, although their position within that clade remains uncertain. We are also uncertain about the position of the ectoprocts, and both issues are discussed below.

Selecting an Outgroup

Accepting that lophophorates are protostomes, there arises the important question of identifying the most appropriate outgroup with which to root the phylogenetic trees and thus to determine the apparent direction of evolution (Hennig, 1966; Farris, 1972; Donoghue & Cantino, 1984; Maddison, Donoghue, & Maddison, 1984; Nixon & Carpenter, 1993; Smith, 1994). Recent analyses of outgroup rooting (Nixon & Carpenter, 1993; Smith, 1994) stress the dangers of remote outgroups and lead to a preference for the closest outgroup, preferably the ingroup's sister group.

How can the ingroup's sister group be identified? Ideally, it would be identified by reference to independent evidence such as comparative zoology. But comparative zoology has decided that the lophophorates are deuterostomes (Brusca & Brusca, 1990; Eernisse, Albert, & Anderson, 1992) in clear conflict with the molecular results (e.g., Fig. 180–182), so we must look elsewhere. No other, independent source of evidence

Fig. 182. High-level phylogenetic relationships of phoronids, ectoprocts, and brachiopods. Maximum-likelihood tree based on the same alignment used for the parsimony analysis in Figure 181. For list of organisms representing nonlophophorate, coelomate protostome taxa, see caption to Figure 180. The fastDNAml program (Olsen & others, 1994) was used with nucleotide frequencies estimated from the data (empirical frequencies) and with global branch exchange. Because this analysis took over 20 days full-time processing on a 70 MHz SUN Sparc5 workstation, no bootstrap analysis was done (new).

FIG. 183. Phylogenetic relationships of brachiopods, ectoprocts, phoronids, and other protostomes in an unrooted weighted parsimony tree based on the alignment of complete SSU sequences used for Figures 181 and 182 but with nonprotostomes omitted. The alignment comprised sequences from 72 protostome taxa and 2,098 sites. For list of organisms representing nonlophophorate, coelomate protostome taxa, see caption to Figure 180. This tree was one of three equally most parsimonious, RCI-weighted trees that differed only in arrangement of the shortest terminal

(Continued on facing page.)

exists, however, and the best we can do is to base outgroup choice on phylogenetic analysis of a large number of available SSU sequences, confining our attention to protostomes so as to avoid the more remote outgroups and seeking as an outgroup the protostome sequence closest to brachiopods plus phoronids. This approach to outgroup rooting minimizes homoplasy, enables alignment of variable regions to be least ambiguous, and minimizes the need to exclude data (SMITH, 1994), in contrast with the approach using evolutionarily remote diploblasts as the outgroup (HALANYCH & others, 1995). We have therefore sought the outgroup in unrooted trees derived from an alignment of sequences from 24 articulated brachiopods, 8 inarticulated brachiopods, 3 phoronids, 3 ectoprocts, 14 molluscs, 12 arthropods, 3 annelids, 2 priapulans, and 1 each of echiuran, pogonophoran, sipunculan, and vestimentiferan, these being all the major protostome lineages from which complete SSU sequences are currently available. The resulting unrooted WP, ML, and NJ trees are presented in Figures 183–185.

Principal conclusions from Figures 183–185 are as follows.

1. All the new inarticulated brachiopod sequences cluster closely with the partial sequence from *Lingula reevii* (FIELD & others, 1988), and the phylogenetic positions of all other brachiopod sequences are consistent with their reputed brachiopod origin. None is an outright contaminant.

2. Brachiopods plus phoronids are monophyletic in all three trees.

3. The phylactolaemate ectoprocts are the sister group of brachiopods plus phoronids in the ML tree but are more distant in the WP and NJ trees.

4. The gymnolaemate ectoproct (*Alcyonidium*) uniformly appears as a long-branched sister group of eulamellibranch molluscs and is distant from brachiopods plus phoronids. The *Alcyonidium* sequence, however, contains a number of unusual sequence motifs in generally conserved regions and may not be truly representative of gymnolaemates. Clearly, given the high taxonomic diversity of ectoprocts (HYMAN, 1959a), the limited species sample currently available, and the disagreement between reconstructions, it is premature to come to any firm conclusion about ectoproct relationships (*contra* HALANYCH & others, 1995); more data are required. It remains possible that ectoprocts may eventually prove to be a sister group of brachiopods plus phoronids.

5. Arthropods, priapulans, the echiuran, the sipunculan, and eulamellibranch molluscs are all distant from brachiopods plus phoronids and therefore are not appropriate outgroups.

6. Ectoprocts apart, the plausible sister groups of brachiopods plus phoronids lie among molluscs or alternatively molluscs and a wider group of taxa including annelids, but quantitative data are needed to determine their relative order of proximity to the ingroup.

Table 6 shows branch lengths extracted from the analyses that generated the trees of Figures 183–185 and list the five closest candidate outgroup taxa in order of proximity to brachiopods and phoronids (the ingroup). All three analyses agree in one respect: the chiton *Acanthopleura* is closest.

In addition, we performed equally weighted and RCI-reweighted (WP) analyses in which each of 22 diverse protostome sequences was used in turn to root a small, representative set of brachiopod plus phoronid sequences, noting the overall tree length and the retention index (RI) in each case. RI measures the proportion of the data that is explained by synapomorphy rather than homoplasy, the higher the better. Since

FIG. 183. *Continued from facing page.*
articulated brachiopod branches. It was based on 685 parsimony-informative sites. See caption to Figure 181 for heuristic search details. The branch-length scale is based on the trees obtained before reweighting and gives an approximate indication of the number of inferred nucleotide base substitutions along each branch (new).

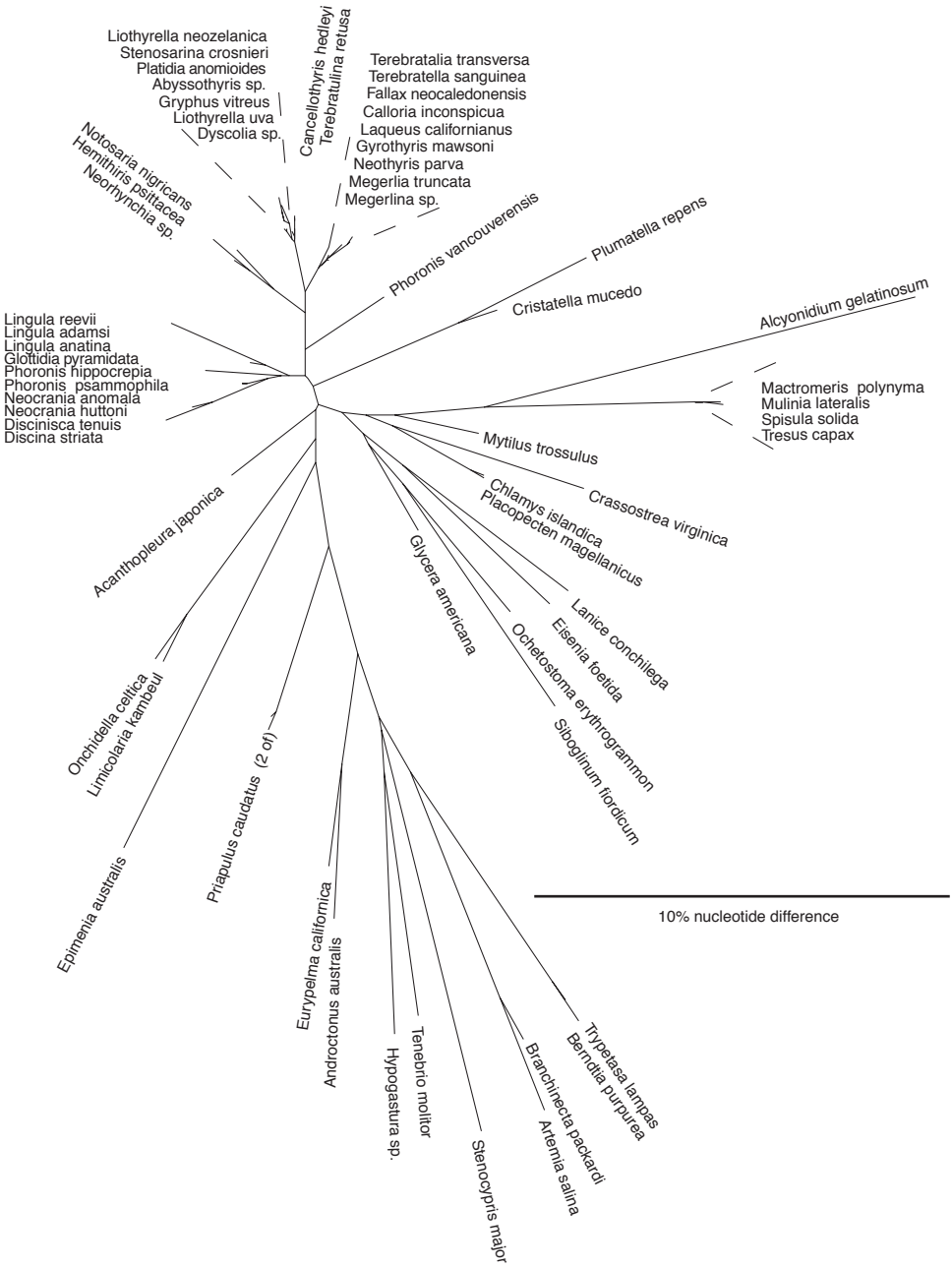

FIG. 184. Phylogenetic relationships of brachiopods, ectoprocts, phoronids, and other protostomes in an unrooted maximum-likelihood tree based on the alignment of complete SSU sequences used for Figures 181 and 182 but with nonprotostomes omitted. The alignment comprised sequences from 72 protostome taxa and 2,098 sites. For list of organisms representing nonlophophorate, coelomate protostome taxa, see caption to Figure 180. This tree was computed using empirical nucleotide frequencies and global rearrangement. All nodes were statistically significant ($P < 0.01$) except for those bearing the shortest articulated brachiopod branches comprising the unresolved *Laqueus, Fallax, Megerlia* plus *Megerlina* node (new).

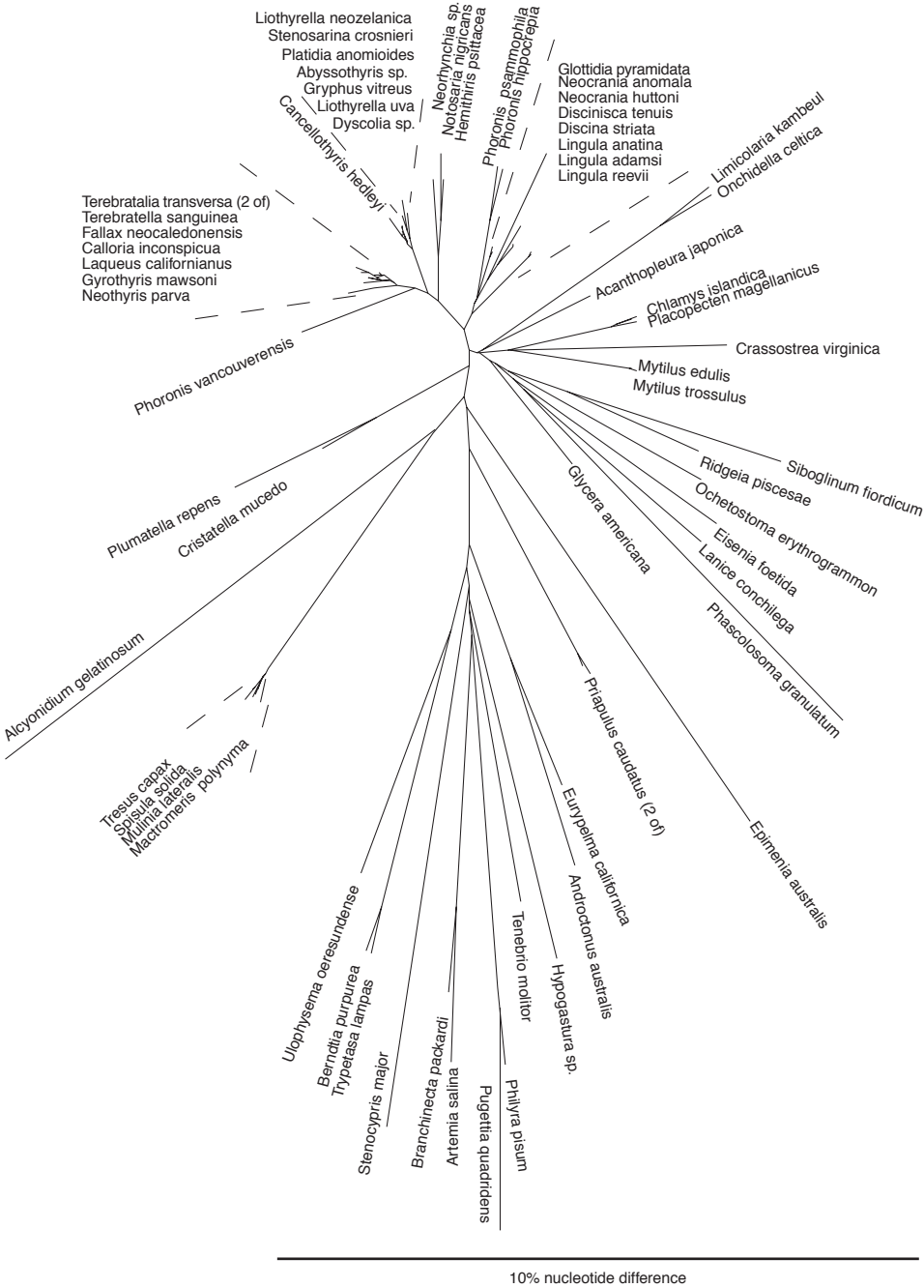

FIG. 185. Phylogenetic relationships of brachiopods, ectoprocts, phoronids, and other protostomes in an unrooted neighbor-joining tree based on the alignment of complete SSU sequences used for Figures 181 and 182 but with nonprotostomes omitted. The alignment comprised sequences from 72 protostome taxa and 2,098 sites. For list of organisms representing nonlophophorate, coelomate protostome taxa, see caption to Figure 180. This tree was based on a distance matrix calculated with the Kimura 2-parameter correction. Alternative correction algorithms and taxon-addition orders did not alter tree topology (new).

TABLE 6. Numerical parameters used to identify the closest outgroup. The methods of analysis used to obtain the parameters shown were also used (with addition of *a posteriori* parsimony character weighting) to construct Figures 183–188 (new).

	Equally weighted parsimony			Maximum likelihood		Neighbor-joining	
Order[1]	Taxon	Tree length[2]	Retention index	Taxon	Nucleotide distance[3]	Taxon	Nucleotide distance[4]
1	*Acanthopleura*	495	0.801	*Acanthopleura*	0.0408	*Acanthopleura*	0.0605 ± 0.0009
2	*Chlamys*	496	0.802	*Glycera*	0.0550	*Glycera*	0.0622 ± 0.0009
3	*Mytilus*	507	0.793	*Chlamys*	0.0598	*Chlamys*	0.0681 ± 0.0014
4	*Cristatella*	509	0.790	*Cristatella*	0.0619	*Mytilus*	0.0686 ± 0.0010
5	*Glycera*	512	0.791	*Eisenia*	0.0943	*Cristatella*	0.0701 ± 0.0009

[1]order of distance from ingroup; nearest = 1.
[2]overall tree length in a heuristic search, with characters equally weighted.
[3]distances estimated by summing branch lengths between the ingroup node and the outgroup terminal taxon. Each branch was shown as being significantly positive, $P < 0.01$, but confidence limits for the summed branch lengths cannot be stated. The ingroup node is the node in Figure 184 at which the branch carrying the inarticulate brachiopods joins the tree.
[4]nucleotide divergence between the outgroup and each ingroup taxon estimated using the Kimura 2-parameter correction and averaged over all ingroups ± standard error of mean.

the ingroup set was constant, tree length depended only on the length of the outgroup branch; and, hence, the shortest tree should identify the phenetically closest outgroup. Again, the tree rooted with *Acanthopleura* was the shortest by a small margin and gave the highest RI (details not shown). Thus, due to some combination of true phyletic position and similarity of evolutionary rate and of nucleotide composition, this chiton is the closest available outgroup for brachiopods and phoronids. While some of the 22 different outgroups yielded topologies showing less resolution, especially of the inarticulated brachiopod plus phoronid clade, none gave a radically different topology from that given by *Acanthopleura.*

The fact that outgroup selection may involve some subjectivity has long been recognized (DONOGHUE & CANTINO, 1984) and is inescapable when, as here, no independent evidence can be used to support outgroup choice. However, we have attempted to minimize the subjective element by basing choice of outgroup upon parameters estimated from the data. The need for selected outgroups arises not only from the theoretical considerations mentioned above but also from practical considerations: an alignment of approximately 80 SSU sequences causes computational difficulties.

The Phylogeny of Brachiopods and Phoronids Analyzed Using the Selected Outgroup

Figures 186–188 illustrate WP, ML, and NJ reconstructions using the selected outgroup, *Acanthopleura,* and lead to the following observations and conclusions.

1. Phoronids are either monophyletic, basal members of the inarticulated brachiopod clade (WP) or diphyletic with *Phoronis hippocrepia* and *P. psammophila* being the sister group of craniids and *P. vancouverensis* joining the base of the articulated brachiopods (ML and NJ with low bootstrap support). The association of *P. vancouverensis* with the articulated brachiopods has been reported elsewhere (HALANYCH & others, 1995).

Diphyly of phoronids is biologically implausible and cannot be accepted, but the conflict is easily resolved by examination of the three phoronid sequences. Those of *Phoronis hippocrepia* and *P. psammophila* show no unusual features when compared with other protostomes, but the *P. vancouverensis* sequence (HALANYCH & others, 1995) lacks several nucleotides in otherwise highly conserved sites. More importantly, all three phoronid sequences share at least three variable-region motifs that are clear

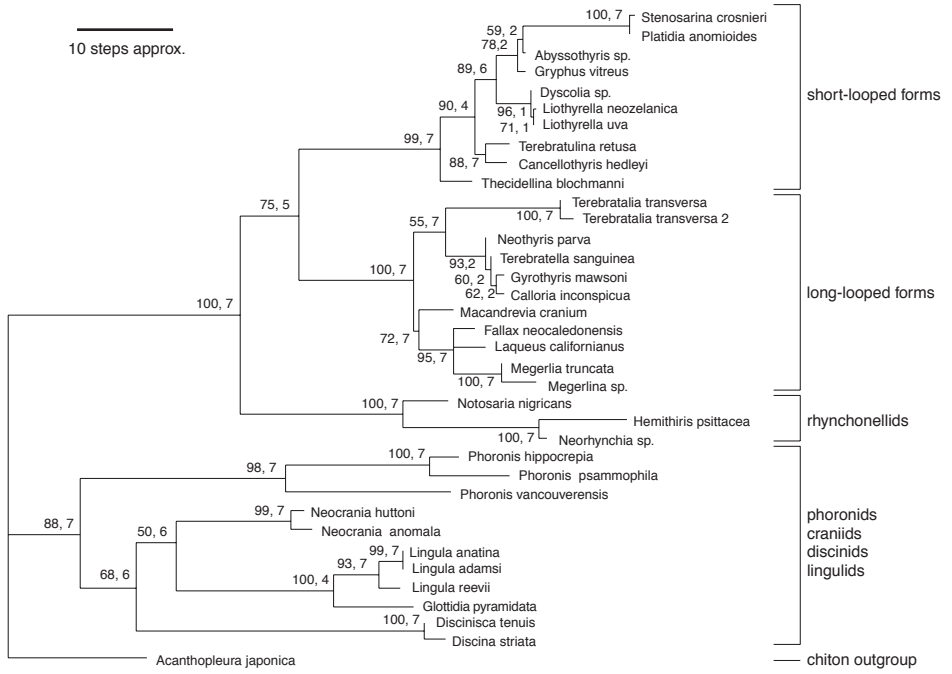

FIG. 186. Relationships of brachiopods and phoronids in phylogenetic trees rooted on the selected outgroup, *Acanthopleura.* This figure shows a weighted parsimony tree based on the alignment of complete SSU sequences used for Figures 181 and 182 but with ectoprocts, nonlophophorate protostomes, and nonprotostomes omitted. The alignment comprised 36 taxa and 1,813 sites, 198 of which were parsimony-informative. The skewness index of 10,000 random trees was g_1 = -0.504, suggesting that WP has an excellent chance of finding the true tree (HILLIS, HUELSENBECK, & CUNNINGHAM, 1994). Heuristic search found 36 equally most parsimonious trees of 495 steps, RI = 0.801. After three cycles of reweighting (base =100) and heuristic search these were reduced to three trees of 20,355 weighted steps, RI = 0.892, differing only in topology at the unresolved *Laqueus, Fallax, Megerlia* plus *Megerlina* node, and one of these trees is shown. See caption to Figure 181 for search details. The numbers adjacent to each node are, first, the frequency with which that node appeared among 100 bootstrap replicates, and, second, the support index for that node (BREMER, 1988; KÄLLERSJÖ & others, 1992). Support indices were calculated as follows: after identifying the length of shortest RCI-weighted trees, a series of heuristic searches was conducted, keeping all trees longer than the shortest by graded proportions. The strict consensus of the trees at each increased length was then calculated, and the collapsed nodes in each consensus tree were identified. Tree length was increased in steps until the number of trees retained reached the level (> 4,000 trees) at which computer memory overflowed, when the search was abandoned. The corresponding support indices are given on a 7-point scale where 1 to 5 identify nodes that collapsed in trees longer than the minimal tree by 0.1 percent to 0.5 percent, 6 identifies nodes that collapsed after an 0.75 percent increase, and 7 identifies nodes that were still intact when the search was abandoned while searching for trees 1.0 percent longer than the minimal tree (new).

synapomorphies of phoronids alone. Since in Figure 186 the support index for the node uniting all three phoronids is relatively high (and this WP analysis is insensitive to apomorphy and plesiomorphy), we reject the suggestion that phoronids are most closely related to articulated brachiopods (HALANYCH & others, 1995); it must be erroneous (CONWAY MORRIS & others, 1996). This conclusion is further supported by the three analyses represented in Figures 180–182 and particularly by Figure 180, which is based on the least variable and hence most reliably aligned nucleotides and in which all three phoronids are again a monophyletic sister group of inarticulated brachiopods.

2. Two of the three reconstructions (Fig. 186, 188) agree in placing the origin of

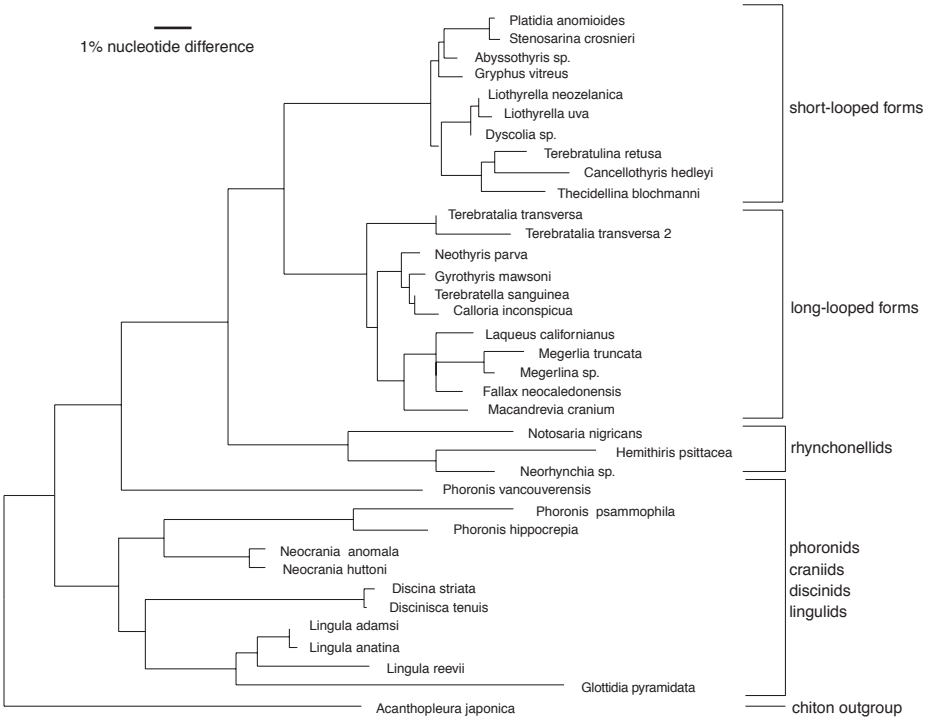

FIG. 187. Relationships of brachiopods and phoronids in phylogenetic trees rooted on the selected outgroup, *Acanthopleura.* This figure shows a maximum-likelihood tree based on the alignment of complete SSU sequences used for Figures 181 and 182 but with ectoprocts, nonlophophorate protostomes, and nonprotostomes omitted. The alignment comprised 36 taxa and 1,813 sites. This tree was computed using empirical nucleotide frequencies and global rearrangement. All nodes were statistically significant ($P < 0.01$) except for those leading to the shortest, articulated brachiopod branches comprising the unresolved *Laqueus, Fallax, Megerlia* plus *Megerlina* node. No bootstrap analysis was performed for computational reasons (new).

discinids before that of lingulids. The long branch and basal position of *Glottidia* among lingulids should be treated with reserve since this sequence (HALANYCH & others, 1995) lacks approximately 14 nucleotides in otherwise highly conserved positions.

3. The WP reconstruction (Fig. 186) joins the craniids and lingulids in a clade, but this has low bootstrap support, indicating that with more data the reconstructed topology might be altered.

4. Rhynchonellids are the sister group of all other articulated brachiopods.

5. Long-looped and short-looped articulated brachiopods are sister groups.

6. Long-looped articulated brachiopods are clearly monophyletic, and at least three subclades are recognized. *Terebratalia* represents either a basal, long-looped form (ML, NJ) or is the sister group of the New Zealand terebratellids (WP). The latter are extremely closely related to one another, with *Neothyris* probably being basal. All three reconstructions (Fig. 186–188) agree that *Macandrevia* is the sister group of a morphologically diverse clade that includes kraussinids.

7. The morphological divergence that gave rise to the genus-level diversity of the New Zealand terebratellids has been accompanied by very little change in the 18S rRNA gene. An adequate molecular phylogeny of the long-looped articulated brachiopods must await results from a wider species sample and a faster-evolving gene.

8. Short-looped articulated brachiopods are clearly monophyletic, and at least four subclades are recognized. The thecideidine is unambiguously a short-looped articulated

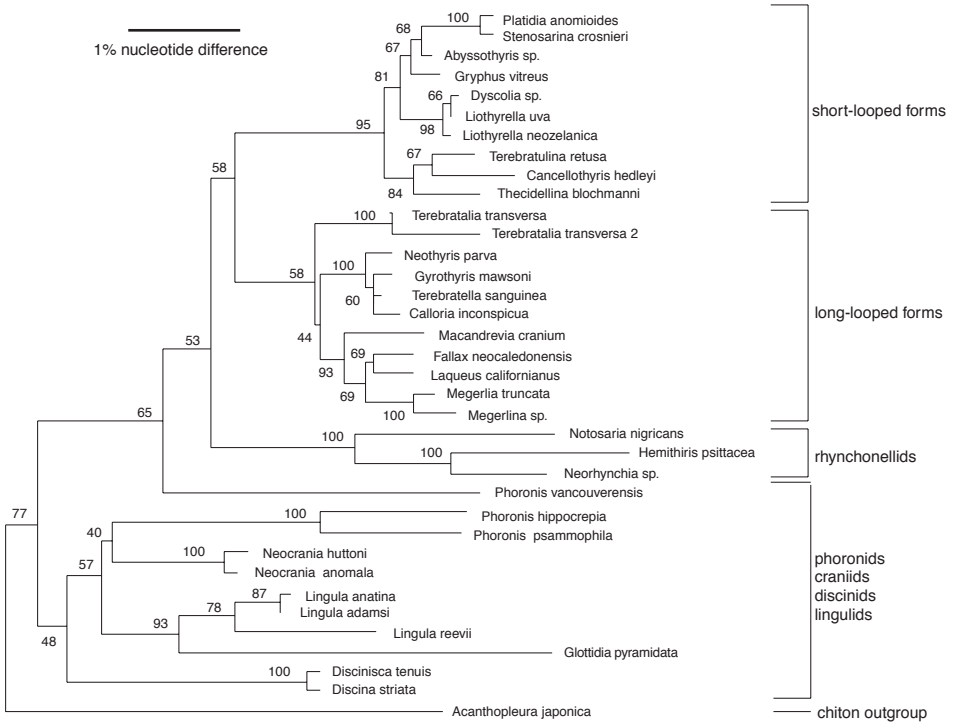

FIG. 188. Relationships of brachiopods and phoronids in phylogenetic trees rooted on the selected outgroup, *Acanthopleura.* This figure shows a neighbor-joining tree based on the alignment of complete SSU sequences used for Figures 181 and 182 but with ectoprocts, nonlophophorate protostomes, and nonprotostomes omitted. The alignment comprised 36 taxa and 1,813 sites. This tree is based on a distance matrix calculated with the Kimura 2-parameter correction. Alternative correction algorithms and taxon-addition orders did not alter tree topology. The numbers adjacent to each node are bootstrap frequencies (%) (new).

brachiopod, either basal (WP, strongly supported) or a sister group of cancellothyrids (ML, NJ). The cancellothyrid subclade shows weak affinity with the *Dyscolia-Liothyrella* subclade. Surprisingly, *Gryphus* is not most strongly associated with the *Dyscolia-Liothyrella* subclade. There is also a surprising association of the undoubted short-looped terebratulid *Stenosarina* with *Platidia* (the sample of which had an atypical history—see above). Some of these nodes, however, are not strongly supported, being based on very few differences. An adequate molecular phylogeny of the short-looped articulated brachiopods must await results from a wider species sample and a faster-evolving gene.

In summary, the topology of relationships among the articulated brachiopods is stable with but minor differences in diverse phylogenetic reconstructions rooted with the closest sister group. It thus appears that the results are reliable and satisfy the aim of providing a first molecular phylogenetic framework for this clade. The topology of the inarticulated plus phoronid clade is less firm; phoronids are either the sister group of all inarticulated brachiopods or the sister group of craniids. The relative position of discinids, craniids, and lingulids is also unsettled. Nevertheless, the results unambiguously indicate that all three inarticulated brachiopod lineages belong together and, to that extent, also provide a secure, high-level framework for their phylogeny and classification.

Although the addition of sequence data from other genes and SSU sequences from

more terminal taxa may improve the robustness of the earliest branch points, an impractically large amount of such data may be required (Philippe, Chenuil, & Adoutte, 1994; Raff, Marshall, & Turbeville, 1994); and the most conclusive evidence will probably come not from additional sequence *per se* but from finding rare, qualitative evolutionary events such as insertions into conserved protein-coding genes or changes in mitochondrial gene order. Clearly, a fully satisfying reconstruction of the historical origins of the deep branches of the radiation that gave rise to the major protostome lineages will depend on the future accumulation of such new types of data. Similarly, a fuller molecular phylogeny of articulated brachiopods below the superfamily or family level will require sequence data from genes that evolve more rapidly than nuclear-encoded SSU.

IMPLICATIONS OF THE SSU rRNA GENE COMPARISON RESULTS FOR BRACHIOPOD SYSTEMATICS

It would be premature and inappropriate to make proposals for taxonomic revision on the basis of phylogenetic reconstructions from a single gene sequence, but it is appropriate to draw attention to the main implications for traditional, cladistic, and immunotaxonomic brachiopod systematics.

1. Regardless of how their embryology may be interpreted, brachiopods, phoronids, and ectoprocts belong in the clade that contains all undoubted protostomes, not in the clade of deuterostomes (Brusca & Brusca, 1990; Nielsen, 1991, 1994, 1995; Schram, 1991; Eernisse, Albert, & Anderson, 1992; Backeljau, Winnepenninckx, & De Bruyn, 1993). The deuterostome hypothesis of brachiopod, phoronid, and ectoproct affinities was also rejected earlier, when the SSU data included fewer sequences (Field & others, 1988; Irwin, 1991; Halanych & others, 1995).

This result presents perhaps the sharpest conflict yet between molecules and morphology (Conway Morris, 1995; Gee, 1995; Patterson, 1985), and it will be interesting to see which relationship will be supported by independent molecular data, such as that from mitochondrial genes.

2. The results are not sufficiently clear-cut to distinguish between alternative proposals for the relationships between discinids, lingulids, and craniids (Rowell, 1982; Gorjansky & Popov, 1986; Bassett & others, 1993; Popov & others, 1993), but they are consistent with grouping all three inarticulated lineages into a single taxon and exclude the arrangement that unites craniids with articulated brachiopods (Gorjansky & Popov, 1986; Bassett & others, 1993; Popov & others, 1993).

3. Articulated and inarticulated brachiopods form a monophyletic group, within which articulated and inarticulated forms belong to separate clades; the traditional system of two brachiopod classes appears to be valid (but see next point below).

4. The results contradict the traditional status of the phoronids as a separate phylum and suggest that they should be included with all three lineages of inarticulated brachiopods in a new taxon, perhaps as a class. If the possible sister group relationship of phoronids and craniids is verified, a taxon comprising craniids plus phoronids may instead be called for, perhaps as a subclass.

5. Within the articulated brachiopods, rhynchonellids fully deserve their distinct taxonomic status.

6. Short-looped and long-looped articulated brachiopods (as so far analyzed) represent distinct clades, but articulated brachiopods such as *Platidia* (note caveat above) and *Megerlia* with atypical, incomplete loops may belong to either clade. Thus, a threefold division of the articulated brachiopods (rhynchonellids, short-looped forms, and long-looped forms), perhaps as orders, may be sufficient to reflect justly the extant sequence diversity. The thecideidines belong within the short-looped articulated brachiopods and are therefore unlikely to be descendants of spiriferids or strophomenids (Williams, 1973; Baker, 1990). One tree raises

the possibility of a sister-group relationship between cancellothyrids and thecideidines, which seems not to have been previously considered (Baker, 1990).

7. A taxonomic method using quantitative immunological cross reaction between shell antigens and antibodies has been described as a practical approach (Collins & others, 1988; Collins, Curry, & others, 1991; Collins, Muyzer, Curry, & others, 1991; Curry, Quinn, & others, 1991; Curry & others, 1993; Endo & others, 1994), although this has been disputed (Cohen, 1992, 1994). Some specific immunotaxonomic proposals, such as the close clustering of *Terebratalia* and *Laqueus* or the grouping of *Macandrevia* relatively close to *Abyssothyris, Gryphus,* and *Liothyrella* are clearly inconsistent with our results.

FUTURE PROSPECTS

GENE EXPRESSION IN DEVELOPMENT AND DIFFERENTIATION

Recognition of homology is the fundamental task of comparative morphology, whether in the egg, the embryo, the larva, or the adult. It is a hazardous undertaking, exemplified by the long-standing uncertainty about whether brachiopods and other lophophorates are protostomes, deuterostomes, neither, or both, and by the uncertain homology of lophophores (Nielsen, 1995 and references therein). The difficulty of correctly interpreting homology in developmental processes is highlighted by claims for the existence of three distinct methods of embryonic coelom formation in brachiopods (references cited by Chuang, 1991), with different methods apparently being employed in two species of one genus. This divergence is inherently unlikely; developmental processes are not so plastic. It is more likely that traditional histological methods are too blunt a tool for the proper interpretation of such dynamic processes. Alternatively, if the multiple methods of coelom formation do occur, that process can have no value as a high-level phylogenetic character. Some implications of lophophorates as protostomes in relation to the evolution of larval forms have been explored (Strathmann & Eernisse, 1994).

The ultimate justification for postulated homology is the identification of orthologous evolutionary relationships between the ancestral and descendant genes that provide the necessary information for anatomy and development. Although this too is fraught with difficulty and largely awaits the future, it has a more objective basis than many anatomical interpretations (Patterson, 1985; Willmer & Holland, 1991). Since the expression of evolutionarily homologous genes has been discovered to underlie the establishment of the embryonic axis and segmentation in both insects and mammals (Holland, 1992; Kimble, 1994; Patel, 1994; Scott, 1994) and three of these genes have been found in a brachiopod (Holland & Hogan, 1986; Holland, Williams, & Lanfear, 1991), the scene is set for very desirable studies of gene expression in brachiopod embryonic development. Furthermore, a recent development even hints at the possibility of an assured supply of embryos (Freeman, 1994), a prerequisite for such work.

The wider paleontological context to which our results must be assimilated has been recently outlined (Conway Morris, 1994). In relation to the latter it is tempting to suggest that the brachiopod-like shells of halkieriids might indicate that the protostome radiation was indeed a matter of combinatorial mixing and matching of a limited number of developmental gene cassettes and might even lead us to invent an (unfashionably *ad hoc*) evolutionary scenario in which predatory selection pressure on diverse *Baupläne* originating by such "stochastic mosaicism" (Schram, 1983, p. 337) favored sessile or infaunal halkieriid-like organisms with more shell and less body and thus led to the emergence of brachiopods. At least this scenario, in which the bivalve shells would both be dorsal but one would have reversed anterior-posterior polarity, might more easily account for the symmetry of the brachiopod shell and the recurved brachiopod,

ectoproct, and phoronid gut than any other yet proposed; and it makes testable predictions about the expression of embryonic axis-determining genes. A much more fully developed hypothesis in which halkieriids play a central part was published after the above was written (Conway Morris & Peel, 1995). Neglecting compositional effects, the sequence similarity demonstrated here between brachiopods plus phoronids and a chiton could reflect a true sister-group relationship, a shared (low) rate of SSU sequence evolution, or convergence in the more rapidly evolving regions of this gene. The fact that chitons appear early in the fossil record (Benton, 1993) is consistent with the possibility of their being a sister group. The possible relationships of chitons and halkieriids have been discussed (Conway Morris & Peel, 1995).

YET MORE GENE SEQUENCE COMPARISONS

Given taxonomic overlap, molecular sequence data is additive (not addictive; it is hard and tedious work!). We can therefore look forward eventually to combining rRNA gene sequences with amino-acid or nucleotide sequences from, for example, highly conserved nuclear protein-coding genes, thus perhaps to resolve the most enigmatic and ancient evolutionary events. Furthermore, a total-evidence approach in which molecular and morphological character-state data are combined is possible (e.g., Eernisse & Kluge, 1993) and may prove informative, although it faces formidable character-weighting and polarization problems. Of more immediate value, the processes underlying speciation, especially population genetic structure, gene flow, and geographical isolation, can be addressed by judicious use of mitochondrial and nuclear gene sequences and other genetic markers (Avise, 1994). The low dispersal potential of articulated brachiopods (but see Peck & Robinson, 1994), their endemicity in oceanic provinces, and the existence of morphological variation between populations over shorter distances (e.g., Aldridge, 1981) suggest that they offer particularly favorable material for the analysis of genomic changes in evolution (Wilson, 1987; Knowlton, 1993) and might, if adequately sampled, provide paradigmatic material for the study of speciation (Templeton, 1989). Preliminary evidence indicates, for example, that both DNA fingerprinting by the comparison of randomly amplified fragments and mitochondrial SSU rRNA gene sequences can be used successfully to resolve divergence between brachiopod individuals, populations, species, and genera (Caryl, 1992; S. Stark, C. Thayer, & B. Cohen, unpublished observations, 1993; B. Cohen & A. Gawthrop, unpublished work, 1995); many golden opportunities lie ahead.

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and M. C. Rhodes, University of Pennsylvania; A. Williams, University of Glasgow; Bamfield Marine Laboratory, University of British Columbia; Portobello Marine Laboratory, Otago University; Scottish Marine Biological Association, Dunstaffnage, Oban; Tasmanian Museum, Hobart; Western Australia Museum, Perth. B. Okamura and J. Vernon, Universities of Bristol and Oxford, respectively, kindly provided DNA from *Cristatella mucedo.* We apologize to anyone whose assistance may have been inadvertently overlooked.

SOURCES OF SEQUENCES

The following sequences were provided by their authors, to whom we are grateful for permission to use them in our analyses while they are still unpublished: *Alcyonidium gelatinosum, Eisenia foetida, Lanice conchilega, Ochetostoma erythrogrammon, Onchidella celtica, Phascolosoma granulatum, Ridgeia piscesae, Siboglinum fiordicum,* B. Winnepenninckx and R. De Wachter, University of Antwerp; *Epimenia australis,* B. Runnegar, University of California; C. Harrison and C. M. Turbeville, Universities of Indiana and Michigan, respectively; *Glottidia pyramidata*, *Phoronis vancouverensis*, *Plumatella repens*, and *Terebratalia transversa* 2, K. Halanych, J. Bachelor, A. M. Aguinaldo, S. Liva, and D. M. Hillis, University of Texas, and J. A. Lake, University of California.

The following sequences were obtained in our own laboratory:

Abyssothyris sp., *Calloria inconspicua, Cancellothyris hedleyi, Cristatella mucedo, Discina striata, Discinisca tenuis, Dyscolia* sp., *Fallax neocaledonensis, Gryphus vitreus, Gyrothyris mawsoni, Hemithiris psittacea, Laqueus californianus, Lingula adamsi, Lingula anatina, Liothyrella neozelanica, Liothyrella uva, Macandrevia cranium, Megerlia truncata, Megerlina* sp., *Neocrania anomala, Neocrania huttoni, Neorhynchia* sp., *Neothyris parva, Notosaria nigricans, Phoronis hippocrepia, Phoronis psammophila, Platidia anomioides, Priapulus caudatus, Stenosarina crosnieri, Terebratalia transversa, Terebratella sanguinea, Terebratulina retusa,* and *Thecidellina blochmanni.* All these sequences will be deposited in GenBank (Benson & others, 1994). DNA aliquots and associated brachiopod shells will be deposited in the Natural History Museum, London. Details of provenance and identification were published (Cohen, Gawthrop, & Cavalier-Smith, in preparation). Copies of the sequence alignment will be available until approximately 1998 on magnetic disk or by FTP by contacting the senior author, e-mail address b.l.cohen@udcf.gla.ac.uk.

The following sequences were obtained from the Ribosomal Database Project electronic archive (Maidak & others, 1994) or from GenBank (Benson & others, 1994): *Acanthoecopsis unguiculata, Acanthopleura japonica, Alligator mississippiensis, Androctonus australis, Anemonia sulcata, Antedon serrata, Argopecten irradians, Artemia salina, Berndtia purpurea, Branchinecta packardi, Branchiostoma floridae, Bufo valliceps, Caenorhabditis elegans, Callinectes sapidus, Chlamys islandica, Crassostrea virginica, Diaphanoeca grandis, Echinorhinus cookei, Eurypelma californica, Fundulus heteroclitus, Gallus gallus, Glycera americana, Herdmania momus, Heterodon platyrhinos, Homo sapiens, Hypogastura* sp., *Lampetra aepyptera, Latimeria chalumnae, Limicolaria kambeul, Lingula reevii, Mactromeris polynyma, Microciona prolifera, Mulinia lateralis, Mytilus edulis, Mytilus trossulus, Myxine glutinosa, Nematodirus battus, Notorhynchus cepedianus, Oryctolagus cuniculus, Petromyzon marinus, Philyra pisum, Placopecten magellanicus, Porocephalus crotali, Priapulus caudatus* 2, *Pugettia quadridens, Raninoides lousianensis, Rattus norvegicus, Rhinobatos lentiginosus, Scypha ciliata, Sebastolobus altivelis, Spisula solida, Squalus acanthias, Stenocypris major, Strongylocentrotus intermedius, Strongylocentrotus purpureus, Strongyloides stercoralis, Styela plicata, Tenebrio molitor, Tresus capax, Trichoplax adhaerens, Tripedalia cystophora, Typetasa lampas, Ulophysema oeresundense,* and *Xenopus laevis.*

PHYSIOLOGY

LLOYD S. PECK[1], MELISSA C. RHODES[2], GORDON B. CURRY[3], and ALAN D. ANSELL[4]

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INTRODUCTION

Prior to the publication of Part H, Brachiopoda, of the *Treatise on Invertebrate Paleontology* in 1965, very few studies dealing with aspects of the physiology of the brachiopods had been published. In that edition, RUDWICK was able to record much of what little information there was, including his own unpublished observations, in the chapter on ecology and paleoecology (1965a), which was later expanded elsewhere into a more comprehensive account (RUDWICK, 1970). Since that date, interest in living representatives of the Brachiopoda has increased, to a large extent as a result of desire by paleontologists for information that could help to interpret the life-styles and habits of fossil brachiopods. Much of the resultant information is discussed in a recent review of the biology of living brachiopods (JAMES & others, 1992), to which readers are referred for a more comprehensive bibliography.

A theme common to many of the recent studies and one of particular relevance to paleontological interpretation is that of scaling or allometry. Scaling concerns particularly the way in which physiological rates and other processes change as organisms grow (intraspecific), or how rates differ between species of differing maximum size (interspecific). Changes in scale, either by growth or through evolution of a larger or smaller body size, inevitably imply some compromises in form or function since not all morphological and functional parameters can increase in proportion while still maintaining the same functional relationships.

A review by LABARBERA (1986a) provided a useful introduction to the vast general literature available on allometric relationships. Following COCK (1966), he distinguished three main categories of primary data in allometric studies—static, cross-sectional, and longitudinal, and four different levels of allometric analysis—ontogenetic, intraspecific, interspecific, and evolutionary. Most reported brachiopod studies are essentially intraspecific, although many authors make interspecific comparisons among groups of allometric exponents and coefficients derived for individual species. In most reported brachiopod studies, allometric analysis has been based on data measured for a group of individuals spanning the whole or part of the size range of the species, usually without the ages being specified or known. This common form of treatment is cross-sectional in COCK's classification (1966). Few sets of data in these brachiopod studies meet all of the criteria regarded as desirable by LABARBERA (1989) either in terms of the choice of variables used, the range of sizes included, or the rigor in choice or reporting of the statistical procedures used. Few reports include all the characteristics of the data or description of the techniques of analysis and results that are included in LABARBERA's (1989) Menu for Scaling Analyses. Finally, most of the studies report empirical relationships without attempting to test specific models derived from theoretical considerations of the relationships involved. A notable exception is the study by ACKERLY (1991, 1992) in which various parameters measured during rapid closure of the shell in articulated brachiopods were compared with values predicted from a theoretical model of the hydrodynamic forces involved. Despite these shortcomings, the variety of size-related data becoming available on many aspects of brachiopod function and physiology constitutes valuable source material for the paleontologist. As many as possible of these quantitative data are therefore summarized here,

although no attempt has been made to rework the original data to minimize the above criticisms.

SENSORY AND NEUROMUSCULAR PHYSIOLOGY AND BEHAVIOR

The sensory and behavioral capabilities of brachiopods are limited. The only sensory structures that have been described are the setae present in the larvae and around the mantle margin in the adult and the statocysts. Although responses to a variety of stimuli have been reported or implied, differentiated sensory structures concerned in such responses have not been identified.

The inarticulated brachiopods include both attached and free-living forms, the former including such species as *Neocrania anomala* (O. F. Müller) in which the pedicle valve is cemented to a hard substratum, further restricting behavioral capabilities. In contrast, the free-living lingulids are the only group to have evolved an infaunal habit, and they have a range of physiological and behavioral features that adapt them for this mode of life. In particular, they are able to burrow into sediment, to maintain their position within the sediment, and to withdraw rapidly into the sediment in an escape response. During burrowing, the pedicle acts as a support or prop, while penetration is achieved by cyclical movements of the shell valves combined with action of the lateral setae. The detailed mechanism of burrowing has been well studied for a number of species (e.g., Trueman & Wong, 1987; Savazzi, 1991; and review in James & others, 1992). During burrowing the coelomic fluid functions as a hydrostatic skeleton facilitating movements of the valves and pedicle, and the lingulids thus differ from other brachiopods in which the coelom persists only as a fluid-transport system. In lingulids, the greatest pressures in the coelom coincide with shell opening during burrowing, indicating that thrust generated by the hydrostatic pressure is required to open the shell against the resistance of the sediment (Fig. 189).

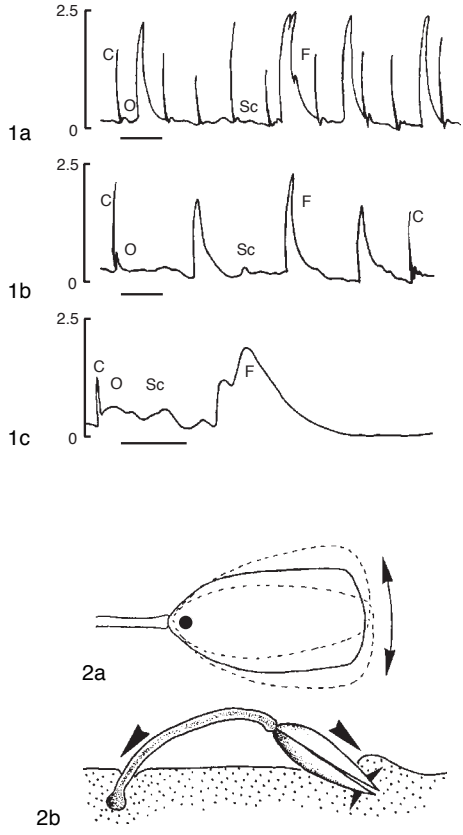

Fig. 189. *1a–c,* Recordings of coelomic pressure (kPa) from the perivisceral coelom of *Lingula anatina* during the early stages of burrowing; recording for *1b* follows immediately after *1a,* showing closing (*C*), opening (*O*), scissors movements of the valves (*Sc*), and the application of greater force (*F*) to effect movement into the sand (time mark, one minute); *1c,* as above; time mark, 10 seconds. *2a–b,* Diagram of the ventral and dorsal valve of *Lingula anatina* showing movement of the valves during *2a,* the scissor motion in burial; the principal pivotal area is represented by a *dot; 2b,* diagram of entry into the sand during high pressure pulse (*large arrows*) that forces valves and pedicle into the substratum with equal force and opens valve simultaneously (*small arrows*) (adapted from Trueman & Wong, 1987).

The articulated brachiopods comprise both attached forms and forms that may become unattached but rely on such passive adaptations as heavy shell thickening to resist physical stress. Activity in the articulated brachiopods is essentially restricted to opening and closing of the valves and, in the attached forms, rotation of the valves around

the pedicle. Rapid closure is accomplished by contraction of the adductor muscles, while opening results from the contraction of the opposing diductor muscles. Cycles of rapid adduction followed by slow abduction occur during normal activity and in response to various stimuli but show little clear rhythmicity (RUDWICK, 1962b; MCCAMMON, 1971). Prolonged shell closure in response to unfavorable conditions is possible and is maintained by contraction of the smooth muscle of the anterior pair of adductors.

Studies of the physiology of the muscle systems involved in these movements of the shell in articulated brachiopods, particularly by J. L. WILKENS and his co-workers (for references see JAMES & others, 1992), have shown that each of the opposing sets of muscles possess features consistent with its individual role. The smooth adductor muscles and the diductor muscles in particular exhibit unique characteristics. In the former, the tension generated in the muscle outlasts the initial neuronal stimulation, allowing the muscle to maintain tension for prolonged periods (WILKENS, 1987). This enables the smooth adductor muscles to eliminate slowly the shell gape remaining after contraction of the striated posterior adductor and to maintain shell closure. The diductor muscles exhibit the phenomenon of slip or slippage in which their tension drops abruptly to zero when they are subjected to vibration or stretching during contraction. This occurs naturally when contraction of the diductor muscles is opposed by the contraction of the striated adductor muscles. After slippage, tension begins to develop immediately but does so very slowly (WILKENS, 1978b). These properties of the diductor muscles allow the valves to be kept open at minimal metabolic cost while allowing for rapid closure in an emergency (ESHLEMAN & WILKENS, 1979a).

Rapid shell closure in the articulated brachiopods is subject to important hydrodynamic constraints related to expulsion of water from the shell. During closure, the principal hydrodynamic forces acting on the shell are inertial reactions, due to the acceleration of water, which dominate the kinematics of shell closure during the initial phases, and pressure forces, which develop as water is expelled from the shell and dominate the later phases of closure. ACKERLY (1991) developed a generalized hydrodynamic model that described the relative magnitude of these forces as functions of the shell's angular acceleration, velocity, and gape. Solutions of the general model predict how variables such as the closing speed and the mass flux of water depend on shell size, initial shell gape, and magnitude of the closing force. Measurements made from actual shell-closure events in *Terebratulina retusa* (LINNAEUS) and *Terebratalia transversa* (SOWERBY), recorded using electronic techniques and high-speed video cameras (Fig. 190), showed close agreement with the predictions of the hydrodynamic model, confirming that hydrodynamic reactions are a fundamental restraint on the closing mechanism (ACKERLY, 1992).

Allometric equations relating features of the kinematics of shell closure (maximum gape, closing velocity, closing time) and of muscle mechanics (moment arm length, muscle length, muscle cross-sectional area, muscle moment force, muscle force) to shell length of *Terebratulina retusa* and *Terebratalia transversa* are summarized in Table 7.

BODY SIZE AND COMPOSITION

RUDWICK (1970, p. 19) stated that "the shell of a brachiopod gives a misleading idea of the real size of the organism." This is because there is little tissue between the valves of a brachiopod shell, and much of the space enclosed by the valves, constituting the mantle cavity, can be viewed as part of the external environment. The problem of how big an animal is superficially appears to be a trivial question, but an organism's size affects almost all aspects of its physiology and ecology. The appropriate measure of size varies with the type of study being conducted and the questions being asked: taxonomists, for example, might be interested in linear

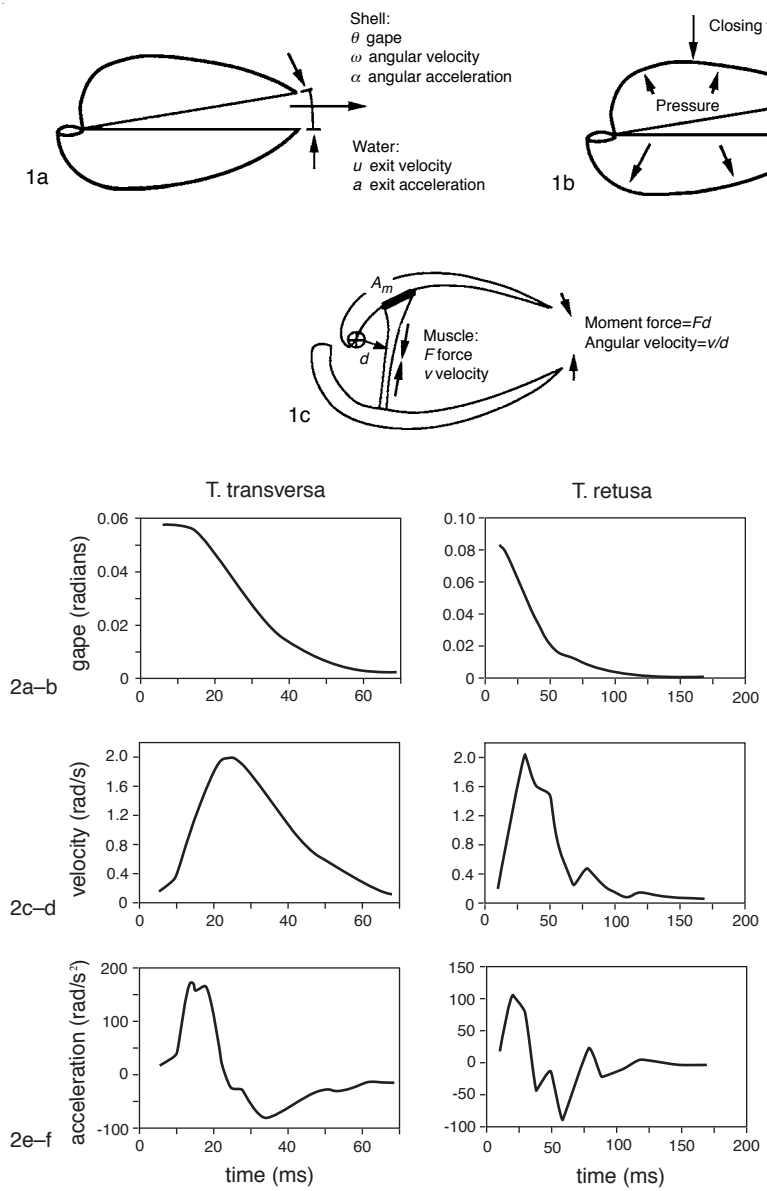

FIG. 190. *1a–c,* Diagrams of the brachiopod shell showing *1a,* the kinematic parameters, *1b,* the forces involved in closure, and *1c,* the quick adductor muscle position; A_m, cross sectional area of the muscle base; *d,* moment arm distance (Ackerly, 1991). *2a–f,* Kinematic data for typical shell closing events in *Terebratalia transversa* and *Terebratulina retusa* showing *2a–b,* the shell gape, *2c–d,* angular velocity, and *2e–f,* angular acceleration as functions of time (James & others, 1992; data from Ackerly, 1992).

dimensions or the ratios of linear dimensions of the shell or parts of the shell to give information on interpopulation or interspecies differences; ecologists might be concerned with the volume of the animal to gain information about competitive interactions between species in terms of space occupied. Physiologists often need to know the

TABLE 7. Regression parameters from equations relating features of the muscle mechanics involved in shell closure to shell lengths (*L,* mm) for the articulated brachiopods *Terebratulina retusa* and *Terebratalia transversa.* Parameters are for the equation $\log_e y = a + b \log_e L$ and were fitted by reduced major axis techniques; *CI,* confidence interval; r^2, coefficient of determination (adapted from Ackerly, 1992).

Species	Dependent variable (y)	Slope (b)	CI_{95}	r^2	Significant allometry ($P < 0.05$)
T. retusa	moment arm length (cm)	1.25	0.114	0.98	positive
	muscle area (cm^2)	2.59	0.73	0.90	-
	muscle force ($\times 10^{-2}$ N)	1.55	0.419	0.81	negative
	muscle length (cm)	1.03	0.349	0.86	-
	muscle moment ($\times 10^{-4}$ N.m)	2.72	0.553	0.94	-
T. transversa	moment arm length (cm)	0.968	0.562	0.66	-
	muscle area (cm^2)	3.04	1.34	0.81	-
	muscle length (cm)	1.37	0.622	0.84	-
	muscle moment ($\times 10^{-4}$ N.m)	3.33	0.107	0.998	positive

amount of organic material present, usually measured as ash-free dry mass (AFDM), while measurements of surface area of body tissues or structures may be important in understanding such processes as the rates of capture of particles by the filter-feeding apparatus or the rates of excretion of the end products of metabolism.

For most biological investigations of brachiopods, linear dimensions (shell length, breadth, and height) are measured, and length is usually used when comparisons with other parameters are made. As a consequence of allometry, ratios of linear dimensions have been shown to vary with size, for example, in the Antarctic brachiopod *Liothyrella uva* (BRODERIP) (PECK, CLARKE, & HOLMES, 1987a). This is a point of some significance to taxonomic studies, which may rely on such ratios for interspecific comparisons.

Mass is the next most common measurement of size taken in brachiopod studies. Here several different measures have been made. Tissue dry mass, which is the mass of soft tissue between the valves weighed after it has been dried to constant weight, has been used in investigations of respiration rate (SHUMWAY, 1982). Ash-free dry mass has been more commonly measured in physiological investigations of brachiopods, and this has also been separated in some cases into amounts present in the shell and in the tissues (CURRY & ANSELL, 1986; PECK, CLARKE, & HOLMES, 1987a).

For the inarticulated brachiopod *Neocrania anomala,* the exponents in the allometric relationship between ash-free dry mass (AFDM) or dry mass (DM) and shell length are close to the value of 3 predicted by geometric isometry (Table 8).

For the articulated brachiopods measured, the exponents in these relationships are generally less than 3, with a mean of 2.71 ± 0.17 SD (Table 8; Fig. 191). Measured exponents in the relationship between AFDM of the tissues only (i.e., neglecting that component of the tissues contained in the caeca) vary between 2.36 and 3.48, with a mean of 2.95 ± 0.42 SD. In the Antarctic brachiopod *Liothyrella uva,* the exponent in these relationships varies systematically through the year, with midsummer exponents as low as 2.69 and late winter values as high as 3.23. Such changes result from differences between young and old individuals in their seasonal cycles of storage and utilization of resources for metabolism during winter and for reproduction. Higher values of the exponent are associated with periods when reserves have been used up and adults have not yet spawned, while lower exponents are found after spawning in the summer and during the period when overwintering reserves are high in all size classes (PECK & HOLMES, 1989b). In *L. uva* also, exponents

TABLE 8. Regression parameters from equations relating dry mass (*DM,* mg) or ash-free dry mass (*AFDM,* mg) to shell length (*L,* mm) for inarticulated (*Neocrania anomala)* and articulated (*Calloria inconspicua, Terebratulina retusa, Liothyrella uva, Liothyrella neozelanica, Neothyris lenticularis,* and *Notosaria nigricans)* brachiopods. Parameters are for the equation $\log_e y = a + b \log_e L$ and were fitted by least squares techniques. The suffixes *t* and *s* refer to measurements of internal tissue and shell respectively; where no suffix is given, data are for measurements of whole animal; *n,* sample size; r^2, coefficient of determination; *SE,* standard error (see also Fig. 191; new).

Species	Dependent variable (y)	Intercept (a)	Slope (b)	SE_b	n	r^2	Source	Size range (mm)
Inarticulated								
N. anomala	DM	-2.12	2.91	-	39	0.95	CURRY & ANSELL (1986)	8–15
N. anomala	AFDM	-4.40	2.96	-	40	0.96	CURRY & ANSELL (1986)	8–15
N. anomala	AFDM(t)	-3.64	2.36	-	9	0.82	CURRY & ANSELL (1986)	8–15
Articulated (punctate)								
C. inconspicua	DM	-3.24	3.25	-	12	0.96	CURRY & ANSELL (1986)	
C. inconspicua	AFDM	-5.04	2.80	-	12	0.97	CURRY & ANSELL (1986)	
C. inconspicua	AFDM(t)	-6.07	2.97	-	12	0.91	CURRY & ANSELL (1986)	8–15
T. retusa	DM	-1.19	2.59	-	100	0.99	CURRY & ANSELL (1986)	8–15
T. retusa	AFDM	-4.22	2.62	-	100	0.98	CURRY & ANSELL (1986)	8–15
T. retusa	AFDM	-4.20	2.67	0.14	18	0.96	PECK & others (1989)	3.4–21.2
T. retusa	AFDM(t)	-4.44	2.55	-	9	0.94	CURRY & ANSELL (1986)	8–15
L. uva	AFDM	-5.22	2.97	0.07	37	0.98	PECK & HOLMES (1989a)	4.2–52.8
L.neozelanica	AFDM(t)	-7.87	3.25	0.12	25	0.91	PECK (1993)	8–50
L.neozelanica	DM(s)	-2.49	2.83	0.06	25	0.99	PECK (1993)	8–50
N. lenticularis	AFDM(t)	-6.17	3.08	0.22	24	0.90	PECK (1993)	8–40
N. lenticularis	DM(s)	-2.60	3.14	0.11	23	0.98	PECK (1993)	8–40
Articulated (impunctate)								
N. nigricans	AFDM(t)	-7.25	3.48	0.18	26	0.94	PECK (1993)	4–25
N. nigricans	DM(s)	-3.31	3.45	0.13	25	0.97	PECK (1993)	4–25

in the relationship between AFDM and length for adults may differ from those for juveniles at certain times of the year (PECK & HOLMES, 1989a) as a result of reproductive activity.

Measured exponents in the relationship between shell mass and shell length range from 2.83 for *Liothyrella neozelanica* (IHERING) to 3.45 for *Notosaria nigricans* (SOWERBY) with a mean of 3.14 ± 0.31 SD, indicating that there is no consistent pattern of positive or negative allometry (Fig. 191). Measured exponents in the relationship between tissue mass (but neglecting that component of the tissues contained in the caeca) and shell mass of the inarticulated brachiopod *Lingula anatina* LAMARCK (as *L. bancrofti* JOHNSON & HIRSCHFELD) and three species of articulated brachiopods are all less than the value of 1 predicted by geometrical isometry, with a mean of 0.82 ± 0.07 SD, indicating that the mass of shell material increases more with age than the mass of internal tissues (Table 9).

Different internal tissues scale differently with brachiopod size. Exponents in the relationship between tissue AFDM and shell length vary not only between tissues, but also seasonally (Table 10). For the digestive gland, the exponent has low values throughout the year, ranging from 2.28 at the end of winter to 2.69 at the end of summer, significantly less than the value of 3 predicted by geometrical isometry. Values for the lophophore are also consistently and significantly less than 3. Exponents for the gonads, in contrast, are more variable, ranging from 2.34 in early summer to 6.21 in late summer.

Absolute amounts of tissue AFDM also vary seasonally in a consistent fashion (Fig. 192). In *Liothyrella uva* from the Antarctic, all tissues show a sharp increase in mass dur-

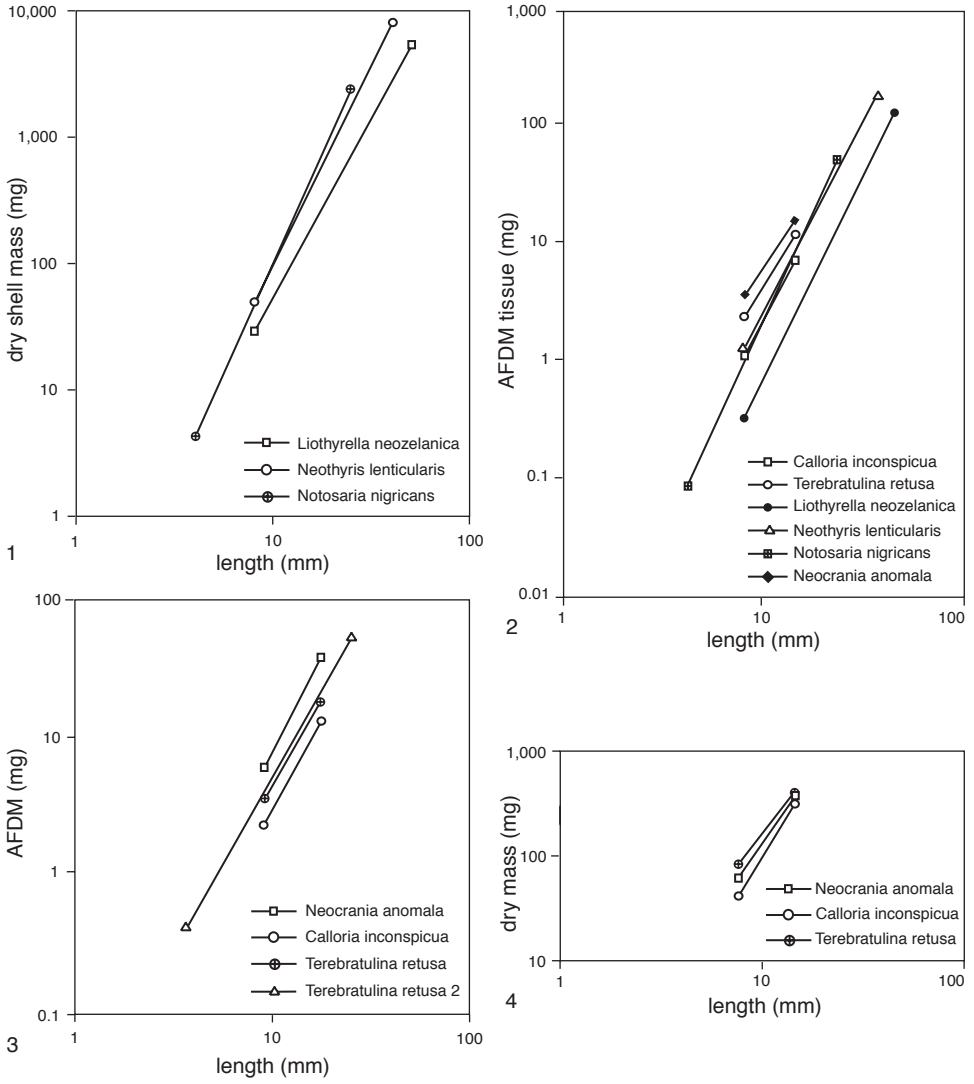

FIG. 191. Relationships between *1,* $\log_e$ dry shell mass (mg) and $\log_e$ shell length (mm); *2,* $\log_e$ ash-free dry mass of tissues (AFDM tissue, mg) and $\log_e$ shell length; *3,* $\log_e$ ash-free dry mass (AFDM, mg) and $\log_e$ shell length; and *4,* $\log_e$ dry mass (mg) and $\log_e$ shell length for inarticulated and articulated brachiopods. Parameters for each regression are shown in Table 8 (new).

ing the period of the summer phytoplankton bloom, the increases in the digestive gland and lophophore probably being related to raised activity levels associated with processing food. The AFDM of the gonad shows a cycle related to growth and proliferation of the gametes and subsequent spawning, while variations in shell AFDM reflect a combination of the storage of reserves for winter and reproductive events combined with the processing of those reserves by intracellular machinery in the caeca (PECK & HOLMES, 1989b; JAMES & others, 1992). Thus brachiopod tissue sizes and proportions change both as the animals grow and with seasonal events.

TABLE 9. Regression parameters from equations relating dry tissue mass (*DM(t),* mg) to dry shell mass (*DM(s),* mg) for inarticulated and articulated brachiopods. Parameters are for the equation $\log_e DM(t) = a + b \log_e DM(s)$ and were fitted by least squares techniques; *n,* sample size; r^2, coefficient of determination (Shumway, 1982).

Species	Intercept (a)	Slope (b)	n	r^2	P (<)	Size range mg DM(s)
Inarticulated						
Lingula bancrofti	0.93	0.78	23	0.93	0.001	40–2,000
Articulated (punctate)						
Neothyris lenticularis	-2.26	0.75	18	0.82	0.001	1,100–12,000
Calloria inconspicua	-3.43	0.88	40	0.98	0.001	50–3,000
Terebratella sanguinea	-3.60	0.89	20	0.93	0.001	50–1,200

Articulated brachiopods generally have a lower overall organic content than many other marine benthic invertebrates, while inarticulated brachiopods do not (SHUMWAY, 1982; CURRY & ANSELL, 1986). The inarticulated brachiopod *Neocrania anomala* has a proportion of inorganic matter in its internal tissues (18.6 percent) similar to crustaceans, polychaetes, and bivalve and gastropod molluscs but a lower proportion than in sponges (PECK, 1993). Values for inorganic content of the internal tissues of articulated brachiopod species are generally about twice those of crustaceans, polychaetes, and molluscs and close to the inorganic contents of sponges. When the shell is also taken into account, 93.9 percent to 97.5 percent of the dry mass of the articulated brachiopods *Liothyrella neozelanica, Neothyris lenticularis* (DESHAYES), and *Notosaria nigricans* is found to be inorganic. These very high values of inorganic content combined with the small amounts of internal tissue found in articulated brachiopod species have profound implications for potential predators (PECK, 1993).

A corollary of the low tissue mass in relation to volume enclosed between the valves in articulated brachiopods is that the size of the mantle cavity and the amount of water held in it is large. The exponent in the allometric relationship between the volume of the shell valves and shell length for *Liothyrella uva* is 2.77, a significant negative allometry (Table 11; PECK & HOLMES, 1989a). In contrast, the volume of the mantle cavity scales with an exponent of 3.34, showing significant positive allometry. The exponents in the relationships of internal tissues and of total animal volume with shell length are 3.06 and 3.12, respectively, and not significantly different from

TABLE 10. Exponents from regressions relating tissue ash-free dry mass (*AFDM,* mg) to shell length (*L,* mm) for the articulated brachiopod *Liothyrella uva* for six dates during the 1982–1983 austral summer. ANCOVA data test for significant variations in exponents among sampling periods, i.e., seasonal changes in the scaling of a given tissue. Exponents are values of *b* in the equation $\log_e AFDM = a + b \log_e L$; *DG,* digestive gland; *GO,* gonads; *LO,* lophophore; *OT,* other internal tissue; *SH,* shell; *TOT,* whole animal (adapted from Peck & Holmes, 1989a).

Tissue	1982			1983			ANCOVA	
	13 Sep	6 Nov	1 Dec	4 Jan	3 Feb	16 Feb	F	P (<)
DG	2.62	2.28	2.30	2.67	2.69	2.58	1.23	0.302
GO	4.36	2.83	2.34	3.86	6.21	3.49	7.00	0.001
LO	2.85	2.83	2.54	2.39	2.80	2.92	2.70	0.024
OT	2.97	3.24	2.83	2.84	3.01	3.03	0.76	0.597
SH	3.13	3.02	2.56	2.56	2.85	2.95	2.68	0.023
TOT	3.16	3.23	2.75	2.69	2.97	2.99	2.03	0.074

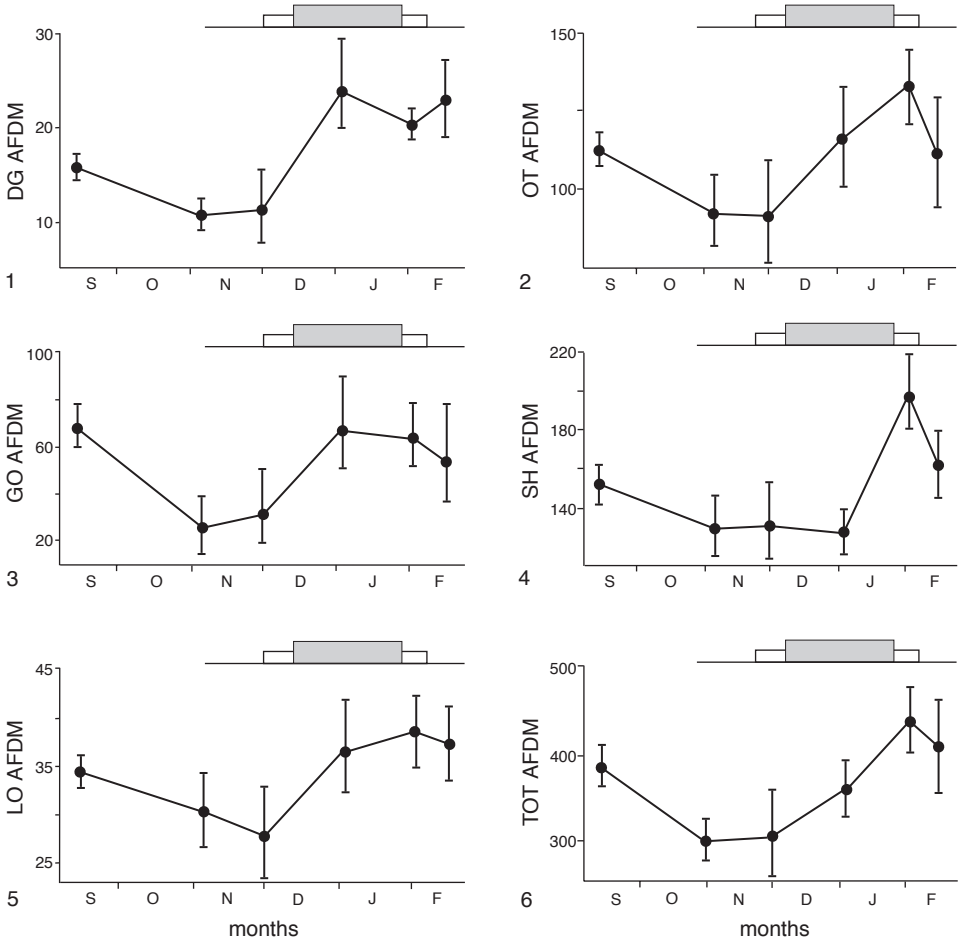

FIG. 192. *1–6,* Seasonal variations in the ash-free dry mass (AFDM, mg) of the tissues of *Liothyrella uva* from Signy Island, Antarctica. Data are for a large adult (45 mm long) from the late winter through late summer of the 1982–1983 season. Points are means with ± 95 percent confidence intervals. Bars at the top of each graph show the duration and intensity of the phytoplankton bloom: *single line,* >1 mg chlorophyll/m^3; *open box,* >5 mg chlorophyll/m^3; *shaded box,* >10 mg chlorophyll/m^3; *DG,* digestive gland; *GO,* gonads; *LO,* lophophore; *OT,* other internal tissues combined; *SH,* shell; *TOT,* total, or whole animal, values (adapted from Peck & Holmes, 1989b).

the value of 3 predicted by geometric isometry (Fig. 193). Thus, with increasing size, a progressively smaller proportion of the total volume is taken up by the shell valves, while relatively more is devoted to the mantle cavity.

The large volume of the mantle cavity may be an adaptation allowing for long periods of closure while utilizing the oxygen stored in the mantle cavity water (SHUMWAY, 1982) or may be a functional requirement set by the architecture of the lophophore. *Liothyrella uva* continues to remove oxygen from water in the mantle cavity for around eight hours during enforced closure of the shell valves (PECK, MORRIS, & CLARKE, 1986a), which supports the former hypothesis. This may not be the sole factor in the evolution of large mantle cavities in articulated brachiopods, however. The architecture of the lophophore, consisting of unfused filaments, may dictate a need for a large mantle cavity for its efficient operation (PECK, 1992). PECK postulated that the resultant

TABLE 11. Regression parameters from equations relating shell valve volume (*V(s),* cm^3), internal tissue volume (*V(t),* cm^3), mantle cavity volume (*V(m),* cm^3), and whole animal volume (*V(tot)*) to shell length (*L,* mm) for the articulated brachiopod *Liothyrella uva.* Parameters are for the equation $\log_e y = a + b \log_e L$ and were fitted by least squares techniques. P values refer to differences between the slope and a value of 3 predicted by geometric isometry. Shell length ranged from 11.5 to 52.2 mm; *n,* sample size; r^2, coefficient of determination; *SE,* standard error (see also Fig. 193; new).

Component	Intercept (a)	Slope (b)	SE_b	n	r^2	Significant allometry (P < 0.05)
V(s)	-9.84	2.77	0.075	45	0.97	negative
V(t)	-9.76	3.06	0.037	45	0.99	-
V(m)	-10.66	3.34	0.091	45	0.97	positive
V(tot)	-9.10	3.12	0.066	45	0.98	-

constraints have a profound influence on brachiopod lifestyles, resulting in low metabolic rates, which in turn contribute to the success of brachiopods in areas of low or highly seasonal food supply.

FEEDING

All brachiopods, both articulated and inarticulated, feed with the lophophore, a ciliated tentacular organ that occupies most of the volume in the mantle cavity. Although the lophophore occurs in a number of configurations, brachiopod species possessing different lophophore types feed in essentially the same manner. When the lophophore tentacles are fully extended for feeding and respiration, the mantle cavity is separated into inhalant and exhalant regions (Fig. 194). Weak, through-going currents are created by the lateral cilia of the tentacles, while the frontal cilia transport food particles along the length of the tentacle toward the brachial groove for transport to the mouth. Undesirable particles are eliminated by a variety of mechanisms (RUDWICK, 1970; THAYER, 1986a; JAMES & others, 1992). Mantle cilia assist in rejection and in the exchange of water through the mantle cavity (WESTBROEK, YANAGIDA, & ISA, 1980; THAYER, 1986a). Water movement through the mantle cavity is generally slow and laminar (Fig. 195), thus minimizing the energy dissipation involved in turbulent-flow regimes (LABARBERA, 1977, 1981, 1990).

The lophophore, in common with other filter-feeding structures, provides a large surface area for the capture of particles suspended in the water being passed across it. As the lophophore is essentially an external surface providing an interface between the tissues and the outside environment, one might expect that its area would scale with exponents of 2 or 2/3 in allometric relationships with length or mass respectively. For

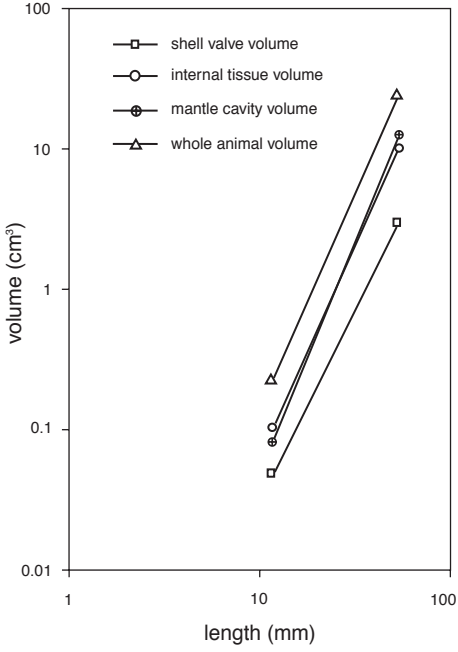

FIG. 193. Relationships of $\log_e$ whole animal volume, $\log_e$ shell valve volume, $\log_e$ mantle cavity volume, and $\log_e$ internal tissue volume (cm^3) with $\log_e$ shell length (mm) for the articulated brachiopod *Liothyrella uva.* Parameters for each regression are shown in Table 11 (new).

two species of inarticulated brachiopods, *Neocrania californica* (BERRY) and *Discinisca strigata* (BRODERIP), measured values for the exponent in the allometric relationships of surface area of the lophophore with AFDM are close to 0.66 (Table 12; Fig. 196). Thus, for inarticulated brachiopods, lophophore area scales in a way that would be expected from a surface increasing in isometric proportion to shell length with growth. Measured values of the exponents in the allometric relationship between lophophore area and AFDM are greater than 0.66 for four species of articulated brachiopods, although for *Terebratalia transversa* and *Laqueus californianus* the difference from 0.66 was not significant. The mean value for the articulated brachiopods, however, is 0.712, indicating significant positive allometry. The mean value for these articulated brachiopods is significantly greater than that of the inarticulated brachiopods.

Epifaunal brachiopods are facultatively active suspension feeders (LABARBERA, 1977, 1981, 1984). They use metabolic energy to produce currents for feeding and respiration, but, when possible, they orient themselves to external water currents so that their ciliary pumping is augmented by the hydrodynamics of the ambient flow regime, and recirculation of previously filtered water is avoided. Some species such as *Terebratalia transversa,* do not reorient themselves after settlement of the larvae. Also such species as *Calloria inconspicua* and *Notosaria nigricans* live in dense, conspecific clusters, where the water pumped by one individual may include a significant component already filtered by closely neighboring individuals or epibionts, resulting in an increase in the total energy needed for pumping water to obtain food. There are no published studies on the hydrodynamics of infaunal inarticulated brachiopods.

Although the sources of nutrition used by brachiopods are still poorly understood for most species, there is little doubt that most of the energy needs of most species are supplied by particulate material filtered from the feeding current. This may include, in various proportions, phytoplankton, bacteria, organic detritus, or organic molecules adsorbed onto inorganic particles. Brachiopods are also able to absorb dissolved carbohydrates and amino acids directly from seawater, but the significance of this process as a source of energy has not been established (for references to nutritional sources, see JAMES & others, 1992).

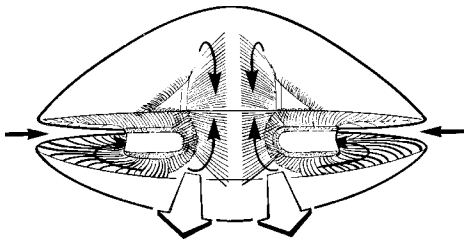

FIG. 194. Diagrammatic representation of the separation of incurrent (*solid arrows*) and excurrent water streams (*open arrows*) through the mantle cavity and lophophore of a typical plectolophous brachiopod (new).

Indirect evidence from measurements of particle-retention efficiencies suggests that brachiopods may fail to capture a large proportion of the suspended particles that are available to them in the water column (JAMES & others, 1992). Absolute particle-retention efficiency, defined as the number of suspended particles in a given size range captured by an organism during one traverse of the feeding structure, is difficult to measure directly without disturbing the animal. More commonly used, therefore, are relative retention efficiencies of differently sized particles, calculated as the percent retention of particles in a given size range relative to the retention efficiency of the most effectively retained size group of particles, taken as 100 percent (MOHLENBERG & RIISGARD, 1978). Particle-retention efficiency versus size has been studied in only three plectolophous articulated brachiopod species, *Terebratulina retusa* (JØRGENSEN & others, 1984), *Terebratulina septentrionalis,* and *Liothyrella uva* (M. RHODES, unpublished data, July, 1989: Newfoundland; December, 1994: Signy). For all the species studied, particles larger than 5 μm are captured more efficiently than smaller

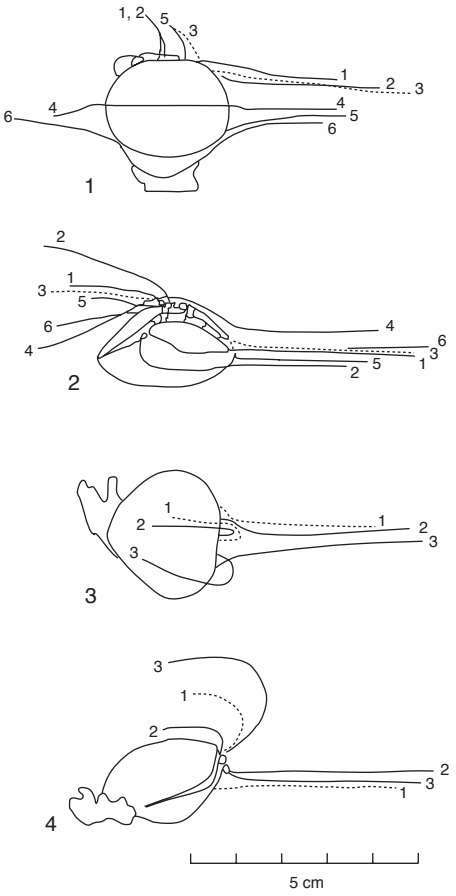

FIG. 195. Dye stream paths (indicated by *numbered lines*) around and through *Terebratalia transversa* in a 2 cm/s current (current flow is from right to left). *1–2, Terebratalia* in the preferred orientation relative to the current direction; *3–4, Terebratalia* in the least favorable orientation for normal flow through the lophophore. Regardless of orientation, the excurrent plume completely bypasses the shell and is never refiltered by the animal. Note that the water entering the anteroventral portion of the downstream incurrent gape (*lines 2 and 5*) passes very close to the dorsal valve and turns through 90° to enter the shell (LaBarbera, 1981).

particles. No comparable information is available for spirolophous articulated brachiopods or inarticulated brachiopods. Unlike bivalve molluscs, which frequently retain 100 percent of all particles above a given size range, articulated brachiopods retain at best only 68 percent of the particles passing through the lophophore (JØRGENSEN & others, 1984). Direct observation of the plectolophous lophophore of undamaged *Terebratalia transversa,* using an endoscope with a high magnification zoom lens, showed that significant leakage of particles occurs through the lateral arms directly into the excurrent stream (THOMPSON, WARD, & RHODES, 1992).

The more efficient retention of larger-sized particles by articulated brachiopod species living in the photic zone (<200 m depth) is consistent with the sizes of particles found in their gut contents, where the most abundant particles are generally 5 to 10 μm in diameter and larger (SUCHANEK & LEVINTON, 1974; DOHERTY, 1976). The preponderance of small inorganic particles less than two microns in the guts of *Hemithiris psittacea* (GMELIN), *Terebratulina septentrionalis* (COUTHOUY), *Glaciarcula spitzbergensis* (DAVIDSON), *Neothyris lenticularis* (DESHAYES), and *Abyssothyris wyvillei* (DAVIDSON) (MCCAMMON, 1969) probably reflects the presence of materials remaining in the gut after digestion.

Water exchange rates through brachiopods are extremely variable and are low relative to such other groups of marine invertebrates as bivalve molluscs or sponges (LABARBARA, 1981; RHODES & THOMPSON, 1992, 1993). In *Terebratulina septentrionalis,* flow rate varies considerably both for the same individual and between individuals of similar size (Fig. 197; MCCAMMON, 1971). Measured rates lie in the range of 0.05 to 0.75 cm/sec for individuals of 19.3 to 21.2 mm shell length. Exhalant flow of *Laqueus californianus* (KOCH), *Terebratulina unguicula* (CARPENTER), *Hemithiris psittacea* (GMELIN), and *Terebratalia transversa* (SOWERBY) (LABARBERA, 1977, 1981) has similarly low, variable, and intermittent rates. Measured mean excurrent rates range from a minimum of 0.2 cm/sec in *Hemithiris psittacea* of 7.9 to 19.8 mm length to a maximum of 1.41 cm/sec in *Terebratalia transversa* of 14.4 mm to 27.8 mm length.

Clearance rate is a measure of the rate at which a suspension feeder filters algae or par-

TABLE 12. Regression parameters from equations relating total lophophore area (*LA,* cm^2) and total ash-free dry mass (*AFDM,* mg) for inarticulated and articulated brachiopods. Parameters are for the equation $\log_e LA = a + b \log_e AFDM$ and were fitted by reduced major axis techniques; *n,* sample size; r^2, coefficient of determination; *SE,* standard error (see also Fig. 196; adapted from LaBarbera, 1986b).

Species	Intercept (a)	Slope (b)	SE_b	r^2	n	Size range mg AFDM
Inarticulated						
Neocrania californica	-0.73	0.65	0.018	0.98	15	0.018–23
Discinisca strigata	-2.21	0.65	0.029	0.94	31	0.1–45
Articulated (punctate)						
Terebratalia transversa	-1.27	0.67	0.008	0.98	99	0.0018–>400
Terebratulina unguicula	-1.06	0.70	0.010	0.98	80	0.026–80
Laqueus californianus	-0.89	0.69	0.023	0.98	22	2.7–80
Hemithiris psittacea	-1.32	0.78	0.019	0.96	74	0.0045–60

ticles from the surrounding water. It thus reflects the ability to acquire food. Clearance rate is defined as the volume cleared per unit time, measured in liters per hour (l/h) or milliliters per hour (ml/h) (WIDDOWS, 1985). Clearance rates for the spirolophous articulated brachiopod *Hemithiris psittacea* and the plectolophous articulated brachiopods *Terebratulina septentrionalis, Neothyris lenticularis,* and *Liothyrella neozelanica* fed

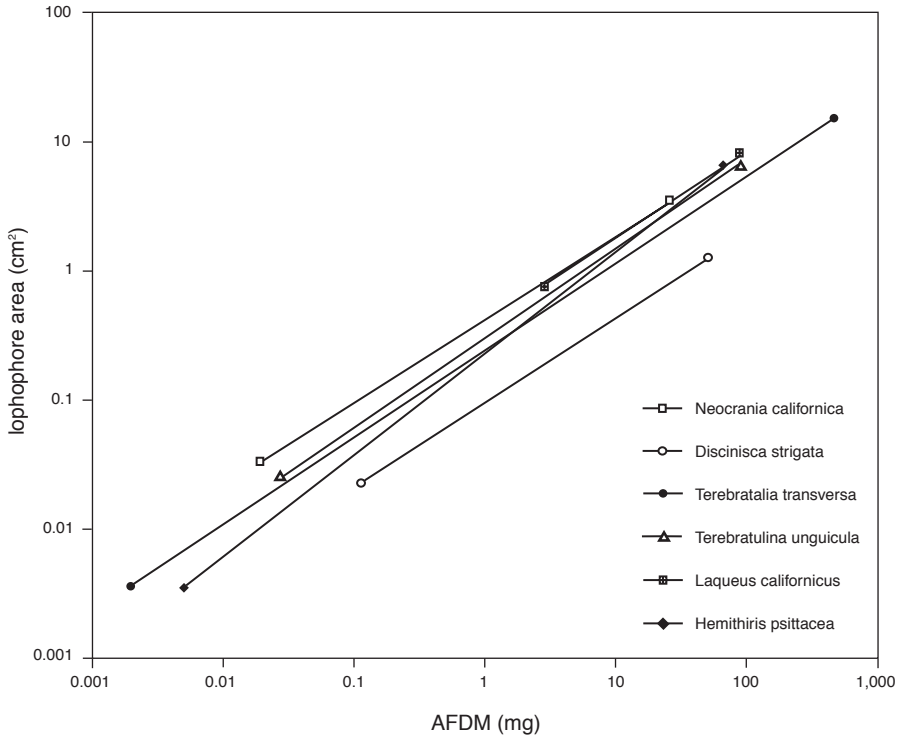

FIG. 196. Relationships between $\log_e$ total lophophore area (cm^2) and $\log_e$ total ash-free dry mass (AFDM, mg) of inarticulated and articulated brachiopods. Parameters for each regression are shown in Table 12 (new; data from LaBarbera, 1986b).

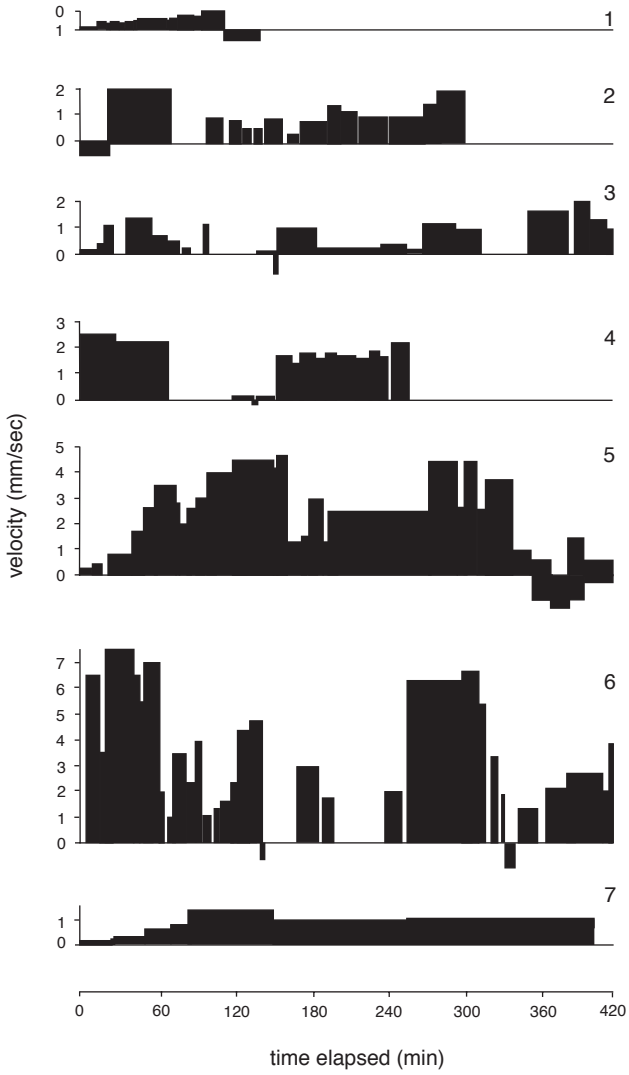

FIG. 197. Flow velocity in seven *Terebratulina septentrionalis* during the first seven hours of recording from a thermistor flow meter positioned in the incurrent flow. Maximum velocity of flow recorded during each cycle is plotted against time. Shell lengths of animals: *1,* 19.4 mm; *2,* 19.3 mm; *3,* 12.7 mm; *4,* 18.3 mm; *5,* 16.0 mm; *6,* 21.2 mm; *7,* 14.8 mm (McCammon, 1971).

unicellular algae in controlled laboratory conditions (Table 13; Fig. 198; RHODES & THOMPSON, 1992, 1993), range from 22.0 ml/h for a 12.8 mg *Liothyrella neozelanica* to 1.20 l/h for a *Neothyris lenticularis* of 183 mg. Individuals have highly variable clearance rates; for example, recorded rates of one *Neothyris lenticularis* differed by a factor of seven to eight during repeated measurements at ten-minute intervals. This variability is thus similar to that noted earlier for water pumping rates. No similar data are available for inarticulated brachiopods.

Exponents in the allometric relationship between clearance rate and ash-free dry mass (AFDM) range from 0.54 for *Terebratulina septentrionalis* to 0.62 for *Liothyrella neozelanica* (Table 13; Fig. 198; RHODES &

TABLE 13. Regression parameters from equations relating clearance rate (*CR,* ml/h) to ash-free dry mass (*AFDM,* mg) for articulated brachiopods. Parameters are for the equation $\log_e CR = a + b \log_e AFDM$ and were fitted by least squares technique. There is no significant correlation between CR and AFDM for *T. septentrionalis* at an algal cell concentration of 11,000/ml, but the correlation is significant at 5,500 cells/ml; *n,* sample size; r^2, coefficient of determination; *SE,* standard error (see also Fig. 198; adapted from Rhodes & Thompson, 1992, 1993).

Species	Concentration algal cells/ml	Intercept (a)	Slope (b)	SE_b	n	r^2	Size range mg AFDM
Hemithiris psittacea	11,000	3.43	0.61	0.108	24	0.59	11–44
Terebratulina septentrionalis	11,000	-	-	-	15	NS	32–102
Terebratulina septentrionalis	5,500	3.52	0.54	0.227	12	0.36	32–102
Liothyrella neozelanica	5,300	1.92	0.62	0.084	20	0.63	13–292
Neothyris lenticularis	5,300	3.57	0.56	0.111	19	0.72	39–251

THOMPSON, 1992, 1993). The measured exponents were all lower than 0.66 but not significantly so. However, the mean value of this exponent for articulated brachiopods was 0.583, suggesting slight negative allometry (RHODES & THOMPSON, 1992, 1993), and is close to but generally lower than the value of 0.66 that would be predicted if clearance increased isometrically with surface area. This contrasts with measured exponents in the relationships between lophophore surface area and tissue mass for plectolophes, which are generally higher than 0.66 (Table 12). Simple measures of lophophore area, however, do not take into account the pathways of particles in the feeding current, which may be equally important in predicting the effectiveness of suspension feeding. The lower exponents found for clearance rates suggest that not all areas of the lophophore are functionally equivalent. The tentacles of the median coil in two plectolophous species, *Terebratalia transversa* and *Terebratulina unguicula,* pump at only 60 percent the rate of the tentacles in the lateral arms (LABARBERA, 1981, 1986b). In *Terebratalia transversa,* however, most particles are captured in the median coil, while sorting and rejection are concentrated in the lateral arms (THOMPSON, WARD, & RHODES, 1992).

In the relationships between clearance rate and AFDM, scaling coefficients (intercepts), which measure the relative levels of activity of different species, range from 1.92 for *Liothyrella neozelanica* to 3.57 for *Neothyris lenticularis* (Table 13). Comparisons between species, using clearance rates for individuals of 50, 100, and 350 mg AFDM, calculated using the relationships in Table 13, are summarized in Table 14. These rates are generally lower than those measured for other ecologically similar suspension feeders, such as bivalve molluscs, and are consistent with other evidence for overall low metabolic rates of brachiopods (JAMES & others, 1992).

Clearance rates of the plectolophous articulated brachiopods *Terebratulina septentrionalis* and *Neothyris lenticularis* are concentration dependent (Table 13–14; RHODES & THOMPSON, 1992, 1993), with low rates of clearance at high algal concentrations (10,500 to 12,600 cells/ml). In contrast, the spirolophous *Hemithiris psittacea* continues to clear algal cells effectively in algal concentrations of 10,500 to 12,600 cells per ml (Table 13–14).

There is little agreement whether brachiopods are selective feeders (JAMES & others, 1992), but sorting activity may be one reason for the reduced filtration rates of particles by plectolophes in high particle concentrations. Experimental evidence for the plectolophe *Terebratalia transversa* (RHODES & THAYER, 1991) suggests that articulated brachiopods can sort particles on the basis of specific gravity, shape, or charge. Sorting and rejection are useful adaptations in areas subject to high loads of suspended particulates but may involve extra expenditure of energy.

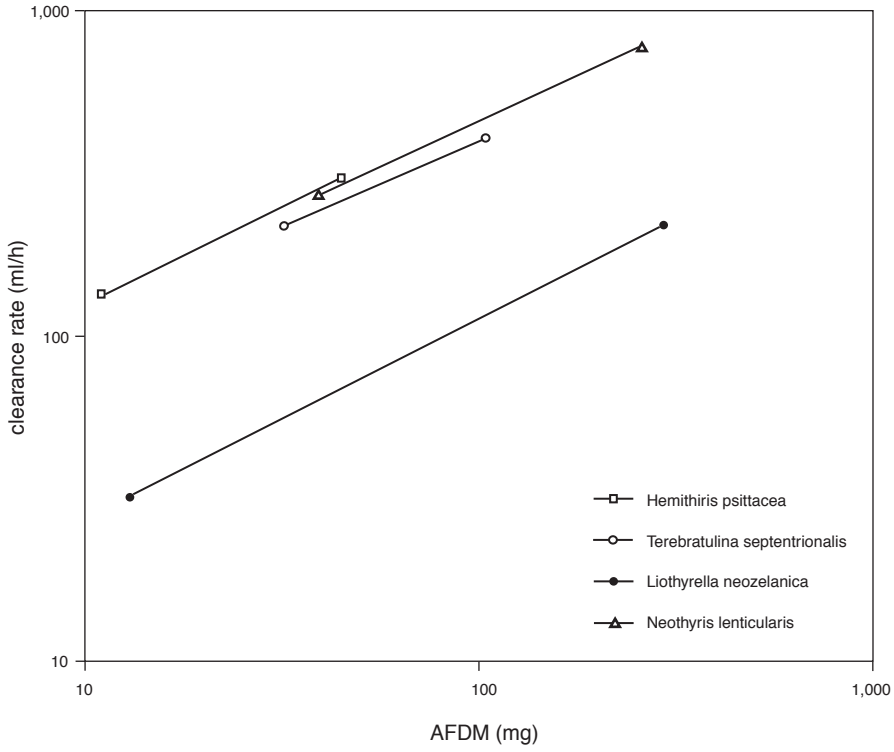

FIG. 198. Relationships between $\log_e$ clearance rate (ml/h) and $\log_e$ total ash-free dry mass (AFDM, mg) for articulated brachiopods. Parameters for each regression are shown in Table 13 (new; data from Rhodes & Thompson, 1992, 1993).

DIGESTION

Brachiopod digestive tracts have two basic configurations. Articulated brachiopods have a blind-ended gut, while inarticulated brachiopods have a separate anus. In both inarticulated and articulated brachiopods, the alimentary tract consists of pharynx, esophagus, stomach, digestive diverticula, and pyloris or intestine (STEELE-PETROVIC, 1976; MCCAMMON, 1981; review in JAMES & others, 1992). Epifaunal inarticulated brachiopods have a short, pouchlike intestine, while the infaunal lingulids have a long intestine (MCCAMMON, 1981). Articulated brachiopods eliminate feces by antiperistalsis through the mouth; inarticulated brachiopods, both epifaunal and infaunal, eliminate feces by peristalsis through the anus. Other than in this respect, the alimentary tracts of both articulated and inarticulated brachiopods are morphologically and histologically similar (STEELE-PETROVIC, 1976; MCCAMMON, 1981).

Brachiopods combine both extracellular and intracellular digestion, depending on the type of material ingested (STEELE-PETROVIC, 1976; review in JAMES & others, 1992). Extracellular digestion and mechanical disruption resulting from contractions of the gut wall break particles down to a size (<2 μm) that the digestive cells can phagocytose (STEELE-PETROVIC, 1976).

The three inarticulated brachiopods *Terebratulina retusa, Gryphus vitreus* (BÖRN), and *Megerlia truncata* (LINNAEUS) and one inarticulated brachiopod, *Neocrania anomala,* for which digestive enzymes have been studied most comprehensively (D'HONDT, 1986) are closely similar in the distribution and level of activity of enzymes in the intestine and digestive gland; the only important difference noted was the strong activity of β-galactoside in the inarticulated brachiopod

compared with the articulated brachiopods. Only acid phosphatase and N-acetyl-β-glucosaminidase showed greater activity in the intestine than in the digestive gland, indicating that only these enzymes are secreted throughout the gut. Most enzyme activities are located in the cells of the digestive gland, reflecting the important role of this organ in digestion and absorption in brachiopods (for review and full references, see JAMES & others, 1992).

RESPIRATION

Respiration refers to the processes involved in the uptake of oxygen by an organism from its proximate environment, the transportation of oxygen to the tissues, and its use as the final electron acceptor in energy-yielding, biochemical pathways. The rate of oxygen consumption (respiration rate) is often taken as a measure of metabolic rate but accurately represents rates of metabolic energy production only when no significant amounts of energy are being produced via anaerobic pathways.

Oxygen enters the body of an animal across soft tissue interfaces with the surrounding medium. These are often specially adapted areas (e.g., gills), where the integument is thin to facilitate the passage of molecules required for metabolism. Waste products of metabolic pathways, including carbon dioxide and nitrogenous wastes in the form of ammonia, urea, or amino nitrogen, may also pass out of the animal via these areas. In brachiopods the transport of oxygen into the body tissues takes place predominantly through the epithelia of the lophophore and mantle lobes. It has been suggested that, under specific conditions, oxygen may be removed from the surrounding seawater by caeca in punctate species (SHUMWAY, 1982). Physical barriers, however, make this unlikely. For example, in the terebratulides, the labyrinthine periostracum would be a formidable barrier even for the diffusion of oxygen, while in the thecideidines the caeca are separated from the subperiostracal cavities by many transverse partitions (A. WILLIAMS, personal communication, 1994). The caeca should not be viewed as respiratory organs because they do not supply oxygen to the internal tissues (PECK, MORRIS, & CLARKE, 1986a, 1986b; JAMES & others, 1992).

TABLE 14. Comparison of clearance rates (ml/h) for similar-sized individuals of the articulated brachiopods *Hemithiris psittacea, Terebratulina septentrionalis, Liothyrella neozelanica,* and *Neothyris lenticularis* (new; data from Rhodes & Thompson, 1992, 1993).

Species	50 mg individual	100 mg individual	Cells/ml
H. psittacea	338	516*	11,000
T. septentrionalis	280	406	5,500
L. neozelanica	77	118	5,300
N. lenticularis	317	468	5,300

*extrapolation slightly beyond range of original data.

Measured rates of oxygen consumption in brachiopods (Table 15–16) are generally low, consistent with the low rates of laminar flow through the brachiopod mantle cavity. They range from 0.1 to 0.9 times those of equivalently sized bivalve molluscs, with a mean value of around 0.5 (JAMES & others, 1992). Most published data on respiration are for articulated brachiopods; those for the inarticulated brachiopods *Lingula anatina* (as *L. bancrofti*) (SHUMWAY, 1982), *L. anatina* (as *L. reevii* DAVIDSON), and *Glottidia pyramidata* (STIMPSON) (HAMMEN, HANLON, & LUM, 1962; HAMMEN, 1969, 1971, 1977) are inconsistent and difficult to compare with the data for articulated brachiopods, partly because of the procedures adopted and partly because different bases were used for measurement of the size of the brachiopods (for detailed discussion see JAMES & others, 1992). At present, good assessments of the oxygen consumption of inarticulated brachiopods based on AFDM are not available.

Exponents in the allometric relationship between rates of oxygen consumption and AFDM or tissue dry weight range from 0.72 for *Terebratella sanguinea* (LEACH), *Laqueus californianus,* and *Liothyrella uva* to 1.00 for *Terebratulina retusa* (Table 15; Fig. 199). The average value of this exponent for the articulated brachiopods (0.78) is greater than the

TABLE 15. Regression parameters from equations relating oxygen consumption ($\dot{V}O_2$, µl O_2/h) with animal ash-free dry mass (*AFDM,* mg) for inarticulated and articulated brachiopods. Parameters are for the equation $\log_e \dot{V}O_2 = a + b \log_e AFDM$ and were fitted by least squares techniques after logarithmic transformation of the data. Coefficients in parentheses were fitted by reduced major axis techniques. Oxygen consumption measurements on brachiopods were also made by HAMMEN (1971) and THAYER (1986b), but it was not possible to extract relevant data from these sources; *F,* fed; *n,* sample size; r^2, coefficient of determination; *S,* starved; *SE,* standard error (see also Fig. 199; new).

Species	Temp (°C)	Intercept (a)	Slope (b)	SE_b	r^2	n	Authority	Size range mg AFDM
Inarticulated								
Lingula bancrofti	10	-0.68	0.71	-	0.85	31	SHUMWAY (1982)	35–2,000
Articulated (punctate)								
Neothyris lenticularis	10	0.26	0.73	-	0.79	22	SHUMWAY (1982)	2–300
Calloria inconspicua	10	0.17	0.74	-	0.88	50	SHUMWAY (1982)	1.5–48
Terebratella sanguinea	10	0.30	0.72	-	0.86	31	SHUMWAY (1982)	1.5–48
Terebratalia transversa	-	(0.40)	(0.73)	0.023	0.96	45	LABARBERA (1986b)	1–360
Terebratulina unguicula	-	(0.26)	(0.74)	0.026	0.96	34	LABARBERA (1986b)	3–60
Laqueus californianus	-	(0.02)	(0.77)	0.039	0.94	22	LABARBERA (1986b)	2.8–80
Hemithiris psittacea	-	(0.58)	(0.72)	0.028	0.96	33	LABARBERA (1986b)	0.38–80
Terebratulina retusa	5.8F	-0.24	0.95	0.089	0.88	24	PECK & others (1989)	38–450
Terebratulina retusa	10.7F	-2.41	0.97	0.055	0.88	45	PECK & others (1989)	5–520
Terebratulina retusa	5.6S	-2.70	1.00	0.055	0.88	46	PECK & others (1989)	12–450
Liothyrella uva	0	-2.16	0.72	0.028	0.88	105	PECK & others (1986)	0.32–660
Liothyrella uva	0	-2.30	0.80	0.033	0.92	45	PECK, CLARKE, & HOLMES (1987b)	1–610

value of 0.66 that would be predicted if oxygen consumption increased isometrically with surface area but close to the exponent of 0.75 commonly found in interspecific scaling comparisons of metabolic rate (see LABARBERA, 1986b).

Exponents in the relationships between rates of oxygen consumption and tissue weight (Table 15) are generally greater than the equivalent exponents for the relationships between particle clearance rates and tissue weight (Table 13). Similarly, when the relationships of rates of oxygen consumption to body mass are compared with those for lophophore surface area (Table 12), three plectolophes, *Terebratalia transversa, Terebratulina unguicula,* and *Laqueus californianus,* all have higher exponents for oxygen consumption than for lophophore area. For the spirolophe, *Hemithiris psittacea,* however, the reverse is the case. As the lophophore is the feeding organ, its area should limit the ability of the brachiopod to capture food particles. Thus, for plectolophes, there is an increasing disparity between ability to obtain food and metabolic energy requirements, and this could limit the maximum attainable size. For spirolophes the limited data available would suggest that no such constraint applies. In this context, LABARBERA (1986b) pointed out that whenever a brachiopod clade has produced species of large size in the fossil record the lophophore was a spirolophe.

Even when the differences in scaling exponent among the relationships between rates of oxygen consumption and tissue mass are taken into account, considerable differences remain between rates for different brachiopod species and between rates measured under different conditions for the same species. Differences in the temperature at which the measurements were made account for part of this variation, but when the data are adjusted to allow for temperature differences between experiments (assuming a Q_{10} value of 2 where

$$Q_{10} = (V_2/V_1)^{10/(t_2-t_1)}$$

TABLE 16. Comparison of rates of oxygen consumption ($\dot{V}O_2$, µl/h) for similar-sized individuals of articulated brachiopods; *F,* fed; *S,* starved (new).

Species	Temp (°C)	$\dot{V}O_2$ (µl/h) 50 mg individual	100 mg individual	Authority
Neothyris lenticularis	10	21.3	37.3	SHUMWAY (1982)
Calloria inconspicua	10	22.1	35.7	SHUMWAY (1982)
Terebratella sanguinea	10	20.3	37.1	SHUMWAY (1982)
Terebratalia transversa	-	26.0	42.9	LABARBERA (1986b)
Terebratulina unguicula	-	23.4	39.1	LABARBERA (1986b)
Laqueus californianus	-	21.1	35.4	LABARBERA (1986b)
Hemithiris psittacea	-	29.7	49.0	LABARBERA (1986b)
Terebratulina retusa	5.8F	3.6	7.1	PECK & others (1989)
Terebratulina retusa	10.7F	4.0	7.8	PECK & others (1989)
Terebratulina retusa	5.6S	3.4	6.7	PECK & others (1989)
Liothyrella uva	0	1.9	3.2	PECK & others (1986)
Liothyrella uva	0	2.3	4.0	PECK, CLARKE, & HOLMES (1987b)

and V_1 and V_2 are the respiration rates at the temperatures t_1 and t_2 respectively) the lowest and highest values recorded for articulated brachiopods are still different by a factor of five.

Rates of oxygen consumption of individual brachiopod species are influenced by other factors besides temperature. These include seasonal factors as metabolic rate in winter is usually lower than in summer even when temperatures are the same, or physiological states, such as reproductive or nutritional condition. The variation in measured rates may also reflect methodological differences among the different studies, in particular the influence of stirring of the experimental chambers (see JAMES & others, 1992). High values tend to be associated with experimental systems using oxygen electrodes and stirred water (SHUMWAY, 1982; LABARBERA, 1986b) and low values with still-water regimes using wet-chemical titration techniques (DOHERTY, 1976; PECK, CLARKE, & HOLMES, 1987b; PECK & others, 1989). Neither accurately reproduce the hydrodynamic conditions in which brachiopods normally live.

Rates of oxygen consumption in articulated brachiopods are relatively insensitive to temperature change. Q_{10} values are generally below 2 when brachiopods are held at temperatures within the range they normally experience (DOHERTY, 1976; SHUMWAY, 1982; PECK, 1989; PECK & others, 1989). For *Calloria inconspicua* (SOWERBY), Q_{10} may be as low as 1.18 within the normal temperature range but greater than 4 when held below the normal temperature range (DOHERTY, 1976; SHUMWAY, 1982). The time course of temperature change affects the apparent effect of temperature on oxygen consumption. Acute (short-term) responses to temperature change in the Antarctic brachiopod *Liothyrella uva* gave Q_{10} values as high as 9.7, while for acclimated (longer-term) responses Q_{10} values were all less than 2 (PECK, 1989).

The rate of oxygen consumption is also affected by the oxygen tension in the surrounding sea water. The inarticulated brachiopod *Lingula anatina* and the articulated brachiopod *Calloria inconspicua* both show strong abilities to remove oxygen from water at low oxygen tension; *Neothyris lenticularis* is poor in this respect, and *Terebratella sanguinea* intermediate (Fig. 200; SHUMWAY, 1982). These different abilities reflect the likelihood in each case that the species would encounter conditions of low oxygen tension in its natural habitat. *Lingula anatina,* living in an infaunal habitat, is the most likely to regularly experience low oxygen tension in the surrounding water; *Calloria inconspicua,* living in fairly shallow areas, is likely to regularly experience low oxygen concentrations during some low tides; while *Neothyris*

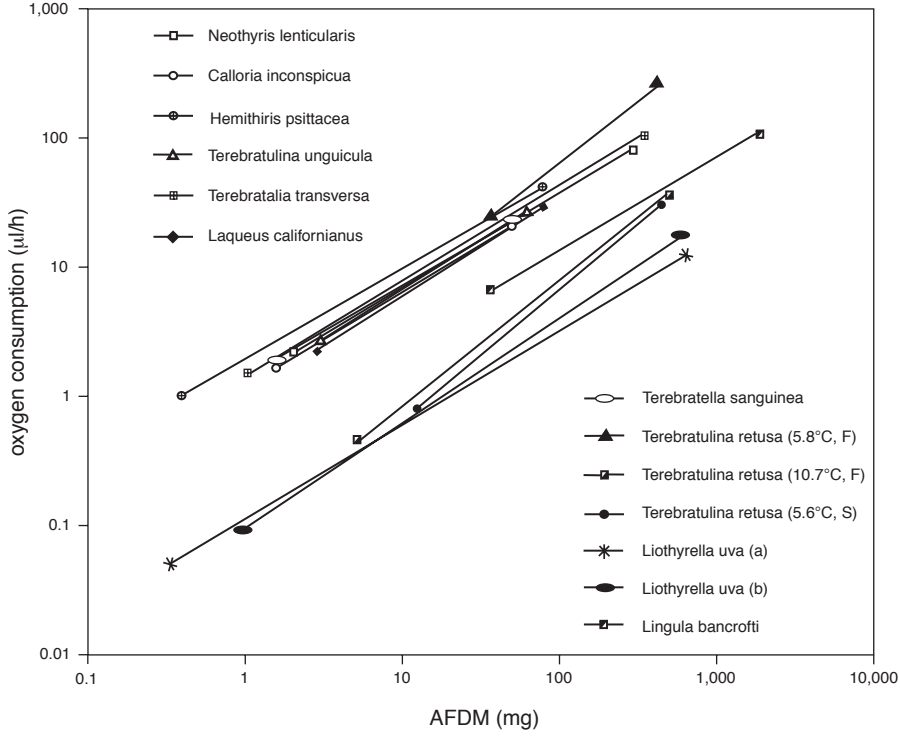

FIG. 199. Relationships between $\log_e$ rate of oxygen consumption (µl/h) and $\log_e$ animal ash-free dry mass (AFDM, mg) for inarticulated and articulated brachiopods. Parameters for each regression and the sources of the data are shown in Table 15; *F,* fed; *S,* starved (new).

lenticularis and *Terebratella sanguinea* both live in deeper-water environments where reduced oxygen regimes are unlikely. *Terebratalia transversa* from subtidal habitats near the San Juan Islands, Washington, USA, are capable of regulating the uptake of oxygen at constant rates down to levels of around 10 percent saturation (0.5 cm^3 O_2/l; THAYER, 1986b).

A further factor affecting rates of oxygen consumption is the nutritional state of the individual. In *Liothyrella uva* and *Terebratulina retusa,* feeding raises rates of oxygen consumption above starved or standard levels by between 20 and 25 percent (PECK & others, 1986, 1989; PECK, CLARKE, & HOLMES, 1987b). Starvation may reduce rates by more than 50 percent in *Liothyrella uva* (PECK, 1989) with reduction to these basal (standard) levels taking between 25 and 30 days to complete from the initiation of starvation. DOHERTY (1976) found no difference in the rates of oxygen consumption of *Calloria inconspicua* held at four different food concentrations, but with his experimental protocol it would not have been possible to detect changes of the order of 25 percent. The above studies do, however, show that the effects of feeding on respiration rate are low. There is no information on the effects of such other parameters as animal density, light regime, turbidity, or salinity on oxygen uptake in brachiopods.

Their low levels of respiration combined with the low tissue densities described earlier (p. 220) suggest that diffusion processes should be sufficient to supply oxygen to the tissues of articulated brachiopods. Calculations based on an equation derived by HARVEY (1928) indicate that diffusion could supply oxygen to the tissues over distances of around 2 mm, which is less than the average thickness of the mantle including the gonads (JAMES, ANSELL, & CURRY, 1991a). There is

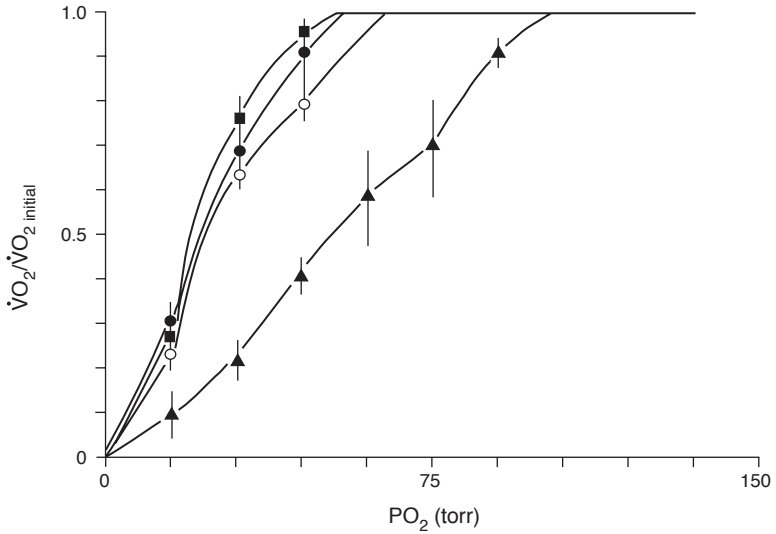

FIG. 200. The effect of declining oxygen tension on oxygen consumption in four species of brachiopods: *Calloria inconspicua* (●, sample size = 15), *Terebratella sanguinea* (o, sample size = 5), *Neothyris lenticularis* (▲, sample size = 5), *Lingula anatina* (as *L. bancrofti*) (■, sample size = 6). Initial $\dot{V}O_2$ (ml O_2/h/g) values are set equal to 1.0 and all subsequent values are expressed as fractions of 1.0. Points on each line represent the mean with 95 percent confidence limits; experimental conditions: 10°C, 33.5 ppt salinity (Shumway, 1982).

thus little requirement for an efficient circulation to transport respiratory gases and waste products. The only brachiopod for which heart-beat rates have been measured, the articulated brachiopod *Liothyrella uva,* shows a rate of less than 1 beat per minute (average = 0.8 beats/min) at 0°C (BUCHAN, PECK, & TUBLITZ, 1988).

Respiratory pigments used to transport oxygen or to store oxygen within the body tissues have not been recorded in articulated brachiopods. The inarticulated brachiopod, *Lingula anatina* (as *L. unguis*), however, contains hemerythrin (KAWAGUTI, 1941), a nonheme, oxygen-carrying Fe-protein that is the rarest of the four respiratory pigments found in the metazoa. Hemerythrin from Japanese *L. anatina* consists of two different subunits, alpha and beta, that are present in equal amounts and have a molecular weight of approximately 12 kDa (SATAKE & others, 1990). In its natural state the molecule has an octameric structure, composed of four alpha helices and four beta helices. In *L. anatina,* both the alpha and beta subunits consist of 117 amino acids, and the primary sequence of both subunits has been determined (Figure 201; YANO, SATAKE, UENO, KONDO, & TSUGITA, 1991). Preliminary investigations have indicated that hemerythrin is also present in *Discina* and *Discinisca* (M. CUSACK, personal communication, 1994). Further information on the distribution of respiratory pigments in brachiopods will require systematic biochemical and genetic investigations.

EXCRETION

Excretion is the process whereby waste products of metabolism, particularly nitrogenous waste, are removed from the body. In marine invertebrates, the main excretory product is ammonia. In brachiopods, metabolic end products are probably mainly excreted across the mantle and lophophore epithelia by diffusion. Brachiopods also have a single pair or, in a few exceptional cases, two pairs of metanephridia situated one on either side of the intestine, which may have some excretory function. The basic form of the nephridium is the same in all brachiopods: a funnel-shaped nephrostome opens into the coelomic cavity, then tapers to a nephridiopore, which opens to the mantle cavity.

	1 10	20	30	40
Lingula alpha	VKVPEPFAWN	ESFATSYKNI	DLEHRTLFNG	LFALSEFNTR
Lingula beta	MKIPVPYAWT	PDFKTTYENI	DSEHRTLFNG	LFALSEFNTQ
	41 50	60	70	80
Lingula alpha	DQLLACKEVF	VMHFRDEQGQ	MEKANYEHFE	EHRGIHEGFL
Lingula beta	HQLNAAIEVF	TLHFHDEQGQ	MIRDNYVNTK	EHTDIHNGFM
	81 90	100	110	117
Lingula alpha	EKMGHWKAPV	AQKDIKFGME	WLVNHIPTED	FKYKGKL
Lingula beta	DTMRGWQSPV	PQKALKDGME	WLANHIPTED	FKYKGKL

FIG. 201. Sequence data for hemerythrin from the inarticulated brachiopod *Lingula unguis* (YANO, SATAKE, UENO, KONDO, & TSUGITA, 1991). Hemerythrin contains two chains of amino acids designated alpha and beta, each composed of 117 amino acids. Single letters are standard abbreviations for individual amino acids in the sequence. The order of amino acids in the protein is determined by the DNA of the genes responsible for coding this protein (Yano, Satake, Ueno, Kondo, & Tsugita, 1991).

RUDWICK (1970) stated that excretory products were ingested by coelomocytes before being moved by ciliary currents to the nephridia. In the nephridia they are bound in mucus and then passed out of the nephridiopore. This view was probably based on the observations of HELLER (1931; reported in HYMAN, 1959b) of the fate of materials injected into the coelom of *Hemithiris psittacea* and *Terebratulina retusa.* It is likely that the role of the nephridia includes the removal of such foreign material as bacteria as well as infected or damaged brachiopod tissues. In general biologists now consider ejection of such solid products as elimination, along with the ejection of feces or pseudofeces, rather than excretion. The nephridia have a major role as the channel through which the eggs and sperm are discharged in spawning.

Of the nitrogen excreted by the inarticulated brachiopod *Lingula anatina* (as *L. reevii*), 94 percent is in the form of ammonia, the remaining 6 percent being amino acids (LUM & HAMMEN, 1964; HAMMEN, 1968). The other major end products excreted by marine invertebrates include urea, uric acid, amino acids, and purines (REGNAULT, 1987). Urea was not detected as a waste product by LUM and HAMMEN (1964), and other products were not investigated. There have been no further investigations using more reliable methods of analysis (see JAMES & others, 1992 for discussion) nor have the nitrogenous products excreted by articulated brachiopods been examined, although they are generally assumed to be predominantly ammonia.

The 6 percent of nitrogen excreted as amino acids by *Lingula anatina* is a low percentage compared with data for molluscs (HAMMEN, 1968; BAYNE, WIDDOWS, & THOMPSON, 1976; BAYNE & NEWELL, 1983) or crustaceans (REGNAULT, 1987). Losses of amino acids may be due to leakage across membrane surfaces along concentration gradients, rather than active excretion. The articulated brachiopod *Calloria inconspicua* is capable of removing glutamic acid and glycine from seawater (DOHERTY, 1981) at rates that vary with animal size and nutritional state, temperature, and the concentration of amino acids in the seawater. The low rate of loss of amino acids by *Lingula,* therefore, probably reflects the ability of membrane pumps in the epithelia of the mantle and lophophore to actively transport amino acids and hence maintain the equilibrium. Tighter junctions between the cell membranes of these epithelia may also be involved. The loss of amino acids to the external medium represents a failure to use them metabolically to build proteins or to gain energy via deamination. The low rates of loss of amino acid by *L. anatina* may indicate that brachiopods are able to efficiently metabolize their nitrogenous energy reserves (JAMES & others, 1992).

Measured exponents in the allometric equations relating rates of ammonia excretion under different conditions to AFDM of *Terebratulina retusa* range from 0.84 to 1.28

TABLE 17. Regression parameters from equations relating rates of NH_3-N excretion (*E,* µg.atom NH_3-N/h) to ash-free dry mass (*AFDM,* g) for the articulated brachiopods *Terebratulina retusa* and *Liothyrella uva.* Parameters are for the equation $\log_e E = a + b \log_e AFDM$ and were fitted by least squares techniques; *n,* sample size; r^2, coefficient of determination; *SE,* standard error (see also Fig. 202; new).

Species	Temp (°C)	Fed/ Starved	Intercept (a)	Slope (b)	SE_b	r^2	n	Authority	Size range mg AFDM
T. retusa	5.6	Starved	-0.99	0.84	0.098	0.68	36	PECK & others (1989)	38–450
	5.8	Fed	-2.40	0.85	0.030	0.45	12	PECK & others (1989)	12–450
	10.7	Fed	0.06	1.28	0.279	0.45	8	PECK & others (1989)	5–520
L. uva	0	Starved	-1.71	0.76	0.042	0.81	78	PECK & others (1986)	0.77–580
	0	Fed	-1.23	0.86	0.040	0.94	45	PECK, CLARKE, & HOLMES (1987b)	1–610

(Table 17; Fig. 202). The differences between exponents are not significant, and the combined data give an exponent of 1.01 (PECK & HOLMES, 1989a). Exponents in this relationship for *Liothyrella uva* are 0.76 in winter conditions and 0.86 in summer conditions. No other comparable data are available for other articulated brachiopods or for inarticulated brachiopods.

Published rates of ammonia excretion by other brachiopods, for which only limited information is available, are compared with those for *T. retusa* in Table 18. If the values are adjusted to take account of temperature differences using a Q_{10} value of 2, the rate for the inarticulated brachiopod *Lingula anatina* is within the range of values for the articulated brachiopod *Terebratulina retusa,* while the results for *Liothyrella uva* were slightly higher than those for *Terebratulina retusa* on the same basis.

Rates of ammonia excretion are strongly dependent on the substrate being respired and the general level of metabolic rate. Excreted ammonia is almost wholly produced from the metabolism of proteins; and, if lipids and carbohydrates are used as the only metabolic energy sources, essentially no nitrogen will be excreted. *Terebratulina retusa* kept in conditions that simulate a winter regime (5.6°C; starved) have 29 percent higher rates of ammonia excretion than in summer conditions (10.7°C; fed). In intermediate conditions (5.8°C; fed) rates were 4.4 times lower than in the winter conditions (PECK & others, 1989). Similar seasonal effects are seen in the Antarctic brachiopod *Liothyrella uva* where the rate of ammonia excretion is 21 percent higher in summer than in winter conditions (PECK & others, 1986; PECK, CLARKE, & HOLMES, 1987b).

The ratio of gram atoms of oxygen consumed to ammonia nitrogen excreted (O:N ratio) provides information on the importance of proteins that yield excreted nitrogen as fuel for metabolism compared to lipids and carbohydrates that yield no nitrogen. The minimum theoretical value of the O:N ratio should approach 7 when the sole metabolic substrate is protein (CONOVER & CORNER, 1968), although MAYZAUD (1973) showed that the ratio could be as low as 4 under some circumstances. O:N ratios of 20 to 30 are obtained when protein forms around 50 percent of the total metabolic substrates (IKEDA, 1974). Brachiopods generally have low values of O:N ratio (Table 19). In *Liothyrella uva,* protein is the dominant substrate under both winter and summer conditions. Protein levels in tissue also have large seasonal variations compared with very small fluctuations in lipids and carbohydrates (PECK & others, 1986; PECK, CLARKE, & HOLMES, 1987b).

In aquaria, *Terebratulina retusa* has O:N ratios of 16 in simulated summer conditions, 8 in winter conditions, and 42 in intermediate conditions (PECK & others, 1989), indicating that protein, almost solely, fuels metabolic costs in winter conditions and is still the dominant respiratory substrate in summer conditions. In intermediate conditions

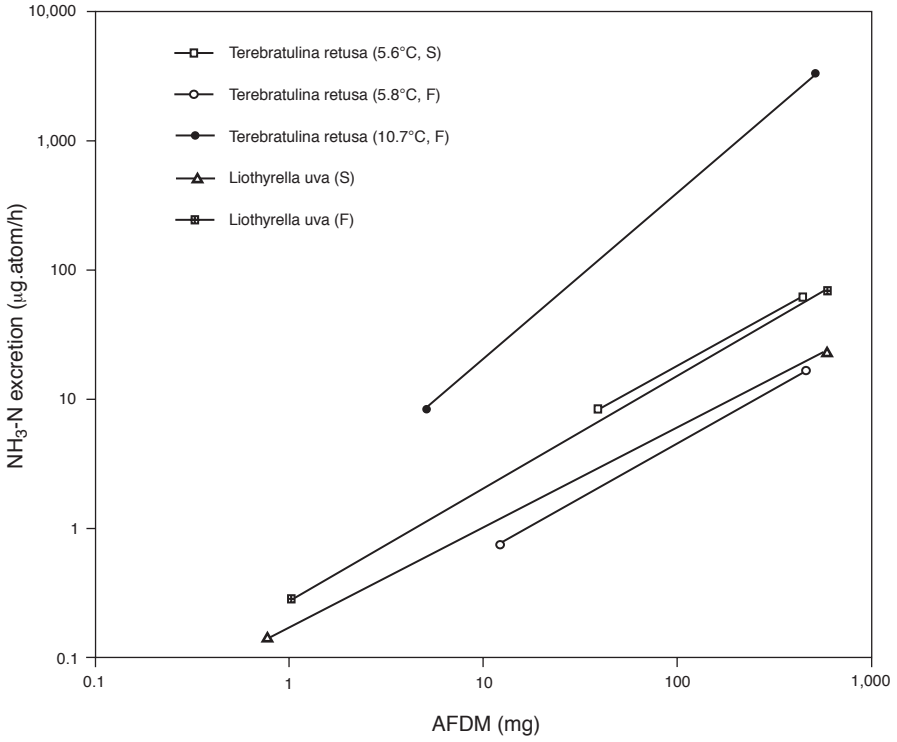

FIG. 202. Relationships between $\log_e$ rate of ammonia excretion (NH_3-N µg/h) with $\log_e$ animal ash-free dry mass (AFDM, mg) of articulated brachiopods. Parameters for each regression and the sources of the data are shown in Table 17; *F,* fed; *S,* starved (new).

Terebratulina retusa uses more lipids and carbohydrates than protein.

The exponents in the scaling relationships between rates of ammonia excretion and tissue weight (Table 17) are not significantly greater than the equivalent exponents for the relationships between rates of oxygen consumption and tissue weight (Table 15), indicating that O:N ratios and hence the proportions of different metabolic substrates used are not size dependent.

One should bear in mind when considering scaling studies of the types discussed above that many of the allometric relationships measured are the end products of evolutionary pressures on many underlying allometries. Metabolic scaling is a case in point, in that the metabolic rate of an organism is the sum of its various requirements for energy in the form of adenosine triphosphate (ATP) at a given time, including, for example, the energy needed for muscular activity, fluid circulation, membrane transport, and osmotic balance. All of these have individual allometric relationships with size, and a rigidly mechanistic approach to the interpretation of the scaling of such complex parameters as metabolism may not always be appropriate.

METABOLIC PATHWAYS

The earliest investigations of metabolic pathways of brachiopods began in the late 1950s and were done by a group of workers led by C. S. HAMMEN (for detailed references see JAMES & others, 1992). Their work, motivated by the very long fossil record of the inarticulated brachiopods, involved mainly linguloids and included studies of aerobic, anaerobic, and nitrogen metabolism, all factors thought to be of importance to species living in infaunal habitats. Other published

TABLE 18. Comparison of rates of ammonia (NH_3-N) excretion by inarticulated and articulated brachiopods (adapted from James & others, 1992).

Species	AFDM (mg)	Temp (°C)	Fed/ Starved	Rate µg.atom NH_3-N/day	Authority
Inarticulated					
Lingula reevii	3580.0[a]	22–25.5	Starved	8.31	HAMMEN (1968)
Articulated					
Terebratulina retusa	572.8[b]	5.6	Starved	5.59	PECK & others (1989)
	572.8[b]	5.8	Fed	1.35	PECK & others (1989)
	572.8[b]	10.7	Fed	12.48	PECK & others (1989)
	30.5[b]	5.6	Starved	0.48	PECK & others (1989)
	30.5[b]	5.8	Fed	0.11	PECK & others (1989)
	30.5[b]	10.7	Fed	0.29	PECK & others (1989)
Liothyrella uva	30.5	0	Starved	0.30	PECK & others (1986)
	30.5	0	Fed	0.35	PECK, CLARKE, & HOLMES (1987b)

[a]mass quoted for *L. reevii* is mean of six individuals; ash-free dry mass (*AFDM*) calculated based on assumptions of 80% tissue water content and 20% dry tissue ash content.

[b]two sets of values are given for *T. retusa* to allow comparison with both inarticulated brachiopod *L. reevi* and articulated brachiopod *L. uva*.

studies of metabolic pathways include those of SCHEID and AWAPARA (1972), ZWAAN and others (1982), LIVINGSTONE (1983), and a group of papers on intermediary metabolism in the mantle of brachiopods in relation to shell growth and free amino acid utilization (HUGHES, ROSENBERG, & TKACHUCK, 1988; ROSENBERG, HUGHES, & TKACHUCK, 1988; TKACHUCK, ROSENBERG, & HUGHES, 1989).

The initial investigations focused on oxidative metabolism. *Lingula anatina* (as *L. reevii*) was shown to be capable of fixing carbon dioxide (HAMMEN & OSBORNE, 1959). In comparisons of enzyme activities and oxygen consumption rates between *L. anatina* and bivalve molluscs (HAMMEN, HANLON, & LUM, 1962), none of the enzyme activities compared were lower in the brachiopod, some (catalase and arginine deaminase) were within the range of those of the bivalves, and others (urease, carbonic anhydrase, succinic dehydrogenase, and arginase) were higher. HAMMEN, HANLON, and LUM (1962) concluded from these comparisons that there are no enzyme deficiencies in *L. anatina* and that the measured low rates of oxygen consumption for whole animals were due to control mechanisms (but see p. 229).

Studies of enzymes important in aerobic and anaerobic metabolic pathways were continued by HAMMEN and LUM (1966) and HAMMEN (1969). They measured activities of the enzymes succinate dehydrogenase (SD) and fumarate reductase (FR) and the rates of pyruvate reduction (PR) and lactate oxidation (LD) for the inarticulated brachiopods *Lingula anatina* and *Glottidia pyramidata* and the articulated brachiopod *Terebratulina septentrionalis;* they then calculated the ratios FR:SD and PR:LD (Table 20; Fig. 203).

The ratio FR:SD is a measure of the strength of the reverse reaction rates, that is, reactions that should proceed in opposite directions during aerobiosis and anaerobiosis. It should be low in highly aerobic organisms and high in anaerobic species (SINGER, 1965; HOCHACHKA & SOMERO, 1973, 1984). Values of this ratio of less than 1 are low, while values greater than 4.5 are high in comparison with other marine invertebrates (HAMMEN, 1969). HAMMEN (1969) suggested that the values of 0.43 and 0.37 obtained for the inarticulated brachiopods indicate that they are rarely faced with conditions that require them to use anaerobic pathways but that the articulated brachiopod *Terebratulina septentrionalis* (collected from the intertidal environment) is highly adapted to anaerobic conditions, perhaps associated with the need to stay tightly closed during low tide to avoid dessication. These results are not totally consistent with expectations based on the different life-styles of these brachiopods, as such infaunal species

TABLE 19. Comparison of oxygen to nitrogen ratios (O:N ratio) for articulated brachiopods; *CI,* confidence interval; *n,* sample size (new).

Species	Temp (°C)	Fed/Starved	O:N ratio	95% CI	n	Authority
Liothyrella uva	0.0	Starved	9.3	7.78, 11.01	78	PECK & others (1986)
Liothyrella uva	0.0	Fed	9.2	7.95, 10.70	45	PECK, CLARKE, & HOLMES (1987b)
Terebratulina retusa	10.7	Fed	16.3	9.1, 29.2	27	PECK & others (1989)
Terebratulina retusa	5.6	Starved	8.0	6.7, 9.7	39	PECK & others (1989)
Terebratulina retusa	5.8	Fed	42.4	23.9, 75.2	12	PECK & others (1989)

as *Lingula anatina* live in habitats where they would be expected to experience anaerobic conditions at regular intervals.

HAMMEN (1969) suggested that the PR:LD ratio could be used as an indication of whether a species is likely to produce the end products of glycolysis in the form of lactate, with a high ratio indicating that the end product was mainly lactate. He postulated that sedentary species would have low ratios and that the ratio would increase in proportion with the scope of the species for muscular activity. The values of 0.68 to 1.76 found for the brachiopods are among the lowest of any marine invertebrates. The articulated brachiopod *Laqueus californianus* has similarly low rates of pyruvate reduction (SCHEID & AWAPARA, 1972), but lactate oxidation in this species was too low to be detectable, so it was not possible to calculate a PR:LD ratio.

Brachiopods possess alternative anaerobic pathways as indicated by the presence of lactate, octopine, alanopine, and taurine dehydrogenases (LDH, ODH, ADH, and TDH) in brachiopod species (ZWAAN & others, 1982; LIVINGSTONE, 1983; DOUMEN & ELLINGTON, 1987). A likely early primitive function of these opine pathways was to provide energy for burrowing (LIVINGSTONE, 1983); but other functions of the pathways have evolved, such as survival during or recovery from anoxia.

HAMMEN (1971, 1977) studied substrate specificity of lactate dehydrogenase in the inarticulated brachiopod *Glottidia pyramidata* and the articulated brachiopods *Notosaria nigricans, Calloria inconspicua,* and *Terebratulina septentrionalis.* With the exception of *T. septentrionalis,* all the brachiopods used only L-lactate; *T. septentrionalis* used L- and D-lactate at approximately equal rates. HAMMEN (1977) used the spread of the above data to suggest that brachiopods had a closer phylogenetic relationship to the deuterostomes (echinoderms and chordates) than to the other branch of the animal kingdom. This type of affinity study is now being done by analysis and sequencing of proteins and DNA, where the data are more robust and the conclusions drawn more specific.

In comparisons between the activities of enzymes involved in nitrogen metabolism in the inarticulated brachiopod *Lingula anatina*

TABLE 20. Enzyme activities and ratios of activities for inarticulated and articulated brachiopods; *FR,* fumarate reductase; *LD,* lactate dehydrogenase; *PR,* pyruviate reductase; *SD,* succinate dehydrogenase (adapted from Hammen, 1969).

	Enzyme activities (μmoles/min/g tissue wet mass)					
Species	PR	LD	PR/LD	FR	SD	FR/SD
Inarticulated						
Lingula reevii	0.311	0.240	1.30	0.087	0.203	0.43
Glottidia pyramidata	0.494	0.280	1.76	0.053	0.142	0.37
Articulated						
Terebratulina septentrionalis	0.098	0.144	0.68	0.067	0.010	6.80

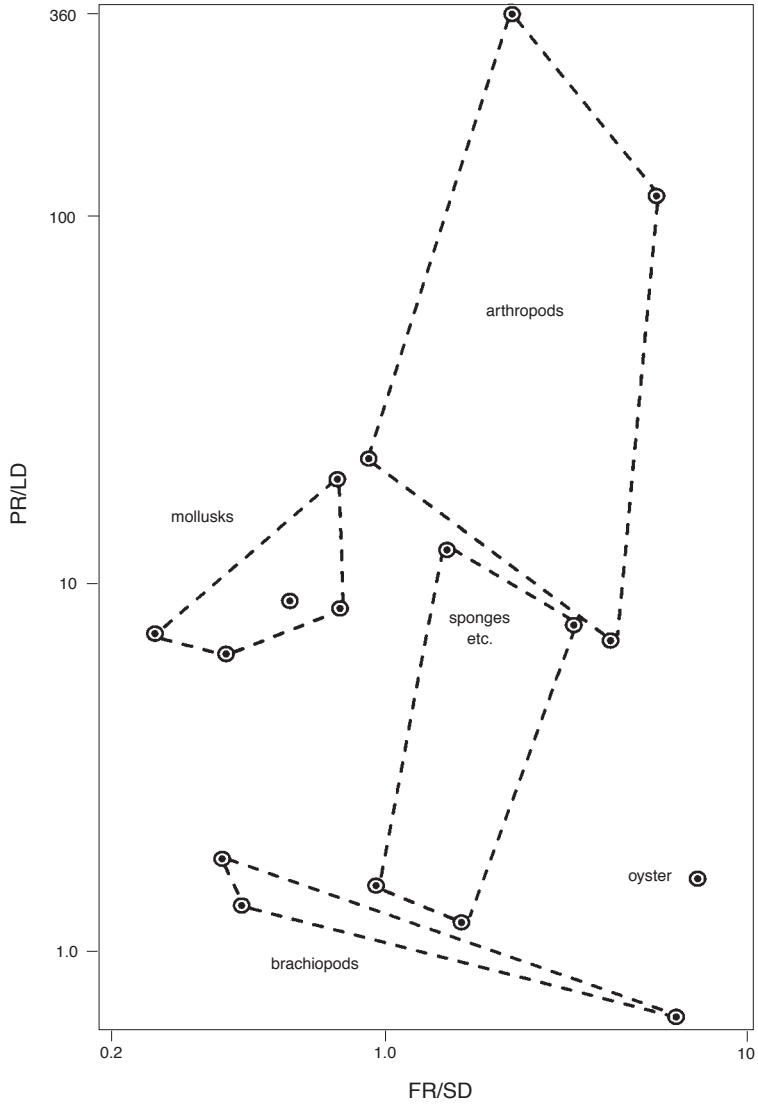

FIG. 203. Relationships between the ratios of rates of fumarate reductase (*FR*) to succinate dehydrogenase (*SD*) activity and the rates of pyruvate reduction (*PR*) to lactate oxidation (*LD*) for brachiopods compared to those of other groups of marine invertebrates (Hammen, 1971).

and those of several bivalve molluscs, aminotransferase activities were found to be lowest in the brachiopod (LUM & HAMMEN, 1964; HAMMEN, 1968).

None of these studies of enzyme activities provides data on the relationship between enzyme activities and dry tissue weight. Expressing results of such metabolic studies on a mass specific basis, for example, the amount of activity per unit mass of tissue does not standardize the measured rates among larger or smaller individuals because metabolic rate rarely scales to body mass with an exponent of 1 (KLEIBER, 1947, 1965; HEMMINGSEN, 1960; SCHMIDT-NIELSEN, 1984; PANDIAN & VERNBERG, 1987). The mean value of 0.78 for the exponent in the relationship between rate of oxygen

consumption and body mass for brachiopods (see p. 229–230) implies that a doubling of body mass will be accompanied by only a 1.70 times increase in metabolic rate. Hence, a doubling of body mass might be expected to be accompanied by a 15 percent fall in enzyme activities expressed on a mass-specific basis. These problems should not, however, affect the ratios of activities discussed above, as these should be more independent of body size.

Isolated portions of mantle tissue from *Terebratalia transversa* were found to be capable of metabolizing 11 of 19 amino acids investigated by measurement of the evolution of carbon dioxide from radioactively labelled amino acids (TKACHUCK, ROSENBERG, & HUGHES, 1989). The most metabolically active amino acid was aspartate, which accounted for 52 percent of the total of 38 μmol/g/h of carbon dioxide evolved. In comparison, in the bivalve *Chlamys hastata,* valine was the most metabolically active amino acid, accounting for 29 percent of the 138 μmol/g/h of carbon dioxide produced.

The metabolic rate of mantle tissue, as assessed by measurement of the rates of use of ^{14}C-labelled carbohydrates, is low in *Terebratalia transversa* (HUGHES, ROSENBERG, & TKACHUCK, 1988; ROSENBERG, HUGHES, & TKACHUCK, 1988). The metabolic rate is 3.7 times greater in the leading marginal edge than in the midportion of the mantle. This compares with a ratio of 1.8:1 in the mantle of the bivalve *Chlamys hastata.* Lengthy periods of anoxia result in a decrease in glucose metabolism in *T. transversa,* and, since the decrease is larger in the leading marginal edge than elsewhere, this results in a fall in the ratio of metabolic rates between sites to 1:1. Organic acids could not be detected in the tissues during these periods of exposure to anoxia (ROSENBERG, HUGHES, & TKACHUCK, 1988), probably because of the very low metabolic rates and not an absence of these end products of anaerobic metabolism. HUGHES, ROSENBERG, and TKACHUCK (1988) thought that periods of alternating aerobic and anaerobic metabolism in mantle tissues might result in cycles of deposition and resorption of calcium carbonate in the shell, with the carbonate being used to buffer the acids produced during anaerobiosis. The same hypothesis was proposed by LUTZ and RHOADS (1977) to explain growth bands in the shells of bivalve molluscs.

More recently, ROSENBERG and HUGHES (1991) have consolidated their earlier work and extended the theory of shell growth and composition being controlled by mantle metabolic rates. This theory contrasts with the generally accepted model that the orientation of the marginal mantle along the commissure is the determinant of shell growth and form (HUXLEY, 1932; WILLIAMS, 1966, 1968a). Part of the mantle-metabolism theory suggests that shell curvature is dictated by the size of the gradient in metabolic rate from the anterior leading edge of the mantle to areas away from the shell edge. Higher shell curvature is thought to be produced by larger gradients in mantle metabolic rate. ROSENBERG, HUGHES, and TKACHUCK (1988) claimed that the deposition of shell material away from the leading edge lends support to their hypothesis. They also concluded that calcium-rich areas of shell were energetically less costly to produce than matrix-rich areas or parts of the shell that are rich in minor elements, which supports the general hypothesis for costs of calcium-carbonate shell production formalized by PALMER (1981, 1983). ROSENBERG and HUGHES (1991) suggested that this was of great significance for paleobiological studies, which could potentially gain much from information on variations in skeletal composition within and between populations of brachiopods and through ontogeny.

ENERGY PARTITIONING

Brachiopods have long been characterized as having low levels of activity. SHIPLEY and MACBRIDE (1920, p. 374) described "the fixed Brachiopod, whose strength is to sit still and sweep little particles of food towards its mouth . . . ." This, with the observation that there was relatively little tissue between the shell valves of articulated brachiopods (HYMAN, 1959b; RUDWICK, 1970), com-

bined to produce the impression that brachiopods had low energy requirements compared with many other marine invertebrates.

Many of the more recent studies of aspects of brachiopod physiology have provided results that strengthen this concept. Ratios of enzyme activities indicative of low levels of muscular activity (HAMMEN, 1969), the long period needed for the diductor muscle to reach tetanus (WILKENS, 1978b), the ability of some articulated brachiopods to facilitate water movement through the mantle cavity by orienting to ambient seawater currents (LABARBERA, 1978), the observation that water flow through the mantle cavity is laminar and speeds of water movement low (LABARBERA, 1981), the low rates of oxygen consumption (SHUMWAY, 1982; PECK, MORRIS, & CLARKE, 1986a; PECK & others, 1986, 1989; THAYER, 1986b; PECK, CLARKE, & HOLMES, 1987b; CURRY & others, 1989) and heartbeat (BUCHAN, PECK, & TUBLITZ, 1988), and the relatively reduced feeding abilities (RHODES & THOMPSON, 1992) have all been proposed to be energy-saving adaptations or indicative that brachiopods have low energy requirements. More recently the concept of an overall, low-energy life-style for brachiopods has been developed (CURRY & others, 1989; PECK & others, 1989; THAYER & ALLMON, 1990). The available evidence for and implications of such a strategy were discussed in detail by JAMES and others (1992). Advantages include an enhanced ability to survive in areas where food supplies are low or highly seasonal, since low metabolic rates require smaller reserves for maintenance through periods of food limitation.

A full assessment of an organism's energy strategy requires quantitative data that can be used to compile a full budget of acquisition and subsequent partitioning of acquired energy between different activities. This is expressed in the energy budget equation:

$$C = F + P_g + P_r + R + U + M$$

where C is food consumed, F is feces produced, P_g is somatic production, P_r is reproductive production, R is respiratory costs, U is excretory losses, and M is mucus produced, all expressed in energy units (BRANCH, 1981; modified from WINBERG, 1956 and RICKER, 1971). The mucus term is often ignored in energetic studies but may be very important, for example, where mucus is used extensively in particle rejection mechanisms, as it is in brachiopods living in turbid areas (RHODES & THAYER, 1991).

Equivalent budgets may also be assessed in terms of biomass, organic carbon, or nitrogen. How closely such budgets balance is not merely an indication of how well the individual parameters have been measured; there can be short-term imbalances in the equation, where seasonal effects are important or individuals are unusually active. Full assessments must therefore take into account longer-term balances between periods of net gain and periods of net loss, in which storage tissues may be implicated.

There are no species of brachiopod for which all the necessary data to compile an energy budget have been collected, and for some of the necessary parameters, such as energy lost in feces and mucous production, there are no published data at all. Data on mucous production are likely to be of less importance to the production of a general energy budget for brachiopods, as mucus appears to be produced only under specific conditions of high turbidity. Measurement of fecal losses, on the other hand, are crucially necessary for the estimation of the amount of energy actually absorbed and hence available to fuel other physiological functions.

On the consumption side of the budget, information on filtration and clearance rates for *Neothyris lenticularis* (RHODES, 1990; JAMES & others, 1992; RHODES & THOMPSON, 1992) may be used to calculate food consumption rates with different concentrations of algal cells in the water. For example, *N. lenticularis* of 200 mg AFDM has a clearance rate of 690 cm^3/h, which converts to a food-consumption rate of around 1,000 algal cells/sec. When feeding on the alga *Dunaliella primolecta,* which has an organic content of 95 pg AFDM per cell, of which 19 percent is lipid (I. LAING, personal communication, 1993), this represents 3.6 mg

AFDM of algae/h, or 79 J/h (1.9 kJ/day) using appropriate energy conversion factors (Schmidt-Nielsen, 1979).

Problems remain, however, in interpreting such data in energetic assessments, as articulated brachiopods cease feeding when the blind-ending gut is full. Useful estimates of consumption therefore require assessments of the proportion of time spent in feeding. Similar needs for estimates of the time spent in other activities affect other components of the budget, but no data on which to base such estimates of activity time budgets are available for brachiopods.

Assessments of growth rates for *Terebratulina retusa* in natural populations (Collins, 1991) provide an estimate of somatic production (P_g). Between the ages of one and six years (approximately 2 mm to 17 mm in length), *T. retusa* grow at a rate of 2.5 mm per year. This converts to a growth rate of 48 mg AFDM per year for a 10 mm length brachiopod (65 mg total body AFDM) using data relating AFDM to shell length (Curry & Ansell, 1986). This is equivalent to 0.26 mg AFDM day, assuming a growing season of six months or 0.18 mg AFDM/day, with a nine-month growing season, which converts to 2.2 and 3.2 J/day using a conversion factor from AFDM to energy content of 12.2 kJ/g AFDM (Peck, 1993).

Similar calculations are possible for the energy requirements of reproductive growth (P_r). The difference in AFDM between empty and full gonads of large adult (45 mm in length) *Liothyrella uva* is some 50 mg (Peck & Holmes, 1989b). Build up of gonads occurs over a three-month period, indicating an increase of about 0.5 mg AFDM per day, or 70 J/day, using a conversion factor of 2.66 kJ/g AFDM for gonad tissues (L. S. Peck, unpublished data, 1992).

More extensive and better data are available on respiratory costs (R). Oxygen-consumption rates calculated for an individual of 50 mg AFDM from the data summarized in Table 15 range from 1.9 to 29.7 µl/h (Table 16). Using an appropriate oxycalorific coefficient (18.8 kJ/l oxygen) based on the assumption that protein is a major respiratory substrate (see p. 235) provides an estimate of respiratory costs ranging from 0.9 to 13.4 J/day.

Estimates of excretory losses (U) based on nitrogen excreted as ammonia (Table 17–18) range from 0.11 to 12.48 µmol/day, equivalent to 3.2×10^{-5} to 3.6×10^{-3} J/day using a conversion factor of 288 J/mol ammonia (Brafield & Solomon, 1972).

Clearly all these calculations have large errors associated with them, and to carry this exercise to the stage of comparing the relative effort or the levels of resource allocation to various components of the energy budget would not be appropriate. Before it will be possible to assess accurately the energy strategies of brachiopods and to identify which components of their energy budgets may be constrained to low levels, much more quantitative data suitable for incorporation into the calculation of energy budgets is needed.