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Yonge 1952

Alternatively, it may be found that *Calliopaea bellula*-like the other European stiligerids *Calliopaea oophaga* Lemche[3] and *Stiliger vesiculosus* Deshayes[4] — is an egg-eater, although eggs were not observed in the gut diverticula. It is apparent that *C. bellula* is not adversely affected by coverage with sediment, but observations on possible burrowing activity were unfortunately not attempted. In addition it was noted that in the live animal the cerata are invariably of an inflated and puckered appearance; this is lost on relaxation and fixation with the result that the cerata adopt an unnaturally long and slender character.

The maximum individual size for the present collection of *C. bellula* was 3.8 mm and this, and similar sized specimens, carried developing oocytes clearly visible through the ventral body wall. Although the individuals were maintained for more than a week no copulatory or spawning activity could be induced, suggesting that they were as yet sexually immature.

In summary the present observations suggest that *Calliopaea bellula* is not as rare as previous records suggest — simply that observers have not examined the correct biotope or habitat. Secondly, it is likely that the diet of this ascoglossan is atypical of the group, perhaps including diatoms or even other molluscs' eggs.

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Mantle margins with a revision of siphonal types in the Bivalvia

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The bivalve mantle is usually bordered by three folds, an outer secretory one bounded internally by the periostracal groove, a middle sensory one and an inner muscular fold (Fig. 1). The two last are associated respectively with cephalic reduction and hypertrophy of the ctenidia. The originally unattached mantle lobes so remain in modern Nuculacea, Arcoida and Pterioida. Later union, first of inner, then of middle, finally of the inner surface of outer, folds produced siphons of increasing complexity[1]. Such fusion was the major factor in the Mesozoic evolutionary radiation largely of deep burrowing bivalves[2]. This note concerns exceptions to the general pattern recently described in the Arcoida and, personally observed, in the Cardiacea. The latter, with certain other alterations, involves revision of the categories of siphonal types proposed in 1957.

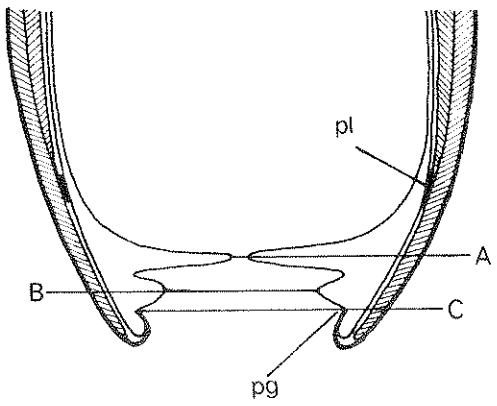

Fig. 1. Mantle margins in typical bivalve showing three folds. **A**, Union of inner folds; **B**, also of middle folds; **C**, addition of inner surface of outer folds. *pg*, periostracal groove; *pl*, pallial line. (Replacing Fig. 3 in Yonge (1957)).

Philobrya[3] with *Arca, Barbata* and *Glycymeris*[4] *possesses two* folds distal to the periostracal groove. There may be one or two inner folds, if the latter the outer of these controls water flow, otherwise this is done by the inner of the outer folds. Photoreceptors occur here (i.e. *under* periostracum). Although regarding these folds as "secondarily derived features", Waller[4] considers they provide evidence against the existence of a basic three fold pattern. But, in conjuncture with the multivincular ligament, they appear rather to emphasize the distinctive characters of the Arcoida, possibly justifying further separation from other Bivalvia.

Observations on Tridacnidae[5], Hemidonacidae[6] and various Cardiidae reveal that in all Cardiacea the middle fold is greatly reduced, sensory tentacles and eyes being carried on the *inner* folds which alone constitute the siphons, designated Type A+[5]. Greatly enlarged and upward directed, these house the endosymbiotic dinoflagellate zooxanthellae. In this family the middle fold is enlarged on the under (umbonal) side, protruding from the byssal gape to assist chemically the mechanical boring of the valves. In *Tridacna maxima* and *T. squamosa* this ensures intimate contact with the substratum of coral rock leading to complete penetration by the smaller *T. crocea*[5]. Similar help in boring by middle folds (*not* inner ones as earlier stated[7]) occurs in *Lithophaga*[8]. In *Fungiacava* these folds enclose the delicate valves and are the *sole* means of penetration through the skeleton of *Fungia* spp.[9]

It is now proposed that, as shown in Fig. 1, bivalve siphons be divided into the following types:-

A, Union of inner folds only, e.g. deposit-feeding Tellinacea with separate, highly mobile siphons.

A+, Special condition in Cardiacea where inner folds carry sense organs, the middle folds (except ventrally in the Tridacnidae) greatly reduced.

B, Union includes middle folds, siphons united with common outer ring of sensory tentacles, inhalant opening fringed with filtering tentacles, exhalant aperture with valvular membrane, e.g. Veneracea.

C, Also involving periostracal groove, united often very long siphons with periostracal sheath, i.e. deep burrowing Myacea, Mactracea, Saxicavacea.

Further fusion involving the outer surface of the outer fold (suggested Type **D**) does *not* occur, such union is confined to the upper (left) valve in the Anomiidae where the mantle margins extend "supradorsally', the valves enclosing the primary ligament[10]. In the Pholadidae the mantle cavity extends beyond the valves forming the base of the the siphons, to this extent covered with periostracum. Formerly classified as Type **B/C**, this condition is better contained **B**. Conditions in the Clavagellacea are obscure but the tube outside the very reduced periostracally covered valves is possibly secreted by enlarged inner folds which can form siphonal tubes in the Teredinidae[11].

As first shown in *Pinna*[12], fusion of the periostracal grooves (or of their secretions) has the highly significant effect of extending the primary ligament, (the separate identity of a postulated "fusion layer" has been denied in many later papers.) Secondarily extended ligaments are increasingly concerned with valve alignment, teeth being reduced (e.g. Saxicavacea[13]). When accompanied by ventral migration of the primary ligament, these extensions unite dorsally forming secondary, periostracal ligaments. The two are attached dorsally in the Lyonsiidae and allied Pandoracea[14] and in Mactracea and Myacea[15]. They are separated in *Placuna* (Anomiacea) and in *Plicatula* and the related Dimyidae[10,16]. Here teeth are lost, the long secondary ligament ensuring valve alignment and all regions of the primary ligament form a resilium. Fusion of the marginal folds has played a major role in bivalve evolution including this change from external to internal ligament with accompanying alterations in form and habit.

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Recent technical modifications of total organic carbon analyses for molluscan growth studies

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Many problems of molluscan ecology and physiology, including currently debated evolutionary questions pertaining to reproductive allocation, require actuarial assessments of bioenergetics. Theoretically, the best of such "balance-sheets" should be based on direct energy-flux data, but in fact, most practical computations involve productivity transfer and growth rates as changes in dry tissue weights or in total organic carbon[1,2]. In a 1968 paper in this journal, we described a colorimetric method of "wet-oxidation" for the analysis of total organic carbon and its use in growth studies of molluscs[3]. Although we claimed[3,4] to be able to provide *real* measures of growth spanning six orders of magnitude, this was based on techniques which could cover samples ranging only from about 90μg to 12.5 mg anticipated organic carbon content, and necessarily employed "batching" of smaller stages. Recent innovations in the techniques not only have extended the range of possible determinations to cover both larger and smaller individual molluscs, but also have utilized more modern (split-beam, digital) spectrophotometers. These technical modifications are summarized here.

All methods still employ the same oxidant, prepared by dissolving 4.84 g potassium dichromate (analytical grade) in 20 ml warmed distilled water

and diluting this to 1 liter with concentrated sulphuric acid (analytical grade), and the same carbon standards, prepared from solutions of anhydrous dextrose in distilled water. Determinations of larger carbon contents (12.5 mg C and above) are possible (utilizing oxidant volumes of 60 ml and more), but become progressively more cumbersome for larger whole molluscs. In our laboratory, Avolizi[5,6] successfully dealt with whole sphaeriids of 41.2 mg C content. More heroic modifications originally devised by Burky[7] for giant *Pomacea* (individual snails up to 12.4 g C content) involve taking for analysis 4 ml aliquots of a bree or homogenate prepared in a tissue grinder or Waring blender. In the course of growth studies on the hydrobiid, *Potamopyrgus jenkinsi*, Simpson[8] independently refined the method to accommodate individual juvenile snails of about 6.5 μg C content, using Eppendorf automatic pipettes with disposable inert plastic tips to dispense oxidant volumes down to 0.5 ml. For even smaller individual eggs, larvae and spat, batching of like-sized cohorts remains necessary in preparing samples for analysis. In studies[4,9] on the salt-marsh pulmonate, *Melampus bidentatus*, we were able to determine mean organic carbon contents of 109 ng for eggs and 33.4 ng for veligers (i.e. levels two orders of magnitude below Simpson's best for individual snails). However, incorporating the innovations of Simpson[8] and Burky[7], we now have techniques covering individual molluscs of from 6 μg to 12.5 g C content.

The original method measured decreased absorption against a blank of acid-dichromate, and although we reported[3] that reproducible measures had been made on several types of colorimeter (including Beckman, Bausch and Lomb, Coleman, and Zeiss instruments) the bulk of our analyses were done using absorptiometers (like the Beckman DU spectrophotometer) designed to measure *increased* extinction values. The somewhat tedious procedure of resetting a base for each unknown sample and then reading an extinction value for the blank (of greater optical density) was shown to be more accurate on these instruments. More recently, split-beam (digital readout) spectrophotometers (such as Beckman's Series 34 and 35) have become available and allow a more straightforward procedure.

On a Beckman Series 34, using 1 cm-path cells and a wave-length setting of 440 mμ, we now read unknown carbons against a known sugar chosen to be near the top of each range of oxidant volumes. For example, we dispense 10 ml units of acid-dichromate for anticipated levels of 125 μg to 1.8 mg C, a somewhat narrower range chosen to be well within the linear, or Beer's law, absorption relationship. At least 2 sugar standards at 1.8 mg C and 1 each at 300 μg, 600 μg, 900 μg and 1.2 mg are set up. After digestion (60 min at 105°C) and dilution (to 50 ml), two cuvettes of the 1.8 mg C standard are placed in the R (reference) and S1 (sample) positions, the baseline is adjusted to 0000 and the instrument placed in the concentration mode. Another cuvette filled with the blank (darkest yellow) is placed in the S2 position and, with this set against R, the concentration calibration is adjusted until the digital display reads 1800. Subsequently, unknowns and sugar standards are placed successively in the S1 position and read off, the blank at S2 being checked (as stable at 1800) every fifth reading. If $x_1, x_2 \ldots x_n$ are the unknown "concentrations" recorded, then $1800/x$, etc. are the corresponding figures for total organic carbon in μg. In cases where all the determinations are likely to fall well within the anticipated range (as would be the case with the individual molluscs in a single growth cohort), a series of say 40 unknowns can be run in about 30% of the time required for the original procedure. On the other hand, with truly unknown samples or ones near the upper limit for that volume of oxidant (more greenish after digestion), we prefer not to use this faster method (reading increased absorption against a sugar of known carbon content) and return to the slower procedure of setting the unknown as the baseline and reading a value for the greater absorption of the blank. In such cases, we can combine the best sensitivity of the instrument with good linearity.

No matter how the spectrophotometer is used, techniques for the determination of total organic carbon by wet-oxidation are much less time-consuming than those of energy content in joules by micro-bomb-calorimetry. Determinations can be made on individual molluscs from 6 μg to 12.4 g C content[7, 8], which roughly correspond to live wet-weights in shell-bearing molluscs of from 135 μg to 279 g. Several publications[2,7,8,9,10,11] attest to the value of data on total organic carbon in studies of the physiology and ecology of molluscan growth, and of actuarial bioenergetics in natural populations of molluscs.

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