RADULAR VARIATION IN TWO SPECIES
OF SPONGE-RASPING DORID NUDIBRANCHS

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ABSTRACT

The damp live weight of specimens of *Archidoris montereyensis* and *Anisodoris nobilis* was found to be positively correlated ($\alpha = 0.05$) to the number of teeth per row, the number of rows in the radula and the length of teeth. Covariance analyses of the regressions of the first two radular characteristics to weight failed to statistically separate the two species. These results argue against the utility of radular information as taxonomic characters in sponge-rasping dorids.

The increase in tooth size with increasing animal size was found to be statistically divergent for these two species and was interpreted as being consistent with the feeding biology of these two species.

INTRODUCTION

Radular characteristics of dorid nudibranchs have been used taxonomically and ecologically but little attention has been paid to radular variation within a species. Radular characteristics such as number, size, shape, and arrangement of teeth are generally recognized to be of taxonomic importance in prosobranch gastropods and have been shown to be ecologically significant in certain gastropod species (Fretter, 1951; Hurst, 1965; Kohn, 1959; Solem, 1974). One criterion of a good taxonomic character is conservatism, i.e. the character remains reasonably invariant within a species while varying between species (Simpson, 1962; Kohn & Riggs, 1975). For many groups of gastropod, the radular characteristics fulfill these requirements. However, for opisthobranchs the radular characteristics cited in the taxonomic literature for a given species can vary widely. While Nybakken & Eastman (1977) have noted a qualitative correlation between changes in radular characteristics and size for *Triopha maculata* MacFarland, to our knowledge the question of the nature and quantitative extent of intraspecific variation in radular characteristics of dorid nudibranchs has never been raised.

Radular characteristics have been used in support of ecological hypotheses dealing with resource utilization (Bloom, 1974, 1976; Nybakken & Eastman, 1977; Young, 1966). Conceivably, variations in radular characteristics within a species or a group of similar species could reflect the patterns of resource utilization and ontogenetic alterations of those patterns.

The most obvious morphological variation within an opisthobranch species is size. In the early literature, little or no useful information is given concerning the size of specimens (Hancock & Embleton, 1852; Odhner, 1934) while other authors list dimensions of preserved (Marcus, 1961; MacFarland, 1966) or dehydrated (Bergh, 1879) specimens. Recently, the dimensions of live specimens have been given (Edmunds, 1971; Kay & Young, 1969; Young, 1967) and occasionally, the dimensions and radular characteristics of the same specimens are listed (Thompson, 1972). Risso-Dominguez (1963) has noted the lack of uniformity and hence the imprecision of many of the linear measurements used for opisthobranchs, but the question of the inherent imprecision of utilizing linear measurements on soft-bodied animals capable of modifying their shape was not convincingly answered. An alternative estimator of size is damp live weight (Carefoot, 1967). While damp live weight is admittedly imprecise, it is nevertheless a more consistent indicator of size than is length.

We propose to examine the relations between various radular characteristics and damp live weight for specimens of two species of sponge-rasping dorid nudibranchs, *Archidoris montereyensis* (Cooper) and *Anisodoris nobilis* (MacFarland) and to discuss the significance of these relations to the taxonomy and ecology of these species.

Bergh's drawing of the central nervous system is reproduced in Fig 4B. He depicted clearly the nerve ring of four main ganglia. However, the abdominal ganglion is shown on the right side, whereas it lies on the left in stiligerids. Nor was this an engraver's error, for Bergh stated that the larger ganglion was on the right side. The buccal ganglia are shown posterior to the nerve ring, whereas they lie anterior to the ring in all sacoglossans. It may be that this was not entirely Bergh's fault. Dr. Graeffe most likely plunged the living animals directly into alcohol without previous treatment. Each animal would then contract and the buccal mass would be withdrawn until it rested on the front of the genital mass. The cerebral and pedal ganglia could not retreat so far, being anchored to the front part of the head and foot by most of the cerebral and pedal nerves. The buccal ganglia, however, would travel with the buccal mass, to which they are attached; hence their posterior position as shown in Fig. 4B. The connectives and visceral loop would also be pulled back and elongated, and it is not surprising that some of their connections appear doubtful. This posterior displacment of the buccal ganglia may be seen in other illustrations of the central nervous system by Bergh, for example in *Placida dendritica* (Alder & Hancock), and it is for this reason that attention has been drawn to it.

NOTES AND OBSERVATIONS

Bergh's papers of 1872 and 1885 were of great assistance to us. A comparison of *C. bellula* with that of *Stiliger mariae* shows that they are one and the same species. Bergh published so many details of *S. mariae* that correspond with those of *C. bellula* that there cannot be any doubt about this. Our paper supports the restoration of the genus *Calliopaea* proposed by Baba & Hamatani (1970). The teeth of *C. bellula* do not exceed a length of 40 μm, its penial style is about 400 μm long and the vagina even longer. These features are not seen in other species of the family Stiligeridae.

We do not know what *C. bellula* feeds on. Its buccal mass is small and it has the smallest teeth of all stiligerids. It has no notch on the anterior border of the foot by means of which most stiligerids hold a filament while piercing it. *C. bellula* may feed on young, thin-walled growths at the bases of algae and it might feed on the eggs of small opisthobranchs.

There remain other gaps to be filled — the degree of retractibility of its tentacles, its defensive reactions, its mode of copulation and egg mass. It has not yet been examined by serial sections. The species is quite hardy; our animal remained active during a fortnight in the laboratory, after which it survived a journey to London.

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METHODS AND MATERIALS

Specimens of *A. montereyensis* and *A. nobilis* were collected in the waters of the San Juan Archipelago, Washington, USA (0 - 40 meters depth). The animals were damp-dried, weighed (see Carefoot, 1967 for procedure) and relaxed in 7.0% $mgCl_2$ solution. The radulas were dissected free of the odontophores and were briefly treated with a dilute solution of $NaOCl$ to remove extraneous tissue. The radulas were dehydrated and embedded in standard balsam microscope mounts. Several teeth from the functional area of the radula (the anterior one-third, center of each half-row) were removed from the ribbon to permit study of individual teeth and were placed in the mounting medium.

Each radula was photographed (using a Wild M5 dissecting microscope with a 35mm camera) and the magnification was adjusted so that the radula filled most of the frame. The resulting negatives were enlarged to 8 × 10 inch (20.3 × 25.4 cm) prints and the number of teeth in a row located in the functional area of the radula as well as the number of rows in the radula were counted. Forty specimens of *Archidoris montereyensis* and sixteen of *Anisodoris nobilis* were examined for the number of teeth in a radular row (an average of four rows counted per radula) and for the number of rows in the radula (an average of two counts per radula).

The teeth which had been removed from the radular ribbon were drawn using a camera lucida attachment on a Wild M-20 compound microscope at 100 magnifications. The size of a tooth was determined by measuring the inner edge of the tooth from the base to the hook tip. Three teeth were drawn and measured from forty-four radulas of *Archidoris montereyensis* and twenty-two radulas of *Anisodoris nobilis*.

The data (number of teeth, number of rows and tooth length in microns as dependent variables and damp live weight in grams as the independent variable) were analyzed by a covariance and least squares linear regression program written by the senior author. A multilinear regression program (BMD02R) was also utilized (Dixon, 1974). All analyses were performed on an IBM 360/65.

RESULTS

The regression analyses are presented in Tables 1 and 2. The linear regression analyses revealed that there are statistically significant increasing relations between the independent variable (the logarithm of dorid damp live weight) and the dependent variables (number of teeth per row, number of rows in the radula and the length of teeth) for both species at the 5% level. A multilinear regression analysis between the logarithm of dorid damp live weight as the dependent variable and the number of teeth per row and the number of rows in the radula as the independent variables revealed that the statistical relationship accounted for over 69% and 81% of the total variance in the data for *A. montereyensis* and *A. nobilis* respectively.

By utilizing a logarithmic transformation, the curvilinear regression can be made quasi-linear (Sokal & Rohlf, 1969) and a comparison of regressions by analysis of covariance can be legitimately applied. Since the method of measuring tooth size is shape-dependent (a characteristic which varies between species), cross-species comparison of this character would be invalid and is not considered. There is no statistical difference ($P>0.25$) between the regressions of log of dorid damp weight to number of teeth per row for *A. montereyensis* and *A. nobilis* (Table 3). The regressions for these two species of log of dorid damp live weight to the number of rows in the radula are not coincident but are parallel ($P>0.05$). *A. montereyensis* tends to have approximately 10 more rows in its radula than a similarly-sized specimen of *A. nobilis* throughout the size range examined.

DISCUSSION

Two characteristics of the radula, the number of teeth per row and the number of rows in the radula, have been shown to be highly correlated to the size of the specimen in two species of sponge-rasping dorid nudibranchs (Table 1 and 2). While the relationships are asymptotic and curvilinear over the range of animal sizes normally encountered in nature, a larger dorid has predictably more teeth per row and more rows in the radula than does a smaller dorid.

Comparisons of the regressions of size to these two radular characteristics (via a covariance analysis) between *A. montereyensis* and *A. nobilis* failed to differentiate these two species on the basis of radular characteristics (Table 3). These two species are neither taxonomically nor ecologically similar. *A. montereyensis* is a member of the subfamily Archidoridinae and of the

Table 1. Linear regression analysis of dorid nudibranch weight in grams (= log (dorid damp live weight + 1.0)) as the independent variable to three radular characteristics as the dependent variables. All slopes were found to be significantly different from zero at the 5% level.

	Intercept B_0	Slope B_1	Degrees of Freedom	F Value	Coefficient of Determination (r^2)
Archidoris montereyensis					
number of teeth per row	74.97	31.37	143	204.8	0.588
number of rows in radula	22.68	9.15	74	92.7	0.557
length of teeth (μm)	226.39	76.84	130	78.7	0.377
Anisodoris nobilis					
number of teeth per row	80.24	27.93	79	85.2	0.518
number of rows in radula	12.51	7.40	24	17.9	0.428
length of teeth (μm)	258.51	294.78	64	91.4	0.588

Table 2. Multiple linear regression analyses of dorid weight in grams (= log (dorid damp live weight + 1.0)) as the dependent variable to two radular characteristics as the independent variables. All results were significant at the 5% level.

	Intercept B_0	Number of Teeth B_1	Number of Rows B_2	Degrees of Freedom	F Value	Coefficient of Determination (r^2)
Archidoris montereyensis	− 1.527	0.0128	0.0377	34	38.4	0.694
Anisodoris nobilis	− 1.609	0.0169	0.0430	13	28.2	0.814

Table 3. Covariance analysis: a comparison of three regression lines (dorid weight to three radular characteristics) between two species of dorid nudibranch. See Table 1 for regression statistics. (* = significant at the 5% level).

	ratio of slopes *Archidoris montereyensis* to *Anisodoris nobilis*	Degrees of Freedom	Coincident Lines F Value	Parallel Lines F Value
Number of teeth per row	1.123	224	0.62	not applicable
Number of rows in radula	1.236	100	112.82*	0.72
Length of teeth (μm)	0.261	194	356.89*	77.243*

caecate guild of sponge-rasping dorids while *A. nobilis* belongs to the Diaululinae and is an acaecate dorid (Bloom, 1976).

The validity of these radular characteristics as descriptive parameters can be questioned. Since there exists a wide range of variation directly related to the size of the specimen, citing radular characteristics without size information is likely to be misleading. Only after numerous specimens of a wide size-range have been examined and the appropriate regression statistics calculated would the radula formula and the number of rows begin to be of any taxonomic use in the opisthobranchs examined here.

Additionally, regressions of those radular characters to size are statistically indistinguishable or are parallel across species. This implies that even if the appropriate regression statistics are known, there is little taxonomic discriminatory power involved in radular comparisons. Taken together, these data argue strongly against the taxonomic utility of radular characteristics in this group of molluscs.

Comparisons of the radular characteristics of these two species can serve to support evolutionary arguments of optimal foraging based on other lines of evidence (Bloom, 1974). The slopes of the regressions of the number of teeth and the number of rows to dorid damp weight are positive for both species and are coincident or parallel respectively while the regression lines of tooth size to damp weight diverge significantly (Table 3). Statistical comparisons of absolute tooth size (as measured here) between species are invalid in that tooth shape is a species-specific character which influences the data. However, comparison of the regressions (the rate of increase of tooth size with increasing animal size) is legitimate when examined by a covariance analysis. The tooth size of *A. nobilis* per gram increases at approximately 4 times the rate of increase for *A. montereyensis*.

The feeding ecology of these two species has been examined (Bloom, 1974; 1976). *A. montereyensis* is a caecate dorid and has been shown to feed preferentially on sponges with little or no skeletal organization. *A. nobilis* belongs to the other guild of sponge-rasping dorid nudibranchs, the acaecate dorids. These animals preferentially feed on sponges with highly organized and robust skeletons.

While an individual dorid is growing and its radula is enlarging, the sponge skeletal structure of the prey species remains constant. For an easily fragmented sponge, a simple increase in number of rows and number of teeth per row would maintain an efficient cropping organ. This growth strategy would be equivalent to boosting the size of the 'broom' while maintaining a constant 'bristle' size. Efficiency would be improved by increasing the surface area of the functional part of the radula while maintaining tooth size at the smallest effective size. The radular characteristics of the caecate dorid *A. montereyensis,* which prefers and exhibits its greatest growth on easily fragmented sponge prey, are consistent with these predictions.

For utilizing a sponge with a skeleton of interconnected spongin-spicule tracts, a large tooth firmly anchored to the radular ribbon would be advantageous in maximizing the amount of prey ingested per unit time while minimizing damage to the radula. Such sponge prey has been shown to be the preferred prey of the acaecate dorid *A. nobilis* (Bloom, 1976) and has also been shown to support higher growth and reproductive rates in adult specimens than a sponge with a poorer skeletal structure (Bloom, 1974). A newly metamorphosed dorid would presumably have a radular musculature unequal to the task of fragmenting a sponge with a highly organized skeleton. By initially feeding on a prey which is more amenable to fragmentation, the young dorid would require a radula adapted to such prey but could achieve a size at which it could successfully exploit the optimal adult prey type. A rapid increase in tooth size would increase the speed (ontogenetically) of the shift from juvenile to adult food types and would result in a larger and more fecund animal.

Qualitative evidence suggests that at least one acaecate dorid *Diaulula sandiegensis* (Cooper) settles out of the plankton on and initially consumes a sponge with a less organized skeleton. After attaining a moderate size, individuals switch their consumption to a sponge with a more highly organized skeleton (Bloom, 1974).

While the necessary data to test the hypothesis advanced above are lacking at this time, the characters of large teeth and a rapid increase in tooth size to animal size are consistent with the optimal growth and foraging strategies suggested for acaecate dorids.

SUMMARY

A statistically significant ($\alpha = 0.05$) curvilinear relationship exists between dorid damp live weight and three radular characteristics, the number of teeth per row, the number of rows in the radula and the length of teeth. Analysis of covariance of the regressions of dorid weight to the number of teeth per row for *A. montereyensis* and *A. nobilis* indicated that the regressions were coincident while the regressions of dorid weight to the number of rows in the radula for these two species were non-coincident but parallel ($P < 0.05$). These data argue that radular characteristics are of little taxonomic use in this group of molluscs and that regression statistics of radular characters are of limited utility in delineating species.

There is a statistically valid difference in the regressions of increasing tooth size to increasing animal size in these two species. This difference is consistent with the feeding biology of these species and can be interpreted as a progressive 'fine-tuning' of their resource utilization abilities.

ACKNOWLEDGMENTS

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