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Form and function of radular teeth of herbivorous molluscs: Focus on the future

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Abstract: The radular apparatus of herbivorous molluscs provides an excellent model for study of form, function, and integration of morphology and function. To date, however, few studies have been conducted in a way that allows us to understand the ecological and evolutionary consequences of different morphologies, and how morphologies differ or are similar in both form and function. I suggest thee areas of focus for future research: (1) Explicit quantification of morphology. (2) Real rather than asserted assessments of function. (3) An integration of structure and function. Taking advantage of new technologies and integrating them with more traditional approaches, experimentation, and quantification of both form and function will prove this to be a rich area for research in the future.

Key words: radula, functional morphology, feeding, herbivore

Although the radula is one of the features characterizing herbivorous molluscs, we know surprisingly little about the ecology and evolution of form and function of this important structure. Hickman (1980) published a very elegant paper directed at paleontologists in which she addressed the evolutionary consequences and considerations of radular tooth form in gastropods and the difficulty of producing a general model of the factors controlling morphology. She categorized these factors as phylogenetic, programmatic, constructional, ecological, maturational, and degenerative (see Hickman 1980: fig. 1). She recognized that not all morphological features of radulae and radular teeth are functionally adaptive or optimized for a specific function and that not all morphology should be viewed in an evolutionary context. Work by Hickman and colleagues (Hickman 1980, 1983, 1984, Morris and Hickman 1981, Hickman and Morris 1985) stressed the importance of understanding how the radular apparatus works, the necessity of examining the radula in its feeding position, and understanding the dynamics of the radula rather than just its static morphology.

Following on work by Littler and Littler (1981), Steneck and Watling (1982) tried to address ecological aspects of the feeding capabilities of herbivorous molluscs. They focused on the feeding capabilities and limitations of herbivores and classified molluscs and seaweeds into “functional groups.” For seaweeds, these groups were based on gross morphology and thallus form. In all cases, function (rates of photosynthesis, successional position in a community, grazer resistance) was assumed to correspond in a one-to-one fashion with gross form (see Padilla and Allen 2000, for a recent review of this hypothesis). For molluscs, functional groups were roughly based on taxonomy and overall radular type. Function was inferred from a few studies of diet or best guesses by the authors. For example, they considered all chitons to be functionally the same whether they had radulae with pointed or broad spade-like cusps on their major teeth, if marginal teeth were reduced or long, or if marginal teeth were few or abundant. Similarly, all patellogastropod limpets were assumed to be functionally equivalent, even species with very diverse numbers, shapes, and sizes of teeth.

The hypothesis of Steneck and Watling (1982) was too general and simplistic to adequately address the complexity of functional morphology of the radulae of grazing molluscs. Padilla (1985, 1989) experimentally tested this model for patellogastropods by developing biomechanical techniques to measure the forces required for limpets with different radulae to remove tissue from different algae. The results were opposite to the predictions by Steneck and Watling (1982), and performance differed significantly for among patellogastropod species (Padilla 1985, 1989). In addition, she found that cusp shape and number affected the mechanical and functional properties of radular teeth when limpets were feeding on macroalgae.

Given the importance of the radula and its function for grazing molluscs, it is surprising how little progress has been made on the functional morphology of this important and

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diverse structure in the past 20 years. This paper is not intended to be a review of all work that has been done on functional morphology of radulae, but rather to suggest factors that need to be considered and approaches that will facilitate progress in our understanding of the ecology and evolution of form and function of the radulae of grazing molluscs.

There are three areas of research with the greatest potential in the next 20 years: (1) Explicit quantification of morphology. This is not an easy task because the shapes of all complex morphological structures are difficult to quantify in ways that will be meaningful for comparisons among shapes. (2) Real rather than asserted assessments of function. We need to develop methods for testing and demonstrating function, rather than assuming or asserting function on the basis of best guesses. Intuition is limited by past experience and can lead to very wrong conclusions. (3) An integration of structure and function. This includes all of the aspects of structures, their dynamics, and use by organisms.

QUANTITATIVE ASSESSMENT OF MORPHOLOGY

Quantification of Shape

The morphology of complex structures is extremely hard to quantify and has been the focus of the field of morphometrics. Morphometric techniques, especially geometric morphometrics, can be used to quantify morphology in a way that allows morphologies to be described and compared in unambiguous ways. Morphometric methodology has undergone a revolution in the last decade (Rohlf and Marcus 1993, Adams *et al.* in press). Morphometric techniques are used for statistical analyses of variation in shape and are needed whenever shapes are to be compared. They can be used to test whether shapes are similar or different, and in some cases, how they differ. Initially, multivariate statistics were used on a variety of quantitative measures. Geometric morphometrics captures the geometry of structures and preserves this information throughout the analysis. Thus, geometric morphometrics allows statistical analysis of structures with complex shapes and forms, including structures lacking landmarks, which are necessary for more traditional analyses (Adams *et al.* in press).

Collecting Morphological Data

Traditionally, studies have focused on the morphology of hard structures associated with the radula, especially radular teeth. Hard structures usually do not require chemical fixation, and therefore maintain their shape when removed from the animal. Even most descriptions of the shape and morphology of hard structures. however, are qualitative rather than quantitative. In addition to the focus on hard structures, soft structures should be studied, especially the morphology of structures auxiliary to the radula that are essential for function. Soft body morphology, including the muscles, muscle attachments, the chitinous ribbon, and other structures such as the odontophore must be integrated into our understanding of the morphology of this whole structure. In addition, an understanding of their development will aid in our interpretations of evolutionary changes in form and function (Guralnick and Lindberg 1999).

Tools such as histology can be very helpful, but, can be extremely difficult. Fixation for histology can create artifacts (Voltzow 1990), as can examining dead rather than living animal, and ultimately confuse the interpretation of critical structures.

Microscopy has been a useful tool for studies of morphology. Scanning electron microscopy (SEM) can be a valuable tool for visualizing morphology. However, as with histology, distortions of features of soft tissue can result from fixation and drying of tissues for SEM. The three dimensional form of structures is difficult to assess or quantify from the two dimensional images of SEM. Confocal microscopy is a relatively new tool that is proving to be very useful for visualizing and quantifying some types of structures. Radular teeth that are transparent can be visualized and optical digital sections can be used to reconstruct three-dimensional structures, allowing morphometric analysis of shapes (Padilla, unpublished data).

Mechanical considerations

Biomechanical properties of radular teeth and accessory structures can be extremely important in understanding the relationships between form and function of the radular apparatus. Mechanical properties set the bounds on the potential functional properties of the radula and its components. Morphometrics is a wonderful tool for describing shape independent of size. However, when considering real function and morphology, size matters. The combination of size and shape are important determiners of potential functional performance of radular teeth, especially for their use as tools for feeding.

Mechanical properties of teeth are influenced most by tooth size, the shape of the tooth where it comes in contact with food items, overall shape of the tooth, the materials that teeth are made of, the material properties of the food items, and the interactions among teeth during feeding. The tooth cusp is usually the part of the tooth that comes in contact with food items and therefore is critically important for determining function. The shape of the cusp and the area of tooth in contact with the substrate affects the amount of force per unit area transferred during feeding. For example, when a limpet is applying its radula to the substrate, a force will be applied at the cusp of each tooth. If the teeth are very pointed, then the total surface area of the tooth in contact with the food item will be less for the total amount of force applied, concentrating the force applied at the tip of each tooth. Thus, pointed teeth concentrate stress and are more effective at piercing and tearing fleshy algae than are blunt teeth (Padilla 1985, 1989). Blunt teeth are broad and have more surface in contact with the substrate when feeding and thus are more effective for rasping and removing loose material from surfaces or broad excavations of brittle materials such as calcified algae. Reductions in the number of teeth per row can also increase the stress experienced at the tip of each of the remaining teeth.

The absolute and relative sizes of the whole tooth are also important, especially the relative size of the length of the tooth to its width. Although long teeth have been proposed to be very effective at excavating algal tissue (Reid 1996), longer teeth should be less stiff and would bend more easily than shorter teeth with the same width. Thinner teeth, for a given length, should also bend more easily. According to beam theory, the ratio of the length to the width dictates how stiff or flexible a tooth (or beam) will be (Wainwright *et al.* 1976, Vincent 1982). Long thin teeth should be flexible and bend when applied to a surface, so are more likely to work in a brush-like fashion. Short, stout teeth should be very stiff and transfer more force to a substrate.

The angle at which a tooth contacts the substrate can also be important. As with machine tools, both cutting angle and clearance angle are important (Padilla 1985). The cutting angle is most important for excavation efficiency. The clearance angle can play an important role in affecting tooth wear with feeding and the potential for changes in tooth shape with use (Hickman 1980). Runham *et al.* (1969) and Vincent (1980) have argued that the structure of patellogastropod teeth enhances their function by increasing wear behind the cusp, which maintains a sharpened edge on the cusp. As a snail feeds and the tooth cusps wear, they work much as a self-sharpening knife, always maintaining their effectiveness. The molluscan radula has even been suggested as a model for improvement of industrial cutting devices (van der Wal *et al.* 2000).

The material properties of teeth are also clearly important. Much work has been done on the mineral properties of patellogastropod radulae and to a certain extent on the radulae of chitons (*e.g.*, Lowenstam 1981). However, surprisingly little work has been done on the material properties or microstructural properties of the radulae of other grazing molluscs.

Morphological variation

The structure of the radula is generally assumed to be constant within a species. However, different radular morphologies have been found within species during ontogeny (Nybakken 1990, Warén 1990, Kawamura *et al.* 2001), among different habitats (Reid and Mak 1999), or when individuals consume different foods (Padilla 1998, 2001). Morphological variability is not expected within all species. For many taxa and for certain aspects of the radula (*e.g.*, numbers of teeth per row of the radula) will be constrained or affected by phylogeny, construction, or use.

Variability within a species may be due to genetic polymorphisms (different genotypes produce alternate morphologies) or phenotypic plasticity (a single genotype can produce different morphologies). Snails in the genus *Lacuna* Turton, 1827 have phenotypically plastic tooth morphologies and individual snails produce different-shaped teeth when exposed to different food environments (Padilla 1998, 2001). Although variability has been found in some other species, critical experiments to demonstrate phenotypic plasticity (the ability of individuals to produce alternative phenotypes) have not been performed. If morphologies are phenotypically plastic, it is important to know whether specific morphologies are triggered by certain conditions or whether the shapes of teeth are merely labile within individuals and are not associated with feeding environment or food (Padilla *et al.* 2000, Padilla, pers. obs.).

Interactions among teeth

The dynamics of how teeth move, interact with substrates, and interact with each other are also important for understanding function (Hickman 1984). Studying the dynamics of feeding permits an assessment of how the radula is used as a tool and what aspects of the morphology are important for function. The functionally important aspects of the morphology of the teeth are not always obvious from observations of their static form. Observations of the radula in motion provide a different perspective on which aspects of morphology are important for feeding. For example, frames from videotapes taken of the inside of the mouth *Katharina tunicata* (Wood, 1815) show that the teeth work by cutting against one another as well as interacting with the benthic substrate (Fig. 1). Their effectiveness depends upon a scissor-like interaction between teeth, not just the action of individual teeth. At present, it is not known how common this mode of feeding is and how important tooth-tooth interactions are in chitons in general let alone in other molluscs. Presently it does not appear that all chitons show similar feeding patterns (Padilla, personal observation). Similar videotapes of the patellogastropod, *Lottia scutum* (= *Tectura scutum* Rathke, 1833) "feeding" on a glass surface shows no interactions among teeth (Fig. 2). The radular teeth move together across the substrate, similar to a wood rasp (Padilla 1985).

ASSESSMENT OF FUNCTION

Many scientists have inferred function from morphology. This is risky because nature can be more clever than scientists; intuition can be misleading. To understand the association between morphology and function, we need to observe and quantify function, and then test the role of morphology in affecting function (*e.g.*, Morris and Hickman 1981).

The function of radular teeth has been examined indirectly through impressions that teeth make on substrates when an animal is feeding (*e.g.*, Hickman and Morris 1985). Direct observations of radular movements are still essential, however, to determine how different impressions are made. Initial efforts to visualize feeding required movie film and high light intensity (Morris and Hickman 1981), and proved to be difficult and costly to obtain. Thus, visualizing radular motion has been a technological challenge. Animals are small, usually covered by hard shells, and many of the structures of interest are difficult or impossible to visualize with x-rays or other techniques that are used with larger animals. Computers and low cost digital video systems, including high speed video, permit the direct observation in real time of the movement of the radulae of grazers. For example, videotapes of the radular motion of patellogastropods (Fig. 2) permitted mimicking their mode of use and the measurement of the forces required to remove tissue from different types of algae (Padilla 1985, 1989). Similar approaches with chitons (Fig. 1) indicate the potential scope of functional possibilities.

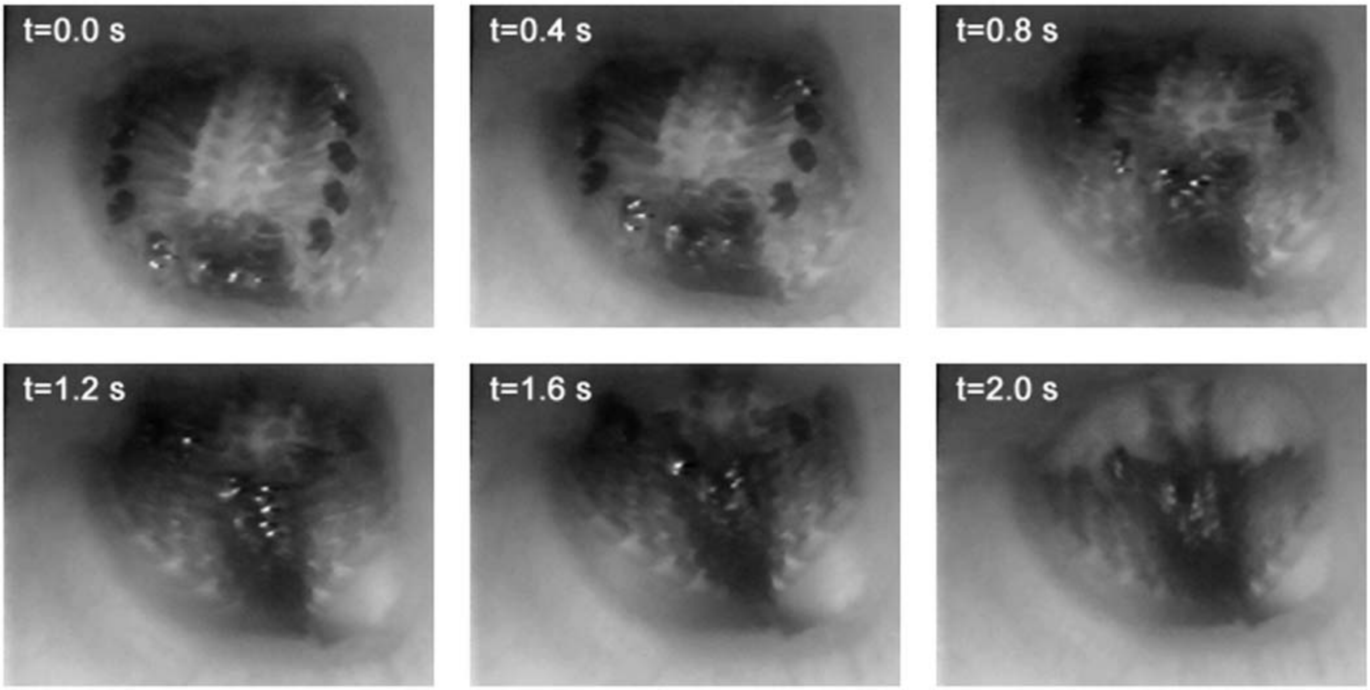

Figure 1. Radular movement of the chiton *Katharina tunicata*. This sequence of the movement of the radula of *K. tunicata* was analyzed by focusing a video camera through the ocular of a dissecting microscope. The chiton was feeding on the side of a glass aquarium. Fiber optic lights were focused on the mouth of the chiton for illumination. The color analog VHS tape was digitally recorded with a video capture system from Pinnacle (DV500). The digital video (avi) file was exported as a image (tif) sequence with Adobe Premiere where it was converted to black and white and the contrast was enhanced. Shown are still black and white frames for every 0.4 seconds during the feeding stroke of the radula.

INTEGRATION OF STRUCTURE AND FUNCTION

Integration of structure and function from all levels of organization is essential (Domenici and Blake 2000). Work on feeding in fishes provides an excellent example of the power of integrating hard and soft morphology, mechanical and material properties and dynamics, and neuromuscular control of feeding (reviewed in Wainwright *et al.* 2000). Most fishes are much larger than herbivorous molluscs and their combination of a soft tissue exterior with a calcified internal skeleton greatly facilitates visualization. Similar types of integration will be more difficult with small, soft-bodied molluscs. Molluscs have been used as models to study the neural control of muscular systems, and much work has already been done on the neuromuscular control of radular movements in gastropods (Elliott and Susswein 2002), especially in terrestrial and sea

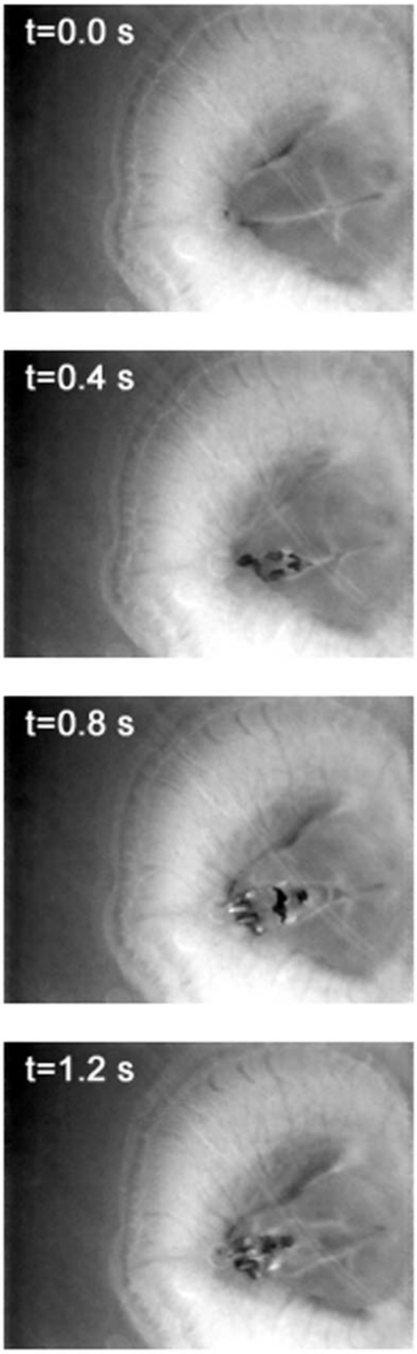

Figure 2. Radular movement of the docoglossan limpet, *Lottia scutum*. This sequence of the movement of the radula of *L. scutum* was analyzed by focusing a video camera through the ocular of a dissecting microscope. The limpet was feeding on a glass slide inverted in a dish of sea water. Fiber optic lights were focused on the mouth of the limpet for illumination. The color analog VHS tape was digitally recorded with a video capture system from Pinnacle (DV500). The digital video (avi) file was exported as a image (tif) sequence with Adobe Premiere where it was converted to black and white and the contrast was enhanced. Shown are still black and white frames for every 0.4 seconds during the feeding stroke of the radula.

slugs. However, unlike fishes, there have been no attempts to integrate physiological mechanisms with radular function and morphology.

The radular apparatus of herbivours molluscs provides an excellent model for study of form, function, and integration of morphology and function. Integration of new technologies with more traditional approaches, coupled with experimentation and quantification of both form and function will prove to be a rich area for research in the future.

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LITERATURE CITED

Adams, D. C., F. J. Rohlf, and D. E. Slice. In press. Geometric morphometrics: Ten years of progress following the 'revolution'. *Italian Journal of Zoology*.

Domenici, P. and R. W. Blake. 2000. *Biomechanics in Animal Behaviour.* BIOS Scientific Publishers, Oxford.

Elliott, C. J. H. and A. J. Susswein. 2002. Comparative neuroethology of feeding control in molluscs. *Journal of Experimental Biology* **205**: 877-896.

Guralnick, R. P. and D. R. Lindberg. 1999. Integrating developmental evolutionary patterns and mechanisms: A case study using the gastropod radula. *Evolution* **53**: 447-459.

Hickman, C. S. 1980. Gastropod radulae and the assessment of form in evolutionary paleontology. *Paleobiology* **6**: 276-294.

Hickman, C. S. 1983. Ecological and phylogenetic implications of the unusual radula of *Laevinesta atlantica* (Mollusca, Gastropoda). *Veliger* **25**: 323-325.

Hickman, C. S. 1984. Implications of radular tooth-row functional-integration for archaeogastropod systematics. *Malacologia* **25**: 143-160.

Hickman, C. S and T. E. Morris. 1985. Gastropod feeding tracks as a source of data in analysis of the functional-morphology of radulae. *Veliger* **27**: 357-365.

Kawamura T., H. Takami, R. D. Roberts, and Y. Yamashita. 2001. Radula development in abalone *Haliotis discus hannai* from larva to adult in relation to feeding transitions. *Fisheries Science* **67**: 596-605.

Littler, M. M. and D. S. Littler. 1980. The evolution of thallus form and survival strategies in benthic marine macroalgae: Field and laboratory tests of a functional form model. *American Naturalist* **116**: 25-44.

Lowenstam, H. A. 1981. Minerals formed by organisms. *Science* **211**: 1126-1131.

Morris, T. E. and C. S. Hickman. 1981. A method for artificially protruding gastropod radulae and a new model of radula function. *Veliger* **24**: 85-89.

Nybakken, J. 1990. Ontogenic change in the *Conus* radula, its form, distribution among the radula types, and significance in systematics and ecology. *Malacologia* **32**: 35-54.

Padilla. D. K. 1985. Structural resistance of algae to herbivores. A biomechanical approach. *Marine Biology* **90**: 103-109.

Padilla. D. K. 1989. Structural defenses of algae: The importance of form and calcification in resistance to tropical limpets. *Ecology* **70**: 835-842.

Padilla, D. K. 1998. Inducible phenotypic plasticity of the radula in *Lacuna* (Gastropoda: Littorinidae). *Veliger* **41**: 201-204.

Padilla, D. K. 2001. Food and environmental cues trigger an inducible offense. *Evolutionary Ecology Research* **3**: 15-25.

Padilla. D. K. and B. J. Allen. 2000. Paradigm lost: Reconsidering functional form and group hypotheses in marine ecology. *Journal of Experimental Marine Biology and Ecology* **250**: 207-221.

Padilla. D. K., E. K. Dilger, and D. E. Dittmann. 2000. Phenotypic plasticity of feeding structures in species of *Littorina*. *American Zoologist* **40**: 1161.

Reid, D. G. and Y. M. Mak. 1999. Indirect evidence for ecophenotypic plasticity in radular dentition of *Littoraria* species (Gastropoda : Littorinidae). *Journal of Molluscan Studies* **65**: 355-370.

Reid, D. G. 1996. *Systematics and Evolution of* Littorina. Ray Society, London.

Rohlf, F. J. and L. F. Marcus. 1993. A revolution in morphometrics. *Trends in Ecology and Evolution* **8**: 129-132.

Runham, N. W., P. R. Thornton, D. A. Shaw, and R. C. Wayte. 1969. The mineralization and harness of radular teeth of the limpet *Patella vulgata* L. *Zeitschrift für Zellforschung und Mikroskopische Anatomie* **99**: 608-626.

Steneck, R. S. and W. L. Watling. 1982. Feeding capabilities and limitation of herbivorous molluscs: A functional group approach. *Marine Biology* **68**: 299-319.

van der Wal, P., H. J. Giesen, and J. J. Videler. 2000. Radular teeth as models for the improvement of industrial cutting devices. *Materials Science and Engineering C-Biomimetic and Supramolecular Systems* **7**: 129-142.

Vincent, J. F. V. 1980. The hardness of the tooth of *Patella vulgata* L. radula: A reappraisal. *Journal of Molluscan Studies* **46**: 129-133.

Vincent, J. F. V. 1982. *Structural Biomaterials.* Halsted Press, New York.

Voltzow, J. 1990. The functional morphology of the pedal musculature of the marine gastropods *Busycon contrarium* and *Haliotis kamtschatkana*. *Veliger* **33**: 1-19.

Wainwright, P. C., M. W. Westneat, and D. R. Bellwood. 2000. Linking feeding behaviour and jaw mechanics in fishes. In: P. Domenici and R. W. Blake, eds., *Biomechanics in Animal Behaviour.* BIOS Scientific Publishers, Oxford. Pp 223-236.

Wainwright, S. A., W. D. Biggs, J. D. Currey, and J. M. Gosline. 1976. *Mechanical Design in Organisms.* Halsted Press, New York.

Warén, A. 1990. Ontogenic changes in the trochoidean (Archaeogastropoda) radula, with some phylogenetic interpretations. *Zoologica Scripta* **19**: 179-187.

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