THE MOLLUSK COLLECTION AND MALACOLOGY AT THE UNIVERSITY OF MICHIGAN

J.B. Burch

SALINITY TOLERANCE OF *RANGIA CUNEATA* (PELECYPoda: MACTRIDAE) IN RELATION TO ITS ESTUARINE ENVIRONMENT: A REVIEW

Rosalind B. Cooper
ABSTRACT. — The mollusk collection of the Museum of Zoology, The University of Michigan, by its size and past and present significance is a major national and international resource. Its importance as a reference collection in freshwater biology, invertebrate zoology, medical and veterinary zoology, and paleontology lies in the fact that it is one of the largest and most complete collections of freshwater mollusks in the world. Although it has only a small (but respectable) marine collection, its land snail collection is large and important, and has, in fact, been the basis of numerous doctoral theses. In regard to land snails, it is one of the top three or four collections in the United States, and this alone marks it as a major national resource. But, it is for its freshwater mollusks that the collection is especially noteworthy.

The mollusk collections contain over 250,000 catalogued lots of dry specimens (approximately five million specimens), at least 5,000 uncatalogued lots of wet specimens (alcohol- and formalin-preserved), about 2,000 live specimens, and sizable collections of radulae, molluscan antiserum and antigens, snail eggs, etc. All of this material is readily available for use by any qualified worker anywhere in the world.

The excellence of its collections and library have enabled the Mollusk Division to provide many services to the scientific community. These services include not only loaning specimens, but also providing facilities, making identifications, collaborating with state, federal and international agencies in regard to conservation, health, and other concerns, and in generating basic data and new research techniques for scientific advancement in malacology.

The Museum of Zoology has long been one of the major research centers in the world on mollusks, and has trained a significant number of the United States' malacologists. Its mollusk collections have played an important part in the doctoral theses of these specialists, and, in turn, these students often have added substantially to the collections. Because of the Division's active research programs, specimens from all over the world continue to arrive, and these and the previous collections continue to provide material for much basic and applied research, including doctoral theses.

THE MOLLUSK COLLECTION

Organization structure

The Museum of Zoology, The University of Michigan

The acquisition of zoological specimens by The University of Michigan began during the first half of the 19th century, and by mid-century
the collections had been organized into a Museum as a distinct University department. By the end of the 19th century the natural history collections had grown so large as to require the appointment of a director and a curator. In 1913 the Board of Regents of the University changed the name of the institution from Museum of Natural History to the Museum of Zoology. At that time and since, with minor adjustments, the Museum has had six semi-autonomous divisions representing various animal groups.

The Museum of Zoology is housed in the Alexander G. Ruthven Museums Building, along with the Museum of Anthropology, the Museum of Palaeontology and the Exhibits Museum. The Museum of Zoology is the largest of the four museums and occupies over half of the Museums building. The Director of the Museum of Zoology is responsible to the Dean of the College of Literature, Science and the Arts. The Museum of Zoology has six divisions, each dealing with specific animal groups: mollusks, insects, fish, amphibians and reptiles, birds, and mammals. Nearly all of the Curators (i.e., all those appointed since 1956) have joint appointments in the Department of Ecology and Evolutionary Biology, Division of Biological Sciences, and thus are required to carry a full teaching load during one semester of the academic year.

The Mollusk Division

The staff of the Mollusk Division consists of two 2/3-time curators, a 1/2-time secretary and a 1/4-time graduate student assistant (paid by the University). Additionally, there is an editor for Malacological Review and varying numbers of pre-doctoral and post-doctoral students, as well as special non-degree students. The latter groups are, for the most part, funded independently of the Mollusk Division. Depending on availability of funds, the Mollusk Division from time to time also hires students as part-time, non-permanent assistants, including students working on work-study programs.

Space and facilities

Space

The Mollusk Division of the University's Museum of Zoology is quartered in 25 rooms, occupying 8,470 square feet of floor space. This
space is divided as follows:

<table>
<thead>
<tr>
<th>Room Type</th>
<th>No. of Rooms</th>
<th>Sq. ft. of space</th>
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<tbody>
<tr>
<td>Specimen storage, dead and preserved specimens</td>
<td>4</td>
<td>3001</td>
</tr>
<tr>
<td>Specimen storage, live specimens</td>
<td>3</td>
<td>1334</td>
</tr>
<tr>
<td>General storage</td>
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<td>540</td>
</tr>
<tr>
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<td>2</td>
<td>482</td>
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<tr>
<td>Laboratories</td>
<td>7</td>
<td>1693</td>
</tr>
<tr>
<td>Offices — curators, secretary, editorial, students</td>
<td>7</td>
<td>1420</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>25</strong></td>
<td><strong>8470</strong></td>
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Specimen storage

The dry collection is located in two rooms and is stored in 254 cases, 145 of which are relatively new metal cases and 109 are older wooden cases. Some of the wooden cases date from the last century; others date from 1928, when the present Museum Building was constructed. Frames to hold metal cases were built over the wooden cases, and it is on these frames that most of the metal cases sit.

The radular slide collection is housed in a special case, in one of the dry collection rooms.

The wet (alcohol- and formalin-preserved) collection is stored in a separate room, in five metal cases and on 1,154 square feet of open shelves and utilized floor space. The Mollusk Division has placed special emphasis for many years on the importance of securing and maintaining preserved animals (rather than only shells) as part of its collections, and in fact the Museum of Zoology is one of the first museums to place special concern on preserving more than just the shells of mollusks. However, the curation of the wet collection still needs to be completed.

Immunological material is stored in three freezers, housed in one of the research laboratories.

Library

The Mollusk Division library contains all of the malacological journals (30) (each complete), about 1,500 other volumes dealing specifically
with mollusks, and many reprints. These are housed in two rooms within the Mollusk Division area. Additionally, the general Museum Library houses nearly all existing journals on natural history (i.e., those not dealing specifically with mollusks, but which contain many malacological articles), and the Graduate, Undergraduate, Biological Sciences and Medical Libraries, all less than three blocks away from the Museums building, are outstanding in their coverage of all biological subjects. In brief, The University of Michigan library system is one of the great libraries of the Western Hemisphere.

Laboratories

The research facilities of the Mollusk Division are excellent and are housed in seven rooms, occupying 1,693 square feet of space, and contain equipment for studies on anatomy, histology, cytology, biochemistry, ultrastructure, etc.

Equipment

A partial list of major equipment items in the laboratories of the Mollusk Division includes autoclaves (3); calculator; balances, analytical (3); balances, general (3); boats and outboard motors (3); cameras (3); centrifuges, refrigerated (2); cryostat; densitometers (2); electrophoresis apparatus, with power supplies (2); flash evaporator; fraction collector; freezers for antigens and antisera (3); immuno-electrophoresis apparatus; lyophilizer; microscopes, stereoscopic (dissecting) (28); microscopes, compound (16); microtomes, rotary (2); muffle furnace; ovens (paraffin, drying, etc.) (8); pH meters (2); oxygen analyzers; refrigerators (8); spectrophotometer; tissue homogenizers (2); ultramicrotome; etc.

Additional equipment is available through the dispensary, stock and equipment rooms of the Division of Biological Sciences, and includes items such as transmission electron microscopes, ultracentrifuges, etc.

The mollusk collection

History and uniqueness of the collection

The University of Michigan began building a collection of mollusks before the first half of the 19th century, but it was with the acquisition
of the Bryant Walker collection that the Museum's Mollusk Division gained international stature. Dr. Bryant Walker of Detroit was one of the leading malacologists of the late 19th and early 20th centuries. He was a wealthy lawyer, who retired early and devoted the rest of his life to the study of mollusks. He collected many mollusks himself, financed expeditions, and exchanged with prominent malacologists all over the world. Dr. Walker was an eminent scholar, publishing many distinguished works on mollusks. He had a very fine library, which included not only complete sets of every existing malacological journal, but most other books and monographs on mollusks, even copies of very expensive, rare and limited editions. In time, Dr. Walker's mollusk collection (containing over 100,000 lots and about two million specimens) and library were moved to The University of Michigan, and became the backbone of the current mollusk collection.

Dr. Walker purchased many significant shell collections, so his collection consisted partly of blocks of subcollections, often those of famous collectors of his day. Examples are the collections of Andrews, Daniels, Lewis, MacAndrew, Pallary, Ponsonby and Wetherby, only to name a few.

The first curator in the Mollusk Division was Mina Winslow, who organized the early collections and published a number of substantial papers, mostly on mollusks of Michigan. She was followed by Calvin Goodrich, during whose time substantial additional collections were added to the Mollusk Division, including large numbers of the Pleuroceridae, a freshwater family of special interest to Mr. Goodrich. Henry van der Schalie joined Mr. Goodrich on the curatorial staff in 1934 and retired in 1977. The two current curators, J.B. Burch and Alex S. Tompa, joined the University curatorial staff in 1963 and 1977 respectively. Both curators have a 1/2-time appointment in the Museum and teach 1/2 time in the University's Division of Biological Sciences during the academic year.

In addition to the Bryant Walker Collection, with its many subcollections, and the mollusks collected on expeditions financed by either Bryant Walker or the University (or both jointly), or by Federally financed research grants, a number of other significant mollusk collections have come to the University over the past years. Several of these are listed below as examples.

(1) Collections of the Royal Ontario Museum. This was the most significant collection of mollusks in Canada, but was in danger of falling to neglect or even being discarded because the departure of its curator,
Dr. John Oughton, left no one to care for the collection, and the rest of the staff of the Royal Ontario Museum was sorely pressed for space. Therefore, in the 1930's, the entire collection was moved to The University of Michigan on "permanent loan". This was an outstanding collection of North American (principally Canadian) freshwater and land mollusks and, like Walker's, contained many important subcollections.

(2) The Stelfox Sphaeriid Collection. One of the most significant collections of the cosmopolitan freshwater bivalve family Sphaeriidae is that gathered by A.W. Stelfox of Dublin, Ireland. When Dr. Stelfox retired he offered to give his collections to the Museum of Zoology, if the recognized authority on the Sphaeriidae, H.B. Herrington, would come to Ireland to get the collection. The Museum of Zoology arranged to send Dr. Herrington there and he incorporated that outstanding collection with the many lots of sphaeriid clams already obtained from North America by our Museum. A major revision of the Sphaeriidae was later prepared by Herrington using these combined collections (Misc. Publ. Mus. Zool., Univ. Mich., (118): 1-74, 1962), and more recently these collections formed the basis for Burch's (1973) manual prepared for the Environmental Protection Agency (Freshwater sphaeriacean clams (Mollusca: Pelecypoda) of North America. Biota of freshwater ecosystems, Identification Manual No. 3, Water Pollution Control Research Series, Environmental Protection Agency, U.S. Government Printing Office, Washington, D.C., pp. 1-31; revised, 1975, Malacol. Publ., Hamburg, Mich., pp. 1-96).

(3) The F. C. Baker Wisconsin Freshwater Mollusk Collection. One of the most extensive studies on North American freshwater mollusks was done by F. C. Baker in the 1920's and monographed in a two-part treatise (The fresh water Mollusca of Wisconsin), and is still one of the most important single publications on North American freshwater mollusks since Bryant Walker's 1918 Synopsis of the freshwater Mollusca of North America. In the late 1950's the Baker Wisconsin collection came to The University of Michigan.

Much more could be written about the history and make-up of The University of Michigan mollusk collections and the prominent malacologists (e.g., H.B. Baker, W. J. Clench, A.E. Ortmann, H.H. Smith, etc., etc.) whose expeditions supplied materials. But the above should give some idea of the events and people that have added significance to the collections, as well as the bulk of physical specimens. But, even more important is how the collections have been instrumental in adding to past and current knowledge of freshwater malacology in North America.
and the rest of the world. This information is contained in the more than 600 books, monographs, research papers and technical reports published from the Mollusk Division since the turn of the century.

Size and scope of the collection

The Mollusk Shell Collection contains over 250,000 catalogued lots of dry specimens. These lots contain approximately five million specimens. Additionally, the Mollusk Division has a catalogued collection of freeze-dried snail antigens and antibodies (over 2,000 lots), as well as a radular collection, and a collection of molluscan eggs representing nearly 40 families. The Mollusk Division’s type specimen collection consists of over 2,400 species housed in two special cabinets. However, holotypes or syntypes are often found in the general collection, having been unrecognized previously as such because types were not segregated separately in the Walker Collection.

The University of Michigan mollusk collection is especially significant because of its large holdings of freshwater specimens; it is probably the largest and most complete collection of freshwater mollusks in the world. The importance of these collections is constantly enhanced because of the continued extinction of our great river systems by dams, and by pollution of our natural waters and other deleterious activities of man. The records of the fauna of many streams only can be known completely by studying museum collections, because these faunas have now largely disappeared.

Acquisitions and loan policies

Acquisitions

Nearly all of the mollusk material currently coming to the Mollusk Division is obtained for research. Some specimens also are added as voucher specimens for research publications, and occasionally collections are added which are historically important or which add significantly to the range of various species.

Loan policy

Loans are made readily to any qualified individuals or institutions requesting them. Qualified persons are staff members of other museums,
universities or research institutions, or exceptional private individuals whose past research history qualifies them as specialists. Loans are made free of charge and for a period of six months. They may be renewed under special arrangements.

Recent curatorial accomplishments (1973-77)

During the recent five-year period for which summarized records are available, over 232,000 lots were rearranged and relocated into a more usable system, to allow for easier use by systematists, and more logical future expansion. While doing this, the taxonomy and nomenclature were up-dated through the family level categories. Additionally, over 18,000 lots were cleaned, sorted, labelled, catalogued, and added to the collections and 1,380 lots were processed (cleaned, sorted, identified and labelled) for the Bishop* and Australian Museums**.

Also during this same period, over 2,000 samples of molluscan antigens and antisera were processed, catalogued and stored (at low temperatures).

Recently, 103 metal cases have been added for storage of dry specimens. These have provided badly needed expansion space and also have served to replace the least usable of the old wooden cabinets.

During this same period of time, the Mollusk Division has provided various services to the outside scientific community, services which, for the most part, are directly related to the mollusk collections. Examples of services are: over 2,400 lots (over 23,000 specimens) of specimens were loaned for scientific studies (to over 50 different individuals or institutions); nearly 80,000 specimens were identified for other scientists; two identification manuals on North American freshwater clams and one on North American freshwater snails were prepared or revised; several outstanding malacologists were trained at the Ph.D. level; and cooperation and collaboration was extended to numerous state, national and international agencies on various malacological problems and projects of special significance to human welfare (public health, environmental quality, etc.).

*Material collected on National Science Foundation-supported Bishop Museum-Museum of Zoology expeditions and initially stored in the Museum of Zoology for processing.

**Synoptic, previously unprocessed local material sent to the Australian Museum on permanent loan.
Collaboration with individuals and institutions

For many years the Mollusk Division has functioned as a state, national and international systematic facility. Like other museums, Mollusk Division personnel make identifications and lend specimens for study. The Division's status as a national and international facility rests in part on the amount of such service, but still more on the nature and extent of its other cooperative activities. The following account, although condensed and incomplete, will give some idea of the scope of the services the Mollusk Division performs. Although in one sense given freely, these are directly or indirectly recompensated in many ways, in particular by additions to the collections and libraries.

(1) Collaboration with individual scientists. This is of various kinds, and given under various arrangements and circumstances. Much of it consists in identification of specimens, either for specialists who cannot themselves come to use the Museum's collections, or for workers in other fields. Among the latter in recent years have been anthropologists, parasitologists, physiologists, fisheries biologists and ecologists. Another important service to individual malacologists is the loan of specimens important to their studies.

The Mollusk Division also provides space, facilities, and sometimes financial support for zoologists who come to work with its collections, and over the years many investigators from other institutions have come to Ann Arbor to use the Division's facilities or collections. They come from various parts of the United States and from Canada, South America, Europe, the Near East, Africa, Southeast Asia, the Far East and Australia.

In recent years, the Mollusk Division has organized four mollusk seminars, with national and international attendance. These seminars drew nearly 100 malacologists to Ann Arbor.

(2) Collaboration with state, federal and international agencies. The services performed by the Mollusk Division fall into the following categories:

(a) contributions to the knowledge of local, national and foreign faunas, by surveys, direct investigation, and publishing both technical and popular reports of its findings;
(b) direct participation in the work of other scientific and regulatory agencies, such as the state and U.S. departments of agriculture, departments of conservation and natural resources,
state and federal water quality agencies, state and national public health services, the Environmental Protection Agency, Energy Research and Development Agency, Armed Forces Epidemiological Board, Agency for International Development, World Health Organization, etc. These activities include identification of specimens, providing consultation services, and participating in research and field surveys.

Auxiliary activities dependent on the mollusk collection

Education services and research

Perhaps the unique feature of the Museum of Zoology at The University of Michigan is the high quality and magnitude of its research collections taken in conjunction with the university environment and the staff which supervises the collections and students who use them. The collections constitute a valuable resource which is matched in few, if any, other institutions offering training in systematic and evolutionary zoology. Accordingly, the Mollusk Division’s instructional and research programs reflect full use of this advantage. This requires a collection-oriented staff which uses the collections and sees that they grow appropriately, as well as a staff heavily oriented toward graduate education.

The Museum of Zoology’s mollusk collection contains one of the largest and most complete assemblages of land and freshwater mollusks in the world. It has been built by the patience and devotion of many students of malacology for nearly 100 years, and it has been an important source of material for the doctoral theses of some 30 doctoral students, many of whom later have become recognized leaders in the field of malacology.

The Museum of Zoology has excellent facilities for research in systematic and evolutionary biology of mollusks, and has been in the forefront in research and graduate education in malacology for many years. The Curators in charge of the mollusk collection traditionally have conducted active research programs, some financed by federal research funds, and most involving field collecting in the United States and abroad. This provides a continuing source of reference material, which needs preparation and curation.

In recent years, the Mollusk Division has been a leader in applying
new methods of research in studying systematics of mollusks. These methods include cytotaxonomy, cell, tissue and organ culture, electrophoresis and serology. But, fundamental to a broadly based systematics program are large research collections. The value of such collections is enhanced rather than diminished by the addition of new and diversified research techniques. Comparative morphology is still the foundation of systematics; biosystematics incorporates and builds on this foundation.

Currently there are several graduate students in the Mollusk Division engaged in an academic program leading to the Ph.D. degree, and using the collections as an important source material. Malacologists receiving degrees here in the past are H.B. Baker, William J. Clench, Alan F. Archer, Elmer G. Berry, Henry van der Schalie, Harold W. Harry, C. Bruce Lee, Aurèle LaRocque, Emile A. Malek, Bruce McCraw, Alan Solem, D.S. Dundee, Paul F. Basch, J.B. Burch, Harold J. Walter, B.C. Dazo, Esther Goudsmit, William H. Heard, A.M. Cvancara, Robert C. Wall, Barry Miller, George M. Davis, C.T. Lo, Gary L. Pace, Louise R. Kraemer, C.M. Patterson, E.S. Upatham, S.K. Wu, S.C. McDonald, Y.-S. Liang, Paul H. Rudolph, P.T. LoVerde, George A. Te, Lyford K. Greene, K.-H. Wurzinger and Viroj Kitikoon. Most of the theses resulting from these students' studies hinged on the mollusk collections, and, accordingly, the value of the collections is greatly enhanced by their representing the data base of much scientific knowledge.

In addition to training American malacologists, the Mollusk Division has provided malacological training, at various levels, for students of other nationalities — from countries such as Canada, China (Taiwan), Egypt, Ethiopia, India, Japan, Kenya, Korea, the Netherlands, Nigeria, the Philippines, South Africa and Thailand. Also, staff of the Mollusk Division have been actively engaged in cooperative programs for training graduates in malacology abroad — in Egypt, India, Jordan, Korea, the Philippines and Thailand.

PUBLICATIONS

Research papers and monographs

Since the turn of the century, over 600 scientific publications on mollusks have come from staff and students working in the Museum of Zoology. Most of these are based, in one way or another, on the mollusk
collections. These represent a great addition to scientific knowledge and increase the value of the Museum’s mollusk collection as a national resource.

While there are too many publications to record here, and it is difficult to select which few publications to list as selected examples, nevertheless a relatively short list is given below to indicate some of the types of studies in which Mollusk Division personnel have been engaged.


Many scientific papers have been based wholly or in part on materials from the Museum of Zoology's mollusk collections. For example, during the 1973-77 period, over 100* such papers were published. Many of these publications were supported with public funds, i.e., grants from the National Institutes of Health (31 papers during the 1973-77 period), the Smithsonian Institution (27 papers), the National Science Foundation (13 papers), U.S. Army Medical Research and Development Command (9 papers), the Environmental Protection Agency (5 papers), the U.S. Navy Bureau of Medicine and Surgery (2 papers), the National Oceanic and Atmospheric Administration (1 paper), and the Australian Public Health and Medical Research Administration (1 paper). Ten of the publications were supported by the Rockefeller Foundation.

A few of these publications from the 1973-77 period are listed below as examples.

1973


*The actual number of publications is probably much greater. Publications often (or usually) result from materials that are loaned from the Mollusk Division collections to other individuals and institutions, but often (or usually) notification regarding publication is not forwarded to the Museum of Zoology. The literature has not been searched for articles acknowledging the use of Museum of Zoology collections.*
1974


1975


1976

Key to the genera of freshwater pelecypods (mussels and clams) of Michigan.


1977


Journals

Two outstanding malacological journals were initiated at the Museum of Zoology, *Malacologia* (in 1962) and *Malacological Review* (in 1968), and the publication of both journals has been materially assisted by the resources of the Mollusk Division.
Alexander G. Ruthven Museums Building, The University of Michigan

B

Mollusk shell collection.
Mollusk aquarium facility.

Mollusk research laboratory.
ABSTRACT. *Rangia cuneata*, the estuarine clam, has only recently become the subject of study for its remarkable salinity tolerance. Possessing the osmoregulatory mechanisms of both marine "osmoconformers" (isosmotic intracellular regulation) and fresh water osmoregulators (anisosmotic extracellular regulation), *Rangia* can adapt to salinities varying from nearly 0 to 33 ppt. The process by which the clam regulates the osmotic potential of its cells has not been completely determined. The young of the species have a much lower salinity tolerance than do the adults; it is this factor, combined with those of inter-specific competition and predation, which restricts the species within the estuarine environment to a salinity range much smaller than its total limit of tolerance.

The coastal estuarine environment is, above all else, variable. It represents an unstable transition zone between stable marine waters on the one side and stable fresh water on the other. Pritchard (1967) defines an estuary as "a semi-enclosed coastal body of water which has a free connection with the open sea and within which sea water is measurably diluted with fresh water derived from land drainage". Gradients along a large number of parameters (salinity, temperature, dissolved gases, density, etc.) are established as salt water rushes in, in accordance with the tidal cycles, to meet fresh water input, which varies with the season (Kinne, 1967). The organisms which have invaded estuarine waters are those able to cope with the frequent, rapid, and often drastic fluctuations in the physical environment.

Salinity variation is one of the abiotic factors most effective in excluding organisms. Estuarine fauna distribute themselves along salinity gradients according to their degree of tolerance. Although salinity gradients based on expected salinity fluctuations may, for the most part,
remain stable, the environment is such that tolerance in some degree is essential, and the wider the tolerance range, the fitter the organism will be for the estuarine environment.

*Rangia cuneata* (Gray), the estuarine clam, is extremely well suited to an estuarine existence. A member of the marine family Mactridae, it inhabits estuaries from Virginia south to Florida, and westward along the Gulf of Mexico (Anderson, 1975). It is one of a small group of organisms, and far smaller group of mollusks, able to adapt and survive indefinitely at salinities that range from those of fresh water (slightly more than 0 ppt (parts per thousand)) to full-strength sea water (approximately 33 ppt) (Hopkins et al., 1974). Only recently has *R. cuneata* been recognized for its exceptional salinity tolerance and become the subject of study. The mechanism by which this tolerance is achieved is still the subject of theory.

External salinity variations are stressful for aquatic organisms, in general, in that they affect the internal ion concentration, osmotic equilibrium and volume regulation of the animal. Aquatic organisms are grouped according to their ability and/or mechanism for dealing with the rigors of salinity variation. The vast majority of marine organisms are classed as osmoconformers because they lack the ability to regulate the osmotic potential of their body fluids in response to changes in the salinity of the medium. The internal fluids, thus, follow or parallel the medium, remaining isotonic across permeable membranes (Robertson, 1964). Osmoconformers range in their tolerance for salinity fluctuation, with the degree of tolerance they have being conferred upon them by the capacity of their cells to adjust to the fluctuations of the internal medium. Since they lack a mechanism for regulation of the blood, however, none can invade fresh water.

The term osmoregulator is used generally to refer to those organisms which are able to maintain a constant osmotic potential of the body fluids despite changes in the salinity of the environment. These tend to be freshwater organisms or those able to adapt to water of very low salinity. Species inhabiting dilute media must regulate continuously, even in water of constant salinity, in order to maintain the body fluids at an osmotic level higher than that of the medium which is required to sustain life.

The division of marine and freshwater organisms into osmoconformers and osmoregulators has found less favor recently, as it has been recognized that a type of regulation also occurs in marine species. Osmoregulation is becoming predominant as a general term for two different regulatory processes: anisosmotic extracellular regulation and isosmotic
intracellular regulation. The first is interchangeable with the term osmo-
regulation in its more restricted usage, i.e., osmotic regulation effected
through the body fluids, as found in organisms common to dilute media.
The second is the term that has been applied to osmotic regulation ef-
acted through the cells; it is this mechanism which permits marine “os-
moconformers” to tolerate modifications of varying degrees in the salin-
ity regime.

*Rangia* is one of a very small group of organisms which possesses
both types of regulatory mechanisms (Gainey & Greenberg, 1977). In
saline solution, its body fluids conform to the medium and it relies on
isosmotic intracellular regulation, but, in an external medium of ap-
proximately 10 ppt or less, it switches over and begins regulating inter-
nal fluids, maintaining blood hyperosmotic to the medium to a maxi-
mum hyperosmolarity at approximately 5 ppt (Bedford & Anderson,
1972b). The transition is smoother going from more saline to dilute
water than from the reverse (Bedford & Anderson, 1972b). This may
reflect environmental occurrences. The upper estuary, where *Rangia*
is found, is far more prone to sudden dilution, due to river run-off or
flooding, than it is to sudden concentration from evaporation (Bedford

Allen (1961) seems to have taken the first exploratory steps toward
an explanation of *Rangia cuneata*’s extreme euryhalinity. He observed
that, when the salinity of the medium was increased, the clams lost
water but their ash weights rose, indicating an increase in inorganic con-
stituents. At the same time, the amino acid concentrations (alanine, in
particular) of the clams increased to a maximum at a salinity of 17-20
ppt. Allen took these results to indicate that some type of relationship
might exist between water balance, inorganic constituents and amino
acid concentration and salinity.

Bedford & Anderson (1972a) were among the next to turn their at-
tention to the strange abilities of the estuarine clam. Their studies re-
vealed that, when placed in an amino-acid-rich medium (glycine-rich) at
salinities less than 5 ppt, *Rangia* was able to maintain the blood osmotic
concentration at approximately 60 milliOsmoles (mOsm)/liter above
that of the medium. At the same time, uptake of glycine and turnover
of accumulated glycine was low, but incorporation of accumulated gly-
cine was high. The results obtained by Bedford and Anderson were
quite different from those of Allen in that they located the plateau for
glycine uptake much lower than 17 ppt. At salinities between 6 and 25
ppt, they found no significant difference in the rate of glycine uptake
by whole animals.
The results of these two studies established the groundwork for most Rangia study that was to follow in that both identified that in Rangia, as in other aquatic invertebrates, the amino acid concentrations of the cells of the organism were, in some way, linked to the salinity response and that the amino acids were being taken up and incorporated at different rates in response to a change in salinity. Because amino acids had been implicated in the past in intracellular isosmotic regulation in other aquatic invertebrates, and with less certainty in mollusks (Bayne et al., 1976), the same assumption was made with Rangia and study proceeded on that basis.

Examination of amino acid participation in the salinity response proceeded, at least initially, on two different tacks. The first type of study, as exemplified by Allen (1961), was based upon measurements of the endogenous concentrations of amino acids in the tissues of specimens placed under osmotic stress. The second method has consisted of observation of the fate of exogenously supplied amino acid. Variations of this type have yielded most of the information that now exists concerning uptake and utilization of the amino acids. A brief overview, using two representative papers of this last type, is useful in forming a base for discussion of theory.

Rangia acclimated to various salinities in media rich with C\(^{14}\)-labeled glycine exhibit rates of uptake that vary. Because of the presence of attached tracer ions, the fate of the glycine can be followed. Anderson & Bedford (1973) studied whole clams and isolated gills. They found that whole animals took up glycine at a fairly constant rate from a salinity of 32 ppt down to 6 ppt, accumulating glycine at levels nearly 75 times that of the medium. The rate of uptake decreased rapidly below 6 ppt. Uptake at 6 ppt was three times the uptake at 2 ppt.

Anderson & Bedford (1973) found that salinity had an effect on the fate of labeled glycine over time. In clams acclimated to 1 ppt, 2 ppt and 5 ppt, over the period of 49 hours after exposure, 75% of the total radioactivity was incorporated into an ethanol-insoluble (80% ETOH) component considered to be protein (Anderson & Bedford, 1973). Clams acclimated to the higher salinities of 10 and 15 ppt, over the same period of time, incorporated only 54% and 32%, respectively. Incorporation in the low salinity clams did not increase appreciably after the 49-hour period, the supply having already been largely depleted, whereas incorporation in clams in the more saline solution increased 58% and 44%, respectively.

Other salinity-related differences in the fate of the glycine were discovered. After three hours of exposure, the low salinity clams (1, 2 and
5 ppt) had incorporated 90% of the ETOH-insoluble glycine into protein synthesis. The higher salinity clams (10 and 15 ppt), however, had only used 75% for that purpose, with a high percentage of the remainder being diverted to nucleic acid synthesis. After 99 hours, the percentage of C\textsuperscript{14} incorporated in the protein of the clams in dilute solution had dropped to 87%, while the clams in the more concentrated solution had incorporated 93%. Small increases in the percentages of labeled glycine in the glycogen and lipid components were also found over the course of 99 hours; however, no strong relationship to salinity was observed.

Exogenously supplied, radioactively labeled glycine has also been used to examine the deposition of amino acids in specific tissues, gill tissue in particular. Building on the earlier work of Anderson and Bedford, Anderson (1975) found that the rate of incorporation of accumulated glycine in the gill tissue was, in general, much greater than that of whole \textit{Rangia}. His results, in fact, indicated that low salinity had much less of an inhibitory effect on uptake and incorporation of exogenous glycine in the gill tissue than it had on the intact animal.

In summation of the work of Anderson & Bedford (1973) and Anderson (1975) with radioactive glycine, the experimental results indicated that the low salinity, or dilution, response consists of a decrease in uptake, but an apparent increase in incorporation, of accumulated glycine, at least during the first 24 hours. The radioactive labels travel via incorporation from the free amino acids, which are ethanol-soluble, into an ethanol-insoluble fraction (Anderson, 1975), thought to be protein (Anderson & Bedford, 1973). The response to increased concentration of the medium consists of an increase in uptake and utilization.

The above studies have provided only a bare framework into which theory on the possible adaptive value of and mechanism behind regulation of an amino acid pool has been fit. There is biochemical justification to explain the observations that internal amino acid concentrations, and the fate of those amino acids, are responsive to salinity variation in the environment. Amino acids can function as solute in times of hyper-osmotic stress, i.e., in environments of high salinity, enabling the cell to nearly maintain its normal volume and osmotic status by supplying particles in concentration to the cell to equalize the increase in particle concentration of the medium, at a low cost in energy (Anderson, 1975). When the medium is diluted and the cell needs to decrease its osmotic potential, it does so by losing solute, i.e., amino acids. Pierce (1971), in his work with \textit{Modiolus} spp., observed that the degree of euryhalinity of a species is directly related to its ability to control its concentration of solute.
Various theoretical mechanisms have been devised to account for the regulation of the amino acid concentration in relation to salinity, some having more support from experimental results than others. The following construct, which might be called a “steady-state” theory, serves more as a conceptual framework for discussion purposes than as a viable theory in its own right. The theory holds that the observed decrease in amino acid concentration in low salinity somehow is linked to the simultaneous increase in the protein concentration in a low salinity medium. Because the amino acids are the precursors of protein, it is possible to assume that the amino acid is used to build the increasing protein component of the cell. Conversely, protein can be catabolized, resulting in an increase in the amino acid concentration in low salinity. The amino acids and protein thus can be visualized as forming opposing pools in steady-state—a decrease in one feeding the other. The adaptive value of such a mechanism is particularly strong when it is recognized that the ability to convert amino acids to protein offers the cell a way to retain the valuable amino acids in an osmotically inactive form during dilution of the medium. Without such a transformation mechanism, the cell would be required to expel the osmotically active amino acids in order to maintain osmotic balance with the medium.

The above theory offers a base for modification as evidence that cannot be accommodated by the theory accumulates. The model must take on more of a unidirectional character, as study indicates that the amino acids present in a cell in high salinity may not be necessarily the result of the breakdown of protein. Allen (1961) commented that the fact that repeated observations have confirmed that only one amino acid, alanine, continues to rise works against a protein degradation theory, such as that included in the “steady-state” theory. Henry & Mangum (1979) attributed the rise in amino acids to de nova synthesis, owing to their observations that use of the glycolytic inhibitor indoacetate inhibits build-up of alanine and the free amino acid pool, in general, in increased salinity. Allen (1961) observed a depletion of cell glycogen and also attributed it to de nova synthesis. Lange (1972) has suggested that the rise in amino acids in increasingly concentrated media may be just a result of the inability of amino-acid-synthesizing enzymes to function in dilute medium.

There also is evidence working against the concept of complete incorporation of amino acid into protein when the cell is exposed to dilute solution. Anderson & Bedford (1973) reported that some leakage of amino acids does occur due to shock. Bayne et al. (1976) and Schoffeniels & Giles (1972) correlated the decrease of amino acids in low
salinity with concurrent increases in nitrogen excretion, which they took to indicate that degradation of amino acids was occurring. Anderson (1975) pointed to an increase in C\textsuperscript{14}O\textsubscript{2} during a decrease in salinity as demonstrating degradation of amino acids. Both observations point to at least some amino acids being lost from the pool with each downward shift in salinity.

Also, the work of Allen (1961) does not fit comfortably into the "steady-state" theory. He found that as the medium concentration increased, the concentration of amino acids increased to a maximum at 17 ppt and decreased above that point. The loss of tissue water and the increase in inorganic ions peaked at salinities of 17-20 ppt. The fact that water loss leveled off and amino acid concentration decreased after a 17 to 20 ppt concentration was reached suggested to Allen that a shift in the osmotic control mechanism occurred at that point. Allen gave no explanation or attempt to further modify theory other than suggesting that a relationship might exist between amino acids, tissue water and inorganic ion concentration with respect to changes in salinity.

Thus, the steady-state theory seems to serve as a model more in a state of negation than anything else. A positive, coherent theory has not yet emerged. The one thing that researchers do seem to agree upon is that, if the mechanism is at all close in type to the component theories that have been tested and have fallen short, the gill appears to be the most likely location for regulation of the amino acid pool (Anderson & Bedford, 1973; Anderson, 1975).

The steady-state theory, and modifications thereof, has not addressed the observation that uptake decreases with dilution. Evidence suggests an endogenous origin for the amino acids. Stephens (1967), in studying the availability and uptake of amino acids in marine, brackish and fresh water species, found that, while the environment was a common and well-documented source of amino acids for marine organisms, brackish water organisms were only able to accumulate amino acids at a much lesser rate, and that fresh water species were totally unable to make use of amino acids in solution. He speculated that the processes underlying the osmotic regulation of the body fluids were incompatible with rapid uptake of amino acids from the medium. Anderson & Bedford (1973) considered part of the uptake rate decrease in low salinity to be a symptom of stress due to osmoregulatory processes. These same authors (1973) suggested that the decrease in some ions, Na\textsuperscript{+} in particular (Anderson, 1975), in less saline water may affect the permeability and/or transport systems of the cell membranes, thus affecting uptake.

A brief discussion of another type and mechanism of osmotic control
in connection with *Rangia cuneata* is warranted. Anisosmotic extracellular regulation is osmotic regulation effected through the blood. It is a case in which the osmotic potential of the blood is regulated at a constant level despite changes in the medium, rather than a case in which the cells are regulated to cope with fluctuations of body fluids which are isotonic with the medium. The control can come through elimination of a waste fluid in which ions are differentially excreted or through controlled absorption of ions and water (Robertson, 1964). Active uptake of at least some ions has been found in all mollusks tested (Robertson, 1949; 1953).

Bedford & Anderson (1972b) seem to have been the first to have observed a fluctuation of the internal concentration of the blood in *Rangia* in relation to the medium. Their studies revealed that at salinities greater than 10 ppt the blood was isotonic to the medium, while below that point the blood was hypertonic. Hyperosmocity increased sharply at 5 ppt and continued increasing to a maximum at approximately 1 ppt. A 55-65 mOsm/l difference was maintained by clams in media of salinities between 5 and 1 ppt.

The major mechanism by which the blood is regulated appears to be through a plasma-membrane-bound enzyme, Na\(^{+}\)+K\(^{+}\)-ATPase, which functions as the equivalent of a sodium pump in transporting ions across the membrane (Saintsing & Towle, 1978a). Recent studies (Henry, 1978; Mangum & Simpson, 1978; Saintsing & Towle, 1978a,b; Mangum et al., 1979) using ouabain, a specific inhibitor of the enzyme, have added greatly to the existing knowledge of Na\(^{+}\)+K\(^{+}\)-ATPase and its role. Some of the strongest support for the importance of Na\(^{+}\)+K\(^{+}\)-ATPase has come from the finding that, in animals subjected to ouabain in a dilute solution, blood Na\(^{+}\) dropped significantly, demonstrating that functional hyperosmotic blood requires Na\(^{+}\)+K\(^{+}\)-ATPase (Mangum & Simpson, 1978). The decrease preceded the hypoxic effect of the enzyme inhibitor and, thus, could be distinguished from a non-specific effect of hypoxia.

Using labeled \(^{3}\)H-oubain, Saintsing & Towle (1978a,b) analyzed various *Rangia* tissues for their role in anisosmotic regulation. Their findings showed that, of the tissue homogenates tested (mantle, gill, kidney, rectum, ventricle and foot), only the first three demonstrated activity. Of these, the highest number of ouabain binding sites, and thus the highest number of enzyme molecules (at a ratio of 1:1 (Kyte, 1972)), were found in the mantle. Because the kidney was discounted for anatomical reasons as having a role in ion regulation (Saintsing & Towle, 1978a), the area of activity was narrowed down to the mantle-
gill complex. Saintsing & Towle (1978a) further narrowed the area of greatest activity to the central skirt region of the mantle.

Labeled ouabain has also been utilized in studying the effects of a change in salinity on Na\(^{+}\)K\(^{+}\)-ATPase activity in *Rangia*. No difference in the number of ouabain molecules bound in various salinities was detected; however, a significant salinity-related difference was found in enzyme activity. In a highly saline medium, this would indicate that turnover is higher but that pre-existing pumps are utilized (Saintsing & Towle, 1978).

In addition to active transport of ions across tissue membranes, the ion concentration of the blood of *Rangia* also may be affected by differential excretion of ions. Henry (1978) found that, in low salinity, ammonia is excreted along a molecular concentration gradient, whereas, in high salinity, there was no evidence that it is excreted by an active process. Because it was inhibited by ouabain, Henry suggested that ammonia excretion in some way might be coupled to the activity of Na\(^{+}\)K\(^{+}\)-ATPase. Thus, it appears that *Rangia* utilizes both uptake of ions and differential excretion of ions to osmoregulate the blood.

As is often the case in organisms of fairly wide tolerance ranges, immature *Rangia* are far less capable than are the adults of withstanding wide salinity variations. According to Cain (1973), the larval stage is the critical link in recruitment to adult populations. Within six or seven days, however, the critical stage passes and the juveniles can survive the same exceptional salinity ranges as can the adults of the species (Hopkins et al., 1974).

Tolerance is lowest in the very earliest stages and increases dramatically within a short time. Cain (1974) found that, for 4 hr old embryos, a drop in salinity from 5 to 1 ppt for embryos acclimated to 5 ppt resulted in 100% mortality. By the 24 hr straight hinge larval stage, the mortality rate for the same salinity change dropped to 45%. The effect of salinity change on the older sample group was of much greater consequence than was temperature change, but simultaneous changes in both parameters accounted for more mortality than either alone.

In an earlier paper, Cain (1973) had explored optimal ranges. At the 4 hr embryo stage, optimum conditions consisted of 18-29°C and 2-20 ppt. Use of a response surface chart demonstrated that salinity was the more limiting factor for the embryo stage, with 85% survival existing over a 12°C change, but over only a 5 ppt salinity variation. Not all salinity-temperature combinations produced equal levels of viability.

It seems certain that correlations exist between adult spawning conditions and viability of the embryos. Field study produced the observation
that, whereas temperature influenced initiation of gametogenesis, salinity was responsible for triggering spawning (Cain, 1975). Adults in the James River estuary were ripe for at least seven months of the year and thus were able to spawn whenever the other piece of the puzzle, salinity, fell into place. Spawning at 5 ppt produced the greatest number of viable young. Cain theorized that salinity may be the exogenous factor which, in activating osmotic regulation, may trigger synchronous spawning of the population. Hopkins et al. (1974) contended that it is the change in salinity which induces spawning, not just the salinity level itself.

More evidence from field observation of *Rangia* population structure suggests a differential between young and old in tolerance levels (Hopkins, 1970; Hoese, 1973). It is not unusual in portions of estuaries that tend toward low salinity for the structure of a particular population to comprise a few very distinct age groups. Correlation with hydrologic records often confirms that conditions for years that correspond with the ages of the population were unusual in having salinity changes which promoted spawning of the adults and which were within tolerance ranges of the young (Cain, 1975). Recruitment also may occur because water movements in particular years were such that veliger stages of nearby populations were able to move with an ambient water mass and settle in a location which otherwise would have been unsuitable for that stage in their life cycle (Cain, 1975). Once settled, the conditions suitable for successful spawning may be few and far between for further recruitment to a "pioneer" population. As long as favorable conditions appear on the average of every eight years, the average life span of *Rangia* (Fairbanks, 1963), the population can be maintained (Cain, 1975). Hopkins et al. (1974) suggested that *Rangia* population structure may be useful as an indicator of salinity climate in a given locality.

This discussion of *Rangia cuneata* would be incomplete if it did not conclude the way it began — by relating the animal to its environment. Despite the great salinity tolerance it has exhibited in the laboratory, *Rangia* has not been found in salinities greater than 25 ppt by Bedford & Anderson (1972a) or greater than 15 ppt by Hopkins et al. (1974). Thus, it is not surprising that *Rangia* has not been shown great interest until fairly recently.

Even though the actual range is much smaller than its potential range, the animal is still afforded a number of advantages over less euryhaline individuals. Estuaries, being the unstable environments that they are, might be considered to be undesirable from the organismic point of
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view. Usually collected in waters of salinity between 1 and 10 ppt (Hopkins & Andrews, 1970), *Rangia* exceeds that generality by specializing in the most undesirable area of the estuary. Classified as the horohalini-
cum by Kinne (1971), the waters of 5-8 ppt salinity have been recog-
nized as an ecological boundary between marine and fresh water species, and, as such, are characterized by low species diversity. Khlebovich
(1968; 1969), in his reviews of the literature on this subject, explained
that the 5 ppt salinity mark is a “critical salinity” for organisms, because pronounced changes in the ion ratios of the water occur at this point.
Marine osmoconformers are restricted to the waters of salinity above
5 ppt, while organisms equipped to regulate the osmotic potential of
their blood fluids inhabit the water of lower salinity. *Rangia*, possess-
ing both mechanisms for coping with salinity change, can easily straddle
the critical salinity line and, thus, decrease inter-specific competition
(Gainey & Greenberg, 1977). It also is thought that the number of
euryhaline predators increases, to a certain degree, with salinity above
the horohalinicum (Gainey & Greenberg, 1977). These two factors of
competition and predation, combined with the more important repro-
ductive requirement for salinity variation, prevail to retain *Rangia*
in a salinity range smaller than its limit of tolerance.

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