

A COMPARISON OF MORPHOMETRIC, BIOCHEMICAL, AND PHYSIOLOGICAL INDEXES OF CONDITION IN MARINE BIVALVE MOLLUSCS

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ABSTRACT

A number of morphometric, biochemical and physiological parameters are compared and evaluated for their potential use as indexes of condition in marine bivalve molluscs. Condition is defined as "the ability of an animal to withstand an adverse environmental stress, be this physical, chemical or biological." Laboratory and field data are compared to determine the effect of one environmental stressor, temperature, on gravimetric and volumetric meat/shell condition indexes; percentages of organic content, carbohydrate, carbon, and nitrogen; C/N and carbohydrate/N ratios; weight-specific ammonia excretion rates; and oxygen-consumption/ammonia-excretion ratios in a number of commercially valuable species of oyster, clam, and mussel.

With the increasing use of the sessile, adult bivalve mollusc as an indicator of environmental quality (Goldberg, 1975) and the considerable interest in culturing commercially valuable bivalve species in thermal effluents, there is a pressing need to determine which of many available morphometric, biochemical, and physiological parameters can be used as indexes of condition in bivalves. Condition is defined as "the ability of an animal to withstand an adverse environmental stress, be this physical, chemical or biological." Stress is defined as "a measurable alteration of a physiological, or behavioral, or biochemical, or cytological steady-state which is induced by environmental change, and which renders the individual (or the population, or the community) more vulnerable to further environmental change" (Bayne, 1975).

Although many stressors, such as disease, starvation, salinity, and exposure, affect the condition of bivalve molluscs, this paper

examines the variation in a number of previously used indexes of condition in relation to only one environmental stressor, temperature. Furthermore, discussion is limited to temperature ranges comparable to those experienced by the species in their natural habitats. The natural occurrence of a species in one particular seawater temperature range should not be taken to indicate that the species is not under temperature stress for at least some portion of the year. For example, the southern geographical limit of *Mytilus edulis* corresponds approximately to a mean summer water temperature of 26.7°C (Wells and Gray, 1960). When water temperature is held constant, however, this species is unable to acclimate its metabolic and feeding rates to temperatures greater than 20 to 22°C (Bayne, Widdows, and Worrall, 1977). Gonzalez and Yevich (1976) made similar observations for populations of *M. edulis* in the effluent canal of a steam generating plant.

INDEXES OF CONDITION

Volumetric and Gravimetric Meat-to-Shell Ratios

Grave (1912) suggested expressing the "fatness" of an oyster as the percentage of the shell-cavity volume occupied by meat. This can be directly related to the ratio used by Baird (1958) to measure the condition of mussels and oysters, i.e.,

$$\frac{\text{Meat volume}}{\text{Shell-cavity volume}} \times 100 = \text{Index of condition} \quad (1)$$

Baird reported that for oysters this index is not affected by the size of the specimen under examination but is size related for mussels. Thus for mussels the index is valid only for comparing animals of the same size (i.e., length). The index should not be used in cases where spawning is in any degree possible, however. In natural populations the spawning period may last for up to 6 months, and in populations exposed to abiotic thermal stimulation it can be even longer. Therefore, the index appears to be of limited value.

Hopkins (in Higgins, 1938) modified the approach of Grave (1912):

$$\frac{\text{Dry weight of meat (g)} \times 100}{\text{Shell-cavity volume (ml)}} = \text{Index of condition} \quad (2)$$

Similar derivations subsequently made by Medcof and Needler (1941) and Korringa (1955) have been used by a large number of

- Millar, R. H., 1963, Breeding and Gonadal Cycle of Oysters in Loch Ryan, Scotland, *J. Cons. Int. Explor. Mer.*, 28: 432-439.
- Nosho, T., and K. K. Chew, 1972, The Setting and Growth of the Manila Clam, *Venerupis japonica* (Deshayes), in Hood Canal, Washington, *Proc. Natl. Shellfish. Assoc.*, 62: 50-58.
- Ohba, S., 1959, Ecological Studies in the Natural Population of a Clam, *Tapes japonica*, with Special Reference to Seasonal Variation in the Size and Structure of Population and to Individual Growth, *Biol. J. Okayama Univ.*, 5: 13-42.
- Porter, H. J., 1964, Seasonal Gonadal Changes in Adult Clams, *Mercenaria mercenaria* (L.), in North Carolina, *Proc. Natl. Shellfish. Assoc.*, 55: 35-52.
- Potts, W. T. W., 1967, Excretion in the Molluscs, *Biol. Rev.*, 42: 1-41.
- Quayle, D. B., 1970, *Pacific Oyster Culture in British Columbia*, Bulletin 169, Fisheries Research Board of Canada, Ottawa.
- Ryther, J. H., and R. Mann, 1977, *Bivalve Mollusc Culture in a Waste Recycling Aquaculture System*, Technical Report 77-79, Woods Hole Oceanographic Institution, Woods Hole, Mass.
- Seed, R., 1968, Factors Influencing Shell Shape in *Mytilus edulis* L., *J. Mar. Biol. Assoc. U. K.*, 48: 561-584.
- , 1975, Reproduction in *Mytilus* (Mollusca: Bivalvia) in European Waters, *Pubbl. Stn. Zool. Napoli*, 39(Suppl.): 317-334.
- Shaw, W. N., 1964, Seasonal Gonadal Changes in Female Soft-Shell Clams, *Mya arenaria*, in the Tred Avon River, Maryland, *Proc. Natl. Shellfish. Assoc.*, 53: 121-132.
- , 1965, *Seasonal Gonadal Cycle of the Male Soft-Shell Clam, Mya arenaria*, in Maryland, Special Scientific Report, Fisheries Series No. 508, U. S. Fish and Wildlife Service, Department of the Interior, Washington, D. C.
- Snow, N. B., and P. J. Le B. Williams, 1971, A Simple Method to Determine the O:N Ratio of Small Marine Animals, *J. Mar. Biol. Assoc. U. K.*, 51: 105-109.
- Strickland, J. D. H., and T. R. Parsons, 1968, *A Practical Handbook of Sea Water Analysis*, Bulletin 167, Fisheries Research Board of Canada, Ottawa.
- Walne, P. R., 1958, Growth of Oysters (*Ostrea edulis* L.), *J. Mar. Biol. Assoc. U. K.*, 37: 591-602.
- , 1970, The Seasonal Variation of Meat and Glycogen Content of Seven Populations of *Ostrea edulis* L. and a Review of the Literature, *Fish. Invest.*, London, Ser. 2, 26(3): 1-35.
- , and R. Mann, 1975, Growth and Biochemical Composition in *Ostrea edulis* and *Crassostrea gigas*, in *Proceedings of the Ninth European Marine Biology Symposium*, H. Barnes (Ed.), pp. 587-607, Aberdeen University Press, Aberdeen, Scotland.
- Wells, W. H., and E. E. Gray, 1960, The Seasonal Occurrence of *Mytilus edulis* on the Carolina Coast as a Result of Transport Around Cape Hatteras, *Biol. Bull. (Woods Hole, Mass.)*, 119: 550-559.
- Widdows, J., 1973, Effect of Temperature and Food on the Heart Beat, Ventilation Rate and Oxygen Uptake of *Mytilus edulis*, *Mar. Biol.*, 20: 269-276.
- Wieser, W., 1972, Oxygen Consumption and Ammonia Excretion in *Ligia baudiana* M. E., *Comp. Biochem. Physiol.*, 43A: 869-876.

investigators since that date. This index is an improvement over the volume-to-volume ratio index in that it accommodates the increase in water content of the soft tissues and the decrease in dry-meat weight which occur on spawning (Masumoto, Masumoto, and Hibino, 1934; Quayle, 1970) and, thus, remains a valid test during periods when spawning is possible. However, Galtsoff (1964) stated that the comparison is valid (for oysters at least) only if specimens of the same species and age are used because oysters from over-crowded natural reefs and young oysters are usually flat, with very little inner space between the valves. Consequently, this index of condition will be relatively high because the soft tissues occupy almost the entire shell cavity.

Recently an alternative gravimetric index was used by Walne and Mann (1975) and Mann and Ryther (1977):

$$\frac{\text{Dry weight of meat (g)} \times 1000}{\text{Dry weight of shell (g)}} = \text{Index of condition} \quad (3)$$

This allows both entities used in the calculation to be assayed by the same method and, thus, facilitates the processing of a large number of samples. The method does not account for possible variations in shell-cavity volume caused by changes in overall shape of an individual animal or in shell thickness, but it does incorporate absolute shell size per se.

Changes in shape of bivalve molluscs have been extensively documented in the scientific literature. Ansell (1965), describing the interrelationship of a number of parameters of growth in mature *Venus (Mercenaria) mercenaria*, concluded that, if only the length is known for a given group of animals, the only transformations that can be accurately made are estimates of total weight, total volume, and shell-cavity volume. Thus, although his data indicate isodiametric shell growth estimates of wet and dry flesh weights, total body nitrogen and carbohydrate levels could not be estimated from length data because of changes related to the gametogenic cycle. Ohba (1959) suggested that growth in mature *Tapes japonica* was not isodiametric since year classes could be separated by significant differences in their breadth-to-length and breadth-to-height ratios. This conclusion was not supported by the data of Nosho and Chew (1972), however, who worked with the same species in Hood Canal, Washington. Seed (1968) documented a number of factors influencing shell shape in *M. edulis*.

A fourth index of condition was used recently by de Wilde (1975) in temperature and growth studies of *Macoma balthica*:

$$\frac{\text{Ash-free dry-meat weight} \times 1000}{\text{Shell length}^3} = \text{Index of condition} \quad (4)$$

Using the weight of ash-free dry meat rather than that of dry meat negates the effects of fluctuations in the ash content of the meat, and using the cube of shell length again assumes isodiametric growth but neglects any potential changes in shell thickness.

Indexes of condition (1 to 4) are all basically similar in that they attempt to compare present meat content with its potential maximum size using the shell as a reference material. This comparison is valid because, although meat content can either increase or decrease, the shell can only increase or remain constant in weight and volume (apart from some very small losses associated with abrasion). All these indexes can be somewhat misleading with respect to meat, however, because only the dry-meat indexes account for fluctuations in water content. None of these indexes give data on the proportionate quantities of available metabolic reserves, structural components, and gonadal material in the soft tissues.

Biochemical Indexes

Within the seawater temperature range experienced annually by a bivalve mollusc, there is generally a close relationship between the absolute temperature and the cycles of storage and gametogenesis in the animal (Masumoto, Masumoto, and Hibino, 1934; Loosanoff, 1936; Chipperfield, 1953; Walne, 1958; Millar, 1963; Porter, 1964; Shaw, 1964; 1965; Holland and Chew, 1974; Dare and Edwards, 1975). A brief overview of this relationship is relevant here before we discuss biochemical indexes per se. Obviously species differ in their overall strategies of storage and gametogenesis, as do different geographical populations of the same species (Seed, 1975). The four phases of the complete gametogenic cycle which can usually be identified are: (1) low metabolic activity, often coincident with cold water temperatures in the winter months; (2) high metabolic activity, often coincident with rising water temperatures in spring and with phytoplankton blooms; (3) rapid gametogenesis or completion of gametogenesis, coincident with depletion of carbohydrate reserves and accumulation of lipid in yolky gametogenic products; and (4) low levels of stored reserves. Spawning follows phase 3, and the subsequent period of low reserve levels (phase 4) is a result of the reserve's having been used in gonad proliferation and lost on spawning and the high metabolic demands associated with high water temperatures. Decreasing water temperatures in the fall months are often accompanied by some replenishing of reserves and,

- de Wilde, P. A. W. J., 1975, Influences of Temperature on Behavior, Energy Metabolism, and Growth of *Macoma balthica* (L.), in *Proceedings of the Ninth European Marine Biology Symposium*, H. Barnes (Ed.), pp. 239-256, Aberdeen University Press, Aberdeen, Scotland.
- Gabbott, P. A., 1975, Storage Cycles in Marine Bivalve Molluscs: A Hypothesis Concerning the Relationship Between Glycogen Metabolism and Gametogenesis, in *Proceedings of the Ninth European Marine Biology Symposium*, H. Barnes (Ed.), pp. 191-211, Aberdeen University Press, Aberdeen, Scotland.
- , and R. R. Stephenson, 1974, A Note on the Relationship Between the Dry Weight Condition Index and the Glycogen Content of Adult Oysters (*Ostrea edulis* L.) Kept in the Laboratory, *J. Cons. Int. Explor. Mer*, 35: 359-361.
- Galtsoff, P. S., 1964, *The American Oyster, Crassostrea virginica Gmelin*, Fishery Bulletin 64, U. S. Fish and Wildlife Service, Department of the Interior, Washington, D. C.
- Goldberg, E. D., 1975, The Mussel Watch. A First Step in Global Marine Monitoring, *Mar. Pollut. Bull.*, 6: 111.
- Gonzalez, J. G., and P. Yevich, 1976, Responses of an Estuarine Population of the Blue Mussel, *Mytilus edulis*, to Heated Water from a Steam Generating Plant, *Mar. Biol.*, 34: 177-189.
- Grave, Caswell, 1912 (Ed.), *A Manual of Oyster Culture in Maryland*, Fourth Report of the Shell Fish Commission of Maryland, 1912, pp. 279-348, reprinted in 1944 by King Brothers, Inc., Baltimore.
- Hammen, C. S., 1968, Amino Transference Activities and Amino Acid Excretion of Bivalve Molluscs and Brachiopods, *Comp. Biochem. Physiol.*, 26: 697-705.
- Higgins, E., 1938, *Progress in Biological Inquiries, 1937*, Report of the Commissioner of Fisheries for the Fiscal Year 1938, Appendix 1, Administrative Report No. 30, pp. 1-70, U. S. Bureau of Fisheries, Washington, D. C.
- Holland, D. A., and K. K. Chew, 1974, Reproductive Cycle of the Manila Clam (*Venerupis japonica*), from Hood Canal, Washington, *Proc. Natl. Shellfish. Assoc.*, 64: 53-58.
- Holland, D. L., and P. J. Hannant, 1974, Biochemical Changes During Growth of the Spat of the Oyster, *Ostrea edulis* L., *J. Mar. Biol. Assoc. U. K.*, 54: 1004-1016.
- , and P. J. Hannant, 1976, The Glycogen Content in Winter and Summer of Oysters, *Ostrea edulis* L., of Different Ages, *J. Cons. Int. Explor. Mer*, 36(3): 240-242.
- Ingle, R. M., 1949, A Comparative Study of Oyster Condition, *Science*, 109: 593.
- Korringa, P., 1955, Quality Estimation of Mussels and Oysters, *Arch. Fischer-eiwiss.*, 46: 189-193.
- Loosanoff, V. L., 1936, Sexual Phases in Quahog, *Science*, 83: 287-288.
- Mann, R., and J. H. Ryther, 1977, Growth of Six Species of Bivalve Molluscs in a Waste Recycling—Aquaculture System, *Aquaculture*, 11: 231-245.
- Masumoto, B., M. Masumoto, and M. Hibino, 1934, The Biochemical Studies of Magaki (*Ostrea gigas* Thunberg). II. The Seasonal Variation in the Biochemical Composition of *Ostrea gigas* Thunberg, *J. Sci. Hiroshima Univ. Ser. A*, 4: 47-56.
- Medcof, J. C., and A. W. H. Needler, 1941, The Influence of Temperature and Salinity on the Condition of Oysters (*Ostrea virginica*), *J. Fish. Res. Board Can.*, 5(3): 253-257.

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REFERENCES

- Allen, J. A., and M. R. Garrett, 1971, The Excretion of Ammonia and Urea by *Mya arenaria* L. (Mollusca: Bivalvia), *Comp. Biochem. Physiol.* 39A: 633-642.
- Ansell, A. D., 1965, Some Parameters of Growth of Mature *Venus mercenaria* L., *J. Cons. Int. Explor. Mer.* 29: 214-220.
- , and P. Sivadas, 1973, Some Effects of Temperature and Starvation on the Bivalve *Donax vittatus* (da Costa) in Experimental Laboratory Populations, *J. Exp. Mar. Biol. Ecol.* 13: 229-262.
- Baird, R. H., 1958, Measurement of Condition in Mussels and Oysters, *J. Cons. Int. Explor. Mer.* 23: 249-257.
- Bayne, B. L., 1973, Physiological Changes in *Mytilus edulis* L. Induced by Temperature and Nutritive Stress, *J. Mar. Biol. Assoc. U. K.*, 53: 39-58.
- , 1975, Aspects of Physiological Condition in *Mytilus edulis* (L.), with Special Reference to the Effects of Oxygen Tension and Salinity, in *Proceedings of the Ninth European Marine Biology Symposium*, H. Barnes (Ed.), pp. 213-238, Aberdeen University Press, Aberdeen, Scotland.
- , 1976 (Ed.), *Marine Mussels: Their Ecology and Physiology*, Cambridge University Press, New York.
- , P. A. Gabbott, and J. Widdows, 1975, Some Effects of Stress in the Adult on the Eggs and Larvae of *Mytilus edulis* L., *J. Mar. Biol. Assoc. U. K.*, 55: 675-689.
- , D. R. Livingstone, M. N. Moore, and J. Widdows, 1976, A Cytochemical and a Biochemical Index of Stress in *Mytilus edulis* L., *Mar. Pollut. Bull.*, 7(12): 221-224.
- , and C. Scullard, 1977a, Rates of Nitrogen Excretion by Species of *Mytilus* (Bivalvia: Mollusca), *J. Mar. Biol. Assoc. U. K.*, 57: 355-369.
- , and C. Scullard, 1977b, An Apparent Specific Dynamic Action in *Mytilus edulis* L., *J. Mar. Biol. Assoc. U. K.*, 57: 371-378.
- , and R. J. Thompson, 1970, Some Physiological Consequences of Keeping *Mytilus edulis* in the Laboratory, *Helgol. Wiss. Meeresunters.*, 20: 526-552.
- , J. Widdows, and C. Worrall, 1977, Some Temperature Relationships in the Physiology of Two Ecologically Distinct Bivalve Populations, in *Physiological Responses of Marine Biota to Pollutants*, F. J. Vernberg, A. Calabrese, F. P. Thurberg, and W. V. Vernberg (Eds.), pp. 379-400, Academic Press, Inc., New York.
- Chipperfield, P. N. J., 1953, Observations on the Breeding and Settlement of *Mytilus edulis* (L) in British Waters, *J. Mar. Biol. Assoc. U. K.*, 32: 449-476.
- Conover, R. J., and E. D. S. Corner, 1968, Respiration and Nitrogen Excretion by Some Marine Zooplankton in Relation to Their Life Cycles, *J. Mar. Biol. Assoc. U. K.*, 48: 49-75.
- Corner, E. D. S., and C. B. Cowey, 1968, Biochemical Studies on the Production of Marine Zooplankton, *Biol. Rev.*, 43: 393-426.
- Dare, P. J., and D. B. Edwards, 1975, Seasonal Changes in Flesh Weight and Biochemical Composition of Mussels (*Mytilus edulis* L.) in the Conway Estuary, North Wales, *J. Exp. Mar. Biol. Ecol.*, 18: 89-97.

in some species (e.g., *M. edulis*), by the initiation of gametogenic processes before low metabolic activity is again evident in relation to low water temperatures.

During the late stages of gametogenesis, glycogen reserves are rapidly depleted (Masumoto, Masumoto, and Hibino, 1934; Mann, unpublished data). Thus a statement like that of Galtsoff (1964): "Oysters of good quality have a relatively large amount of meat in relation to their total volume. Their glycogen content is high, and the meat has a creamy color . . ." can be misleading. The creamy color, caused by developing gonadal material, is produced at the expense of the glycogen reserves. Once gonad development is initiated and a temperature stimulus is maintained, development continues at the expense of stored reserves whatever the physiological state of the animal. This was well demonstrated for *M. edulis* by Bayne, Gabbott, and Widdows (1975). Also, Mann (unpublished data) observed gonad proliferation in juvenile *C. gigas* starved for a period of 3 weeks at 14.5°C.

A simple gross biochemical analysis for major components (carbohydrate, protein, lipid, and ash) or calorie content during late gametogenesis can also be misleading because the possibility exists that a considerable proportion of the energy-rich lipid component is stored in gametogenic products and is not generally available for energy metabolism by the animal.

Larval forms of oysters (and probably other bivalves) have a lipid-protein-based respiratory metabolism, but there is a gradual change to a carbohydrate-protein-based respiratory metabolism after settlement (Holland and Hannant, 1974). In an ecological sense this is a useful strategy since a high lipid content provides buoyancy in a pelagic larval form. This is unnecessary in a sessile adult in which the requirement for a readily mobilized reserve material is adequately served by glycogen. If the initial effect of a temperature stress is a drain of such reserve material, then, by definition, the quantity of available reserves can be used as an index of condition:

$$\% \text{ Carbohydrate content} = \text{Biochemical index} \quad (5)$$

As for the gravimetric and volumetric indexes (1 to 4), some problems are evident when we apply this index to a wide size range of animals. Metabolic functions, which will inevitably influence the rate of utilization of reserves, are generally related allometrically to the absolute size of the animal. Thus, when Holland and Hannant (1976) examined the summer and winter glycogen contents of five age groups of *Ostrea edulis* L., they found that, on a percentage

basis, summer levels were similar in all groups but winter levels increased with increasing age.

Since carbohydrate and protein are the major biochemical components of adult bivalve soft tissues (Masumoto, Masumoto, and Hibino, 1934; Dare and Edwards, 1975; Walne and Mann, 1975), an increase in the percentage content of one is usually reflected in a decrease in percentage content of the other. Since the depletion of carbohydrate is accompanied by an increased use of protein as a respiratory substrate, the ratio of these two components can also be used as an index of condition:

$$\frac{\text{Carbohydrate}}{N} = \text{Biochemical index} \quad (6)$$

The recent development of analytical instrumentation to both carbon and nitrogen contents simultaneously stimulated Ansell and Sivadas (1973) to suggest that the carbon-to-nitrogen ratio may be useful as an index of condition in the bivalve *Donax vittatus* (da Costa). This ratio gives a measure of the total organic content of the soft tissues in relation to protein content and thus, by inference, a measure of nonprotein organic material available for use as a respiratory substrate. Thus

$$\frac{C}{N} = \text{Biochemical index} \quad (7)$$

As in other gross biochemical components, there is a seasonal temperature-related cycle in the ash (inorganic) content of soft tissues (ash is defined as the material remaining after ignition at 500°C or higher for a period of 12 hr). In the oysters *Crassostrea gigas* and *Ostrea edulis*, percentage ash content is usually highest in the winter months and lowest in summer (Walne and Mann, 1975). The decrease in relative organic content in winter partially reflects the utilization of stored reserves during this period and, thus, can be considered a further index of condition:

$$\% \text{ Organic (100-ash) content} = \text{Biochemical index} \quad (8)$$

Physiological Indexes: Ammonia Excretion and the Oxygen-to-Ammonia Ratio

All the indexes discussed previously are based on quantitative measurements of a potentially utilizable reserve material, and all suffer from inadequacy because they do not account for rates of accumulation and depletion of such material. In this context certain

oysters, found a positive correlation between the two parameters ($r = 0.69$), but omitting the three highest values for glycogen content reduced the r value to 0.38. Ingle's general conclusion was that the relationship between these two parameters was poor, but it is notable that the glycogen contents of the oysters used in the study are very low (1 to 2% in most cases), and this probably is not a representative sample. By contrast, Walne (1970) and Gabbott and Stephenson (1974) found a good correlation in *O. edulis* between the dry-weight/shell-cavity ratio (index 2) and a glycogen condition index calculated as glycogen (g)/internal shell volume (ml) ($P < 0.01$ and $P < 0.001$, respectively).

Despite the constraints involved in the use of each regressed parameter, the present data suggest that any of these parameters can be used as equally valuable indexes of condition in the bivalves described here. Only further studies of other species of bivalves can confirm or eliminate these general relationships, but it is indeed reassuring to suggest that an easily affected volumetric or gravimetric assay of condition could be equally as meaningful as a more complex biochemical one. The use of physiological indexes, such as the O/NH_3 ratio, must await further investigation for a complete assessment of value.

In a more general context, the use of any index of condition to predict the bivalve's ability to withstand stress must rest upon an understanding of basic life processes in the animals. Despite many decades of intensive research, there are still areas markedly depauperate in documentation. For example, Bayne et al. (1976) recently noted changes in the taurine/glycine ratio in mussel tissue as a response to temperature stress. A complete physiological explanation of this phenomenon is not yet forthcoming, however, let alone an explanation that incorporates the effect of environmental stresses. In studies of the response of bivalves to thermal perturbation, further research is required at both the laboratory and field levels. In this respect the recent studies of Gonzalez and Yevich (1976) and Bayne, Widdows, and Worrall (1977) serve as excellent examples of the use of modern techniques to provide evidence of thermal stress at the biochemical, physiological, and cytological levels and will undoubtedly stimulate similar studies in the near future.

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TABLE 1
LINEAR REGRESSIONS OF BIOCHEMICAL INDEXES FOR
NATURAL POPULATIONS*

Biochemical index	Species		r	df	P
% Carbohydrate	<i>C. gigas</i>	$Y = +0.28X - 1.597$	+0.805	24	<0.001
	<i>O. edulis</i>	$Y = +0.665X - 5.087$	+0.812	10	<0.001
	<i>M. edulis</i>	$Y = +0.093X + 2.277$	+0.610	23	<0.001
Carbohydrate/N ratio	<i>C. gigas</i>	$Y = +0.043X - 0.595$	+0.833	24	<0.001
	<i>O. edulis</i>	$Y = +0.075X - 0.857$	+0.831	10	<0.001
	<i>M. edulis</i>	$Y = +0.0115X - 0.025$	+0.541	23	<0.02
% Organic content	<i>C. gigas</i>	$Y = +0.059X + 89.3$	+0.783	24	<0.001
	<i>O. edulis</i>	$Y = +0.526X + 64.01$	+0.728	10	<0.01
	<i>M. edulis</i>	$Y = +0.035X + 80.62$	+0.784	23	<0.001

*All data take the form $Y = MX + b$. $Y =$ % carbohydrate, carbohydrate/N ratio, and % organic (100-ash) content and $X =$ dry-meat (mg)/dry-shell (g) ratio in natural populations of *C. gigas* (Masumoto, Masumoto, and Hibino, 1934), *O. edulis* (Walne and Mann, 1975), and *M. edulis* (Dare and Edwards, 1975).

TABLE 2
LINEAR REGRESSIONS OF BIOCHEMICAL INDEXES
FOR LABORATORY POPULATIONS*

Biochemical index	Species		r	df	P
% Carbohydrate	<i>C. gigas</i>	$Y = +0.192X - 1.832$	+0.811	38	<0.001
	<i>O. edulis</i>	$Y = +0.576X + 10.37$	+0.701	38	<0.001
	<i>T. japonica</i>	$Y = +0.097X + 2.71$	+0.564	38	<0.001
Carbohydrate/N ratio	<i>C. gigas</i>	$Y = +0.032X - 0.791$	+0.802	38	<0.001
	<i>O. edulis</i>	$Y = +0.092X + 1.784$	+0.725	38	<0.001
	<i>T. japonica</i>	$Y = +0.014X + 0.062$	+0.558	38	<0.001
C/N ratio	<i>C. gigas</i>	$Y = +0.021X + 3.308$	+0.804	38	<0.001
	<i>O. edulis</i>	$Y = +0.054X + 2.73$	+0.803	38	<0.001
	<i>T. japonica</i>	$Y = +0.008X + 3.612$	+0.688	38	<0.001
% Organic content	<i>C. gigas</i>	$Y = +0.201X + 64.12$	+0.528	38	<0.001
	<i>O. edulis</i>	$Y = +0.306X + 70.66$	+0.844	38	<0.001
	<i>T. japonica</i>	$Y = +0.059X + 80.07$	+0.855	38	<0.001

* $Y =$ % carbohydrate, carbohydrate/N ratio, C/N ratio, and % organic (100-ash) content and $X =$ dry-meat (mg)/dry-shell (g) ratio in populations of *C. gigas*, *O. edulis*, and *T. japonica* maintained at 12, 15, 18, and 21°C for 19 weeks. Data, pooled for all temperatures, take the form $Y = MX + b$.

physiological parameters can be of value. Oxygen consumption rate can be usefully employed as an index of physiological rate. In bivalve molluscs, however, the respiratory response to temperature stress is complex; both short-term acute and long-term acclimation responses may be involved. These have been extensively described for *M. edulis* by Bayne and co-workers (Bayne, 1973; Widdows, 1973; see also a comprehensive review in Bayne, 1976). Furthermore, these responses can be superimposed on short-term fluctuations caused by feeding activity (Bayne, 1973; Bayne and Scullard, 1977b). The nature of such responses may vary somewhat between species, however; e.g., Ansell and Sivadas (1973) stated that *Donax vittatus* does not exhibit thermal acclimation of its respiratory processes.

It is evident that the use of respiration data alone can provide some distinct interpretive problems. Its use in conjunction with other physiological parameters, however, can alleviate some of these problems. The cycles of accumulation and depletion of carbohydrate reserves in bivalves and the increasing use of protein as a respiratory substrate after depletion have been briefly discussed earlier. Increased use of protein as a respiratory substrate inevitably results in an increased rate of ammonia excretion. A combination of oxygen consumption rate (O) and ammonia excretion rate (NH_3) can be used in the form of an O/ NH_3 ratio to assess what proportion of the respiratory substrate is protein and, thus, to infer the status of an animal's reserves:

$$\frac{\text{O}}{\text{NH}_3} = \text{Physiological index} \quad (9)$$

The O/ NH_3 ratio was used previously in studies of isopods (Wieser, 1972) and the bivalves *M. edulis* (Bayne, 1973; Bayne and Scullard, 1977a) and *Donax vittatus* (Ansell and Sivadas, 1973). Note that the ammonia excretion rate, not total nitrogen elimination, should be used to evaluate the nature of the substrate being oxidized (see Ansell and Sivadas, 1973). Total nitrogen includes a variable amount of amino acids and urea (Potts, 1967; Hammen, 1968; Allen and Garrett, 1971; Bayne, 1976) which may, in part, be connected with osmoregulatory rather than excretory functions. The ratio of oxygen consumed to total nitrogen excreted has been used in studies of copepods (Conover and Corner, 1968; Corner and Cowey, 1968), prawns (Snow and Williams, 1971), and *M. edulis* (Bayne and Thompson, 1970).

A seasonal variation in the O/ NH_3 ratio occurs in conjunction with the natural gametogenic cycle, but using a ratio rather than the absolute physiological rates can partially eliminate some of the

problems involved in comparing animals of differing sizes. Physiological functions can be used to compare animals of differing sizes if we know the allometric relationship of that function to size. Thus, if

$$P = aW^b$$

where P is the specific rate function, W is the weight, and a and b are constants, animals of differing sizes can be compared by expressing the rate function using the "physiological weight" (W^b) rather than the absolute weight (W). Thus comparisons of weight-specific ammonia excretion rates change from

$$\mu\text{g NH}_3 - \text{N g dry } W^{-1} \text{ hr}^{-1}$$

to

$$\mu\text{g NH}_3 - \text{N g dry } (W^b)^{-1} \text{ hr}^{-1}$$

The nature of the allometric relationship may also change with the seasonal gametogenic cycle. Bayne and Scullard (1977a) described this change for ammonia excretion in *M. edulis*. Ryther and Mann (1977) described a similar increase in weight-specific ammonia excretion rates and a change in the allometric relationship of this rate to dry-meat weight after spawning in the oysters *Crassostrea gigas* and *Ostrea edulis*.

COMPARING GRAVIMETRIC AND BIOCHEMICAL INDEXES: MATERIALS AND METHODS

Literature Data

Data from Masumoto, Masumoto, and Hibino (1934) for *C. gigas* (Kusatsu stock grown at Kusatsu), from Dare and Edwards (1975) for 65-mm-long *M. edulis*, and from Walne and Mann (1975) for *O. edulis* were recalculated to obtain values for percentage carbohydrate, carbohydrate/N ratios, percentage organic content, and dry-meat/dry-shell ratios for the shellfish stocks examined. Data for each biochemical index for each species were compared to the gravimetric condition index by linear regression analysis.

Laboratory Studies

An experiment was performed to delineate the effect of temperature on growth, biochemical storage cycles, and gameto-

genesis in three species of bivalves when all other environmental factors (salinity, water flow, and phytoplankton concentration) were constant. Four populations of each of three species (*Crassostrea gigas*, *Ostrea edulis*, and *Tapes japonica*), matched by numbers of animals and mean individual live weight, were selected from common parental stocks. Populations of each species were maintained at sustained temperatures of 12, 15, 18, and 21°C for 19 weeks. Samples of 15 to 20 animals of each species were sacrificed at biweekly intervals for assay of live weight, freeze-dried meat weight, dry-shell weight (100°C for 24 hr), and the following biochemical components:

1. Carbon and nitrogen assayed by a Perkin-Elmer CHN analyzer
2. Carbohydrate assayed by the anthrone method of Strickland and Parsons (1968) after extraction in cold 5% wt/vol trichloroacetic acid
3. Ash, defined as the material remaining after ignition for 12 hr at 500°C

Within each species-temperature combination, data for each of the biochemical indexes (% carbohydrate, carbohydrate/N ratio, C/N ratio, and % organic content) were compared to the gravimetric dry-meat/dry-shell condition index by linear regression analysis. Since no significant differences in values of both slope and intercept were found between temperatures within each species, data for all temperatures were pooled and recalculated to obtain one regression line for each biochemical-condition index combination for each species.

RESULTS AND DISCUSSION

Linear regressions of biochemical and gravimetric indexes for natural and laboratory populations are summarized in Tables 1 and 2, respectively. A good correlation is evident in all regressions for all species tested. It is notable, however, that, although slope and intercept values are similar for *C. gigas* in both natural and laboratory populations for percentage carbohydrate and carbohydrate/N ratio computations, only slope values are similar for natural and laboratory populations of *O. edulis*. This indicates a consistently higher carbohydrate content in laboratory animals. A complete explanation of this phenomenon must await further study, however.

Previously a number of investigators examined the use of glycogen content as an index of condition in bivalve molluscs, usually by direct comparison of dry-meat/shell-cavity ratios (index 2) with percentages of glycogen. Ingle (1949), comparing 34 samples of