THE USE OF PHYSIOLOGICAL CONDITION INDICES IN MARINE BIVALVE AQUACULTURE

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ABSTRACT


The physiological condition indices most commonly used in bivalve aquaculture are reviewed and evaluated. Two general categories of indices may be distinguished: static and dynamic. Of the static indices reviewed, only one is recommended for use in the culture of early developmental stages — the dry ash weight : total dry weight ratio. In the case of juveniles and adults, the easily-measured dry tissue weight : dry shell weight is recommended for most routine work.

The dynamic indices reviewed are based on production estimates, and hence reflect physiological changes over specified time intervals. Of these indices, only the net growth efficiency is recommended for use in bivalve aquaculture, and it is applicable to all life stages. While this index gives the most information about the physiological state of the animals comprising a population, it is very difficult to evaluate and is thus most appropriate as a research tool.

INTRODUCTION

Various condition indices have been used in bivalve aquaculture, and it appears desirable at this point to review these and to make specific recommendations for the use of the more appropriate ones. Engle (1949) and Mann (1978) have previously reviewed some of the more common condition indices; however, recent developments in this area have made a comprehensive critical review necessary.

Condition indices in aquaculture may serve two purposes. The first is an economic one, in which the index is used to designate the quality of a marketed product. The second is an ecophysiological one, in which the index is used to characterize the apparent “health” of a cultured stock; or, in other words, to summarize the physiological activity of the animals (growth, reproduction, secretion, etc.) under given environmental conditions. The present work is concerned only with the latter utilization of condition indices. Consequently, those indices involving variables with little or no physio-
logical significance will not be considered here. Such variables include the following:

(a) size of shell, in those species of variable shell form, such as oysters — e.g., the condition index of Imai and Sakai (1961): thickness × \[1/2 (\text{length} + \text{width})\]^{-1};

(b) the cube-length, even for those species of constant form — e.g., the conditions index of Beukema and De Bruin (1977): ash-free dry flesh weight × \(L^{-3}\), or that of Ansell et al. (1980): percent dry weight × \(L^{-3}\);

(c) the total weight, due to the extreme variability in the amount of intervalval (or mantle) fluid in the majority of bivalves — e.g., the condition indices of Ansell et al. (1964), Lee et al. (1975) and Booth (1983): wet flesh weight × total weight^{-1}.

All of the preceding types of indices give an assessment of the market quality of bivalves. There are, however, a number of indices which incorporate meaningful physiological or biochemical variables, indicative of the metabolic state of the animal. Two such types of indices may be distinguished:

(a) Those which are determined at a single point in time, thus giving information about the physiological state of the animal at a given moment. These may be termed static indices.

(b) Those indices which are determined over a period of time in a given population, and which therefore give information about the physiological changes in the individuals comprising the population. These may be called dynamic indices.

1. STATIC INDICES

1.1. Generalized indices applicable to most animals or organs

1.1.1. The dry weight : wet weight ratio. This index may be calculated for a whole animal, or for the flesh in the case of bivalves. It may also be calculated for an organ, e.g., the gonad.

This ratio expresses the proportion of dry matter in the whole tissue, which thus also denotes the proportion of water. Since it is known that a high proportion of water in tissue often signifies a state of depleted energy reserves, as observed in starvation experiments (Wilkins, 1967; Johnston and Goldspink, 1973) or winter conditions (Barnes et al., 1963; Ansell, 1975; Taylor and Venn, 1979; Shafee, 1981; Beninger and Lucas, 1984), this easily-determined ratio may be used to indicate the energy balance of a tissue or an animal. Its utilization in aquaculture is thus recommended.

1.1.2. The RNA : DNA ratio. It has been demonstrated in bivalves and other aquatic organisms that the RNA level, and in particular the RNA : DNA ratio, is directly related to ongoing tissue growth (Sutcliffe, 1965, 1970; Bulow, 1970; Haines, 1973; Holland and Hannant, 1973). The greatly-increased enzyme-protein synthesis during growth probably explains this
phenomenon. However, the method has been reported to lack sensitivity (Dagg and Littlepage, 1972), and in some cases no positive relationship between RNA levels and growth rates may be found (Helm et al., 1973). In addition, RNA and DNA levels may only be measured using laboratory techniques, which are seldom available to aquaculturists. Until more universal and reliable results are obtained using this method, it should not be recommended as a condition index.

1.1.3. Adenylate energy charge. Atkinson and Walton (1967) proposed the use of the adenylate energy charge (AEC) as a biochemical indicator of environmental stress. This index, of the form

\[ AEC = \frac{(ATP + 1/2 ADP)}{(ATP + ADP + AMP)} \]

has a maximum value of 1, but is maintained between 0.87 and 0.94 in most unstressed organisms studied (Vetter and Hodson, 1982). Its use is becoming widespread and the AEC has been utilized by Wijsman (1976) in a study on *Mytilus edulis*.

While very sensitive, this index poses two major problems: the first is that adenylate levels are quite time-consuming and difficult to measure at the present time; and the second is one of sample collection. As noted by Vetter and Hodson (1982), violent muscle contractions may drastically alter cellular ATP levels; this compromises the validity of AEC measurements in bivalves, which are likely to contract their muscles frequently and violently during manipulation.

For these reasons, the use of the AEC is not recommended as a condition index in bivalves.

1.2. Indices particular to bivalves

In juvenile or adult bivalves, the presence of a relatively large quantity of both a mineral shell and a tissuey body greatly facilitates estimates of condition. In effect, the shell represents cumulative growth, being the secretory product of the animal's metabolism, whereas the amount of body tissue may vary greatly depending on the current sexual and metabolic activity of the organism. It is thus possible to evaluate the extent of current metabolic or reproductive activity by comparing the amount of tissue to the amount of shell.

In bivalve larvae and postlarvae, however, the small quantity of shell and tissue renders the utilization of condition indices based on the separation of each component impractical. Special indices have thus been proposed for these life stages and these will be discussed where appropriate.

1.2.1. The wet flesh weight : wet shell weight ratio. Although by far the easiest index to measure, since most bivalves are shucked when fresh, there are several problems with the use of the wet flesh weight : wet shell weight
ratio (Walne, 1976). Firstly, the notion of "drained" flesh is physiologically vague and practically very difficult to standardize. This is also true of the "wet" shell weight. Secondly, as mentioned previously, in certain states of poor physiological condition, such as prolonged starvation, bivalves tend to compensate organic losses with water uptake; hence giving a false impression of their real condition. For these reasons, the following index is greatly preferred.

1.2.2. The dry flesh weight : dry shell weight ratio. This widely-used condition index, because of the nature of the measurements involved, is easily standardized and is thus much more universal. In addition, as previously mentioned, the use of dry tissue weights eliminates the bias due to water content fluctuations of whole tissue. A low value for this index indicates that a major biological effort has been expended, either as maintenance energy under poor environmental conditions or disease, or in the production and release of gametes. Thus, as an indicator of stress, or sexual activity, this index gives meaningful information about the physiological state of the animal.

Two types of dry flesh measurements have been used to calculate this index: dry flesh weight (Walne, 1976; Mann and Glomb, 1978), and ash-free dry weight, or AFDW (Walne and Mann, 1975). In theory, the latter index is more precise, since ash content, like that of water, has been known to increase under unfavorable physiological conditions (Wilkins, 1967; Mayzeaud, 1976), and hence partially mask these conditions. In practice, however, the two ratios follow each other quite closely, as demonstrated in a recent study involving two clam species raised under unfavorable environmental conditions (Beninger and Lucas, 1984).

Due to its easy standardization and measurement as well as its physiological validity, this probably represents the best adult static condition index now available.

In the case of larvae and postlarvae, where dissection is impractical, Walne and Millican (1978) have demonstrated that the ratio dry ash weight : total dry weight of whole powdered oyster spat corresponded quite well to the more conventional dry tissue weight : dry shell weight ratio. Although a quantitative relationship was not presented, a linear regression of their data yields a correlation coefficient ($r^2$) of 0.91 ($P \leq 0.001$). It may thus be concluded that such a technique could prove useful for evaluating the condition of spat in a hatchery or nursery. However, it should be emphasized that the statistical significance and the exact relationship between the two indices should be established for each species under given hatchery conditions, before using this method in a routine manner.

At present, this index constitutes the only practical, quantitative static estimator of spat condition; its simplicity and validity make it highly recommendable for use in bivalve aquaculture.
1.2.3. Gross biochemical component indices — glycogen : dry flesh weight, glycogen : protein, C : N, and lipid staining. Glycogen has long been recognized as the principal energy reserve in juvenile and adult bivalves (Giese, 1967, 1969), both as an energy reserve under unfavorable environmental conditions (Reid, 1969; De Zwaan and Zandee, 1972; Beukema and De Bruin, 1977), and also for the formation of gametes, as suggested by Reid (1969), Walne (1970), Gabbott (1975), Mann (1979a,b), and Barber and Blake (1981). It may thus be concluded that a knowledge of the proportion of glycogen in the animal is a good index of its metabolic state (Gabbott and Stephenson, 1974).

This does appear to be the case for the ratio glycogen : dry weight, as evidenced by the numerous studies of the seasonal variation of bivalve biochemical composition (see preceding references this section), often in conjunction with studies of the reproductive cycle (Williams, 1969; Ansell, 1972, 1974, 1975; Shafee, 1978, 1981; Ansell et al., 1980). However, the related index glycogen : protein (G : P) does not appear to be a good indicator of the physiological state of bivalves, as demonstrated by Gabbott and Walker (1971). In this study, both glycogen and protein levels declined in hatchery-reared oysters, while both components increased in field-reared animals. As a result, the G : P ratios were not significantly different, even though the condition of the two groups was very different.

It is thus safe to assume that while the glycogen : dry flesh weight is probably a good indicator of bivalve condition, the more tediously-measured G : P index should be avoided. The same argument applies to the C : N ratio, which has been recommended or used as a type of condition index in bivalves (Bayne and Thompson, 1970; Ansell, 1974).

In contrast to juveniles and adults, lipid has been shown to constitute the main energy reserve in early developmental stages of bivalves (Holland and Spencer, 1973; Holland and Hannant, 1974; and review by Holland, 1978). A simple technique has recently been developed to qualitatively assess the condition of bivalve larvae based on the Sudan Black B lipid stain (Gallager and Mann, 1981). Although quite useful, this method is qualitative, and quantitative evaluations have not yet been performed. However, the approach used is promising, and further studies in this area are to be encouraged.

1.2.4. Volumetric indices. The dry tissue weight to intervalval volume ratio has been used in bivalve studies, chiefly in oysters (Higgins, 1938; Walne, 1970, 1976; Gabbott and Stephenson, 1974; Lee et al., 1975; Giguère and Poirier, 1980; Lawrence and Scott, 1982; Paul, 1983). In general, these indices have suffered from poor precision due to the displacement methods used to measure internal shell volume. Recently, however, Lawrence and Scott (1982) have proposed a method based on the assumption that the density of oyster tissue is very close to that of water; the precision and reliability of measurement was reported to be quite good. While the index
proposed by Lawrence and Scott (1982) would certainly be useful in oyster studies, it is unlikely that the underlying assumptions are applicable to all bivalves.

In any event, there is no physiological significance in the quantity of mantle fluid of a submerged bivalve, and in fact this quantity remains unknown (although it is included in the measurement of "intervalval volume"). For these reasons, the use of condition indices incorporating intervalval volumes is not recommended. Chemical condition indices based on intervalval volume have also been proposed (Whyte and Englar, 1982). In addition to the problems previously mentioned, these indices do, of course, require laboratory techniques and are thus impractical as routine static indices. Moreover, their correlation with the dry tissue indices vary according to the constituent chemical. Therefore, the simple, easily-interpreted and more universal dry tissue : dry shell weight ratio is recommended for use as a static condition index.

2. DYNAMIC INDICES

The numerator and denominator of most dynamic indices are calculated by taking the difference in the values of the two parameters measured, the values being separated by a chosen time interval. If the data collection requires the animals to be sacrificed, great care should be taken to minimize sampling bias at each successive sampling.

2.1. Net growth efficiency

This index has been developed from the analysis of energy budgets and must be expressed in energy units. According to the recommendations of the IAPSO (1979), the use of the calorie is "strongly discouraged". The SI unit for energy is the joule and the conversion factor is 1 cal = 4.18 J.

Net growth efficiency is the efficiency with which the assimilated energy is utilized for tissue growth

\[ NGE = \frac{P_g}{A} \]

\( P_g \) represents tissue growth or, more precisely, the variations in biomass during the period considered. These variations can be positive, zero or negative.

Calculation of \( A \), as shown by Lucas and Shafee (1983), depends on the sign of \( P_g \).

If \( P_g > 0 \)

\[ A = C - FU = P + R \]

Assimilation = consumption — (feces urine) = production + respiration,
where

\[ P = P_g + Pr + Ps + Pe \]

Production = tissue growth + reproduction + secretions + eliminated tissues, thus:

\[ A = C - FU = (P_g + Pr + Ps + Pe) + R \]

If \( P_g < 0 \)

\[ A = (C - FU) - P_g = (Pr + Ps + Pe) + R \]

According to Lucas and Shafee (1983), \( Pr + Ps + Pe \) can be considered as "non-biomass production" and \( P_g \) negative (thus \( -P_g \) positive) as part of assimilated biomass. In this case, assimilation \((A)\) has a double origin: external \((C - FU)\) and internal \((-P_g)\). If starvation is complete, no external food is assimilated and \( A = -P_g \). The NGE index becomes \( P_g/-P_g \) or \(-1\), which represents the minimal value of this index.

In summary, a NGE value of 0 indicates that metabolic requirements are just balanced by food intake, and therefore no tissue growth takes place. Values greater than 0 (but less than 1) indicate a state of positive energy balance, and their magnitude is an indicator of the intensity of tissue growth. Conversely, values less than 0 reflect a state of negative energy balance, indicating that the organism is using its own energy reserves to meet metabolic demands.

Production measurements, such as growth \((P_g)\) and secretions (e.g., byssus, shell organic matter) should be made at regular time intervals, scaled to the developmental stage being studied: monthly for adults, fortnightly for juveniles, weekly for postlarvae, and daily for larvae. Estimations of feces production \((F)\), excretion \((U)\), oxygen consumption \((R)\) and food consumption \((C)\) are usually performed over some hours in vitro, and these data are extrapolated in situ for 1 month in the case of adults. Therefore, the validity of these data strongly depends upon the precision of the laboratory measurements.

The estimation of organic matter energy content \((P, FU, C)\) may be performed either by direct bomb calorimetry or by determining the biochemical composition of the organic matter and converting this to the energy values. In the latter case, particular attention should be paid to the conversion factor used. For carbohydrate, the conversion factor of 17.1 kJ g\(^{-1}\) is generally accepted (Paine, 1971; Ansell, 1972, 1974, 1975; Beukema and De Bruin, 1979; Ansell et al., 1980). The protein conversion factor has recently been modified from 23.6 to 17.8 kJ g\(^{-1}\) in ammoniotelic animals (Beukema and De Bruin, 1979) due to the incomplete oxidation of protein by such organisms. Conversion factors of lipids vary with the extraction method, the greatest values (39.5 kJ g\(^{-1}\)) being associated with incomplete extraction techniques, yet often applied even when more complete extractions are performed (Winberg, 1971; Ansell, 1972, 1974, 1975;...
Shafee, 1978, 1981; Ansell et al., 1980). More accurate values for bivalves have since been determined, ranging from 35.2 kJ g\(^{-1}\) (Beukema and De Bruin, 1979) to 32.9 kJ g\(^{-1}\) (Beninger and Lucas, 1984).

The estimation of oxygen consumption (\(R\)) in energy units is obtained using the oxycalorific factor, for which the generally accepted value is 20.3 J ml O\(_2\)\(^{-1}\) (Ivlev, 1935; Dame, 1972).

Other efficiencies (net production efficiency \(PA\)^{-1}, gross growth efficiency \(PgC\)^{-1} and gross production efficiency \(PC\)^{-1}) have been defined for annual energy budgets. Lucas and Shafee (1983) have shown that these efficiencies cannot be calculated over short periods, when \(Pg\) is negative. Thus, only the \(PgA\)^{-1} efficiency is ecophysically meaningful, providing it is calculated according to the above-mentioned recommendations and applying equations (1) and (2).

Such calculations have been performed for a wild population of Chlamys varia in the Bay of Brest (Lucas and Shafee, 1983), and also for a cultivated population of Crassostrea gigas in the Marennes-Oléron basin (Deslous-Paoli and Heral, 1984). In both studies, negative values were obtained for certain months of the year. It is thus clear that this type of analysis can be used to detect periods of physiological weakness in a given population, therefore constituting an invaluable indicator in aquaculture.

An important advantage of this index is that it is easily interpreted, regardless of the species or the geographical location; its meaning is universal. This is a considerable improvement over most of the previously-mentioned condition indices, which are often species- and location-specific, necessitating comparisons with previous publications in order to interpret their meaning.

Another equally important advantage of this ratio as a condition index is that it constitutes a sensitive method for assessing and quantifying the physiological condition of an organism in a given environment for the aquaculture of that species. The major drawback of this index is that it is difficult to calculate, as it requires several types of sensitive laboratory determinations.

### 2.2. Scope for growth

This concept has been widely used by a number of workers (Warren and Davis, 1967; Bayne et al., 1975; Bayne and Widdows, 1978; Widdows, 1978), but will be mentioned only briefly here. In practice, the term “scope for growth” is synonymous with the bioenergetic term “production”, since

\[ Sc = A - R = P \] (Worrall et al., 1983)

Consequently, \(Sc\) is not an index and, moreover, its designation is inappropriate because it leads to confusion between “growth” and “production” (since, in this context, growth is regarded as the net change in energy content of the animal in unit time — Bayne et al., 1975). As reported by Lucas (1982), this confusion has been made by most authors who have
studied the bioenergetics of bivalves, beginning with Crisp (1971) and continuing through to Shafee and Lucas (1982).

2.3. O : N ratio and relative maintenance cost

Physiological parameters such as oxygen consumption and nitrogen excretion may be measured over a given period of time to assess the physiological state of cultivated bivalves. The O : N ratio is defined as the ratio, by atomic equivalents, of oxygen consumed to ammonia-N excreted. This index does not require the sacrifice of the animals studied, and so may be evaluated at given time intervals or under varying environmental conditions using the same group of organisms. It is thus possible to follow the physiological changes in a given group of organisms over a given time period or under varying environmental conditions. Values less than 30 signify a considerable rate of protein catabolism, generally associated with a condition of stress (Widdows, 1978), whereas values greater than 50 suggest a high rate of lipid and carbohydrate metabolism. Food availability is reported to greatly influence the O : N ratio; starvation and very low ration levels result in low O : N values of approximately 20, but these values increase to 40 or 50 at high food concentrations (Bayne, 1973a,b; Gabbott and Bayne, 1973; Ansell and Sivadas, 1973; Widdows, 1978). Although the O : N ratio does not sum up the physiological condition of an animal as well as does the net growth efficiency, it does provide meaningful data on the metabolic state of an organism and it may thus be used to complement the NGE index.

Fisher's (1977) "relative maintenance cost" represents another physiological index which does not require the sacrifice of the animals under study. This index is used to evaluate the extent to which the environmental food supply meets the suspension-feeders' requirement to elaborate new tissue. As in the case of the O : N ratio, the relative maintenance cost may be evaluated at various time intervals for a given group of individuals. Bayne and Widdows (1978) used this index in a comparative seasonal study of two Mytilus edulis populations.

Several considerations render the relative maintenance cost unsuitable as a generalized condition index: the measurements required are quite difficult to perform and there are no reference values. In addition, poor condition may sometimes be independent of environmental food supply (disease, temperature, etc.), and these states would not be reflected in the relative maintenance cost.

CONCLUSION

None of the physiological condition indices herein reviewed may be measured in the field, and those involving the measurements of tissue weights require the sacrifice of the sampled animals. For field evaluations of the
apparent health of a cultured stock, it may suffice to simply observe the
temporal variations in shell size or total weight. However, these variables
are greatly influenced by the chronological age of the animal, while true
condition indices summarize the overall physiological status of the organism.
It is thus clear that there is no real substitute for appropriate physiological
condition indices. An index is appropriate only if it accurately and faith-
fully reflects the variations in the intensity of anabolic activity, and in
particular tissue growth.

Of the condition indices reviewed in the present paper, the net growth
efficiency undoubtedly represents the most informative physiological
condition index. However, measurement of the NGE requires precision
instruments and skilled workers, especially for the measurements of respira-
tion, and hence it is of most value as a research tool.

The remaining condition indices are all static. Only one is practical and
appropriate for use in larvae and spat — the dry ash weight : total dry weight
ratio. Of the three static indices recommended for adult bivalves, the dry
tissue weight : dry shell weight ratio, with its comparative ease of measure
and universal application, should be adopted for most routine aquaculture
work, as suggested by Mann (1978) in his earlier review of static condition
indices. The ash-free dry tissue weight : dry shell weight and glycogen : dry
tissue weight ratios, although good indicators of bivalve condition, are more
tedious to measure and, in most cases, probably do not significantly increase
our knowledge of physiological condition.

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