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COMMENT

N-specific metabolic data are not relevant to the 'visual interactions' hypothesis concerning the depth-related declines in metabolic rates: Comment on Ikeda et al. (2006)

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ABSTRACT: Ikeda et al. (2006; Mar Ecol Prog Ser 322:199–211) declared that they were testing the 'visual interactions' hypothesis for the decline in metabolic rates with depth in midwater animals, but their data and data analysis are irrelevant to this hypothesis for 4 reasons: (1) they do not show changes relative to the live mass of the animals studied; (2) they do not compare species-specific data; (3) they do not use evolutionarily significant descriptors of habitat depth; and (4) there are significant differences in the respirometry methods within their own data sets, and between those data and the data that support the 'visual interactions' hypothesis. Live weight mass-specific expression of rates is necessary when considering hypotheses concerning the evolution and function of living animals, although N-specific expressions may be useful in other contexts.

KEY WORDS: 'Visual interactions' hypothesis · Locomotor decline hypothesis · Midwater habitat · Mesopelagic · Bathypelagic · Crustacea · Metabolic rates

Ikeda et al. (2006a) presented a dataset of O_2 consumption rates in deep-living copepods, and they compared these data with other data on metabolic rates of deep-living pelagic animals. Ikeda et al. (2006a) used methods that are completely different from those in previous studies, and claimed to refute a large and consistent body of literature carried out with carefully defined and consistent methods. Their data have no relevance to the hypotheses which they purport to reject. Their erroneous conclusions have resulted in confusion in modeling studies of oceanic zooplankton carbon demand (Steinberg et al. 2008). Below, we provide a brief history of the hypotheses in question and describe 4 principal errors which invalidate the conclusions in Ikeda et al. (2006a).

Hypotheses on metabolic declines with depth

The decline in metabolic rates with depth was first documented in midwater crustaceans by Childress (1969, 1971, 1975) and in fishes by Torres et al. (1979). The realization that this decline was related to declines in muscle power was put forward by Childress & Somero (1979), and Childress et al. (1980, p. 38) put forward an overarching hypothesis identifying reduced locomotor capacity as the source of metabolic decline:

> Evidently, the critical selective factor(s) is not energy conservation but selection for well-developed locomotion in species which live near the surface and the relative weakness and progressive diminution of such selective

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pressure at greater depths. The following factors might select for locomotory abilities and decrease progressively with depth: visual predation, water turbulence and whatever selects for vertical and horizontal migrations. If one were to find an animal occupying a niche in the deep sea that required high locomotory activities one would expect it to have a correspondingly high metabolic rate. In other words, patterns of structural and metabolic reduction with depth in pelagic animals are made possible by diminution with depth of selective factors which are strongest at the surface, and these selective factors have little to do with energy supply per se, but rather with the locomotory abilities of the organisms.

Subsequent studies found a correlation between the presence or absence of image-forming eyes in a taxon and the magnitude of metabolic decline: metabolism declines by several orders of magnitude with depth in cephalopods, while groups without image-forming eyes show negligible declines, if any. The 'visual interactions' hypothesis (a sub-hypothesis of the locomotory decline hypothesis) was postulated by Childress & Mickel (1985) to explain these patterns. It attributes large declines in metabolic rates with depth in fishes, crustaceans and cephalopods having image-forming eyes to the relaxation of selection for strong locomotor abilities for both predators and prey as the visual reactive distances decline with increasing depth. The corollary is that the absence of large declines in other groups such as cnidarians, chaetognaths, and copepods reflects the lack of such depth-related selection for species without image-forming eyes. Many studies reviewed in Childress & Thuesen (1992), Childress (1995) and Seibel & Drazen (2007), have added data that support these hypotheses.

Importance of using live weight in evolutionary studies

The crux of the issue is that Ikeda et al. (2006a) expressed their metabolic rate data relative to N content of the copepods. In biological oceanography, O_2 consumption rates are often expressed relative to dry weight, N, or C. This has been driven by difficulties in weighing samples at sea and a primary interest in questions of elemental cycling. This tradition has not been concerned with the characteristics of individual organisms, species and the evolution of those properties.

In contrast, previous studies on metabolic rates in midwater animals have been cast in terms of the properties of intact living animals and the selective factors producing the observed rates. The decline in metabolic rates (O_2 consumption rate per unit wet wt) in pelagic fishes, crustaceans and cephalopods is based on a decline in locomotor abilities, as expressed by enzyme activities (Childress & Somero 1979, Seibel 2007), protein content (Torres et al. 1979, Seibel et al. 2004), water content in locomotory muscles (Childress & Nygaard 1973, 1974), and swimming abilities (Cowles & Childress 1988, Seibel et al. 1998). To be meaningful for understanding the nature of this decline and the factors selecting for it, metabolism must be expressed relative to the mass of living animals.

Since much of the decline in metabolic rates is due to a reduction in structures supporting locomotion, the dry weight or N content of these animals declines in parallel to metabolic rates. Expressing metabolic rates relative to these fractions in whole animals thus minimizes any decline with depth (Childress 1977), confusing the description of the basic phenomenon with the analysis of its nature and causality. The extreme of this approach would be to compare O_2 consumption in a fish and a jellyfish relative to body N. Such an expression would show relatively similar rates for the fish and the jellyfish, a conclusion clearly at odds with the ecology of these organisms and not useful from an evolutionary or ecological perspective.

In Fig. 1, data on pelagic cephalopods illustrate the difference between using live weight or some fraction of the animals to standardize metabolic rates. The data standardized to live weight show a 35-fold decline in

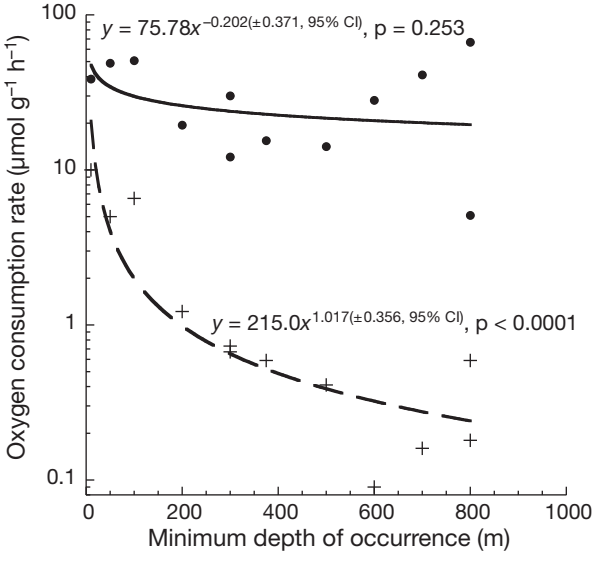

Fig. 1. Oxygen consumption rates (µmol O_2 g^{-1} h^{-1}), expressed on a live weight basis or protein basis in pelagic cephalopods. Crosses and dashed line: live weight specific rates; closed circles and solid line: protein specific rates. Rates adjusted to a common body size of 10 g wet wt using measured scaling coefficients for each species or assuming a mass specific scaling coefficient of −0.25. Measured at 5°C or corrected to 5°C. Rates and scaling coefficients from Seibel et al. (1997) and Seibel (2007); protein contents from Seibel et al. (2004). Species include *Abraliopsis felis*, *Histioteuthis heteropsis*, *Chiroteuthis calyx*, *Bathyteuthis abyssicola*, *Dosidicus gigas*, *Gonatus onyx*, *Japetella heathi*, and *Vampyroteuthis infernalis*

metabolic rate with depth, as the deeper living species are fragile and gelatinous and the shallowest species are robust and active. However, when the data are standardized to protein content there is no significant change with depth. This indicates that protein contents decline in parallel to the metabolic rates, reflecting reductions in locomotory capacity underlying the metabolic decline. Protein or N-specific metabolic rate data cannot be used to investigate relaxed selection for robust structure and metabolic potential at greater depths.

Therefore, expressing O_2 consumption relative to N at a body size of 1 mg N and analyzing this as a function of depth (Ikeda et al. 2006a) is not the same as considering O_2 consumption relative to live weight. It is inappropriate to use N-specific data to evaluate the locomotor and 'visual interactions' hypotheses. The data presented by Ikeda et al. (2006a) cannot refute these hypotheses because they do not concern the metabolic rates of live animals that are at the basis of these hypotheses.

The same issues of dry versus live weight apply to chemical composition as a function of depth. Making conclusions about the functioning of living animals requires the expression of composition as fractions of live weight, not as fractions of dry weight, which can be <10% of wet weight for midwater organisms (Childress 1977). This problem is evident in the study on composition of copepods (Ikeda et al. 2006b), repeatedly cited in Ikeda et al. (2006a) as supporting their conclusions. Their discussion of compositional changes with depth reported in the literature confuses this issue by reanalyzing published data, originally expressed relative to live weight, on the basis of dry weight. Such discussions are irrelevant and confusing with regard to the hypotheses under consideration.

Depth and averaging of data

The analysis by Ikeda et al. (2006a) is also not comparable to analyses in the cited papers on metabolic declines because of the way they parameterize habitat depth. They use the capture depth for each individual and then average the rates of all the individuals captured at fixed depth intervals, plotting the mean against the middle of the depth interval. As a result, they often have the same species represented in more than 1 depth interval and a given species may have many values in the averages. There were no significant differences in any of the parameters measured (Ikeda et al. 2006a, their Tables 2 & 3 and Fig. 2) between the 3 data sets collected for deep specimens. However, many of the same species were used in all deep intervals. For example, 8 of the 27 species in their lower bathypelagic dataset were also included in the upper bathypelagic and mesopelagic datasets. Since species are not connected to a given depth interval there is an implicit hypothesis in their work that evolution is irrelevant and acclimation to the capture depth is paramount. Therefore, no conclusions regarding evolutionary differences in relation to depth can be made using their methods.

In contrast, the cited literature on metabolic decline uses an estimate of the minimum depth of occurrence plotted against the average of all measurements of a given parameter on a given species. Childress & Nygaard (1973) defined minimum depth of occurrence (MDO) as the shallowest depth below which 90% of the individuals in a population live. Using MDO means that species that migrate vertically are analyzed in the shallower part of their overall depth distribution. This is an ecologically appropriate approach to examining changes with depth, since resources and light are most abundant at shallower depths. In addition to the studies on crustaceans and fishes cited above, species-specific MDOs have been used to examine the effects of depth on chaetognaths (Thuesen & Childress 1993), medusae (Thuesen & Childress 1994) and cephalopods (Seibel et al. 1997). Thuesen et al. (1998) used minimum capture depths for each copepod species. These methods give each species only 1 depth and 1 averaged metabolic rate in any analysis, as is appropriate for evolutionary questions concerned with the properties of species.

Proxies for metabolic rates

In the literature on metabolic rates as a function of depth, proxies of metabolic rate — primarily citrate synthase activity — have been used as controls for artefacts due to damage to the specimens or unrealistic activity levels in captivity. These proxies have been significantly correlated ($r^2 > 0.5$, often well above) and have shown the same patterns with depth as the metabolic rates in all cases. Ikeda et al. (2006a) dismiss enzymes of intermediary metabolism as proxies for metabolic rates because their correlations are not extremely high. They tout the 'electron transport system assay' (ETS) as superior to other proxies for O_2 consumption in ways that are both misleading and incorrect.

The original ETS studies cited by Ikeda et al. (2006a) used samples of a wide size range. Since they plotted total ETS versus total O_2 consumption across a wide range of masses, they effectively plotted body mass against body mass, thus obtaining a very high correlation (King & Packard 1975). Had they plotted mass-specific metabolic rates and enzyme activities, the correlations would have been much lower. Limits

to correlation between metabolic rate and ETS for copepods have been discussed in Mayzaud (1986), who found that ETS could be useful, but is not an exact predictor of metabolic rate. Childress & Thuesen (1992, 1995) discussed the use of enzymatic proxies for metabolic rate, concluding that it is a useful technique, but one which has considerable inherent variability. This is because, as for ETS, it measures metabolic potential that will only be realized at maximal activity levels. Both are useful proxies, but neither is a precise indicator of routine metabolic rates.

Instead of testing the relation between ETS and metabolic rate in their data, e.g. by plotting metabolic rates against ETS values for the same individuals or species, Ikeda et al. (2006a) presented means of each depth group in their Fig. 3 and claimed excellent agreement with theory. However, the wide error bars show that correlation between ETS and metabolic rates in individual copepods is not good in their dataset. Given the smaller differences between groups and greater overlap in ETS values between groups compared to N-specific metabolic rate, as shown in their Fig. 3, the difference in ETS values as a function of depth in their data may not even be significant.

Measurement of O_2 consumption

Ikeda et al. (2006a) measured O_2 consumption using methods that are not comparable to methods used in previous studies on the decline in metabolic rates with depth. Our studies and Ikeda's deep studies (Ikeda et al. 2006a) were done with single individuals in closed chambers. However, Ikeda's shallow species were all measured in groups of 5 to 20 individuals in the same chamber (Ikeda et al. 2001). This likely results in higher metabolic rates, due to stimulation of the copepods by each other. Supporting this interpretation is the fact that the ratio of ETS activity to metabolic rate is substantially lower in the epipelagic polar species studied (Fig. 3 in Ikeda et al. 2006a), as expected if the copepods were at higher activity levels than usual. The argument of Ikeda et al. (2006a) rests on this dataset on shallow-living polar animals, which is not appropriate for comparison with their newer measurements on deeper-living species, because the former were collected under conditions which apparently resulted in higher metabolic rates.

A second problem is that the water used for deeper-living species had low O_2 content to simulate the low O_2 habitat from which they were captured (Ikeda et al. 2006a). The literature on midwater animals, reviewed by Childress & Seibel (1998), shows that crustaceans, including copepods, living in midwater are good regulators of their O_2 consumption rates, generally maintaining a stable rate until about the lowest O_2 content in their environment. Ikeda et al. (2006a) began incubation at or near the O_2 content where the copepods live, and it is likely that during the experiments the copepods consumed the O_2 to levels that limit O_2 consumption, thus biasing the measurements on deep-living copepods toward lower values.

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