Estimation of mortality for stage-structured zooplankton populations: What is to be done?

Mark D. Ohman *

California Current Ecosystem LTER site, Scripps Institution of Oceanography, 9500 Gilman Dr., La Jolla, CA 92093-0218, USA

ABSTRACT

Estimation of zooplankton mortality rates in field populations is a challenging task that some contend is inherently intractable. This paper examines several of the objections that are commonly raised to efforts to estimate mortality. We find that there are circumstances in the field where it is possible to sequentially sample the same population and to resolve biologically caused mortality, albeit with error. Precision can be improved with sampling directed by knowledge of the physical structure of the water column, combined with adequate sample replication. Intercalibration of sampling methods can make it possible to sample across the life history in a quantitative manner. Rates of development can be constrained by laboratory-based estimates of stage durations from temperature- and food-dependent functions, mesocosm studies of molting rates, or approximation of development rates from growth rates, combined with the vertical distributions of organisms in relation to food and temperature gradients. Careful design of field studies guided by the assumptions of specific estimation models can lead to satisfactory mortality estimates, but model uncertainty also needs to be quantified. We highlight additional issues requiring attention to further advance the field, including the need for linked cooperative studies of the rates and causes of mortality of co-occurring holozooplankton and ichthyoplankton.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

It is challenging to find a problem in evolutionary ecology, population dynamics, functional morphology, trophic dynamics, or climate change science that does not turn on understanding the risks and rates of loss experienced by natural populations. Yet for scientific generations, zooplankton ecologists have eschewed the problem of quantifying such losses in favor of other processes that are more experimentally tractable and convenient, even if of uncertain significance. A litany of objections is commonly raised to attempts to estimate mortality for planktonic organisms, which are used to justify this lack of attention. Among the more common objections include assertions that: it is impossible to sample the same planktonic population over time; advection always predominates over all other sources of loss or gain; patchiness generates poor precision of abundance and rate measurements; unbiased sampling across the entire life history is unattainable; organism ages or stage durations, which are usually needed for mortality estimates, are unknown; and the underlying population models are biased and contain hidden assumptions and unmeasureable parameters to which they are unduly sensitive. In a word, mortality rates are inherently unknowable. So, faced with the dilemma of the importance of quantifying mortality,

yet the conventional wisdom that the problem is intractable, what is to be done?

Beginning at the beginning, it is reasonable to assume that non-clonal organisms do die. It is also reasonable to infer that metazoans experience different risks and rates of loss at different points in their life history. Body mass can vary by 3 orders of magnitude or more from egg to adult for many types of holozooplankton, with attendant changes in Reynolds number, swimming behavior, escape responses, hydrodynamic disturbances, and visibility. Hence it is reasonable to expect that sources and rates of mortality will change with ontogeny. Mortality rates thus likely vary with developmental stage or size of the organism and, as an important corollary, the developmental stage composition of a natural population is expected to contain an imprint of the points in the life history where higher or lower rates of mortality have acted. It is also likely that sources and rates of mortality vary through time, especially in mid- to higher latitude environments where many environmental variables, both biotic and abiotic, show strong seasonality. Furthermore, mortality risks are unlikely to be uniform across the entire geographic range of a species, thus also to vary in space.

Armed with the expectation that differential, stage-specific mortality varies in time and space in the planktonic realm and that the action of such processes should leave an imprint on the developmental stage or size structure of the population, the question becomes whether it is ever practical to detect and interpret such an imprint in natural zooplankton populations. If the critiques in the first paragraph were found to be unequivocally true, then the answer would be no, the problem is

* Tel.: +1 858 534 2754; fax: +1 858 534 0738.
  E-mail address: mohman@ucsd.edu.

intractable and we should not waste either time or money. However, are there circumstances or conditions in which some types of methods, even if imperfect, can be applied with limited bias? Are there approaches, including indirect ones, that are sufficiently robust that they can extract the predominant underlying patterns, even in a complex fluid environment? Is it possible to estimate the bias associated with a given method, such that the uncertainty of the solutions can be quantified and made explicit? If any of these questions are answered in the affirmative, a pathway forward presents itself.

Toward this end, one of a family of *inverse models* is commonly employed in order to solve for the mortality rates that would give rise to the observed stage structure of a population. Also needed is knowledge or assumptions about the rates of transfer between successive developmental stages. These inverse models are commonly classified as *horizontal methods* that follow the developmental stage progression of a population through successive time points, and *vertical methods* that rely on the stage structure of a population sampled at one point in time, with assumptions about the temporal stability of that stage structure (Aksnes et al., 1997). A number of different horizontal (Aksnes et al., 1997; Caswell, 2001; Manly, 1990; Wood, 1994) and vertical (Aksnes and Ohman, 1996; Mullin and Brooks, 1970; Peterson and Kimmerer, 1994; see Aksnes et al., 1997) methods have been proposed. Application of these methods requires careful attention to the underlying assumptions and selection of appropriate field sites, as discussed further below.

As an alternative to site-specific demographic study employing inverse models, some authors have suggested that life (and death) processes are governed principally by body size, or by body size and an environmental co-variate such as temperature (Hirst and Kiørboe, 2002). If true, the appropriate mortality coefficients could be approximated by size-scaling (usually allometric) relationships, together with environmental measurements. Of course, the hypothesis of allometric scaling cannot be parameterized or tested without a suite of robust field estimates of mortality for organisms across a broad spectrum of body sizes. So the allometric approach also remains dependent upon a body of empirical estimates and does not by itself resolve the problem. Another alternative is the use of the average annual production:biomass (P:B) ratio, which can be used to approximate the average annual mortality rate, provided it is assumed that a population is at steady-state from year to year. Such an approach does not resolve within-season variations in mortality that can be fundamental to population dynamics (e.g., Ohman and Hirche, 2001). Another proposed indirect approach would equate the mortality at trophic level$_i$ to the consumption at trophic level$_{i+1}$, but this method ignores a variety of sources of non-predatory mortality.

This article will now examine more closely some of the arguments summarized briefly above that, if found to be absolutely true, would preclude resolution of stage-specific rates of loss and condemn this line of inquiry (Hugo, 1829). We will analyze each of the major critical assertions that has been made and briefly offer relevant recommendations. We will then seek to clarify some misconceptions in this line of research, and finally point to key issues requiring attention to improve future studies.

2. Examination of assertions

2.1. It is impossible to sample the same planktonic population over time

While sometimes true, it is not universally so. In addition to closed systems such as some freshwater or marine lakes, in some semi-closed systems such as lagoons, embayments, or fjords there may be protracted time periods of limited flushing that permit the sequential development of a planktonic population to be sampled by conventional Eulerian sampling designs. A number of examples from such environments illustrate coherent, seasonally reproducible patterns of

population changes that can only sensibly be explained as reflecting repeated sampling of the same population (Eiane et al., 2002; Landry, 1978; Ohman, 1990; Uye et al., 1983). In addition, some regions of recurrent, retentive ocean circulation offer the potential for repeated temporal sampling. For example, persistent mesoscale or sub-mesoscale eddies and their associated biota may be followed as they translate through space. Some specific regions of the coastal ocean have topographically restricted, tidally rectified retentive circulation that permits sampling of individuals derived from the same population at successive points in time (Durbin and Casas, 2006). While there may be only specific time periods when following temporal progressions is feasible, and diffusive losses/gains remain difficult to quantify, there have been demonstrable advances in such studies in recent years (e.g., Bi and Benfield, 2006; Eiane and Ohman, 2004; Li et al., 2006; Ohman et al., 2008; Bi et al., 2011).

In addition, the "population" of interest needs to be defined carefully. Some numerical methods (e.g., vertical life table methods) do not require that individuals of the same specific cohort be followed through time, rather that patches having similar developmental stage composition (as proportional stage ratios) and development rates be followable, even if absolute abundances vary among patches.

Studies that employ a Lagrangian sampling design have been pursued for many years (e.g., Heron, 1972), though newer technologies present opportunities for Lagrangian studies that are potentially more effective at following water parcels and entrained plankton populations. The development of carefully engineered drifters with limited slip relative to the surrounding fluid has made it possible to continuously follow water parcels centered on the surface mixed-layer for protracted time intervals (e.g., Lee and Niiler, 2010). Following populations is, of course, more challenging than following fluid parcels, as organisms can be broadly distributed vertically and different developmental stages may reside at different depths and therefore experience different flows. Diel vertical migration (DVM) introduces further challenges to efforts to follow populations in sheared flows. However, some approaches that mimic the DVM of zooplankton have been introduced (e.g., De Robertis and Ohman, 1999) and the profiling SOLOPC (Checkley et al., 2008) could be adapted to this purpose. New generations of Lagrangian floats are in development (Jaffe and Franks, 2010). Other technologies such as autonomous gliders and Moving Vessel Profiler surveys have been recently used to improve Lagrangian studies by initially mapping the three-dimensional field of a water parcel, thereby avoiding fronts and other strong gradient features that otherwise make parcel tracking difficult (e.g., Landry et al., 2009).

2.1.1. Recommendations

Therefore, in certain circumstances with closed or semi-closed circulation, it can be feasible to take sequential samples of members of the same population using Eulerian sampling designs. Vertical life table methods may be applied when the stage composition of a population is not changing rapidly in time or space. Lagrangian studies, particularly aided by newer technologies, can make it possible to track populations through time, at least for limited time periods. It is equally important to recognize that in some field situations it is not presently practical to carry out sequential sampling and thus solve for mortality.

2.2. Advection always predominates over all other sources of loss or gain

This assertion usually results from improperly designed field studies. Basic consideration of the relevant time scales makes clear that the spatial scale of the field site must be chosen so that the residence time of the fluid is greater than the time scale of change in the population. If the study site is too small or the rate of fluid transport too large, changes due to fluid flow will exceed rates of biological changes. Consider a study site with fluid residence time τ;

in general the smaller the study site, the smaller τ will be. Now consider the time scale of change in the population of interest, here taken as the generation time (T_{gen}) of the organisms. For a given T_{gen}, increasing the spatial scale of study will lead to progressively smaller effects of advection. A pre-condition that therefore should be met in the design of a field study is that the spatial domain of the study site should ensure that the residence time, τ, is greater than or equal to the generation time T_{gen} (Aksnes et al., 1997; Ohman et al., 2008). Note that this condition does not imply that there is no fluid loss from the study domain, only that the average advective and diffusive flux is no greater than the rate of biological change from generation to generation.

For rapidly growing organisms like protists (short T_{gen}) the spatial scale of study can therefore be small, while for multicellular organisms sampled across their entire life history, the study domain must be correspondingly larger (or located in a region whose circulation is more retentive). Since spatially representative sampling must always be conducted within the study domain, both the spatial scale and the sampling effort required for larger, more slowly growing metazoan organisms increase substantially. However, if losses for only a specific phase of the life history are of interest (e.g., from egg to early larval, or from larval to juvenile stages), then the combined duration of these life history phases, which is a considerably shorter time period than T_{gen}, establishes the appropriate time scale for comparison with τ.

It should also be obvious that whether there is a gain or loss of organisms associated with fluid flow depends on the sign of the horizontal spatial gradients of animal abundance; advective and diffusive fluxes can have either a positive or negative sign.

An alternative solution to increasing the residence time of the organisms in the study domain, beyond increasing the size of the region sampled in a Eulerian design, is to conduct Lagrangian sampling, as described above.

An additional approach to addressing changes due to transport is to explicitly model the advective and diffusive fluxes of the fluid and entrained organisms, in addition to the population growth trajectories. Complete 4-dimensional measurement of such physically-induced fluxes is unlikely to be attainable, but in some instances can be modeled. An example is the study of Li et al. (2006), who explicitly solved for advective, diffusive, and molting fluxes, and resolved the difference as copepod mortality. Care must be taken because uncertainties in the modeled flow fields, however slight, will propagate as error in the resulting mortality rates.

2.2.1. Recommendations

With sufficient forethought in the design of field studies, biologically-induced mortality is resolvable. The spatial scale over which mortality is being resolved should be defined explicitly (e.g., fjord, bight, marginal sea, etc.). The spatial scale of sampling should be sufficiently large that the residence time of the fluid, τ, is longer than the time scale of change in the population (e.g., the generation time, T_{gen}). Lagrangian design field studies again can be advantageous, for studies of limited duration.

2.3. Patchiness generates poor precision of abundance and rate measurements

Spatial variability in abundance and rate measurements across many spatial scales is a ubiquitous feature of pelagic ecosystems, presenting a particular challenge for population dynamic studies. In many instances the underlying causality of the patchiness is not resolved, in which case patchiness is treated merely as an expression of noise leading to poor precision. The usual remedies are to markedly increase measurement replication and the spatial density of samples. However, often the limitation of finite resources restricts sampling and sample analysis effort and the poor precision remains.

The underlying mechanisms leading to patchiness may be both biotic and abiotic (e.g., Décima et al., 2010). Increasingly, measurement technologies confer the ability to resolve some of the physical causal agents of patch structure, e.g., discontinuities associated with fronts and mesoscale eddies, internal wave-associated features, Langmuir cells, etc. Rather than representing unexplained sampling "noise," such horizontal discontinuities may be a key environmental "signal." Such gradients may be disproportionately significant as sites of elevated growth rates, aggregation rates, or mortality rates. In the vertical plane, thin layers and other sharp vertical gradients may be loci of enhanced zooplankton feeding and reproductive success. As technologies evolve that permit such features to be measured and sampled repeatedly over time, the spatial gradient regions will likely become sites of directed study, rather than unwanted noise.

In some environments, zooplankton may exhibit less horizontal patchiness than others, conferring the ability to not only sample a population over time, as noted above in Section 2.1, but also to do so with reasonable precision (e.g., Ohman, 1990). Also, some numerical methods have been found to be relatively robust to modest levels of sampling error (e.g., Aksnes and Ohman, 1996; Wood, 1994), hence underlying rates can sometimes be recovered from such methods with relatively small bias. However, no analytical method will compensate for undersampling in patchy environments.

An additional approach has been to utilize inverse methods that rely on the proportional stage composition of the population, rather than absolute numerical abundances (e.g., Aksnes and Ohman, 1996). It is frequently the case that the proportions of different developmental stages are less variable either in space or time than the absolute abundances. If the stage ratios are relatively uniform in space, then the observed average stage composition can be used to infer the rates of mortality that give rise to them (this is the essence of the Vertical Life Table [VLT] method). However, even the VLT method requires adequate replication to ensure stable estimates of mean stage composition (Aksnes and Ohman, 1996).

2.3.1. Recommendations

Low sampling precision must be addressed with sufficient spatial sampling coverage and adequate replication, because errors in abundance will propagate as errors in mortality. The adequacy of sampling coverage and replication can be determined through precision analysis from pilot field studies. Field study design should explicitly consider the presence of fronts, eddies, and other physical discontinuities in the water column. Numerical methods should be tested to ascertain sensitivity to sampling error.

2.4. Unbiased sampling across the entire life history is unattainable

A single sampling device is invariably inadequate to obtain quantitative, unbiased measurements of abundance of all developmental stages across the life history of planktonic organisms. In the case of copepods, for example, the size change from egg to adults can represent 1.5 orders of magnitude by length and 3 orders of magnitude by carbon biomass. For euphausiids, salps, and many other zooplankton taxa, comparable changes occur. Although it is true that no single sampling device, or optical or acoustic method, can provide quantitative samples across such ranges, solutions have been adopted. These generally entail sampling with multiple devices, selecting the most appropriate for each life history stage, and then forming composites of abundance based on more than one method. As the depths and volumes of water sampled may vary according to the method used, this practice introduces additional uncertainty into population abundance information.

Another form of sampling bias has been suggested when there are carcasses, empty exoskeletons, or other organismal remains suspended in the water column (e.g., Haury et al., 1995). If enumerated as a part of the population, these injured individuals will artificially

inflate stage abundances and potentially lead to either severe over- or underestimates of mortality (Elliott and Tang, 2011; Tang et al., 2006). Vital dyes have been proposed to ascertain the fraction of the population that was alive at the time of collection (Elliott and Tang, 2009), although the approach seems most applicable to short plankton tows.

At times sampling methods are found a posteriori to contain a bias against some developmental stages. Durbin and Casas (2006) switched from a centrifugal pump to a diaphragm pump for sampling naupliar stages when it was realized that the youngest naupliar stages were undersampled (probably damaged) by the centrifugal pump, yet later recognized a bias against N1 stages even by the diaphragm pump. This necessitated solving for mortality from the egg to the egg to the N2 stage, bypassing the first naupliar stage, with some attendant loss of life history resolution (Ohman et al., 2002; Todd et al., 2011).

2.4.1. Recommendations

Mortality and other demographic rate estimates will only be as good as the sampling upon which the calculations are based. If care is taken to intercompare methods used for different life history stages, it can be feasible to reconstruct abundances for the entire population. In some instances, analysis of stage-specific losses across only a restricted portion of the life history may be possible, in which case a single sampling device may prove suitable.

2.5. Organism ages or stage durations, which are usually needed for mortality estimates, are unknown

Although some matrix methods can be used to infer both molting and mortality probabilities from stage-specific census information (Twombly, 1994), for most inverse methods an independent measure of molting rate or stage duration is required. For copepods, the duration of a given developmental stage is strongly dependent upon temperature, which has led many to utilize laboratory-determined relationships such as a Bělehrádek function (e.g., McLaren, 1978) to provide estimates of stage durations based on in situ temperatures. However, food concentration also influences rates of development (Campbell et al., 2001; Vidal, 1980), hence functional forms that include food as well as temperature are needed (Li et al., 2006; Neuheimer et al., 2009; Ohman and Hsieh, 2008). An additional, but as yet poorly quantified factor is the effects of food quality on development rates.

In addition to a need for appropriate laboratory-derived functions describing rates of development for the entire life history as joint functions of food and temperature, there is uncertainty about the temperature and food regimes experienced by each developmental stage in situ. Often vertical distributions change markedly with ontogeny. In addition, as noted above, even a single developmental stage may be distributed broadly in the vertical plane in the water column, leading to a range of different temperature and food concentrations experienced in the natural environment. Considerable sampling effort may be required to determine the temperature and food conditions appropriate to different developmental stages, especially if frequency distributions rather than mean conditions need to be resolved. The expected rate of development can be highly sensitive to assumptions about in situ temperature and food concentration.

An additional approach, beyond extrapolation from laboratory-derived functions to conditions experienced in the water column, is to conduct direct measurement of molting rates of field-collected animals in simulated in situ conditions. Such measurements, carried out for all relevant stages, would provide the best estimates of the molting fluxes that are required for mortality estimation. Shipboard mesocosms have been successfully used for this purpose and it has been suggested that RNA/DNA ratios, if carefully calibrated, may provide a suitable surrogate measure (Wagner et al., 1998). Egg

production rates of adult females can also be used as an independent indication of intervals of food limitation (e.g., Runge and Roff, 2000), although adults and juveniles may demonstrate different sensitivities.

2.5.1. Recommendations

Mortality rate estimates can be quite sensitive to the values of stage durations and this issue requires careful attention. Temperature- and food-dependent relationships derived from laboratory study can be combined with field estimates of vertical distributions in relation to vertical gradients of temperature and food. It is preferable to also make independent estimates of development rates in mesocosm-based molting rate measurements, or to use growth rates of juveniles or egg production rates of adults as indicators of episodes of food limitation.

2.6. The underlying population models are biased and contain hidden assumptions and unmeasureable parameters to which they are unduly sensitive

Some formulation of expected abundances and population stage structure at time $t+1$, given observations at time t together with expected rates of development, is required for most mortality estimation methods. However, models for population growth differ widely with respect to underlying assumptions, the number of parameters required, and frequency of sampling necessary for application. A variety of both parametric and nonparametric methods have been proposed, some of which may achieve mathematical elegance but have limited applicability to natural populations that are sampled with error or with irregular sampling intervals. Aksnes et al. (1997) observed that there are probably more solution methods proposed in the literature than data sets available to validate them.

Because of the expense of sampling in the ocean, it is difficult to obtain high frequency time series of sufficient temporal resolution to meet the requirements for some methods. Delay-differential equation methods require regular temporal sampling at high frequency. Matrix-based population projection methods generally require that temporal sampling intervals be shorter than the shortest stage durations within the population. This can imply daily or even more frequent sampling, in warmer tropical environments. While daily sampling has been attainable in some studies (e.g., Hirche et al., 2001; Krause and Trahms, 1983), or even sustained twice daily sampling (Bi et al., 2011), such studies are the exception. Other numerical methods may make less restrictive assumptions about sampling intervals.

While some methods make explicit assumptions about the functional form of the population growth response others may make a more limited suite of assumptions. For example, the Population Surface Method (Wood, 1994) is a nonparametric approach requiring only that changes occur relatively smoothly between successive developmental stages and over time, without specifying the specific form of the change.

The Vertical Life Table approach has been proposed as an alternative to the time-dependent methods noted above, although the assumptions must be closely met for it to be applicable. It assumes stationarity of the observed stage structure of a population for a period of time equal to the duration of the stage pairs being considered. If there is a rapidly changing cohort structure in the population, this method is not appropriate and should be avoided (Aksnes and Ohman, 1996). It should be noted that the modified VLT method (Ohman et al., 2008) does not assume abundances are constant (as asserted by Neuheimer et al., 2009, whose analysis assumed egg production remained invariant through time), but that stage ratios and mortality rates are constant over this time interval. In addition, an inherent limitation is that the method resolves the mortality of successive stage pairs, but not individual stages. It also requires several replicate estimates of stage ratios in order to obtain stable estimates of mortality (Aksnes and Ohman, 1996).

2.6.1. Recommendations

Reviews of methods are available (Aksnes et al., 1997; Caswell, 2001; Manly, 1990) and these and the current literature should be consulted closely before designing a field study. Paying close attention to the assumptions of different methods is imperative. It is also highly advisable to test the robustness of a method with simulated data prior to field applications (e.g., Aksnes and Ohman, 1996; Wood, 1994), in order to assess the sensitivity of the model to the assumptions. In addition, it is recommended to explicitly estimate the uncertainty associated with numerical solutions by propagating the errors of the assumptions (e.g., Ohman et al., 2002). Finally, methods can sometimes be applied for short time intervals when the assumptions do apply (e.g., Bi et al., 2011; Plourde et al, 2009), even if this is not possible for the entire duration of a field study.

3. Common misconceptions

Some misconceptions seem to persist in the scientific community, which we will attempt to clarify. It has been stated at scientific conferences that mortality rates can only be estimated for populations at steady state $(dN/dt = 0)$. Fortunately this is not true. Neither the time-dependent nor vertical life table methods make this assumption. Horizontal methods were devised to resolve the time-dependent variations in birth and mortality rates and make no assumption about whether a population is increasing, decreasing, or remains constant. Similarly, vertical methods in general make no such assumption. The VLT approach assumes the population has attained a stable stage distribution, which elementary textbooks (e.g., Gotelli, 2008) show occurs when a population is growing exponentially, with growth coefficients that can be positive, negative, or zero.

It is also stated that mortality coefficients should never be less than zero. Some numerical methods apply the constraint that the mortality rates must be non-negative, which obviously must apply if a population is sampled without error. However, given random error (and patchiness) associated with estimates of abundance, when comparing such abundances in successive time points or between successive developmental stages, in many circumstances some mortality solutions will inevitably be less than 0. Aksnes and Ohman (1996) argued that to obtain an unbiased measure of mortality rates when applying the VLT approach, it is best to obtain a sizable number of replicate measures of abundance and stage proportions, from which solutions can then be averaged to obtain an unbiased measure of the mean mortality rate. In such cases, all solutions need to be averaged, including those less than zero, in order to obtain an unbiased estimate of the mean mortality rate.

It has also been stated that mortality need not be solved for explicitly because it is simply equal to predation by the next trophic level, i.e., mortality at TL_i = consumption by TL_{i+1}. It is assumed that consumption can be determined simply by analysis of predator gut contents or predator energetic requirements. Such a statement assumes there is no non-predatory source of mortality and that consumed prey are always identifiable. It also assumes that the system is closed, such that none of the secondary production is imported/exported either vertically or horizontally beyond the study region. These assumptions are unlikely to be true. While predators are often responsible for the majority of losses, they do not account for all (e.g., Ohman et al., 2008). Mass mortality events induced by parasites may cause high fluxes of dead euphausiids to the seafloor (Gómez-Gutiérrez et al., 2003), and parasite-induced mortality generally remains poorly quantified. Sizable numbers of copepod carcasses have been found in some field samples (Haury et al, 1995), whose cause is unknown. Predators may fatally injure prey without consuming them (Ohman, 1986) and organisms may cross physiological thresholds of salinity, temperature, pH, and dissolved oxygen that may lead to abrupt mortality. Starvation-induced mortality may lead to sinking and remineralization by bacteria. The presence of secondary compounds such as polyunsaturated aldehydes and other oxylipins in phytoplankton may induce hatching failure and deformed larvae (Ianora and Miralto, 2010). The analysis of gut contents is well known to be biased toward organisms that leave discernible hard parts or macromolecules. Furthermore, the assumption that predator consumption equates to prey mortality assumes that all relevant predators have been sampled. Calculations of vertical carbon export suggest that a sizable fraction of secondary production may be exported vertically from surface layers into the mesopelagic zone or deeper (Steinberg et al., 2008), where consumers are rarely sampled quantitatively.

The issue of density-dependent mortality is of considerable theoretical and practical significance, as it introduces an important nonlinear term into population growth. Many models have been parameterized with a quadratic closure term for the zooplankton (e.g., Fasham et al., 1990; Steele and Henderson, 1992), which is an indirect means of expressing density-dependent mortality without explicitly describing the underlying mechanism. Others have included more explicit functions (e.g., Speirs et al., 2006). It should be clear that "density-dependent" population growth, in the sense of the population dynamics literature, implies that the parameters describing birth, development, or death rates vary as a function of the numerical density of the organisms within the population. Positive density-dependence (e.g., Allee effects) and negative density-dependence have been documented for various populations. The most sensitive way to demonstrate such density-dependence is to show that a key population parameter (e.g., instantaneous per capita birth rate or mortality rate) varies with population density. Comparisons made in this manner have illustrated either density-dependence (e.g., Ohman and Hirche, 2001) or conditional density-dependence (Ohman et al., 2002) for *Calanus finmarchicus* in different field sites. It should be noted that the independent variable in these comparisons is to be considered the density of animals in the population. Such comparisons bear no direct relationship to predator functional responses (sensu Holling, 1959), which describe individual per capita consumption rates of predators as a function of prey density (e.g., Heath et al., 2008) and should not be confused with the use of "density-dependence" from the population dynamics literature.

4. Key issues for the future

The analysis above indicates that, despite many challenges associated with the estimation of mortality for field populations of zooplankton, specific situations exist when sampling, experimental, and numerical methods make the goal feasible, given appropriate attention to the assumptions involved. So, at this time, what are the primary needs that would advance this field further, faster? What is (yet) to be done?

4.1. Techniques for aging zooplankton

A method to ascertain ages of different development zooplankton developmental stages collected in situ has been a significant impediment to constraining mortality estimates. Copepods, euphausiids, and many other types of zooplankton are not known to have mineralizable hard parts with discernible growth increments such as otoliths or statoliths that would permit direct aging of individuals. While revised analytical methods for the age pigment lipofuscin have revealed an association between age and lipofuscin content for both blue crabs (Ju et al., 1999) and euphausiids (Harvey et al., 2010), there is considerable variability in lipofuscin-at-age (Harvey et al., 2010) and the method has not yet been calibrated for a sufficient number of planktonic crustaceans to ascertain its general applicability. Novel approaches are needed that will permit animals to be aged in situ.

4.2. Tagging methods

Many challenges associated with indirect methods for mortality estimation would be eliminated with direct tagging methods that would permit the fate of individuals to be followed through time. While such a prospect would have seemed unattainable for the zooplankton a few years ago, recent developments in miniaturization of acoustic and other tags have begun to bring this prospect within the realm of feasibility. Individual jellyfish have been tagged (Hays et al., 2008) and short-term tracking of individual decapod shrimp has been accomplished (Steig and Greene, 2006). We expect continued developments in microelectronics to open the door to tagging and perhaps even mark/recapture studies in the future.

4.3. Cohort tracking in the open ocean

Lagrangian approaches have developed rapidly in recent years, but the challenges associated with tracking zooplankton are considerably greater than those associated with tracking fluid parcels. Because of the directed behavior of the animals, especially diel vertical migration in regions of vertical current shear, the problem is more complex than following a single density surface. Since depth distributions often change with ontogeny, this means that methods are needed to follow the behavior of different developmental stages occupying different depth strata.

4.4. Resolution of subpopulation differences in mortality rates

Many behavioral and life history traits of zooplankton are exhibited by part, but not all of the population. Consider, for example, intrapopulation differences and variability among individuals in propensity to exhibit dormancy, diel vertical migration, accumulate lipid stores, and other traits. At present we have limited ability to resolve intraspecific differences in mortality risks associated with such different trait states. Understanding the variations in fitness associated with such differences will depend on developing a quantitative understanding of the costs and benefits of different variants within a population. Furthermore, spatial variations in mortality, even within small geographic areas, have substantial effects on spatial patterns of distributions (Eiane et al., 2002; Ohman et al., 2008; Plourde et al., 2009). Representing such variations with individual-based modeling approaches requires estimates of frequency distributions of traits, not mean values alone.

4.5. Integrated studies by holozooplankton and ichthyoplankton workers

Mortality rates associated with early life histories of marine fishes have been investigated for many years in a variety of field studies (Houde, this volume), as have, to some extent, mortality rates for meroplanktonic larvae of benthic invertebrates. Such studies have traditionally been carried out by different groups of scientists than those focused on holozooplankton population dynamics. Yet both groups share many common needs, including vertically stratified sampling for different developmental stages, knowledge of the circulation, temperature and food environment, etc. In addition, there are often significant predator–prey relationships between holozooplankton and meroplanktonic fish and invertebrates. There may also be common sources of predation on both holo- and meroplanktonic organisms, an additional reason to link such studies. An important need for the future is explicitly integrated, cooperative field studies that seek to jointly estimate the patterns (and causes) of mortality of co-occurring ichthyoplankton and holozooplankton.

5. Conclusions

This analysis suggests that each of the principal assertions made above that challenge the feasibility of in situ mortality estimation has important exceptions that can render the problem tractable. Section 2.1. In judiciously selected field sites it is possible to use Eulerian sampling to follow the same population through time; technological developments are enhancing the capabilities of Lagrangian approaches. Section 2.2. Proper design of field studies such that the residence time of the fluid is at least as long as the time scale of change in the population sampled can make it possible to resolve biologically-mediated losses. Section 2.3. Sufficient replication, combined with targeted sampling that resolves significant mesoscale and submesoscale features, can lead to improved precision in abundance and rate measurements. Section 2.4. Carefully intercalibrated methods that sample different life history phases can make it possible to reconstruct abundances across the life history. Section 2.5. Estimates of stage durations that take into account the functional dependence on both temperature and food, as well as in situ distributions of temperature and food, can help constrain mortality estimates. Simulated in situ molting rate experiments are highly advisable. Section 2.6. Different models will be appropriate in different field circumstances. It is essential to plan sampling effort according to the needs of different model designs, and to carefully assess when model assumptions are and when they are not met. Errors should be propagated and uncertainty associated with mortality estimates should be quantified.

In addition we point toward several areas warranting development in the near future, including techniques for aging zooplankton, zooplankton tags, Lagrangian methods suitable for vertically migrating zooplankton, and resolution of intraspecific differences in mortality associated with different traits and spatial habitats. Finally, we point to the need for integrated, cooperative studies involving joint analyses of mortality rates of co-occurring holozooplankton and ichthyoplankton.

References

Aksnes, D.L., Ohman, M.D., 1996. A vertical life table approach to zooplankton mortality estimation. Limnology and Oceanography 41, 1461–1469.

Aksnes, D.L., Miller, C.B., Ohman, M.D., Wood, S.N., 1997. Estimation techniques used in studies of copepod population dynamics — a review of underlying assumptions. Sarsia 82, 279–296.

Bi, H.S., Benfield, M.C., 2006. Egg production rates and stage-specific development times of *Clausocalanus furcatus* (Copepoda, Calanoida) in the northern Gulf of Mexico. Journal of Plankton Research 28, 1199–1216.

Bi, H., Rose, K.A., Benfield, M.C., 2011. Estimating copepod stage-specific mortality rates in open ocean waters: a case study from the northern Gulf of Mexico, USA. Marine Ecology Progress Series 427, 145–159.

Campbell, R.G., Wagner, M.M., Teegarden, G.J., Boudreau, C.A., Durbin, E.G., 2001. Growth and development rates of the copepod *Calanus finmarchicus* reared in the laboratory. Marine Ecology Progress Series 221, 161–183.

Caswell, H., 2001. Matrix Population Models : Construction, Analysis, and Interpretation. Sinauer Associates Inc., Sunderland, MA.

Checkley Jr., D.M., Davis, R.E., Herman, A.W., Jackson, G.A., Beanlands, B., Regier, L.A., 2008. Assessing plankton and other particles in situ with the SOLOPC. Limnology and Oceanography 53, 2123–2136.

Décima, M., Ohman, M.D., DeRobertis, A., 2010. Body size dependence of euphausiid spatial patchiness. Limnology and Oceanography 55, 777–788.

DeRobertis, A., Ohman, M.D., 1999. A free-drifting mimic of vertically migrating zooplankton. Journal of Plankton Research 21, 1865–1875.

Durbin, E.G., Casas, M.C., 2006. Abundance and spatial distribution of copepods on Georges Bank during the winter/spring period. Deep-Sea Research Part II — Topical Studies in Oceanography 53, 2537–2569.

Eiane, K., Ohman, M.D., 2004. Stage specific mortality of *Calanus finmarchicus*, *Pseudocalanus elongatus* and *Oithona similis* on Fladen Ground, North Sea, during a spring bloom. Marine Ecology Progress Series 268, 183–193.

Eiane, K., Aksnes, D.L., Ohman, M.D., Wood, S., Martinussen, M.B., 2002. Stage-specific mortality of *Calanus* spp. under different predation regimes. Limnology and Oceanography 47, 636–645.

Elliot, D.T., Tang, K.W., 2009. Simple staining method for differentiating live and dead marine zooplankton in field samples. Limnology and Oceanography: Methods 7, 585–594.

Elliot, D.T., Tang, K.W., 2011. Influence of carcass abundance on estimates of mortality and assessment of population dynamics in *Acartia tonsa*. Marine Ecology Progress Series 427, 1–12.

Fasham, M.J.R., Ducklow, H.W., McKelvie, S.M., 1990. A nitrogen-based model of plankton dynamics in the oceanic mixed layer. Journal of Marine Research 48, 591–639.

Gómez-Gutiérrez, J., Peterson, W.T., DeRobertis, A., Brodeur, R.D., 2003. Mass mortality of krill cased by parasitoid ciliates (Brevia). Science 301, 339-339.

Gotelli, N.J., 2008. A Primer of Ecology, 4th ed. Sinauer, Sunderland, MA.

Harvey, H.R., Ju, S.J., Son, S.K., Feinberg, L.R., Shaw, C.T., Peterson, W.T., 2010. The biochemical estimation of age in euphausiids: laboratory calibration and field comparisons. Deep-Sea Research Part II—Topical Studies in Oceanography 57, 663–671.

Haury, L., Fey, C., Gal, G., Hobday, A., Genin, A., 1995. Copepod carcasses in the ocean. I. Over seamounts. Marine Ecology Progress Series 123, 57–63.

Hays, G.C., Doyle, T.K., Houghton, J.D.R., Lilley, M.K.S., Metcalfe, J.D., Righton, D., 2008. Diving behaviour of jellyfish equipped with electronic tags. Journal of Plankton Research 30, 325–331.

Heath, M.R., Rasmussen, J., Ahmed, Y., Allen, J., Anderson, C.I.H., Brierley, A.S., Brown, L., Bunker, A., Cook, K., Davidson, R., Fielding, S., Gurney, W.S.C., Harris, R., Hay, S., Henson, S., Hirst, A.G., Holliday, N.P., Ingvardsdottir, A., Irigoien, X., Lindeque, P., Mayor, D.J., Montagnes, D., Moffat, C., Pollard, R., Richards, S., Saunders, R.A., Sidey, J., Smerdon, G., Speirs, D., Walsham, P., Waniek, J., Webster, L., Wilson, D., 2008. Spatial demography of *Calanus finmarchicus* in the Irminger Sea. Progress in Oceanography 76, 39–88.

Heron, A.C., 1972. Population ecology of a colonizing species: the pelagic tunicate *Thalia democratica*. I. Individual growth rate and generation time. Oecologia 10, 269–293.

Hirche, H.-J., Brey, T., Niehoff, B., 2001. A high-frequency time series at ocean weathership station M (Norwegian Sea): population dynamics of *Calanus finmarchicus*. Marine Ecology Progress Series 219, 205–219.

Hirst, A.G., Kiørboe, T., 2002. Mortality of marine planktonic copepods: global rates and patterns. Marine Ecology Progress Series 230, 195–209.

Holling, C.S., 1959. The components of predation as revealed by a study of small-mammal predation of the European sawfly. The Canadian Entomologist 91, 293–320.

Hugo, V., 1829. Le dernier jour d'un condamné. reprinted 1989. Le Livre de Poche 1–153.

Ianora, A., Miralto, A., 2010. Toxigenic effects of diatoms on grazers, phytoplankton and other microbes: a review. Ecotoxicology 19, 493–511.

Jaffe, J.S., Franks, P.J., 2010. Observing sub-mesoscale structure and dynamics using swarms of miniature vehicles. ASLO Meeting, Portland, Oregon, 22–26 February 2010, IT43A-06. .

Ju, S.J., Secor, D.H., Harvey, H.R., 1999. Use of extractable lipofuscin for age determination of blue crab *Callinectes sapidus*. Marine Ecology Progress Series 185, 171–179.

Krause, M., Trahms, J., 1983. In: Sündermann, J., Lenz, W. (Eds.), Zooplankton Dynamics during FLEX '76. Springer Verlag, Berlin, pp. 632–661.

Landry, M.R., 1978. Population dynamics and production of a planktonic marine copepod, *Acartia clausii*, in a small temperate lagoon on San Juan Island, Washington. International Revue der gesamtem Hydrobiologie und Hydrographie 63, 77–119.

Landry, M.R., Ohman, M.D., Goericke, R., Stukel, M.R., Tsyrklevitch, K., 2009. Lagrangian studies of phytoplankton growth and grazing relationships in a coastal upwelling ecosystem off Southern California. Progress in Oceanography 83, 208–216.

Lee, D.K., Niiler, P., 2010. Surface circulation in the southwestern Japan/East Sea as observed from drifters and sea surface height. Deep-Sea Research Part I—Oceanographic Research Papers 57, 1222–1232.

Li, X.W., McGillicuddy, D.J., Durbin, E.G., Wiebe, P.H., 2006. Biological control of the vernal population increase of *Calanus finmarchicus* on Georges Bank. Deep-Sea Research Part II—Topical Studies in Oceanography 53, 2632–2655.

Manly, B.F.J., 1990. Stage-Structured Populations: Sampling, Analysis, Simulation. Chapman and Hall, New York.

McLaren, I.A., 1978. Generation lengths of some temperate marine copepods: estimation, prediction, and implications. Journal of the Fisheries Research Board Canada 35, 1330–1342.

Mullin, M.M., Brooks, E.R., 1970. The ecology of the plankton off La Jolla, California, in the period April through September, 1967. Bulletin of the Scripps Institution of Oceanography 17, 89–102.

Neuheimer, A.B., Gentleman, W.C., Galloway, C.L., Johnson, D.L., 2009. Modeling larval *Calanus finmarchicus* on Georges Bank: time-varying mortality rates and a cannibalism hypothesis. Fisheries Oceanography 18, 147–160.

Ohman, M.D., 1986. Predator-limited population growth of the copepod *Pseudocalanus* sp. Journal of Plankton Research 8, 673–713.

Ohman, M.D., 1990. The demographic benefits of diel vertical migration by zooplankton. Ecological Monographs 60, 257–281.

Ohman, M.D., Hirche, H.-J., 2001. Density-dependent mortality in an oceanic copepod population. Nature 412, 638–641.

Ohman, M.D., Hsieh, C.-H., 2008. Spatial differences in mortality of *Calanus pacificus* within the California Current System. Journal of Plankton Research 30, 359–366.

Ohman, M.D., Runge, J.A., Durbin, E.G., Field, D.B., Niehoff, B., 2002. On birth and death in the sea. Hydrobiologia 480, 55–68.

Ohman, M.D., Durbin, E.G., Runge, J.A., Sullivan, B.K., Field, D.B., 2008. Relationship of predation potential to mortality of *Calanus finmarchicus* on Georges Bank, northwest Atlantic. Limnology and Oceanography 53, 1643–1655.

Peterson, W.T., Kimmerer, W.J., 1994. Processes controlling recruitment of the marine calanoid copepod *Temora longicornis* in Long Island Sound: egg production, egg mortality, and cohort survival rates. Limnology and Oceanography 39, 1594–1605.

Plourde, S., Pepin, P., Head, E.J.H., 2009. Long-term seasonal and spatial patterns in mortality and survival of *Calanus finmarchicus* across the Atlantic Zone Monitoring Programme region, Northwest Atlantic. ICES Journal of Marine Science 66, 1942–1958.

Runge, J.A., Roff, J.C., 2000. The measurement of growth and reproductive rates. Ch..9. In: Harris, R.P., Wiebe, P., Lenz, J., Huntley, M., Skjoldal, H.R. (Eds.), ICES Zooplankton Methodology Manual. Academic Press, pp. 401–454.

Speirs, D.C., Gurney, W.S.C., Heath, M.R., Horbelt, W., Wood, S.N., de Cuevas, B.A., 2006. Ocean-scale modeling of the distribution, abundance, and seasonal dynamics of the copepod *Calanus finmarchicus*. Marine Ecology Progress Series 313, 173–192.

Steele, J.H., Henderson, E.W., 1992. The role of predation in plankton models. Journal of Plankton Research 14, 157–172.

Steig, T.W., Greene, C.H., 2006. Real time three-dimensional behavioral results of acoustically tagged shrimp. EOS, Transactions of the American Geophysical Union 87 OS44O-01.

Steinberg, D.K., Van Mooy, B.A.S., Buesseler, K.O., Boyd, P.W., Kobari, T., Karl, D.M., 2008. Bacterial vs. zooplankton control of sinking particle flux in the ocean's twilight zone. Limnology and Oceanography 53, 1327–1338.

Tang, K.W., Freund, C.S., Schweitzer, C.L., 2006. Occurrence of copepod carcasses in the lower Chesapeake Bay and their decomposition by ambient microbes. Estuarine, Coastal and Shelf Science 68, 499–508.

Todd, R.E., Rudnick, D.L., Davis, R.E., Ohman, M.D., 2011. Underwater gliders reveal rapid arrival of El Nino effects off California's coast. Geophysical Research Letters 38, L03609. doi:10.1029/2010gl046376.

Twombly, S., 1994. Comparative demography and population dynamics of two coexisting copepods in a Venezuelan floodplain lake. Limnology and Oceanography 39, 234–247.

Uye, S., Iwai, Y., Kasahara, S., 1983. Growth and production of the inshore marine copepod *Pseudodiaptomus marinus* in the central part of the inland Sea of Japan. Marine Biology 73, 91–98.

Vidal, J., 1980. Physioecology of zooplankton. II. Effects of phytoplankton concentration, temperature, and body size on the development and molting rates of *Calanus pacificus* and *Pseudocalanus* sp. Marine Biology 56, 135–146.

Wagner, M., Durbin, E., Buckley, L, 1998. RNA:DNA ratios as indicators of nutritional condition in the copepod *Calanus finmarchicus*. Marine Ecology Progress Series 162, 173–181.

Wood, S.N., 1994. Obtaining birth and mortality patterns from structured population trajectories. Ecological Monographs 64, 23–44.