CHAPTER 6

Dynamics of marine ecosystems: observation and experimentation

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6.1 Sampling and technological advances in support of GLOBEC science

Global Ocean Ecosystem Dynamics (GLOBEC) research has used a nested set of observations, experiments, and models in space and time to address the question of how climate change may affect marine populations. One of the great challenges has been to use observations effectively that span roughly 10 orders of magnitude spatially and temporally to understand variability in physical and biological environments. A major achievement has been the fostering of a coupled modelling and observational programme in a number of well-selected ecosystems globally. The advances in interdisciplinary observation and experimentation over the past decade, reviewed in this chapter, have led to significant progress in understanding the structure and functioning of ocean ecosystems. The concept of target organisms (Gifford *et al.*, Chapter 4, this volume) has been central to this approach and is linked to advances in the understanding of individual organism behaviours and population processes. New sampling and observation systems have been developed, particularly acoustic and optical. Shipboard, laboratory, and *in situ* process studies have been linked with new approaches to understanding trophic complexity. New approaches, applying techniques to retrospective studies, have contributed to understanding past ecosystem states and widespread use of a comparative approach has revealed new insights into the role of target species and the dynamics of marine ecosystems globally.

In this chapter, observational (field and laboratory) approaches and how they have helped broader advances in the field are presented. The unique challenges of sampling marine ecosystems and quantifying key ecosystem process are considered, and the significant developments are highlighted. How the methods adopted worked in different regions and on different species are evaluated. New methods for observation and experimentation represent one of the significant contributions to the legacy of GLOBEC.

While the focus is on methodology there is a clear contribution to advances in understanding the structure and dynamics of marine ecosystems. The material reviewed relates in particular to physical-biological processes (De Young *et al.*, Chapter 5, this volume) and food web processes (Moloney *et al.*, Chapter 7, this volume).

6.2 New approaches to the trophic complexity of marine ecosystems

Research on new methods to understand trophic links has not resulted in major advances during GLOBEC. However, a number of issues that will need to be addressed in the future have been

clearly identified during the programme. These include the identification of digested and visually unrecognizable remains in gut contents, measuring real food concentrations in the field, the effects of food quality and mechanisms like intraguild predation.

Sampling trophic complexity has been a challenge at two main levels: (1) understanding who eats whom, and (2) understanding the effect of eating different prey both for the predator and prey populations.

Understanding who eats whom has posed a surprising number of methodological challenges. Methods can be classified into two main groups (Bamstedt *et al.* 2000), quantification of gut contents (the usual method for fish and using gut fluorescence for zooplankton) on the one hand, and incubation methods on the other (zooplankton). A third alternative is markers such as stable isotopes (e.g. Bode *et al.* 2003) or lipids (Dalsgaard *et al.* 2003) that, although not providing detailed information on the diet, do provide an integrative view of the average diet of the organism.

Methods based on gut contents offer the advantage of allowing feeding to be estimated under natural conditions, without biases resulting from incubation conditions. Therefore the estimates obtained with these types of methods are more likely to be closer to the real situation. However, methods based on gut contents have the limitation of not being able to estimate feeding rates for food items that do not resist digestion or leave unidentifiable remains in the gut. For fish this is the case with, for example, ciliates and other soft-bodied organisms that are likely to be important for the first larval stages but that cannot be reliably identified in gut contents (Fukami *et al.* 1999). The same situation occurs for zooplankton where ciliates have been identified as an important food source (Calbet and Saiz 2005) but cannot be identified directly in the gut in contrast to chlorophyll *a* by fluorescence. However, considering the methodological problems involved, and that incubations are not realistic for fish above a certain size, it is clear that examination of gut contents remains a key technique to understand trophic interactions. In this sense a very promising tool is the application of molecular methods to identify digested items in the gut (e.g. Nejstgaard *et al.* 2003); Blankenship and Yayanos (2005); Sheppard and Harwood (2005); Durbin *et al.* (2008); and see Box 6.1). The application of microarray techniques that allow gut contents to be tested for the presence of a large number of potential prey items should make molecular approaches one of the main tools for understanding trophic links in future (King *et al.* 2008). However, at the moment this field is limited by the low number of primers and sequences available for marine organisms (in particular for small organisms and invertebrates).

A further problem in estimating what are the actual *in situ* ingestion rates of zooplankton that became evident during the GLOBEC programme is how to define food concentration and what proportion of the *in situ* food field is really available. Even for something easy to measure such as chlorophyll the presence of thin layers (McManus *et al.* 2003) results in available concentrations that vary dramatically within the water column. It is therefore important to evaluate the vertical position of zooplankton and fish larvae in relation to the food distribution to estimate the real ingestion rates. Furthermore, it is also important to establish which fraction of the apparent food field is really available to the predator, either because of size (too small) or other reasons limiting availability. This has been an obvious problem when defining food preferences for fish, where selectivity indices may be more dependent on the mesh size of the net and the layers sampled to evaluate the prey field (zooplankton) than on the real fish behaviour. Estimating the prey distribution with traditional methods involves an enormous amount of work (analyses of net samples for different size ranges and different layers) that is beyond the practical reach of most studies. However, a very promising approach based on image analysis (*in situ* and in the lab) and automatic recognition (Benfield *et al.* 2007 and see Box 6.2) has been developing during the last decades. This new technology is opening a completely new approach to characterizing the spatial distribution of pelagic organisms, offering the possibility of a much better estimate of the three-dimensional distribution of both predators and prey (see Section 6.5).

Box 6.1 Molecular techniques to establish trophic links

A promising new strategy for assessing feeding in small invertebrates is the use of molecular methods to detect prey-specific nucleic acid molecules as biomarkers of trophic interactions (Sheppard and Harwood 2005). Various different assays have been developed, but the general strategy of these methods is to purify DNA from stomach contents followed by detection and possible quantification using Polymerase Chain Reaction (PCR) amplification-based methods targeting gene fragments associated with prey organisms. Increasingly, this approach is being utilized to tease apart food webs, establish tropic links, and to estimate *in situ* feeding rates. Molecular approaches provide a means by which stomach content analyses can be conducted directly on field-caught animals without the potential of bias from incubation-based methodologies (Nejstgaard *et al.* 2003, 2007). A distinct advantage of a DNA-based molecular approach compared to gut fluorescence and direct microscopic observation is the ability to detect non-pigmented and macerated prey. Two general approaches have been used. The first is to use end-point PCR to qualitatively identify prey species in the guts of predators, while the second is to use real-time quantitative PCR (qPCR) to quantify the amount of DNA of a prey species in the stomach of a predator.

In the first approach PCR amplification primers with different specificities for prey have been used for amplification of genetic markers including species-specific (Bucklin *et al.* 1998; Nejstgaard *et al.* 2003; Vestheim *et al.* 2005), group-specific (Jarman *et al.* 2006), and universal (Blankenship and Yayanos 2005). Species-specific primers only amplify gene fragments associated with a prey species of interest and require a priori knowledge of the marker gene sequence of the prey species to design primers. In contrast, universal primers for a particular genetic marker amplify DNA of all of the different prey species. These amplification products may be separated through the use of clone libraries or other DNA profiling techniques (Troedsson *et al.* 2008a) and sequenced, enabling prey species to be identified (Blankenship and Yayanos 2005). Because predator DNA is always abundant compared to prey DNA, PCR amplification using universal primers is typically biased towards amplification of predator DNA and rarer prey sequences from the stomachs may fail to amplify. Different approaches have been utilized to attempt to overcome this problem. Blankenship and Yayanos (2005) attempted to minimize it by dissecting the stomachs to reduce the amount of predator DNA in their DNA purifications. They were able to further reduce the amount of predator DNA amplified by digesting with a restriction enzyme that cut only predator DNA within the target PCR amplicon prior to amplification with universal primers (Blankenship and Yayanos 2005).

qPCR offers a method for quantifying the amount of prey DNA present in the stomachs of predators and provides a basis for determining predator feeding rates. This approach allows quantification of the starting amount of DNA template and is based on the detection of a fluorescent reporter molecule that increases exponentially as PCR amplicons accumulate with each cycle of amplification. Fluorescence is measured at each cycle and an amplification plot is generated from the fluorescence data for standards and samples. Samples with higher amounts of target DNA exhibit increases in fluorescence after fewer number of amplification cycles than samples with less target DNA.

During the past decade qPCR has begun to be applied widely in ecological studies including the quantification of algal species in marine planktonic and sediment environments and for investigations of protist parasites and pathogens of marine metazoans (Frischer *et al.* 2006; Handy *et al.* 2006; Lyons *et al.* 2006). PCR-based assays are

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Box 6.1 ***continued***

now becoming routine in marine ecology studies, especially to detect and quantify free-living organisms. Typically, standard curves are prepared using a dilution series of organism numbers from which DNA is extracted so prey abundance can be expressed as organism concentrations. However, quantitative estimates of target prey or parasite species in predator or host organisms presents a unique set of methodological challenges including the development of efficient quantitative DNA extraction and purification protocols, minimization of DNA digestion and degradation, minimization of PCR artefacts associated with the detection of the target organism in the environment of a host organism, and importantly, the use of appropriate quantitative calibration standards.

The first application of qPCR to measure feeding rates of marine organisms was in a laboratory study of the appendicularian *Oikopleura dioica* feeding on several different algal species (Troedsson *et al.* 2008b). In this study algal DNA in the guts and filtering apparatus was quantified using species-specific primers targeting the 18S gene. In these experiments ingestion rates were measured over short time intervals and digestion of DNA was not apparent. To calculate ingestion rates and filtering apparatus trapping rates, standard curves were prepared from cloned genes as well as cultured algal cells that the animals were fed so prey abundance could be expressed both in units of gene copy numbers and cell concentration.

More recently, a similar approach was applied to investigate feeding of different copepod species in laboratory and field studies (Nejstgaard *et al.* 2007). Results were compared directly with feeding rates derived from parallel studies utilizing gut pigment methodology (laboratory studies) and rates based on direct microscopic analysis of simultaneously conducted bottle incubation experiments (field studies) (Nejstgaard *et al.* 2001a,b). Both laboratory and field studies demonstrated robust quantitative relationships between gut DNA content and independently obtained gut content or feeding rate estimates for the specific prey. However, when absolute estimates of prey algae recovered from copepods based on DNA were compared to independent estimates of ingested algae, they suggested that algal consumption was underestimated by the DNA-based qPCR assays. In these studies it was hypothesized that the underestimation by qPCR was due to digestion of prey DNA either after consumption or during post-handling steps associated with DNA purification.

A more general application of this method would be to combine information on gut contents in the field over time with a measure of how rapidly food disappears from the guts either through digestion or evacuation (Durbin and Campbell 2007). Ingestion rate is calculated from the equation: $C = 24\,SR$, where C is consumption over a 24 h period, S the mean gut content over 24 h and, R the exponential digestion rate. If there is strong diel migration, or indications of diel feeding periodicity in non-migrating copepods, ingestion is calculated for each time interval and then summed over 24 h (Durbin *et al.* 1995).

Methods have been developed to apply this approach in measuring copepod ingestion of multicellular organisms (nauplii), (Durbin and Campbell 2007); specifically predation by *Calanus finmarchicus* in the Georges Bank/Gulf of Maine region. In this region there is a limited number of potential prey species (Durbin and Casas 2006) making it possible to design species-specific primers for the mtCOI gene for each prey. Laboratory experiments were carried out with *Acartia tonsa* N1 and N2 as prey and adult female *Centropages typicus* as predator. The relationship between *A. tonsa* mtCOI gene copy numbers copepod^{-1} for stages N2-C1 copepod carbon was similar across stages indicating that copy number could be used as a measure of copepod biomass. *A. tonsa* DNA was detectable in the guts of the predators for as long as 3 h. Exponential rates of decline in prey DNA from the stomachs of the predators are similar to those measured for gut pigments.

Conversion of the copy number ingestion rates to numbers of each naupliar stage ingested is complicated by the fact that copy numbers change with

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Box 6.1 ***continued***

stage. In order to apportion these copy number ingestion rates amongst different stages it was suggested that estimates of the clearance rates of each stage by the predator determined in laboratory experiments be used together with the abundance of each stage in the field, to calculate the relative proportions of the copy number of each stage ingested. The actual numbers of each stage ingested in the field are calculated from these proportions and the *in situ* measurements of total mtCOI copies of each prey species ingested. At present this work is still in the development stage and there is a need to actually calibrate it in the laboratory against more traditional methods. One disadvantage is that this method cannot be used to measure cannibalism, which may be significant in some copepods (e.g. Bonnet *et al.* (2004); Ohman *et al.* 2004).

There are clear advantages and disadvantages of DNA-based methods compared to many of the classical approaches for investigation of zooplankton trophic interactions. The primary advantage of the DNA-based methods is the ability to obtain species-specific information of the trophic interaction, both qualitatively as well as quantitatively. However, due to digestion problems, the technique is at best semi-quantitative at this point, although there are some promising assays aiming at profiling digestion to obtain absolute quantification. Further, for organisms with complex trophic interactions, DNA-based techniques are still laborious and expensive. There are a number of promising high throughput sequencing as well as profiling techniques available today, but they are still relatively expensive. Therefore, we believe that the strength of the DNA-based techniques will be in the combination with classical approaches mainly because DNA-based techniques offer much better resolution of specific trophic interactions when such resolution is necessary in the data analysis. However, biotechnological companies are making rapid advances in user-friendly, high-throughput and affordable assays, and we predict that many of the methods reviewed here will be standard in most studies only a few years from now.

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The other general method used to estimate trophic relations of zoo- and phytoplankton involves incubations. Incubations also present a large number of yet unsolved problems. Other than their intrinsic limitations such as wall effects and enclosure (see Section 6.5) the main problem of incubations is the enhancement of trophic cascades that bias feeding estimations. The classic example is that of copepods, ciliates, and phytoplankton (Nejstgaard *et al.* 2001a,b). The copepod concentrations needed to estimate feeding rates on phytoplankton are often high enough to strongly reduce the ciliate population in the container and hence their feeding pressure on phytoplankton, resulting in an increase of phytoplankton in the incubation bottle instead of a decrease. Different methods have been proposed to eliminate this type of error (Nejstgaard *et al.* 2001a,b) but in general all involve much more laborious procedures multiplying the number of samples to be analysed. In this sense, future improvements may be expected through the use of image analysis systems allowing the enumeration of organisms in a rapid and effective way (see Box 6.1). A further problem of incubations is their limited capacity to estimate predation on prey present at low concentrations. As an example, laboratory experiments have shown that copepods are able to ingest a number of large organisms such as meroplanktonic larvae (Kang *et al.* 2000; Lopez-Urrutia *et al.* 2004) and copepod eggs and nauplii (Bonnet *et al.* 2004). The ingestion of such large organisms in the field may be occasional but important in terms of contribution to diet because of their large size. However, standard incubation experiments under field conditions do not allow the quantification of such trophic links. Again, the most promising technique in the future seems to be based on the identification of prey in the gut through molecular techniques (Box 6.1).

A more generalized problem in obtaining a generic understanding of who eats whom is the lack

Box 6.2 RAPID visualization of zooplankton predator/prey distributions

Collecting, identifying, and counting zooplankton has traditionally been a time-consuming, labour-intensive process. For these reasons, there is normally quite a long lag between sample collection and the visualization and analysis of distributions of biological taxa and corresponding hydrographic properties. The advent of imaging systems capable of recording the contents of defined volumes of water holds great promise for advancing our ability to describe the three-dimensional spatial distributions of zooplankton. This is particularly true for taxa that either avoid conventional nets and pumps, or for fragile organisms that are not well preserved during net sampling.

While imaging systems deliver massive volumes of data about zooplankton, exploiting their true potential is hampered by an inadequate ability to process this torrent of information. The development of exciting new software tools that semi-automatically and automatically process image data to extract zooplankton target information has begun to reveal the enormous power of *in situ* imaging systems. The same software tools can also be applied to digitized images of preserved or live plankton in order to accelerate their processing.

Automated image classification begins with an image data set from one of the many innovative *in situ* and laboratory systems developed to study plankton. See Benfield *et al.* (2007) for a review of currently operational systems. The first step is to isolate valid plankton targets from the background of each image. This process is termed segmentation and requires algorithms to locate the external boundary of each object. A somewhat larger bounding box is usually applied to the target to ensure that subtle features such as antennae or tentacles, that may not have been part of the perimeter of the object, are included. Segmentation may also include a screening process for focus detection to ensure that only targets imaged within the in-focus depth of field of the camera are included in the analysis.

Once a valid, in-focus object has been isolated from the background image, most software packages employ a step called feature extraction. Features are characteristics of the plankton image and its metadata that contain taxonomically useful information. These may include many of the morphological features that conventional taxonomy employs to distinguish different taxa as well as length to width ratios, circumference, and other allometric measurements. More often, features include a diverse suite of optical characteristics of the image. These optical features can include things such as the range of greyscale and colour levels in an image, brightness, texture, color, specularity, contrast gradients, and a host of optical characteristics that the human eye may or may not perceive. The completion of the feature extraction stage results in a collection of images of plankton, each isolated from their parent image, and associated with a series of characteristic features.

Computer-based classifiers require training before they can attempt to identify the contents of a series of unknown images. This requires that an individual or group constructs a training data set. A training set consists of taxonomic categories that each contains a series of representative images of each taxon. There must be a training category corresponding to each taxon that the researcher wishes the software to attempt to distinguish. Each category should include a large number of representative images of that taxon. A training set consisting of the best examples of each taxon will not be useful because such images may not share features that are common with the majority of images in the unknown sample. If organisms of the same taxa are imaged in two or more typical orientations (e.g. copepods in a lateral and dorsal view), then it may be necessary to dedicate a category to each orientation.

Once a training set has been assembled, it can be used to build a classifier. Classifiers are mathematical algorithms that learn to classify unidentified plankton images by constructing

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Box 6.2 *continued*

decision mechanisms. These decision mechanisms use relationships between features associated with the images in a training set and the label provided by a human expert to classify images of unknown identity. Misclassification errors must be quantified with a confusion matrix. It should be noted that automated classification systems are at best, expected to perform as well as a human expert. Experts do make mistakes (Culverhouse *et al.* 2003). When mistakes are incorporated into the training set, boundaries between features associated with each taxon will be less well defined and accuracy will likely suffer. Ideally an expert system should be capable of learning from misclassification errors to improve overall classification accuracy.

There are currently several examples of software tools that incorporate most or all of the above activities to enable computerized classification. These include ZOOIMAGE (www.sciviews.org), ZooProcess and Plankton Identifier (www.zooscan.com), Visual Plankton (www.whoi.edu/instruments/vpr), and SIPPER software (http://figment.csee.usf.edu/~shallow/sipper/papers/SipperSoftwareManual.pdf). At present, each of these packages is primarily designed to function with a single instrument type, although ZOOIMAGE has the capability of functioning with both scanner-based instruments such as ZOOSCAN (Grosjean *et al.* 2004) and the FlowCAM (Sieracki *et al.* 1998).

Considerable progress is being made using computers to conduct the labour-intensive classification of plankton samples. Accuracies of 70–80% or better have been demonstrated for 10–20 class problems. A notable success has been the demonstration of an accuracy of 88% for a 22-class phytoplankton problem with individual class accuracies ranging from 69 to 99% (Sosik and Olsen 2007). Based on work with the Video Plankton Recorder (VPR) and other systems such as SIPPER, it appears likely that similar performance is achievable for mesozooplankton using support vector machine (SVM) and other classification algorithms.

Once we are able to employ computers to advance past our current image bottleneck, the oceanographic and plankton ecology communities will be able to tap into the wealth of information that imaging systems can provide about planktonic predators and their prey. Far too often the time lag between data collection and interpretation is unacceptably long. When computers are able to do the hard work while oceanographers are at sea collecting the data, we will be able to observe plankton ecology on time scales that permit real-time responses to interesting predator-prey interactions.

An example of the type of interactions that could be visualized while at sea is provided by Global Ocean Ecosystem Dynamics (GLOBEC) data collected with the VPR in Wilkinson Basin, Gulf of Maine. During 1998 and 1999, there were dramatic changes in the abundances of diapausing *Calanus finmarchicus* and their invertebrate predators (see Box 6.2, Fig. 1). For example, in 1998, relatively few *C. finmarchicus* were present while physonect siphonophores were very abundant. In contrast, *C. finmarchicus* were very abundant during 1999 while siphonophores were relatively sparse. This relationship may have been partially a consequence of predation pressure by siphonophores on *C. finmarchicus* because their regions of high densities were inversely distributed in 1998 (see Box 6.2, Fig. 1) while there was no obvious spatial relationship in 1999.

Although these spatial patterns illustrated in Box 6.2, Fig. 1 were not obtained using semi-automated techniques, we have begun using a new interactive sorting tool called Plankton Interactive Classification Tool (PICT) to rapidly separate VPR images into their constituent taxa. PICT was developed by the University of Massachusetts Computer Vision Laboratory as part of a project to develop flexible software tools to classify plankton images. PICT combines segmentation and feature extraction with a classifier to semi-automatically sort images into taxonomic categories. As images are placed into these by a human operator, all unknown images that share features with those

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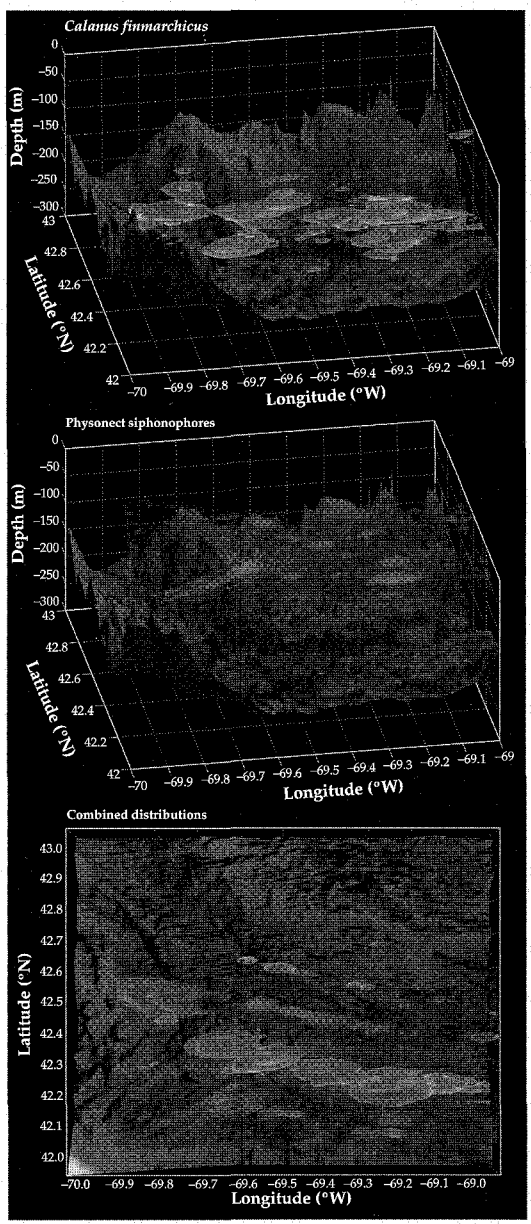
Box 6.2 *continued*



classified objects are then allocated to appropriate categories. In this manner, a training set can be rapidly assembled for construction of a classifier.

There is clearly a need for flexible software classification tools that bring automated image classification capabilities to the broad constituency of users who employ a diverse suite of imaging systems. Many current research imaging systems do not have associated image classification software. Research on Automated Plankton IDentification (RAPID) is a global initiative designed to bring zooplankton ecologists, engineers, software and hardware developers together to develop new tools that seamlessly work with imaging systems to locate, extract, learn, classify, and count zooplankton in near-real-time. Associated with the work of Scientific Committee on Oceanic Research (SCOR) Working Group WG130 (http://www.scor-wg130.net/index.cfm), RAPID is committed to developing practical and flexible software tools for the oceanographic and plankton ecology communities.

Box 6.2, Figure 1 Spatial distributions of diapausing *Calanus finmarchicus* (top panel) and physonect siphonophores (middle panel) in Wilkinson Basin during December1999 as measured with a towed Video Plankton Recorder (VPR). Observations of each taxon were converted to abundances and interpolated in three-dimension using GLOBEC EasyKrig 3.0 software. Isosurfaces in this visualization correspond to the highest densities. For *C. finmarchicus* these densities were 100–300 individuals per m^3 and for siphonophores (1–4 colonies m^{-3}). Combined distributions in plan view are illustrated in the bottom panel, which demonstrates that the patches of *C. finmarchicus* are generally absent from regions of high siphonophore densities (Christian Briseno). (See Plate 20).

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of a theoretical basis allowing the design of robust experiments to test the predictions of theory. Most studies have been opportunistic experiments where the trophic links in one area for a target species are measured. Field experiments are usually observational rather than aimed at testing a hypothesis. The extrapolation of such results and incorporation in a general synthesis is difficult without a theoretical basis. However, there is a developing body of theoretical research on encounter rates between predator and prey (Visser and Kiørboe 2006; Visser 2007b) providing such a theoretical basis (see Section 6.5). There is a need for future studies to link field studies with the predictions of theory.

Understanding the effects of the different trophic links between predator and prey populations also offers a number of challenges. On the one hand there is the effect of food composition on the physiology of the organisms. Both the stoichiometric composition of the food (Jones and Flynn 2005) and the presence of toxins through physiology or feeding inhibition (Miralto *et al.* 1999; Selander *et al.* 2006) have been shown in the laboratory to have the potential to influence population dynamics. The relevance of such effects in the field is still unclear (Irigoien *et al.* 2002; Irigoien *et al.* 2005; Pierson *et al.* 2005) and needs further field research combining high-resolution sampling with food composition and diet studies. One further problem related to this issue is how to incorporate the complexity of the food composition, where the effects can be not only species but also strain dependent (Wichard *et al.* 2008), into models. A possibility emerging from a better understanding of biodiversity (Irigoien *et al.* 2004) could be to use a statistical approach. At low food concentrations food limitation is more likely to have an effect than food composition. At medium phytoplankton concentrations diversity is high and zooplankton may be expected to find an appropriate diet in terms of composition through selection. It is at high phytoplankton concentrations when diversity is low that food composition could result in a decrease of zooplankton production below the values expected from food concentration alone. Because phytoplankton diversity is related to phytoplankton concentration (Irigoien *et al.* 2004) an index of probability of reduced production could be developed for high concentrations. This would not be based on the idea that a diverse diet is necessarily better than a diet based on a single food source, but on the idea that as diversity decreases the probability of food choice being toxic or nutritionally deficient increases. This approach could simplify the inclusion of food quality in models because experiments would not need to test all species and strains but rather estimate the number of times where a single diet decreases production in comparison with a mixed diet.

Predator effects may also have important impacts on prey populations. Top-down control and trophic cascades are increasingly being demonstrated for the marine environment (e.g. Worm and Myers 2003; Frank *et al.* 2005). However, the predatory effect of fish on zooplankton populations has rarely been convincingly demonstrated (Möllmann and Köster 2002). Other mechanisms such as intraguild predation and cannibalism (Polis *et al.* 1989; Köster and Möllmann 2000; Ohman and Hirche 2001) can have an important effect on the population dynamics of the species as well. Intraguild predation (competitors that eat each other) releases pressure on the basic prey and favours dominance when resources are abundant. An example of such an interaction is that of copepods, ciliates, and phytoplankton (Gismervik and Andersen 1997). Although phytoplankton are their main food source, by having high feeding rates on ciliates copepods might actually release pressure on phytoplankton, therefore affecting carbon fluxes. Similar mechanisms may occur with predation by copepods on eggs and nauplii that could determine the success of cohorts (Ohman and Hirche 2001) or the succession of species (Irigoien and Harris 2006). Similarly planktivorous fish have been shown to limit their own mortality rates by preying on early-life stages of their predators (Köster and Möllmann 2000).

GLOBEC associated programmes have highlighted such mechanisms, but our understanding of the relevance of those interactions is still very limited. Recent studies strongly suggest that predation is more likely to influence population dynamics in the field than food composition (Pierson *et al.* 2005). Therefore trophic complexity should be considered in a wider view, and for an organism one should consider both prey and predators, including the often ignored predators on the early stages. To have

such a wider view of the trophic links of organisms we urgently need new approaches allowing better estimates of *in situ* trophic links in the field.

6.3 Sampling and observation systems

GLOBEC programmes have developed and exploited new sampling and *in situ* optical, video, and acoustical methods, together with satellite tracking of individual organisms. These observational advances have provided comprehensive measurements of ecosystem properties on time scales from minutes to years and on space scales from less than millimetres to the global.

At the beginning and during the GLOBEC field programmes, considerable effort was expended to develop and exploit a number of new sampling tools and techniques as well as to use mature technologies to sample the zooplankton target species and their predators and prey, and to measure important environmental variables concurrently. Programmes studying marine ecosystems worldwide recognized that existing sampling technologies were not sufficient to meet the objectives. As a result a meeting was held 'to discuss the existing capabilities and potential developments in acoustical and optical technology, methodology, and instrumentation for measuring spatial and temporal distributions and assessing the behaviour of animals in the sea' (US GLOBEC 1991; see also GLOBEC 1993).

The resultant field work consisted of broad-scale and process surveys of the study sites at designated station locations, along the transect lines between stations, or in the vicinity of drogues or dye patches used to track water parcels. Fixed location moorings with a combination of physical and biological instrumentation and near-surface drogues have been used to provide continuous data to fill in the time gaps between survey and process cruises. In addition, in some studies, tags on large mammals and sea birds were used to acquire environmental data as well as information about animal location and behaviour. The data from tags, buoys, and drogues were frequently transmitted to land via satellite telemetry. The special issue, Southern Ocean GLOBEC (Hofmann *et al.* 2004c) provides examples and illustrations of these methodologies applied at the US GLOBEC study sites.

Sensors that operate on quasi-continuous spatial and temporal scales were viewed as essential if GLOBEC was to link small-scale process measurements to population parameters in the quest to understand the dynamics of zooplankton target species populations. Although seawater transmits visible light poorly because it is absorbed, scattered, and reflected more than in air, increasingly powerful video and camera chip technology made it possible to develop a number of new optically based sensors for zooplankton research. Transmission of sound in the moderate- to high-frequency range (38 to 1,000 kHz) is much greater and suitable for studies of zooplankton, which can be detected 10s to 100s of metres from the sound source. Thus, it was recognized that the integration of acoustical and optical technology would be highly beneficial and that the technologies were both complementary and synergistic in their potential utility. The need for synoptic sampling of both the biological and physical characteristics of the water column was stressed at the outset of the programme. The following is a summary of some of the technological developments and their application. There are two aspects to be considered: the sensors themselves and their modes of deployment.

6.3.1 Optical systems

Three optical sensor systems were principally used in the GLOBEC field programmes to study the distribution and abundance of zooplankton and fish eggs and larvae: the Video Plankton Recorder (VPR), the Continuous Underway Fish Egg Sampler (CUFES), and the Optical Plankton Counter (OPC) (Table 6.1). Other optical systems were also used to study phytoplankton distributions based principally on fluorometry and light attenuation (e.g. Barth *et al.* 2005), and they will not be discussed further here.

VPR: The VPR is a high-magnification underwater video microscope that images and identifies plankton and seston undisturbed in their natural orientations and that can quantify their abundances at sea in real time (Davis *et al.* 1992, 2005). The original VPR had four analog video cameras and a strobe light; each camera imaged concentrically located volumes of water ranging from less

Table 6.1 Regional GLOBEC programmes that used optical or acoustical senor systems and the net systems deployed in the field work. GLOBEC programmes that did not employ optical or acoustical sensors are not included in this table.

GLOBEC Programs ≥>		Georges Bank, Gulf of Maine	NEP California Current	NEP Alaska	SO GLOBEC	Arabian Sea	Canada GLOBEC	ICOS	TASC	Mare Cognitum	UK GLOBEC Marine productivity	Baltic Sea German GLOBEC
Instrumentation												
Acoustical systems	HTI	x	x	x	x							
	BioSonics	x										
	Simrad		x		x				x	x		
	TAPS		x	x								
	ADCP				x	x						
Imaging systems	OPC		x		x		x	x			x	
	VPR	x			x			x				x
	CUFES		x									
Net systems	MOCNESS	x	x	x	x	x			x	x		
	BIONESS						x		x			
	MultiNet								x			x?
	ARIES										x	
	Ocean Sampler										x	
	WP2								x	x		x
	Bongo net	x	x			x	x		x			
	CPR						x				x	
	Other (RingNets)	x	x				x		x	x		
	CalVET		x									

Note: NEP, North-East Pacific; SO, Southern Ocean; ICOS, Investigation of *Calanus finmarchicus* migrations between Oceanic and Shelf seas; TASC, Trans-Atlantic Study of *Calanus finmarchicus*.

than 1 to 1,000 ml, but it was subsequently modified to a one or two-camera system. The systems typically imaged a volume of about 5.1 ml at 60 Hz (3×10^{-4} m^3 s^{-1}). An image processing system was also developed that was capable of digitizing each video field in real time and scanning the fields for targets using user-defined search criteria such as brightness, focus, and size (Davis *et al.* 1996; Tang *et al.* 1998; Davis *et al.* 2004; Hu and Davis 2006; see also Box 6.2). The targets are identified using a zooplankton identification programme to provide near-real-time maps of the zooplankton distributions. Targets that meet the criteria are sorted into different taxonomic categories, enumerated, and measured together with the location, time, and depth at which they were observed. The software can also be used to post-process data from internally recording VPRs that are deployed autonomously. The VPR has typically been deployed as the primary zooplankton sensor along with environmental sensors in a V-fin vehicle that is undulated from the surface to some depth (100 m or greater). Recently, the VPRII has been developed that substantially improves the original version through use of a high-resolution digital camera, an automatically undulating towfish capable of tow speeds up to 12 knots on a trackline offset from the wake of the ship, and an improved software interface for automatic identification and display of plankton taxa together with hydrographic data (Davis *et al.* 2005).

A number of VPR-based systems were used in GLOBEC programmes. The original version and modified versions were used in surveys of Georges Bank and the Gulf of Maine (Benfield *et al.* 1996; Gallager *et al.* 1996; Norrbin *et al.* 1996; Ashjian *et al.* 2001; Davis *et al.* 2004). A one-camera system was used on the Bio-Optical Multifrequency Acoustical and Physical Environmental Recorder (BIOMAPER-II) vehicle (described below, see also Box 6.3) to map the vertical and horizontal structure of zooplankton and nekton in the deep basins of the Gulf of Maine (Benfield *et al.* 2003; Lavery *et al.* 2007). A VPR was mounted on a 1 m^2 Multiple Opening/Closing Net and Environmental Sampling System (MOCNESS) net system to map the fine-scale distributions of larval cod prey items (Broughton and Lough 2006; Lough and Broughton 2007) on Georges Bank. In the Southern Ocean GLOBEC Programme a two-camera system mounted on BIOMAPER-II was used on four survey cruises (Ashjian *et al.* 2008) to map the distribution of larval krill and other zooplankton. In addition, euphausiid furcilia populations living under sea ice were quantified using a stereo VPR mounted on a Remotely Controlled Vehicle (ROV; Gallager *et al.* 2001). In the Baltic Sea GLOBEC programme, a VPR was used by Schmidt *et al.* (2003) to examine the distribution of *Pseudocalanus*.

CUFES: Image resolution constraints inherent in the use of standard video formats have driven the development of optical systems that utilize higher-resolution formats. Development of the CUFES (see Box 6.4) utilizes a line-scanning digital camera to quantify the abundance of fish eggs (Checkley *et al.* 1997, 1999). Mounted on shipboard, seawater from a surface intake is channelled through a fish egg concentrator and viewport. A digital camera creates images of the water that are recorded on a microcomputer- based image processor. Near-real-time estimates of egg abundances are possible with this system. CUFES has been used by a number of countries involved in GLOBEC Small Pelagic Fish and Climate Change (SPACC) projects (Hunter and Alheit 1997; Checkley *et al.* 1999, 2000).

OPC: The OPC, a non-image-forming device, has been used widely. The OPC was developed during the mid-1980s (Herman 1988) and was redesigned in the 1990s (Herman 1992). This instrument measures changes in the intensity of a light beam that occurs when a particle crosses the beam. Light intensity attenuation caused by the passage of a particle across the light sheet is detected and counted, and the magnitude of the change in light intensity is used to determine the size of the particle. The detectable size range is nominally between 250 um and 14 mm. A more sophisticated version of the OPC, the Laser OPC (LOPC) was developed to provide higher sampling frequency and improved information about the shapes of particles as they pass through the laser sheet beam (Herman *et al.* 1998).

As part of the US GLOBEC Northeast Pacific Study, an OPC and a fluorometer mounted on a vertically undulating SeaSoar were used to survey zooplankton and phytoplankton in the California Current between 42.5 and 44.7°N in spring and

Box 6.3 BIOMAPER-II

Sampling of plankton communities historically has been a costly, labour-intensive activity, due in large part to the effort needed for sorting and identifying organisms collected by nets, pumping systems, or water bottles. Thus, in the planning phases of the Global Ocean Ecosystem Dynamics (GLOBEC) programme, more efficient, higher-resolution samplers were designed, tested, and deployed in the field sampling at many of the study sites. Video and acoustic technologies employed have demonstrated the capability for cost-efficient plankton sampling and identification. One such system is the Bio-Optical Multifrequency Acoustical and Physical Environmental Recorder, or BIOMAPER-II. This is a towed system capable of conducting quantitative surveys of the spatial distribution of coastal and oceanic plankton/nekton (Wiebe *et al.* 2002).

BIOMAPER-II consists of a multi-frequency sonar, a Video Plankton Recorder (VPR-Davis *et al.* 1992) system (Davis *et al.* 2005) and an environmental sensor package (CTD, fluorometer, transmissometer). The latter sensor set is used to describe the hydrographic and environmental characteristics of the water column that then can be related to plankton distributions and abundances.

The acoustic system collects backscatter data from a total of 10 echo sounders (5 pairs of transducers with center frequencies of 43 kHz, 120 kHz, 200 kHz, 420 kHz, and 1 MHz), half of which are mounted on the top of the towbody looking upward, while the other half look downward. This arrangement enables acoustic scattering data to be collected for much of the water column.

These acoustic frequencies were chosen to bracket the transition from the Rayleigh to geometric scattering regions for zooplankton and micronekton in the range of 1 to 200 mm. The software enables data aquisition on five frequencies with each pair of transducers. The range of the 0.5 m depth strata allocated for each transducer is dependent on frequency with the lowest frequencies given the longest range and highest frequency the shortest range (i.e. 43 kHz = 200 m, 120 kHz = 200 m, 200 kHz = 149 m, 420 kHz = 100 m, 1,000 kHz = 35 m). Echo integration is normally conducted at 12 s intervals to provide volume backscattering data at all five frequencies. Split-beam data are normally collected at the four lower frequencies, which enables individual targets to be identified and target strength (TS) determined.

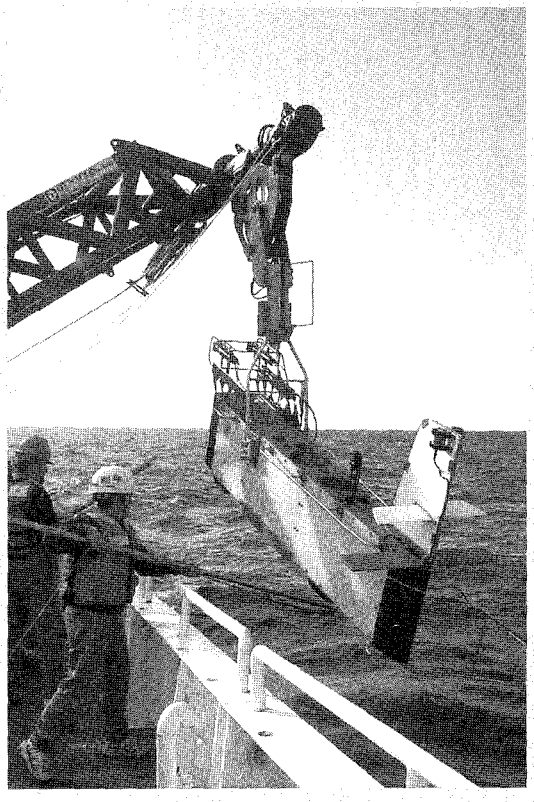
Acoustic data from the up- and down-looking transducers are processed in real time and combined to provide a vertically continuous acoustic record extending from the surface to at least 200 m, and at most 350 m, depending on the position of the BIOMAPER-II along its undulating towyo path.

The VPR is an underwater video microscope that images and identifies and counts plankton and seston in the size range 0.5–25 mm, often in real time. The VPR video data augments the high-resolution acoustical backscatter data. The two systems together allow high-resolution data to be obtained on zooplankton in the water column. The range-gated acoustical data provides distributional data at a higher horizontal resolution than is possible with an independent VPR, while the video data provides high-resolution taxa-specific abundance patterns along the towpath and allows for direct identification, enumeration, and sizing of objects in acoustic scattering layers, so that the VPR data can be used to calibrate the acoustical data.

BIOMAPER-II in combination with a Multiple Opening/Closing Net and Environmental Sampling System (MOCNESS) was used on a series of five US GLOBEC cruises in the Gulf of Maine in a project to examine the overwintering stock of *Calanus finmarchicus*. The high-frequency volume backscattering data provided the most complete coverage of the Gulf of Maine basins on the cruises. Although the backscattering data did not reflect the distribution of the zooplankton and micronekton biomass directly, patterns in the

continues

Box 6.3 ***continued***



Box 6.3, Figure 1 Deployment of BIOMAPER-II at sea (Peter Wiebe).

acoustics data can augment the interpretation of the net tow data taken concurrently. There were clear day/night shifts in some of the profiles of volume backscattering indicating diel vertical migration. During the day, depths below 100 m generally had higher backscattering and surface values were lower. The reverse generally occurred at night. Ground truthing the acoustics data to provide biologically meaningful information has been a significant aspect of the work (see papers by Chu and Wiebe (2003); Warren *et al.* (2003); Benfield *et al.* (2007); Lavery *et al.* 2007).

BIOMAPER-II was also used on the four Southern Ocean GLOBEC broad-scale surveys on the Western Antarctic continental shelf region in the Marguerite Bay environs. In this work, krill distribution and abundance were determined on two austral fall cruises and two winter cruises when pack ice covered the entire survey region. Acoustic volume backscattering was used as an index of the overall biomass of zooplankton. Distinct spatial and seasonal patterns were observed that coincided with advective features (Lawson *et al.* 2004). The general pattern of backscattering across most of the survey area involved low backscattering in the surface mixed layer, moderate backscattering in the pycnocline, a midwater zone that typically had faint scattering, and when the bottom was within range of the transducers, a well-developed bottom scattering layer extending 40 to 100 m above the bottom. More sophisticated methods that capitalize on the full multi-frequency data set were developed. These distinguished the scattering of krill from that of other zooplankton taxa, delineating krill aggregations in the acoustic record, and then estimated krill length, abundance, and biomass in each acoustically identified aggregation (Lawson *et al.* 2008a,b). The distribution of krill was characterized by many small aggregations closely spaced relative to one another, punctuated by much fewer aggregations of very large size that accounted for the majority of overall biomass in the region. The greatest number of aggregations was found at depths less than 100 m, but aggregation biomass was usually greatest at deeper depths. There was little association between the characteristics of individual aggregations and the mean length of krill estimated acoustically, and thus little evidence for any size- or age-related changes in aggregative behavior.

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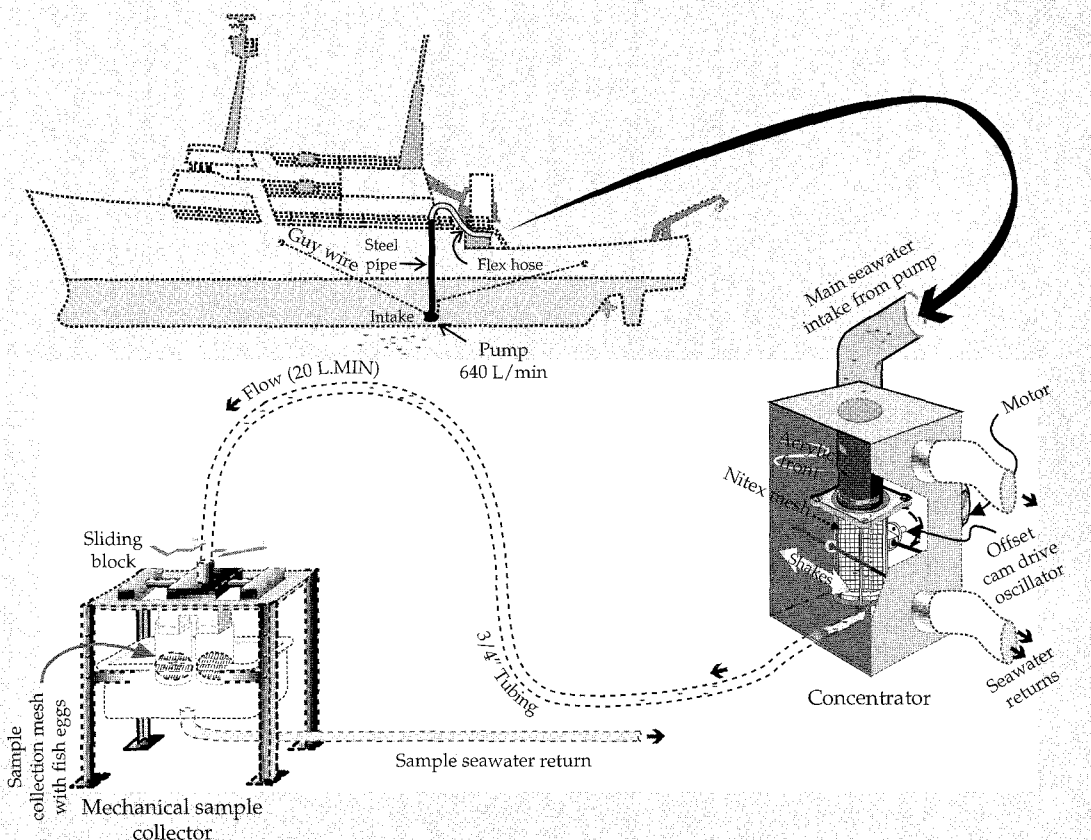
Box 6.4 Continuous, Underway Fish Egg Sampler

The Continuous, Underway Fish Egg Sampler (CUFES) was developed during the 1990s to improve sampling of the typically highly patchy distributions of pelagic fish eggs, and was first applied to test the hypothesis that spawning by Atlantic menhaden (*Brevoortia tyrannus*) occurs during storms along the western wall of the Gulf Stream. Over the past decade the CUFES has been used in many regions, principally to study the distribution of eggs of small pelagic fishes such as anchovy and sardine. The CUFES has now become the standard sampling tool for mapping spawning habitat used by participants in the Small Pelagic Fishes and Climate Change (SPACC) regional programme of Global Ocean Ecosystem Dynamics (GLOBEC), which ensures compatibility for inter-ecosystem comparisons.

A CUFES system consists of a high-volume (ca. 0.5 m^3 min^{-1}), submersible pump either fixed rigidly to the ship's hull or pumping through the hull via a sea-chest; a sample concentrator; and a mechanical sample collector. Water is pumped from pump depth (around 3 m for the external configuration or 6 m for the through-hull configuration) to the concentrator, where particles retained by a 500 µm mesh (or occasionally smaller) are concentrated in a reduced flow. This flow is then directed to the mechanical sample collector, which allows for



Box 6.4, Figure 1 Schematic of the CUFES system (David Checkley).

continues

Box 6.4 *continued*

sequential collection of samples which are generally examined immediately after collection and hence provide near-real-time information on egg abundance. Because the pump can be used while the vessel is both on-station and underway, the CUFES collects many more samples and provides much higher spatial resolution than is possible using standard, on-station ichthyoplankton samplers such as a California Cooperative Oceanic Fisheries Investigations Vertical Egg Tow (CalVET) net. In contrast to the CalVET net which collects a vertically integrated sample at a single location, however, the CUFES only samples at a fixed depth and thus collects horizontally integrated samples at that depth when used while underway, or point-samples from that depth when used while on-station.

The major disadvantage of the CUFES is its inability to sample the entire egg vertical distribution range, but because the eggs of pelagic fishes are typically positively buoyant and abundant near the surface, near-surface sampling allows inference about the areal abundance (i.e. the number of eggs under 1 m^2 of sea surface) and distribution of pelagic fish eggs. Such inference depends on a statistically significant relationship between egg concentration at CUFES pump depth and areal egg concentration, which is the case in most published studies using CUFES and demonstrates the efficacy of CUFES as a sampler of pelagic eggs.

CUFES samples are used to characterize spawning habitat in terms of space and time, and environmental data (temperature, salinity, fluorescence, etc.) collected concurrently with CUFES samples have been used to characterize spawning habitat in terms of hydrography, such characterizations being subsequently used to develop models of spawning habitat. The high spatial resolution of CUFES-derived data has been used to examine the fine-scale spatial structure of egg patches and to optimize sampling design, including the location and spacing of transects and stations for net collections. CUFES has also been incorporated into the Daily Egg Production Method (DEPM) of estimating spawner biomass, with on-board egg counts being used to determine when full water column samples should be taken with a CalVET net. However this adaptive sample allocation is critically dependent on a thorough understanding of the relationship between the abundance of eggs at the CUFES pump depth and vertically integrated egg abundance, and how this may change under different oceanographic conditions. This has stimulated significant research effort aimed at deriving realistic egg vertical distribution models.

Future development involves automation of the counting and staging of eggs of target fish species in CUFES through the use of progressive-scan cameras and line scan video (see Box 6.2). Such automation would reduce cost and provide real-time data on egg abundance and distributions under all conditions. More details on the CUFES are available at http://cufes.ucsd.edu.

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autumn 2000 (Zhou and Zhu 2002). An OPC mounted on a MOCNESS was used in Southern Ocean GLOBEC on autumn and winter process and survey cruises (Zhou *et al.* 2004) to examine size spectra changes between these two seasons and to estimate zooplankton growth and mortality. The OPC has also been used in GLOBEC-related projects in the northern North Atlantic. In the investigation of *Calanus finmarchicus* migrations between oceanic and shelf seas off north-west Europe (Heath 1999b), the Autosampling and Recording Instrumental Environmental Sampler (ARIES; Dunn *et al.* 1993) was equipped with a Mark II OPC and used in conjunction with the serial plankton collector on ARIES to assess the vertical distribution of *Calanus* and other zooplankton in the Faroe-Shetland Channel (Heath *et al.* 1999b, 2000). The NERC Marine Productivity programme (UK GLOBEC) also used ARIES equipped with an OPC to study the dynamics of zooplankton in the Iceland Basin and Irminger Sea on four cruises during 2001 and 2002 (Heath *et al.* 2008b). Sampling extended to depths greater

than 2,500 m. The OPC has also been incorporated into the along-track surface sampling CUFES system used in the SPACC surveys.

6.3.2 Acoustic systems

High-frequency acoustics played a significant role in a number of the GLOBEC field programmes in part due to the rapid pace of technological development of high-speed microprocessors, accessory electronic components, and concomitant software that made a new generation of acoustical instruments possible in the 1990s. Several different acoustic systems have been used. Essentially all of the acoustic sensors were made by commercial companies either as standard off-the-shelf units or as special units configured to meet programmatic requirements. They included single-frequency systems (dual-beam [BioSonics Inc.], split-beam [Hydroacoustic Technologies Inc.-HTI, Simrad Inc.], multiple-beam [Acoustic Doppler Current Profilers, ADCP-Teledyne RDI Inc]) and dual or multiple frequency systems (BioSonics Inc.; HTI Inc.; Tracor Acoustic Profiling System-TAPS, Tracor Inc.; Simrad Inc.). Some were hull mounted systems (Simrad-typically EK500 with 38, 120, and 200 kHz; ADCP-typically 153 kHz), while others were deployed over the side in towed vehicles or profiling systems as described below.

There are two fundamental measurements relevant to the acoustic detection of zooplankton: volume backscattering (integration of the energy return from all individuals in a given ensonified volume, i.e. echo integration) and target strength (TS-echo strength from an individual; Foote and Stanton 2000). Depending upon the construction of the echo sounder and transducers, either or both of these measurements can be obtained. With a single-frequency single-beam transducer, only volume backscattering can be determined directly. A given return cannot be used to discriminate individual size, although statistical procedures have been developed to provide estimates of the animal assemblage size distribution using the data from single-beam transducers (Clay 1983; Stanton 1985a,b). With a series of single-beam transducers operating at different frequencies (e.g. TAPS) and a frequency-dependent theoretical model(s) of backscatter from individual animals, it is possible to estimate animal size distribution in addition to volume backscattering (Greenlaw and Johnson 1983; Holliday and Pieper 1989, 1995). Both dual-beam (Ehrenberg 1974) and split-beam (Ehrenberg 1979) systems provide a direct means of determining individual TS. With a dual-beam system, the TS of an animal that is detected with both beams can be estimated directly, but not its angular position. In contrast, a split-beam system, with a receiving transducer array divided into four quadrants, provides both TS and angular position (Chu and Wiebe 2003).

No single echo sounder or acoustic data processing methodology was used in all of the GLOBEC study areas (Table 6.1). Several echo sounders were used in surveys of Georges Bank and the Gulf of Maine: a 420 and 1,000 kHz BioSonics system was mounted in a towed V-fin, the 'Greene Bomber', for early GLOBEC work on the Bank (Wiebe and Greene 1994; Wiebe *et al.* 1996); a more advanced digital BioSonics system was used in the towed vehicle 'BIOMAPER' to survey the bank until lost at sea (Wiebe *et al.* 1997); its replacement, BIOMAPER-II, was used principally to survey the Gulf of Maine and carried an HTI multiple frequency sonar with pairs of up- or down-looking split-beam transducers operating at 43, 120, 200, 420, and 1,000 kHz (Wiebe *et al.* 2002, see Box 6.3 for more details). The Greene Bomber, outfitted with an HTI sonar operating at 120 and 420 kHz, was used to complete the bank surveys.

A variety of echo sounders were used to survey zooplankton in the Southern Ocean GLOBEC programme. BIOMAPER-II was used on all four of the US survey cruises to map zooplankton distributions and krill patchiness (Lawson et al. 2004, 2008a,b). Independently, another 120/420 kHz HTI towed system and a hull mounted ADCP were used on the Southern Ocean process cruises to survey krill and study their larval development and patch dynamics (Daley 2004; Zhou et al. 2004). A Simrad EK 500 (38/120/200 kHz) was also used to observe krill layers while they were being sampled by a MOCNESS to study net avoidance behaviour (Wiebe et al. 2004).

In the north-east Pacific California Current studies, a four-frequency (38, 120, 200, and 420 kHz) HTI

system with the transducers oriented in a down-looking configuration in a fixed-depth towbody (15 m) was used to measure the fine-scale backscattering from zooplankton (Sutor *et al.* 2005). This same system was used by Ressler *et al.* (2005) to qualitatively map the distribution of euphausiids along the Oregon and California coast using the difference in backscattering at 38 and 120 kHz to identify euphausiid aggregations. A similar approach was used by Swartzman *et al.* (2005) to study euphausiid distributions along the Pacific Coast using a SIMRAD EK-500 split-beam echo sounder operating at 38 and 120 kHz. In the Gulf of Alaska, a TAPS acoustic system was deployed on moorings on the Seward line.

In the northern North Atlantic, there were two major programmes that utilized acoustics as an integral part of their sampling programmes. The Trans-Atlantic Study of *Calanus* (TASC), which focused on experimentation, modelling, and field sampling of *Calanus finmarchicus*, employed an EK 500 operating at 38 and 120 kHz (Kaartvedt *et al.* 1996; Dale *et al.* 1999, 2001; Bagoien *et al.* 2001). During Mare Cognitum, a regional GLOBEC programme in the Nordic Seas (Greenland, Iceland, and Norwegian Seas;Fernö *et al.* 1997) extensive surveys of zooplankton and fish stocks (principally herring and cod) were conducted in the 1990s also using a 38/120 kHz EK 500 (Kaartvedt *et al.* 1996; Torgersen *et al.* 1997; Misund *et al.* 1998; Melle *et al.* 2004).

In the Arabian Sea, the two GLOBEC cruises used a hull mounted 153 kHz ADCP as the principal acoustic instrument to study diel migration of zooplankton and mesopelagic fish and their spatial distribution (Luo *et al.* 2000; Hitchcock *et al.* 2002). A 12 kHz echo sounder was also used on an ancillary basis (Luo *et al.* 2000).

6.3.3 Conventional zooplankton collection systems

Although imaging and acoustical systems such as those described above provide substantially increased sampling frequency and ease of analysis thus allowing biological and physical gradients in the ocean to be examined at high resolution, they do not eliminate the need to collect animals for species and stage identification, rate process experimentation (e.g. feeding, growth and development, egg production-see Section 6.5), or biomass determination. Thus, net systems and/or pumping systems were used in all of the GLOBEC programmes to collect animals to determine their spatial distributions and for rate process measurements (Table 6.1). In addition, the net collections were used to ground-truth or calibrate the optical or acoustic measurements, or for inter-comparison purposes.

For quantitative depth-specific sampling, the principal sampling systems used were the MOCNESS (Wiebe *et al.* 1985), Bedford Institute of Oceanography Net and Environmental Sampling (BIONESS; Sameoto *et al.* 1980), Multi-net (Weikert and John 1981), ARIES (Dunn *et al.* 1993), and Ocean (Dunn *et al.* 1989) samplers (Table 6.1). A number of other non-opening/closing nets were also used either for quantitative vertically integrated or oblique sampling, or for collecting animals for experimental purposes. The Bongo net (McGowan and Brown 1966; Posgay and Marak 1980) was used in many of the Pacific and western North Atlantic studies and the WP-2 net was principally used in northern North Atlantic work (Table 6.1). The Continuous Plankton Recorder (CPR, Hardy 1926) was used in the Canadian and UK GLOBEC programmes. A variety of other ring-nets with varying mesh sizes were also used to sample the zooplankton. Also in the north-west Pacific surface tows were made with a Nordic 264 rope trawl with a mouth opening of approximately 30 × 18 m to capture juvenile salmon (Brodeur *et al.* 2004).

Field comparisons between optical/acoustical sensors and net collections: Inter-comparison of optical and acoustical data with net tow collections is an essential part of the process of assuring that the data from a given instrument can be related to data produced by the other instruments and under what conditions they may be valid. A number of studies have been conducted with the sensors typically used during the GLOBEC programmes. The VPR has been the subject of two inter-comparisons with MOCNESS (Benfield *et al.* 1996; Broughton and Lough 2006). The OPC has been the subject of significantly more calibration work (Heath 1999b; Zhou and Tande 2002; Nogueria *et al.* 2004). High-frequency acoustics data produced by the Biosonics and HTI systems has also been used in a

number of calibration studies using net collections (e.g. Wiebe *et al.* 1996; Lawson *et al.* 2004; Ressler *et al.* 2005; Sutor *et al.* 2005). In most of these comparisons, taxon-specific abundance and size data were used with appropriate acoustic backscattering models (Lavery *et al.* 2007) to predict the volume backscattering. The predictions were then compared to the observed backscattering. This approach has also been used once to examine the biological interpretation of mean volume backscattering strength of ADCP data (Fielding *et al.* 2004). The VPR data combined with MOCNESS data have also been used to interpret acoustic backscattering data (Benfield *et al.* 1998, 2003; Lavery *et al.* 2007; Lawson *et al.* 2008). A clear message that comes from virtually all of these inter-comparisons is that each sensor or sampler has distinct built-in biases and a high degree of caution and in many cases ground-truthing is required in using them to make inferences about the quantitative distribution and abundance of zooplankton.

6.3.4 Animal tags and telemetry

While a number of GLOBEC programmes have included studies of top predators, most of this work employed traditional ship-based survey methods where the distribution of top predators was correlated with oceanographic features (Chapman *et al.* 2004; Ainley *et al.* 2005; Bluhm *et al.* 2007; Ribic *et al.* 2008). This approach has been critical to developing an understanding of trophic relationships and the importance of biophysical forcing of their distribution. This work has shown that apex predators occur in areas where oceanographic features such as currents, frontal systems, thermal layers, sea mounts, and continental shelf breaks increase the availability of prey (Hui 1979; Haney 1986; Ainley and DeMaster 1990; van Franecker 1992; Hunt 1997; Tynan 1998). All these features and processes are thought to impact predator distributions by physically forcing prey aggregations and, thus, creating areas where foraging efficiency can be increased (Ainley and Jacobs 1981; Croxall *et al.* 1985; van Franecker 1992; Veit *et al.* 1993). Indeed, for many predators, regions of highly localized productivity may be essential for reproduction and survival (Haney 1986; Costa *et al.* 1989; Fraser *et al.* 1989; Hunt *et al.* 1992; Veit *et al.* 1993; Croll *et al.* 1998, Croll *et al.* 2005).

However, the survey approach has limitations as the associations are limited to population-level studies where the distribution of animals is correlated with oceanography. Although these studies have been and continue to be quite informative, they do not provide insights into the strategies employed by individual animals, nor can they provide information on the spatial or temporal course of these interactions. Advances in satellite telemetry, electronic tags, and remote sensing methods are providing new tools that allow us to follow the movements and behaviour of individual animals. These studies provide insights into the links between predators, prey, and the oceanic environment (Boustany *et al.* 2002; Block 2005; Crocker *et al.* 2006; Shaffer *et al.* 2006; Biuw *et al.* 2007). These new tools make it possible to extend our understanding beyond linkages of prey and predator distributions with environmental features (Biuw *et al.* 2007). The key to understanding the processes that lead to high predator abundance is the identification of the specific foraging behaviours associated with different environmental conditions (Guinet *et al.* 1997).

These new tools or electronic tags have provided field biologists with a new form of 'biotechnology' that allows the study of complex behaviour and physiology in freely ranging animals (Costa and Sinervo 2004). This technology has produced data loggers small enough to be attached to animals while they freely go about their activities (Block 2005; Shaffer and Costa 2006). Information on the movement patterns, depth utilization, and/or diving behaviour are obtained when the tags are recovered (archival tags) or when transmitted via satellite. Archival and satellite linked tags have made possible the study of ocean basin-scale movements, oceanographic preferences, and behaviours of many pelagic species (Delong *et al.* 1992; McConnell *et al.* 1992a,b; Costa 1993; Block *et al.* 1998; Klimley *et al.* 1998; Lutcavage *et al.* 1999; Block *et al.* 2001; Gunn and Block 2001; Boustany *et al.* 2002; Metcalfe 2006). Further advances in data compression have made it possible to get significantly more information through the limitations of the ARGOS system, including detailed oceanographic and behavioural information (Fedak *et al.*

2001). As these new techniques and tools became available they have been incorporated into GLOBEC programmes such as Southern Ocean GLOBEC (Burns *et al.* 2004; Burns *et al.* 2008; Costa *et al.* 2008) and the CLimate Impacts On TOp Predators (CLIOTOP) programme. These new tools used in conjunction with established survey methods are providing an understanding of the distribution of oceanic organisms in relationship to their changing physical and biological environments. There are a variety of new devices that can be used to track fish and other marine organisms such as miniature Global Positioning System (GPS) devices (Rikardsen *et al.* 2007) and GPS tags that can be deployed on marine organisms that frequently come to the surface (www.wildlifecomputers.com). There are a number of programmes that are using these new technologies to gain an understanding of the large-scale movements of marine organisms, such as the Tagging of Pacific Pelagics programme (www.topp.org; Block *et al.* 2003), the Pacific Ocean Tracking Project (www.postcoml.org), and the Ocean Tracking Network (http://oceantrackingnetwork.org).

A comparison of the advantages and disadvantages of the two approaches of studying top marine predators can be seen in Table 6.2. While *in situ* environmental data can be collected using both methods, electronic tags allow us to follow the animals wherever they go. In contrast, survey data are limited to areas where the observation platform can go. In some cases this can lead to a significant bias in our understanding of the distribution of a species. For example, prior to the deployment of electronic tags, northern elephant seals were thought to range just offshore along the west coast of North America (Fig. 6.1a), whereas, tracking data showed that they travel across the entire north-eastern Pacific Ocean (Fig. 6.1b). Tagging data provide a time series that can last from months to in some cases years, and provide behavioural information that can be used to identify behaviours and associated habitats. Depending on the type of tag deployed data acquired can range from a simple surface track (Fig. 6.2a), to a surface track with a dive profile (Fig. 6.2b) or a surface track and dive profile with associated environmental data (Fig. 6.2c; temperature, salinity, and/or light level). Such behavioural data are important to identify differences in the movement patterns and habitat utilization of different species. For example, some species may travel over considerable distances (southern elephant seals), while others may remain within a smaller home range (Weddell seals, Fig. 6.3). Such differences in behaviour would not be apparent with traditional survey methods. However, tagging data have some significant limitations. Foremost among these is that data can only be collected from animals that can be tagged and that there is as yet no way to derive estimates of animal abundance. For these reasons, Southern Ocean GLOBEC employed both approaches and is in the process of integrating these complementary approaches to obtain a more complete picture of the ecology of top predators (Burns *et al.* 2004; Ribic *et al.* 2008).

Table 6.2 Comparison of survey and tagging methods to determine the distribution of marine animals.

Measure of animal distribution and abundance	
Survey	*Electronic Tags*
Advantages:	Advantages:
Can sample; hard to study species	Long time series
Environmental data	Animal behaviour
Physical environment	Dive pattern
CTD, chlorophyll	Animal movements
	Home range
	Habitat utilization
	Environmental data
	Physical environment
	CTD, chlorophyll
Disadvantages:	Disadvantages:
Snapshot	Must be able to tag animal
Only know about area surveyed	No direct measure of abundance
Biased measure of range	
Sample bias	
Animal behaviour	

Archival tags: Archival tags are data logging tags that record data as a time series from sensors that measure depth (pressure), water temperature, salinity, animal body temperature, and light level. The major limitation of archival tags is that they must be recovered in order to obtain the data. However, judicious choice of animals or use on exploited species where a reward is offered has provided a wealth of information on the foraging behaviour and habitat utilization of a large group of marine organisms (Block 2005; Shaffer and Costa 2006;

Shaffer *et al.* 2006). Archival tags have provided tracks covering up to 3.6 years (Block *et al.* 2001).

Movement patterns can be derived with archival tags by examining changes in light level to establish local apparent noon. In turn, longitude and day length can be estimated from time of sunrise and sunset to determine latitude (Ekstrom 2004). These locations can be further corrected using sea surface temperatures (SST; Teo *et al.* 2004; Shaffer *et al.* 2006). Salmon researchers have also been using depth and temperature archival tags to discern more about the behaviour and movement of salmonids in relationship to their environment. The data intensity of these devices allows studies of both fine- and large-scale behavioural patterns, migratory routes, and physiology, all in relation to the environment (Boehlert 1997).

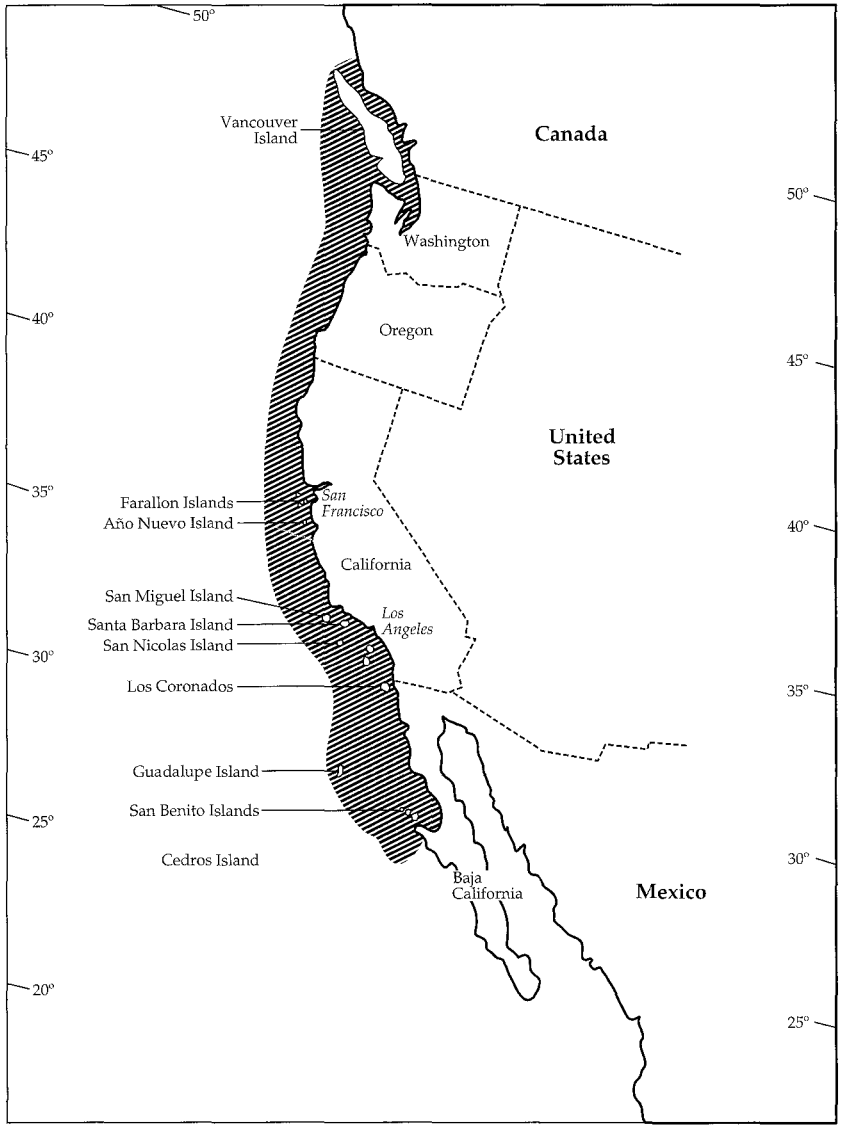

Figure 6.1 (a) Distribution of northern elephant seals as determined using boat and plane based surveys. (continues). Reproduced with permission of Ecological Society of America, from Foraging ecology of northern elephant seals, Le Boeuf, B. J., Crocker, D. E., Costa, D. P., *et al.* Ecological Monographs, **70**, 2000; permission conveyed through Copyright Clearance Center, Inc.).

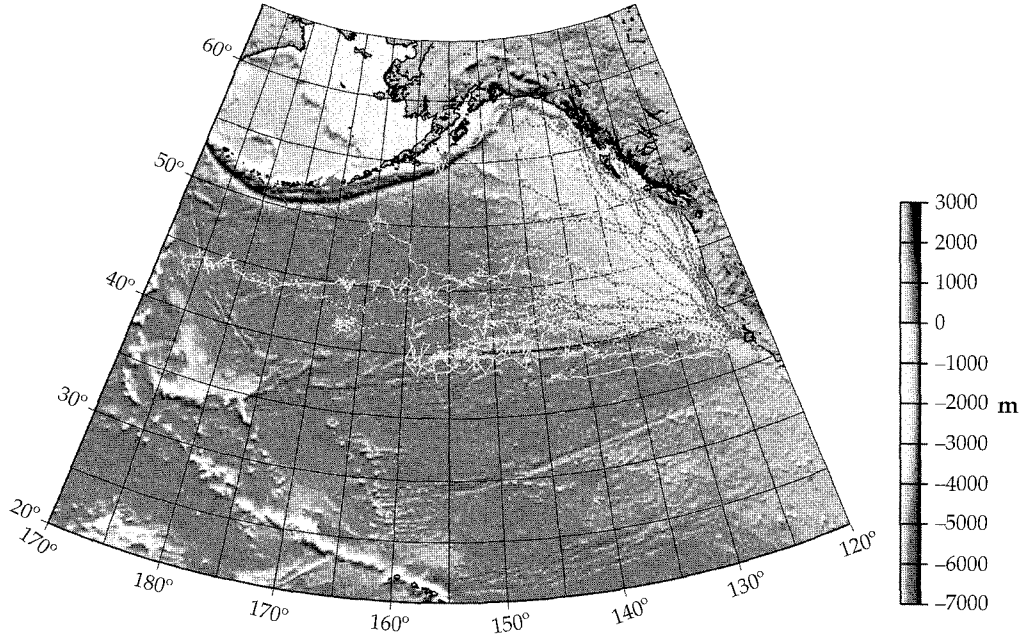

Figure 6.1 (continued) (b) Distribution of northern elephant seals determined using satellite telemetry. Reproduced with permission of Ecological Society of America, from Foraging ecology of northern elephant seals, Le Boeuf, B. J., Crocker, D. E., Costa, D.P. *et al.* Ecological Monographs, **70**, 2000; permission conveyed through Copyright Clearance Center, Inc.). (See Plate 16).

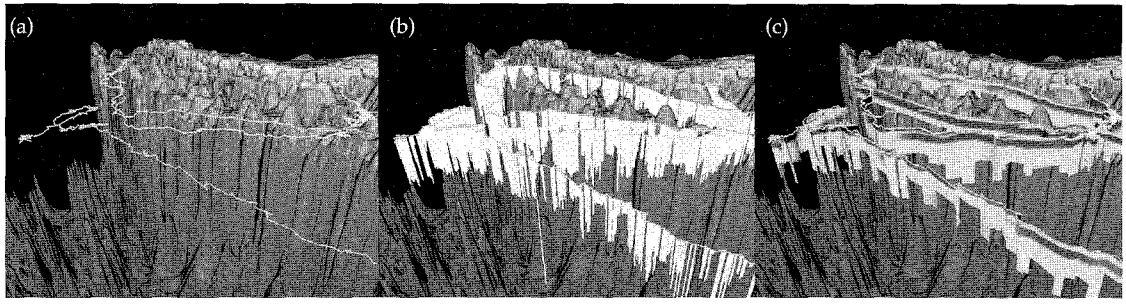

Figure 6.2 Track of southern elephant seals in the western Antarctic Peninsula obtained using the SMRU CTD-SRDL 9,000 tag. (a) Surface track. (b) Shows the surface track and with diving behaviour. (c) The temperature and salinity profiles obtained from animal dives. (From Costa, Goebel, and McDonald, unpublished data.) (See Plate 17).

Argos satellite tags: Satellite tags provide at sea locations and have the advantage that the data can be recovered remotely without the need to recover the tag. Satellite-linked data recorders have expanded our understanding of the fine-scale movements of marine birds (Weimerskirch *et al.* 1993, 2000; Burns and Kooyman 2001), sea turtles, (Renaud and Carpenter 1994; Polovina *et al.* 2000), sharks (Eckert *et al.* 2002; Weng *et al.* 2005), and marine mammals (McConnell *et al.* 1992a,b; Le Boeuf *et al.* 2000; Shaffer and Costa 2006). Since the antenna on the satellite transmitter must be out of the water to communicate with an orbiting satellite, the technology has mainly been used on air-breathing vertebrates that surface regularly. For large fish and other animals that remain continuously submerged, the ability to transmit at the surface is not possible. For these organisms, a pop-up satellite archival tag (PSAT) has been

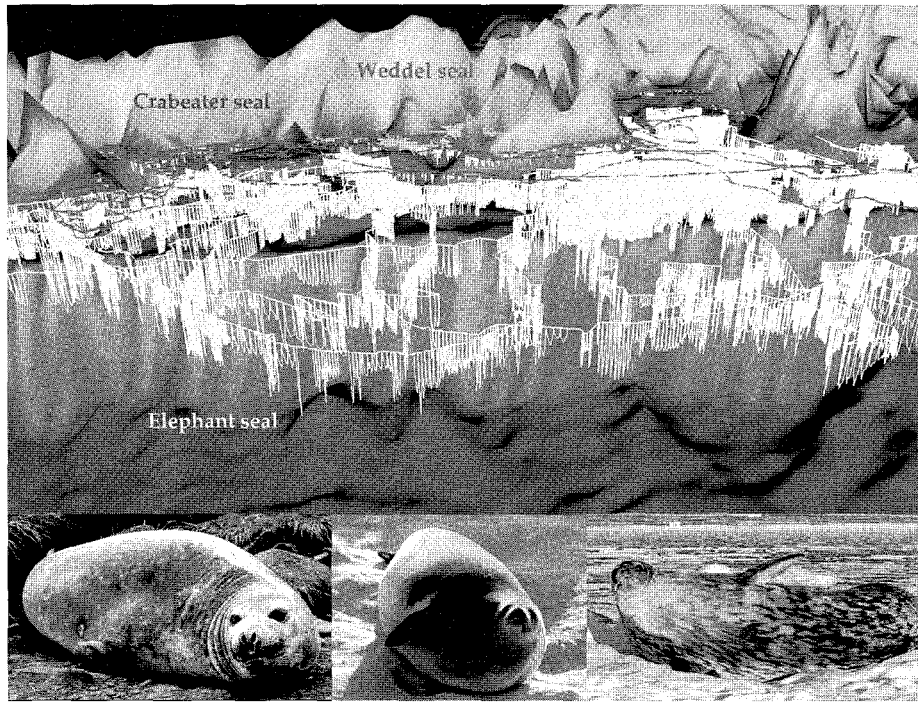

Figure 6.3 Differences in the movement patterns of southern elephant seals (yellow), crabeater seals (red), and Weddell seals (green) along the Antarctic Peninsula. The tracks cover the same time period during 2007. (From Costa, Goebel and McDonald, unpublished data). (See Plate 18).

developed (Block *et al.* 1998; Lutcavage *et al.* 1999; Block *et al.* 2001; Boustany *et al.* 2002). Pop-up satellite tags combine data storage tags with satellite transmitters. The pop-up satellite device communicates with the ARGOS satellites that serve to both uplink data and calculate an end-point location. Importantly, the tags are fisheries independent in that they do not require recapture of the fish for data acquisition.

GPS tags: Development of a GPS tag has increased the precision of animal movement data to within 10 m compared to the 1–10 km currently possible with ARGOS satellite tags. Such precision is allowing measurements of animal movements relative to the mesoscale features and will provide higher-resolution locations for the physical oceanographic data collected by the animals. However, standard navigational GPS units require many seconds or even minutes of exposure to GPS satellites to calculate positions and the on-board calculations required consume considerable power. A GPS system that can obtain GPS satellite information in less than a second and can transmit the location information within the narrow bandwidth of the ARGOS system has now been developed. The Fastloc system uses a novel intermediate solution that couples brief satellite reception with limited on-board processing to reduce the memory required to store or transmit the location.

Marine animals as oceanographers: An exciting, recent development from observing diving predators such as marine mammals, fish, and birds has been the realization that electronic tag-bearing animals can be employed as autonomous ocean profilers to provide environmental data in diverse ocean regions (Costa 1993). A significant advantage of such oceanographic data is that they are collected at a scale and resolution that matches the animals' behaviour (Fig. 6.2). As more environmental information is gathered and delivered from the tagged animals, new insights will be obtained about their individual behaviours, as well as how they respond to environmental variability on daily, seasonal, and interannual

time scales. Animal-collected oceanic data can complement traditional methods for assimilation into oceanographic models. The feasibility of marine animals as autonomous ocean profilers has been proven by deployments of temperature and salinity tags on a variety of marine species, such as marine mammals (e.g. Boehlert *et al.* 2001; Hooker and Boyd 2003; Campagna *et al.* 2006; Biuw *et al.* 2007; Costa *et al.* 2008), seabirds (e.g. Weimerskirch *et al.* 1995; Charrassin *et al.* 2002), turtles (McMahon *et al.* 2005), and fish (Weng *et al.* 2005). While the acquisition of such environmental data has been ongoing, only recently have these data begun to be used to address specific oceanographic questions (Charrassin *et al.* 2002; Costa *et al.* 2008).

The most advanced oceanographic tag is the Sea Mammal Research Unit 9000 CTD~SRDL (Satellite Relay Data Logger; www.smru.st-andrews.ac.uk). In addition to collecting data on the animal's location and diving behaviour it collects conductivity, temperature, and depth (CTD) profiles. The tag looks for the deepest dive for a 1- or 2-hour interval. Every time a deeper dive is detected for that 1–2-hour interval, the tag begins rapidly sampling (2 Hz) CTD from the bottom of the dive to the surface. These high-resolution data are then summarized into a set of 20 depth points with corresponding temperatures and conductivities. These 20 depth points include 10 predefined depths and 10 inflection points chosen via a 'broken stick' selection algorithm. These data are then held in a buffer for transmission via ARGOS. Given the limitations of the ARGOS system, all records cannot be transmitted; therefore a pseudo-random method is used to transmit an unbiased sample of stored records. If the SRDLs are recovered, all data collected for transmission, whether or not they were successfully relayed, can be recovered. An example of the kind of coverage provided by these tags can be seen in Figure 6.4.

6.4 Advances in shipboard, laboratory, and *in situ* process studies

GLOBEC process studies have required new experimental approaches to investigating specific mechanisms which are thought to link ecosystem responses with environmental variability. Innovative methods to understand key components of the population dynamics of target species, both zooplankton and fish, have been used, focusing particularly on reproduction, growth and mortality, and between-species interactions. An extensive programme of laboratory experimentation on zooplankton and fish maintained under controlled conditions has been fostered. These experiments have focused on determining vital rates, such as feeding, growth, and reproduction of target species and this information has been especially valuable for model parameterization.

The GLOBEC focus on the influence of global change on marine animal populations required investigation of processes controlling abundance and productivity and how these processes are affected by environmental variability. The abundance

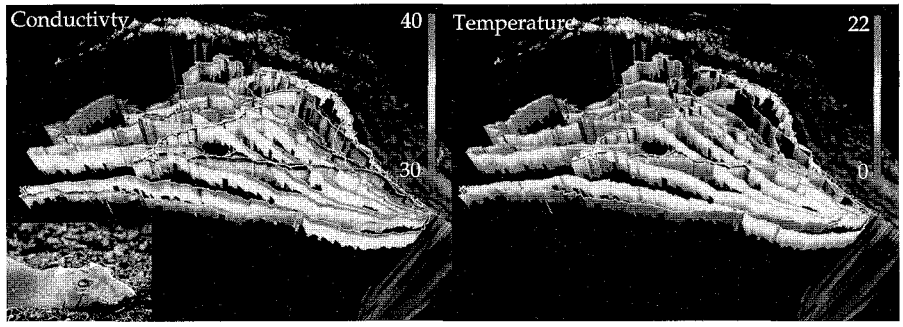

Figure 6.4 Left: conductivity and right: temperature profiles obtained from seven female elephant seals migrating across the North Pacific Ocean. The different coloured lines refer to the tracks of individual seals and the 'curtain' effect shows the depth over which the CTD data were obtained. The coloured bars are the scale for conductivity (mS/cm) and temperature (°C). Inset lower left: female elephant seal with CTD tag on her head. (From Costa, unpublished data). (See Plate 19).

of a pelagic population distributed in some defined volume of the ocean can be expressed as:

1. $dn/dt = (b - d)n - \varepsilon n + \iota n_b$,

where n is the number of individuals per unit volume, b and d are the instantaneous population birth and mortality rates, and ε and ι are the emigration and immigration rates and n_b is the abundance of individuals in the surrounding water (Aksnes *et al.* 1997). In a population of planktonic copepods, primary target organisms in GLOBEC studies (see Gifford *et al.*, Chapter 4, this volume), the change in abundance is a function of the recruitment rate (R_i) into each life stage, i, the hatching or moulting rate, M_i, into the next stage and the instantaneous mortality rate, d_i, excluding advective terms, as follows (Aksnes *et al.* 1997):

2. $dn_i/dt = R_i(t) - M_i(t) - d_i(t)n_i(t)$.

The variable, M_i, is determined by the stage-specific development rates and the initial input into the population, R_1, can be estimated from the measurement of egg production rates. Understanding change in productivity also involves processes influencing change in mass (typically as carbon or nitrogen), requiring investigation of influences on rates of growth and feeding in planktonic populations (e.g. Omori and Ikeda 1984).

In the following sections, we describe approaches used to measure and investigate processes determining birth, growth, feeding, and mortality rates in the GLOBEC-related studies. A common feature across many of these approaches is the ability to capture and observe living zooplankton in controlled settings, either in shipboard or shore laboratories or in mesocosms. These approaches contributed to and continue to be a source of quantitative understanding and parameterization of the rate processes determining population dynamics and production for incorporation into coupled physical-biological models that simulate effects of climate forcing on the secondary and higher levels of the pelagic ecosystem (e.g. deYoung *et al.* 2004a; Runge *et al.* 2005).

6.4.1 Zooplankton reproduction

6.4.1.1 Reproduction of planktonic copepods

Climate forcing may directly impact R_1, the rate of production of new individuals, through the bottom-up influence of ambient temperature and food supply. In the 2 decades leading up to the start of the GLOBEC programmes, laboratory studies of the effect of temperature, food concentration, and food quality on rates of egg production of planktonic copepods (e.g. Corkett and McLaren 1969; Runge 1985b; Kleppel 1992; reviewed for calanoid copepods in Mauchline 1998) had already indicated that these environmental variables could have a strong influence on copepod birth rates. New techniques for estimating female-specific egg production rates (eggs per female per day) of broadcast-spawning planktonic copepods, involving incubation of females immediately after capture (e.g. Dagg 1978; Durbin *et al.* 1983; Runge 1985b), expanded capabilities for measurement of *in situ* egg production rates of both broadcast spawners and copepods bearing eggs until hatching, for which egg production can also be estimated by the egg ratio method (Edmondson 1960; Aksnes *et al.* 1997).

The measurement and understanding of factors determining variability of copepod egg reproductive rates increased enormously during the GLOBEC years. While not all these studies were conducted with GLOBEC support, clearly research from GLOBEC national programmes both stimulated and contributed significantly to advancement of knowledge of zooplankton reproduction. Further assessment (e.g. Laabir *et al.* 1995; Saiz *et al.* 1997) and refinements to the incubation method were made (see methodology and procedures review in Runge and Roff 2000) and the method was applied to a wide diversity of copepod species in habitats ranging from the tropics to the polar seas (e.g. Plourde *et al.* 2001; Durbin *et al.* 2003; Hopcroft *et al.* 2005; Napp *et al.* 2005; Stenevik *et al.* 2007; Peters *et al.* 2007). A review of studies of copepod egg production rates collected between 1977 and 1999 showed significant Michaelis-Menten type relationships between egg production rate and chlorophyll *a* for broadcast spawners but not for egg-bearing species (Bunker and Hirst 2004). Increasing temperature was found to have little effect on broadcast spawners and more so in egg-bearing species (which must wait for temperature-dependent hatching before producing a new clutch of eggs). Considerable focus in North Atlantic and north-east Pacific GLOBEC programmes was on target species

in the genera *Calanus*, *Neocalanus*, and *Pseudocalanus*. In the north-west Atlantic in spring and early summer, the egg production rates of *Calanus finmarchicus* followed a hyperbolic relationship with chlorophyll *a*, but with a critical concentration corresponding to an average chlorophyll concentration in the upper 50 m of 1.6–1.8 $\mu g\ l^{-1}$, above which food does not generally limit egg production (e.g. Campbell and Head 2000; Runge *et al.* 2006). This is considerably lower than the critical concentration indicated by the general equations in Bunker and Hirst (2004). In stratified summer waters, however, the relationship with chlorophyll *a* breaks down (e.g. Runge and Plourde 1996; Jonasdottir *et al.* 2005), suggesting different trophic connections to microzooplankton (e.g. Ohman and Runge 1994), and there is clearly high variability in the relationship between *Calanus* species egg production and chlorophyll in the North Atlantic (e.g. Gislason 2005) as well as the upwelling coastal regions of the north-east Pacific (e.g. Peterson *et al.* 2002). Moreover, there may be genotypic variability in the use of internal lipid stores to fuel egg production between populations across basins (cf. Runge *et al.* 2006 and Mayor *et al.* 2006).

A number of GLOBEC-related studies have investigated the composition and nutritional quality of food and the connection to microzooplankton as prey, providing insight into sources of variability in the chlorophyll-egg production relationship. In the laboratory, studies combined culture techniques for maintaining copepods in laboratory settings with sophisticated manipulation and analysis of nutritional composition, including concentrations of essential amino acids (e.g. Helland *et al.* 2003), lipids (e.g. Shin *et al.* 2003; Peters *et al.* 2007), and nitrogen (e.g. Augustin and Boersma 2006).

New methods for estimating egg production rate by assessment of the state of gonadal maturity in copepod females were also developed (e.g. Niehoff and Hirche 1996; Niehoff 2007). By calibrating the state of gonadal maturity in a female population with incubation measurement of egg production in the same population, the female-specific egg production rate can be determined from preserved net samples (Niehoff and Runge 2003). The product of the female-specific rate with the corresponding female abundance (individuals m^{-2}) yields the population egg production rate (eggs $m^{-2}\ day^{-1}$). This variable has been used to estimate mortality rate of the eggs and early naupliar stages of *Calanus* species (e.g. Ohman *et al.* 2002; Hirst *et al.* 2007) and can be applied broadly to measure the production of copepod eggs and naupliar stages as prey for early life stages of fish.

6.4.1.2 Hatching success

The measurement, on-board ship or in shore-based laboratories (methodology discussed in Runge and Roff (2000) and Pierson *et al.* (2005); see also Buttino *et al.* (2004) for application of fluorescent probes), of the fraction of eggs spawned that successfully hatch into nauplii took on important significance for understanding the control of copepod populations after the suggestion that feeding by female copepods on diatom diets in the laboratory can result in production of deformed nauplii or complete inhibition of egg hatching (Poulet *et al.* 1995; Ban *et al.* 1997; Ianora *et al.* 2003). Causative agents have been found to be volatile unsaturated aldehydes transformed from diatom derived polyunsaturated fatty acids (Pohnert *et al.* 2002); the ability to form reactive aldehydes varies among diatom species and even among isolates of the same species. This mechanism in diatoms for inhibition of embryogenesis and nauplius development has been argued to be a plant-herbivore interaction that prevents copepod grazers from efficiently utilizing spring diatom blooms and suppresses copepod recruitment (Ianora *et al.* 2004). In a global comparative study, hatching success across copepod species and ocean habitats was found to be high most of the time, even during diatom blooms (Irigoien *et al.* 2002). There were, however, occasional periods of low hatching success, and viability of early naupliar stages was not observed. Shipboard experiments to observe feeding behaviour, reproduction, and hatching success of *Calanus pacificus* in a coastal Pacific ocean habitat revealed that female copepods are capable of avoiding ingestion of harmful diatoms when feeding on a natural mixture of phytoplankton and microzooplankton prey (Leising *et al.* 2005), but that harmful effects of low hatching success and naupliar viability can occur during unicellular diatom

blooms when there are few other food choices (Pierson *et al.* 2005).

6.4.1.3 Euphausid reproduction

The euphausids, *Euphausia pacifica* and *Thysanoessa inermis*, were target organisms (see Gifford *et al.*, Chapter 4, this volume) in the US GLOBEC Northeast Pacific programme and *Euphausia superba* was a target organism in the Southern Ocean. Incubation methods have been employed to observe spawning characteristics and egg production of these species. Findings indicate species-specific responses in spawning strategies to differences in food supply in specific environments. Pinchuk and Hopcroft (2006) observed a spawning period during April and May, coinciding with the spring bloom, for *T. inermis* and from early July through October, coinciding with development of seasonal stratification, *for E. pacifica* in the Gulf of Alaska. They found a strong, hyperbolic relationship between brood size of *E. pacifica* and chlorophyll *a*, in which the average chlorophyll concentration at which brood size was maximal was approximately 0.7 μg chlorophyll *a* l^{-1}. In contrast, the absence of a significant relationship between brood size of *T. inermis* and chlorophyll *a* concentrations indicates either reliance on stored lipid reserves or feeding on prey other than phytoplankton. In laboratory observations of spawning behaviour, Feinberg *et al.* (2007) found high variability in egg production characteristics among individuals of *E. pacifica*, suggesting that this species has a very plastic reproductive strategy in different environments. Quetin and Ross (2001) used direct observations of *E. superba* egg production in their analysis of the role of spring sea ice retreat and the extent of spring sea ice in determining the intensity and timing of reproduction and subsequent recruitment into populations along the western Antarctic Peninsula.

6.4.2 Growth and development rates

During the GLOBEC programmes both laboratory and field studies were employed to investigate the growth and development rates of target zooplankton species for the ecosystems under investigation.

6.4.2.1 Laboratory studies

Studies of growth and development rates in the laboratory were undertaken to assess growth in the field (e.g. Campbell *et al.* 2001b), to aid in investigations of mortality (Eiane *et al.* 2002; Durbin *et al.* 2003; Eiane and Ohman 2004; Ohman *et al.* 2004), to calibrate new techniques for estimating growth rates *in situ* (Wagner *et al.* 2001), for use in population and biophysical coupled models (e.g. Lynch *et al.* 1998; Miller *et al.* 1998; Stegert *et al.* 2007), and to allow for estimates of secondary production rates through data integration techniques. A laboratory study of *Calanus finmarchicus* growth and development rates by Campbell *et al.* (2001a) was a key component of the US GLOBEC Georges Bank programme. It was identified early on that these data were sorely needed, especially for the construction of biophysical coupled models that would be used to guide the development of the programme and future studies. The work was designed to investigate the growth and development rates of this target species under a range of temperatures and food concentrations that spanned the environmental conditions encountered on Georges Bank. There were several major findings from the study: (1) Maximum stage-specific development rates as a function of temperature were fully described by a series of Belehrádek functions. (2) The effect of food limitation on development and growth rate was determined. (3) Food requirements for growth were greater than those for development. (4) Growth rates were not equivalent for all stages; it was unwise to estimate secondary production rates of the population from egg production rates alone.

Another series of experiments was undertaken to investigate growth and development rates of *Calanus helgolandicus* under the auspices of the European Trans-Atlantic Study of *Calanus finmarchicus* (TASC) initiative (Harris *et al.* 2000; Rey *et al.* 2001; Rey-Rassat *et al.* 2002b). These studies focused on the effects of food quality on the growth and development rate of naupliar stages and on food concentration with respect to copepodite stages. Rey *et al.* (2001) found that naupliar growth and development rates were different when grown on different algal species and that factors influencing development were different than those for growth.

In a related experiment, Rey-Rassat *et al.* (2002b) described a new method for estimating growth rates in laboratory studies based on the initial weight of each stage that better described the growth within a stage compared to earlier studies. Also, these authors found that food requirements for growth were greater than those for development for copepodite stages, the same conclusion reached by Campbell *et al.* (2001a) for *C. finmarchicus*.

6.4.2.2 Incubations with natural populations

Over the course of the GLOBEC programmes, two main methods were used to determine moulting and/or growth rates for naturally occurring populations of copepods. The first was the artificial cohort method and variations thereof. This method was first proposed by Kimmerer and McKinnon (1987) and involves construction of artificial cohorts from naturally occurring populations by sequential sieving of the catch and a following incubation under ambient environmental conditions. Moulting rates can then be determined from the change in the stage frequency distribution between the initial sample and final sample collected after the incubation period (e.g. Liu and Hopcroft 2006a) or from a series of samples collected over time (Campbell *et al.* 2001b; see papers for details of methods). The main criticism of the method is that non-uniform age distributions within a stage can bias estimates of development rate from moulting rate. Growth rates can be estimated from knowledge of initial and final stage distributions, stage-weights, and incubation time (Liu and Hopcroft 2006a, 2007b). The artificial cohort technique is useful when numerous stages/species are present and it is not practical to sort for single stage incubations, but care must be taken when interpreting results. A second approach is the direct measurement of moulting/growth from incubations (e.g. Renz *et al.* 2007, 2008). This method has the advantage that a direct measurement of growth can be determined from initial and final weight measurements (e.g. Campbell *et al.* 2001b), although it has the same potential bias for estimating development rate as the artificial cohort technique (Hirst *et al.* 2005). To estimate the growth and moulting rates of euphausiids, incubations with individual animals were the method of choice (e.g. Daly 2004; Pakhomov *et al.* 2004; Ross *et al.* 2004; Pinchuk and Hopcroft 2007). In these experiments moulting rates were determined in the same manner as for the copepod experiments, and growth rate from the incremental length increase between the euphausiid and its moult, and length:weight relationships.

Incubation studies require a substantial effort to obtain even a very few measurements, but they have been the cornerstone for understanding the variability in growth and development processes of target zooplankton species. They have provided knowledge on the relationships between temperature and food on the growth and development of naturally occurring populations that would otherwise be unattainable (e.g. Liu and Hopcroft 2006a). Comparisons with laboratory measurements and ambient and enriched incubation treatments have provided important insights into the role that food limitation may play in limiting secondary production rates (e.g. Campbell *et al.* 2001a). Although rate measurements from laboratory experiments are often used in biophysical coupled models, the field measurements are necessary for ground-truthing.

6.4.2.3 In situ methods

Several new techniques for estimating growth rates *in situ* have been under investigation for some time (see Runge and Roff 2000). One of the more promising techniques uses nucleic acid ratios, specifically total RNA:DNA ratios measured with a microplate fluorescent assay technique (e.g. Wagner *et al.* 1998, 2001). The obvious advantage of using this technique is the ability to obtain estimates of growth rates of individuals in naturally occurring populations without having to worry about the potential bias of 'bottle effects' associated with incubation techniques. However, it was found that the RNA:DNA ratios were sensitive to temperature, food, and stage of development of the species of interest and therefore, extensive laboratory calibration was required before the approach could be applied to field populations. The technique was used successfully to demonstrate the importance of food limitation on growth rates of *Calanus finmarchicus* on Georges Bank and the Gulf of Maine (Campbell *et al.* 2001b; Durbin *et al.* 2003) and was also shown to be a very good predictor of egg production rates for this same species (Durbin *et al.* 2003). Another approach, employing measurement

of aminoacyl-tRNA synthetase enzyme activity level, has been shown to be a significant index of somatic growth of copepodid stages in laboratory experiments, but has yet to be worked out as reliable measure of growth rates in the sea (Yebra *et al.* 2005).

6.4.3 Feeding studies

In the GLOBEC programmes, studies of ingestion of key species of copepods and euphausiids were undertaken in order to understand their feeding behaviour under natural conditions, including selectivity, ingestion rates, and daily food requirements. Generally, two approaches were used: the gut pigment method (Irigoien *et al.* 1998; Pakhomov *et al.* 2004) and bottle incubations (Irigoien *et al.* 1998, 2000; Meyer-Harms *et al.* 1999; Harris *et al.* 2000; Liu *et al.* 2005; Dagg *et al.* 2006). The gut pigment method has best been used to estimate grazing impacts on phytoplankton or as a complement to the bottle incubation approach, but it is not adequate to estimate total food intake because of the importance of non-pigmented microzooplankton in the diets of mesozooplankton. It does however have the advantage of being an *in situ* method and by definition eliminates the question of 'bottle effects'. The method had been criticized in the past because of a belief that pigment degradation in the gut was variable and could destroy up to 90% of the pigment resulting in substantial errors in estimates of chlorophyll ingestion. However, it has recently been demonstrated that the 'disappearance' of pigment both by degradation and evacuation processes is accounted for during gut evacuation rate measurements and therefore the method is valid as long as concurrent estimates of pigment disappearance are determined (see Durbin and Campbell 2007).

The bottle incubation method was the gold standard approach for measuring feeding rates in the GLOBEC studies and probably will continue to be for the foreseeable future. This approach allows for the estimate of total ingestion including both phytoplankton and microzooplankton food sources. Phytoplankton ingestion rates were determined through changes in chlorophyll (Liu *et al.* 2005; Dagg *et al.* 2006), pigment composition by High Performance Liquid Chromatography (HPLC; Irigoien *et al.* 1998; Meyer-Harms *et al.* 1999), cell counts by automated (flow cytometer, FlowCAM), or microscopic counting methods (Liu *et al.* 2005; see papers for methods). Ingestion of microzooplankton was estimated by the Utermöhl microscopic approach (Irigoien *et al.* 1998; Liu *et al.* 2005). In general, it was found that the target mesozooplankton species fed selectively, sometimes preferring certain phytoplankton groups (Meyer-Harms *et al.* 1999) or microzooplankton (Liu *et al.* 2005). However, microzooplankton were not an important food source when they were not abundant (Irigoien *et al.* 1998). In addition, the method allowed for the determination of predictive relationships between food concentration and ingestion (Dagg *et al.* 2006) and for the estimation of food requirements and seasonal changes in total ingestion (Irigoien *et al.* 1998; Liu *et al.* 2005). The method does not, however, adequately estimate the ingestion of large rare food sources such as small metazoans or particle aggregates. This will most likely come from the future development of new techniques that can quantify rates *in situ* through genetic analysis of stomach contents (e.g. Nejstgaard *et al.* 2003; see Box 6.1).

6.4.4 Zooplankton mortality

The comprehensive GLOBEC field studies made it possible to address zooplankton mortality, an important process affecting zooplankton behaviour, population growth rates (see equation (1) above), abundances, and spatial distributions. Unlike many other rate processes, however, mortality rates relevant to natural populations cannot be measured in incubations in the laboratory or shipboard because it is impractical to include all sources, or even the dominant sources, of mortality within a container. Mesocosm experiments have proven useful for assessing background mortality rates when most predators are excluded (e.g. Hygum *et al.* 2000). Direct measurement of mortality *in situ* has not been done because of the challenges associated with tracking individual zooplankton, although recent technological advances may open the door to this possibility (Steig and Greene 2006). However, several important developments in the realm of indirect means to estimate zooplankton mortality have occurred during the GLOBEC era.

One approach utilizes general life history principles to solve for steady-state mortality rates over a broad range of environmental temperatures and copepod body sizes (Hirst and Kiørboe 2002). These authors observed a negative size-dependence of development rates of planktonic copepods, which is generally consistent with allometric scaling of other biological processes (e.g. Peters 1983). Combining this observation with average egg production rates, Hirst and Kiørboe (2002) solved for the average mortality over a generation. For broadcast-spawning species, they suggested that average mortality rates of copepods living in the epipelagic zone could be described by body size together with ambient temperature. For egg-sac-bearing copepods, average mortality rates were not related to body size but varied with environmental temperature. Another approach based on allometric principles is the use of plankton biomass size spectra to infer rates of growth and mortality (Edvardsen *et al.* 2002; Zhou *et al.* 2004). In the absence of immigration, emigration, and patchiness, the biomass spectrum is defined primarily by growth, which leads to propagation from smaller- to larger-size classes, and mortality, which reduces abundance within a size class. This approach assumes that all organisms of the same size grow and die at the same rate. Commonly, OPCs (see Section 6.3.1) have been used to assess the biovolume size spectrum, assuming that all particles sensed are living zooplankton, which is not the case in all ocean regions (e.g. Heath *et al.* 1999b; Checkley *et al.* 2008). Both steady-state and non-steady-state applications of biovolume spectra have been reported (e.g. Zhou 2006).

By definition, methods that assume equilibria, such as some allometric methods or the use of Production:Biomass ratios to approximate average lifespan mortality, cannot resolve the time-dependent variations that affect seasonal and interannual variations in populations. Averaged over a growing season or a year mortality may balance birth, but it is the variability in both that determines the timing of population variations and the temporal variability of abundance and secondary production. During the GLOBEC years, different inverse methods have been developed and refined to solve for time-dependent rates in stage-structured populations (Wood 1994; Aksnes and Ohman 1996; Caswell 2001; Li *et al.* 2006). Such inverse methods utilize the observed abundances and stage structure of a field population, usually together with independent estimates of development rates, to estimate mortality rates that would be consistent with the observed stage structure. These inverse methods are commonly described as either *horizontal* life table methods, referring to changes in demographic structure of a population followed sequentially over time, or *vertical* methods, referring to the static stage structure of a population measured at a single point in time. Although both horizontal and vertical methods were under development prior to GLOBEC, they advanced and were applied more extensively during the GLOBEC years. Some of the comprehensive GLOBEC field studies provided unusual opportunities where all essential measurements needed to make these estimates (including ocean circulation, egg production rates, stage-specific abundances and vertical distributions, measurements of food concentration and temperature) were available.

Of the inverse horizontal methods, the Population Surface Method (Wood 1994) was successfully applied to subpopulations of *Calanus* in two Norwegian fjords that were geographically close (ca. 20 km apart), but had markedly different predation regimes. One fjord (Sørfjorden) was dominated by zooplanktivorous fish, while the other (Lurefjorden) had few fish and high population densities of carnivorous zooplankton (Eiane *et al.* 2002). Eiane and co-authors found pronounced differences in the stage-specific patterns of mortality in the two fjords, apparently a consequence of different size/stage preferences of the two groups of predators. McCaffrey (2000) showed that even if the final abundances of adults were the same in the two fjords, the observed differences in stage-specific mortality significantly alter rates of secondary production. A delay-difference method was used to investigate the time-dependent mortality of *Calanus finmarchicus* at Weathership M in the central Norwegian Sea (Ohman and Hirche 2001). These authors suggested that the spring onset of population growth of *C. finmarchicus* may be affected as much by reductions in mortality rate as by increased birth rate. They uncovered a density-dependent mortality relationship for *C. finmarchicus* in the open

Norwegian Sea, apparently caused by cannibalism on eggs, a mechanism demonstrated in the laboratory by Bonnet *et al.* (2004). Density-dependent mortality of eggs and early nauplii was subsequently observed for the same species on Georges Bank in the north-west Atlantic (Ohman *et al.* 2004; Ohman *et al.* 2008). Heath *et al.* (2008b) suggested that cannibalism is perhaps a factor explaining egg mortality of *C. finmarchicus* in the Irminger Sea. Density-dependent mortality of zooplankton has been shown to be a key stabilizing mechanism for plankton predator-prey interactions (Steele and Henderson 1992b). Hirst *et al.* (2007) found a positive correlation between egg mortality and abundance of adult female *Calanus helgolandicus*, but in this case suggested that there were not sufficient females present to account for the observed egg mortality.

Another GLOBEC-related development of inverse methods was the use of an adjoint method to solve for mortality rates of late naupliar and copepodid stages of *Calanus finmarchicus* on Georges Bank (Li *et al.* 2006). In this approach an explicit model of the climatological mean circulation on Georges Bank (Naimie *et al.* 2001) was combined with observed stage structure (Durbin and Casas 2006) and temperature- and food-dependent development rates (Campbell *et al.* 2001b) to estimate the expected moulting fluxes of successive developmental stages, together with mortality rates. The approach resulted in time- and space-dependent mortality rates in such a way that their effects could be compared with the corresponding fluxes from both advection and diffusion (Li *et al.* 2006).

Applications of vertical life table (VLT) methods have proven illuminating in a number of field situations where following the sequential development of a population through time is impractical. The method requires that observed ratios of different developmental stages, as well as their rates of development, are constant for a period of time at least equivalent to the duration of each stage pair and that there be no rapidly passing cohort that causes stage structure to change quickly (Aksnes and Ohman 1996). Such methods were used to compare mortality rates of *Pseudocalanus* spp. and *Calanus finmarchicus* co-occurring on Georges Bank (Ohman *et al.* 2002). This study revealed that the high fecundity of broadcast-spawning *Calanus* is compensated by very high early stage mortality, while the low-fecundity *Pseudocalanus* has correspondingly low egg mortality (Fig. 6.5). Understanding such trade-offs, which have also been modelled theoretically (Kiørboe and Sabatini 1994), is key to forecasting differential responses of different species to changes in climate forcing.

In a study in the California Current, VLT methods revealed that upwelling regions of elevated food supply can also be regions of elevated mortality of *Calanus pacificus* (Ohman and Hsieh 2008), suggesting that there are trade-offs between regions of enhanced food supply and enhanced predation risk.

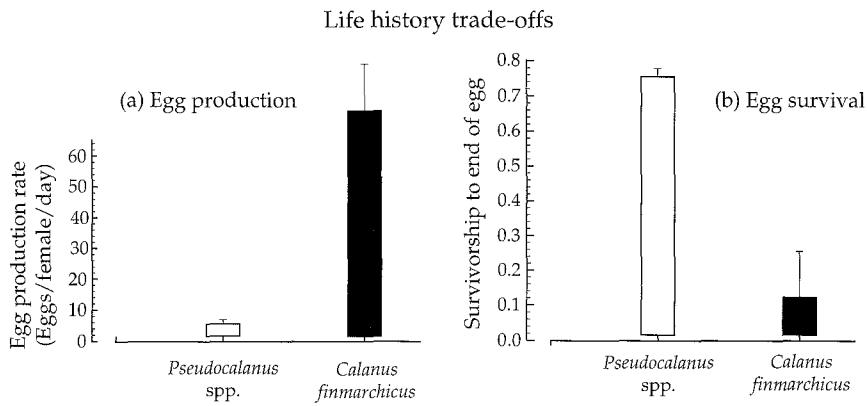

Figure 6.5 Trade-offs between egg production and egg survival for the broadcast-spawning copepod *Calanus finmarchicus* and the egg-carrying *Pseudocalanus* spp. co-occurring on Georges Bank, north-west Atlantic. (From Ohman *et al.* 2002 with kind permission of Springer Science and Business Media.).

In the Irminger Sea, a modified VLT method suggested that nauplius 3 and 4 *Calanus finmarchicus* may have elevated mortalities below a threshold chlorophyll concentration (<0.6 mg m^{-3}, Heath *et al.* 2008b), and that spatial differences in mortality may be key to explaining spatial patterns in recruitment. South-west of Iceland, deep overwintering C5 *C. finmarchicus* show remarkably low mortality rates, which increase in April and June when animals enter near-surface waters (Gislason *et al.* 2007). This study suggests that *C. finmarchicus* successfully minimizes predator encounter through deep dormancy. Another development in the GLOBEC era was the establishment of guidelines for reasonable bounds of mortality rates (Dam and Tang 2001), which have led to reinterpretation of some earlier results.

During GLOBEC, a new perspective emerged on some of the causal agents of zooplankton mortality. The presence of large numbers of resuspended benthically derived hydroids of the genus *Clytia* was rediscovered on Georges Bank (Madin *et al.* 1996; Concelman *et al.* 2001) and their potential to ingest copepod eggs, nauplii, and fish larvae was established (Madin *et al.* 1996). These hydroids are especially abundant in the shallow bank crest region (Concelman *et al.* 2001) where they are resuspended by vigorous tidal shear and associated with mortality of *Calanus finmarchicus* eggs and nauplii (Ohman *et al.* 2008). In the California Current, mass mortality of euphausiids was found to be linked to infestations by a parasitic ciliate (Gomez-Guteirrez *et al.* 2003). Cannibalism by *Calanus* on its own eggs and early nauplii is now recognized to be widespread (Bonnet *et al.* 2004; Ohman *et al.* 2008), and likely occurs in other abundant species of broadcast-spawning copepods (e.g. Landry 1978) and perhaps other types of zooplankton as well.

In addition to the development and application of inverse and allometric methods to field situations, some of the new conceptual insights about mortality that have developed during the GLOBEC era include:

- The importance of timing of mortality in affecting seasonal population dynamics.
- The existence of spatial differences in rates and stage-specific patterns of mortality that lead to unequal risks in different localities.
- The role of density-dependent mortality, operating through cannibalism on early life history stages, as a mechanism altering population growth and ecosystem dynamics.

The focus of much of this work has been on copepods, the biomass dominant in the mesozooplankton in the upper pelagic ocean. General trends and parameters describing growth and reproduction relationships to food and temperature have been analysed and described (e.g. Hirst and Bunker 2003, 2004). Despite its limitations, chlorophyll *a* concentration, which is widely measured and can be estimated remotely, has been shown to have utility as a proxy of food availability.

These results form the foundation for quantitative analysis and parameterization of population rate processes, which in GLOBEC programmes has especially advanced development of life cycle population models of copepod species in the genus *Calanus* and *Pseudocalanus* (e.g. Stegert *et al.* 2007) and coupling to physical circulation models (e.g. Speirs *et al.* 2006; Moll and Stegert 2007).

6.4.5 Estimating growth of fish larvae

Growth and development of marine fish larvae are linked. Both proceed at rates that are dependent on temperature, food availability, and quality, among other environmental and intrinsic factors. There are several approaches to estimating growth rate in marine fish larvae, falling into three general categories: (1) serial sampling of a cohort; (2) otolith microstructure analysis; and (3) biochemical approaches. While the latter two approaches were employed widely in the GLOBEC programme, results from the most widely used of the biochemical approaches, RNA:DNA ratio analysis, are considered here.

Serial sampling of a cohort, while routinely used with cultured larvae and often used to calibrate other methods, is limited in the field due to difficulties in identifying and following individual cohorts, since most marine fish species spawn over several weeks to months. In most instances otolith microstructure analysis is used to age the larvae and to identify cohorts.

Since the 1970s it has been recognized that most marine fish deposit daily rings in their otoliths

(Brothers *et al.* 1976). Otolith microstructure analysis has provided a wealth of information on larval age, growth rate, hatch size, and environmental history (Campana 2005). Estimates of larval age and birth date were critical to estimating mortality rates from field surveys in several of the GLOBEC field programmes (Mountain *et al.* 2008). Growth rate integrated over the life of a larva can be estimated from size-at-age. Based on the assumption that otolith diameter is proportional to larval length, growth history can be back calculated from ring diameter and growth rate over periods as short as a day can be estimated from ring width.

Numerous biochemical and molecular approaches to estimate larval growth rates have been employed with varying degrees of success and acceptance (Ferron and Leggett 1994). The underlying concept is that the concentration or activity of certain constituents, such as enzymes, lipids, hormones and nucleic acids, vary in proportion to food availability and growth rate. The challenge is to identify a constituent that varies reproducibly on the appropriate timescale and to rigorously test and calibrate the method with larvae of known environmental history and growth rate. Bulk ribonucleic acid (RNA) concentration has been related to growth rate in a wide variety of organisms ranging from elephants to viruses. The three classes of RNA, ribosomal, messenger, and transfer, are key components of the molecular machinery for protein synthesis and are regulated in response to the availability of nutrients and the need for protein synthesis. For purposes of estimation of growth rate or nutritional condition in fish larvae, RNA is usually normalized to DNA content, although dry weight and protein have also been used to account for the effect of size (Buckley *et al.* 1999). DNA is the carrier of genetic information and DNA content per cell is usually considered constant. The RNA:DNA ratio is an index of the protein synthetic machinery per cell.

Larval RNA:DNA ratio responds to changes in feeding conditions within about a day or two depending upon water temperature (Buckley *et al.* 1999). This time frame is appropriate to the persistence of many features of the physical and biotic environment important to growth and survival of fish larvae. The relations among larval RNA:DNA ratio, water temperature, and growth rate have been calibrated for a range of species of interest to the GLOBEC programme, including Atlantic cod and haddock. Also, GLOBEC facilitated the comparison of results among species and it now appears that there may be a single relationship among RNA:DNA ratio, water temperature, and growth rate in temperate marine fish larvae that can be used to estimate growth of species for which no species-specific calibration is available (Buckley *et al.* 2008). This development should greatly increase the utility of the approach.

While RNA:DNA ratio analysis requires special handling including sorting at sea and storage at low temperatures, large numbers of larvae can be processed and the analysis can be completed at sea if necessary. Over 10,000 individual cod and haddock larvae and early juveniles were analysed for RNA, DNA, and protein content as part of the Georges Bank programme. This unprecedented sampling effort revealed seasonal and interannual trends in recent growth rate that were related to photoperiod, water temperature, and food availability (Fig. 6.6) (Buckley and Durbin 2006; Buckley *et al.* 2006). While the relationship between photoperiod and growth rate were similar among years, the response to temperature varied among years with distinct maxima in growth rate observed near 6 to 7°C in some years (Fig. 6.6). In other years, when prey was abundant, no temperature optimum was observed. At times growth of larvae was food limited (Fig. 6.8). Although estimates of starvation mortality of young cod and haddock larvae were usually low (<2% day^{-1}), starvation mortality of larvae was particularly high (5 and 9% day^{-1} respectively) in 1995 when their copepod prey were scarce.

Results from the German GLOBEC programme revealed similar seasonal trends in recent growth of sprat larvae in the Baltic Sea (Petereit *et al.* 2008). Maximum growth of sprat larvae occurred between 7 and 8°C shortly before the photoperiod maximum in June. Again, trends in growth rate were related to food availability.

6.5 Zooplankton individual behaviours and population processes

Innovative new approaches have enabled GLOBEC researchers to investigate small-scale behaviour of

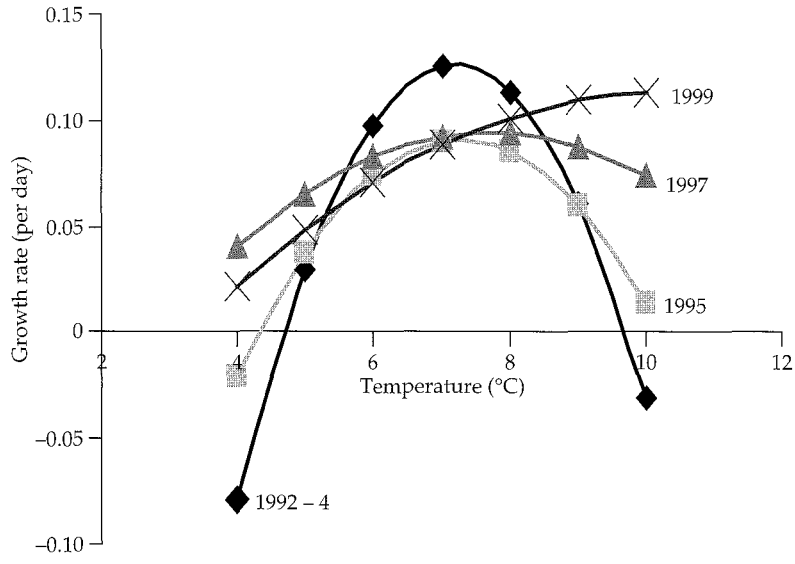

Figure 6.6 Growth rate of larval cod versus water temperature on Georges Bank. (From Buckley *et al.* 2004, 2006.)

both zooplankton and fish and to survey the diversity in behavioural responses. These studies have tackled the effects of physical (e.g. turbulence and light) and biological variables (e.g. behaviour and size) on predator-prey encounter rates, capture success, and feeding efficiency, eventually determining competitive interactions among organisms.

One approach to achieving the GLOBEC aim of predicting zooplankton population processes in the ocean is to make inferences from studies of the behaviour of individuals. This is not a common approach. While a respectable number of studies of individual behaviour in zooplankton have been conducted during the past, few have attempted extrapolations to population processes in more than very general terms. The dynamics of a population, that is, its variation in numbers, age-composition, birth and death rates, and vertical and horizontal distributions are the result of events happening at the level of the individuals. Thus, essential features of the dynamics of zooplankton populations can be understood only in the context of individual behaviours, and descriptions of individual behaviours may, in turn, be used to predict properties of the population. In this section we first briefly summarize past studies of zooplankton individual behaviours and then, through a few examples, demonstrate how this reductionist mechanistic-behavioural approach to population dynamics may be applied to marine zooplankton populations.

6.5.1 Brief review of behavioural studies

To illustrate the evolution in research focus and the improvement in knowledge over the last 30 years, we will review five major topics where most contributions on zooplankton behaviour fall. We restrict ourselves to larval fish and copepods, with emphasis on the latter, and emphasize studies that directly observe behaviour, but note that it has become increasingly common to couple pure observational studies with 'black-box'-type incubation experiments and modelling.

6.5.1.1 Feeding behaviour

The development of high-speed cinematography and copepod tethering techniques in the early 1980s allowed the very detailed observation of copepod feeding at the smallest scales (e.g. Alcaraz *et al.* 1980; Koehl and Strickler 1981; Paffenhöfer *et al.* 1982; Strickler 1982, 1984; Price *et al.* 1983; Price and

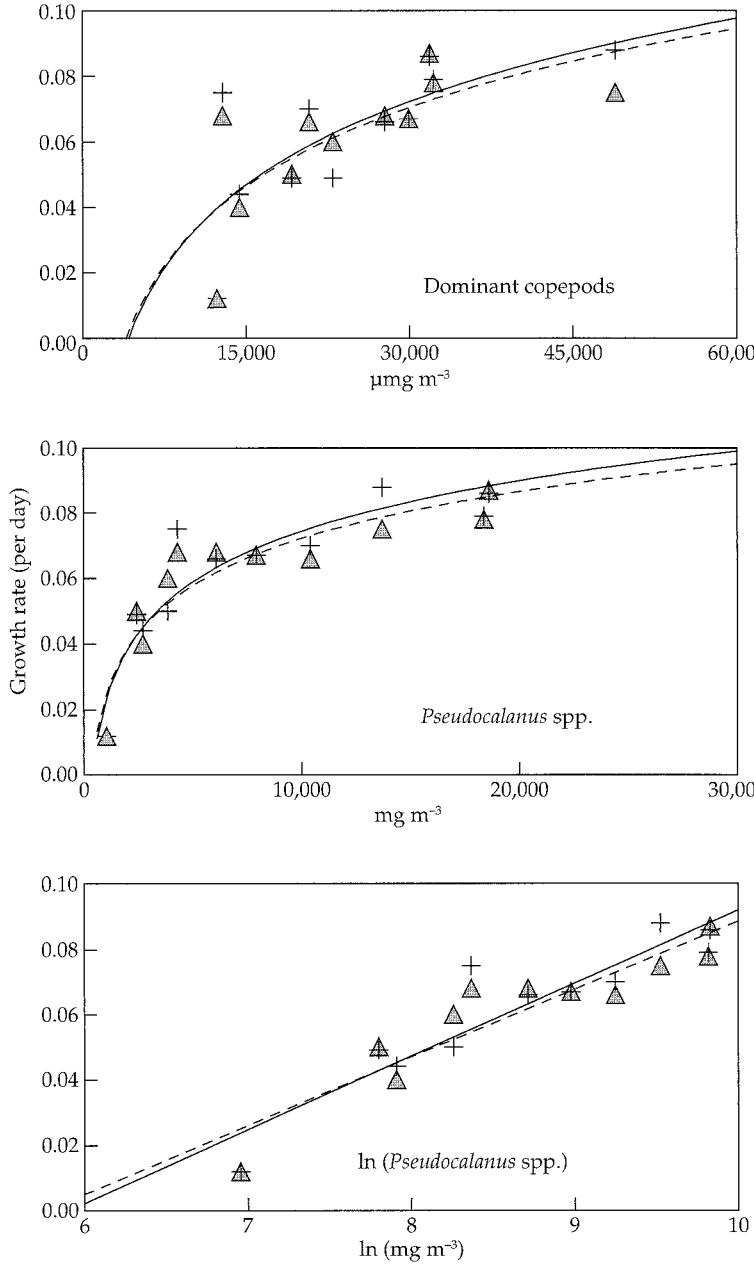

Figure 6.7 Relationship between potential prey biomass and growth rate for 7mm cod larvae on Georges Bank. (Reprinted from Buckley and Durbin 2006 with kind permission from Elsevier).

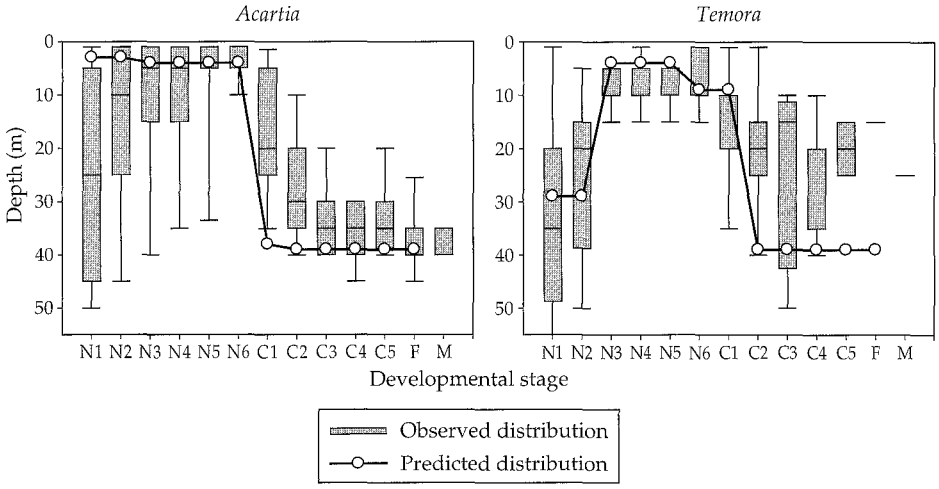

Figure 6.8 Observed and predicted vertical distributions of different developmental stages of two copepod species in a Swedish fjord. Box plot shows median residence depth, the 25 and 75% (boxes), and the 5 and 95% (whiskers) fractiles of the observed depth distributions. The open symbols and lines show the median depth predicted from a habitat optimization model based on mechanistic insights into individual behaviours. The plots also demonstrate the general trend found in other species, of small stages residing near the surface, and larger stages deeper down. (Modified from Titelman and Fiksen 2004.) (Modified from Kiorboe 2006. Copyright 2006 by the American Society of Limnology and Oceanography).

Paffenhöfer 1984). Issues like prey perception (chemo- versus mechanoreception), the generation of feeding currents to enhance prey encounter, and the mechanisms of prey selection and actual capture were thoroughly studied (Paffenhöfer and Lewis 1989, 1990; Yen *et al.* 1991). At somewhat larger scales (millimetres to centimetres and seconds to hours), and with the easy access to video, since the late 1980s a large body of research has been conducted with free-swimming animals. These studies have revealed important aspects of the diversity of copepod foraging strategies (ambush, cruising, and suspension feeding), swimming behaviours and time budgets in response to environmental variables (e.g. Jonsson and Tiselius 1990; Tiselius and Jonsson 1990; Saiz 1994), often combined with incubation experiments to test mechanistic models (Svensen and Kiørboe 2000; Henriksen *et al.* 2007). Regarding visual predators like fish larvae, a similar development has taken place (observational studies: e.g. Munk and Kiørboe 1985; Munk 1992; MacKenzie and Kiørboe 1995b, 2000; Hunt von Herbing and Gallager 2000; Hunt von Herbing *et al.* 2001; modelling studies: Fiksen and Mackenzie 2002; Galbraith *et al.* 2004).

6.5.1.2 Effect of turbulence on feeding

The impact of turbulence on zooplankton feeding is a topic that stems from the papers by Strickler (1985) and especially Rothschild and Osborn (1988), who showed theoretically that small-scale turbulence can increase encounter rates between particles, with implications for predator-prey relationships and ecosystem processes as well. Regarding feeding, subsequent studies conducted on copepods and fish larvae have shown that the enhancement of encounter rates due to turbulence (Marrasé *et al.* 1990; MacKenzie and Kiørboe 1995a) interacts strongly with predator behaviour resulting in a dome-shaped relationship which appears to be species-specific (MacKenzie and Kiørboe 1995b; Saiz and Kiørboe 1995; Saiz *et al.* 2003). Due to technical difficulties, only a few studies have actually observed the behaviour of zooplankters under turbulent conditions, either free-swimming (Saiz and Alcaraz 1992; Saiz 1994; MacKenzie and Kiørboe 1995a; MacKenzie and Kiørboe 2000; Seuront *et al.* 2004b) or tethered (Costello *et al.* 1990; Marrasé *et al.* 1990; Hwang *et al.* 1994). In this regard, incubation experiments (Saiz *et al.* 1992; Saiz and Kiørboe 1995; Caparroy *et al.* 1998), and modelling exercises

(MacKenzie *et al.* 1994; Kiørboe and Saiz 1995) have been a required complement.

6.5.1.3 Predator escape behaviour

Although some early studies demonstrated the different ability of copepods to perceive fluid disturbances and showed how water mixing could affect it (Singarajah 1969, 1975; Haury and Kenyon 1980), it was not until the 1990s and later that this topic was thoroughly addressed. Two major lines of research have been followed. One line has focused on the morphological adaptation and the neurophysiological performance of mechanoreceptors in copepods (e.g. Yen and Nicoll 1990; Yen *et al.* 1992; Bundy and Paffenhöfer 1993; Weatherby and Lenz 2000; Fields *et al.* 2002; Fields and Yen 2002; Fields and Weissburg 2004), and on the determination of the hydrodynamic signals that trigger the escape response in front of an approaching predator (e.g. Yen and Fields 1992; Kiørboe *et al.* 1999; Doall *et al.* 2002). The second line of research focuses on the perceptive performance and escape ability of copepods (e.g. Buskey and Hartline 2003; Titelman and Kiørboe 2003), and how, from an ecological point of view, the swimming and perceptive performance of copepods may affect predation risk through the effects of enhanced encounter (due to higher motility, higher hydrodynamical conspicuousness) and escape success (which integrates perceptive performance and escape ability) (e.g. Viitasalo *et al.* 1998; Broglio *et al.* 2001; Titelman 2001; Waggett and Buskey 2006).

6.5.1.4 Motile behaviour

There have been two major topics in the study of motility behaviour in copepods at the individual level. One of them relates to the ability of copepods to form swarms or aggregations in relation to microstructures in the water column (e.g. Cassie 1959; Owen 1989; Ambler 2002), and has focused on behavioural processes that allow copepods to find and stay in patches (triggered by either physical or chemical gradients, food patches, etc.; e.g. Tiselius 1992; Saiz *et al.* 1993; Buskey *et al.* 1996; Lougee *et al.* 2002; Bochdansky and Bollens 2004; Woodson *et al.* 2005). The second line of research has described swimming patterns and examined their ecological implications (e.g. Buskey *et al.* 1993; van Duren and Videler 1995; van Duren and Videler 1996; Mazzocchi and Paffenhofer 1999; Gallager *et al.* 2004). Several studies have assessed the adaptive value of motile behaviour by means of modelling, for example, effects on encounter rate, optimal foraging behaviour, risk of predation, etc. (Bundy *et al.* 1993; Tiselius *et al.* 1993; Visser and Thygesen 2003; Seuront *et al.* 2004a; Visser and Kiørboe 2006). At a different level, changes in motile behaviour of zooplankton result in patterns of diel vertical migration and swarming behaviour with important demographic consequences in populations (e.g. Batchelder *et al.* 2002a; Zhou and Dorland 2004).

6.5.1.5 Mate-finding behaviour

Early studies demonstrated remote mate detection by chemical signals in copepods (Katona 1973; Uchima and Hirano 1988; Yen 1988; and several earlier studies), and later studies provided the details (Doall *et al.* 1998; Strickler 1998; Tsuda and Miller 1998) necessary for modelling mating signals and mate finding behaviour (Yen *et al.* 1998; Bagoien and Kiørboe 2005a,b). Although the number of actual species studied is still limited, very different mechanisms and strategies have been demonstrated (chemical trails: Tsuda and Miller 1998; Weissburg *et al.* 1998; Bagoien and Kiørboe 2005a,b; pheromone clouds: Kiørboe *et al.* 2005; hydromechanical cues: Strickler 1998; Bagoien and Kiørboe 2005b). Despite these differences, a general size-dependent pattern in mate finding capacities has emerged (Kiørboe 2006, 2007).

6.5.2 Individual behaviours and population properties

We consider three examples below, which, respectively, demonstrate how vertical distribution patterns, mortality rates, and population densities can be predicted from a mechanistic description of individual behaviours.

6.5.2.1 Vertical distribution

The ocean is stratified, typically such that the temperature and food availability for zooplankton is high in the well-illuminated surface layer, whereas

predation risk from visual predators is low at depth and varies diurnally at the surface. Consequently, many zooplankters undertake diurnal vertical migration, such that they always reside at a depth where the ratio of gain to risk, or some other measure of fitness, is maximized. Vertical migration is well documented and is a classical example of how individual behaviours dictate the distribution of the population. Several authors have elaborated on the general idea of fitness optimization with respect to vertical distribution in zooplankton attempting to predict vertical distributions from behaviour (e.g. Aksnes and Giske 1990; Ohman 1990; De Robertis 2002). The study by Titelman and Fiksen (2004) is particularly illuminating in the present context as it combines detailed mechanistic descriptions of predator encounter risk from visual (fish) and tactile (zooplankton) predators as functions of individual prey behaviours, individual predator avoidance capability, and temperature-dependent growth rates in a habitat optimization model to predict the ontogenetic vertical distribution pattern of various copepods. The general prediction from this exercise is that nauplii and small copepods will reside near the surface, while later developmental stages and larger copepods should reside deeper, consistent with observations (Fig. 6.8).

Similar considerations of the trade-off between feeding opportunities and predation risk would predict that zooplankters should reside shallower in the water column when feeding conditions are poor and deeper when they are better. Such variation in feeding opportunity may be a simple function of food concentration, but may also be mediated by variation in small-scale turbulence. If turbulence enhances the predator-prey contact rate, as suggested by some laboratory studies (see above) elevated levels of turbulence during wind events should lead to a deeper optimal zooplankton residence depth. Although other hypotheses lead to a similar prediction (Pringle 2007), observations of vertical distributions of copepods (Mackas *et al.* 1993; Lagadeuc *et al.* 1997; Incze *et al.* 2001; Visser *et al.* 2001) and fish larvae (Heath *et al.* 1988; Reiss *et al.* 2002) consistently show that these zooplankters reside deeper in the water column during wind events than during calm weather. This prediction is robust, because even in cases where turbulence has a negative effect on feeding, deeper residence should be preferred in turbulent environments because turbulent intensities typically decline with depth.

6.5.2.2 Motility and mortality

Most zooplankters move, either by passive sinking or active swimming, and/or they produce feeding currents. There are gains and risks associated with moving. Specifically, moving enhances the chance of encountering food and mates, but moving also elevates the risk of meeting predators and has energetic costs. The optimal motility is that which maximizes gains over risks, in whatever units are relevant for the situation considered. Here we examine the case of mortalities in mate-searching pelagic copepods. Because it is typically the male that has to find the female, rather than vice versa, males often swim faster and with more directional persistence than females. This implies a higher mortality in males than in females and leads to female-biased adult sex ratios in field populations. The male should swim at the speed which optimizes the number of females he will encounter during his adult life. That speed may depend on the feeding strategy of the male. Some adult males do not feed at all (common among calanoid copepods), others cruise through the water while feeding and thus may feed and search for females simultaneously (most copepods of the superfamily Centropagoidea), while others again are ambush feeders and, thus at any point in time *either* feed *or* search for females (common among Oithonid copepods). Analytical predictions of the swimming velocity that optimizes the trade-offs between mate encounters, predation mortality, and energetics as well as empirical evidence suggest that the optimal swimming velocities of males with these different feeding strategies are dramatically different: ambush feeders swim at very high velocities when they swim, and at orders of magnitude faster than the females; non-feeding and cruise-feeding males swim quite slowly and at speeds that are within a factor of 2 of those of the females (Kiørboe 2008; Table 6.3). Simple models allow one to estimate the ratio of male to female mortalities (or average longevities) from differences in energetics (feeding or not) and swimming speed and, in turn, to predict adult sex ratios in field populations (Kiørboe 2008). The correspondence between observed and

Table 6.3 Observed swimming speeds and predicted male:female sex ratios in pelagic copepods with various male feeding strategies.

Male feeding strategy	Relative swimming speed (body lengths s^{-1})		Observed male:female swimming speed	Predicted male:female sex ratio
	Male	Female		
Ambush (*Oithona davisae*)	27	1.5	18	≥0.1
Non-feeding (*Pseudocalanus elongatus*)	4.4	2.5	1.8	≥0.25
Cruise-feeding (*various Centropagoidea*)	4.0–7.4	1.9–3.3	1.2–2.4	≥0.4–0.8

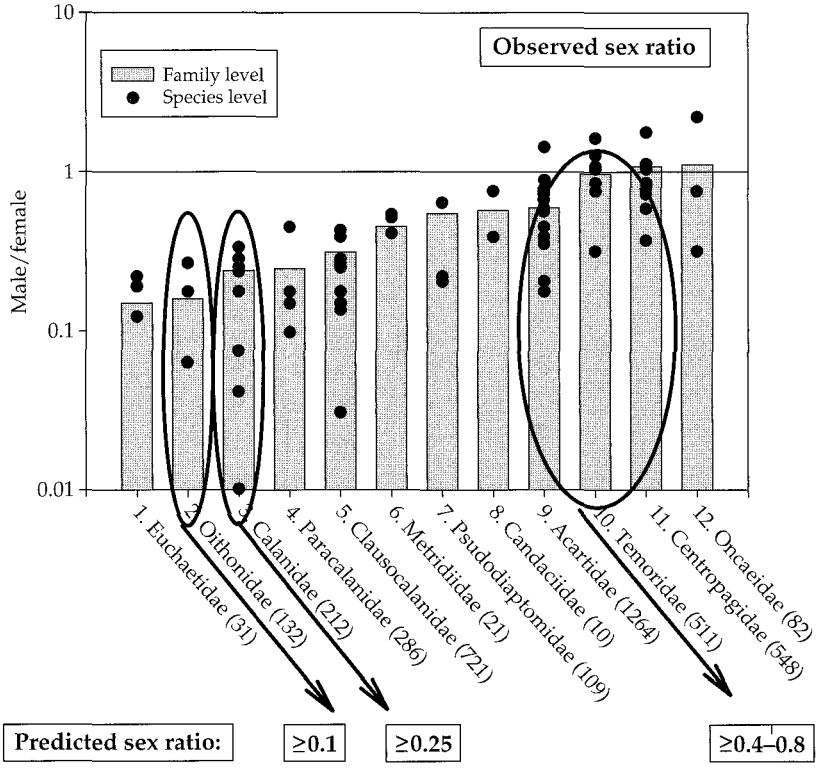

Figure 6.9 Observed and predicted sex ratios of copepod field populations. Sex ratios were predicted from observations and models of individual behaviours as described in the text and detailed in Kiørboe (2008). Field observed sex ratios were based on more than 4,000 samples that were compiled by Hirst and Kiørboe (2002) and taken from Kiørboe (2006). (Modified from Kiørboe 2006. Copyright 2006 by the American Society of Limnology and Oceanography).

predicted sex ratios is sufficient to provide yet another example of how important properties of the population can be predicted from observations of individual behaviours (Fig. 6.9).

6.5.2.3 Mate-finding and population dynamics

In organisms with sexual reproduction there must be a minimum critical population size below which mate encounters are too rare to allow population maintenance and the population will go extinct. Similarly, at low population densities, population growth may vary in proportion to population density, leading to negative density dependence (Allee effect). These population phenomena may in particular be relevant to small zooplankters that live in a big three-dimensional world where finding a

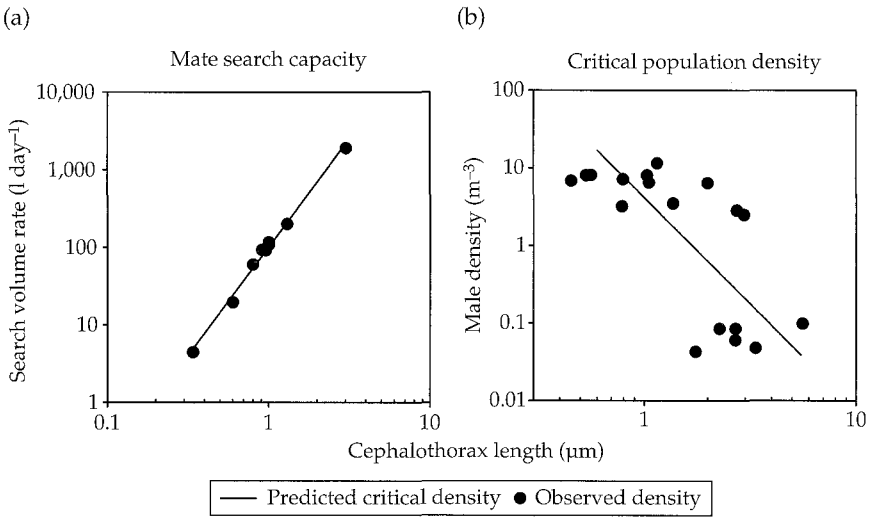

Figure 6.10 (a) Mate search capability of pelagic male copepods expressed as volume searched for female per day. (Modified from Kiørboe 2006. Copyright 2006 by the American Society of Limnology and Oceanography). (b) Predicted critical density of adult copepod males required to maintain a population (line) compared to observed seasonal minimum densities of adult males in the eastern North Sea (closed symbols). (Modified from Kiørboe 2006. Copyright 2006 by the American Society of Limnology and Oceanography).

mate is a challenge. Zooplankton have developed ways to enhance mate encounters: they may aggregate at certain depths for mating (Tsuda and Miller 1998), they may form mating swarms (Ambler 2002), and they may advertise their presence and position to potential mates using hydromechanical or chemical signals (Katona 1973; Strickler 1998). In pelagic copepods the latter appears to be the most widespread means of enhancing mate encounters, and mate search capacities are substantial, yet finite (Fig. 6.10). Critical population densities can be predicted from estimates of mate search capacities and mortality rates (Gerritsen 1980; Kiørboe 2006; Choi and Kimmerer 2008) and appear to fit seasonal minimum densities pretty well (Fig. 6.10). It also follows from these considerations that there may be negative density-dependent population regulation at low but typical densities of pelagic copepods, which can explain why winter population densities appear to have such a strong impact on population densities during the subsequent summer, several generations later (Kiørboe 2006). Critical population densities and negative density dependence may in particular be pronounced in populations with very biased sex ratios, as seen for many pelagic copepods (see above). In fact, the relative scarcity of males in some populations may lead to fertilization limitation and substantially reduced population growth, even when food is plentiful (Kiørboe 2008).

6.6 Methods applied to retrospective studies on past ecosystem states

GLOBEC programmes have used retrospective analyses and time series studies to identify and understand the characteristic, natural, modes of historical forcing and marine ecosystem variability over a range of temporal scales. Early studies detected global synchrony of fish abundance and its significant correlation with various climatic indices in the twentieth century, while studies using palaeoclimate proxies such as fish scales have revealed centennial-scale variations. Many recent studies have conducted community-level or functional-level analyses on historically collected zooplankton samples rather than comparing biomass alone. The aim has been to elucidate mechanisms responsible for observed variations in abundance. These approaches have revealed biogeographical shifts and changes in phenology at lower trophic levels induced by climate and physical forcing, and subsequent match-mismatch with higher trophic levels. Multivariate analyses to investigate spatio-temporal variation of

plankton communities have been applied, and intercalibration of sampling gear has been necessary to deal with historical samples collected using different methods.

It is widely known that the catch of commercially important fish, such as sardine, salmon, herrings etc., many of them target species (see Gifford *et al.*, Chapter 4, this volume), fluctuates on a multi-decadal scale. Retrospective studies in the early years of GLOBEC detected significant correlations between regional ecosystem changes and large-scale climatic forcing indicated by the various climatic indices, for example North Atlantic Oscillation (NAO), Pacific Decadal Oscillation (PDO), Southern Oscillation Index (SOI), etc. (see Drinkwater *et al.*, Chapter 2, this volume). The Kawasaki diagrams (Kawasaki 1991) exhibited global synchrony at a multi-decadal scale of variations in the abundance of common fish species, providing evidence of the influence of large-scale climatic forcing on regional ecosystems. Analysis of fish scales in anoxic sediments has revealed the fluctuation of fish abundance over the past 2 millennia far before commercial exploitation started (see Box 6.5 for the details). All these facts demonstrate obvious climate-ecosystem links, but what mechanisms lie behind these links?

Box 6.5 Fish debris indicate many modes of variability

As GLOBEC-related studies have the overall objective of understanding the mechanisms of physical and biological change, it is worthwhile to review palaeo studies when considering recent observations of ecosystem change. Fishery catches in the twentieth century suggest several paradigms of ecosystem variability. There are alternations in the catch of sardines and anchovies in various regions of the world as well as opposing variations in abundance between salmon catch in the Gulf of Alaska and salmon and pelagic fish in the California Current and these fluctuations appear to have a 50–60-year periodicity (Lluch-Belda *et al.* 1989; Kawasaki 1991; Mantua *et al.* 1997; Chavez *et al.* 2003). However, the palaeo-archives from sedimentary records, such as fish scales, nitrogen isotope signatures in Alaskan lakes, and other palaeoclimate proxies, indicate that the modes of variability observed in the twentieth century only exemplify a small portion of the total range of past variability. Seasonal variations in sediment flux to hypoxic sediments results in the presence of laminae or annual varves in the bottom sediments. The lack of oxygen both inhibits benthic organisms from disturbing the sediment layers as well as preserves the remains of fish scales, which sink to the seafloor.

Box 6.5, Figure 1 Fish scales typical of an upwelling community, mostly sardines and anchovies from sediment core off Namibia (diameter of fish scales around 5–10 mm). (From Struck *et al.* 2002.)

continues

Box 6.5 *continued*

Within Santa Barbara Basin in the California Current, there can be a persistent high abundance, or complete absence, of sardine scales in sediment layers corresponding to nearly 100 years duration (Baumgartner *et al.* 1992). Assuming that fish scale deposition off central California, which is near the center of the population ranges, generally reflects small fish pelagic populations in the California Current, then the 50–60 year periodicity is not likely to be a good predictor of future changes.

There are also co-occurring periods of high- and low-scale abundance for sardines and anchovies for decades at a time, both within the California Current (Baumgartner *et al.* 1992) and within the Humboldt Current (Valdés *et al.* 2008). Thus, while sardines and anchovies within a given region vary out of phase at times (e.g. the twentieth century), this pattern is not consistent through time and offers little chance of predictability.

Furthermore, centennial-scale periods of high sardine-scale fluxes in the California Current coincide with low inferred salmon returns to the Gulf of Alaska, whereas the two species co-varied in abundance during the twentieth century (Finney *et al.* 2002). Also, sardines did not appear to co-vary off California and Japan during the nineentieth century (Field *et al.*, in press), as they did during the twentieth century. Therefore, the alternations between different regions observed in the twentieth century may not be typical of all modes of climate variability.

Thus, many of the patterns of variability in fish populations, as well as other palaeoclimate records of sea surface temperature (SST) in different regions of the Pacific (Gedalof *et al.* 2002; D'Arrigo *et al.* 2005), indicate that the Pacific Decadal Oscillation (PDO)-like interdecadal variability observed in the twentieth century is not consistently observed in the palaeo records. Work in progress off Peru and in the Benguela Current is also revealing modes of variability that have not been observed in the twentieth century. Inferred SST from the alkenone unsaturation index, in the Benguela Current showed an abrupt shift nearly 1,000 years ago. The percent of anchovy scales is apparently higher during warmer SST periods, a pattern not observed in other boundary currents.

Research programmes that test the paradigms and establish the mechanisms of change offer hope for interpreting complex ocean processes and histories. Rigorous testing of existing paradigms and hypotheses is essential for understanding and predicting future marine ecosystem changes.

David Field

To answer this question, and to construct future ecosystem change scenarios, GLOBEC has both developed and applied various analytical methods for historically collected samples and data.

6.6.1 Community- and functional-level analysis

Long-term zooplankton collections, which were historically sampled during the mid to late twentieth century mainly as a part of fisheries surveys, are to be found in institutes worldwide. While early analyses of these considered the annual average of total plankton biomass, most recent studies have applied community-level and functional-level analyses. Classification of zooplankton species based on their geographical distribution range has revealed biogeographical shifts of zooplankton communities induced by climate and physical forcing. The Fourth IPCC Report pointed out that northward shifts of southern species are a globally observed phenomenon corresponding to the warming trend over recent decades. Although direct influences of global warming are not certain, the northward shift of southern plankton species associated with northern intrusion of warm water has been reported both in the eastern North Pacific (Mackas *et al.* 2004) and eastern North Atlantic (Beaugrand *et al.* 2002a).

Various statistical and multivariate analysis methods have been used for time series decomposi-

tion not only of zooplankton but also phytoplankton communities. However, selection of the methods and application of them in an appropriate manner is crucial to extract a 'pattern' of temporal and spatial variation. Beaugrand *et al.* (2003b) reviewed and discussed a series of multivariate methods, including a range of Principal Component Analyses (PCA), non-metric multidimensional scaling (MDS), cluster analysis, and spectral analysis (Table 6.4). These methods were used to effectively extract information from the data collected by the CPR survey in the North Atlantic, which are a highly extensive, both temporally and spatially, 50-year zooplankton data set. Biodiversity of the zooplankton community can be used as an indicator of variation in physical and climatic conditions. Using a similar approach based on species richness and the Shannon-Weaver Index, Hooff and Peterson (2006) detected a relationship between copepod biodiversity and transport of coastal subarctic waters into

Table 6.4 Types of multivariate analysis performed on CPR data.

Multivariate techniques	Ecological goal	Authors
Standardized PCA	Identification of species assemblages. Examination of the relations between species. Geographical locations of species associations.	Colebrook (1964, 1984)
Centred PCA	Determination of seasonal and diel patterns of months to hour diversity of calanoid copepods. Quantification of two scales of variability of diversity of calanoids at a mesoscale resolution in the North Atlantic. Examination of the spatial variation of the diversity of calanoids at diel and seasonal scales.	See Table 6.2 in Beaugrand *et al.* (2003b)
Seriation	Examination of the relations between species based on their annual fluctuation in abundance.	Colebrook (1964) Colebrook and Robinson (1964) Colebrook (1969)
Cluster analysis, single linkage agglomerative (nearest-neighbour) clustering method	Grouping of species or taxa.	Lindley (1987) Lindley and Williams (1994)
Cluster analysis, hierarchical agglomerative flexible clustering technique (Lance and Williams 1967)	Clustering of pixels or geographical areas to identify regions with similar year-to-year or annual patterns in the abundance of species.	Planque and Ibňez (1997) Beaugrand *et al.* (2000a)
Cluster analysis, complete linkages agglomerative clustering	Partition of the North Atlantic Ocean based on the diel and seasonal patterns of diversity of calanoid copepods.	Beaugrand *et al.* (2000b)
Indicator-value method (Dufrêne and Legendre 1997)	Determination of species associations based on the relative abundance and presence of species in distinct areas in the North Atlantic.	Beaugrand *et al.* (2000b)
Non-metric MDSg	Ordination of species or taxa based on the similarity of their spatial distribution.	Lindley (1987) Lindley and Williams (1994)
Mantel correlogram	Study of relationships between the size of spatial structures and their temporal variability.	Planque and Ibaňez (1997)
Generalized additive models	Spatial and temporal modelling of the abundance of species.	Beare and McKenzie (1999a, 1999b)
Three-mode PCA	Analysis of biological tables structured in space and time. Evaluation and quantification of the interactions between biology, space, and time.	Beaugrand *et al.* (2000)

Source: From Beaugrand *et al.* (2003b).

the northern California Current, which was driven by a basin-scale climatic forcing.

At higher trophic levels, studies have shown that long-term variations of fish abundance are species-specific, and the mechanisms of variation have been investigated for the respective target species in a number of ecosystems. It is thought that life history strategy is a key determinant of productive success and survival of the year class when an environmental perturbation occurs. With the aim of providing a conceptual framework for fisheries management, King and McFarlane (2003) classified fish species based on their life history strategies, Periodic, Equilibrium, Opportunistic, Salmonic and Intermediate, and examined how long-term variation patterns could differ among these groups.

6.6.2 Detecting phenology

The fourth IPCC report mentioned changes in seasonality, phenological change, also as a result of the impact of the warming trend on global ecosystems. Community-level or species-level analysis of zooplankton populations enables phenology to be detected at lower trophic levels of marine ecosystems. These changes, induced by climatic and physical forcing, affect productivity of higher trophic levels through match-mismatch mechanisms, bottom-up, or top-down controls.

The Cumulative Sum (CuSum) technique is a simple method developed to visualize the extent and duration of change of time series variables by cumulating the value at each data point consecutively in a temporal order. When the temporal resolution of data is high enough (e.g. monthly or more), CuSum has been applied to the time series abundance/biomass of target species to detect shifts of peak abundance/biomass and reproductive timing. Greve *et al.* (2001) studied the phenological shift of appendicularians in the North Sea in the late twentieth century using the CuSum technique, by setting the interval between the 15 and 85% percentiles of the annual cumulative abundance as the productive season of each year (Fig. 6.11).

Interannual variation in seasonal developmental stage composition of target species is useful in understanding lower trophic level phenology. Calculating the timing at which the CV copepodid stage reached 50% of the total abundance and assuming this to be an indicator of peak reproductive timing of *Neocalanus* species, Mackas *et al.* (1998) detected a decadal-scale shift of the peak in the North Pacific which was closely related to the water temperature anomaly associated with the PDO.

Remote sensing techniques are relatively new and an extensive data set only exists since the late 1990s. However, this tool is extremely useful for better understanding of detailed spatio-temporal variations of marine ecosystems, such as phenology, due to its high-observation frequency. Yamada *et al.* (2006) developed a method to estimate timing and duration of the spring bloom during recent decades based on chlorophyll *a* variation from satellite ocean color, ship and buoy data obtained for the period 1998–2003 in the Japan Sea. These authors found a decadal-scale phenology of phytoplankton productivity: an early and short productive season during the 1980s, as inferred from seasonal ship observation-based study. This method will be applicable for other regions as remote sensing data accumulate.

6.6.3 Stable isotope analysis

Compared to zooplankton, information on primary production other than total chlorophyll *a* data are scarce for the past decades, and community-level and functional-level analyses of phytoplankton were limited in most of the GLOBEC regions. This makes it difficult to clarify the response of phytoplankton to long-term environmental variation. However, chemical properties of secondary and higher trophic level organisms sometimes tell us cumulative information about phytoplankton they have fed on: its availability, physiological conditions etc. The nitrogen stable isotope ratio ($\delta^{15}N$) indicates the trophic level of organisms, and carbon stable isotope ratio ($\delta^{13}C$) of higher trophic level organisms mirrors the condition of primary producers. Therefore, $\delta^{15}N$ and $\delta^{13}C$ of target species of secondary producers and the higher trophic levels can be a proxy of primary productivity and its response to environmental variation in the past.

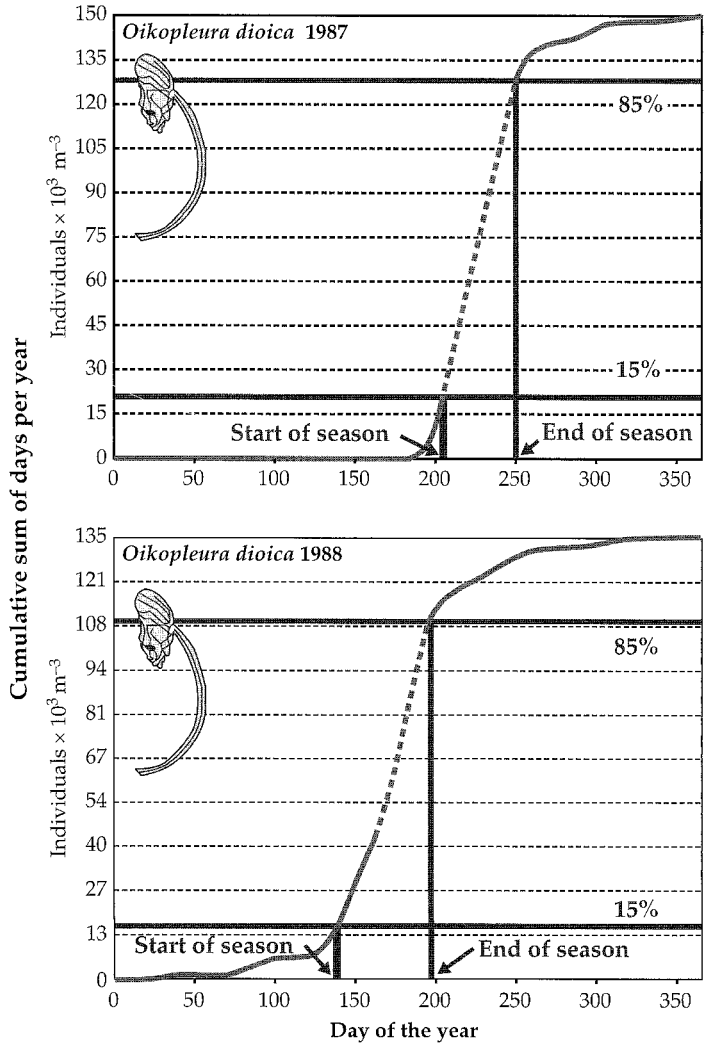

Figure 6.11 CuSum methods of detecting phenology; phenological determination on the season of *Oikopleura dioica* in 1987 (cold) and 1988 (warm). (From Greve *et al.* 2001.)

This method is useful for detecting changes in a food web structure and mechanisms of bottom-up control.

Observing a 50 year decreasing trend of $\delta^{13}C$ in the baleen of bowhead whales, even after considering the degree of $\delta^{13}C$ decline due to anthropogenic fossil fuel consumption Schell (2000) suggested a decline of primary productivity in the Bering Sea during the latter half of the twentieth century. In the California Current system, long-term variation of $\delta^{15}N$ in several key zooplankton species has been examined together with physical and climate indices (Rau *et al.* 2003). Although no trend was observed in $\delta^{15}N$ of those species, *El Niño*-related $\delta^{15}N$ enrichment was conspicuous, which was considered to be a result of (1) reduced nutrient supply due to weak upwelling, or (2) increasing advection of $\delta^{15}N$-enriched nitrate from southern water. Stable Isotope ratios were also applied to fish scale analysis for the past 2 millennia (Finney *et al.* 2002).

6.6.4 Making various time series comparable

Regional information on long-term changes at lower and higher trophic levels has accumulated during the last decades of the twentieth century. The next phase of GLOBEC retrospective programmes is integration and synthesis of regional ecosystem responses to common, large-scale climatic forcing. Having recognized synchrony in the long-term variation in commercial fish species (Kawasaki *et al.* 1991), scientists next attempted basin- to global-scale comparison of existing zooplankton time series to understand regionally specific mechanisms of ecosystem change. There were, however, a number of impediments for comparison of these time series (Perry *et al.* 2004), which were collected at various sampling frequency with a wide variety of sampling gear and at different target depths. Many time series were based on a single-season (mostly during the high-productivity period) or seasonal observation, and sometimes temporarily intermittent. Sampling gear and methods were sometimes changed during a single time series. Systematic solutions are required to tackle these impediments to inter-time-series comparison.

One of the most extensive zooplankton time series, the Station Papa time series in the Gulf of Alaska was collected with three different sampling gears, the NORPAC net and SCOR net, from 1956 to 1981, and the Bongo net after 1997. Mckinnell and Mackas (2003) recalibrated these three nets and found that previous biomass estimation based on the early calibration method overestimated the SCOR net catch. According to the new criteria, the average time series biomass reduced especially in the early period, resulting in changes in the long-term trend in the zooplankton time series.

A simple solution for comparison of regional time series with different characteristics is to standardize annual biomass data and environmental variables, for example, water temperature, for each time series. Mackas *et al.* (2004) applied log-scale anomalies for comparison of interannual variation of three independent zooplankton time series covering 850 km distance along the continental margin of the eastern North Pacific. They found spatial coherence in low-frequency variation of the zooplankton community in the three regions with a marked transition coinciding with the North Pacific Regime Shift (1988–91, 1998/9) and/or the ENSO event (Fig. 6.12). Standardization methods other than the log-scale anomalies have been applied for other regional comparison studies depending on characteristics of the time series.

6.6.5 Time series analysis: red-noise or shift, linear or non-linear

Although long-term changes in marine ecosystems and the influence of climatic forcing on these changes are now widely recognized, determination of the 'type' of change (oscillation, shift and/or trend) has been an area of debate in retrospective studies.

By composite analysis of 100 physical and biological time series, Hare and Mantua (2000) suggested that environmental and ecological regime shifts occurred in the mid-1970s and the late 1980s in North Pacific. Rudnick and Davis (2003) challenged the definition of the regime shift based on the composite of time series by demonstrating that composite analysis of random, independent red-noise time series could generate such a step-like change. However, non-linear, step-like changes were indeed demonstrated to be a characteristic of biological time series by Hsieh *et al.* (2005). They tested non-linearity of a series of physical and biological time series in the California Current system by comparing the out-of-sample forecast skill of a linear and equivalent non-linear models, and concluded that, while all of the climatic and physical time series showed a linear red-noise pattern, biological time series responding to such linear changes in physical and climatic forcing almost exclusively varied in a non-linear manner. This result suggests the capacity for dynamic changes in marine ecosystems responding to the low-frequency fluctuation of physical environments. To statistically test the timing of the 'shift', Rodionov and Overland (2005) developed an improved regime shift detection method based on the sequential t-test analysis (STARS) (Fig. 6.13), and an application tool is available at (http://www.beringclimate.noaa.gov/regimes/).

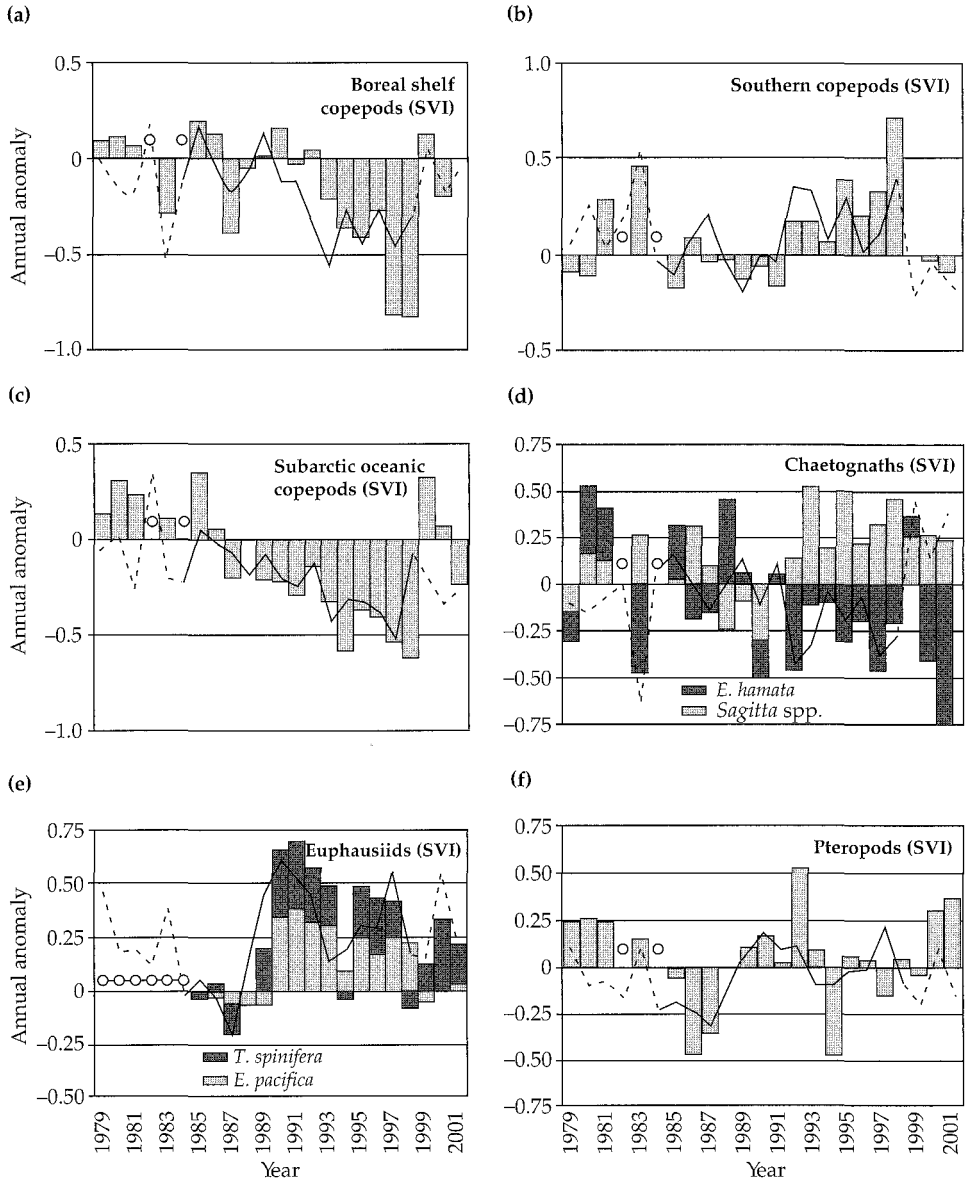

Figure 6.12 Zooplankton anomaly time series, 1979–2001, for the southern Vancouver Island continental margin region (latitude 48–49°N). Species groups for averaging and/or comparison are (a) 'boreal shelf' copepods, (b) 'southern' copepods, (c) 'subarctic oceanic' copepods, (d) chaetognaths (*Sagitta* spp. vs. *E. hamata*), (e) euphausiids (*Euphausia pacifica* and *T. spinifera*), and (f) thecosomatous pteropods (*L. helicina* and *Clio pyramidata*). Bar graphs are the annual zooplankton anomalies, averaged over the entire southern Vancouver Island region (anomalies in shelf and offshore regions are highly correlated). See Mackas *et al.* (2001, 2004) for calculation and year averaging methods. SVI anomalies include samples from all seasons, however about two-thirds of data are from spring and summer (April-September). Circles show years with no anomaly estimates due to low sample numbers or gear bias. Lines show regression fits of the anomalies to ocean climate indices; solid lines are the 'predicted' anomalies for a 'learning set' of years (1985–98) used to estimate the regressions; dashed lines show 'predictions' for the remaining years (1979–84 and 1999–2001). Note the strong inverse correlation of the 'southern' versus the 'boreal shelf' and 'subarctic' copepod groups, and the rapid change in sign of the anomalies 1998/9.(Reprinted from Mackas *et al.* 2004 with kind permission from Elsevier).

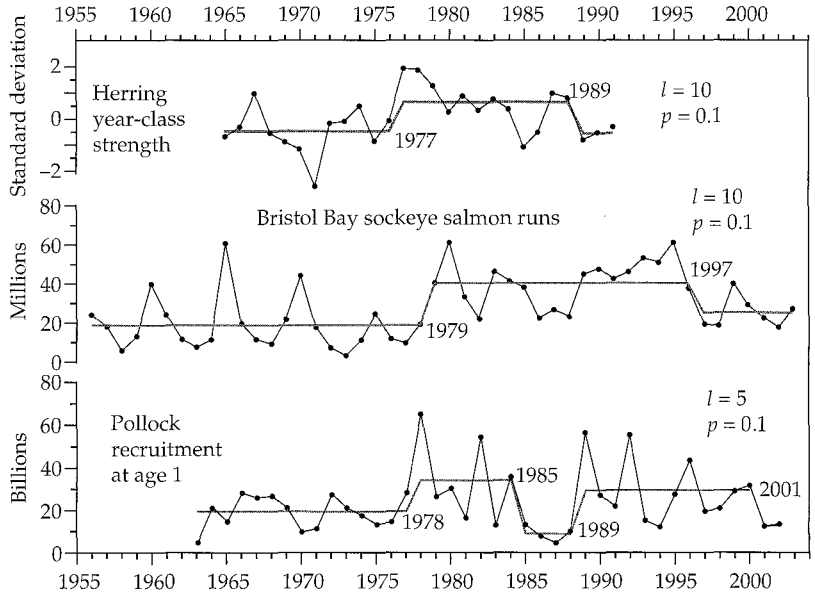

Figure 6.13 Regime shifts in herring year-class strength (*top*), sockeye salmon runs (*middle*) and pollock recruitment (*bottom*) in the Bering Sea. (From Rodionov and Overland 2004. Application of a sequential regime shift detection method to the Bering Sea ecosystem. ICES Journal of Marine Science, **62**, 328–32. By permission of Oxford University Press).

6.7 Future directions

The need for programmes such as GLOBEC and comparable future programmes is becoming ever more compelling on the international stage as the public becomes evermore aware of the effects of global change on marine ecosystems. Increasing occurrences of red tides, the collapse of major fisheries, loss of coral reef communities, global warming effects on ecosystems, and ocean acidification, are just some of the emerging problem areas (e.g. Dickey and Bidigare (2005): table 6.2). Thus, there is strong justification for increases in financial resources that can be directed to fundamental and applied oceanographic research, ocean observing systems, and predictive oceanographic models that bear on these issues. It will be important for marine ecologists and oceanographers to utilize research funding most effectively to solve these problems and to assist in management decisions.

A wide variety of technological advances have contributed to improved understanding of the structure and functioning of the global ecosystem as it is affected by and in turn affects the physics and chemistry of the ocean. GLOBEC research, spanning the past decades has been influential in this regard (e.g. Dickey 1993). This chapter has highlighted many relevant technological and methodological advances either as a direct result of the programme, or stimulated by it. Clearly, the groundwork has been laid for future breakthroughs that will benefit succeeding research. GLOBEC has also contributed significantly to the general science of life in the ocean and how it may change in the future as the physical and chemical environments evolve in response to natural and anthropogenic forcing. We conclude this chapter with some conceptual ideas concerning future directions for sampling and observing ocean systems, experimentation, as well as the necessary linkage with models.

At the outset of GLOBEC, it was recognized that a very large number of interdisciplinary variables needed to be sampled on space- and time scales covering over 10 orders of magnitude (e.g. Dickey 1988, 1990). Several new *in situ* sampling platform technologies emerged and/or became more available during GLOBEC. These include autonomous sampling fixed-depth and profiling moorings (e.g. Dickey *et al.* 2006), profiling floats (e.g. Davis *et al.*

2001; Argo Science Team 2001; Bishop *et al.* 2002; Perry and Rudnick 2003), autonomous underwater vehicles (Griffiths 2003; Perry and Rudnick 2003), and gliders (e.g. Davis *et al.* 2003; Perry and Rudnick 2003). Many of these platforms include near-real-time data telemetry systems. Looking towards the future, miniaturized, low-power biological, optical, chemical, and acoustic 'chip-based' sensors will be developed and will be suitable for interfacing to these and other novel platforms (e.g. Dickey and Bidigare 2005). Already, there is great promise as indicated by microelectromechanical systems (MEMS; Tokar and Dickey 2000) and nanotechnologies (e.g. Bishop *et al.* 2001), which are being developed at a rapid pace for a host of applications. While there will always be a need for ship platforms for certain observations, oceanographers will need to be especially vigilant in following these developments and will need to form partnerships to facilitate the widespread availability and application in field programmes of these new sensors and telemetry methods.

While GLOBEC developed much new technology that is reviewed in the chapter it is interesting to reflect that many of the key advances in understanding came from conventional approaches to sampling and experimentation. 'Working with the animals' through traditional net sampling and shipboard and laboratory experimentation has been a fundamental foundation of the programme. The advances based on these approaches are well represented in the studies reviewed.

Particularly, promising future directions involve the application of molecular and biochemical techniques to complex species assemblages and their interactions within marine ecosystems (e.g. DeLong *et al.* 1999). A specific example is provided in Box 6.1 demonstrating the potential of the application of techniques from the rapidly advancing field of molecular biology.

The shipboard, laboratory and *in situ* process studies have provided fundamental insight and data needed to formulate and parameterize essential processes controlling abundance of zooplankton and ichthyoplankton species targeted in GLOBEC programmes. Coupled physical-biological modelling is still at an early stage. Nevertheless it shows the potential to extract from the complexity of the ecosystem and population dynamics processes, simplifying formulations and approaches. These will allow evaluation of effects of environmental forcing on species abundance and distribution and on processes determining ichthyoplankton survival, with implications for use as a tool in ecosystem approaches to management. The challenge for the future is to build on this foundation of methodological approaches and integrative tools. A key issue is to establish appropriate observing systems and gather knowledge of population dynamics and ecosystem production processes that will provide us with useful predictions of change in the coastal and open ocean ecosystems.

The utilization of satellites and aircraft for biological applications has been well developed for phytoplankton and primary productivity. Recent advances in hyperspectral optical sensing of the ocean for both *in situ* and satellite platforms bode well for identifying at least groups if not species of phytoplankton (e.g. Dickey 2004; Dickey *et al.* 2006). However, major development is needed in order to capitalize on these platforms, which can in principal provide large-scale upper-ocean sensing, for studies of higher trophic level organisms and their distributions. Satellite-based data telemetry (i.e. for near-real-time data transmission) and positioning information will continue to advance both in quality and quantity. Tracking of organisms from aircraft and satellites can be especially powerful as has been demonstrated (see Section 6.3.4.). Sound transmission in the sea remains one of our most important *in situ* sensing methods for zooplankton and higher trophic level organisms (Foote *et al.* 2000; Chu and Wiebe 2003; Wiebe and Benfield 2003). In analogy to hyperspectral optics, broadband, multi-frequency acoustics has perhaps one of the greatest potentials and can in principle allow studies of trophic interactions, especially if deployed in conjunction with video and holographic methodologies.

There is growing consensus that observationalists and modellers need to coordinate their efforts and the studies reviewed in this chapter illustrate good progress in this regard (e.g. Robinson and Lermusiaux 2002). For example, development of sampling strategies, adaptive sampling, and more traditional inter-comparisons of data and model

results are key to future breakthroughs in understanding as well as to prediction (e.g. Dickey 2003). Predictions of the state of global ecosystems on short as well as very long time scales are clearly needed for a host of societally relevant issues involving the stewardship of the world ocean resources and human health. Advances in computational capabilities will offer modellers opportunities to make high temporal and spatial simulations of complex ecosystems and the physical and chemical environment. Education of the next generation of oceanographers would be well served by not only interdisciplinary training, but also exposure of students to both theoretical and observational research modes, regardless of thesis emphasis.

Finally, it is important to recognize that international cooperation and coordination has been a hallmark of GLOBEC. Globalization of ocean sciences through expanded efforts to share remote sensing and *in situ* data and models as well as predictions is especially important for the future of interdisciplinary oceanography and its applications for societal benefit.