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