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Variation in assimilation efficiencies of dominant *Neocalanus* and *Eucalanus* copepods in the subarctic Pacific: Consequences for population structure models

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ABSTRACT

The assimilation efficiency of zooplankton is an essential parameter required to estimate energy transfer to higher trophic levels in marine ecosystems. However, little information is available for large oceanic copepods, especially the *Neocalanus* and *Eucalanus* species dominant in the subarctic Pacific. In this study, the assimilation efficiencies of the C5 stages of *Neocalanus cristatus*, *Neocalanus flemingeri* and *Eucalanus bungii* were evaluated using eight phytoplankton species as food. The average assimilation efficiencies of *N. cristatus*, *N. flemingeri* and *E. bungii* ranged between 45 and 66%, 44 and 66% and 34 and 65%, respectively. The assimilation efficiency was highly variable depending on the food phytoplankton species. In all species, the assimilation efficiency showed a significant negative relationship with the ash content of the phytoplankton ($r^2 = 0.79–0.87$, $p < 0.001$). The assimilation efficiency of large-body sized *N. cristatus* for large-sized diatoms was higher than for the other copepod species. In population models of *N. cristatus*, changes in assimilation efficiency affect the growth and survival rates of the population. The Lagrangian ensemble model (LEM) for *N. cristatus* showed that, for assimilation efficiencies less than 57%, the population could not be maintained. Because variations in assimilation efficiency may have significant effects on the copepod population, their variability should be incorporated into marine ecosystem models in the future.

1. Introduction

In marine ecosystems, copepods feed on phyto- and microzooplankton and are preyed upon by pelagic fishes, sea birds and whales; thus, they play an important role in energy transfers to higher trophic organisms (Beamish et al., 1999; Hunt et al., 1998; Ikeda et al., 2008; Nemoto, 1963). In the summer zooplankton community of the subarctic Pacific, large oceanic copepods, *Neocalanus* and *Eucalanus* species, are predominant and form 85–90% of the total zooplankton biomass (Vinogradov, 1970). Because *Neocalanus* and *Eucalanus* species egest large faecal pellets and perform seasonal vertical migrations, they play an important role in transporting organic material from the surface layer to the deep sea, called the "biological pump" (Kobari et al., 2003, 2008).

Among the various copepod parameters that affect the material flux, the assimilation efficiency is an essential parameter required to estimate the energy transfer to higher trophic levels in marine ecosystems (Conover, 1966a,b). From the 1960s to the present, many studies have been performed on the assimilation efficiencies of copepods. Through these studies, much information has been accumulated, e.g., assimilation efficiency varies with the ash content of the food (Conover, 1966a,b), and the carbon assimilation efficiency is correlated with the concentrations of soluble carbohydrates in diets (Head, 1992). However, our knowledge about assimilation efficiency has mainly come from coastal species (cf. Berggreen et al., 1988; Besiktepe and Dam, 2002; Gottfried and Roman, 1983; Katechakis et al., 2004), and little information is available for large oceanic copepods, especially the *Neocalanus* and *Eucalanus* species that are dominant in the subarctic Pacific. Even in marine ecosystem models such as NEMURO, a constant value (70%) is applied for the copepod assimilation efficiency (Kishi et al., 2007; Terui and Kishi, 2008; Terui et al., 2012). As mentioned above, a few large oceanic copepods (*Neocalanus* and *Eucalanus* species) are dominant in the zooplankton biomass of the subarctic Pacific; therefore, information on their assimilation efficiency is very important for increasing the accuracy of ecosystem models (e.g., NEMURO) in this region.

The assimilation efficiency of copepods is measured by several methods; the Ratio method, the Radio tracer method and the Calculation method, based on ingestion rate, evacuation rate and faecal pellet volume (Besiktepe and Dam, 2002; Conover, 1966a; Sorokin, 1968). Among these methods, the Radio tracer method is mainly used for minimally metabolised materials, such as heavy metals. For common materials (e.g., carbon and phosphorus), the Radio tracer method cannot be applied because they are readily metabolised, which prevents

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accurate measurement (Båmstedt et al., 2000). The Calculation method requires the quantitative collection of faecal pellets, so this method is also difficult (Conover and Francis, 1973; Omori and Ikeda, 1984). The Ratio method by Conover (1966a) does not require quantitative collection of faecal pellets, is readily applicable to various animals and is an effective method, even though nearly half a century has passed since it was originally described (Azad et al., 2011; Enríquez-Ocaña et al., 2012; Nelson et al., 2012). Recently, most studies on assimilation efficiency of copepods have mainly concerned marine heavy metal pollution in coastal small copepods as evaluated using the Radio tracer method (Chang and Reinfelder, 2000; Fisher and Reinfelder, 1991; Hutchins et al., 1995; Stewart and Fisher, 2003; Wang and Fisher, 1998; Wang et al., 2007; Xu and Wang, 2001, 2002; Zheng et al., 2011). However, little information is available on the assimilation efficiencies of common materials (organic material, carbon and nitrogen) by large oceanic copepods.

In this study, the assimilation efficiencies of three large oceanic copepods (*N. cristatus, N. flemingeri* and *E. bungii*), which are dominant in the zooplankton biomass in the subarctic Pacific, were measured by applying the Ratio method considering eight phytoplankton species (diatoms, a dinoflagellate and a raphidophycean) as food. Phytoplankton cell size, colony formation, swimming ability and ash contents were also analysed as factors that may affect the assimilation efficiency of copepods. The effects of food carbon concentration on copepod assimilation efficiency were also evaluated. By applying the observed assimilation efficiency of one species of copepod (*N. cristatus*), the effects of changes in the assimilation efficiency on copepod population structure were evaluated using the LEM (Lagrangian ensemble model) population model.

2. Materials and methods

Live specimens of the C5 stages of *N. cristatus, N. flemingeri* and *E. bungii* were collected using vertical hauls of a 80-cm ring net from 0 to 30 or 0 to 150 m deep at several stations in the subarctic Pacific from March to July 2011 and May to August 2012. Because adult (C6) *Neocalanus* spp. degrade feeding appendages and cease feeding (Miller, 1988; Miller et al., 1984), we used the C5 stage as experimental specimens. Seawater was collected from 30 m deep using Niskin bottles, filtered through a GF/F filter and used in the subsequent experiments. Ten live specimens were transferred into a 1-L bottle filled with filtered seawater (FSW). Up to 100 specimens of each species were kept at 2 °C and then carried to the land laboratory.

To obtain sympatric phytoplankton species, 5 ml of unfiltered seawater was added to a flask containing 300 ml of modified SWM-3 medium (Chen et al., 1969; Imai et al., 1996), then incubated at 15 °C under illumination of 100 to 120 μmol photons m^{-2} s^{-1} with a 14 h light and 10 h dark photocycle. Three diatoms (*Chaetoceros* sp., *Ditylum brightwellii* and *Thalassiosira nordenskioeldii*) were isolated from this treatment. Six diatoms (*Attheya septentrionalis*, *Chaetoceros* sp., *D. brightwellii*, *Pauliella taeniata*, *Skeletonema* sp. and *Th. nordenskioeldii*), one dinoflagellate (*Alexandrium tamarense*) and one raphidophycean (*Heterosigma akashiwo*) were incubated under the same conditions as food for the copepods. The carbon contents of *Chaetoceros* sp., *D. brightwellii* and *Skeletonema* sp. were estimated by multiplying 0.43 (for the former two species) or 0.51 (for the latter species) with the ash-free dry weight (AFDW) (Parsons et al., 1961). For *Th. nordenskioeldii* and *A. tamarense*, the carbon contents were obtained by multiplying 0.108 or 0.173 with the dry weight (DW), respectively (Liu and Wang, 2002). Information regarding cell size, carbon and ash contents, colony formation, swimming ability and initial cell density of each phytoplankton is summarised in Table 1.

Before the experiments, no food was added for the copepod specimens for at least one day (24 h). For the experiments, each phytoplankton species was adjusted to a density of 5.0×10^2–2.0×10^4 cells ml^{-1} (110–2577 μg C L^{-1}) (Table 1). For each experiment, 15 individuals of *N. cristatus* or *E. bungii* or 20 individuals of *N. flemingeri* were added to each phytoplankton species in 1-L bottles and incubated for 24 h under dark conditions at 3 °C. Experiments were carried out in triplicate along with one control bottle with no added copepods. During the experiments, the bottles were rotated every 3 h to prevent the phytoplankton from sinking. After the 24-h experiment, the copepods were transferred to new bottles containing FSW. Faecal pellets were pipetted from the incubation bottles using sterile Pasteur pipettes, placed in Petri dishes filled with chilled FSW and rinsed 5–10 times by

Table 2
The volume of the faecal pellets of *Neocalanus cristatus*, *Neocalanus flemingeri* and *Eucalanus bungii* observed with various phytoplanktons as food. For comparison possible, faecal pellet volumes (*FPV* in μm^3) predicted by prosome length (PL in mm) (Log FPV = 2.474 log PL + 5.226, Mauchline, 1998) are shown in the bottom column. Values are mean ± 1 sd.

Taxa/Species	Faecal pellet volume (10^6 μm^3)		
	Neocalanus cristatus	*Neocalanus flemingeri*	*Eucalanus bungii*
Diatoms			
Attheya septentrionalis	16.06 ± 14.60		
Chaetoceros sp.	17.16		21.52
Ditylum brightwellii	12.53 ± 4.44	5.95	10.33 ± 2.84
Pauliella taeniata	29.38		
Skeletonema sp.	12.23 ± 4.09		
Thalassiosira nordenskioeldii	49.67	5.20	13.24 ± 2.64
Dinoflagellates			
Alexandrium tamarense	16.94 ± 9.80	3.77	5.20 ± 1.94
Raphidophyceae			
Heterosigma akashiwo	20.98 ± 1.74		
FPV predicted by *PL*	38.88	5.64	16.06

Table 1
Data on phytoplankton (cell size, carbon, ash contents, colony formation and movement ability) used as food for copepods in the laboratory experiments. Owing to size, cell density of phytoplankton was changed for assimilation experiments. For carbon and ash contents, values are mean ± 1 sd.

Taxa/Species	Cell size (μm)	Carbon content (pg C $cell^{-1}$)	Ash content (pg ash $cell^{-1}$)	Colony formation	Movement ability	Concentration	
						(cells ml^{-1})	(μg C L^{-1})
Diatoms							
Attheya septentrionalis	5 – 10		190 ± 99	+		5.0×10^3 – 1.0×10^4	
Chaetoceros sp.	10 – 40	249 ± 3	754 ± 105	+		1.0×10^3	249
Ditylum brightwellii	25 – 100	2596 ± 1540	9400 ± 582	+		5.0×10^2	1298
Pauliella taeniata	25 – 30			+		5.0×10^2 – 1.0×10^4	
Skeletonema sp.	2 – 21	48 ± 16	105 ± 76	+		1.0×10^4 – 2.0×10^4	480 – 960
Thalassiosira nordenskioeldii	10 – 50	252 ± 62	1389 ± 540	+		1.0×10^3	252
Dinoflagellates							
Alexandrium tamarense	30 – 40	2577 ± 543	6796 ± 1747		+	5.0×10^2 – 1.0×10^3	1289–2577
Raphidophyceae							
Heterosigma akashiwo	10 – 20	100	528 ± 85		+	5.0×10^3	500

immersion in FSW to avoid contamination of the remaining phytoplankton cells. Then, the faecal pellets were checked under a stereomicroscope. The faecal pellet volume (*FPV*, μm^3) was quantified using the following equation:

$$FPV = \frac{4}{3}\pi\left(\frac{FPW}{2}\right)^2\left(\frac{FPL}{2}\right) \quad (1)$$

where *FPW* and *FPL* are faecal pellet width and length in μm, respectively. Both FPL and FPW were measured to a precision of 10 μm under a dissecting microscope fitted with an eye-piece micrometer.

Previously, GF/F filters were combusted at 480 °C for 5 h, then weighed with a microbalance (Mettler Toledo MT5) to a precision of 1 μg. Food phytoplankton and faecal pellets were gently filtered with pre-weighed GF/F filter, briefly rinsed with small amount of distilled water and then dried at 60 °C for 5 h. Their DW was then measured

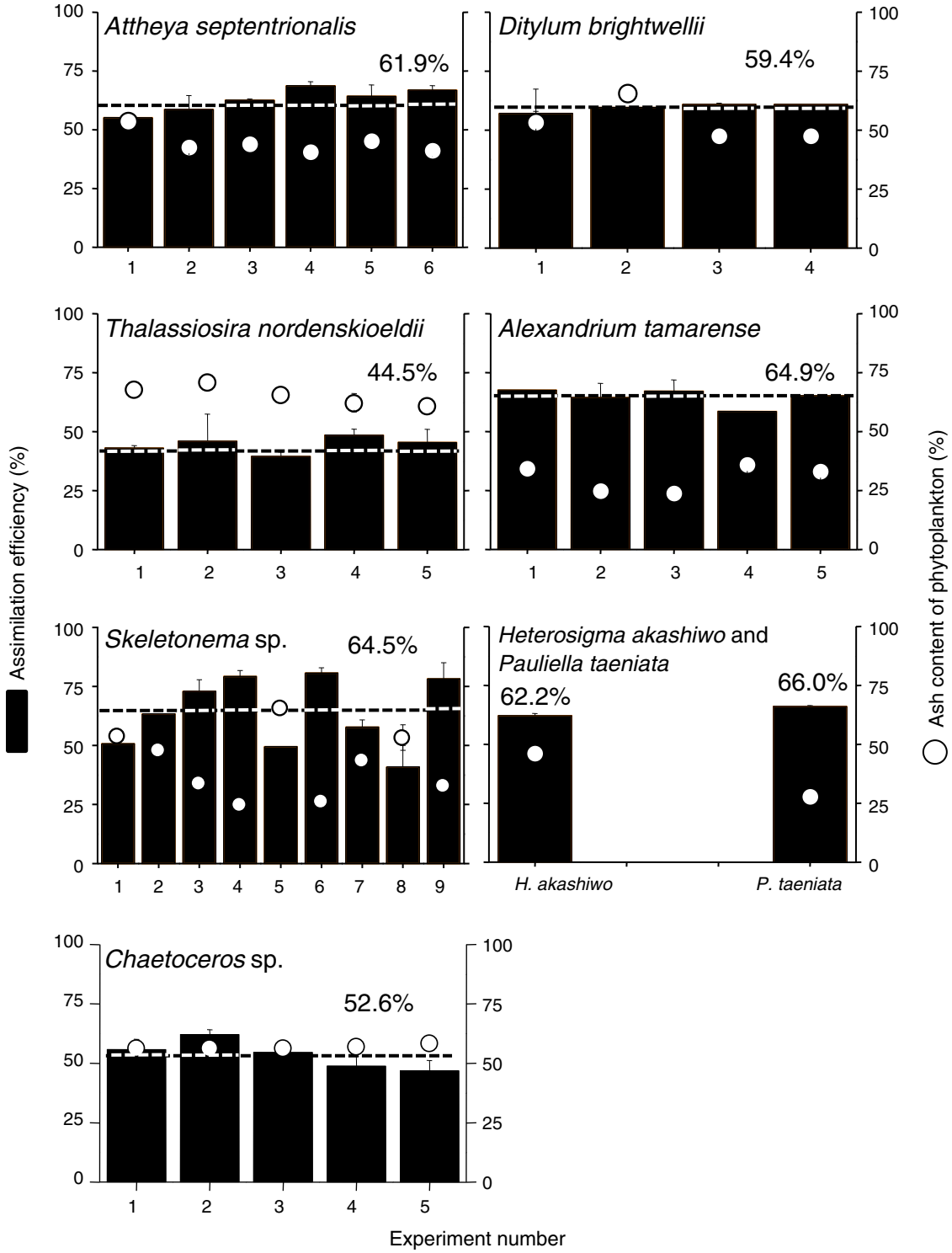

Fig. 1. Assimilation efficiency (solid column) of *Neocalanus cristatus* C5 for various food phytoplankton. Experiments were performed in triplicate, and the bars indicate standard deviations. Open circles are the ash contents of the phytoplankton in each experiment. Horizontal lines indicate the means of the assimilation efficiencies. The percentage values in the panels indicate the means of the assimilation efficiencies.

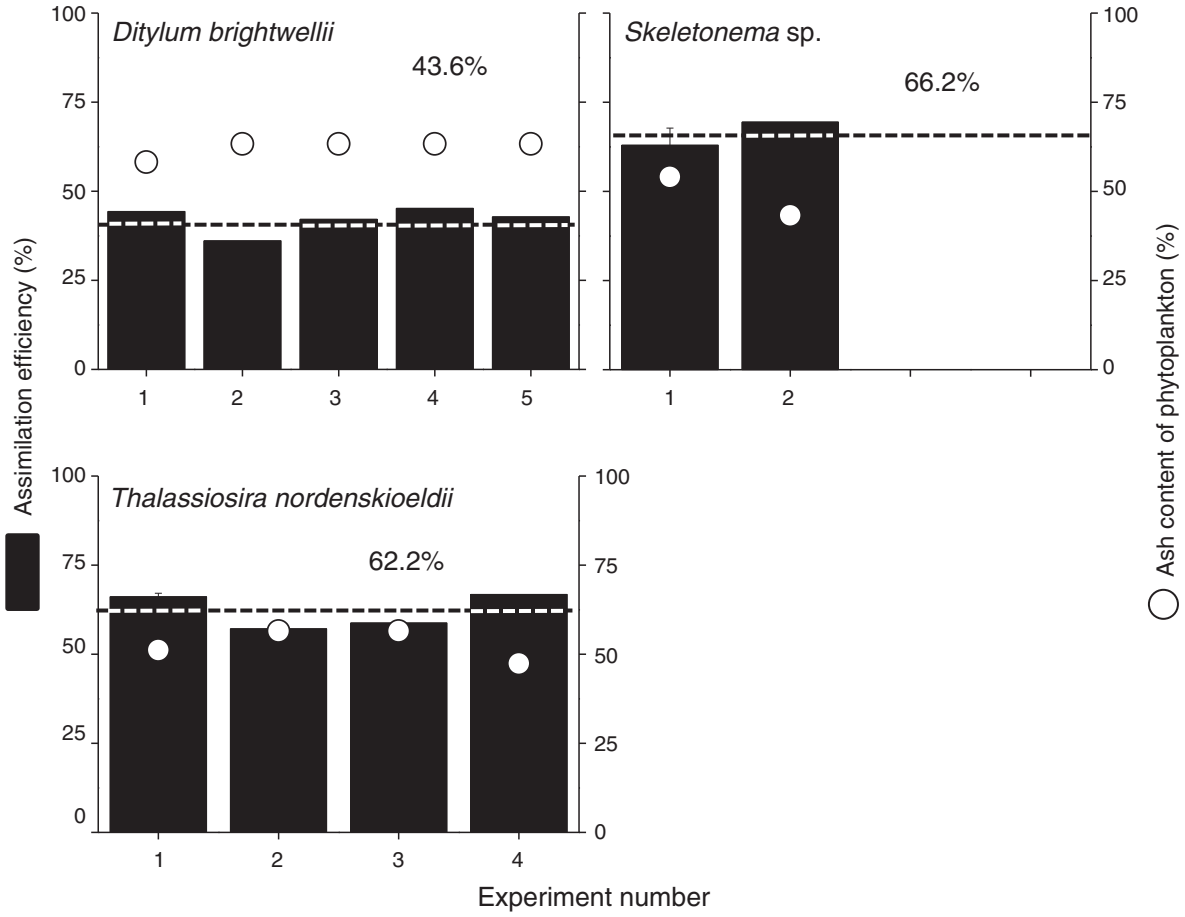

Fig. 2. Assimilation efficiency (solid column) of *Neocalanus flemingeri* C5 for various food phytoplankton. Experiments were performed in triplicate, and the bars indicate standard deviations. Open circles are the ash contents of the phytoplankton in each experiment. Horizontal lines indicate the means of the assimilation efficiencies. The percentage values in the panels indicate the means of the assimilation efficiencies.

with a microbalance. Dried filters were combusted at 480 °C for 5 h, and the ash weight (*ASH*) was measured with a microbalance. The organic contents of the phytoplankton and faecal pellets were calculated as *DW* − *ASH*. The assimilation efficiency was determined by the Ratio method (Conover, 1966a,b):

$$U' \; [(F'-E')/(1-E')\,(F')] \times 100 \qquad (2)$$

where U' is the assimilation efficiency (%), F' is the organic fraction of the food, and E' is the organic fraction of the faecal pellets.

To evaluate differences among species in the assimilation efficiencies and phytoplankton ash contents, an ANCOVA was applied using the copepod species and phytoplankton ash contents as independent variables.

The relationship between assimilation efficiency and phytoplankton carbon concentration was analysed as another factor that controls the copepod assimilation efficiency. To express the nonlinear regression models between assimilation efficiency and carbon concentration, a generalised additive model (GAM) was applied using the free software "R" and the multivariate smoothing parameter estimation package "mgcv".

3. Results

The faecal pellet volumes observed in this study are summarised in Table 2. The faecal pellet volumes varied with phytoplankton species, and their means were 12.23–49.67, 3.77–5.95 and 5.20–21.52 × 10^6 μm^3 for *N. cristatus, N. flemingeri* and *E. bungii*, respectively (Table 2). These values corresponded well with the predicted values from the prosome length of each copepod species predicted by Mauchline's equation (1998): 38.88, 5.64 and 16.06 × 10^6 μm^3 for *N. cristatus, N. flemingeri* and *E. bungii*, respectively (Table 2).

Experiments on the assimilation efficiency of *N. cristatus* were performed with eight phytoplankton species. The assimilation efficiency of *N. cristatus* ranged between 45% and 66% and varied with the phytoplankton species. The highest and lowest assimilation efficiencies were observed for the diatoms *P. taeniata* and *Th. nordenskioeldii,* respectively (Fig. 1).

Many specimens of C5 *N. flemingeri* moulted to C6 during the experiments. If moulting was observed, the whole replicate was thrown out. Because of this limitation, assimilation efficiency data for this species were only obtained for three diatom species. The assimilation efficiency of *N. flemingeri* ranged between 44% and 66% and varied with the phytoplankton species. The highest and lowest values of assimilation efficiency were recorded for the diatoms *Skeletonema* sp. and *D. brightwellii,* respectively (Fig. 2).

Experiments on *E. bungii* were performed using four phytoplankton species. The assimilation efficiency of *E. bungii* ranged between 34% and 65% and varied with the phytoplankton species. The highest and lowest values of assimilation efficiency were recorded for the diatoms *Skeletonema* sp. and *D. brightwellii,* respectively (Fig. 3).

A common trend for the three copepod species was that the assimilation efficiencies had a significant negative correlation with the ash contents of the food phytoplankton (r^2 = 0.79–0.87, $p < 0.001$, Fig. 4A–C), and the AFDW content showed no relationship with assimilation efficiency (Fig. 4D–F). From the ANCOVA analysis with

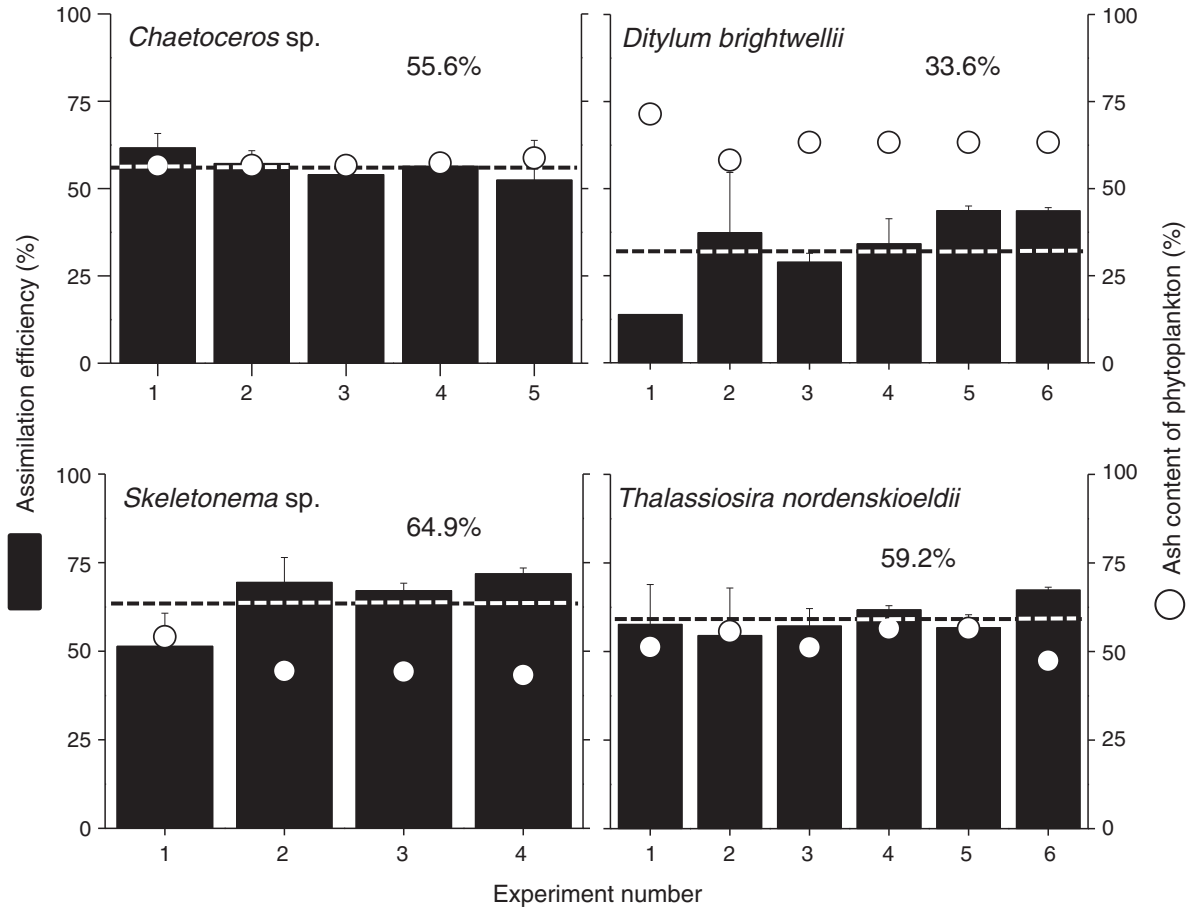

Fig. 3. Assimilation efficiency (solid column) of *Eucalanus bungii* C5 for various food phytoplankton. Experiments were performed in triplicate, and the bars indicate standard deviations. Open circles are the ash contents of the phytoplankton in each experiment. Horizontal lines indicate the means of the assimilation efficiencies. The percentage values in the panels indicate the means of the assimilation efficiencies.

assimilation efficiency as the dependent variable and covariate with the ash content of phytoplankton, the effect of the interaction of copepods with ash contents was highly significant (Table 3). Thus, the parallelism of the regression lines for the three copepods (Fig. 4A–C) was rejected (i.e., the slopes of the regression lines varied with species).

The assimilation efficiency was stable for carbon concentrations in the range of 100–830 μg C L^{-1} in the phytoplankton species, and it decreased with increasing carbon concentration above 830 μg C L^{-1} (Fig. 5).

4. Discussion

According to Båmstedt et al. (2000), potential sources of bias in the Ratio method are the following: (1) non-homogenous food material, (2) food selectivity, (3) sloppy feeding, (4) losses from faecal material, (5) absorbance of inert tracers in the digestive tract and (6) production of non-faecal material mixed with faeces. In the present study, because we applied only one phytoplankton species as food for each experiment, sources (1) and (2) seem to be eliminated. For (3) – (6), we rotated the treated incubation bottles, pipetted faecal pellets by sterile Pasteur pipette, rinsed the pellets 5–10 times within FSW and then checked the faecal pellets under stereomicroscope. Because the FPV observed in this study corresponded well with the predicted values (Table 2), we believe that the effects of coprophagy or coprohexy were negligible, and the biases on the Ratio method were minimised for this study.

The most important result of this study is that the assimilation efficiencies of the three oceanic copepods showed highly significant negative correlations with the ash content of the food phytoplankton (Fig. 4A–C). These findings correspond well with a previous study (Conover, 1966b). Among the phytoplankton species, the highest ash content was observed for diatoms (cf. Fig. 1). As a specialised characteristic of diatoms, their cell walls are made of silica. Although silica is ingested by copepods, 79–90% of the silica is egested as faecal pellets (Conover et al., 1986; Cowie and Hedges, 1996; Tande and Slagstad, 1985). Because copepods do not utilise silica, the highly significant negative relationship between copepod assimilation efficiency and ash content of phytoplankton is a common pattern. The slope (b) of the regression ($Y = a + bX$) between copepod assimilation efficiency (Y: %) and the ash content of the phytoplankton (X: %) in this study (−0.72) is very close to that found by Conover (1966b) (−0.73). Conover (1966b) mainly used the large oceanic copepod *Calanus hyperboreus* as the experimental species, which is dominant in the northern North Atlantic. *N. cristatus* can be considered as the counterpart species in the North Pacific of *C. hyperboreus* in the North Atlantic (Parsons and Lalli, 1988), and the similar slopes of the regression formula between this study (*N. cristatus*) and Conover (1966b) (*C. hyperboreus*) indicate that they have similar ecological roles.

To clarify the factors that determine the copepod assimilation efficiency, we calculated the adjusted copepod assimilation efficiency (U'_{adj}: %) with phytoplankton ash contents using the following equation (Sokal and Rohlf, 2012):

$$U'adj = U' - b(1 - F') \times 100 \quad (3)$$

where U' is the assimilation efficiency (%), (1-F') is the ash fraction of the phytoplankton, and b is the fitted constant of the regression for

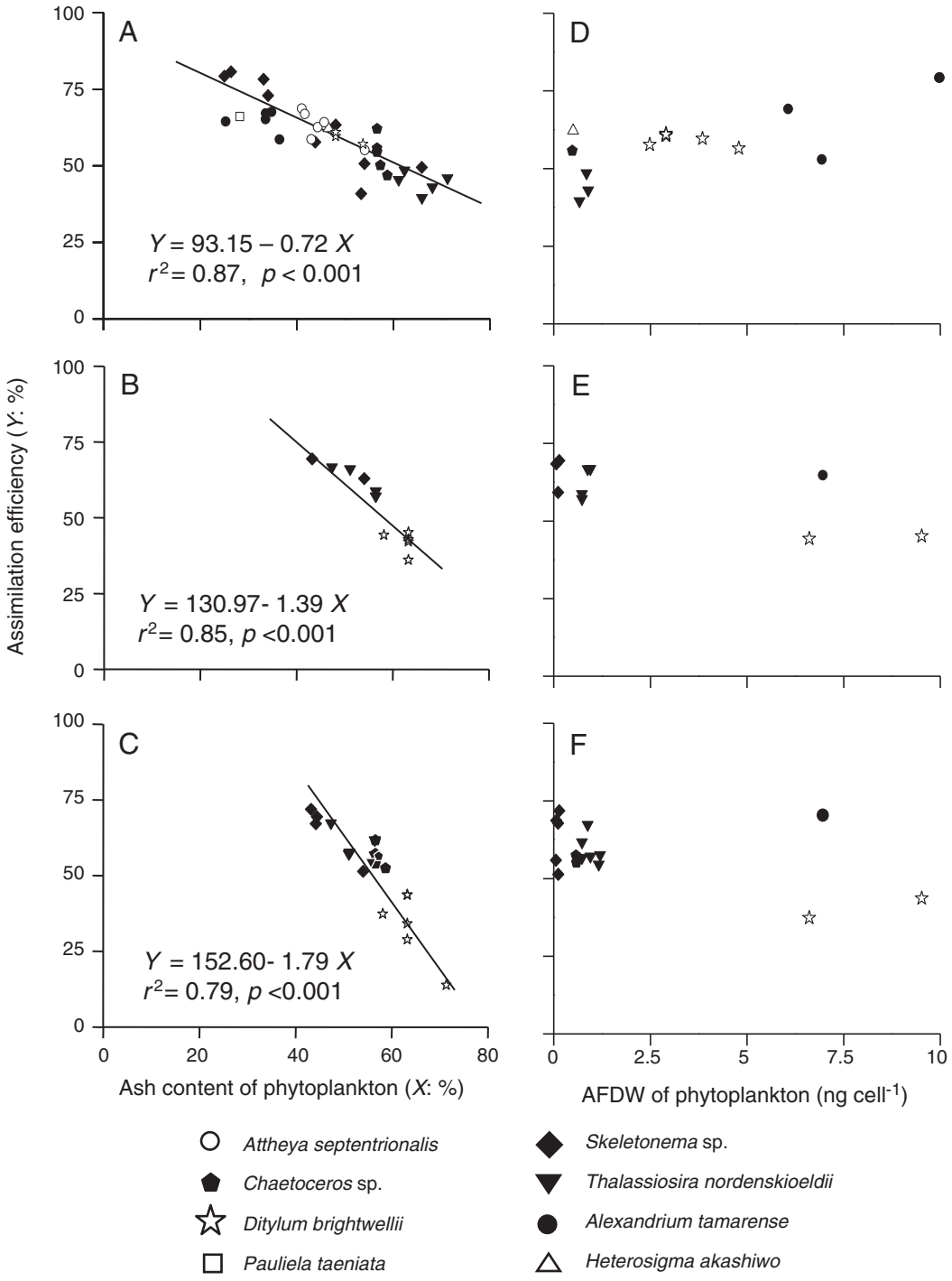

Fig. 4. Relationships between assimilation efficiency and ash content of food phytoplankton: (A) *Neocalanus cristatus* C5, (B) *Neocalanus flemingeri* C5 and (C) *Eucalanus bungii* C5. Relationships between assimilation efficiency and ash free dry weight (AFDW) contents of food phytoplankton: (D) *N. cristatus* C5, (E) *N. flemingeri* C5 and (F) *E. bungii* C5. Regression equations are shown for each species.

Table 3
Result of ANCOVA for the adjusted assimilation efficiencies. For this analysis, copepod species and phytoplankton ash contents applied as independent variables. df: degree of freedom, SS: sum of squares, ***: $p < 0.001$.

Parameter	df	SS	F-value	p
Copepods	2	1385.26	13.93	***
Ash contents	1	31,176.81	626.88	***
Copepods × Ash contents	2	7110.58	71.49	***
Error	86	4277.08		

each copepod species (cf. Fig. 4A–C). The relationships between the adjusted copepod assimilation efficiency and each parameter are listed in Table 1 (i.e., phytoplankton cell size, colony formation and movement ability) and were analysed along with copepod body size (Ueda et al., 2008) (Fig. 6). For phytoplankton cell size, both *N. flemingeri* and *E. bungii* had high assimilation rates for small-sized *Skeletonema* sp. (2–21 μm) and low assimilation rates for large-sized *D. brightwellii* (25–100 μm). A negative correlation between assimilation efficiency and phytoplankton cell size was detected ($p<0.001$) (Fig. 6A). However, changes in assimilation efficiency with the phytoplankton cell size were

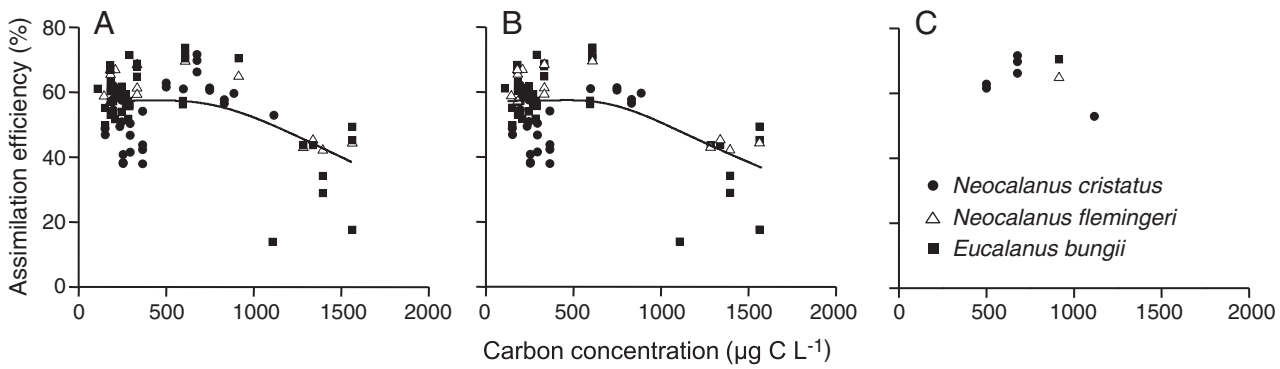

Fig. 5. The effects of variable phytoplankton carbon concentrations on copepod assimilation efficiency for whole data (A), diatoms (B) and dinoflagellates (C). To express the nonlinear regression model between the assimilation efficiency and the carbon concentration, a generalised additive model (GAM) was applied using the free software "R" and the multivariate smoothing parameter estimation package "mgcv".

not detected for *N. cristatus* (Fig. 6A). This is because the assimilation efficiency of the large-sized *D. brightwellii* was higher for *N. cristatus* than for the other two species (Fig. 6C). Thus, the large-sized copepod *N. cristatus* had a high assimilation efficiency for large-sized phytoplankton, so there was no relationship between the assimilation efficiency and phytoplankton cell size for *N. cristatus* (Fig. 6A).

Colony formation and movement ability are closely related among the phytoplankton species treated in this study, i.e., colony-

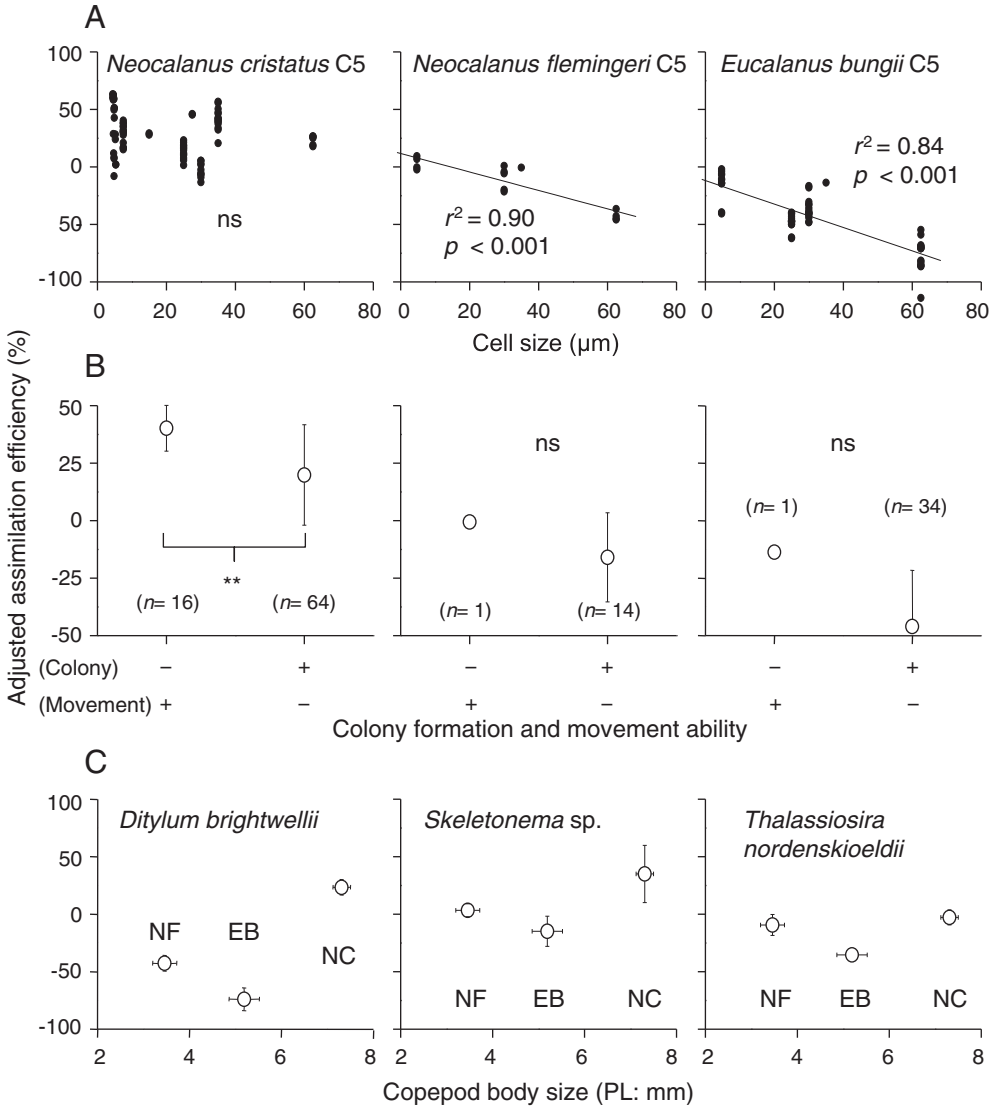

Fig. 6. Relationships between the adjusted assimilation efficiency and phytoplankton cell size (A), colony formation/movement ability (B) and copepod body size (C). **: $p < 0.01$, ns: not significant. NF: *Neocalanus flemingeri*, EB: *Eucalanus bungii*, NC: *Neocalanus cristatus*.

forming species have no movement ability (diatoms), whereas non-colony-forming species have movement ability (dinoflagellates and raphidophyceans) (Table 1). In this study, a significant difference was only detected for *N. cristatus*: the assimilation efficiency was higher for non-colony-forming phytoplankton with movement ability (dinoflagellates and raphidophyceans) than for the colony-forming phytoplankton with no movement ability (diatoms) (Fig. 6B). However, this pattern was not detected for the other two copepods, which was partly because the number of experiments with dinoflagellates and raphidophyceans was extremely limited ($n = 1$) for these species (Fig. 6B). The limitation pattern of *N. cristatus* for this study (assimilation efficiency of dinoflagellates was higher than those of diatoms, $p < 0.01$, Fig. 6B) corresponds well with the results of previous studies (Besiktepe and Dam, 2002; Conover, 1966a).

Concerning the effects of food concentration on assimilation efficiency, Conover (1966b) reported that the assimilation efficiency did not change, regardless of the food carbon concentration. However, it is recognised that the assimilation efficiency decreases with increasing food concentration (Besiktepe and Dam, 2002; Gaudy, 1974; Landry et al., 1984; Thor and Wendt, 2010). The decrease of assimilation efficiency under high food concentrations (>830 μg C L^{-1}) was also the case in this study (Fig. 5). Two factors are considered as possible causes of the decrease of assimilation under high food concentrations: (1) the shortened gut passage time under high food concentration (Besiktepe and Dam, 2002) may prevent sufficient digestion and assimilation (Landry et al., 1984; Lehman, 1976) or (2) changes in the activities of digestive enzymes with food concentration (high under low food concentration) (Hassett and Landry, 1983), where the digestive enzyme activity decreases under high food concentrations, may reduce the assimilation efficiency (Landry et al., 1984). Based on this information, Pahlow and Prome (2010) created an ecosystem model that incorporates the decrease of assimilation efficiency with increasing food concentration. Montagnes and Fenton (2012) also developed an ecosystem model in which assimilation efficiency varied with food concentration and compared it to the model with constant assimilation efficiency. Because the decrease of assimilation efficiency under high food carbon concentrations was observed for an anomalously high food concentration in the experimental condition (Fig. 5), it should be questioned whether this phenomenon would occur under natural food concentrations in the field. Applying the same carbon contents of food is recommended for future studies of incubation experiments on assimilation efficiency.

Marine ecosystem models, such as NPZD models (e.g., NEMURO), PDM (population dynamics model) and LEM, apply a constant assimilation efficiency (70%) for zooplankton (mainly considering copepods) (Kishi et al., 2007; Terui et al., 2012). This study, however, showed that the assimilation efficiency of large oceanic copepods was lower, in the range of 34%–66%, and varied depending on food ash contents. The low assimilation efficiency observed in this study may be caused by the food applied (phytoplankton). Under natural food conditions, these copepods may prefer to feed on microzooplankton rather than on phytoplankton (cf. Dagg et al., 2009 and references therein). The assimilation efficiency of microzooplankton may be higher than the phytoplankton, especially for the diatoms, which is why the observed assimilation efficiency of this study (34–66%) was lower than the commonly used value (70%).

Next, we tested the effects of changes in assimilation efficiency by applying the LEM for *N. cristatus* (Terui et al., 2012). In this test, the environmental settings (e.g., water temperature and solar radiation) were the same as in the original model, and only the assimilation efficiency (P6 in LEM of Terui et al., 2012) was changed. We ran a model using an assimilation efficiency (as 70% in the original model) between 45% and 66% (i.e., the range experimentally observed for *N. cristatus*, Fig. 1). After a run of 50 years, the results reached a steady state after more than 51 years and are shown in Fig. 7. Under the 66% assimilation efficiency, *N. cristatus* could maintain the population

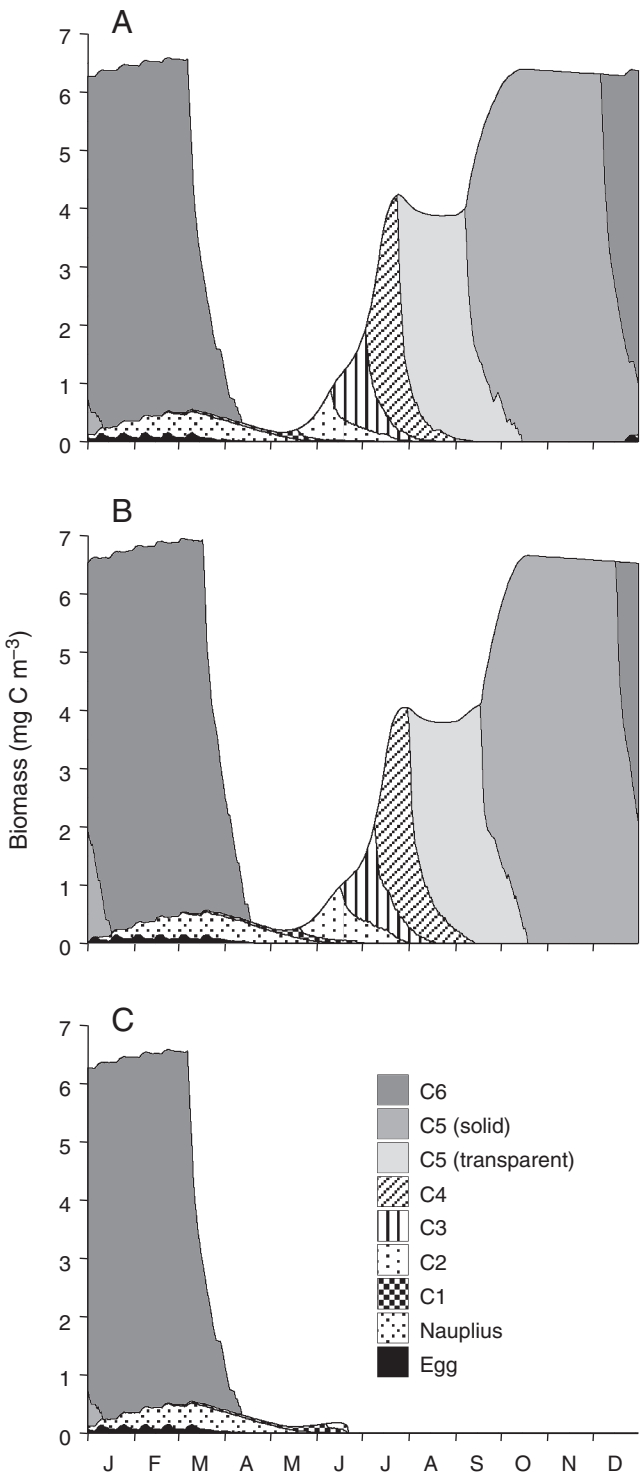

Fig. 7. Simulated biomass of each stage of *Neocalanus cristatus* by LEM from Terui et al. (2012) applying a modification of the assimilation efficiency to 70% (A), 66% (B) and 45% (C).

(Fig. 7B). However, *N. cristatus* could not maintain its population under the lowest 44% assimilation efficiency (Fig. 7C). When the runs changed for every 1%, it was demonstrated that *N. cristatus* could not maintain its population for <57% assimilation efficiency, and the survival of the population was possible for >58% assimilation efficiency. In addition to population survival, changes in assimilation efficiency affected development time, i.e., 139 days were required for individuals born on 22 February to reach C5 (solid) under 70% assimilation

efficiency, and 150 days were required for same hatch date individuals under 66% assimilation efficiency. Thus, the changes in assimilation efficiency have significant effects on copepod population survival and growth and variations in assimilation efficiency should be incorporated into marine ecosystem models in the future. Because copepod assimilation efficiency is highly significantly correlated with the inorganic content of phytoplankton (Fig. 4), the assimilation efficiency in the model should be estimated using parameters based on the composition of the food phytoplankton taxa.

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