



**HAL**  
open science

# Sources of seasonal variability in mesozooplankton aspartate transcarbamylase activity in coastal waters off Plymouth, UK

Isabelle C. Biegala, Roger Harris

► **To cite this version:**

Isabelle C. Biegala, Roger Harris. Sources of seasonal variability in mesozooplankton aspartate transcarbamylase activity in coastal waters off Plymouth, UK. *Journal of Plankton Research*, Oxford University Press (OUP), 1999, 21 (11), pp.2085-2103. 10.1093/plankt/21.11.2085 . ird-03759415

**HAL Id: ird-03759415**

**<https://hal.ird.fr/ird-03759415>**

Submitted on 24 Aug 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## Sources of seasonal variability in mesozooplankton aspartate transcarbamylase activity in coastal waters off Plymouth, UK

I.C.Biegala<sup>1,2</sup> and R.P.Harris<sup>2</sup>

<sup>1</sup>Station Biologique de Roscoff, place Georges Teissier, BP 74, F-29680, Roscoff, France and <sup>2</sup>Plymouth Marine Laboratory, Prospect Place, Plymouth PL1 3DH, UK

**Abstract.** Some investigators have proposed aspartate transcarbamylase (ATCase) activity as an overall index of mesozooplankton productivity. However, seasonal changes in mesozooplankton species composition have never been investigated as a possible source of variation in ATCase activity. In this study, we investigate mesozooplankton composition in terms of (i) developmental stages, (ii) species and developmental stages body mass and (iii) species composition, and their relationship to ATCase activity. In controlled laboratory conditions, ATCase activity variability was closely related to changes in somatic growth rate of the copepod *Calanus helgolandicus*, but was not related to changes in nucleic acid concentrations. It can be argued, however, that the activity of this enzyme is partially involved in copepod somatic productivity, and should be a good index of embryogenesis. In addition, changes in ATCase activity were not significantly influenced by variability in mesozooplankton biomass, when investigated both on inter- and intraspecific levels. Finally, when a complete seasonal cycle was investigated at a fixed station off Plymouth (English Channel), ATCase activity was not correlated with the abundance of any mesozooplankton species apart from copepodites and adult *C.helgolandicus*. Furthermore, ATCase activity measured both on mesozooplankton and female *C.helgolandicus* was significantly correlated ( $R^2 = 0.72$ ,  $n = 33$ ,  $P < 0.001$ ) throughout the year, apart from April. At that particular time of the year, ATCase activity was in phase with the peak of abundance of copepod eggs and nauplii. It is suggested that mesozooplankton peaks of ATCase activity reflect two periods in the life history of the copepod: embryogenesis and terminal moult. We propose further experiments to test this hypothesis and to promote the development of molecular biomarkers in order to characterize specific zooplankton metabolic processes.

### Introduction

Knowledge of zooplankton production is essential for monitoring marine pelagic ecosystems for signs of environmental changes (Banse, 1995; Roemmich and McGowan, 1995). Copepods mainly dominate zooplankton biomass and composition, and play a key role in the marine pelagic food web. Thus, monitoring changes in copepod biomass and productivity (i.e. their ability to produce organic matter) is essential for defining the production of these key organisms.

The classical methods for estimating copepod productivity or relative growth rate involve counting, weighing or incubating the different developmental stages of dominant species (Shushkina, 1968; Bougis, 1974; Sekiguchi *et al.*, 1980; Kimmerer and McKinnon, 1987; Biegala, 1998). These methods are time consuming and do not allow the gathering of data on large scales with the same frequency as other environmental descriptors (e.g. temperature, salinity, nutrient and chlorophyll *a* concentrations). A suitable alternative would be to estimate the relative rate of metabolic processes involved in organic matter biosynthesis. With that objective, marine biologists have proposed measuring biochemical correlates of protein synthesis, cellular multiplication and moult processes, such as nucleic acid (Sutcliffe, 1965), DNA polymerase (Sapienza and Mague, 1979), NDPKase (nucleoside diphosphate kinase) (Berges *et al.*, 1990) and chitobiase (Espie and

Roff, 1995a,b). Until now, none of these methods have been adopted as a comprehensive index of both germinal and somatic productivity. With a similar perspective, Bergeron and Buestel (1979) and Bergeron (1983) proposed the measurement of aspartate transcarbamylase (ATCase) activity as an index of relative growth rate or zooplankton productivity.

ATCase in eukaryotes specifically catalyses the second step of the *de novo* synthesis of pyrimidine nucleotides, the fundamental units of nucleic acid (Jones, 1980). In eukaryotic cells, the ATCase active site is not under allosteric control (Jones, 1980), and therefore measurement of the *in vitro* optimal activity may indicate the amount of enzyme present. The activity of this enzyme has been shown to be particularly important in plant or animal tissues with intense mitotic activity, such as embryonic (Nordman *et al.*, 1964; Kim and Cohen, 1965; Stein and Cohen, 1965; Hertzfeld and Knox, 1972), tumour (Calva *et al.*, 1959) or regenerating tissues (Calva *et al.*, 1959; Kim and Cohen, 1965; Young *et al.*, 1967). Consequently, ATCase activity was found to be much higher when measured in primary developmental stages than in juveniles or adults (Brothers *et al.*, 1978; Patnaik and Patnaik, 1990). In addition, Alayse-Danet (1980) and Bergeron (1982) showed that ATCase activity was significantly correlated with the relative growth rate of *Artemia salina* and fish larvae. However, Hernández-León *et al.* (1995) did not find any correlation between ATCase activity and the relative growth rate of *Acartia clausi* copepodites, when reared with different food concentrations. Furthermore, Biegala *et al.* (1999) showed that in the female copepod *Calanus helgolandicus*, ATCase activity was significantly correlated with egg production (an index of female copepod productivity) in autumn and winter, but when a complete seasonal cycle was considered no significant correlation was observed. To explain this lack of correlation, the authors suggested (i) the involvement of ATCase activity in anabolic processes other than egg production (e.g. tissue synthesis during female copepod post-ecdysis), and (ii) the involvement of salvage pyrimidine nucleotide biosynthesis, which uses pre-existing pyrimidine bases directly supplied by the diet or coming from nucleic acid catabolism. The measurement of ATCase activity seems, therefore, to be only partially related to female copepod productivity or relative growth rate.

Until now, mesozooplankton ATCase activity has been measured in samples collected in spring from coastal European and Antarctic waters (Bergeron, 1986, 1990, 1995; Hernández-León *et al.*, 1995). Changes in the activity of this enzyme have been compared by these authors to environmental parameters and mesozooplankton biomass, but the variability in ATCase activity has never been investigated in relation to species composition. Mesozooplankton are composed of different stages of holoplankton and meroplankton species that overlap and follow one another through a seasonal cycle (Bougis, 1974). Therefore, changes in the activity of this enzyme in mesozooplankton could reflect variations in the proportion of mesozooplankton species and/or developmental stages. In addition, it has been shown in small crustaceans that many metabolic rates ( $T$ ) and enzyme activities decrease with species biomass ( $W$ ) according to the following allometric relationship (Schmidt-Nielsen, 1984; Berges and Ballantyne, 1991):

$$T = aW^b \text{ or } TW^{-1} = aW^{b-1}$$

with  $a$  a constant and  $b$  the allometric coefficient. Similar allometric relationships were also observed for many enzyme activities during the development of *Artemia salina* (Berges *et al.*, 1990). These authors could distinguish the influence of body mass on enzyme activities from its effect on growth rate, because the development of *Artemia salina* juvenile stages is exponential and the growth rate constant. It is, therefore, possible that the seasonal changes in mesozooplankton ATCase activity could be related to the variation in the proportion of the smallest species.

The aim of this work was to investigate the source of variability of ATCase activity in mesozooplankton, mainly in relation to seasonal changes in species composition. This study was also supported by detailed information concerning changes in ATCase activity in the copepod *C. helgolandicus*, both reared in laboratory controlled conditions and sampled in the field during a seasonal cycle. The laboratory cultures were used to understand the changes in ATCase activity during the development of this species. The activity of the enzyme was then compared with the relative growth rate and nucleic acid content. RNA and DNA can be seen as intermediates between ATCase and cellular multiplication and protein synthesis, as ATCase takes part in the synthesis of the fundamental units of nucleic acids. Finally, the presence of an inter- and intraspecific allometric relationship between mesozooplankton body mass and ATCase activity was investigated.

## Method

### *Seasonal sample collection and storage*

Samples were collected at weekly intervals from May 1995 to June 1996, from a coastal station (Station L4: 50°15'N, 4°13'W) ~10 km off Plymouth (English Channel). The CTD records showed that the water column was well mixed throughout the year, except from April to June when a thermocline developed at 15 m in both 1995 and 1996 (data not shown; see Biegala, 1998). Surface temperature was measured and samples of subsurface (–10 m) sea water were taken for further chlorophyll  $a$  measurements in the laboratory. Zooplankton samples were collected from 5 m above the bottom (~50 m) to the surface, in three replicates, with 200 and 500  $\mu\text{m}$  WP2 vertical net hauls. The contents of the non-filtering cod ends were diluted with sea water and kept in 2 or 5 l plastic buckets at sea temperature until further treatment in the laboratory.

To quantify total particulate chlorophyll  $a$ , triplicates of 250 ml sea water were filtered through an ashed glass fibre filter (Whatman GF/F) 25 mm in diameter. The filters were placed in Petri dishes, wrapped in aluminium foil and stored at –25°C. Two replicates of 200  $\mu\text{m}$  WP2 vertical net hauls were poured in plastic bottles and preserved in 4% formalin for later analysis of zooplankton species composition. Three replicates of the 200  $\mu\text{m}$  WP2 vertical net hauls were pre-filtered through a 2000  $\mu\text{m}$  mesh, which retained macrozooplankton, and mesozooplankton were collected on a 200  $\mu\text{m}$  mesh. Mesozooplankton wet weight was

then measured and the animals were poured into cryogenic vials and stored in liquid nitrogen ( $-196^{\circ}\text{C}$ ) for later biochemical assays. Two replicates of 30 female *C.helgolandicus* were picked out under a stereomicroscope, from the 500  $\mu\text{m}$  WP2 vertical net haul, and placed in cryogenic vials in liquid nitrogen for further biochemical assays. *Calanus helgolandicus* was chosen as a reference copepod species because of its large size and abundance off Plymouth.

#### *Culture of Calanus helgolandicus developmental stages*

Zooplankton were collected, with 500  $\mu\text{m}$  WP2 vertical net hauls, in October 1996, over three consecutive days. The animals were maintained under the same conditions as previously described. Within a few hours after landing, 600 females were sorted under a stereomicroscope, with flexible forceps. The females were then placed in glass beakers containing 0.6- $\mu\text{m}$ -filtered sea water and Plexiglas tubes equipped with 500- $\mu\text{m}$ -mesh bottoms, to separate the eggs and faecal pellets from the females. Female concentration was 30 individuals  $\text{l}^{-1}$ . The dinoflagellate *Prorocentrum micans* was introduced as an algal food source at a concentration of 600 cells  $\text{ml}^{-1}$  ( $\sim 240 \mu\text{g C l}^{-1}$ ). Algal densities were determined using a Coulter Multisizer. The females were acclimatized to these conditions for 4 days and were laying  $6000 \pm 500$  eggs  $\text{day}^{-1}$  at the end of the acclimatization period.

Every day, the beakers and food suspension were changed, and the eggs were counted and separated from faecal pellets under a stereomicroscope with a fine glass pipette. The eggs were sampled and either stored for subsequent analysis, or allowed to develop in 5 l glass beakers, containing a similar food suspension as mentioned above. A total of 12 beakers were set up under these conditions. During the first 6 days of incubation, the food suspension was not changed due to the fragility of the animals and because they only start feeding at nauplius stage III (Marshall and Orr, 1972). After day six, the nauplii were introduced into Plexiglas tubes with bottoms of 80  $\mu\text{m}$  mesh, to separate the faecal pellets from the copepods. When the cultures reached copepodite II, tubes equipped with 200- $\mu\text{m}$ -mesh bottoms were employed, which were subsequently increased to 500  $\mu\text{m}$  for copepodite V. Each day, the beakers and the sea water were changed and *P.micans* added, allowing a final concentration of 600 cells  $\text{ml}^{-1}$ . After 3, 6, 10, 17, 23 and 24 days of incubation, respectively,  $\sim 2000 \pm 500$  nauplii dominated by stage II,  $\sim 2000 \pm 500$  nauplii dominated by stage IV, 500 copepodites dominated by stage I, 100 copepodites dominated by stages III and IV, 32 copepodites V and 30 adult females were sampled. The stages nauplii II and IV and copepodite II and IV were grown and sampled in triplicate, whereas copepodites V and adults were grown from the remaining copepodites IV. The required numbers of individuals for each stage were then concentrated on a mesh, pipetted into 1 ml of sea water and stored in liquid nitrogen for later biochemical assays. To determine the exact developmental stages of nauplii and copepodite cohorts, subsamples of 30 ml were taken in triplicate and preserved in formalin for subsequent identification.

The complete experiment was conducted at  $15^{\circ}\text{C}$ , using a diurnal cycle of 9 h light:15 h dark.

*Chlorophyll a analysis*

Chlorophyll *a* was quantified according to Yentsch and Menzel (1963), with a Turner fluorimeter, and acetone (90%) was used as pigment extraction solvent.

*Preparation of zooplankton homogenates*

Samples of *C.helgolandicus* and total mesozooplankton were homogenized as described by Biegala and Bergeron (1998) to produce 1 g mesozooplankton wet weight ml<sup>-1</sup> and a total protein concentration per sample ranging from 2 to 55 mg ml<sup>-1</sup>. This concentration of material was chosen because ATCase activity, expressed in terms of protein, was found to be constant within that range for most of the samples investigated.

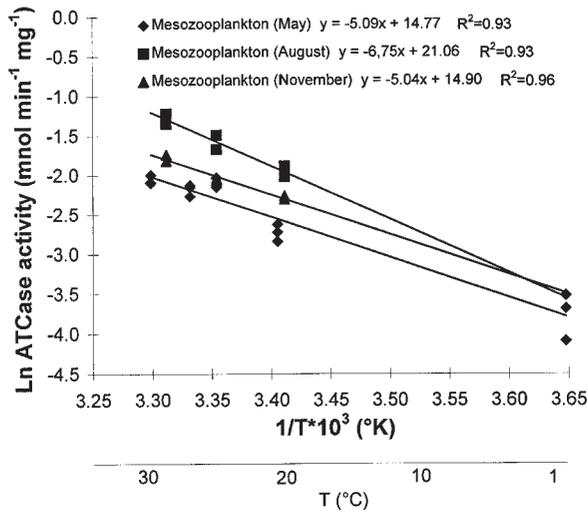
*Biochemical assays*

Total protein was determined using a modification of the Lowry *et al.* (1951) method described by Markwell *et al.* (1978), where sodium dodecyl sulphate (SDS) was added to the alkaline reagent. This method allows the measurement of membrane proteins and lipoproteins in addition to soluble proteins. Bovine serum albumin (BSA) (Sigma) was used as a standard. Nucleic acids were assayed according to the tissue homogenates ethidium bromide method of Karsten and Wollenberger (1972), where pronase was replaced by heparin (purified from porcine intestinal mucosa; Sigma) as suggested by Karsten and Wollenberger (1977). Calf thymus DNA (Sigma) was used as standard. The assay of ATCase (EC 2.1.3.1.) activity followed the radiochemical method of Bresnick and Mossé (1966), and was conducted under the same optimum conditions as those described by Biegala and Bergeron (1998) (i.e. 30 min of incubation, at 35°C, at pH 9).

ATCase activity was expressed relative to protein levels and was not corrected for *in situ* temperature, as no significant changes in mesozooplankton ATCase activity were observed between values uncorrected and corrected through the Arrhenius relationship (Table I; Owens and King, 1975; Mayzaud, 1986):

$$\ln \frac{\text{ATCase}_{\text{in situ}}}{\text{ATCase}_{\text{inc}}} = \frac{E_a}{R} \frac{1}{T_{\text{inc}} - T_{\text{in situ}}}$$

where ATCase<sub>inc</sub> and ATCase<sub>in situ</sub> are the ATCase activities (nmol min<sup>-1</sup> mg<sup>-1</sup>) measured *in vitro* at 35°C and corrected to *in situ* temperature, respectively,  $T_{\text{inc}}$  and  $T_{\text{in situ}}$  are the *in vitro* and *in situ* temperature (°K),  $R$  is the gas constant (1.987 cal °K<sup>-1</sup> mol<sup>-1</sup>) and  $E_a$  is the energy of activation (kcal mol<sup>-1</sup>), which is equal to  $-RS$ , with  $S$  the slope of the Arrhenius plot (Figure 1).



**Fig. 1.** Arrhenius plot for ATCase activity (Ln ATCase activity) from mesozooplankton samples collected at Station L4 at different times of the year.  $E_a$ , the energy of activation, was calculated from the slopes ( $S$ ) and is presented in Table I ( $E_a = -RS$ , with  $R$  the gas constant:  $1.987 \text{ cal } ^\circ\text{K}^{-1} \text{ mol}^{-1}$ ).

**Table I.** Arrhenius correction of mesozooplankton ATCase activity to *in situ* temperature

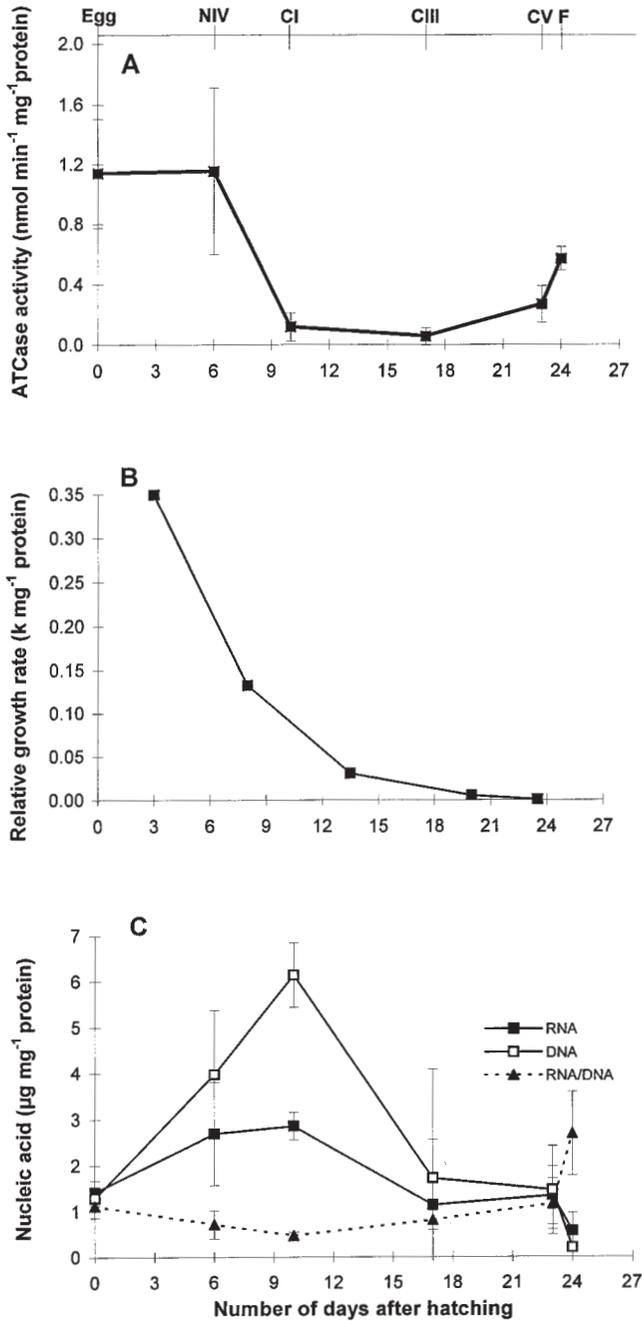
Mesozooplankton samples	$E_a$ (kcal mol <sup>-1</sup> )	$T_{\text{inc}}$ (°C)	$T_{\text{in situ}}$ (°C)	ATCase <sub>inc</sub> activity (nmol min <sup>-1</sup> mg <sup>-1</sup> ) mean ± SD ( $n$ )	ATCase <sub>in situ</sub> activity (nmol min <sup>-1</sup> mg <sup>-1</sup> ) mean ± SD ( $n$ )
May	10.11	35	11	$0.1207 \pm 0.0125$ (3)	$0.1198 \pm 0.0125$ (3)
August	13.41	35	16	$0.3294 \pm 0.0153$ (2)	$0.3295 \pm 0.0153$ (2)
November	10.01	35	13	$0.1690 \pm 0.0348$ (2)	$0.1698 \pm 0.0348$ (2)

$E_a$  is the energy of activation calculated from Figure 1.  $T_{\text{inc}}$  and  $T_{\text{in situ}}$  are the *in vitro* temperature and the environmental *in situ* temperature. ATCase<sub>inc</sub> and ATCase<sub>in situ</sub> are the ATCase activities measured *in vitro* and corrected to *in situ* temperature.

## Results

### Calanus helgolandicus laboratory cultures

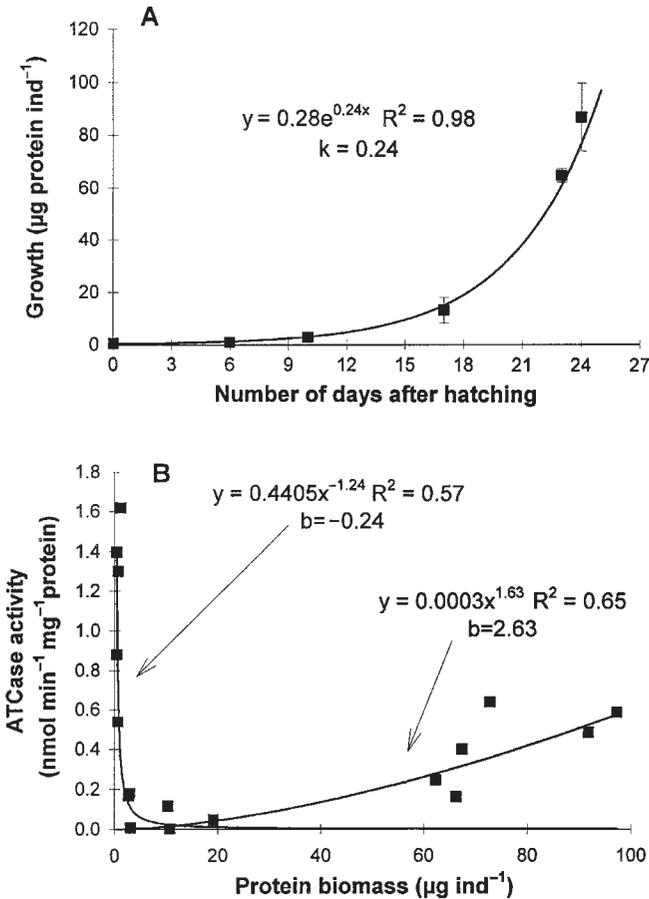
The highest ATCase activity was observed in eggs and nauplius stage IV, and the lowest in younger copepodite stages (Figure 2A). The enzyme activity increased again in copepodite stage V and adult females. Similarly, the highest relative growth rate was observed in nauplius stages and decreased exponentially during the development to reach a minimum value in adult females (Figure 2B). On the other hand, changes in nucleic acid contents and in RNA–DNA did not follow the trend of either the ATCase activity or the relative growth rate (Figure 2C). However, a similar increase in ATCase activity and RNA–DNA ratio was observed in copepodite V and adult females.



**Fig. 2.** Comparison between ATCase activity (A), relative growth rate (B) and nucleic acid concentration and RNA–DNA ratio (C) during the development of the copepod *C. helgolandicus* (N, nauplius; C, copepodite; F, adult female). The different cohorts were reared at 15°C and fed with the dinoflagellate *P. micans*. The relative growth rate is expressed in term of *k*, the coefficient of daily exponential growth calculated from Figure 3A. Error bars are SD, *n* = 3.

Individual protein biomass ( $W_t$ ) increased exponentially during development ( $W_t = 0.28 \times e^{0.24t}$ ,  $R^2 = 0.98$ ) (Figure 3A). Therefore, the population of *C.helgolandicus* grew at a constant rate ( $k = 0.24$ ). The allometric relationship between ATCase activity ( $T$ ) and the protein biomass ( $W$ ) changed during the development of *C.helgolandicus* (Figure 3B). From the egg to the copepodite stage III, this relationship was described by the equation:  $TW^{-1} = 0.44W^{-1.24}$ ,  $R^2 = 0.57$ , with the allometric coefficient  $b = -0.24$ ; and from the copepodite stage III to the adult female by the equation:  $TW^{-1} = 0.0003W^{1.63}$ ,  $R^2 = 0.65$ , with  $b = 2.63$  (Figure 3B).

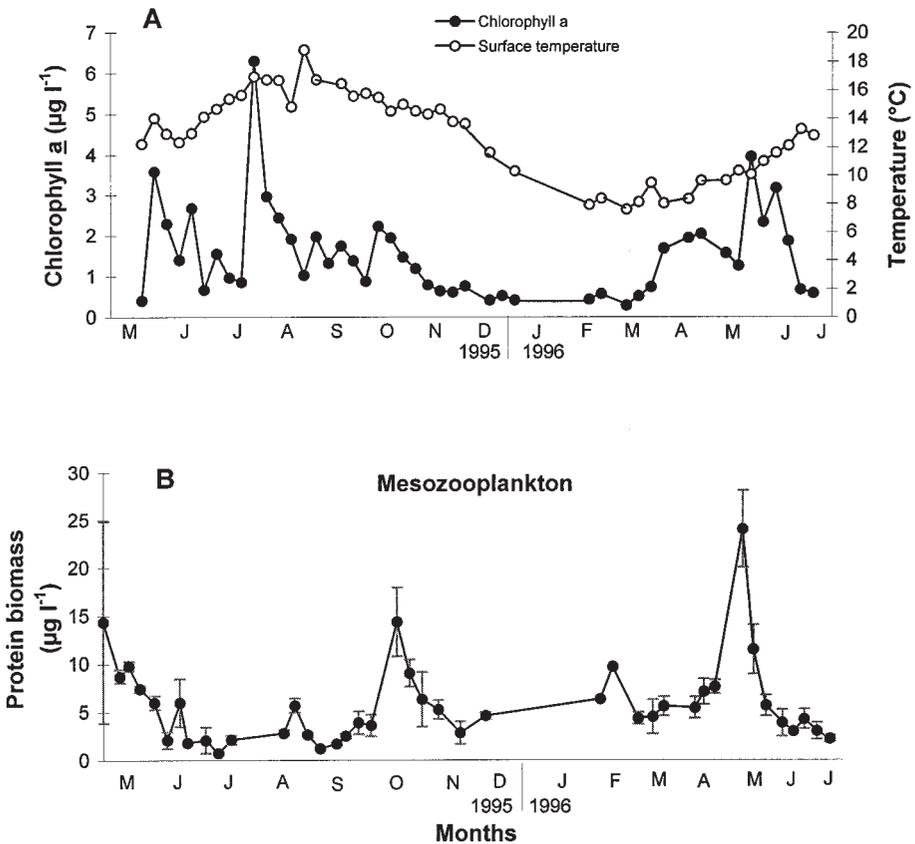
The data on the nauplius stage II are not presented because the protein concentration was not sufficient ( $<2 \text{ mg ml}^{-1}$ ) to rely on ATCase activity data (Biegala and Bergeron, 1998).



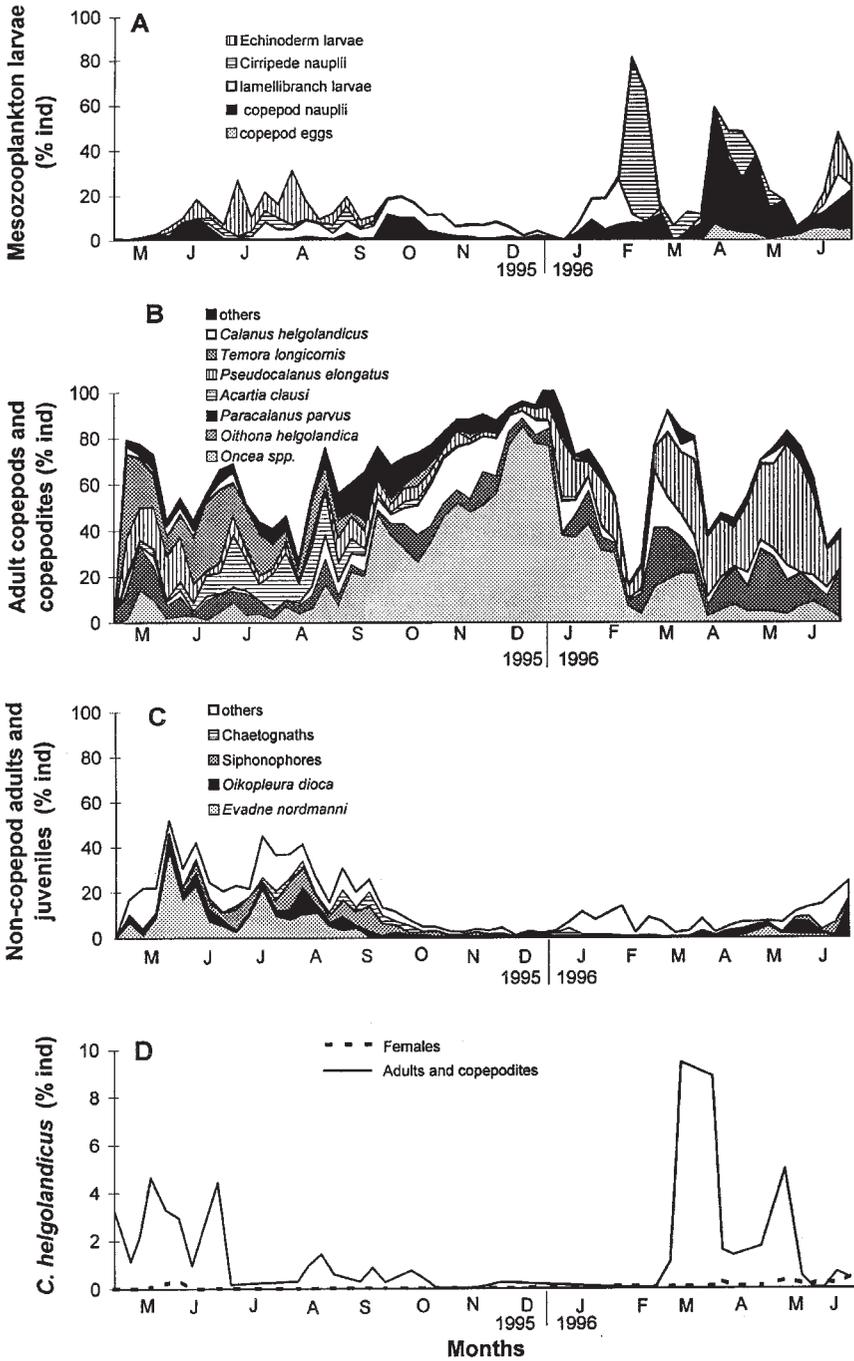
**Fig. 3.** Evolution of exponential protein growth (A) and allometric relationships between ATCase activity and protein biomass (B) during the development of *C.helgolandicus*, reared at 15°C and fed with the dinoflagellate *P.micans*.  $k$  is the coefficient of daily exponential growth calculated from  $W_t = W_0 \times e^{kt}$  (Mullin and Brook, 1965) and  $b$  is the allometric coefficient calculated from  $TW^{-1} = aW^{b-1}$  (Schmidt-Nielsen, 1984) with  $W$  the protein biomass and  $T$  the enzyme activity. Error bars are SD,  $n = 3$ .

Seasonal field studies

Sea surface temperature and the surface chlorophyll *a* concentration typically ranged from 7.5 to 18.5°C and from 0.3 to 6.3 µg l<sup>-1</sup>, respectively (Figure 4A), and the seasonal changes in mesozooplankton biomass were typically characterized by two peaks at the beginning of spring and autumn, respectively (Figure 4B). Throughout the seasonal study, copepodites and adult copepods represented 65% of mesozooplankton, meroplanktonic and holoplanktonic larvae represented 25%, and the adults and juveniles of the other mesozooplankton species (non-copepod) represented 10% (Figure 5). However, these proportions changed with season. In summer, the three mesozooplankton components were well represented (55% adult copepods and copepodites, 30% adult and juvenile non-copepods, 15% larvae). In autumn, the mesozooplankton were dominated (>80%) by copepodites and adult copepods. At the end of the winter and in spring, the zooplankton composition was dominated alternatively by either



**Fig. 4.** Evolution of the surface temperature and phytoplankton biomass, expressed in terms of chlorophyll *a* concentration, at Station L4 (English Channel) (A) and of mesozooplankton biomass (B). Error bars are SD, *n* = 3.



copepodites and adult copepods or larvae (i.e. cirripede nauplii in February and copepod nauplii in April).

During the study period, ATCase activity did not correlate significantly with the abundance of any of the non-copepod species, or with the proportion of the smallest copepod species, such as *Oncea* spp. and *Oithona helgolandica* (Todd *et al.*, 1996), which are very abundant in the mesozooplankton (Table II; Figure 5B). However, ATCase activity was significantly correlated with *C.helgolandicus* adults and copepodites, even though the correlation coefficient was low ( $R^2 = 0.185$ ,  $n = 40$ ,  $P < 0.001$ ) (Table II). In addition, mesozooplankton ATCase activity was significantly correlated with the activity measured in female *C.helgolandicus* ( $R^2 = 0.72$ ,  $n = 33$ ,  $P < 0.001$ ) (Table II; Figure 6A) throughout the seasonal cycle, apart from April. *Calanus helgolandicus* is one of the largest copepod species observed at L4 (~3.5 mm), and even though adult *C.helgolandicus* and copepodites represented up to 10% of mesozooplankton composition, females never exceeded 0.5% (Figure 5D). In addition, the female *C.helgolandicus*

**Table II.** Summary statistics on the relationship between mesozooplankton ATCase activity and environmental, biological and biochemical variables

Variable	$R^2$	$P$	$n$
Temperature	0.264	>0.05	43
Chlorophyll <i>a</i>	0.002	>0.05	43
Mesozooplankton biomass	0.025	>0.05	43
Total adult copepod and copepodites	0.013	>0.05	40
<i>Oncea</i> spp.	0.023	>0.05	40
<i>Oithona helgolandica</i>	0.015	>0.05	40
<i>Paracalanus parvus</i>	0.023	>0.05	40
<i>Acartia clausi</i>	0.000	>0.05	40
<i>Pseudocalanus elongatus</i>	0.013	>0.05	40
<i>Temora longicornis</i>	0.032	>0.05	40
<i>Calanus helgolandicus</i>	0.186	<0.001	40
Total adult and juveniles non-copepod	0.013	>0.05	40
<i>Evadne nordmanni</i>	0.051	>0.05	40
<i>Oikopleura dioica</i>	0.011	>0.05	40
Siphonophores	0.010	>0.05	40
Chaetognaths	0.000	>0.05	40
Total larvae	0.088	>0.05	40
Lamellibranch larvae	0.002	>0.05	40
Cirriped nauplii	0.021	>0.05	40
Echinoderm larvae	0.002	>0.05	40
Copepod eggs	0.011	>0.05	40
Copepod nauplii	0.091	>0.05	40
Female <i>C.helgolandicus</i> ATCase activity	0.72	<0.001	33

**Fig. 5.** Comparison of the proportion of the different species and stages within the total mesozooplankton at Station L4 (English Channel). (A) Meroplankton and holoplankton larvae. (B) Adult copepods and copepodites. (C) Adults and juveniles of the species other than copepods. (D) A detailed representation of adult females and pooled adults and copepodites of *C.helgolandicus*. Others represent species which were <2% of the total mesozooplankton abundance.

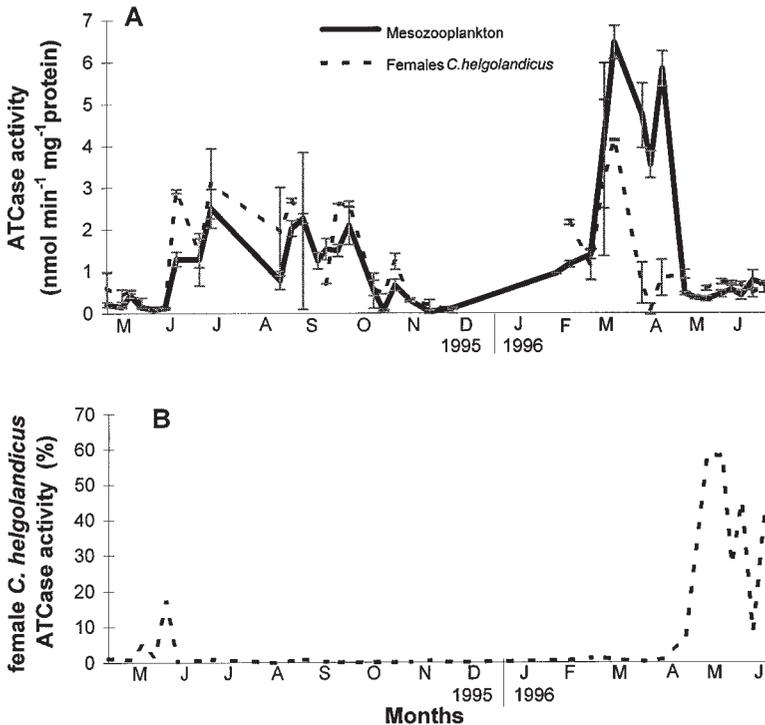
ATCase activity related to that of the mesozooplankton was lower than 5% during most of the year and was up to 20–60% in spring periods (Figure 6B). In April, the peak observed in mesozooplankton ATCase activity was absent in female *C.helgolandicus*, but was in phase with the peaks of the copepod eggs and the nauplii (Figures 5A and 6A).

**Discussion**

*Calanus helgolandicus* development

The highest ATCase activity was observed in *C.helgolandicus* eggs and naupliar stages, and the lowest in young copepodites. Enzyme activity increased then in pre-adult and adult stages (Figure 2A). This pattern of ATCase activity seems to be common to many organisms, since similar observations have been reported in animals as different as the rat (Nordman *et al.*, 1964; Weichsel *et al.*, 1972), the sea bass *Dicentrarchus* spp. (Bergeron, 1982), *Drosophila melanogaster* (Brothers *et al.*, 1978) and *Artemia salina* (Alayse-Danet, 1980).

Changes in ATCase activity were similar to those observed for *C.helgolandicus* relative growth rate, at least during the first 17 days of development (Figure



**Fig. 6.** Comparison of the mesozooplankton ATCase activity at Station L4 (English Channel) (A) with ATCase activity in female *C.helgolandicus* and (B) with the proportion of mesozooplankton ATCase activity due to female *C.helgolandicus*. Error bars are SD, *n* = 3 (mesozooplankton), *n* = 2 (female *C.helgolandicus*).

2B). These observations are similar to those reported by Alayse-Danet (1980) and Bergeron (1982), who both found a significant correlation between ATCase activity and the relative growth rate of *Artemia salina* and fish larvae. However, Hernández-León *et al.* (1995) did not observe such a correlation using *Acartia clausi* copepodite stages, reared at different food concentration. These authors suggested that the samples might have been taken at different stages of the copepodites' moult cycle. We propose an alternative hypothesis to explain the lack of correlation observed. The uncoupling between ATCase activity and the copepodites' relative growth rate could be due to the presence of a pyrimidine nucleotide biosynthetic pathway other than the *de novo* pathway, in which ATCase is involved. The presence of a second pathway for pyrimidine nucleotides biosynthesis has already been suggested by Biegala *et al.* (1999) as an explanation for the lack of correlation between ATCase activity and egg production in female *C.helgolandicus*. Prokaryotes and eukaryotes can synthesize purine and pyrimidine nucleotides through both a *de novo* and a salvage pathway, which use pre-existing pyrimidine bases and nucleotides directly provided by the diet and nucleic acid catabolism (Weichsel *et al.*, 1972; Ryszard *et al.*, 1988; Iriberry *et al.*, 1990; Zubay *et al.*, 1995). Even though the presence of the salvage pathway has not been demonstrated in copepods, it has been described in *Artemia salina*, another planktonic crustacean (Emerson, 1964). This author showed that [ $H^3$ ]thymidine, a pyrimidine nucleotide, was incorporated into the DNA of *Artemia salina* developmental stages that could feed from an exogenous food source. On the other hand, this radiochemical was not incorporated into the eggs or the first developmental stages, which use yolk reserves as a source of energy and metabolites. In addition, Hartenstein (1970) showed that bicarbonate, [ $^{14}C$ ]HCO $_3^-$ , was incorporated in pyrimidine nucleotides during the entire development of *Artemia salina*. Bicarbonate is the precursor of carbamyl phosphate, one of the ATCase substrates (Zubay *et al.*, 1995). As changes in ATCase activity during *Artemia salina* development were similar to those observed in *C.helgolandicus*, it is probable that (i) the *de novo* biosynthetic pathway is the only source of pyrimidine nucleotide during embryogenesis and (ii) both the *de novo* and the salvage pathways are involved in the pyrimidine nucleotide biosynthesis as soon as nauplii start feeding.

The presence of such a salvage pathway could also partially explain the lack of covariance between ATCase activity and nucleic acid concentrations, especially in copepodite stages (Figure 2C). In addition, nucleic acids might accumulate in those stages, when ATCase is probably quickly renewed. Ribosomal RNAs, which constitute the bulk of cellular RNA, have a turnover of ~48 h (Zubay *et al.*, 1995), while ATCase has a turnover of ~3 h in *Bacillus subtilis* (Bond *et al.*, 1983).

#### *Inter- and intraspecific size scaling relationships*

In this study, *C.helgolandicus* growth in controlled conditions was exponential and thus the growth rate was constant (Figure 3A). This is a necessary condition to study the effect of body mass on ATCase activity and distinguish it from the effect

of growth rate, since growth rate has been shown to scale the body mass of many organisms (Von Bertalanffy, 1957). The relationship between ATCase activity and protein biomass was described by two allometric relationships (Figure 3B). These relationships were characterized by allometric coefficients ( $b$ ) different from those that have been previously observed with other enzyme activities in small crustaceans (i.e.  $0.5 < b < 1$ ; Berges *et al.*, 1990; Berges and Ballantyne, 1991). During the first 17 days of development, the allometric coefficient was slightly negative and close to zero, which means that the ATCase activity per individual is nearly constant and independent of changes in biomass. On the other hand, during the last week of *C.helgolandicus* development, ATCase activity increased with a much higher rate than for protein biomass. A similar anomaly, with regard to allometric relationships between organism body mass and metabolism, has already been mentioned by Somero and Childress (1980). These authors showed that, in fish muscle, glycolytic enzyme activities (i.e. lactate dehydrogenase and pyruvate kinase) increased much more rapidly than the organism's body size. These authors explained this observation by the adaptation of anaerobic metabolism to strength requirements for burst-swimming behaviour in front of predators. Concerning this study, it is possible that the increase in ATCase activity in copepodite V and adult is due to an important anabolic activity related to oogenesis, as the gonads can already be well identified in copepodite V. A similar hypothesis has already been mentioned by Biegala *et al.* (1999) in relation to the increase in ATCase activity during gonad maturation in female *C.helgolandicus*.

Therefore, it does not seem that, among stages, changes in ATCase activity can be explained by body mass scaling relationships. This observation is in agreement with Biegala *et al.* (1999), who showed that the activity of this enzyme was not related to changes in protein body mass of female *C.helgolandicus*. On the other hand, many enzyme activities have been shown to scale with respect to body size among similar stages of different crustaceans (Berges and Ballantyne, 1991). Thus, even though body size scaling relationships were not examined similarly in this study, a general comparison between changes in mesozooplankton ATCase activity and the size of the species composition should indicate the presence of an obvious body size scaling relationship. According to Todd *et al.* (1996), adult stages of mesozooplankton found around the British Isles range from 0.5 to 23 mm in length, and can be classified according to their size in the following order: *Oncea* spp. < *Oithona helgolandica* < *Paracalanus parvus* < *Acartia clausi* < *Pseudocalanus elongatus* < *Temora longicornis* < *Calanus helgolandicus* < siphonophores < *Oikopleura dioica* < chaetognaths. At L4, ATCase activity was very low in autumn and winter, when mesozooplankton were largely dominated by the smallest holoplanktonic species *Oncea* spp. (Figures 5 and 6). Therefore, in this study, it has not been demonstrated that ATCase activity was influenced by species body mass.

#### *Mesozooplankton stage and species composition*

During most of the seasonal study at L4, mesozooplankton ATCase activity was significantly correlated with female *C.helgolandicus* ATCase activity (Figure 6).

This correlation is surprising because female *C.helgolandicus* represented <0.5% of mesozooplankton individuals. In addition, the percentage of mesozooplankton ATCase activity due to female *C.helgolandicus* was <5% for most of the year, apart from May 1996, when it represented 60%. It is, therefore, possible that other species or developmental stages underwent the same seasonal changes in ATCase activity as those observed in female *C.helgolandicus*.

Among all the mesozooplankton species present at Station L4, mesozooplankton ATCase activity was significantly correlated with the changes in percentage of adults and copepodites of *C.helgolandicus*. However, the correlation coefficient is very low and the significance of this correlation seems to be mainly explained by the fact that in March the peak of mesozooplankton ATCase activity is in phase with the peak of abundance of *C.helgolandicus* adults and copepodites. The peaks of adults and copepodites of *C.helgolandicus* represent changes in copepodite abundance, as the percentage of female *C.helgolandicus* is <0.5 throughout the year and the males are usually three times less abundant than the females at L4 (Pond *et al.*, 1996). In addition, it is quite probable that in May and June *C.helgolandicus* copepodites are dominated by stages I–IV, and that in March and summer they are dominated by stage V. The seasonal development cycle of *Calanus* in temperate waters, and more precisely of *C.helgolandicus* in coastal waters off Plymouth, is well known (Marshall and Orr, 1952; Mullin and Brooks, 1967; Grigg and Bardwell, 1982; Tande, 1982; Conover, 1988; Green *et al.*, 1993). After the summer, the lipid-rich *C.helgolandicus* population overwinter, as copepodite V. In March, at the end of the winter, the copepodite V realize their terminal moult and gametogenesis, fuelled by lipids stored during the previous summer (Gatten *et al.*, 1979; Sargent and Falk-Petersen, 1988). The newly moulted females lay a large quantity of eggs in April and May during the phytoplanktonic bloom (Bautista *et al.*, 1994; Pond *et al.*, 1996; Biegala *et al.*, 1999). It then takes <3 weeks for the eggs to develop into young copepodites, which dominate the population in May and June. At the beginning of the summer, copepodites V appear in large quantities, some will then overwinter, whereas others will moult to adults, mainly females. On the other hand, when ATCase activity was measured in different development stages of *C.helgolandicus*, the lowest values were found in young copepodites and the activity of this enzyme rose in copepodite V and adult females. Therefore, it is possible that seasonal changes in mesozooplankton ATCase activity partially correlate with the proportion of *C.helgolandicus* copepodite V in mesozooplankton. This hypothesis is in accordance with the observed correlation between mesozooplankton and female *C.helgolandicus* ATCase activities. Biegala *et al.* (1999) explained the peaks of female *C.helgolandicus* in March and summer by the involvement of ATCase activity in germinal and somatic tissue synthesis during female copepod post-ecdysis. A high proportion of newly moulted females appear in the environment when the population is dominated by pre-adults (i.e. copepodite V).

In April, high values of mesozooplankton ATCase activity were observed, whereas this was not the case in female *C.helgolandicus* and a very low percentage

of copepodites was recorded. However, this peak of mesozooplankton ATCase activity is in phase with the peak of copepod eggs and nauplii in the mesozooplankton. This observation is in accordance with the high ATCase activity measured in eggs and nauplii during the development of *C.helgolandicus* under controlled conditions. It could then be hypothesized that the activity of this enzyme is particularly high during embryogenesis.

In conclusion, this study shows good evidence that the seasonal peaks in mesozooplankton ATCase activity reflect the presence of a high proportion of copepods undergoing embryogenesis and copepodites which are about to undergo or which have finished their terminal moult. This hypothesis should, however, be checked by measuring both the *de novo* synthesis in which ATCase is involved and the salvage pyrimidine nucleotide pathways during the development of several copepod species. Such experiments should be carried out after improvement of enzymatic assays, such as the development of enzyme immunoquantification (Orellana and Perry, 1992; Vrieling and Anderson, 1996) which will allow both a direct measurement of enzyme concentration and an increase in sensitivity. This method should overcome the problems of assessing enzyme concentration via enzyme maximal activity and the use of a large quantity of small-stage organisms (i.e. 2000 eggs and nauplii) to assess ATCase concentration.

Such investigations need to be encouraged and developed to provide a group of molecular biomarkers that could be used in field studies in order to characterize some particular metabolic events within the life cycle of mesozooplankton species.

### Acknowledgements

We especially thank the crew of the RV 'Sepia' and RV 'Squilla' for collecting the samples, R.Head for analysing chlorophyll *a*, and Dr S.A.Poulet and Dr J.-P.Bergeron for helpful advice and comments. Thank also to Dr S.K.Malham for English corrections. This research was partially supported by a European Human Capital and Mobility Programme Fellowship (N°ERB4001GT940181), the IFREMER grant (N°96064) and the Ministry of Agriculture Fisheries and Food (MAFF).

### References

- Alayse-Danet,A.M. (1980) Aspartate transcarbamylase in *Artemia salina* during early stages of development. In Persoone,G., Sorgeloos,P., Roels,O. and Jaspers,E. (eds), *The Brine Shrimp Artemia. Vol. 2. Physiology, Biochemistry, Molecular Biology*. Universa, Belgium, pp. 259-275.
- Banse,K. (1995) Zooplankton pivotal role in the control of ocean production. *ICES J. Mar. Sci.*, **52**, 265-277.
- Bautista,B., Harris,R.P., Rodriguez,V. and Guerrero,F. (1994) Temporal variability in copepod fecundity during two different spring bloom periods in coastal waters off Plymouth (SW England). *J. Plankton. Res.*, **16**, 1367-1377.
- Bergeron,J.-P. (1982) L'aspartate transcarbamylase, indice de croissance des organismes marins: perspectives et limites. *Publ. CNEXO (Actes et Colloq.)*, **14**, 177-192.
- Bergeron,J.-P. (1983) Approche systémique des potentialités de production secondaire pélagique: l'évolution printanière en province néritique. *Oceanol. Acta*, **No. Spec.**, 15-19.
- Bergeron,J.-P. (1986) Approche systémique des potentialités de production secondaire pélagique: inférences de fluctuations interannuelles. *Oceanol. Acta*, **9**, 291-303.

- Bergeron, J.-P. (1990) Activité de l'aspartate transcarbamylase et variations temporelles de biomasses des systèmes mésozooplanctoniques en provinces néritiques tempérées. *CR Acad. Sci. Paris*, **310**, 495–502.
- Bergeron, J.-P. (1995) Aspartate transcarbamylase activity for the assessment of mesozooplankton production: new aspect from oceanic areas. *ICES J. Mar. Sci.*, **52**, 305–313.
- Bergeron, J.P. and Buestel, D. (1979) L'aspartate transcarbamylase, indice de l'activité sexuelle de la coquille Saint-Jacques (*Pecten maximus* L.). Premiers résultats. In Naylor, E. and Hartnoll, R.G. (eds), *Cyclic Phenomena in Marine Plants and Animals*. Pergamon Press, Oxford, pp. 301–308.
- Berges, J.A. and Ballantyne, J.S. (1991) Size scaling of whole-body maximal enzyme activities in aquatic crustaceans. *Can. J. Fish. Aquat. Sci.*, **48**, 2385–2394.
- Berges, J.A., Roff, J.C. and Ballantyne, J.S. (1990) Relationship between body size, growth rate, and maximal enzyme activities in the brine shrimp, *Artemia franciscana*. *Biol. Bull.*, **179**, 287–296.
- Biegala, I.C. (1998) Zooplankton productivity in marine ecosystems: biochemical approach (ATCase). Thesis, University of Aix-Marseille II, France, 147 pp.
- Biegala, I.C. and Bergeron, J.-P. (1998) Optimal assay conditions for aspartate transcarbamylase (ATCase) activity in mesozooplankton. *J. Plankton Res.*, **20**, 1205–1218.
- Biegala, I.C., Harris, R.P. and Bergeron, J.-P. (1999) ATCase activity, nucleic acid content and egg production in the copepod *Calanus helgolandicus*. *Mar. Biol.*, in press.
- Bond, R.W., Schmulbach Fiel, A. and Switzer, R.L. (1983) Nutritional regulation of degradation of aspartate transcarbamylase and of bulk protein in exponentially growing *Bacillus subtilis* cells. *J. Bacteriol.*, **153**, 253–258.
- Bougis, P. (1974) *Ecologie du plankton marin*. Masson et Cie, Paris, Vol. II, 190 pp.
- Bresnick, E. and Mossé, H. (1966) Aspartate carbamoyl transferase from rat liver. *Biochem. J.*, **101**, 63–69.
- Brothers, V.M., Tsubota, S.I., Germeraad, S.E. and Friston, J.W. (1978) Rudimentary locus of *Drosophila melanogaster*: partial purification of a carbamylphosphate synthetase-aspartate transcarbamylase-dihydroorotase complex. *Biochem. Genet.*, **16**, 321–331.
- Calva, E., Lowenstein, J.M. and Cohen, Ph.P. (1959) Carbamyl phosphate-aspartate transcarbamylase activity in tumors. *Cancer Res.*, **19**, 101–103.
- Conover, R.J. (1988) Comparative life histories in the genera *Calanus* and *Neocalanus* in high latitude of the northern hemisphere. *Hydrobiologia*, **167/168**, 127–142.
- Emerson, D.N. (1964) Incorporation of tritiated thymidine by *Artemia salina*. *Proc. S. D. Acad. Sci.*, **XLIII**, 90–93.
- Espie, P.J. and Roff, J.C. (1995a) Characterisation of chitobiase from *Daphnia magna* and its relation to chitin flux. *Physiol. Zool.*, **68**, 727–748.
- Espie, P.J. and Roff, J.C. (1995b) A biochemical index of duration of the molt cycle for planktonic crustacea based on the chitin-degrading enzyme, chitobiase. *Limnol. Oceanogr.*, **40**, 1028–1034.
- Gatten, R.P., Corner, E.D.S., Kilvington, C.C. and Sargent, J.R. (1979) A seasonal survey of the lipids in *Calanus helgolandicus* Claus from the English Channel. In Naylor, E. and Hartnoll, R.G. (eds), *Cyclic Phenomena in Marine Plants and Animals. Proceedings of the 13th European Marine Biology Symposium*. Pergamon Press, Oxford, pp. 275–284.
- Green, E.P., Harris, R.P. and Duncan, A. (1993) The seasonal abundance of the copepodite stages of *Calanus helgolandicus* and *Pseudocalanus elongatus* off Plymouth. *J. Mar. Biol. Assoc. UK*, **73**, 109–122.
- Grigg, H. and Bardwell, S.J. (1982) The seasonal observations on moulting and maturation in stage V copepodites of *Calanus finmarchicus* from the Firth of Clyde. *J. Mar. Biol. Assoc. UK*, **62**, 315–327.
- Hartenstein, R. (1970) Nitrogen metabolism in non-insect arthropods. In Campbell, J.W. (ed.), *Comparative Biochemistry of Nitrogen Metabolism*. Academic Press, London, pp. 299–492.
- Hernández-León, S., Almeida, C. and Montero, I. (1995) The use of aspartate transcarbamylase activity to estimate growth rates in zooplankton. *ICES J. Mar. Sci.*, **52**, 377–383.
- Hertzfeld, A.M. and Knox, W.E. (1972) Aspartate transcarbamylase concentrations in relation to growth rates of fetal, adult and neoplastic rat tissue. *Cancer Res.*, **22**, 1246–1251.
- Iriberrí, J., Unanue, M., Ayo, B., Barcina, I. and Egea, L. (1990) Bacterial production and growth rate estimation from [<sup>3</sup>H]thymidine incorporation for attached and free-living bacteria in aquatic systems. *Appl. Environ. Microbiol.*, **56**, 483–487.
- Jones, M.E. (1980) Pyrimidine nucleotide biosynthesis in animals: genes, enzymes, and regulation of UMP biosynthesis. *Ann. Rev. Biochem.*, **49**, 253–279.
- Karsten, U. and Wollenberger, A. (1972) Determination of DNA and RNA in homogenized cells and tissues by surface fluorometry. *Anal. Biochem.*, **46**, 135–145.
- Karsten, U. and Wollenberger, A. (1977) Improvements in the ethidium bromide method for direct

- fluorometric estimation of DNA and RNA in cell and tissue homogenates. *Anal. Biochem.*, **77**, 464–470.
- Kim,S. and Cohen,P.P. (1965) Transcarbamyase activity in fetal liver of partially hepatectomised parabiotic rats. *Arch. Biochem. Biophys.*, **109**, 421–428.
- Kimmerer,W.J. and McKinnon,A.D. (1987) Growth, mortality, and secondary production of the copepod *Acartia tranteri* in westernport Bay, Australia. *Limnol. Oceanogr.*, **32**, 14–28.
- Lowry,O.N., Roseborough,N.J., Farr,A.L. and Randall,R.J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.
- Markwell,M.-A.K., Haas,S.M., Bieber,L.L. and Tolbert,M.E. (1978) A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Ann. Biochem.*, **87**, 206–210.
- Marshall,S.M and Orr,A.P. (eds) (1972) *The Biology of Marine Copepods*. Springer-Verlag. Oliver and Boyd, Edinburgh.
- Mayzaud,P. (1986) Enzymatic measurement of metabolic processes concerned with respiration and ammonia excretion. In Corner,E.D.S. and O'Hara,S.C.M. (eds), *The Biological Chemistry of Marine Copepods*. Oxford Science, UK, pp. 226–259.
- Mullin,M.M. and Brooks,E.R. (1967) Laboratory culture, growth rate, and feeding behavior of a planktonic marine copepod. *Limnol. Oceanogr.*, **12**, 657–666.
- Nordman,Y., Hurwitz,R. and Kretchmer,N. (1964) Activity of aspartate transcarbamyase in heart and liver of the developing rat. *Nature*, **201**, 616–617.
- Orellana,M.V. and Perry,M.J. (1992) An immunoprobe to measure Rubisco concentrations and maximal photosynthetic rates of individual phytoplankton cells. *Limnol. Oceanogr.*, **37**, 478–490.
- Owens,T.G and King,F.D. (1975) The measurement of respiratory electron-transport-system activity in marine zooplankton. *Mar. Biol.*, **30**, 27–36.
- Patnaik,S.K and Patnaik,R. (1990) Tissue-specific differential induction of aspartate transcarbamyase by hydrocortisone in rats of various ages. *Biochem. Int.*, **20**, 641–647.
- Pond,D.W., Harris,R.P., Head,R.N. and Harbour,D. (1996) Environmental and nutritional factors determining the seasonal variability in the fecundity and egg viability of *Calanus helgolandicus* in coastal waters off Plymouth, U.K. *Mar. Ecol. Prog. Ser.*, **143**, 45–63.
- Roemmich,D. and McGowan,J. (1995) Climatic warming and the decline of zooplankton in the California Current. *Science*, **267**, 1324–1326.
- Ryszard,J.C., Overbeck,J. and Weislo,R. (1988) Evaluation of the [<sup>3</sup>H]thymidine method for estimating bacterial growth rates and production in lake water: re-examination and methodological comments. *Acta Microbiol. Pol.*, **37**, 95–112.
- Sapienza,C. and Mague,T.H. (1979) DNA polymerase activity and growth rate in *Artemia salina* nauplii. *Limnol. Oceanogr.*, **24**, 572–576.
- Sargent,J.R. and Falk-Petersen,S. (1988) The lipid biochemistry of calanoid copepods. *Hydrobiologia*, **167/168**, 101–114.
- Schmidt-Nielsen,K. (ed.) (1984) *Scaling: Why is Animal Size so Important?* Cambridge University Press, Cambridge.
- Sekiguchi,H., McLaren,I.A. and Corkett, C.J. (1980) Relationship between growth rate and egg production in the copepod *Acartia clausi hudsonica*. *Mar. Biol.*, **58**, 133–138.
- Shushkina,E.A. (1968) Calculation of copepod production based on metabolic features and the coefficient of the utilisation of assimilated food for growth. *Oceanology*, **8**, 98–110.
- Somero,G.N. and Childress,J.J. (1980) A violation of the metabolism-size scaling paradigm: activities of glycolytic enzymes in muscle increase in large-size fish. *Physiol. Zool.*, **53**, 322–337.
- Stein,L.I. and Cohen,Ph.P. (1965) Correlation of growth and aspartate transcarbamyase activity in higher plants. *Arch. Biochem. Biophys.*, **109**, 429–433.
- Sutcliffe,W.H. (1965) Growth estimates from ribonucleic acid content in some small organisms. *Limnol. Oceanogr.*, **10(Suppl.)**, R-253–R-258.
- Tande,K.S. (1982) Ecological investigation on the zooplankton community of Balsjorden, Norway: generation cycle, and variations in body weight and body content of carbon and nitrogen related to overwintering and reproduction in the copepod *Calanus finmarchicus* (Gunnerus). *J. Exp. Mar. Ecol.*, **62**, 129–142.
- Todd,G.D., Laverack,M.S. and Boxshall,G.A. (eds) (1996) *Coastal Marine Zooplankton: A Practical Manual for Students*, 2nd edn. Cambridge University Press, New York.
- Von Bertalanffy,L. (1957) Quantitative laws in metabolism and growth. *Q. Rev. Biol.*, **32**, 217–231.
- Vrieling,E.G. and Anderson,D.M. (1996) Immunofluorescence in phytoplankton research: applications and potential. *J. Phycol.*, **32**, 1–16.
- Weichsel,M.E., Hoogenraad,J.C., Levine,R.L. and Kretmer,N. (1972) Pyrimidine biosynthesis during development of rat cerebellum. *Pediatr. Res.*, **6**, 682–686.

- Yentsch, C.S and Menzel, D.W. (1963) A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. *Deep-Sea Res.*, **10**, 221–231.
- Young, J.E., Prager, M.D. and Atkins, I.C. (1967) Comparative activities of aspartate transcarbamylase in various tissues of the rat. *Proc. Soc. Exp. Biol. Med.*, **125**, 860–862.
- Zubay, G.L., Parson W.W. and Vance D.E. (eds) (1995) *Principles of Biochemistry*. Brown WMC Press, Dubuque.

*Received on April 12, 1999; accepted on June 17, 1999*

