

Acrocalanus gracilis (Copepoda: Calanoida) development and production in the Timor Sea

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Received October 8, 2008; accepted in principle May 24, 2009; accepted for publication May 27, 2009; published online 26 June, 2009

Corresponding editor: Mark J. Gibbons

*We investigated the juvenile development of the calanoid copepod Acrocalanus gracilis in the Timor Sea. The development was characterized by the calculation of stage-specific duration, median development time, moulting period duration, moult and growth rates. The morphometry of the experimental and wild populations were compared. Food concentrations in the experiment were lower than those in the field, and resulted in smaller animals than those in the field. Somatic growth (0.44 day^{-1}) was log-linear throughout the development, but moulting rates slowed with time. The development of *A. gracilis* was similar to other paracalanids, and conforms to the equiproportional model. Trophic resources seem to play an important role in controlling the developmental features and morphometry of Acrocalanus in tropical environments.*

INTRODUCTION

Among the many zooplankton taxa in the oceans, copepods are the most abundant and ubiquitous group, accounting for up to 80% of the ocean's mesozooplankton biomass (Verity and Smetacek, 1996) and linking pelagic microbial processes to higher trophic levels (Kiørboe, 1997). They are important in the ocean's biogeochemical cycles, playing a key role in nutrient regeneration and in the flow of matter and energy. Only by quantifying these fluxes can the complex dynamics of pelagic marine systems be unwoven (Banse, 1995). Studies on copepod development and growth rates (and ultimately, their productivity) are amongst some of the best ways to quantify and understand these fluxes, and therefore have become more common in recent marine zooplankton research (Poulet *et al.*, 1995; Runge and Roff, 2000). Moreover, in order to fully understand the role of copepods in structuring pelagic food webs, it is necessary to understand their biology and life history traits (Kiørboe, 1997, 2006).

In this study, we investigated the juvenile development of the small paracalanid, *Acrocalanus gracilis*. The Paracalanidae are represented by the genera *Paracalanus*,

Parvocalanus, *Bestiolina*, *Calocalanus*, *Delius* and *Acrocalanus* (Boxshall and Halsey, 2004), and are often the most common copepods in tropical and subtropical coastal waters (Milstein, 1979; McKinnon and Thorrold, 1993; McKinnon, 1996; McKinnon and Ayukai, 1996; Hopcroft *et al.*, 1998; Araujo, 2006). However, their biology is poorly known in comparison with families common in temperate seas. In order to address this issue, we investigated the juvenile development and growth of a population of *Acrocalanus gracilis* in the Timor Sea in June 2005. Stage and moulting duration, growth and moult rates and development time were estimated, and the morphometry of animals of an experimentally incubated population compared to those in the field.

METHOD

Study site

This study was conducted during a cruise of the R.V. *Southern Surveyor* in the Timor Sea in June 2005, at a

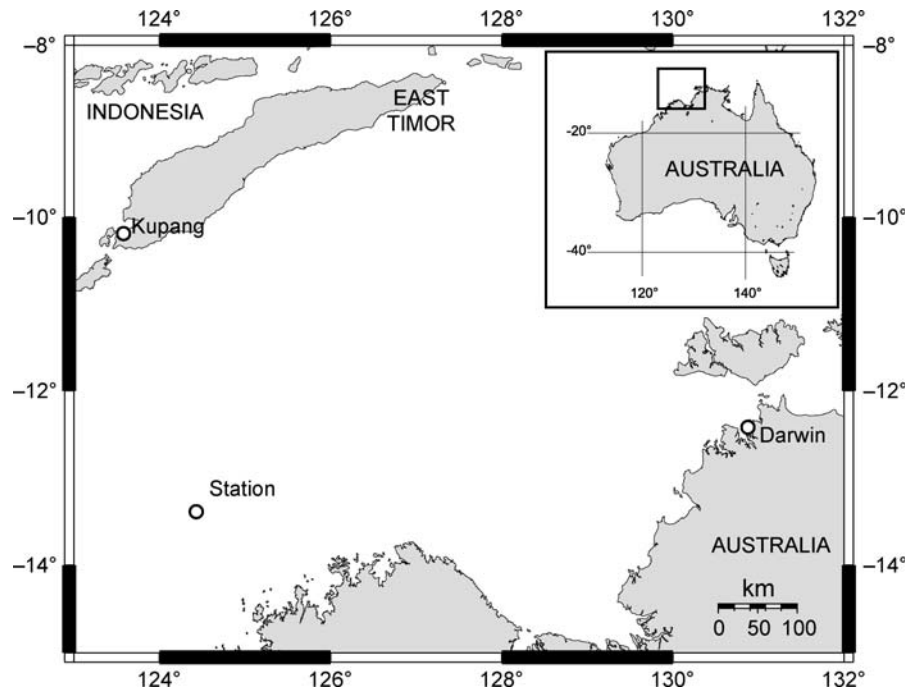


Fig. 1. Location of the sampling station in the Timor Sea.

mid-shelf station of 90 m depth (Fig. 1). Prior to the zooplankton sampling, we made a CTD cast (Seabird SBE911 equipped with a Chelsea chlorophyll fluorometer) to describe field conditions of temperature and fluorescence.

Zooplankton sampling

Zooplankton for abundance estimation was sampled with a 150 μm net, 50 cm diameter, with an electronic flowmeter (Hydrobios) mounted in its mouth. Immediately after sampling, the sample was preserved in formalin for subsequent description of the *Acrocalanus gracilis* population.

For the onboard experiment, live zooplankton was sampled using a 73 μm mesh WP2 net with a non-filtering cod end. The contents of the cod end were gently emptied into a bucket filled with surface seawater and closed with a lid to avoid strong temperature variation and direct sunlight. The live zooplankton was processed in a controlled temperature room set at the same temperature as in the field (26–28°C).

Juvenile development experiment

Juvenile development of *Acrocalanus gracilis* was studied following the development of a cohort hatched from eggs (McKinnon, 1996). Intact and active females of *A. gracilis* were selected with a pipette and transferred to a

1 L beaker containing 37 μm filtered surface seawater, in which all copepodites and nauplii had been removed but in which food particles <37 μm were retained. After isolation of several *A. gracilis* females, the beaker with the animals was gently poured inside a PVC cylinder (~30 cm aperture and ~50 cm height) with a 73 μm mesh screen on the bottom (a reverse concentrator, Kimmerer and McKinnon, 1987) that was immersed in a bucket filled with 37 μm filtered surface seawater. The start time of the experiment ($t = 0$) used for the calculation of the growth rate and development indices (see below) was the time when the first egg was laid by these females, considered to have occurred just after the females were transferred to the reverse concentrator. The females were left in the reverse concentrator to produce eggs for the next 24 h.

The next day, three 10 L flexible wall cubic containers were filled with 37 μm reverse-filtered surface seawater. The cohort was then isolated from the adults by gently removing the reverse concentrator containing the female *Acrocalanus gracilis* sorted on the previous day, and allowing only the eggs and nauplii to pass through the 73 μm mesh. The animals remaining in the bucket therefore comprised individuals as old as 24 h. Equal volume aliquots of the cohort were gently poured into each cubic container, and three aliquots were preserved in formalin, representing the “day 1” sample. The containers were then tightly closed and immersed in deck incubators with surface seawater constantly flowing

through, and covered with a neutral density mesh that filtered 70% of the incident light. Temperature was constantly monitored.

The development of the cohort was followed for the next 12 days. The cohort was sampled on days 1, 3, 5, 7, 9, 10 and 11 and the experiment was terminated on day 12 by harvesting the entire content of the containers. Each cubic container was sampled by carefully filtering one-fourth to one-third of its contents through a 37 μm mesh, which was then rinsed into a scintillation vial and preserved with buffered formalin. During each sampling, three 100 mL aliquots of water from the containers were filtered through GF/F filters and frozen (-10°C) for later chlorophyll *a* analysis by fluorometry after extraction in 90% acetone (Parsons *et al.*, 1984). After each harvest, the containers were topped up with 37 μm filtered surface seawater.

Laboratory analysis

The 150 μm net sample was analysed for the estimation of abundance and stage composition of *Acrocalanus* in the field. Aliquots from the sample were taken with a Stempel pipette and all *A. gracilis* from copepodite (hereafter C) stage 4 (C4) to adult were counted, staged, sexed and retained for morphometric analysis. Due to the high abundance and diversity of paracalanids in the Timor Sea, distinguishing single species at stages younger than C4 proved to be impractical. In the samples taken from the juvenile development experiment, all individuals present in each sample were counted, staged, sexed and measured (see below). Intersex individuals were observed in both experimental and field samples, and were treated independently of the other sexes.

Morphometry

Acrocalanus gracilis developmental stages were photographed with a high definition digital camera fitted to a Zeiss AxioVision Microscope. These images were then analysed using ImageJ open source software (Rasband, 2007) in a procedure modified from that of Alcaraz *et al.* (Alcaraz *et al.*, 2003). In our study, each area to be measured was selected by hand using the polygon selection tool. For each individual, a polygon was drawn to describe the region to be measured (the whole body in eggs and nauplii, and the prosome in copepodites and adults). Then, an ellipse of similar area was fitted to the polygon and its major and minor axes determined. For eggs and nauplii, the measurements represented the length and width, and for copepodites and adults the prosome length and width. The biovolume of each individual was then calculated from these axes values

assuming that the whole body or prosome shape is equivalent to an ellipsoid (Alcaraz *et al.*, 2003) using the equation:

$$v = \frac{\pi \times A \times a^2}{6} \quad (1)$$

where v is the biovolume, A the major axis and a the minor axis values.

We also directly measured the length of the fifth leg of males and intersexes.

Juvenile growth

The mean individual biomass in each sample was calculated as:

$$\bar{w}_T = \frac{\sum_{i=1}^n (\bar{w}_i \times D_i)}{\sum_{i=1}^n D_i} \quad (2)$$

where \bar{w}_T is the mean individual biomass in a sample taken at time T , \bar{w}_i the mean individual biomass for stage i , D_i the abundance of stage i and n the total number of stages in the sample. The mean stage-specific carbon content for *Acrocalanus gibber* from McKinnon (McKinnon, 1996) was used for \bar{w}_i (previously unpublished values are now presented here in Tables I and II). We considered these values to be a good index of biomass for *A. gracilis* because both species are similar in size. The growth rate was determined as the slope of the least-squares regression generated by the ln transformed values of the mean individual weight \bar{w}_T ($\mu\text{gC ind}^{-1}$) against time for each container. Using this procedure, the linearity of growth can be checked by the coefficient of determination (R^2) of the curve.

Table I: Morphometry of eggs and nauplii stages of *Acrocalanus gracilis* from the growth experiment

Stage	Length	Width	Volume	$\mu\text{gC ind}^{-1}$
Eggs (44)	97.8 \pm 9.8	94.2 \pm 11	0.47 \pm 0.16	0.05
N1 (11)	101 \pm 4.8	63.5 \pm 3.1	0.21 \pm 0.02	0.05
N2 (302)	113.4 \pm 9.6	71.9 \pm 5.5	0.31 \pm 0.07	0.07
N3 (2)	152.3 \pm 5.3	65.8 \pm 1.1	0.34 \pm 0.02	0.08
N4 (20)	169.2 \pm 14.8	88.8 \pm 6.5	0.7 \pm 0.12	0.1
N5 (95)	193.3 \pm 11.4	100.9 \pm 6.4	1.04 \pm 0.14	0.14
N6 (20)	225.4 \pm 17.5	102.3 \pm 10.3	1.24 \pm 0.23	0.2

Mean whole body length and width (10^{-3}mm) and volume (10^{-3}mm^3) (± 1 standard deviation). The number of animals measured is inside the brackets beside the stage name. Mean individual carbon content was taken from the October 1993 experiment from McKinnon (1996) for *A. gibber*. N1 was considered to have the same biomass of eggs.

Table II: Morphometry of copepodites and adults of *Acrocalanus gracilis*

Stage and sex	N		Prososome length		Prososome width		Prososome volume		Fifth leg length	
	Exp.	Field	Exp.	Field	Exp.	Field	Exp.	Field	Exp.	Field
C1	80	-	267 (±18)	-	98 (±9)	-	1	-	-	-
C2	152	-	357 (±19)	-	129 (±13)	-	3 (±1)	-	-	-
C3	128	-	455 (±30)	-	160 (±16)	-	6 (±2)	-	-	-
C4 ♀	91	9	575 (±37)	***	202 (±18)	***	13 (±3)	***	18 (±3)	***
C4 ♂	38	6	586 (±35)	NS	204 (±17)	***	13 (±3)	***	18 (±2)	***
C5 ♀	66	30	718 (±40)	***	253 (±21)	***	24 (±5)	***	48 (±10)	***
C5 Int.	33	1	745 (±35)	***	264 (±20)	***	27 (±5)	***	57	64
C5 ♂	11	25	779 (±54)	***	273 (±19)	***	31 (±6)	***	51 (±12)	NS
Adult ♀	13	49	870 (±49)	***	311 (±17)	***	44 (±8)	***	101 (±17)	-
Adult Int.	5	1	894 (±48)	-	318 (±21)	-	48 (±8)	-	53 (±12)	-
Adult ♂	-	14	-	-	951 (±60)	-	-	-	119	60
									56 (±13)	219 (±33)

Mean prososome length and width (10⁻³ mm) and volume (10⁻³ mm³) of animals from the field and from the growth experiment are represented. N indicates the number of animals measured in each stage and sex. (♀) indicate normal females, (Int.) intersexes and (♂) males. Differences in morphometry between animals from the field and the experiment were tested with the nonparametric Mann-Whitney U-test. Levels of significance are indicated by *** P < 0.001, and ns, not significant. Adult intersexes and C5 intersexes from the field were excluded from the analysis because only one individual of each were recorded.

The growth calculation included all samples up to the last point before any adults appeared. After this point, the growth rate was not calculated due to the presence of non-moulting adults.

Stage development, moulting and duration

The development of *Acrocalanus gracilis* was characterized by the calculation of four indices: the median development time (MDT), the moulting period duration (MPD), the stage duration (STD) and the simplified moult rate (SMR).

The MDT of a stage is the time when 50% of the population has moulted to that stage (Peterson and Painting, 1990). The MDT was calculated according to Cook *et al.* (Cook *et al.*, 2007), where the cumulative percentage of animals in a certain stage over time was fitted to the sigmoidal Hill function:

$$y = \frac{Mx^p}{c^p + x^p} \tag{3}$$

where, for a given stage, *y* is the percentage of animals in a later developmental stage and *M* its highest value (i.e. 100% when all the animals are in later developmental stages); and *x* is the time since females began to lay their first eggs. The coefficient *p* is the gradient of the curve between 25 and 75%, and the coefficient *c* corresponds to the MDT. The curve was fitted using SigmaPlot 9 automatic regression curve fitting procedure (equation Hill, 3 parameter). The MPD (Campbell *et al.*, 2001) of a stage was calculated as the time interval (the x axis range) between the beginning and end of that stage (defined by the y axis values of 5 and 95%, respectively) from the fitted Hill function. The STD was calculated as the time interval between the MDT of a given stage and the MDT of the following stage.

Finally, we calculated a SMR to serve as a simple index of moulting in the population. Each stage was assigned a sequential value, from N1 (value 1) to Adult (value 12), and the mean stage value (MSV) of the cohort was calculated for each date substituting \bar{w}_i by the value for stage *i* in equation (2). SMRs were calculated from days 1 to 9, the period representing the development of juveniles until the first occurrence of adults. MSV was assumed to change linearly between every two sequential sampling dates (McKinnon, 1996), and the SMR represents the rate by which the MSV changes over time. It was calculated as $(S_T - S_{T0}) \times T^{-1}$, where *S_{T0}* is the initial mean stage value, *S_T* the mean stage value at time *T* and *T* is the time between the two samplings.

Secondary production

Total and stage-specific secondary production were estimated for C4, C5 and adults as the product of the growth rate times the biomass of each stage in the field. Growth rate of C4 and C5 was considered to be the same and equal to the growth rate estimated in the juvenile development experiment. The growth rate of adult females was considered to be equivalent to the females' carbon specific egg production rate estimated from the same population in the field [0.15 day^{-1} ($22.1 \text{ eggs female}^{-1} \text{ day}^{-1}$), unpublished data]. Adult males were considered to have the same growth rate as adult females. Intersexes were considered to have the same growth rate of females in C5 and adults. The mean individual carbon content for *A. gibber* nauplii (Table I), and copepodites and adults (Table II) from the October 1993 experiment from McKinnon (McKinnon, 1996) was used for the calculation.

Statistical analyses

All logarithms applied to the data are natural logarithms. The Mann–Whitney U (M–W U) test of pairwise comparisons was used to compare the median lengths and biovolumes for each stage in animals from the field and the growth experiments. The Kruskal–Wallis test (K–W) was used for comparisons of more than two cases, subsequently applying the M–W U-test for pair-wise comparisons. When not otherwise specified, the level of significance used was $P < 0.05$. All data analyses were performed using SPSS version 15 (SPSS, Chicago, IL, USA) and R 2.7.1 (R Development Core Team, 2008), and curves were fitted using SigmaPlot version 9.

RESULTS

Field and experimental conditions

At the time of sampling, the surface seawater temperature was 28°C with a total chlorophyll *a* (Chl *a*) content of $0.554 \mu\text{g L}^{-1}$. The water column was well mixed, with temperature differing by only 0.14°C with depth. In the incubators, the temperature during the experiment was stable, between 27.7 and 28.6°C (mean 28°C). During the 12-day time course of the experiment, the concentration of the $<37 \mu\text{m}$ fraction of Chl *a* within the experimental containers ranged from 0.05 to $0.14 \mu\text{g L}^{-1}$ (mean $0.1 \mu\text{g L}^{-1}$), and was highest on days 3 and 12 (Fig. 2).

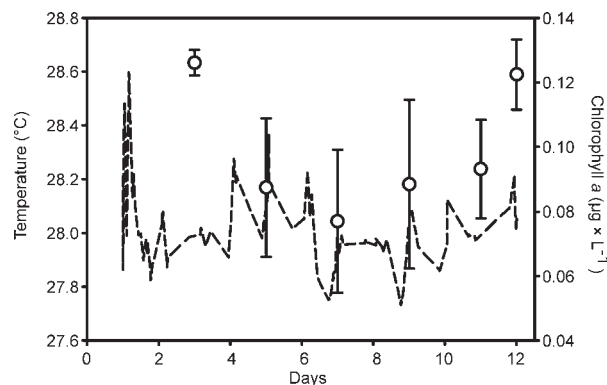


Fig. 2. Variation in temperature ($^\circ\text{C}$) (dashed line) and mean $\leq 37 \mu\text{m}$ Chlorophyll *a* concentration ($\mu\text{g L}^{-1}$) (± 1 standard deviation) in the containers during the experiment.

Morphometry

Animals from the experiment were usually smaller than the same stages and sexes from the field (Table II). Significant differences in the median size of all morphometric features were observed for most stages and sexes for animals from the field and from the experiment, with the exception of the C4 male prosome length and the C5 male fifth leg length. The relationships of size between sexes were similar in animals from the field and from the experiment. In the field, C4 males and females showed no significant difference in prosome length (M–W U, $P > 0.05$, all measurements). In addition, prosome lengths of C5 wild males were greater than C5 wild females (M–W U, $P < 0.05$), and adult females were significantly larger than adult males (M–W U, $P < 0.001$, all measurements).

In the experiment, there was no significant difference between the prosome size of normal adult females and adult intersexes (M–W U, $P > 0.05$ for all measurements). The median prosome length of normal C5 males was significantly larger than C5 intersexes (M–W U, $P < 0.01$) and C5 females (M–W U, $P < 0.01$). The median prosome length of C5 females was significantly smaller than C5 intersexes in the experiment (M–W U, $P < 0.01$). As was the case in the field, C4 males and females also showed no differences in their sizes (M–W U, $P > 0.05$, all measurements). The median fifth leg length of C5 males was significantly greater than C5 intersexes (M–W U, $P < 0.001$). However, the fifth leg length of adult intersexes was not significantly different from C5 intersex and C4 males (M–W U, $P = 0.142$ and $P = 0.97$, respectively).

The prosome length of most copepodite stages and sexes in the experiment varied with age within stage (Fig. 3). In most copepodite stages, the median prosome length of older animals was significantly smaller (M–W

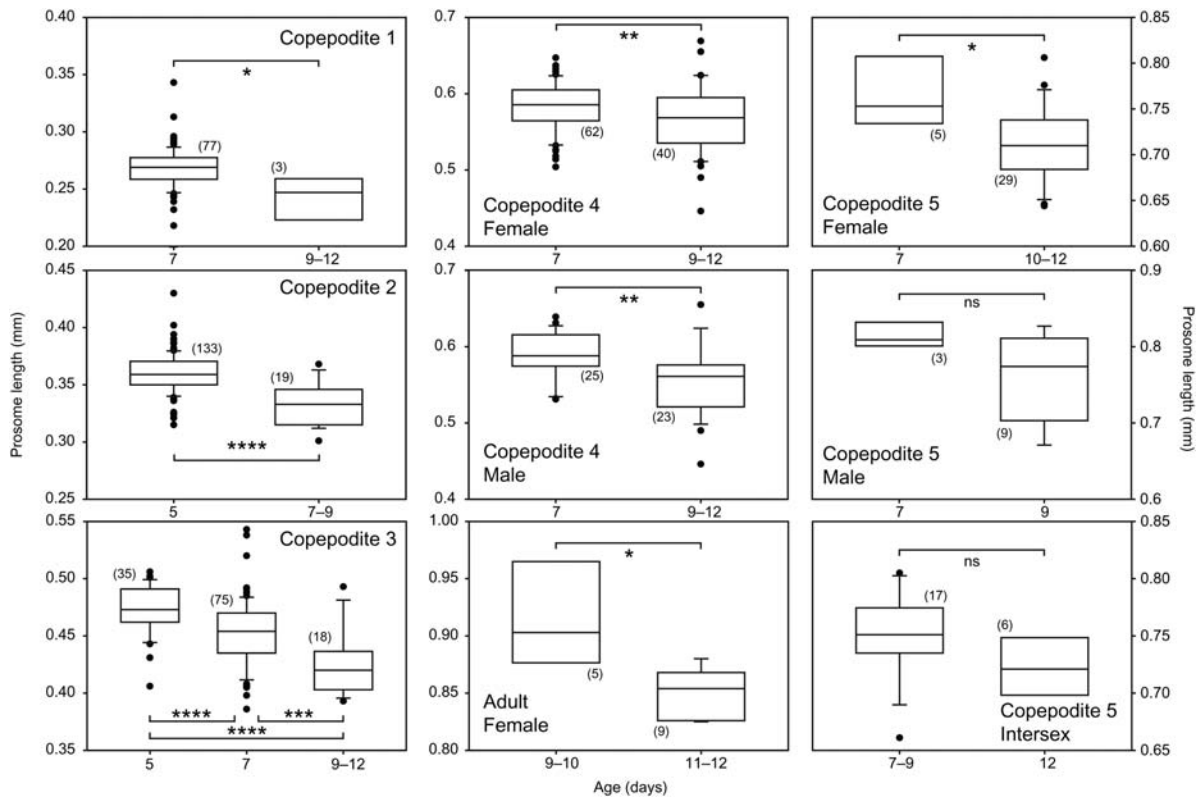


Fig. 3. Box plots for comparison of the median prosome length of some stages and sexes of *Acrocalanus gracilis* with different ages in the growth experiment. The box indicates the 25th and 75th percentiles, the whiskers the 90th and 10th percentiles, the line within the box the median and the black circles the outlying points. Differences in the median prosome length were tested with the non-parametric Mann–Whitney U-test. The number of individuals measured is presented inside parenthesis. Levels of significance are: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; ns, not significant.

U-test) than younger ones. The only exceptions were the C5 males and intersexes, where young and old animals showed no significant difference in prosome length.

Juvenile development and growth rate

It was only possible to calculate MDT, MPD and STD for stages later than N5 because the second sample of the experiment was taken only 2 days after the commencement of the incubation, and most animals had moulted to nauplius (hereafter N) stage 4 at this time. Similarly, since the experiment was terminated prior to all animals becoming adult, these parameters could not be calculated for the adult stage. Since MDT for N4 and adults could not be calculated, it was not possible to calculate STD for N5 and C5.

At day 1, the cohort was composed mainly of N2 ($85 \pm 2\%$; MSV: 1.78 ± 0.05) (Fig. 4). At day 3, most of the cohort reached N5 ($64.3 \pm 11.5\%$; MSV: 5.18 ± 0.11), and the first copepodites were observed. All naupliar stages were well represented in the samples, with

the exception of N3, for which only a few individuals were observed. The cohort had a relatively fast SMR from days 1 to 3 (1.7 ± 0.03 stages day^{-1}), and N3 was probably abundant on day 2, which was not sampled. On day 9, the first adults were observed and after that point C5 was the most abundant stage until the end of the experiment.

N6 had a lower STD than any copepodite stage, C1, C2 and C3 had similar values, and C4 had a STD twice that of previous copepodite stages (Fig. 5). The lowest MPD times were observed in naupliar stages. MPD times increased from C1 to C5, C2 and C4 presented a slightly lower MPD from the immediate previous stage and C3 MPD was twice the duration of C2. Finally, MDT increased log linearly with each stage from C1 to C5.

The juvenile somatic growth rate of *A. gracilis* was 0.44 day^{-1} (Fig. 6A). The development of the mean individual weight was log-linear with a coefficient of determination (R^2) of 0.99, with no obvious difference in growth rates between nauplii and copepodites. However, the MSV in the cohort did not show a linear relationship

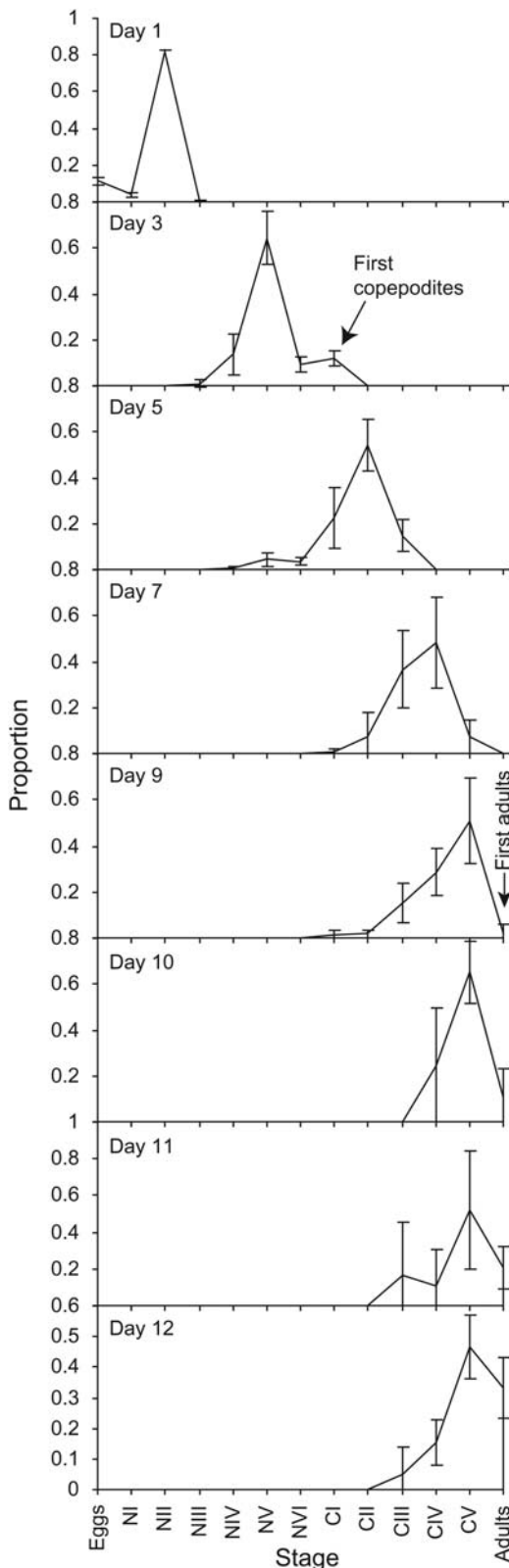


Fig. 4. Development of *Acrocalanus gracilis* represented as the mean relative proportion of each stage in each sampling date. Bars indicate ± 1 standard deviation.

with time (Fig. 6B). Consequently, the SMR was not constant with time, and a faster SMR was observed in younger individuals than in older ones (Fig. 6C).

Finally, our experiment produced a relatively high proportion of intersexes and an absence of adult males (Table II). Moreover, C5 intersexes were more frequent than C5 males in the experiment. We believe that these intersexes are actually males that changed sex during the development. The analysis of sex ratios from this experiment, and a detailed discussion about intersexuality and sex change in *A. gracilis* can be found elsewhere (Gusmão and McKinnon, in press).

Abundance and secondary production

Adults were as abundant as late copepodites (C4 and C5) in the field (53 and 56 m^{-3} , respectively), but contributed more than the latter to the total secondary production (62 and 38% , respectively) (Table III). Adult females were the most abundant stage in the field, contributing to 52.6% of the total secondary production. Among the copepodites, C4 was less abundant than C5 (11 and 45 m^{-3} , respectively) and also contributed less to the total secondary production than the latter (6.6% and 31.5% , respectively). Intersexes were the least abundant sex (1.4% of the total) and the smallest contributor to the total secondary production in the field (1.5% of the total).

DISCUSSION

Linearity of somatic growth was maintained throughout the whole juvenile development in our experimental containers, despite food concentrations being lower than those in the field. However, moulting rates of experimental animals slowed during development, and resulted in smaller animals at a given stage than those in the field. The proportion of intersex individuals was higher in our experiment than in the field. Finally, our results also show that older animals were smaller than younger ones at a given stage of development.

Experimental conditions and food environment

The low Chl *a* content in the incubation water used in the experiment suggests that *A. gracilis* was food-limited during the experiment. The low levels of Chl *a* in the experiment are an indication of a poor trophic environment in the containers: Chl *a* in the containers was lower than the total Chl *a* in the field and also below the theoretical saturation level for the growth of calanoid copepods ($0.6\text{ }\mu\text{g L}^{-1}$, Hirst and Bunker, 2003).

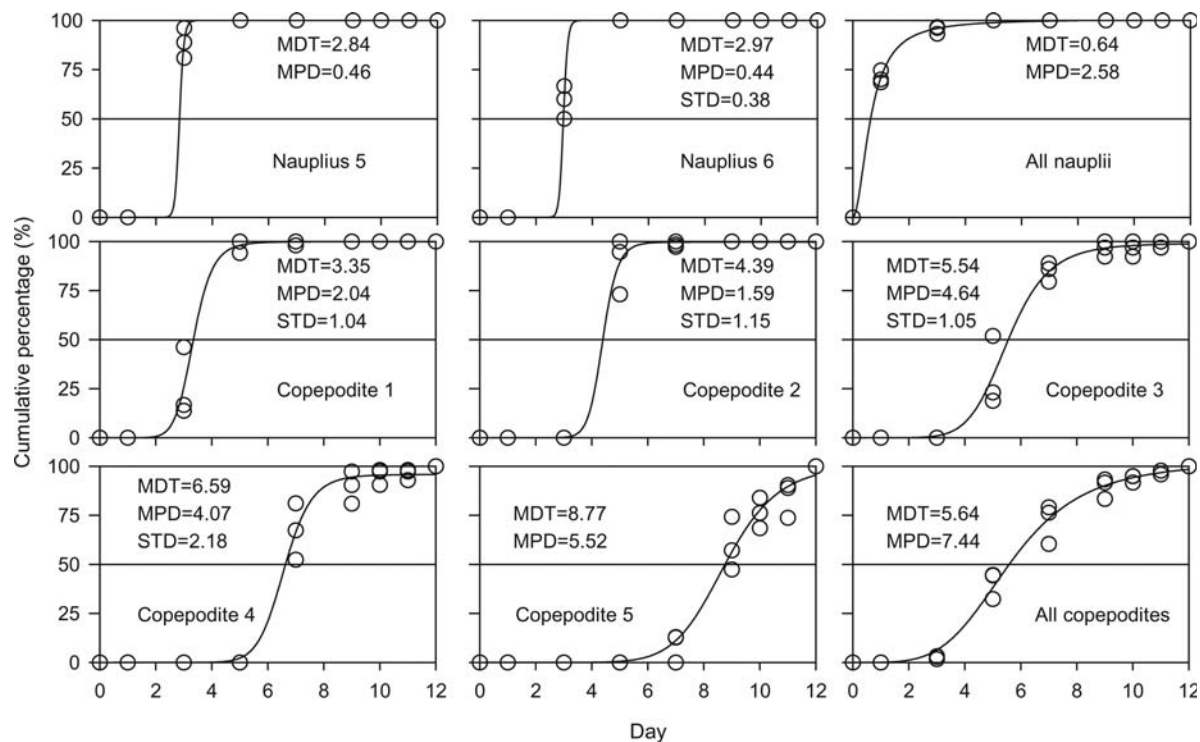


Fig. 5. *Acrocalanus gracilis* stage progression represented as the cumulative percentage of individuals greater than or equal to a given stage at each sample time for all three replicates of the experiment. The line is the fitted Hill function. Values for nauplii and copepodites were calculated by pooling the data of all stages of each type. MDT, median development time (days), MPD, moulting period duration (days) and STD, stage duration (days).

The Chl *a* content in the incubation water declined up to the 7th day, suggesting that the naupliar and early copepodite stages were actively feeding on the <math><37\ \mu\text{m}</math> fraction of the phytoplankton. The lowest values of Chl *a* in the containers were registered in the period when the cohort was composed mainly of C3–C4, and is consistent with the observation that the weight-specific ingestion rates of the closely related genus *Paracalanus* increase from nauplius to C3 and decrease towards adulthood (Paffenhöfer, 1984b). Calanoid nauplii are known to feed on smaller particles than copepodites and adults (Mauchline, 1998), and a change in the selectivity of food particles with age was observed in *Paracalanus* (Paffenhöfer, 1984a). The increase in Chl *a* content in the containers towards the end of the experiment may be an indication of this shift in food preference with stage, associated with an increased uptake of NH_4 from copepod excretion by the phytoplankton.

Unfortunately, in an incubation experiment, it is impossible to simulate the diversity of food particles found in the field, and a reduction of food items by the reverse filtering process is inevitable (McKinnon and Duggan, 2003). Food quality is important for the nutrition of calanoid copepods (Kleppel, 1993), and may be independent of food quantity (Gifford, 1991). It has

been shown that adult *Paracalanus sp.* feed actively on microprotozoans, especially heterotrophic dinoflagellates and ciliates (Suzuki *et al.*, 1999). It is likely that *Acrocalanus* adults and late copepodites may also feed on microzooplankton (see McKinnon, 1996), which were likely to occur in lower abundances in our experiment than in field conditions.

Morphometry

The smaller size of copepods in the experiment than in the field most likely resulted from differences in trophic conditions. Though temperature is important in regulating body size in copepods (Mauchline, 1998), it is unlikely that it was responsible for the smaller size of the experimental animals because the temperature was similar to that in the field. However, both food quality and quantity are known to have an influence on the body size of calanoid copepods (Mauchline, 1998). The occurrence of undersize copepodites and adults has been related to poor feeding conditions in other calanoid species, and there is evidence that food conditions can be even more important than temperature in determining adult size (Klein Breteler and Gonzalez, 1982). In our experiment, the prosome length of the animals

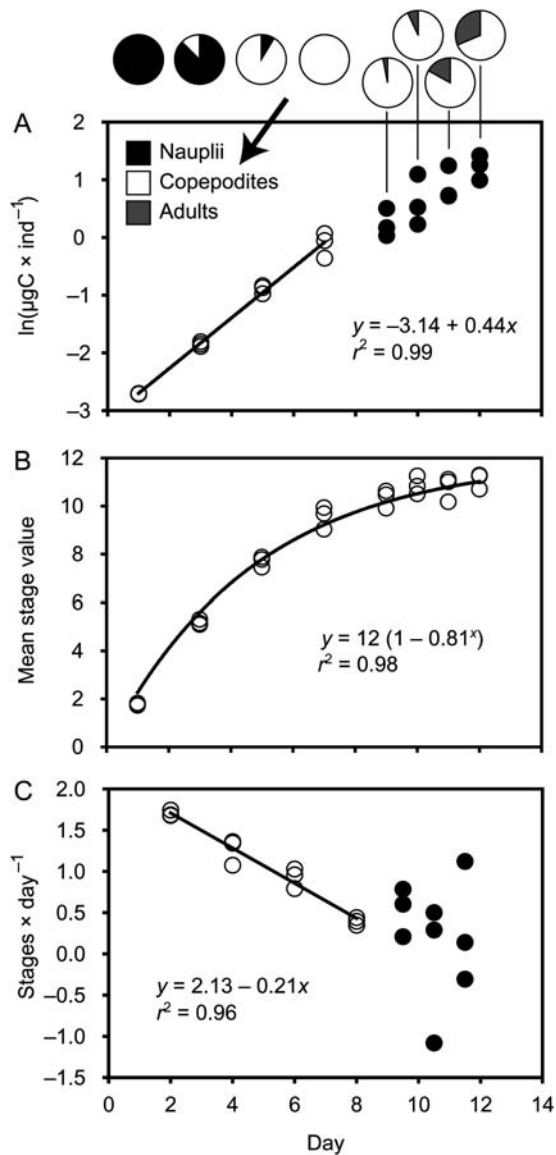


Fig. 6. Descriptors of development of *Acrocalanus gracilis*. Pie graphs represent the total proportion of nauplii, copepodites and adults on each sampling date (legend inside graph A). (A) Growth represented as the variation of the mean individual carbon content ($\mu\text{gC ind}^{-1}$) during the experiment. The last 4 days (black points) are not considered in the calculation of the regression due to the presence of adults. (B) Variation of the mean stage value in the population in relation to the days of experiment. (C) Variation of the moult rate with age (the black points are not included in the regression).

(870 μm) was not only smaller than wild females of *A. gracilis* in the Timor Sea (1092 μm), but was also smaller than *A. gracilis* populations in the Great Barrier Reef in two consecutive austral summers (920 and 1032 μm) under similar temperatures (26–28°C) (McKinnon and Thorrold, 1993).

Variability in individual growth rate is usually observed during the development of artificial cohorts

Table III: Abundance (m^{-3}) and production ($\mu\text{gC m}^{-3} \text{ day}^{-1}$) of *Acrocalanus gracilis* in the Timor Sea

Stage and sex	Biomass ($\mu\text{gC ind}^{-1}$)	Abundance (m^{-3})	Growth rate (day^{-1})	Secondary production ($\mu\text{gC m}^{-3} \text{ day}^{-1}$)
C1	0.3	–	–	–
C2	0.45	–	–	–
C3	0.57	–	–	–
C4 ♀	1.16	6.58	0.44	3.36
C4 ♂	1.16	4.38	0.44	2.24
C5 ♀	1.35	21.92	0.44	13
C5 Int.	1.35	0.73	0.44	0.43
C5 ♂	1.39	21.92	0.44	13.41
Adult ♀	7.88	38	0.15	44.92
Adult Int.	7.88	0.73	0.15	0.86
Adult ♂	3.4	13.88	0.15	7.08
Total		108.14		85.31

Values for mean individual carbon content were taken from the October 1993 experiment from McKinnon (1996) for *A. gibber*. C4 ♀ and ♂ were considered to have the same biomass, C5 and adult intersexes were considered to have the same biomass as the females of these respective stages. C: Copepodites; A: Adults; N indicates the number of animals measured in each stage and sex; (♀) indicate normal females, (Int.) intersexes and (♂) males.

(McKinnon and Duggan, 2003; Kimmerer *et al.*, 2007), and more slowly growing copepods generally have larger body sizes than those growing faster (see Mauchline, 1998). Consequently, it is reasonable to expect that animals entering a certain stage later than others in the same cohort would be larger because of their slower growth rate. However, in our experiment, older animals of most copepodite stages were smaller than younger ones (Fig. 3). This was probably caused by the continuous exposure of the cohort to poor food conditions during the incubation. The smaller or non-significant differences in size with age observed in C5 and adult stages may be an indication that all animals were experiencing a similar degree of food limitation independent of their age at these stages, which agrees with the hypothesis of a shift in feeding habits in late copepodite stages in paracalanids (Paffenhöfer, 1984a).

Juvenile growth, development and secondary production

The only other *Acrocalanus* species for which development has been studied is *A. gibber* (McKinnon, 1996), which shares some similarities with *A. gracilis*: fast N1–N2 development, total naupliar development shorter than that of the copepodites, and increasing development time with copepodite stage. However, all copepodite stages of *A. gracilis* had a relatively longer STD time than *A. gibber* under similar temperature conditions,

particularly C4. This difference might have been caused by the enrichment of the *A. gibber* experiments with cultured phytoplankton, while the present experiment had only $<37\ \mu\text{m}$ filtered natural seawater. McKinnon (McKinnon, 1996) also attributed variation in the development times of *A. gibber* in different experiments, especially in C1 and C5, to differences in food conditions.

Initial mean moult rates of *A. gracilis* (day 2; $\text{SMR} = 1.7 \pm 0.03\ \text{stages day}^{-1}$) were similar to those observed in *A. gibber* (1.55–1.92 stages day^{-1}) at the same temperature range (27.6–29°C). However, the moult rates of *A. gracilis* showed a clear deceleration over time (Fig. 6C), and in older animals it was lower (day 8; $\text{SMR} = 0.4 \pm 0.05\ \text{stages day}^{-1}$) than *A. gibber*. This difference might have occurred because the moult rates were estimated in *A. gibber* considering only the initial and final mean stage values for the whole incubation period, a calculation that is unable to detect the deceleration in the moult rate. This may have been the reason why McKinnon (McKinnon, 1996) could not find a correlation between total moult rate and somatic growth in *A. gibber*.

The STD estimations of *Acrocalanus* spp. (McKinnon, 1996; present work) are similar to those of *Paracalanus* spp. (Landry, 1983; Uye, 1991) and other paracalanids (see Peterson, 2001). The similar pattern of development of paracalanids reported in different incubation conditions, and the longer STD of older stages (see Hart, 1990; Peterson, 2001), conforms to the equiproportional model of development. This model of development, previously suggested for *Paracalanus* sp. (Uye, 1991), seems to be the rule for the Paracalanidae. However, Chisholm and Roff (Chisholm and Roff, 1990) suggested an isochronal model of development for *P. aculeatus*, but because they used an approach that did not estimate SDT values for single stages, their results cannot be compared to other studies. Paracalanids appear to have a total naupliar development that is shorter than the total copepodite development, and the development of the initial naupliar stages (pre-feeding instars) is very rapid ($<24\ \text{h}$). With the exception of *Paracalanus* sp. (Uye, 1991), C1 has a longer duration than N6, and in most cases it is two times longer. Initial copepodite stages up to C3 show a similar or slightly decreasing STD, and from C3 onwards the development time increases with stage. Although we were unable to calculate the STD value for C5 of *A. gracilis*, it was obviously longer than the previous stages, as shown by the longest MPD of all copepodites and the long time needed for most of the C5s to become adults.

In contrast to the moult rates and STDs, the somatic growth rate of *A. gracilis* and *A. gibber* (McKinnon, 1996)

juveniles was log-linear throughout the whole development and there were no differences between naupliar and copepodite growth rates. Linearity of somatic growth was observed under similar temperatures in both species, but in very different trophic environments: both in a non-limiting food condition for *A. gibber*, and in a limiting food environment for *A. gracilis*. It seems that the linearity of the somatic growth can be maintained under different food conditions by altering other developmental features, such as the moult rate. If we consider that the moult to a subsequent stage occurs when “the net accumulated carbon allocated to carapace building bricks during the current instar reaches a fixed fraction of the body mass at the beginning of the current instar” (the “moulting rule”, van den Bosch and Gabriel, 1994, p. 1529), larger developmental stages would need to accumulate more biomass in proportion to their body size in order to moult to the next stage, therefore spending more time in a certain stage the older (and larger) they are. This could explain the deceleration of the moult rate observed in *A. gracilis* and the variability among experiments in the development time of C1 and C5 in *A. gibber*.

McKinnon (McKinnon, 1996) hypothesized that the linearity in somatic growth in *A. gibber* indicated that food conditions were equivalent in his experiments for all juvenile stages. However, our results indicate that this linearity may be maintained independently of the food condition at the cost of varying other developmental traits. In fact, this variation in developmental features was observed in *A. gibber*, manifested mainly by variable STDs and length–weight relationships. Due to the shift in feeding behaviour of paracalanids with age, we may expect this variability to be more pronounced in late copepodites, shown in *A. gracilis* by the deceleration of the moult rate. This observation is consistent with the observation that juvenile stages are less food-limited than late copepodites and adults (Chisholm and Roff, 1990; Peterson *et al.*, 1991; Webber and Roff, 1995; Richardson and Verheye, 1999), also a common trait in most calanoids (Hart, 1990). Nonetheless, the variability in developmental traits supports the idea that the food quality is an important control on the development of *Acrocalanus* (McKinnon, 1996).

Secondary production

The growth rate estimated for *A. gracilis* was close to the lowest value observed for *A. gibber* ($0.49\ \text{day}^{-1}$, April 1994) (McKinnon, 1996). Once again, the artificial enrichment and richer food environment of some of the *A. gibber* experiments could be the explanation for this difference. Due to food limitation during the incubation, it is possible that the growth rate estimated in our

experiment might have been lower than the actual growth rate in the field, contributing to an underestimation of secondary production. Nevertheless, *A. gracilis* growth rate was similar to the mean growth rates of juvenile calanoids (0.43 day^{-1}) near Australia's North West Cape (range: $0.32\text{--}0.51 \text{ day}^{-1}$, McKinnon and Duggan, 2001, 2003), and higher than in the Great Barrier Reef ($0.33\text{--}0.36 \text{ day}^{-1}$, McKinnon *et al.*, 2005). Furthermore, the estimations of secondary production of *A. gracilis* were comparable to previous estimations using similar methods around Australia, but lower than other shelf environments around the world. The total areal daily production of *A. gracilis* in the Timor Sea was $7.68 \text{ mg C m}^{-2} \text{ day}^{-1}$ or $406.8 \text{ J m}^{-2} \text{ day}^{-1}$, calculated according to McKinnon and Duggan (McKinnon and Duggan, 2003), assuming a water depth of 90 m. The production of *A. gracilis* was within the range reported for the total copepod production in Australia's North West Cape shelf (McKinnon and Duggan, 2003) and the Great Barrier Reef in the summer (McKinnon *et al.*, 2005), and similar to *Acartia tranteri* in Westernport Bay, Victoria (Kimmerer and McKinnon, 1987), but it was lower than the copepod production in other tropical and upwelling shelf systems around the world (reviewed in Table III by McKinnon and Duggan, 2003). Differences in trophic resources between tropical Australia and other shelf environments would explain why the secondary production of *A. gracilis* was relatively low. In fact, food limitation appears to be an important control of copepod growth in tropical Australia (McKinnon and Duggan, 2003; McKinnon *et al.*, 2005).

The developmental biology of tropical copepods is still poorly understood in comparison to subtropical and temperate species. The present work helps to elucidate peculiarities of the developmental biology of the ubiquitous but poorly studied genus *Acrocalanus*. Our data suggest that the often scarce trophic resources in tropical Australia seems to play a major role in controlling the developmental features of *Acrocalanus*. The linearity of somatic growth of *Acrocalanus* species may be an indication of an efficient use of these trophic resources, an important adaptation that could explain the success of this genus in tropical waters. Moreover, our observations suggest that trophic conditions can have a strong effect on the morphometry of these copepods, which could be used as an indicator of the environment that these animals were exposed to during their development.

ACKNOWLEDGEMENTS

We are grateful to Dr Gregg Brunskill for allowing us to participate in Cruise SS0605 of Australia's National Research Facility, the RV Southern Surveyor. We thank

Samantha Duggan, two anonymous reviewers and the associate editor for comments on the manuscript.

FUNDING

L.F.M.G. received a scholarship from the National Council for Scientific and Technological Development (CNPq – Brazilian government).

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