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RESEARCH NOTE

First measurements of euphausiid growth rates in the northern Humboldt Current (23ºS)

Primeras mediciones de tasas de crecimiento de eufáusidos en el norte de la Corriente de Humboldt (23ºS)

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Abstract.- One hundred and five juveniles and adults of *Euphausia mucronata* were incubated in order to estimate their daily growth rates (DGR) using the standard instantaneous growth rate method. From 77 krill that survived after 2 days of incubation under laboratory conditions, 70 individuals molted (35.1% males, 64.9% females). *E. mucronata* mean intermolt period was 5.2 days (range = 4-9 d) with mean DGR of 0.01 mm d^{-1} for males and -0.04 mm d^{-1} for females. Overall mean DGR was -0.02 mm d^{-1}. DGRs were positive (31%), zero (12%) and negative (57%). DGR<0 evidenced body shrinking process suggesting that environmental conditions in austral autumn 2010 were unfavorable for the krill population. However, because females had DGR<0 values more frequently than males it is probable that females also used part of their energy on gonad development rather than body growth.

Key words: *Euphausia mucronata*, intermolt period, Bahía Mejillones, Southeastern Pacific, Chile

INTRODUCTION

Euphausiids (or krill) are small pelagic crustaceans similar in shape to shrimps. Several species form immense aggregations being a key component in the marine pelagic food web by channeling organic matter from lower to higher trophic levels (Everson 2000, González *et al*. 2009, Antezana 2010). Their ecology and biology have been broadly studied in the world ocean (Brinton *et al*. 2000, Everson 2000), but most knowledge on ecology and biology come mainly from studies of the Antarctic krill (*Euphausia superba*) or krill species in the northern hemisphere. In the past it was though that euphausiid always increase size (somatic growth) between successive ecdysis events; however, since the 1980's decade it was demonstrated that euphausiids do not growth or even shrink between two successive molting events. Ikeda & Dixon (1982) were the first scientists to report shrinking (they called negative growth) in the subtropical euphausiid *Nyctiphanes australis* based on body length and physiological rate measurements using incubated specimens under laboratory conditions. This phenomenon has usually been interpreted as a response of krill to suboptimal environmental conditions (temperature, food) and/or as an alternative source of energy for gonad development (reproduction) and growth during periods of scarce food (as an overwintering strategy) (Ikeda & Dixon 1982, Quetin & Ross 1991). However, experimental evidence suggests that shrinking in krill occur throughout the year round in polar (Nicol 2000, Tarling *et al*. 2006), temperate (Pinchuck & Hopcroft 2007, Feinberg *et al*. 2007, Shaw *et al*. 2010, Pond *et al*. 2012, Riquelme-Bugueño *et al*. 2016), and subtropical krill species (Gómez-Gutiérrez *et al*. 2012). These studies, among others, provide evidence that: 1) growth in krill is not necessarily limited by food but in occasions caused by anomalous changes in temperatures (Marinovic & Mangel 1999), 2) growth is highly variable at intraspecific and interspecific levels and body growth rates in females is negative correlated with brood size and interbrood periods (Feinberg *et al*. 2007), and 3) positive, zero and negative growth should occur in any krill species because this physiological process have been observed in species from distinct biogeographic affinities (Atkinson *et al*. 2006, Tarling *et al*. 2006, Shaw *et al*. 2010, Gómez-Gutiérrez *et al*. 2012, Pond *et al*. 2012).

Euphausia mucronata is the most abundant, widely distributed and ecologically relevant krill species in the Humboldt Current System (HCS) (Antezana 2010, Riquelme-Bugueño *et al*. 2012, 2013). Riquelme-Bugueño *et al*. (2013) estimated

the *E. mucronata* intermolt period from specimens collected during austral spring 2007 and summer 2008 using the 1/MR standard method (Tarling *et al.* 2006). They estimated secondary production (body and egg production rates) using the cohort analysis from a monthly time series at a station off Dichato (2002-2007). However, these methods are, by definition, mean population estimations of growth rates and intermoult period rather than individual measurements representative of euphausiid physiological status under certain local environmental conditions. Thus, the magnitude and variability of individual *E. mucronata* growth rates has not been yet estimated. The aim of the present study was to do the first report in the HCS of the intermolt period and instantaneous growth rate of *E. mucronata* measured under laboratory conditions.

MATERIALS AND METHODS

Euphausia mucronata specimens were captured at night onboard the boat Marlin in Bahía Mejillones, Antofagasta, Chile (23ºS, Fig. 1) during May 10-30, 2010. Krill sampling was made using a conical net (1 m diameter mouth and 333 µm mesh net) with a non-filtering cod-end (31.5 L). The net was deployed at a depth of 20-50 m and trawled to the surface during 10 to 20 min. Once animals were onboard, they were gently placed in a cooler and transported to a land-based laboratory equipped with cold rooms. The incubation was conducted either onboard or at land <2 h after sampling. In total 105 *E. mucronata* individuals were incubated from 3 nocturnal samplings. The euphausiids were incubated individually in ~800 ml transparent plastic jars containing filtered seawater (100 µm). The jars were kept in darkness in a cold room at 11-12°C, which was the sea surface temperature recorded *in situ* with a portable temperature sensor (SevenGo, Mettler Toledo). Incubations lasted 48 h to avoid laboratory artifacts with food deprivation (Shaw *et al.* 2006, 2010; Gómez-Gutiérrez *et al.* 2012) and each incubated euphausiid was checked for molting at 12 h intervals. Males were identified by examining the presence of spermatophores or the petasma located in the first pair of abdominal appendages and females were identified by observing their thelycum or presence of spermatophores attached on their thelycum (Brinton *et al.* 2000). Seawater was changed every 24 h. When molts were present these were separated together with animals and later measured the telson and uropod lengths. The instantaneous growth rate (IGR) technique was used to estimate growth rates (Ross *et al.* 2000). This technique consists of measuring the difference between the length of the telson (and/or uropod) of the animal and of the molt during the laboratory incubation (Shaw *et al.* 2006, 2010, Fig. 2). The telson of the animal and the molt are generally used because the telson is usually the best preserved structure in comparison with the rest of the structures of the discarded molt (also known as exuvia). The telson lengths of the animal and the molt were measured immediately after molt was detected in the incubation bottles. When it was not possible to do measurements when molting occurred, the animal and molt were preserved in a 10% formalin solution for later measurements. Measured molt length (ML) was transformed to total length (TL) following Melo & Antezana (1980) equation. The standard IGR method (Tarling *et al.* 2006) was used to estimate the *E. mucronata* growth increment (*GI*, in %) as follows:

$$GI = \frac{T_a \times T_m}{T_m} \times 100$$

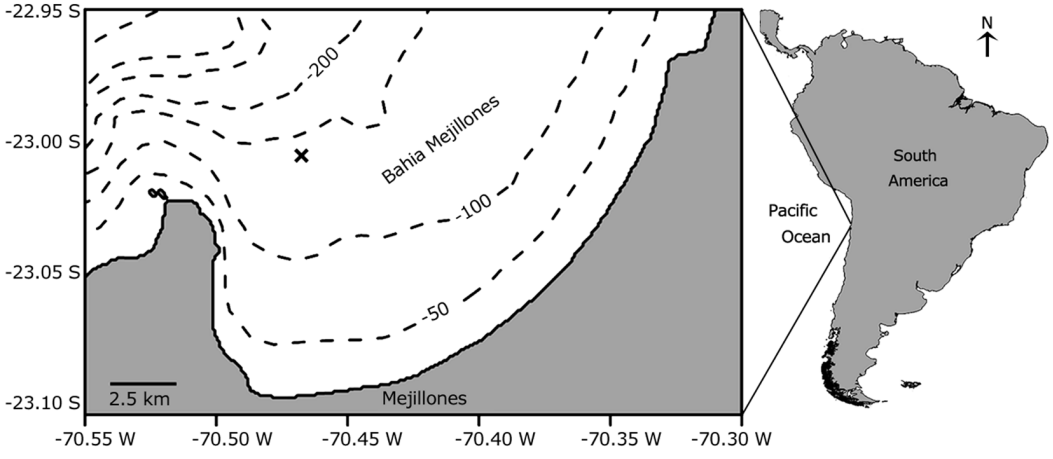

Figure 1. Study area in Bahía de Mejillones, located northern region of the Humboldt Current System (Antofagasta, Chile), showing the site (black cross) where specimens of *Euphausia mucronata* were sampled in May 2010 / Área de estudio en Bahía de Mejillones, localizado en la región norte de la Corriente de Humboldt (Antofagasta, Chile), mostrando el sitio (cruz negra) donde especímenes de *Euphausia mucronata* fueron muestreados en mayo 2010

where T_a and T_m are the telson length of the animal and molt, respectively. T_a and T_m were measured with a lateral view from the posterior part of the sixth abdominal segment to the tip of telson. DGR (mm d^{-1}) was then calculated based on Tarling *et al*. (2006) method as:

$$DGR = \frac{L_{pre} \times GI}{IMP \times 100}$$

where L_{pre} is the estimated total length (*TL*) before molt of the animal (mm), GI is the growth increment, and IMP is the intermolt period. L_{pre} was derived from a *TL*-telson equation from Melo & Antezana (1980). IMP was calculated using the 1/MR method (Tarling *et al*. 2006):

$$IMP = \frac{N \times t}{N_m}$$

where N is the total number of krill that were alive at the end of the experiment plus krill that molted during the incubation period, N_m is the number of krill specimens that molted, and t is the duration of the incubation period (2 days). All length measurements were done using a stereomicroscope equipped with a calibrated micrometer.

The instantaneous growth rate (IGR) method assumes that 1) field sampling (with the net of big cod-end and drifting tow) and field-to-laboratory transport conditions do not cause significant stress, 2) growth rates measured under laboratory reflect what animals feed on a natural conditions (phytoplankton and microzooplankton) before zooplankton sampling, and 3) that incubated individuals were not under starvation for a significant period of time. Thus, we assume that measured instantaneous growth rates are representative of the growth rates that occur at nature (Tarling *et al*. 2006).

We tested statistical differences in DGR between male and female using an ANOVA test and descriptive summary is presented by mean of a box-and-whisker plots. Analyses and plots were done using STATISTICA 8 software.

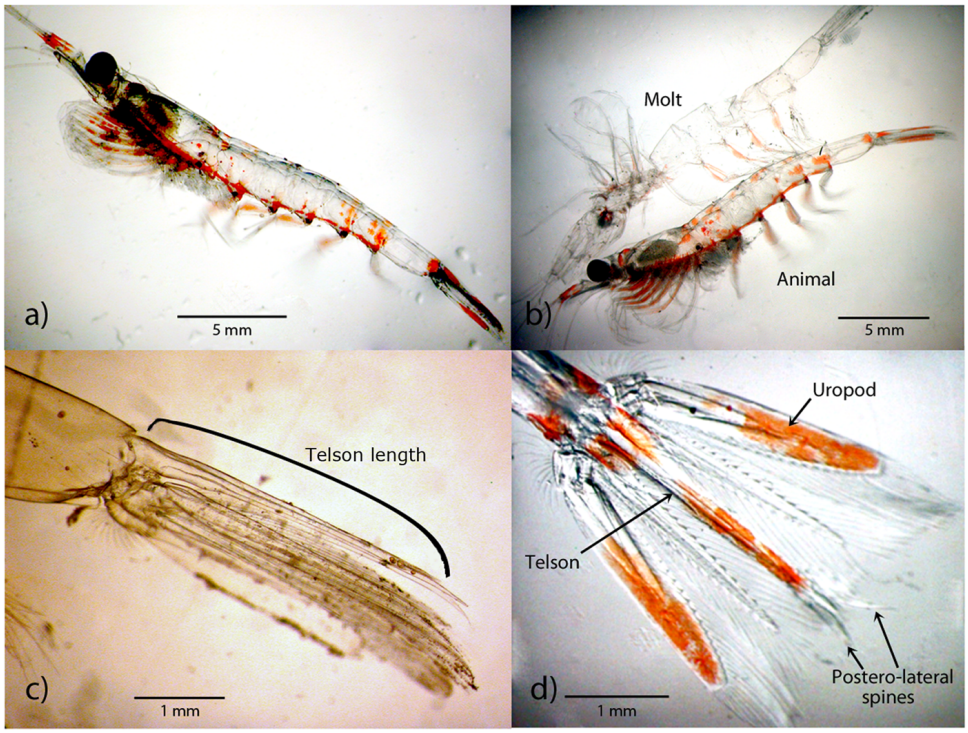

Figure 2. *Euphausia mucronata*. a) incubated specimen, b) specimen showing its molt after the molting event, c) lateral view showing how the telson length of each specimen was measured, d) dorsal view of measurements of the telson length / *Euphausia mucronata*. a) espécimen incubado, b) espécimen mostrando su muda después del evento de ecdisis, c) vista lateral mostrando cómo se realizaron las mediciones de la longitud del telson en cada individuo, d) vista dorsal de las mediciones de la longitud del telson

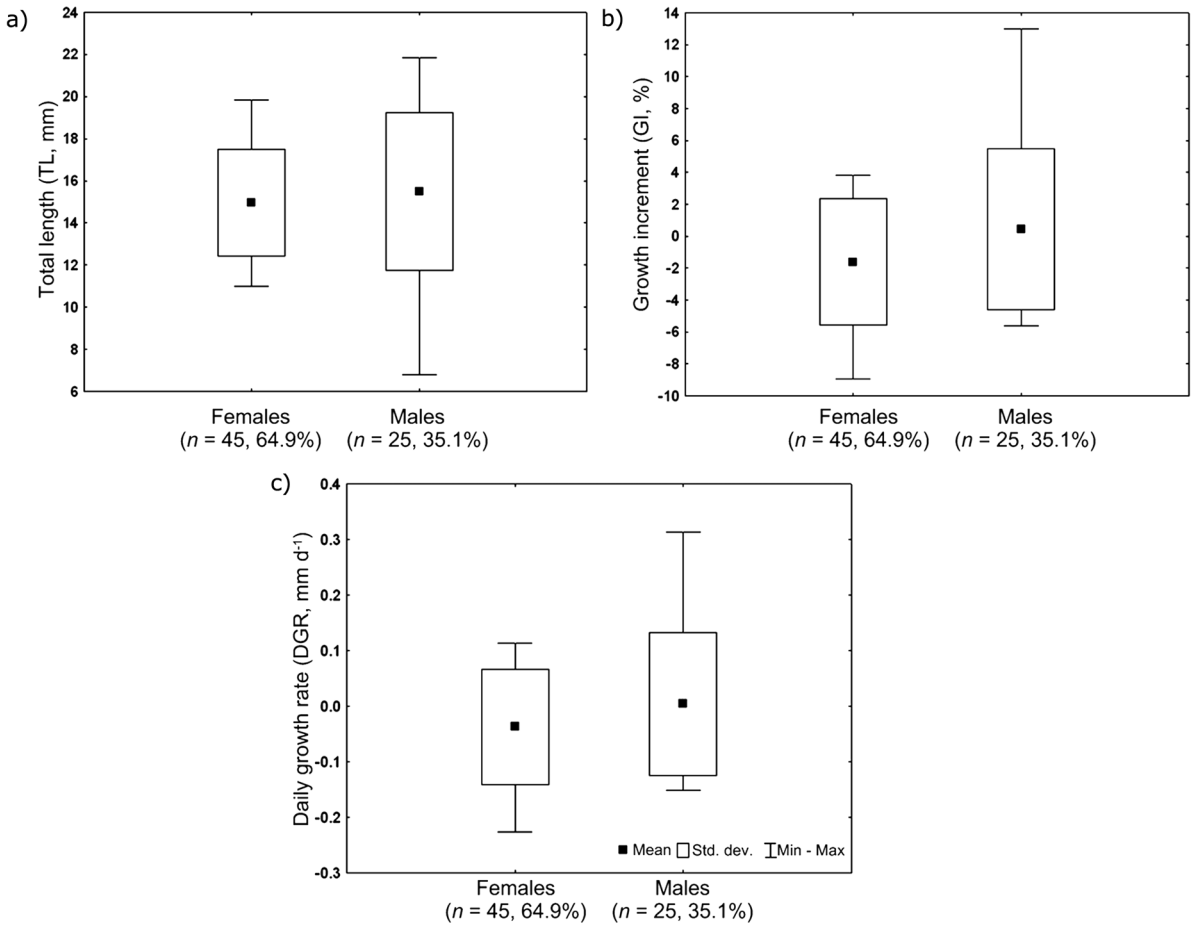

Figure 3. Box-and-whisker plots indicating the descriptive statistical (mean, standard deviation and range) of *Euphausia mucronata*: a) total length (TL), b) growth increment (GI) and c) daily growth rate (DGR), measured in females and males during incubations under laboratory conditions carried out in May 2010 / Diagrama de caja indicando la estadística descriptiva (promedio, desviación estándar y rango) de: a) longitud total (TL), b) incremento en crecimiento (GI) y c) tasa de crecimiento diaria (DGR) de machos y hembras de *Euphausia mucronata* durante incubaciones bajo condiciones de laboratorio realizadas en mayo 2010

RESULTS AND DISCUSSION

From 105 *E. mucronata* incubated specimens, 77 of them survived (73.3%) after 2 days of incubation. From these survivors, 70 individuals molted during incubations including 25 males (35.1%) and 45 females (64.9%). Mean total length was similar for males and females (~15 mm TL), although the standard deviation (SD) and range were wider for males than for females (Fig. 3a). Mean IMP was 5.2 days (± 1.5 SD), ranging from 4 to 9 days, which is within the mean of 4 days (range: 4-6 days) estimated by Antezana & Melo (2008). Our IMP estimations were similar to those reported for the temperate species *E. pacifica* estimated during upwelling (mean = 8 days) and downwelling (mean = 11 days) seasons from a 7-years' time series carried out off the Oregon coast (Shaw *et al.* 2010).

E. mucronata mean GI was 0.73% (± 5.12 SD) for males and -1.62% (± 3.96 SD) for females. Mean DGR was 0.01 mm d^{-1} (± 0.13 SD) in males and -0.04 mm d^{-1} (± 0.10 SD) in females (Figs. 3b, c). Shaw *et al.* (2010) reported mean *E. pacifica* DGRs of 0.02 mm d^{-1} for the upwelling and 0.01 mm d^{-1} for the downwelling seasons. Pooling all the males and females data from our experiment, mean GI was -0.83% (± 4.46 SD) and mean DGR was -0.02 mm d^{-1} (± 0.11 SD). All our measurements were comparable to those reported in other studies on krill growth rates (Shaw *et al.* 2010, Gómez-Gutiérrez *et al.* 2012). No significant differences of TL, GI and DGR were found between *E. mucronata* males and females (ANOVA, $P > 0.05$). A high degree of individual variability was observed in

our measurements corresponding 31% to individuals growing (DGR>0), a 12% with DGR= 0 and, a 57% with DGR<0 (*i.e.*, shrinking) evidencing how the population has individuals with different capabilities to obtain energy from its habitat and how respond to local environmental conditions. Hosie & Ritz (1989) also observed shrinkage in *Nyctiphanes australis*, with >70% of incubated individuals experimenting decreased body size, apparently because of poor food quality or lack of feeding in nature. Negative DGR has been attributed to krill populations under unfavorable environmental conditions, such as unusually high temperature (Marinovic & Mangel 1999) and limited food availability (Virtue *et al*. 2010, Gómez-Gutiérrez *et al*. 2012), or as an overwinter strategy (Ikeda & Dixon 1982, Daly 2004). However, experimental evidence suggests that negative growth is commonly observed in krill during their life cycle and occurring in any season of the year in polar (Tarling *et al*. 2006), temperate (Marinovic & Mangel 1999, Shaw *et al*. 2010), and subtropical ecosystems (Gómez-Gutiérrez *et al*. 2012). Most recent field work (2011–2014) off Dichato coast provided a comparative frame of reference of *E. mucronata* growth rates (Riquelme-Bugueño *et al*. 2016) with our measurements observed in the northern HCS. In our study, DGR did not show a significant relationship with TL (data not shown). Body shrinkage in females may reflect the allocation of energy to gonad development (reproduction) rather than somatic growth (Atkinson *et al*. 2006, Feinberg *et al*. 2007). However, during our sampling effort we did not observe gravid females (females with blue or purple gonad) or spawned.

Direct measurements of vital rates of individuals incubated under laboratory conditions, such as the body growth rate and intermolt periods, have improved estimations of total secondary production because provide credible data input to models about how animals respond to local conditions (Tarling *et al*. 2006, Gómez-Gutiérrez *et al*. 2012, Riquelme-Bugueño *et al*. 2013). Ongoing monitoring efforts will help to better understand the ecological function of *E. mucronata* in the secondary productivity and biomass contribution in this coastal upwelling ecosystem and its co-variation with spatio-temporal environmental conditions. In the future, studies using the hepato-somatic index (relative size of the digestive gland in the relation of cephalothorax size) and lipid content analyses will provide also useful information to know the recent nutritional history in this krill species. Because respiration accounted for the major portion of the assimilation of carbon of *E. pacifica* (62-87%) and molting, growth, and reproduction accounted for the remainder (Lasker 1966) as was done for this species in Japan (Iguchi & Ikeda 1995), future studies should also estimate respiration rates of *E. mucronata*. The results of the present study confirm how krill populations display considerably high individual variability in observed krill growth rates independently of the species, sex, regions and seasons. This challenges the integrative understanding about how krill respond to local conditions and how they can transform their energy into biomass available to their natural predators, several of them of commercial interest for human consumption.

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