SHORT COMMUNICATIONS

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Effect of formalin preservation on the body length of copepods

Maria Põllupüü

Estonian Marine Institute, University of Tartu, Mäealuse 10a, 12618 Tallinn, Estonia; maria.pollupuu@ut.ee

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Abstract. Two copepod groups – *Acartia bifilosa* adults and nauplii – were examined for the effect of preservation with 4% formalin solution on their total body length. The length of the same individual was measured before, immediately, one week, and two months after formalin fixation. There was no statistically significant difference in live and preserved copepod body length at any time of measurement either in the case of adults or nauplii. Therefore it is suggested that the body length of copepods drawn from preserved samples can be used as an unbiased measure for the calculation of copepod biomass from the length–weight relationship.

Key words: formalin preservation, copepods, body length.

INTRODUCTION

Studies on crustaceans and other planktonic organisms have shown that the preservation with formaldehyde generally results in weight loss caused by the leaching of body substances into the surroundig fixation fluid (Hopkins, 1968; Morris, 1972). Direct weight measurement of mesozooplankton individuals is complicated because of their small size. Therefore, a geometrical method has been used to determine individual weight of zooplankton. Commonly, measurements are made using preserved animals but not fresh material. Zooplankton samples are generally preserved with 4% formalin solution. Several authors have recommended using ethanol instead of carcinogenic formalin, and demonstrated that the choice

of the preservative is irrelevant for biomass estimation (Mauchline, 1998; Wetzel et al., 2005). In addition, Black & Dodson (2003) showed that there are no significant differences in the body length of *Daphnia* (Cladocera) when the measurements of fresh and formalin or ethanol preserved animals are compared. The aim of the present paper is to evaluate the effects of formalin preservation on zooplankton by evaluating its impact on the body length of adults and nauplii of *Acartia bifilosa*. Knowing the impact of formalin on copepod size helps us to obtain reliable estimates of copepod biomass and thus to better assess the energy flow to higher trophic levels.

MATERIALS AND METHODS

The material for this study was collected in the north-eastern part of the Gulf of Riga. During the sample collection water salinity was 3–5 and water temperature 10–20 °C. Zooplankton samples were collected with a standard Juday net (mouth surface area 0.1 m^2, mesh size 90 μm) by vertical hauls. Each collected sample was put in a 0.5 L bottle filled with sea water. Samples were brought to the laboratory within one hour. Living undamaged animals were pipetted under the microscope and placed in filtered sea water. *Acartia bifilosa* adults and nauplii were chosen for further investigations. Changes in the length of each individual were followed separately before and after formalin (4%) preservation. First the length of an individual was measured in sea water and then immediately in formalin. After that each individual was placed separately in a multi-dish plate. After a week, every individual was separately pipetted out, measured again, and put back in the multi-dish. Measurements were repeated the same way after two months.

The total body length of adult copepods was measured from the beginning of the cephalothorax to the end of the caudal rami, without taking into account the caudal setae. For the nauplii the total body length was measured. Length measurements were performed with microscope equipped with a micrometer eye-piece with a precision of 0.01 mm. Altogether 91 adults and 95 nauplii were examined.

The significance of differences in the body length between the living and the preserved animals of varying periods was estimated by one-way ANOVA. The differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

The average length of living *A. bifilosa* adults was 0.947±0.012 mm (avg ±SE) and that of living nauplii 0.190±0.004 mm (avg ±SE) (Table 1). Based on the size change assessed separately for each individual, the body length of *A. bifilosa* adults varied (in comparison with living individuals) within 0.01 mm (i.e. within measurement precision) in over 65% of the cases of measurements immediately after, one week, and two months after formalin fixation. The corresponding figure

Table 1. Average body length of living and preserved copepods

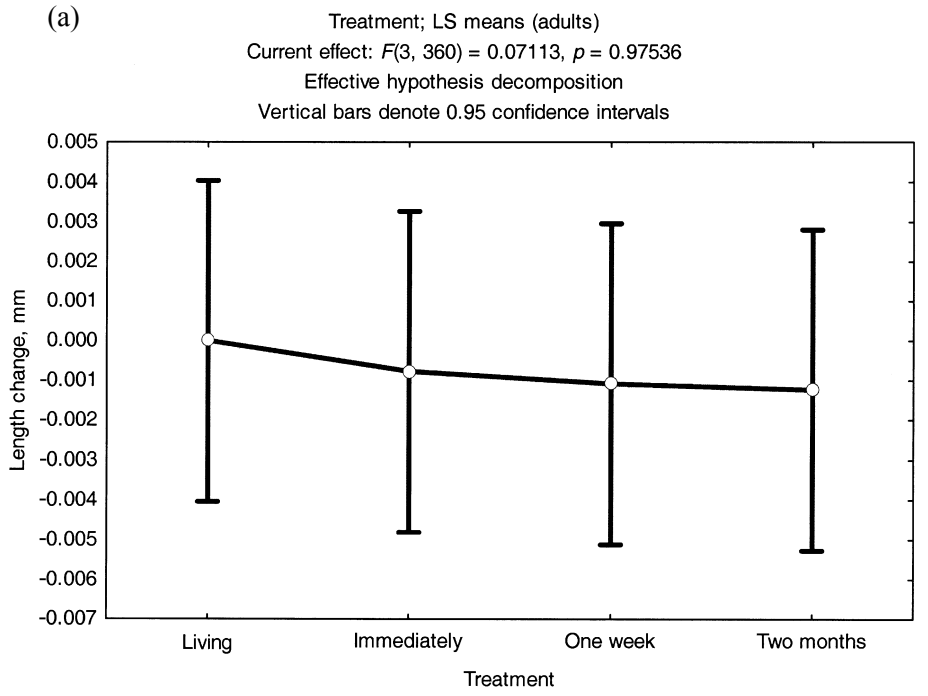
Acartia	Average body length, mm ±SE			
	Living	Immediately	After 1 week	After 2 months
Adults	0.947±0.012	0.944±0.012	0.944±0.012	0.942±0.012
Nauplii	0.190±0.004	0.190±0.004	0.190±0.004	0.192±0.004

for nauplii was 80%. The proportion of nauplii whose body length (in comparison with living individuals) decreased or increased more than 0.01 mm at different preservation times was estimated at 2% to 10%. The values were higher for adults, being mostly between 14% and 17% (Table 2). The differences between the subsequently measured lengths were not statistically significant at $P > 0.05$ either in the case of adults or nauplii (Fig. 1).

Earlier literature data suggest that preservation with formalin results in weight loss of planktonic organisms (Omori, 1970; Durbin & Durbin, 1978; Landry, 1978; Champalbert & Kerambrun, 1979; Böttger, 1984; Kapiris et al., 1997; Wetzel et al., 2005). However, the effect of formalin preservation on the body length of copepods is not thoroughly investigated. Published results regarding possible changes in copepod length caused by formalin preservation (Landry, 1978; Durbin & Durbin, 1978) indicate that this effect, if present, is minor (Kuhlmann et al. 1982; Viitasalo et al., 1995). Böttger (1984) compared the length–weight relationships estimated for fixed and unpreserved material and showed that the weight of fixed copepods (*Eurytemora affinis*) is significantly smaller than the weight of unpreserved copepods of the same length. However, comparison of the regression lines for the two treatment series showed that the slopes were not significantly different, and it was concluded that there was no size-specific effect on the relative loss of weight. In contrast, Kapiris et al. (1997) found that formalin causes significant shrinkage of all biometrical characters of *Acartia clausi* with the length loss being larger for the abdomen than for the cephalothorax or for total length. The results of the present study showed that there was no significant

Table 2. Distribution of body length change (in comparison with living individuals): number of individuals (%) whose body length decreased and increased more than 0.01 and whose body length change was within 0.01 mm (i.e. within measurement precision)

Treatment	Adults			Nauplii		
	Decreased	Within measurement precision	Increased	Decreased	Within measurement precision	Increased
Immediately	14.3	71.4	14.3	5.3	92.6	2.1
After 1 week	17.6	67.0	15.4	9.5	83.2	7.4
After 2 months	17.6	68.1	14.3	4.2	85.3	10.5



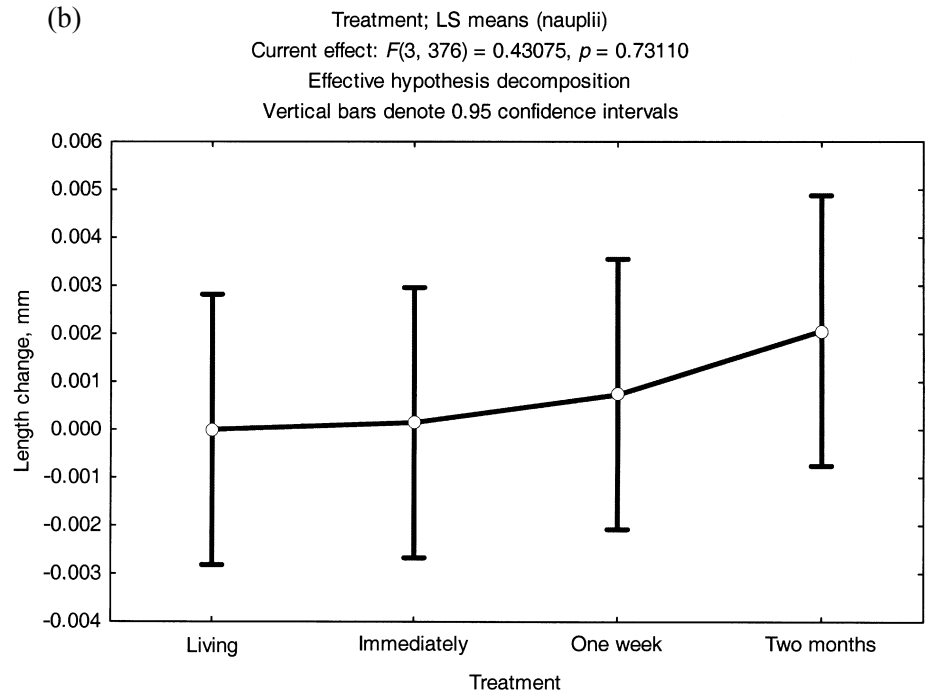


Fig. 1. Body length change (in comparison with living individuals) of *Acartia bifilosa* adults (a) and nauplii (b) measured immediately after, one week and two months after 4% formalin fixation.

change in the body length of living and preserved animals either in the case of different length groups, adults, or nauplii. The considerably larger fluctuations in the body length of the adults are probably induced by the larger body size compared with the nauplii. Therefore, it can be argued that the chitin crust of copepods is either not affected by formalin preservation or the effect is too weak to be detected.

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Formaliini mõju aerjalgsete keha pikkusele

Maria Põllupüü

On uuritud formaliini mõju aerjalgse *Acartia bifilosa* täiskasvanute ja naupliuste keha pikkusele. Sama isendi pikkust on mõõdetud enne, kohe, üks nädal ja kaks kuud pärast formaliiniga (4%-line lahus) fikseerimist. Elusate ja pärast fikseerimist eri aegadel mõõdetud täiskasvanute ning naupliuste pikkuses ei esine olulist statistilist erinevust. Käesoleva artikli tulemuste alusel võib väita, et fikseeritud loomade pikkuste põhjal arvutatud individuaalsed kaalud on õiged ja annavad usaldusväärse biomassi hinnangu.