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Dry weight and chemical composition (CHN) in relation to population density of cultivated *Tisbe holothuriae* (Copepoda, Harpacticoida)

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ABSTRACT: The harpacticoid copepod *Tisbe holothuriae* was cultivated at different densities in a running-water system. Dry weight and chemical composition (CHN) of both females and egg-sacs have been determined. Dry weight decreased significantly with increasing density from 3.48 to 1.94 µg in egg-sacs, and from 10.80 to 6.58 µg in females. Increasing density in both egg-sacs and females results in decrease of carbon, nitrogen and hydrogen contents, expressed as a percentage of dry weight of egg-sacs and females, respectively. Carbon decreases from 52.56 to 50.57 % (egg-sacs) and 45.80 to 39.95 % (♀), nitrogen from 12.63 to 11.94 % and 10.72 to 9.73 % and hydrogen from 9.08 to 7.78 % and 7.47 to 6.17 %. The dry weight and elemental compositions of the egg-sacs varied in accordance with that of the females, however, a higher percentage of elemental content was observed in egg-sacs. The energy equivalents were calculated from the carbon content. This indicates that more energy was transferred from the maternal body into egg-sacs. The ratio of carbon to nitrogen did not show any marked variations; no clear relation appeared between C:N and density, indicating a relatively constant chemical composition in both females and egg-sacs.

INTRODUCTION

In the past few decades, many studies have been carried out on the biomass and chemical composition of marine plankton to understand the reproduction and biogeochemical circulation of elements in the ocean (Omori, 1969; Båmstedt, 1976; Nemoto et al., 1976; Champalbert & Kerambrun, 1978; Finlay & Uhlig, 1981, and Ikeda & Skjoldal, 1989). However, most of this data is derived from natural populations where practical problems arise due to the difficulties in isolating certain species of the net plankton and obtaining relatively large amounts of pure and dry material for analyses. The available data on field material from such studies always offers a high degree of variation. From this point of view, laboratory analyses from live, known material, reared under controlled conditions, are much more precise and can be used for a better interpretation and estimation of the production in the field. Lindley (1988) for instance, used laboratory data to estimate biomass and production of brachyuran larvae in the pelagic ecosystem of the Irish Sea.

The harpacticoid copepod *Tisbe holothuriae* is considered to be a well-suited, living

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food organism for growing fish larvae (Uhlig, 1981). This copepod has been successfully cultivated in the Helgoland laboratory since 1963. A variety of studies have been carried out under the aspect of optimizing conditions for mass cultivation of this harpacticoid copepod (Uhlig, 1984; Schwenzer, 1985; Zhang & Uhlig, 1991). Data about the biomass and chemical composition of *Tisbe holothuriae* is very little, scattered about in literature (Schwenzer, 1985; Guérin & Gaudy, 1977). In this paper, the dry weight and carbon, nitrogen and hydrogen of the egg-sac and the maternal body were studied in the light of female productivity in cultures of different densities.

MATERIAL AND METHODS

The experimental material derived from the stocks of our mass cultures. The nauplii were collected by sieving the females through 125 μm gauze and concentrating them on a 50 μm gauze. Only freshly hatched nauplii were selected for experiment (not older than 6 h, size ranging from 60–80 μm). The nauplii were cultivated in millipore-filtered and pasteurized (96 °C) seawater of 28 ‰ salinity within a temperature-controlled (20 ± 1 °C) running-water system (for details see Zhang & Uhlig, 1993). After hatching the nauplii, stages I and II were fed with *Dunaliella tertiolecta*; later on some *Skeletonema costatum* were added. The copepodite stages were offered a nutritious mixed diet of *Dunaliella tertiolecta, Skeletonema costatum* and some fine grains of dehydrated mantle tissue of *Mytilus edulis*. The population densities were divided into four levels: 20, 60, 180 and 540 nauplii/cm^2. The experiments of each density range were run with three replications.

Samples for measurement of dry weight, carbon, nitrogen and hydrogen were taken after the animals matured. The three replications were pooled and in one series of experiments 50 ovigerous females and in another series 200 egg-sacs were counted per sample, with usually 5 parallel samples being made for each density. The pre-counted females or egg-sacs were pipetted onto Waterman fibre-glass filters, which had been previously ashed in a muffle furnace at 500 °C for 4 h and weighed on an electronic autobalance (Perkin-Elmer, AD-2). Immediately after filtering off the remaining seawater with a vacuum pump, the females or egg-sacs were rinsed briefly with distilled water and transferred with filters into pre-weighted silver cartridges for freeze-drying at < 10 mbar for at least 12 h, using a GT-2 (Leybold-Heraeus™) apparatus. Dry weight and content of carbon, nitrogen and hydrogen were determined in ovigerous females, as well as in egg-sacs, by means of an Elemental Analyser (Model 1106, Carlo Erba Science). Energy equivalents were calculated from carbon by the N-corrected formula given by Salonen et al. (1976) and expressed in Joule units (1 J = 0.239 cal.).

RESULTS

The values for dry weight, carbon, nitrogen and hydrogen of ovigerous females and egg-sacs of *Tisbe holothuriae* are summarized in Table 1.

The dry weight of both egg-sacs and females depends significantly on the culture density during their larval development. For egg-sacs dry weight decreases with increasing density, from 3.48 to 1.94 μg; for ovigerous females, from 10.80 to 6.58 μg (Fig. 1). The percentage of the dry weight of egg-sacs in relation to that of ovigerous females ranges from 27 % to 32 % (30 % on average), obviously with a tendency to decrease with

Table 1. The values of dry weight (DW), content of carbon (C), nitrogen (N) and hydrogen (H), C/N and C/H ratio and energy equivalents (Joule) per individual and per mg DW in the egg-sac and the maternal body of ovigerous females reared at different densities. The values are expressed as a form of mean ($\bar{x}$) ± standard deviation. The percentages are related to the dry weight. As a rule, five analyses were taken as replicates, and one sample contained 200 egg-sacs, or 50 females, respectively

Density	(ind./cm^2)	Egg-Sac				Ovigerous Female			
		20	60	180	540	20	60	180	540
DW(μg)	($\bar{x}$)	3.4841	2.8846	2.1633	1.9399	10.7965	9.5205	7.9495	6.5800
	±	0.0740	0.1360	0.1510	0.1643	0.4361	1.3533	0.6902	0.5765
C(%)	($\bar{x}$)	52.5564	51.3859	51.0742	50.5666	45.7988	44.9255	41.4876	39.9465
	±	1.4074	2.3649	3.1534	2.4811	2.0891	2.7512	2.7006	2.7345
N(%)	($\bar{x}$)	12.6306	12.5657	12.0250	11.9410	10.7208	10.5567	9.7574	9.7269
	±	0.5232	0.4903	0.7277	0.6771	0.6377	0.6237	0.6674	0.6189
H(%)	($\bar{x}$)	9.0796	8.3298	7.8267	7.7846	7.4738	7.2506	6.6366	6.1667
	±	0.2959	0.3404	0.5018	0.3721	0.5342	0.4239	0.4837	0.3161
C(μg)	($\bar{x}$)	1.8307	1.4799	1.1013	0,9794	4.9382	4.2548	3.2846	2.6168
	±	0.0391	0.0276	0.0274	0.0717	0.0833	0.4553	0.1102	0.0938
N(μg)	($\bar{x}$)	0.4398	0.3620	0.2593	0.2314	1.1556	1.0010	0.7723	0.6375
	±	0.0104	0.0082	0.0071	0.0205	0.0369	0.1200	0.0229	0.0279
H(μg)	($\bar{x}$)	0.3163	0.2400	0.1688	0.1510	0.8053	0.6889	0.5255	0.4045
	±	0.0109	0.0066	0.0057	0.0152	0.0330	0.0955	0.0276	0.0198
C/N	($\bar{x}$)	4.1635	4.0885	4.2478	4.2366	4.2749	4.2556	4.2526	4.1060
	±	0.0972	0.0332	0.0979	0.0768	0.0707	0.0581	0.0362	0.0459
C/H	($\bar{x}$)	5.7902	6.1682	6.5267	6.4973	6.1317	6.1979	6.2584	6.4736
	±	0.1276	0.0644	0.0642	0.1729	0.1741	0.2063	0.2799	0.1392
J/mg DW	($\bar{x}$)	21.9768	21.2474	21.0664	20.7355	17.8595	17.3645	15.4376	14.6086
	±	0.9018	1.5089	1.9842	1.5598	1.2008	1.5860	1.4923	1.4597
J/ind	($\bar{x}$)	0.0765	0.0611	0.0453	0.0401	0.1925	0.1640	0.1220	0.0955
	±	0.0025	0.0019	0.0017	0.0031	0.0066	0.0160	0.0032	0.0036

increasing culture density. On the other hand, the dry weight in egg-sacs varies in accordance to that in ovigerous females. There is a linear relationship of dry weight between the egg-sac and ovigerous female. The regression equation is shown in Figure 2. It is suggested that about 0.3 μg dry weight is gained in the egg-sac, with an increase of 1 μg dry weight in the ovigerous female.

The carbon content, expressed either as the absolute weight per individual or the relative percentage of the body dry weight in the ovigerous females, decreases with increasing density from 4.94 to 2.62 μg or, respectively, from 45.80 to 39.95 %. Similarly, the absolute and the relative content of nitrogen and hydrogen in ovigerous females decreases with increasing density, from 1.16 to 0.64 μg and 10.72 to 9.73 % for nitrogen, and from 0.81 to 0.40 μg and 7.47 to 6.17 % for hydrogen. Females reared at a lower density offer higher contents of all three elements than those reared at a higher density (Fig. 3a).

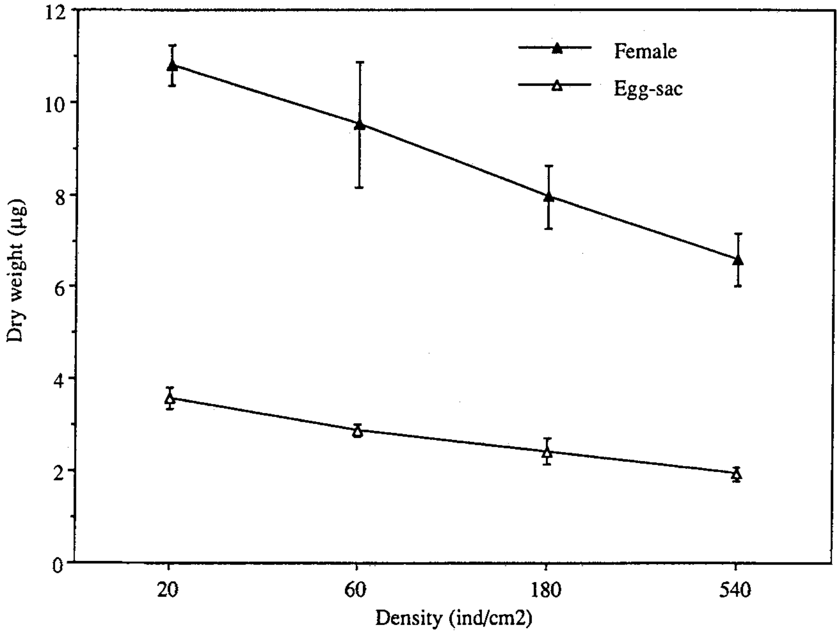

Fig. 1. Density-dependent variations of dry weight in ovigerous females and egg-sacs of *Tisbe holothuriae*

Also, the energy equivalents in ovigerous females show an apparent decrease with increasing density. They range from 17.86 to 14.61 J/mg dw, and 0.19 to 0.09 J/ind. However, there is no indication that the C/N ratio of ovigerous females decreases with increasing density.

In comparison with the ovigerous females, the carbon content in egg-sacs, expressed as absolute and relative contents, displays a similar tendency. With increasing density, the absolute contents of carbon per individual drops markedly from 1.83 to 0.98 µg, while the relative contents of carbon per mg dry weight in egg-sacs ranges from 52.56 to 40.57 % of dry weight. This value is much higher than that in ovigerous females.

With increasing density the nitrogen content changes in relation to dry weight, from 12.63 to 11.94 %, and in absolute weight per individual, from 0.44 to 0.23 µg. The hydrogen content in the egg-sacs is also reduced from 9.08 to 7.78 % of the dry weight, and from 0.31 to 0.15 µg per individual. As in carbon, the relative content of nitrogen and hydrogen is much higher than that in females.

The C/N ratio in the egg-sacs does not show any tendency with regard to the density. It has a similar value for the ovigerous females, fluctuating between the range of 4.09 and 4.27. The energy equivalents in the egg-sacs are much higher than those in ovigerous females. It is about 21 J per mg dry weight; however, the differences of energy equivalents of egg-sacs appeared obscure among all four densities (Fig. 3b and Table).

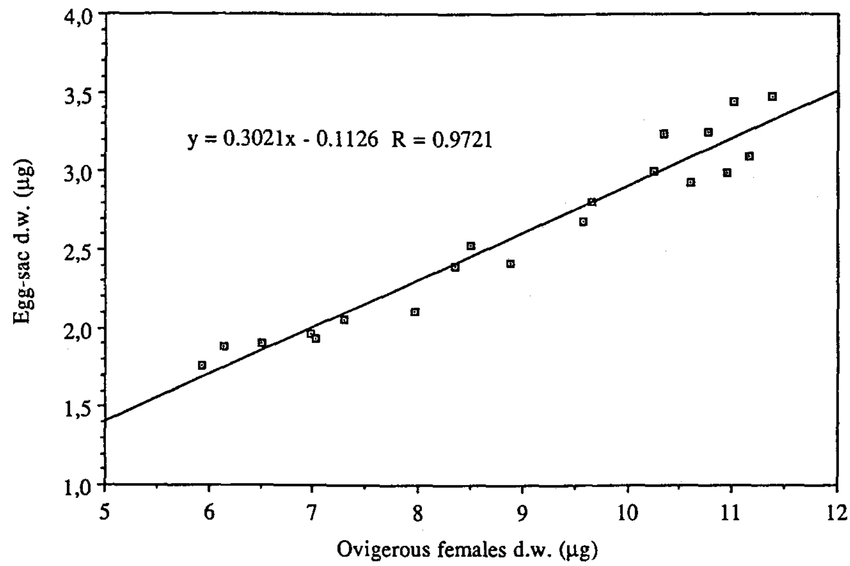

Fig. 2. Relationship of dry weight between egg-sac and ovigerous female

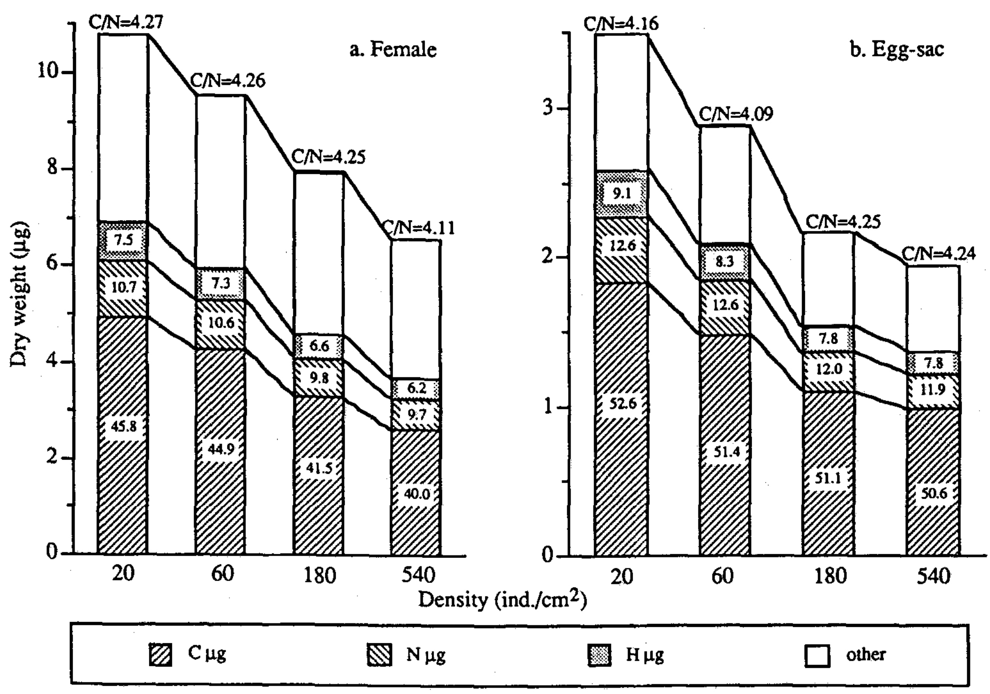

Fig. 3. Elementary composition of ovigerous females (a) and egg-sacs (b) of *Tisbe holothuriae*. The length of the blocks corresponds to the weight fraction of body dry weight at different densities. The number in the blocks indicates the CHN percentage of dry weight

DISCUSSION

According to previous literature, the dry weight and chemical composition of copepods can be influenced by various factors, for example, by season and life cycle (Båmstedt, 1976), food (Guerin & Gaudy, 1977), temperature and nutritional conditions (Schwenzer, 1985), and starvation (Ikeda, 1971). The results presented here show that culture density has a marked effect on the dry weight and chemical composition in both egg-sacs and their maternal ovigerous females of *Tisbe holothuriae*. High density causes a decrease in dry weight and contents of carbon, nitrogen, and hydrogen in egg-sacs and ovigerous females. According to our experiments, the number of nauplii hatched per egg-sac also decreased with increasing density (Zhang & Uhlig, 1993). In combination with this data, it is suggested that crowding conditions affect female productivity by reducing not only the size, but also the quality of egg-sacs produced, as reflected by the chemical contents and composition. It depends largely on the maternal body. Similar results were obtained in *Parenchaeta norvegica* (Nemoto et al., 1976).

In general, the range of dry weight and chemical composition as found in *Tisbe holothuriae* agrees with the data presented in literature for marine copepoda (Ikeda, 1974; Guérin & Gaudy, 1977; Schwenzer, 1985). Dry weight and chemical composition in the egg-sac depends on the maternal body. However, a much higher percentage of carbon, nitrogen and hydrogen was found in the egg-sac. Besides, the energy equivalence per weight unit (mg) was higher in the egg-sac than in the ovigerous female. This indicates that the total organic material was reassigned during the period of egg-sac production, and that more energy is stored in the egg-sac. Nemoto et al. (1976) presented in *Parenchaeta norvegica* some examples of the partitioning of chemical components of the maternal body into the egg-sac. A relatively higher carbon content was observed in the egg-sac (63.6 %) than in the females (53.0 %). Also, he found a higher C/N ratio in the egg-sacs (11.0) than in the females (5.2). In our experiment, however, we did not find obvious differences of C/N ratio between the egg-sacs and ovigerous females: the contents of both carbon and nitrogen increased equally during egg-sac formation. Furthermore, with increased density, there are no obvious changes in the C/N ratio in egg-sacs and ovigerous females. Obviously, the chemical composition is not affected by the density. On the contrary, as a general rule, the C/N ratio is strongly affected by nutritional conditions (Omori, 1969; Guérin & Gaudy, 1977; Schwenzer, 1985). In our experiment, apart from variations in density, all the cultures were kept under identical environmental conditions, always offered the same kind of food, and also a surplus of this. The relatively steady value of C/N ratio indicates that the population density exerts its influence equally on the total amount of the organic material, but not on the relative proportions of this.

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