

Chemical composition and caloric content of egg and zoea of the hermit crab *Eupagurus bernhardus*

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KURZFASSUNG: Chemische Zusammensetzung und Kaloriengehalt von Ei und Zoea des Einsiedlerkrebse *Eupagurus bernhardus*. Im Laufe der vollständigen Eientwicklung findet eine Zunahme von Wasser um 31,5 %, Asche um 5,7 %, Eiweiß um 8,8 % und Nicht-Eiweiß-Stickstoff um 0,2 % statt. In der gleichen Zeit sinkt der Fettgehalt von 29,5 % auf 14,8 % und der Energiegehalt von 6292 auf 5282 cal/g organischer Substanz. Der Kumulativ-Nutzeffekt der Entwicklung von einem Ei zur Zoea-Larve beträgt 70,0 %, 55,3 %, 79,4 % bzw. 35,0 % bezogen auf Trockengewicht, Kaloriengehalt, Eiweiß bzw. Fett. Von den 0,0722 cal, die sich auf den Stoffwechsel der gesamten Entwicklung eines Eies beziehen, werden nur 28,4 % aus Eiweiß gewonnen; 66,6 % werden durch Oxydation von Fett beigetragen.

INTRODUCTION

Studies on the rate and efficiency of transformation of matter and energy in the developing eggs of crustaceans have received considerable attention in recent times (URBANI 1959, KINNE 1960, 1961, McLAREN 1966, TAUBE & NAUWERCK 1967, PANDIAN 1967a). It has been shown that freshly hatched protozoa larvae of the shrimp *Crangon crangon* have utilized about 55 % of the energy contained in the egg and that oxidation of fat was the main energy source for metabolism of the embryo (PANDIAN 1967a). This paper reports on the chemical composition and caloric content of the egg and zoea of the common hermit crab *Eupagurus bernhardus*.

MATERIAL AND METHODS

A collection of *E. bernhardus* LINNAEUS (Paguridae, Anomura) was made near Helgoland in March 1967. All specimens were maintained under laboratory conditions. Immediately after copulation, the mother animals were transferred into suitable aquarium cylinders containing running sea-water maintained at about 12° C. The fresh eggs were round, with an average diameter of about 750 μ , and deep violet in color. As the development proceeded, they became more or less oval and lighter in color; after a 35 to 40 day-incubation period (SCHUMANN unpublished) the zoea hatched. Eggs freshly attached to the pleopods and fresh hatched zoeae were collected for further studies.

Details on the methods used have been published by PANDIAN (1967a). A definite number of eggs or zoeae was counted, washed free from adhering salt particles, weighed and dried at 80° C for a period of 4 hours. All weighings were made using a Sartorius balance with a sensitivity of ± 0.01 mg. Ash content was determined by incinerating the sample at 500° C for 5 hours. Protein content was estimated by nitrogen analysis following a standard micro-Kjeldhal procedure described by ROTH (1958). Caloric content was determined in a semi-micro bomb calorimeter (Parr Instrument Co., Model 1412). Three zoea samples were not completely combusted; the respective results were discarded.

Except for water content all percentages given hereafter are based on dry weight.

RESULTS

Chemical composition and caloric content

Water content: Due to technical difficulties water content of the samples could not be determined precisely. Results based on crude estimations suggest that water content increased from about 45.6 % in the fresh egg to 77.1 % in the zoea.

Dry weight: Total number of eggs and zoeae counted to determine the dry weights of a single egg and zoea are given in Table 1. A single egg weighed 26.7 μ g, a zoea 18.7 μ g; this indicates a net dry weight loss of 8.0 μ g during the entire development.

Table 1

Estimation of dry weight of a single egg of *Eupagurus bernhardus* at the beginning and end of the embryonic development

Developmental stage	Number of mother animals	Total number of eggs counted	Mean dry weight of one egg (μ g)	Standard deviation (μ g)	Coefficient of variability (%)
Fresh egg	7	7496	26.7	± 2.3	8.6
Zoea larva	5	8725	18.7	± 2.5	13.4

The samples collected in August 1967 gave very low values: 19.2 μ g for the egg and 12.5 μ g for the zoea (based on 1000 counts in each case). It is likely that late summer eggs were less heavy than early spring eggs. Some information from literature supports this assumption. CRISP (1959) reported that the eggs of *Balanus balanoides* of warm waters were smaller than those of cold waters (see also BARNES & BARNES 1965). Maintaining warm water eurythermal barnacles at low temperatures, PATEL & CRISP (1960) found that these produced larger eggs than at higher temperatures. PATEL & CRISP further reported that nauplii hatched from eggs developed *in vitro* outside the mantle cavity were larger at low temperature. These results suggest that temperature affects both size of the egg and embryo.

Ash content, which was 3.8 % in the fresh egg, increased to 9.5 % in the zoea, amounting to about a 250 % increase from its initial value (Table 2). Since salts are added to the dry weight of a zoea, it is obvious that the net dry weight (organic substance) loss by a developing egg is greater than 8.0 μg . Therefore, the dry weight of organic substance per egg and zoea was calculated, using the values given in Tables 1 and 2; it amounted to 25.7 μg per egg and 16.9 μg per zoea (see Table 4); thus the net loss of (dry) organic substance amounts to 8.8 μg during the entire embryonic development.

All estimations were made on samples collected in early spring, except those on nitrogen which were conducted on late summer material. As pointed out above, egg and zoea had different sizes in early spring and late summer, respectively. It is,

Table 2

Chemical composition of the eggs of *Eupagurus bernhardus* at the beginning and end of the embryonic development. The numbers enclosed in brackets indicate the number of estimations made

Developmental stage	Ash (%)	Protein (%)	Nonprotein nitrogen (%)	Fat (%)
Fresh egg	3.8 (4)	66.0 (3)	0.7 (3)	29.5
Zoea larva	9.5 (6)	74.8 (3)	0.9 (3)	14.8

however, assumed that these differences did not affect the chemical composition of either. Protein content increased from 66.0 % in the egg to 74.8 % in the zoea. In the corresponding stages non-protein nitrogen increased from 0.7 % to 0.9 % (Table 2).

No direct estimation of fat content was made. Since the weights of organic substance and protein as well as non-protein nitrogen contents are known, and assuming that carbohydrates were in trace quantities, fat contents of egg and zoea were calcu-

Table 3

Caloric content of the eggs of *Eupagurus bernhardus* at the beginning and end of the embryonic development

Developmental stage	Number of estimates made	Energy content (cal/g dry wt.)	Standard deviation	Energy content (cal/g dry organic substance)
Fresh egg	5	6053	± 388	6292
Zoea larva	5	4780	± 496	5282

lated. Fat content, which was 29.5 % in the egg, decreased to 14.8 % in the zoea (Table 2). Correspondingly, the caloric value also decreased from 6292 cal/g organic substance in the egg to 5282 cal/g organic substance in the zoea (Table 3).

Efficiency of yolk utilization

From the values presented in Tables 1 to 3, average chemical composition and caloric content of a single egg at the beginning and end of embryonic development are obtained (Table 4). The cumulative efficiency values (the ratio of: body formed to

Table 4

Chemical composition and caloric content of a single egg of *Eupagurus bernhardus* at the beginning and end of the embryonic development. All the values, except those of caloric contents, are given in micrograms

Substance	Fresh egg	Zoea larva
Dry weight	26.70	18.70
Ash content	1.01	1.78
Organic substance	25.69	16.92
Protein	17.62	13.99
Non-protein nitrogen	0.19	0.17
Fat	7.88	2.76
Caloric content (cal)	0.1616	0.0894

yolk used for metabolism + body formed, GRAY 1928) were 70.0 %, 65.9 %, 55.3 %, 79.4 % or 35.0 % for dry weight, organic substance, energy content, protein or fat. These values are in agreement with those reported for other crustaceans as *Crangon crangon*, *Ligia oceanica*, and *Homarus gammarus* (PANDIAN 1967a, b, c). The species specific differences observed in regard to the efficiency values indicate variations in efficiencies – possibly also rates – with which the different substances are utilized by the embryo. *E. bernhardus* expended 0.0722 cal on metabolism during its development from egg to zoea. Of this amount only 28.4 % ($3.63 \mu\text{g protein} \times 5650 \text{ cal/g protein} = 0.0205 \text{ cal}$, Table 4) was drawn by protein oxidation, while as much as 66.6 % ($5.12 \mu\text{g fat} \times 9400 \text{ cal/g fat} = 0.0481 \text{ cal}$) was supplied from fat. Thus, it may be concluded that oxidation of fat is the main energy source for the embryonic metabolism of *E. bernhardus*.

DISCUSSION

Water content (31.5 %) and inorganic salts (5.7 % or 0.77 $\mu\text{g/egg}$) increase considerably during the embryonic development of the hermit crab *Eupagurus bernhardus*. Such increases seem to be a common feature in developing crustacean eggs, e. g., in *Crangon crangon*, *Ligia oceanica*, *Homarus gammarus* and *Artemia salina* (PANDIAN 1967a, b, c, d). While salts must have been absorbed from the surrounding seawater, it is not clear whether increase in water content is due to absorption of water and/or to the production of (metabolic) water by the oxidation of fat and protein.

Percentage values obtained for protein and fat contents of egg and zoea of *E. bernhardus* agree with those found in *C. crangon* (PANDIAN 1967a), except for some minor variations. The eggs of the former contain a higher protein content (66.0 %) and lower fat content (29.5 %) than those (58.7 % protein and 32.6 % fat) of the latter.

These variations are further reflected in the caloric contents of the eggs. The values were 6443 cal/g and 6292 cal/g dry organic substance of the eggs of *C. crangon* and *E. bernhardus*, respectively. They do not appear to be related to the different feeding habits. Completely herbivorous crustaceans like *Artemia salina* (feeding mainly on green algae) and *Ligia oceanica*, said to feed exclusively on *Fucus vesiculosus* (NICHOLLS 1931), produce eggs with quite different caloric properties; the caloric values per gram dry organic substance amount to 5637 and 6000 in the eggs of *A. salina* and *L. oceanica*, respectively. Thus, these variations appear to have some other ecophysiological significance, which will be discussed elsewhere.

The main energy source for the embryonic metabolism of *E. bernhardus* was fat oxidation (66.6 %); only 28.4 % of the total energy was derived from protein. Eggs of *Crangon crangon* use only 20.8 % protein (PANDIAN 1967a). Fat serves as main energy source for the developing eggs of *Ligia oceanica* and *Homarus gammarus* (PANDIAN 1967b, c). In fact, it appears that marine molluscs also use fat as main energy source during their embryonic development. Thus, as much as 61.5 % of the total energy used was drawn from fat oxidation in developing eggs of the squid *Loligo vulgaris* (STOLFI 1933). The slipper limpet *Crepidula fornicata*, has also been found to use larger quantities (66 %) of fat (PANDIAN 1967e). According to OCKELMANN (personal communication), in many marine lamellibranchs, the dense fat globules of fresh eggs gradually disappeared as development proceeded. This suggests again utilization of considerable quantities of fat during development. Upon oxidation, fat releases large quantities of water (1 g fat releases 1.07 g water, 1 g protein only 0.41 g water; BALDWIN 1964, p. 52) and unlike protein, fat oxidation does not result in ammonia production, the removal of which costs considerable quantities of water. These two properties of fat, namely production and conservation of water, obviously represent advantages for eggs of marine organisms to which water is not as readily available as is the case in freshwater species. Such a generalization may, however, be premature; it would differ from the concept of NEEDHAM (1950, pp. 39-41). Depending on the nature of the substance used as energy source during the development, NEEDHAM distinguished between terrestrial animals with fat as main energy source, and aquatic animals, which derive their energy primarily by oxidizing protein. Further work in this field is urgently needed.

SUMMARY

1. Chemical composition and caloric content of egg and zoea of the hermit crab *Eupagurus bernhardus* have been determined and the cumulative efficiencies of yolk utilization calculated.
2. The cumulative efficiencies of yolk utilization for different constituents were 70.0 %, 55.3 %, 79.4 % or 35.0 % for dry weight, caloric content, protein or fat.
3. Since the efficiencies with which the different constituents of yolk utilized vary, the chemical composition of the developing eggs undergoes considerable changes. While fat and caloric content showed remarkable decreases, water, ash and protein contents of the eggs increased.

4. Of 0.0722 cal expended on metabolic processes of the embryo, only 28.4 % was drawn from the oxidation of protein, while fat contributed as much as 66.6 %.
5. Considerable quantities of inorganic salts (0.77 $\mu\text{g}/\text{egg}$) are absorbed from the sea-water by the developing egg.

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