Changes in chemical composition and caloric content of developing eggs of the shrimp *Crangon crangon*[1, 2]

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KURZFASSUNG: **Änderungen in der chemischen Zusammensetzung und im Kaloriengehalt sich entwickelnder Eier der Garnele *Crangon crangon*.** Pro Trockengewichtseinheit ergeben sich während der gesamten Eientwicklung von *Crangon crangon* L. folgende relative Zunahmen: Wasser 16,8 %, Asche 5,9 %, Eiweiß 10,6 %, Nicht-Eiweiß Stickstoff 0,5 %. In gleichem Zeitraum sinken die entsprechenden Werte für den Fettgehalt von 32,6 % auf 15,6 % und für den Energiegehalt von 6443 auf 5287 cal/g organische Substanz. Für die Entwicklung der aus dem Ei geschlüpften Protozoea beträgt der Kumulativ-Nutzeffekt 70,3 % für Trockengewicht, 54,0 % für Energie, 83,0 % für Eiweiß und 33,6 % für Fett. Während der ganzen Entwicklung eines Eies werden im Mittel 0,0453 cal für den Stoffwechsel aufgewendet; davon stammen 20,8 % aus dem Eiweiß und 75,0 % aus der Fettoxydation. Im Verlauf der Eientwicklung werden offensichtlich beträchtliche Mengen anorganischer Salze (0,29 μg pro Ei) aus dem umgebenden Meerwasser absorbiert.

INTRODUCTION

Our knowledge on the chemical embryology of vertebrates and insects has been summarized by NEEDHAM (1931, 1950). SMITH (1957) reviewed the pertinent publications on the early development and hatching of fishes; since then, contributions on rates and efficiencies of yolk utilization have been made by KINNE & KINNE (1962), LASKER (1962), FAUSTOV & ZOTIN (1964), BLAXTER & HEMPEL (1966) and FLÜCHTER & PANDIAN (1967). The fact that WATERMAN's (1960) "The Physiology of Crustacea" does not contain a corresponding chapter, indicates how sadly this aspect has been neglected thus far. Comparative studies on chemical composition and caloric content of crustacean eggs and larvae may indeed greatly stimulate and enhance our appreciation of the different ecological niches occupied, the adaptive changes in numbers and sizes of crustacean eggs and the varying morphological stages at which their larvae hatch. The present paper reports on changes in the chemical composition and caloric content observed in developing eggs of the shrimp *Crangon crangon*.

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[1] This paper is based on a lecture presented during the Annual meeting of the "International Council for the Exploration of the Sea" in Hamburg, October, 1967.

[2] Dedicated to Prof. R. V. SESHAIYA, the founder of the Marine Biological Laboratory, Porto Nova, South India, on his 70th birthday.

MATERIAL AND METHODS

Material

Crangon crangon LINNAEUS (Family: Crangonidae) carry developing eggs during June and July (50 to 70 % frequency of egg-carrying females), although females with eggs can be seen in small numbers (about 5 %) during winter. For further details on the bionomics of *C. crangon* consult LLOYD & YONGE (1947). Our shrimps were collected near Helgoland (Southern North Sea) during April and May, 1967 and transferred into large aquaria (capacity 2500 l). The egg bearing females were later transferred into smaller aquarium cylinders (capacity 5 l) containing non-circulated but vigorously aerated sea-water of 32 ‰ salinity maintained at 12.0° ± 0.1° C. The shrimps were fed with cod flesh every other day.

For chemical analyses, the following arbitrary stages were chosen:

S t a g e I : Undeveloped eggs and cleavage stages. These were the earliest stages obtainable since transfer of fertilized eggs to the appendages takes 24 hours or more; during this period of time the eggs seem to have undergone as many as eight divisions and are approaching the blastula stage. Eggs of stage I have an average diameter of 425 μ, are creamy white in color and round to oval in shape.

S t a g e I I : Oval eggs with diameters of 488 μ; the embryo can be seen on the yolk; they have clearly recognizable brownish, elliptical eye spots with a vertical diameter of 13 μ.

S t a g e I I I : Eggs with diameters of about 575 μ, filled by a transparent larva with appendages; eye spots are broader and oval in shape with a vertical diameter of 150 μ; a pumping heart can be seen. Females carrying eggs of stage III can easily be recognized; they become restless moving about the water surface; eventually protozoeae hatch within the same or the next night.

S t a g e I V : Freshly hatched protozoeae larvae of about 1.8 mm body length. In order to prevent caloric losses by the actively swimming protozoeae, they were collected immediately after hatching for analyses.

Methods

Of each of the above mentioned four stages 1000 to 4000 eggs or larvae were counted. As sea-water adhering to the egg surface may increase ash weight by about 20 % (FLÜCHTER & PANDIAN 1967), eggs and larvae were washed free from the adhering sea-water by exposing them three times to distilled water for 1 minute each time. After drying at 80° C for 4 hours, the test materials were weighed in a Sartorius balance (type 2604) sensitive to ± 0.01 mg.

Ash content was estimated keeping the sample in a crucible of known weight in a muffle furnace at 500° C for a period of 5 hours, as recommended by PAINE (1964).

Protein was precipitated by grinding the sample with 0.5 ml of cold trichloroacetic acid in a glass mortar; the contents were subsequently centrifuged. The supernatant contained the non-protein nitrogen, while the precipitate was protein (GIESE

et al. 1959). Nitrogen contents were determined following a standard micro-Kjeldahl procedure described by ROTH (1958). Protein contents were determined as albumin equivalent by estimating the nitrogen and multiplying the values obtained by 6.25. (Nitrogen occurs in the different proteins in a fairly constant percentage – 16 % on the average, and the factor 6.25 is taken by dividing 100 by 16.)

Caloric contents were determined with a Parr 1412 semi-micro bomb calorimeter. Since the sample available was less than 20 mg in most cases, a known amount of benzoic acid was added as trigger substance.

RESULTS

Changes in chemical composition

Water content. Although eggs and protozoeae were blotted carefully and always in the same manner, it was realized in advance, that varying quantities of water would be retained both on the surfaces of individual eggs and between the neighbouring eggs. The values obtained indeed showed considerable variations. However, the results indicate a remarkable increase in water content from about 68.5 % in the egg stage I to about 87.3 % in the protozoea.

Dry weight. Table 1 gives the mean dry weight of a fresh egg and the changes in its weight during subsequent development. The mean dry weight of an egg decreased from 16.63 μg in stage I to 12.38 μg in stage III. The mean dry weight of a single protozoea is 11.69 μg; thus the total loss amounts to 4.94 μg during the whole embryonic development. Although the body size range of the egg-bearing females chosen was restricted (7.0 to 8.5 cm in length), egg size deviated up to 10.8 % from the mean value. Such variation in egg size does not seem to be uncommon among crustaceans; it has, for example, been demonstrated in cirripedes by BARNES & BARNES (1965). In *C. crangon* eggs, the range of variations became, however, less (8 %) in stages II and III. It seems possible that embryos in the smaller eggs utilized the available yolk more efficiently than those in the larger ones. Such a situation has been shown to exist in developing herring eggs (BLAXTER & HEMPEL 1966).

Table 1

Dry weight estimations of different developmental stages of *Crangon crangon*

Developmental stage	Number of mother animals	Number of eggs counted	Mean dry weight of one egg (μg)	Standard deviation	Coefficient of variation
Stage I	6	13782	16.63	± 1.79	10.8 %
Stage II	2	6000	15.15	± 1.07	7.1 %
Stage III	3	9000	12.38	± 1.03	8.3 %
Stage IV	6 to 10	16425	11.69	± 0.94	8.0 %

Ash content. Changes in chemical composition of the four developmental stages have been reported in Table 2. Ash content, which was 8.2 % in fresh eggs,

increased to 14.1 % in stage IV; this amount is a relative increase of 72 %. Ash content of all four stages were black in color even after 5 hours incineration at 500° C; further heating at 600° C for an additional 5 hours did not change the color of the ash of egg stage I. This may indicate the presence of lead containing ommochrome pigment in these developing eggs (see GOODWIN 1960, p. 134).

Table 2

Changes in chemical composition of developing eggs and freshly hatched protozoea (stage IV). Percentage values are based on dry weights. Brackets indicate the number of estimations made

Developmental stage	Ash (%)	Protein (%)	Non-protein nitrogen (%)	Fat (%)
Stage I	8.2 (7)	58.7 (4)	0.5 (4)	32.6
Stage II	9.2 (2)	58.7 (2)	0.6 (2)	31.5
Stage III	11.2 (3)	71.3 (2)	1.1 (2)	16.0
Stage IV	14.1 (7)	69.3 (4)	1.0 (4)	15.6

Protein content of the egg increased from 58.7 % in the stage I to 69.3 % in the protozoea (Table 2). Similarly, non-protein nitrogen increased about 0.5 %; this may be due to utilization of nitrogen for chitin formation; chitin, a non-protein substance, contains 6.9 % nitrogen (RICHARDS 1951).

Fat content. No direct determinations of fat contents have been made. Since dry weight, ash, protein and non-protein nitrogen contents are known (assuming carbohydrates etc. were in trace-quantities, as is the case in many fish eggs, SMITH 1957), fat contents were calculated. Unlike the other constituents, fat content decreased from 32.6 % in stage I to 15.6 % in the protozoea, amounting to a heavy loss of 52.2 % from the initial value.

Table 3

Changes in caloric contents of the developing eggs and freshly hatched protozoea of *Crangon crangon*. S. D.: standard deviation, C. V.: coefficient of variation

Developmental stage	No. of estimates made	Energy content (cal/g dry weight)	S. D.	C. V.	Energy content (cal/g dry organic substance)
Stage I	5	5915	± 254	4.3 %	6443
Stage II	2	5602	± 117	2.1 %	6170
Stage III	3	5165	± 315	6.1 %	5856
Stage IV	6	4544	± 205	4.1 %	5287

Caloric content per g dry weight and per g dry organic substance are given in Table 3. Caloric content per unit dry weight decreased progressively from 6443 cal/g in stage I to 5287 cal/g dry organic substance in the protozoea.

Efficiency of yolk utilization

From the values presented in Tables 1 to 3, average changes in chemical composition and caloric content of a single egg from stage I to stage IV have been calculated; the values obtained are shown in Table 4. Except for ash and non-protein nitrogen, the constituents of the egg decrease, as development progresses (Fig. 1). The

Table 4

Average changes in chemical composition and caloric content in a developing egg and a freshly hatched protozoea of *Crangon crangon*. All weight units are given in μg.
(Data based on Tables 1 to 3)

Substance	Stage I (egg)	Stage II	Stage III	Stage IV (protozoea)
Dry weight	16.63	15.15	12.38	11.69
Ash	1.36	1.39	1.44	1.65
Organic substance	15.27	13.76	10.94	10.04
Protein	9.76	8.89	8.83	8.10
Non-protein nitrogen	0.08	0.09	0.14	0.11
Fat	5.42	4.77	1.98	1.82
Energy (cal/egg)	0.0984	0.0849	0.0639	0.0531

ratio "body formed/body formed + yolk used for metabolism" may be used as a measure of the efficiency of development at any particular stage (GRAY 1928). It is well known that with advancing age or body weight the efficiency to convert yolk or nutrient matter decreases (IVLEV 1939, PANDIAN 1967a). Following BLAXTER & HEMPEL (1966), I have focussed my attention on the "cumulative efficiency" of the total development. Wet weight, dry weight, organic substance, caloric content, protein content, and/or fat content may be used as indices. The efficiency values were 70.3 %, 65.7 %, 54.0 %, 83.0 % and 33.6 % for dry weight, organic substance, caloric content, protein and fat, respectively. The differences in the values show that the efficiency, with which different substances of the yolk are utilized, varies considerably. The last two values are especially significant in that only 17.0 % of the total protein content of the fresh egg is spent on metabolic processes during the entire embryonic development, while as much as 66.4 % of the fat content is used for that purpose. Thus, during embryonic development, fat was the main energy source for metabolic processes.

DISCUSSION

The results presented bring out several interesting aspects in regard to changes in the relative proportions of yolk constituents during the embryonic development of *Crangon crangon* eggs. A net increase of 16.8 % water explains the increase in egg diameter from 425 μ in the fresh egg to 575 μ in stage III. This observation agrees particularly well with findings of NEEDHAM & NEEDHAM (1930) and RAMULT (1930); the first named authors found an increase in water from 63.3 % in the egg to 83.7 % in the freshly hatched zoea of the sand crab *Emerita analoga*. RAMULT (1930), working

on cladocerans, assumed that the egg swells progressively and that the larva finally hatches due to the bursting of its membranes and owing to increasing hydrostatic pressure. In *C. crangon,* it is not clear, whether the entire increase in water content is due to the absorption of water and/or to the retention of metabolic water released by the oxidation of fat.

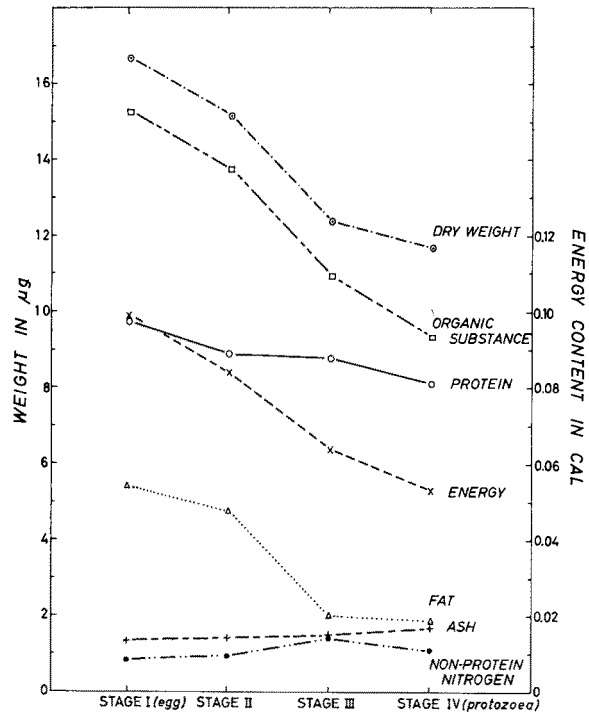

Fig. 1: Changes in chemical composition and caloric content of a single developing egg and protozoea of the shrimp *Crangon crangon*

The increase in ash amounted to about (0.29 µg per egg) 72 % of the initial content. BALDWIN (1964, p. 12) reported that an egg of *Sepia* contains only 0.8 mg at the beginning of its development and no less than 3.3 mg at the end. Likewise, developing sea urchin eggs obtain additional salts from the surrounding sea-water. Among crustaceans, developing eggs of *Ligia oceanica, Homarus gammarus, Artemia salina* (PANDIAN 1967b, c, d) and *Eupagurus bernhardus* (PANDIAN & SCHUMANN 1967) have been shown to obtain considerable quantities of salts from the sea-water.

No data on protein and non-protein nitrogen contents of the developing crustacean eggs are available, except for some scattered values on the total nitrogen content of eggs collected by VINOGRADOV (1953); 9.1 % for eggs of *Homarus gammarus* (Table 231, p. 379) and 6.3 % for portunid megalopas, 7.2 % for portunid zoea and crangonoid larvae and 9.7 % for nauplii of *Balanus balanoides* (Table 232, p. 380).

The corresponding values obtained for *C. crangon* are higher: 9.9 % in the fresh eggs and 12.1 % in the protozoea.

In regard to fat content, it is necessary to justify the values obtained by calculation. The caloric value decreased from 6443 cal/g dry organic substance in the fresh egg to 5287 cal/g dry organic substance in the protozoea. This decrease of 17.9 % in caloric content suggests that a substance containing large amounts of biologically usable energy has been expended during the course of development. This substance is obviously fat, as it alone contains 9400 cal/g. This assumption is supported by NEEDHAM & NEEDHAM (1930, p. 334) reporting that "this crustacean" *(Emerita analoga)* "egg is very lecithic, ... and" (lecithicity) "regularly declines as development proceeds". Moreover, in *C. crangon* the total energy expended by a single egg during the development was 0.0453 cal (Table 4). According to the estimated value, the loss of protein was 1.66 μg (1.66 μg protein $\times$ 5650 cal/g protein = 0.0094 cal); the derived value for the loss of fat was 3.6 μg or 0.034 cal (3.6 μg fat $\times$ 9400 cal/g fat = 0.034 cal). If the fat values calculated are correct, the energy loss of 0.0453 cal must be accounted for by the energy contents of protein and fat loss. Thus, the two substances accounted only for 0.0434 cal (out of 0.0453 cal), i. e. the accountable energy loss would be only 95.8 % of the total energy loss, and the "experimental error" 4.2 %.

The caloric values of the different developmental stages of *C. crangon* lie within the ranges reported for different micro-crustaceans by COMITA & SCHINDLER (1963). As has been said above, the caloric value per g dry organic substance decreased from 6443 cal in stage I to 5287 cal in stage IV (protozoea). A single value reported by SLOBODKIN & RICHMAN (1961) for the nauplii of *Artemia salina* amounts to 6737 ($\pm$ 863 cal) cal/g dry organic substance. PAFFENHÖFER (1967), concentrating on the study of the caloric losses of starving *A. salina*, found that the caloric value of the freshly hatched nauplii is 5953 ($\pm$ 60 cal) cal/g dry organic substance. Using the same stock as PAFFENHÖFER, PANDIAN (1967d) found the caloric value of *A. salina* eggs to be 5637 ($\pm$ 21 cal) g dry organic substance. Thus, per unit weight, *A. salina* nauplii contain about 5.3 % more energy than that of the eggs. A considerable decrease in caloric values have, however, been found in larvae of other crustaceans such as *Ligia oceanica*, *Homarus gammarus* (PANDIAN 1967b, c) and *Eupagurus bernhardus* (PANDIAN & SCHUMANN 1967); thus similar results were obtained in these species as in *C. crangon*. Working on the phosphorus metabolism of invertebrate eggs, NEEDHAM & NEEDHAM (1930, p. 343) have shown that the lipoid phosphorus content is high in unfertilized eggs of the sand crab *Emerita analoga* and declines during the development, whereas it is low in *Artemia salina* eggs in which it remains constant or may even increase as the development proceeds. In the light of the above mentioned results, it seems that *A. salina* uses a different energy source for its embryonic metabolism than *C. crangon* and the other crustaceans mentioned.

In *C. crangon* the cumulative efficiencies of embryonic development were 54.0 % for total energy, 83.0 % for protein and 33.6 % for fat. Corresponding values have been found in fishes, e. g. 67 % for energy in *Siluris glanis* (IVLEV 1939) and 62 % for protein in *Sardinops caerulea* (LASKER 1962). The efficiency values obtained in *C. crangon* are in good agreement with those reported for *Eupagurus bernhardus* by PANDIAN & SCHUMANN (1967).

It is important to know which substance is being oxidized to meet the metabolic needs of the embryo. Of the 0.0453 cal lost during the development by a single *C. crangon* egg, only 20.8 % (1.66 µg protein or 0.0094 cal) was drawn from protein, while as much as 75.0 % (3.6 µg fat or 0.034 cal) was supplied by fat. Depending on the nature of the substance used as source of energy during the embryonic development, NEEDHAM (1950, pp. 39 to 41) distinguished the terrestrial eggs with fat as main energy source, from all aquatic eggs, which derive the required energy by oxidizing their protein. The eggs of *C. crangon,* although undergoing development in aquatic environment, use fat as main energy source; this finding is in contrast to NEEDHAM's concept. The oxidation of fat in *C. crangon* eggs appears to represent an adaptation to the marine environment which is characterized by poor availability of water. The oxidation of nutrient matter releases metabolic water; 100 g fat releases 107.1 g water; an equal amount of protein or carbohydrate releases 41.3 g or 55.5 g water (BALDWIN 1964, pp. 52). Furthermore, oxidation of protein results in the production of ammonia, the removal of which costs much water.

SUMMARY

1. Changes in chemical composition and caloric content as well as the cumulative efficiencies of yolk utilization have been studied in the developing eggs and freshly hatched protozoea of the shrimp *Crangon crangon* L.
2. Per unit dry weight of the fresh egg the following relative increases were observed during the development: 16.8 % water, 5.9 % ash, 10.6 % protein, and 0.5 % non-protein nitrogen. During the same period (fresh egg to freshly hatched protozoea) fat content decreased from 32.6 % to 15.6 % and energy content from 6443 to 5287 cal/g dry organic substance.
3. The cumulative efficiencies of yolk utilization for the different constituents varied; they were 70.3 % for dry weight, 54.0 % for total energy, 83.0 % for protein, and 33.6 % for fat.
4. Of the 0.0453 cal expended on the metabolic processes of the embryo, only 20.8 % was drawn from the oxidation of protein, while fat oxidation contributed as much as 75.0 %.
5. Considerable quantities of inorganic salts (0.29 µg/egg) were absorbed from the surrounding sea-water by the egg during its development.

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