Caloric content of larvae of the brine shrimp *Artemia salina*[1]

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KURZFASSUNG: **Kaloriengehalt von Larven des Salinenkrebses *Artemia salina*.** Der Energiegehalt frisch geschlüpfter Nauplien (5953 cal/g organische Substanz) und 2 mm langer Larven (Metanauplius IV, 5854 cal/g organische Substanz) von *Artemia salina* LEACH wurde mit einem Parr 1412 Bombenkalorimeter ermittelt. Ferner wurde der Einfluß verschieden langer Hungerperioden auf den Kaloriengehalt dieser Tiere untersucht. Nach 72stündigem Hungern wurden für die Nauplien 5430 cal/g organische Substanz und für die 2 mm langen Larven 5115 cal/g organische Substanz ermittelt. Der Aschegehalt der Tiere stieg mit zunehmender Dauer der Hungerperiode. Anhand des Gewichts der geschlüpften und hungernden Nauplien wurde der Kaloriengehalt einzelner Tiere bestimmt: er reicht von 0,00924 cal pro Tier (gerade geschlüpfter Nauplius) bis 0,00459 cal pro Tier (Nauplius, 96 Stunden ohne Futter).

INTRODUCTION

The brine shrimp, *Artemia salina* LEACH, is frequently used as a food source for various animals kept under laboratory conditions for maintenance, as well as for experimental purposes. In experiments devoted to quantitative assessments of food intake and food conversion, exact information on the caloric content of the *Artemia* larvae fed is desirable. SLOBODKIN & RICHMAN (1961) determined the caloric content of *Artemia* nauplii with a bomb calorimeter. URBANI (1959) investigated the amount of glucose, protein and lipids in eggs and nauplii of *Artemia*, from which the caloric contents can be calculated. Caloric values of Crustacea vary from 4427 cal/g dry weight for immature crayfish to 7672 cal/g for *Calanus* copepodites (COMITA & SCHINDLER 1963).

This paper deals with the caloric contents of *Artemia* larvae of different ages and considers the effect of starvation.

MATERIAL AND METHODS

In autumn 1964, *Artemia* eggs were bought from DR. BAENSCH, 452 Melle, Germany. They were poured into a glass container with filtered sea-water of 32 ‰ salinity

[1] Dedicated to Professor Dr. FRIEDRICH KRÜGER on his 65th birthday, August 18, 1967.

and kept there for 10 hours at 30° C. The temperature was then lowered to 20° C and hatching occurred 15 hours later. The hatching nauplii were pipetted into a glass dish filled with 500 ml of filtered sea-water kept at 20° ± 0.3° C in the dark. The starving nauplii were transferred after 48 hours into newly filtered sea-water. At 20° C most nauplii died after 100 hours of starvation.

For calorimetration, *Artemia* nauplii were dried and the externally adhering salt was washed off twice by exposing them for periods of 25 seconds to distilled water. Thereafter they were transferred onto a glass slide, dried for 60 minutes at 105° C (LOVEGROVE 1962) and kept in a desiccator for 2 to 7 days until calorimetration.

The 7-day-old (2 mm long) *Artemia* larvae (Metanauplius IV) were raised and fed at 20° ± 0.3° C. Immediately after hatching, 3000 nauplii were collected and transferred into 1500 ml of filtered sea-water; 30 hours after hatching they had nearly consumed their yolk and obtained their first food (see also REEVE 1963). After this the nauplii were fed daily 1500 ml of a solution (350 cells/microliter) of the unicellular alga *Dunaliella*. The *Dunaliella* concentration was determined with a counting chamber. The culture water of the 2 mm long starving larvae was renewed daily.

To determine the ash content of *Artemia* the dried larvae were pressed to a pellet and weighed with a semi-microbalance (accuracy ± 0.01 mg). They were then burned at 520° C in a muffle-furnace during a period of 4 hours.

The caloric content was determined with a Parr 1412 semi-microcalorimeter (PARR 1958, 1960). The *Artemia* samples weighed between 12 and 40 mg. Benzoic acid was used as a trigger substance. After 8 to 10 determinations, the water equivalent of the calorimeter was measured using benzoic acid. It must be added that three times the combustion in the calorimeter was incomplete, resulting in values which were 10 to 20 % too low in comparison to samples burned up completely.

RESULTS

Before calculating caloric contents the amounts of ash per sample were determined. The subtraction of ash values made the estimation of the absolute caloric values

Table 1

Ash content of (a) hatched nauplii and (b) 7-day-old (2 mm long) larvae of *Artemia salina* as a function of the length of the starvation period

	Length of starvation period	Ash content (% of dry weight)	Number of estimations
(a)	0 hours	5.91	3
	24 „	9.84	2
	48 „	11.49	3
	72 „	13.47	2
	96 „	16.56	2
(b)	0 hours	11.68	8
	24 „	13.01	1
	48 „	15.00	2
	72 „	16.27	2

(cal/g organic substance) possible. Table 1 shows that the ash content of dried nauplii, expressed as percentage of dry weight, increases as a function of starvation time.

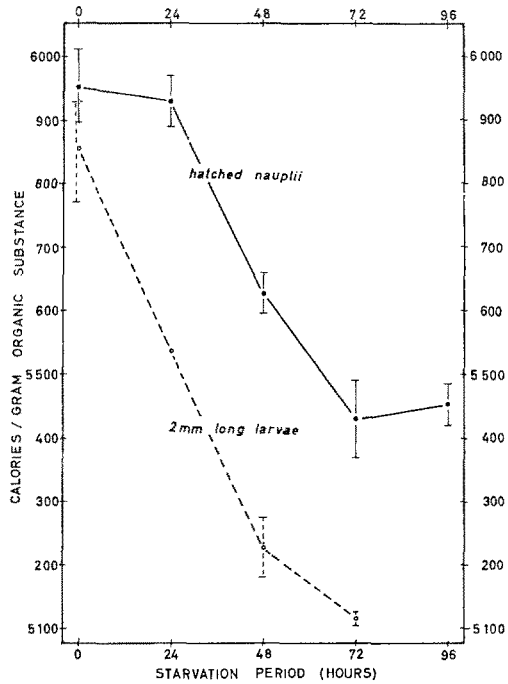

Fig. 1: Loss of energy of freshly hatched nauplii and 2 mm/long larvae of *Artemia salina* after varying starvation periods

Table 2

Caloric values of (a) hatched nauplii and (b) 2 mm long larvae of *Artemia salina as* a function of the length of the starvation period

Length of starvation period	cal/g organic substance	Number of estimations	Standard deviation	Coefficient of variation
(a) 0 hours	5953	4	± 60.03	1.01 %
24 „	5929	4	± 39.48	0.67 %
48 „	5627	4	± 32.62	0.58 %
72 „	5430	3	± 58.28	1.07 %
96 „	5454	3	± 31.91	0.59 %
(b) 0 hours	5854	10	± 77.93	1.33 %
24 „	5535	1	—	—
48 „	5227	2	± 45.19	0.87 %
72 „	5115	2	± 12.05	0.24 %

Table 2 and Figure 1 give the caloric values per organic substance (dry weight minus ash weight). These decrease during starvation. The 2 mm long *Artemia* larvae exhibit a steady decrease during the first 48 hours of starvation; thereafter the curve tends to

level off. The nauplii lose only small amounts of energy during the first day; thereafter caloric contents decrease more rapidly; after 72 hours, however, further losses are rather small.

Table 3

Weight and caloric content of single nauplii of *Artemia salina* after different periods of starvation

Starvation period	dry weight (mg)	Standard deviation	Coefficient of variation	Weight of organic matter (mg)	Caloric content/ individual (cal)
0 hours	0.00165	± 0.000038	2.3 %	0.00155	0.00924
24 „	0.00158	± 0.000027	1.7 %	0.00145	0.00854
48 „	0.00142	± 0.000041	2.9 %	0.00126	0.00725
72 „	0.00105	± 0.000041	3.9 %	0.00090	0.00489
96 „	0.00100	± 0.000020	2.0 %	0.00084	0.00459

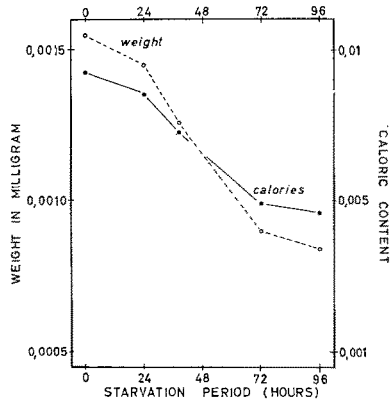

Fig. 2: Caloric content and organic dry weight of a single nauplius of *Artemia salina* after different periods of starvation

Figure 2 and Table 3 summarize the results obtained in regard to the energy and weight losses of individual *Artemia* nauplii during starvation. In contrast to the loss of organic material (Fig. 1), caloric contents and weight per individual decrease over the whole test period from 0 to 96 hours. During the first and last 24 hours, however, the decline is less marked. While the nauplii metabolize 50 % of their energy during the 96 hour test period, their weight loss amounts to about 39 %.

DISCUSSION

At a temperature of 20° C, the yolk of *Artemia* nauplii is used up 36 hours after hatching (REEVE 1963). The information obtained in the present study shows, however, that the nauplii possess enough energy to sustain high metabolic activities for an addi-

tional period of 36 hours. During the last quarter of the starvation tests (72 to 96 hours after hatching) swimming speed and movements of the nauplii become slower, possibly resulting in a more economic use of their energy reserves. A similar behaviour was observed after 48 hours of starvation in the 2 mm long *Artemia* larvae.

The rather small caloric loss during the first 24 hours (Figures 1, 2) can be explained by the fact that hatching nauplii are much smaller than 1- or 2-day-old individuals; consequently their energy consumption for maintenance and swimming is likely to be significantly lower.

URBANI (1959) states that during the first 20 hours after hatching the loss of glucose, and especially that of lipids, is relatively small; thereafter it increases rapidly. He terminated his experiments 50 hours after hatching. His results, although based on biochemical data, are in general agreement with the data presented in this paper.

STEPHENS & SCHINSKE (1961) point out that Crustacea do not take up amino acids from sea-water. According to PROVASOLI & SHIRAISHI (1959) *Artemia* larvae are unable to take up organic material from filtered sea-water in the absence of particulate matter. Heavy aeration may cause the formation of particulate matter from dissolved organic substances in sea-water (BAYLOR & SUTCLIFFE 1963). During our experiments *Artemia* larvae were kept in calm, non-aerated water. On the basis of the information cited above, it may be assumed, therefore, that our *Artemia* did not take up organic material from the culture medium.

The average caloric content of our newly hatched *Artemia* nauplii is 5953 cal/g organic substance. In nauplii of *Artemia* spec SLOBODKIN & RICHMAN (1961) found 6737 cal/g ash-free material. This difference may be due to different "egg" quality or to different breeding conditions.

Drying at 105° C for one hour did not cause a significant loss of volatile substance e. g. lipids: calorimetrations of nauplii dried at 80° C gave the same results as those of nauplii dried at 105° C. The drying period was one hour in all cases.

SUMMARY

1. Caloric contents of *Artemia salina* nauplii and larvae have been determined by employing a combustion calorimetry. Freshly hatched nauplii contain on an average 5953 cal/g organic substance; the respective value for 7-day-old (2 mm long) larvae is 5854 cal/g.
2. The effect of starvation on the caloric contents was investigated. After a 72 hour starvation period, nauplii contain 5430 cal/g organic substance, 2 mm long larvae 5115 cal/g.
3. The ash contents (expressed as % of body dry weight) of *Artemia* larvae increase as a function of the length of the starvation period.
4. After determining the weight of the nauplii, the caloric content of a single nauplius was calculated after different periods of starvation. It ranges from 0.00924 cal per freshly hatched nauplius to 0.00459 cal per nauplius starved for 96 hours.

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LITERATURE CITED

BAYLOR, E. R. & SUTCLIFFE, W. H., 1963. Dissolved organic matter in seawater as a source of particulate food. *Limnol. Oceanogr.* **8**, 369–371.

COMITA, G. W. & SCHINDLER, D. W., 1963. Calorific values of microcrustacea. *Science*, N. Y. **140**, 1394–1396.

LOVEGROVE, T., 1962. The effect of various factors on dry weight values. *Rapp. P.-v. Réun. Cons. perm. int. Explor. Mer* **153**, 86–91.

PARR Instrument Company, 1958. Instructions for the No. 1411 combustion calorimeter. Moline, Ill. (Manual No. **128**).

— 1960. Oxygen bomb calorimetry and combustion methods. Moline, Ill. (Manual No. **130**).

PROVASOLI, L. & SHIRAISHI, K., 1959. Axenic cultivation of the brine shrimp *Artemia salina*. *Biol. Bull. mar. biol. Lab., Woods Hole* **117**, 347–355.

REEVE, M., 1963. Growth efficiency in *Artemia* under laboratory conditions. *Biol. Bull. mar. biol. Lab., Woods Hole* **125**, 133–145.

SLOBODKIN, L. B. & RICHMAN, S., 1961. Calories/gm in species of animals. *Nature, Lond.* **191**, 299.

STEPHENS, G. C. & SCHINSKE, R. A., 1961. Uptake of amino acids by marine invertebrates. *Limnol. Oceanogr.* **6**, 175–181.

URBANI, E., 1959. Protidi, glucidi e lipidi nello sviluppo di *Artemia salina* LEACH. *Acta Embryol. Morph. exp.* **2**, 171–194.