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Yolk utilization and hatching time in the Canadian lobster *Homarus americanus**

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Abstract

During the course of its embryonic development, the Canadian lobster *Homarus americanus* MILNE-EDWARDS exhibits steady increases in water content (56.2 to 86.8%) and ash (5.8 to 21.2%), and a progressive decrease in energy content from 6636 to 4292 cal/g dry weight. Mean dry weight of a single egg is 965 µg, equivalent to 6.4 cal; a freshly hatched larva utilizes as much as 60.3% of the energy contained in the egg. The lobster hatches about 1,500 larvae per night over a period of 4 to 5 days. Dry weight, ash and calorific contents of larvae hatched on different days show considerable variations. After larvae hatch on the first day, continuous salt absorption by eggs to hatch on subsequent days leads to a steady increase in ash content from 143 µg/larva hatched on the first day to 255 µg/larva hatched on the fourth day, and consequently, to an increase in dry weight from 854 to 956 µg/larva. Metabolism of embryos (0.1 cal/day), which are yet to be hatched on subsequent days, depletes the calorific content per unit weight (from 4637 to 3837 cal/g dry weight) as well as per larva (from 3.98 to 3.67 cal).

Introduction

The female lobster *Homarus gammarus* carries about 15,000 fertilized eggs for a period of some 10 months; at the termination of successful incubation, hatching takes place; hatching occurs usually in the night and lasts for a period of 7 to 10 days (APPELLÖF, 1909). Hatching larvae in batches at intervals, is not uncommon among other decapod crustaceans although the duration of intervals between the successive hatchings may be shorter. Since it is just prior to hatching, that a considerable amount of energy is expended on embryonic metabolism, the larvae hatched in subsequent batches are likely to contain less energy than those hatched previously. This paper reports on yolk utilization efficiency and effects of hatching time in *Homarus americanus*.

Material and methods

A collection of *Homarus americanus* MILNE-EDWARDS (Nephropsidae; Macrura) was sent (via air) by a Canadian firm to the Biologische Anstalt Helgoland. A few of these Canadian lobsters were found to carry freshly attached eggs; one egg-carrying female was kept separately in a large aquarium (2,000 l capacity) with running sea water (30‰ S) at an ambient temperature of 14 °C ± 2 °C, and fed crabs and small fishes. Her eggs were used for chemical analyses and the following, arbitrarily chosen, developmental stages studied:

Stage I: November 8, 1967; early cleavage stage; dark green round eggs, with an average diameter of 1.5 mm.

Stage II: December 12, 1967; oval eggs with an average length of 1.7 mm and an average breadth of 1.5 mm; a disc-like milky green-blue embryo spot was visible on the animal pole of the dark green egg.

Stage III: June 16, 1968; orange-yellow colored, oval eggs (2.3 × 2.0 mm). Embryo fills up the entire egg; dark oval eyes; appendages well developed; heart beating (138 times/min).

Stage IV: June 20 to 24, 1968; freshly hatched young larvae; hatching occurred at the rate of 1,500 individuals per night for a period of 5 days.

Details on the methods used have been published earlier (PANDIAN, 1970a). A definite number of eggs or larvae was counted, washed free from adhering sea water by exposure to distilled water (twice, 45 sec each time) and weighed; they were then dried at 80 °C for a period of 5 h. All weighings were made in a Sartorius balance (Type 2604; sensitivity 10 µg). Ash was estimated by incinerating the sample at 560 °C for 5 h. Calorific content was determined in a semimicro bomb calorimeter (Parr Instrument Co.; Model 1412).

Results

Changes in composition and calorific content

Water content amounts to 56.2% of the living matter, ash to 5.8% of the dry matter in the freshly attached egg (stage I); the freshly hatched larvae

* Dedicated to my colleague K.-H. SCHUMANN (Biologische Anstalt Helgoland) who died on December 12, 1969 due to a diving accident.

(stage IV) contains 86.8% water and 21.2% ash (Table 1). Increase in water is rather slow during the early stages (from 56.2% in stage I on November 8 to 57.4% in stage II on December 12); a few days prior to hatching, there occurs a remarkable increase in water content (from 73.9% in stage III on June 16 to 86.8% in stage IV on June 20). Like water, ash also increases markedly in later stages (from 8.5% in stage III to 21.2% in stage IV) than in earlier stages (5.8 and 6.6% in stages I and II, respectively).

Table 1. *Homarus americanus. Average changes in water and ash contents in developing eggs and larvae. Brackets indicate the number of estimations made*

Developmental stage	Mean water content (% of live weight)	Mean ash content (% of dry weight)
Stage I (egg)	56.2 ± 0.6 (3)	5.8 ± 0.1 (3)
Stage II	57.4 (2)	6.6 ± 0.4 (2)
Stage III	73.9 ± 0.9 (3)	8.5 ± 0.8 (3)
Stage IV (larva)	86.8[a] ± 0.2 (5)	21.2 ± 5.1 (8)

[a] Data based on larvae hatched on first day only.

Table 2. *Homarus americanus. Average changes in calorific content of developing eggs and freshly hatched larvae*

Developmental stage	No. of estimates	Energy content (cal/g dry weight)	Coefficient of variation (%)
Stage I (egg)	3	6636 ± 116	1.7
Stage II	4	5963 ± 146	2.5
Stage III	3	5619 ± 55	1.0
Stage IV (larva)	10	4292 ± 417	9.7

Table 2 shows changes in calorific content of lobster eggs during the various developmental stages. Calorific content is 6636 cal/g dry weight in freshly laid eggs (stage I); it progressively decreases to 5619 cal/g dry weight in stage III, and to 4292 cal/g dry weight in stage IV. During the period from June 16 to June 20, rates of net water and salt intake considerably increased (Fig. 1); such active absorption of water and salt seems to mark a very high metabolic activity, since it is accompanied by heavy depletion of calorific contents of the egg (from 5619 to 4292 cal/g dry weight).

Yolk utilization

For a better understanding of embryonic growth and metabolism, it is necessary that the data presented are related to the weight of a single egg or larva. The average dry weight of a single egg is 965 μg, that of a larva 900 μg (Table 3); thus, total loss during the whole development amounts to 65 μg. Of this, a major portion is oxidized in order to meet the energy requirements of embryonic metabolism, and the remainder — i.e. the egg membrane — is sloughed off during eclosion. In the European lobster *Homarus gammarus* the egg membrane weighs 5.3% of the dry weight of an egg ready to hatch, and the membrane contains 4049 cal/g dry weight (Pandian, 1970a). Using these two values for the egg membrane of the corresponding egg stage of *H. americanus*, it was calculated that the dry weight of a single egg membrane is 48.8 μg which is equivalent to 0.18 cal.

From the values presented in Tables 1 to 3, average changes in composition and calorific content

Table 3. *Homarus americanus. Average changes in dry weight of a single developing egg*

Developmental stage	No. of estimates	No. of eggs or larvae	Mean dry weight of one egg or larva (μg)	Coefficient of variation (%)
Stage I (egg)	3	300	965 ± 16.2	1.7
Stage II	3	150	956 ± 7.0	0.7
Stage III	6	300	920 ± 16.2	1.8
Stage IV (larva)	10	550	900 ± 52.0	5.8

Table 4. *Homarus americanus. Average changes in composition and calorific content of a single egg. All weights in μg. Data based on Tables 1 to 3*

Parameter	Stage I (egg)	Stage II	Stage III	Stage IV (larva)
Live weight	2203	2245	3524	6815
Water	1238	1289	2604	5915
Dry weight	965	956	920	900
Ash	56.0	63.1	78.2	190.7
Organic substance	909.0	892.9	841.8	709.3
Energy (cal/egg)	6.40	5.70	5.17	3.86

of a single egg from stage I to stage IV are shown in Table 4. During embryonic development their occurs a steady increase in water (from 1238 to 5915 µg) and ash (from 56.0 to 190.7 µg) contents, and a progressive decrease in energy content (from 6.4 to 3.86 cal).

The cumulative yolk utilization efficiency during the total development is 93.3% for dry weight; the corresponding values for organic substance and energy are 78.0 and 60.3%, respectively.

Effects of hatching time

The values obtained for ash (Table 1), calorific content (Table 2) and dry weight (Table 3) of the freshly hatched larvae (stage IV) deviate considerably from the respective means, indicating a greater variability among the different samples of larvae. For instance, the deviation range from the mean calorific content of stages I to III is less than 2.5%, while that of freshly hatched larvae is 9.7%, i.e. nearly 5 times more than those of all previous stages.

How can we explain the increased variability in stage IV? The Canadian lobster hatches at the rate of 1,500 larvae per night (around 10 p.m.) for a period of 4 to 5 days. Larvae which hatched on the first, second and fourth day were separately subjected to dry weight, ash and calorific content analyses; the values obtained are given in Table 5. The larvae which hatched on the first day contain 4656 cal/g dry weight, while those hatched on the second and fourth day contain 4383 and 3837 cal/g dry weight, respectively. Ash content, which is only 16.7% in larvae of the first hatching day increases, it amounts to 20.2 and 26.7% in larvae hatched on the second and fourth day, respectively. In other words, while the ash content of a larvae hatched on the first day amounts to only 143 µg, it is 180 and 255 µg for larvae hatched on the second and fourth day (Fig. 1). A considerable part of the salts absorbed seems to be deposited on the integument; for the integument, when felt with a needle, proved to be harder in larvae hatched on subsequent days than in those hatched during the first day.

A day or two prior to hatching, a continuous rapid inflow of salts into the developing eggs considerably alters the dry weight of the larvae hatching on different days. Thus, the mean dry weight of a single larva increases from 854 µg in first-day hatchers to 889 and 956 µg for second and fourth-day hatchers, respectively. Calorific content, which is 3.98 cal for a single larva hatched on the first day, decreases to 3.90 and 3.67 cal in a larva hatched on the second and fourth day; i.e. as much as 0.08 and 0.31 cal must have been expended on metabolism by the larva hatched on the second and fourth day.

Energy expended on metabolism by a single embryo from stage III to IV amounts to 1.19 cal (Tables 4 and 5); of this, substances equivalent to about 0.18 cal are removed in the form of the egg membrane during hatching. During the 4-day period from June 16 to 20, each embryo expended about 0.25 cal/day on metabolism. However, after the first batch of larvae has hatched on the first day (June 20), this high metabolic rate decreases considerably in the embryos yet to hatch during the subsequent days. From June 20 to June 23, an embryo expended only 0.3 cal, or 0.10 cal/day.

Table 5. *Homarus americanus. Average changes in composition and calorific content of freshly hatched larvae hatched on different days. Brackets indicate the number of estimations made*

Larvae hatched on	Dry weight of one larva (µg)	Ash content (%)	Energy content (cal/g dry wt)	Calories per larva
1st day	854 ± 28.6 (5)	16.7 ± 1.95 (3)	4656 ± 104 (4)	3.98
2nd day	889 ± 27.1 (3)	20.2 ± 2.03 (3)	4383 ± 134 (3)	3.90
3rd day	—	—	—	—
4th day	956[a] (2)	26.7[a] (2)	3837 ± 64[a] (3)	3.67
5th day	—	—	—	—

[a] Dr. G.-A. PAFFENHÖFER (Biologische Anstalt Helgoland) estimated and communicated to me these values.

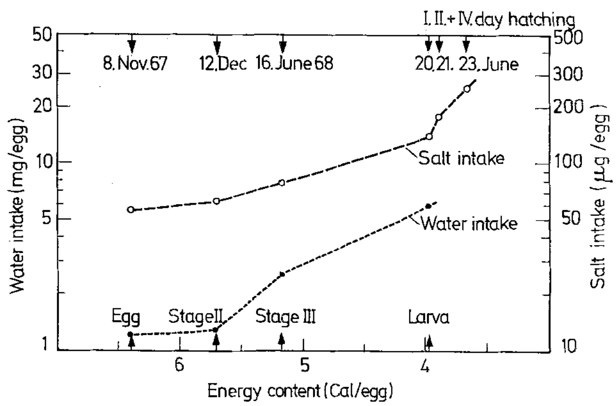

Fig. 1. *Homarus americanus.* Water and salt contents of a single developing egg as a function of the incubation period (expressed in calorific content of the developing egg)

Changes in permeability and hatching

Water metabolism: A single lobster egg (2.20 mg wet weight) requires as much as 4.68 mg water for successful completion of embryonic development. During the period from November 8 (stage I) to December 12 (stage II), there is little change in the water content, indicating that the egg membrane has a low permeability to water (Fig. 1). Just a few days prior to June 16 (stage III), the egg membrane seems to have become more permeable, allowing 1.315 mg water to enter the egg; the permeability of the egg membrane seems to attain maximum values during the ensuing 4 days (from June 16 to 20) since as much as 3.311 mg water is being taken up. Unfortunately, no estimation of water content of the larvae hatched on the subsequent days has been made. Water content of *Balanus balanoides* eggs of (hatching) H^{II} stage is known to reach the maximum level (about 3,300 mg/10^6 eggs) in February and to remain at the same level until hatching occurs in April (BARNES, 1965). It is therefore tempting to suggest that the water content in eggs of *Homarus americanus*, prior to hatching, has not changed any further.

Salt metabolism: Salt is more or less continuously taken up throughout the embryonic development, especially so from stage III onwards. After the first batch of larvae has hatched on June 20, salt is rapidly absorbed by the embryos that will hatch on the subsequent days; the larvae hatched on the fourth day contain almost double the amount of salt (255 μg/larva) as those hatched on the first day (143 μg/larva; Fig. 1).

According to DAVIS (1964), imbibition of water leading to increased internal pressure results in bursting of the egg membrane initiating hatching in the eggs of *Homarus americanus*. The rapid inflow of salts, even after June 20, into the eggs, should have considerably increased the internal osmotic pressure; but why should only 1,500 eggs (with less salts and hence less internal osmotic pressure) hatch on June 20, while the others (with higher internal osmotic pressures due to their increased salt content) hatch on the subsequent days? Obviously, in addition to the internal osmotic pressure, other factors are involved in the initiation of hatching in lobsters. A detailed discussion of this problem has been presented elsewhere (PANDIAN, 1970a).

Discussion

Water, ash and fat are three important variables during the embryonic development of many marine demersal crustaceans. Absorption of water and salts from the surrounding sea water raises the levels of these constituents during development, while (due to oxidation) fat is heavily depleted. Fat depletion is reflected in the decrease of calorific content per unit weight of the egg. The magnitude of dependence on the environment varies considerably among the marine demersal eggs belonging to different species (Table 6). Lobsters accumulate considerable quantities of water and salts during their development, while eggs of shrimps and hermit crabs require only small quantities of water and salts. Like lobsters, *Ligia oceanica*, an inhabitant of the supra tidal zone, requires more than about 60 μg salts per egg weighing 1 mg; however, water requirement of the *L. oceanica* egg is low. While the initial water level of lobster eggs is about 50 to 60 %, that of the *L. oceanica* egg is significantly higher, i.e. 76 %; furthermore, the inflow of water into attached lobster eggs leads to a reduction in specific gravity and floats the larvae. The egg of *L. oceanica*, on the other hand, releases a fully developed juvenile, which is semi-terrestrial; obviously, there is no need to imbibe water and to reduce the specific gravity. Lastly, of the total amount of water required, as much as 90 % is made available through fat oxidation in the *L. oceanica* egg, while metabolic water production amounts to only about 10 % of the total water required in the lobster *Homarus gammarus* (PANDIAN, 1970a, b). Presumably, *L. oceanica*, primarily through initial provision of water in the egg, minimizes its dependence upon environmental water. Nevertheless, it remains dependent upon environmental salts and hence is restricted to the supralittoral zone. Water require-

Table 6. *Water and salt requirements of some marine demersal eggs of crustaceans for successful completion of their embryonic development. Values based on 1 mg wet weight per egg*

Species	Required quantity of water (mg)	Required quantity of salt (μg)	Authors
Homarus americanus	2.12	61.1	PANDIAN (this paper)
Homarus gammarus	1.32	50.4	PANDIAN (1970a)
Crangon crangon	0.84	5.5	PANDIAN (1967)
Eupagurus bernhardus	0.81	15.7	PANDIAN and SCHUMANN (1967)
Ligia oceanica	0.07	67.5	PANDIAN (1970b)

Table 7. *Percentages of depletion of fat and calorific contents in demersal eggs of marine crustaceans during embryonic development*

Species	Calorific content (cal/g dry weight)		Percentage of depletion from the respective initial values	
	eggs	larvae	energy	fat
Homarus americanus	6636	4292	64.7	—
Homarus gammarus	6172	4524	73.3	47.4
Crangon crangon	5915	4544	76.8	33.6
Eupagurus bernhardus	6053	4780	79.0	35.0
Ligia oceanica	5956	4175	70.1	57.8
Average:	6146	4463	72.8	43.5

References as cited in Table 6.

ment of the lobsters is more than 1.3 mg for successful completion of embryonic development of a single egg of 1 mg wet weight, that of the shrimp and the hermit crab is less than 0.9 mg/1 egg of 1 mg wet weight, although the (attached) demersal eggs of all these crustaceans produce planktonic larvae. Possibly, the small larvae of shrimp and crab (less than 100 µg) require less reduction in specific gravity than the heavier lobster larvae (about 7 to 8 mg).

On an average, fat is depleted to about 44% of its initial value (Table 7); the corresponding value for the calorific content is 73%. Since the calorific content of *Homarus americanus* eggs is depleted to 65% of its initial value (Table 7), it is conceivable that the primary energy source for the embryonic metabolism of this lobster is fat oxidation. During the development of a number of marine crustaceans fat serves as main energy source, e.g. *Crangon crangon*, *Homarus gammarus*, *Ligia oceanica* (PANDIAN, 1967, 1970a, b) and *Eupagurus bernhardus* (PANDIAN and SCHUMANN, 1967). The advantages of employing fat as main energy source during embryonic development of marine demersal eggs have been discussed elsewhere (PANDIAN, 1969, 1970a, b, c).

Hatching of *Homarus americanus* over a period of about 5 days results in variations in composition and calorific content of the larvae. The two major variations are: (1) Accumulation of salts in the eggs hatching on subsequent days results in increases of ash contents from 16.7 to 26.7% (from 143 to 255 µg/larva); hence the dry weight of a single larva increases from 854 to 956 µg (Table 5; Fig. 1). A considerable part of the salts accumulated seems to be deposited in the integument; consequently, these larvae are harder. (2) Metabolic rate of embryos hatching during the subsequent days depletes the calorific content both per unit weight (from 4656 to 3837 cal/g) and per larva (from 3.98 to 3.67 cal).

Such variations due to hatching in batches over a period of 7 days has also been reported in *Homarus gammarus* (PANDIAN, 1970a). Hatching of larvae in batches (the interval between successive hatching periods may vary) is not uncommon in other crustaceans. It is customary for fish physiologists to consider the time span as incubation period, during which 50% of the eggs hatch; even at constant conditions, all eggs spawned by the same fish and at the same time, do not hatch simultaneously (e.g. FLÜCHTER and PANDIAN, 1968). Fish or crustacean larvae hatched early contain more energy and, hence, may be able to withstand adverse situations better than the ones hatched at a later date. In regard to the lobster, a larva that hatched earlier contains more energy but has a softer integument, while a larva hatched later has less energy but a hard (strong) integument (with greater amounts of salt deposited) to withstand critical situations.

Summary

1. Yolk utilization efficiency and effects of hatching time have been investigated by estimating the changes in composition and calorific content in developing eggs and freshly hatched larvae of the Canadian lobster *Homarus americanus* MILNE-EDWARDS.

2. During embryonic development water content of the egg increases progressively (from 56.2 to 86,2%); the same applies to ash (from 5.8 to 21.2%); the energy content decreases (from 6636 to 4292 cal/g dry weight).

3. Average dry weight of a single egg is 965 µg (equivalent to 6.40 cal). On an average, a freshly hatched larvae utilizes as much as 60.3% of the energy contained in the egg.

4. The lobster hatches at the rate of about 1,500 larvae per night (around 10 p.m.) over a period of 4 to 5 days. Larvae hatched on different days display considerable variations in dry weight, ash and calorific contents. After some of the larvae hatch on the first day, absorption of salts by remaining eggs leads to a steady increase in ash content from 16.7 to 26.7% (or from 143 to 255 µg salts/larva); hence, the dry weight

of a single larva increases from 854 to 956 μg. Metabolic rate of embryos (0.1 cal/day) that hatch on subsequent days depletes the calorific content per unit weight (from 4656 to 3837 cal/g) as well as per larva (from 3.98 to 3.67 cal).

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